Public Written Comments

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As a follow-up to his oral Public Comments at the April 4, 2014 PCAST meeting, Dr. Price provided the following attachments for PCAST Members (Pages 3 – 81).

Topic: The Scientific Evidence Linking the Overuse of Antibiotics in Animal Agriculture to Antibiotic Resistance

CHANGES IN INTESTINAL FLORA OF FARM PERSONNEL AFTER INTRODUCTION OF A TETRACYCLINE-SUPPLEMENTED FEED ON A FARM

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Abstract A prospective study was undertaken to determine whether feeding farm animals antibiotics in feed caused changes in the intestinal bacterial flora of farm dwellers and their neighbors. Chickens were fed tetracycline-supplemented feed (tet-feed), and, as expected, within one week their intestinal flora contained almost entirely tetracycline-resistant organisms. Increased numbers of resistant intestinal bacteria also appeared, but more slowly, in farm members, but not their neighbors. Within five and six months, 31.3 per cent of weekly fecal samples from farm dwellers contained >80 per cent tetracycline-resistant bacteria as compared to 6.8 per cent of the samples from the neighbors ($P<0.001$). Seven of the 11 farm members, but only three of the 24 neighbors, had two or more fecal samples containing >80 per cent tetracycline-resistant coliforms ($P<0.01$). These resistant bacteria contained transferable plasmids conferring multiple antibiotic resistances. Selective pressure by tet-feed for antibiotic-resistant bacteria in chickens extends to human beings in contact with chickens and the feed. (N Engl J Med 295:583-588, 1976)

THE prevalence of antibiotic-resistant bacteria in the environment has caused widespread concern.[1,2] Besides the difficult problem of treating clinical infection caused by antibiotic-resistant organisms, there is the threat posed by the potential transfer of resistances from nonpathogenic to pathogenic bacteria. The genetic information for antibiotic resistance resides primarily on extrachromosomal DNA elements, called R plasmids (R factors), many of which can be transferred from bacterium to bacterium even between different bacterial species.[3] Numerous reports in the literature have demonstrated the worldwide prevalence of resistance plasmids in bacteria in hospitalized and ambulatory persons,[1,4-6] in domestic and farm animals[1,7,8] and in food products.[9-12]

Antibiotics select for resistant bacteria in the animals and human beings taking the drug.[1,13,14] Many of these resistances are present on transferable plasmids. It has been suggested that one factor contributing to increased resistant bacteria in human beings is the selective pressure of antibiotics in animal feeds.[1,2,7,15,16] Anderson and Lewis linked an antibiotic-resistant *Salmonella typhimurium* to infection in cattle and human beings.[17] Evidence has suggested animal-to-human spread of resistant bacteria,[1,2,7,15] and this possibility has recently been documented with use of a biochemically marked plasmid and host bacterium.[18] However, a cause-and-effect relation between animal drug feeds and antibiotic-resistant strains in human beings is unclear, since antibiotics in different forms are used so frequently in animal and human groups.

To examine in a controlled manner what effect an antibiotic-supplemented feed given to animals had on the farm inhabitants, we designed the present study and recorded the changes in bacterial intestinal flora of chickens, farm dwellers and their neighbors before and after the introduction of a tetracycline-supplemented feed to the farm.

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MATERIALS AND METHODS

Animals

Three hundred and twenty-five one-day-old Leghorn chickens purchased from SPAFAS (Connecticut) were received on a private farmstead in Sherborn, Massachusetts, on July 1, 1974. No antibiotic-containing feed products had been used in the area during at least the previous seven-year period. The chickens were raised to one month in a small brooder (1.4 m²) heated with accessory lamps. They were maintained in the absence of antibiotics. Over 90 per cent of the chickens lived and were vaccinated twice against respiratory virus during this time. The vaccine was prepared without the antibiotics (penicillin and streptomycin) that are routinely added to commercial vaccines. Animals were fed commercial starter mash (Farm Bureau brand) for the first month. This feed contained the coccidiostat amprolium (0.005 per cent) and bacitracin. After the first month, the chickens were given a pullet developer (Purina) that contained no drugs. Each new lot of feed was tested for the absence of antibiotic activity by extraction of 1 g of feed with 5 ml of water and study of this extract for inhibition of growth of sensitive *Escherichia coli* on nutrient agar plates. From the age of three months, chicken feed with and without tetracycline (oxytetracycline) (100 g per 909 kg) (Pfizer) was given. Only extracts of the tet-feed showed antibacterial activity.

Human Subjects

The resident farm family consisted of two parents and nine children (4 to 20 years old). Five families (24 members, including 10 adults and 14 children) living within an 8-km radius of the farm, without direct contact with the chickens or with any antibiotic-supplemented feed, acted as a control group. Two people in the laboratory, and 10 volunteer Tufts University Medical School students also participated in the study; none of them were taking antibiotics.

Distribution of Chickens on the Farm

At two months of age the chickens were placed into six newly-constructed cages (9.4 m², approximately 50 chickens per cage). Four groups were raised inside a barn of 900 m², and two groups were housed outside. At each end of the 60-meter long barn two cages were constructed approximately 15 meters apart. Males and females were evenly distributed. Each chicken was identifiable by a number tagged to its foot. Chickens housed in two cages at one end of the barn and in one cage outside the barn were placed on tet-feed during the course of the study.

Stool Sampling and Plating

We obtained samples of chicken feces by introducing and rotating a sterile moistened swab (Handi Swab, Fisher Scientific) 6 to 8 cm into the cloacus of the chicken. The swab was placed in holding medium (Stuart's medium) for delivery to the laboratory for plating. These specimens were streaked onto MacConkey, phenyl ethyl alcohol and Hektoen plates and incubated at 30 to 37°C for 18 to 24 hours. Approximately one quarter to one half of the chickens in a cage were sampled each week. In general each chicken was sampled every other week. Human fecal specimens were also obtained with use of the sterile swab. Chicken fecal samples were plated within two to six hours after being obtained; human specimens were plated within two to 12 hours. Prior studies in the laboratory indicated that samples plated during this period did not differ from those plated immediately. Since most of the stool samples were taken from the anal canal, not directly from a stool sample, this method of sampling was compared with direct sampling of feces. No difference in numbers or kinds of bacteria was observed between two fecal samples taken from the same chicken and held in holding medium: one taken immediately after excretion and the other obtained by cloacal swab. Likewise, no difference was observed in the bacterial flora or the antibiotic-resistance pattern when three fecal specimens from the same human subject were compared: one plated immediately after excretion; one stored in holding medium for one hour at 25°C; and one taken from the rectum before the stool was passed and also stored in holding medium for one hour at 25°C. Within the limits of individual stool specimens, then, the method of obtaining the specimen appeared valid for that time and day.

To test the possibility that R plasmid-containing bacteria were unable to convert from anaerobic in vivo conditions to aerobic culture conditions for testing, animal fecal specimens were incubated in duplicate under aerobic or anaerobic conditions. The same numbers of colonies and the same resistance patterns were found aerobically and anaerobically on MacConkey agar plates. Aerobic and anaerobic analysis of fecal samples containing large numbers of R plasmid-containing *Esch. coli* showed no differences. R plasmids did not influence the ability of *Esch. coli* to convert to aerobic conditions.

Bacteriology

Routine bacteriologic analyses to identify organisms were performed on all fecal specimens from the chickens and human beings living on the farm.[19] In the initial bacterial studies two to four morphologically different colonies representing the predominant organisms were isolated from each of the three selective plates. These organisms were identified and classified by means of biochemical methods and were subsequently characterized for antibiotic sensitivity with use of antibiotic disks (Pfizer Diagnostics) on Mueller–Hinton plates.[20] Antibiotic sensitivity was tested to ampicillin (10 μg), carbenicillin (50 μg), chloramphenicol (5 μg and 30 μg), cephalothin (30 μg), streptomycin (10 μg), tetracycline (5 μg and 30 μg), sulfonamides (300 μg), trimethoprim-sulfamethoxazole (25 μg), gentamicin (10 μg) and neomycin (5 μg). When tet-feed was introduced on the farm, bacterial samples were replica-plated[21] onto MacConkey agar supplemented with tetracycline (25 μg per milliliter). By two-dimensional dilutional spreading of the initial stool specimen on the MacConkey plate, several hundred colonies could be observed and replica-plated, and the percentage of tetracycline-resistant organisms could be determined.

Characterization of Antibiotic Resistance

The patterns and types of antibiotic resistance were checked in coliforms. The transferability of drug resistances in *Esch. coli* was determined by overnight bacterial mating. The recipient was DO-11, a nalidixic acid-resistant derivative of *Esch. coli* CSH-2. Donor (final concentration 1.5×10^7 per milliliter) and recipient (final concentration 1.5×10^8 per milliliter) were incubated together overnight at 37°C in 10 ml of nutrient broth. The mixture was subsequently plated onto different selective plates to examine transfer of individual resistances. Recombinants were checked for coincident transfer of other resistance markers.

Results

Changes in Intestinal Flora after Introduction of Tet-Feed

Before tet-feed was given, *Esch. coli, Proteus mirabilis* and enterococci were the predominant organisms obtained from aerobic cultures of chicken and human feces. The relative quantities of each varied, but in both chicken and human specimens, *Esch. coli* was predominant (80 to 90 per cent) among the enterobacteria. Other enterobacteria in the chicken cultures included *Klebsiella pneumoniae, Enterobacter agglomerans,* pseudomonas and acinetobacter. No salmonella was detected. Other organisms obtained from human beings were *K. pneumoniae, Citrobacter freudi, Enterobacter cloacae* and *hafniae, Proteus rettgeri* and acinetobacter.

The feces of chickens contained low numbers of organisms resistant either to tetracycline, streptomycin or sulfonamides. Of the *Esch. coli* generally less than 10 per cent were resistant to tetracycline, and many fecal samples contained less than 0.1 per cent tetracycline-resistant organisms. Among farm personnel and neighbors the numbers of tetracycline-resistant organisms were relatively low from the beginning of the study. Over 90 per cent of the fecal samples contained less than 10 per cent tetracycline-resistant *Esch. coli,* and most of them had levels less than 0.1 per cent. All proteus and enterococci were resistant to tetracycline.

The tet-feed was received on the farm in September, 1974, and was given to the chickens during the second week of October. The amount of tetracycline in the feed was at a level used for therapy or prophylaxis. The intestinal flora of chickens changed rapidly within 36 to 48 hours from low numbers of resistant organisms to high numbers (>60 per cent) with tetracycline resistance. Within two weeks 90 per cent of the chickens were excreting essentially all tetracycline-resistant organisms. No change in the frequency of organisms was observed: *Esch. coli* remained predominant. Chickens not fed tet-feed maintained low numbers of resistant intestinal bacteria until four months after tet-feed was introduced into the barn, when the intestinal flora of over 30 per cent of the chickens inside the barn contained >50 per cent resistant bacteria. Bacteria were observed that were resistant to antibiotics other than tetracycline, including ampicillin, carbenicillin, streptomycin and sulfonamides. All chickens in the same cage had *Esch. coli* with similar antibiotic-resistance patterns, but patterns and frequencies differed among the cages.

During the first three months after introduction of tet-feed, monthly samples from the family and neighbors showed little difference in the number of individual samples containing high numbers (>80 per cent) of tetracycline-resistant organisms. However, the average number of tetracycline-resistant bacteria was

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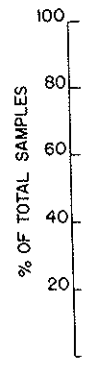

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higher in the farm group. In January the fecal samples from three of eight family members contained >80 per cent tetracycline-resistant bacteria with an average number of resistant organisms in fecal samples from the family members of 36 per cent. In contrast, only one of 15 neighbors sampled was excreting high numbers of resistant bacteria, and the average number of resistant organisms in this group was <10 per cent.

To control for seasonal variation and to assess at a period more regular than once a month, weekly samples were taken from the farm family and neighbors during the fifth and sixth months (March and April) after introduction of tet-feed to the chickens. Each subject was sampled at least six times, and in most cases for nine weeks. Ten members of the first-year Tufts medical-school class and two members of the Tufts laboratory in Boston were similarly tested and served as controls outside the community. The results showed increased tetracycline resistance in the intestinal bacteria from farm personnel (Fig. 1). Of the 83 fecal samples received from the 11 members of the farm family, 31.3 per cent contained >80 per cent tetracycline-resistant organisms, primarily *Esch. coli*. In contrast, 0 to 12.1 per cent of the samples from any neighboring family showed high numbers (>80 per cent) of resistant organisms, and the average from all the neighboring families was 6.8 per cent. Furthermore, the overall number of tetracycline-resistant organisms in the samples from the neighbors was lower than that in those from the farm. Samples from the Boston group closely resembled those of the Sherborn neighbors in that only 3.8 per cent of the samples showed >80 per cent organisms resistant to tetracycline. Assuming that each sample is independent, the probability of getting a result this extreme is <0.001.

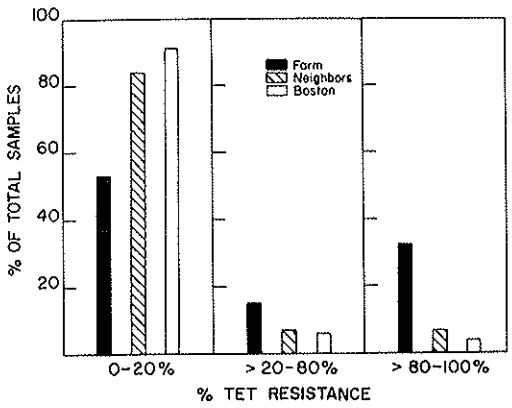

Figure 1. Proportion of Tetracycline-Resistant Bacteria in Fecal Samples from Farm Family (83), Neighbors (189) and Boston Group (79) during a Nine-Week Period, Five and Six Months after Tetracycline Was Introduced onto the Farm.

Resistance of 0 to 20 per cent is regarded as within the normal range.

Seven of the farm personnel and family members produced two or more fecal samples with >80 per cent tetracycline-resistant organisms as compared to only three of 24 neighbors ($P<0.01$). Moreover, four of the subjects on the farm, but only one of the neighbors, had consecutive weekly elevated numbers (>80 per cent) of tetracycline-resistant bacteria.

Emergence of Multiply Resistant Bacteria in Chickens and Human Beings after Exposure to Tet-Feed

With lengthened time of exposure of chickens to tet-feed, there was an increased frequency of *Esch. coli* carrying resistance to unselected antibiotics, including streptomycin, ampicillin, carbenicillin and sulfonamides (Fig. 2). Resistance to ampicillin and carbenicillin was not detected in chickens before their ingestion of tet-feed and was found only in chickens housed inside the barn. Multiple resistance was found in more than 50 per cent of the *Esch. coli* strains from chickens eating tet-feed for more than 10 weeks. After two months on tet-feed, over 20 per cent of the *Esch.*

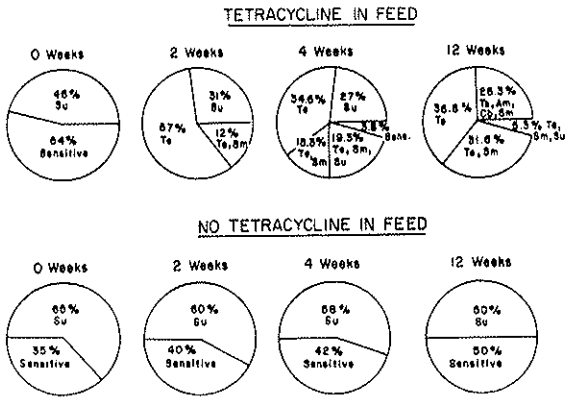

Figure 2. Effect of Time of Exposure to Tet-Feed on Predominant *Esch. coli* in Intestinal Flora of Chickens Housed inside the Barn.

From weekly samples from 10 to 20 chickens, predominant coliforms were isolated on MacConkey plates and were tested for antibiotic sensitivity. Resistances were noted to sulfonamides (Su), tetracycline (Te), streptomycin (Sm), ampicillin (Am) and carbenicillin (Cb).

coli organisms from chickens inside the barn carried the pattern of resistance to tetracycline, ampicillin, carbenicillin and streptomycin. In the outside cage, over 25 per cent of bacteria cultured from tetracycline-fed chickens showed resistance to tetracycline in association with streptomycin and sulfonamides.

Similarly, increased multiply resistant *Esch. coli* were noted in the farm dwellers, but not in neighbors, during the later months of tet-feed administration on the farm (Table 1). Over the period examined, 36 per cent of bacteria from the farm family showed resistance to three or more antibiotics, as compared to 6

Table 1. Antibiotic-Resistance Pattern of *Esch. coli* Strains Cultured from Feces.*

Mo	No. of People Sampled	Total Samples	Sensitive Strains	No. of Resistant Strains																
				Te	N	Sm	Te Sm	Te Su	Sm Su	Am Cb	Te Sm Su	Te Sm N	Sm Cm Su	Te Sm Su N	Te Am Cb	Am Cb Sm Su	Te Am Cb Sm Su	Te Am Cb N Sm Su	Te Am Cb Sm SXT Su
Farm family:																				
Oct	6	6	2	2		1	1									1	1	1		
Nov	8	13	6	1	1	2										3	2	2	2	
Dec	9	17	5		1		2									2	3			
Jan	10	20	9		2	1	1					1			1	4	1			
Feb	8	14	4	3		1							1			10	7	3	2	
Totals		70	26	6	4	5	4	0	0	0	0	1	1	0	1					
Neighbors:																				
Oct	12	14	10				2		1					1						
Nov	3	4	2							2										
Dec	7	13	8					1		4										
Jan	15	26	22				1		1	1	1									
Feb	5	9	6							1	2									
Totals		66	48	0	0	0	3	1	2	8	3	0	0	1	0	0	0	0	0	

*Te denotes tetracycline, N neomycin, Sm streptomycin, Su sulfonamides, Am ampicillin, Cb carbenicillin, Cm chloramphenicol, & SXT trimethoprim + sulfamethoxazole.

per cent from the neighbors. Resistance to more than four drugs was found only in farm dwellers. During October to February only 18 of 41 (44 per cent) of predominant *Esch. coli* strains isolated from the farm family were sensitive to antibiotics in contrast to 34 of 42 (81 per cent) from neighbors.

Transferability of Antibiotic Resistance in Chickens and Human Beings

Bacterial matings were performed to determine whether the genes mediating resistance were transferable to other *Esch. coli*. Tetracycline resistance when found alone in *Esch. coli* in chickens or human beings was rarely transferable. Only four of the 41 isolates from chickens tested showed transfer, and none of 17 isolates from human subjects. As this resistance gene became associated with other resistances, its transfer became more easily detected. When associated with streptomycin resistance in *Esch. coli* from chickens, tetracycline resistance transferred from about 70 per cent of the donors, approximately half the time with streptomycin resistance (Table 2). Transfer of the tetracycline resistance gene was not detected when in combination with sulfonamide resistance, but transfer was detected from about 30 per cent of the donor bacteria when in combination with resistance to streptomycin and sulfonamides. When associated with resistance to streptomycin, ampicillin and carbenicillin, tetracycline resistance was transferred from every donor tested. All resistant organisms were transferred from the donor en bloc, indicating their presence on a single R plasmid.

A more varied pattern of antibiotic resistance was seen in the human samples (Table 1); however, *Esch. coli* strains were found with resistance patterns similar to those observed in chickens. In general, one or more of the resistances were transferable; in many, resistances were transferred separately. Tetracycline and streptomycin resistances were transferred together, as was seen in the *Esch. coli* from chickens. Ampicillin resistance, found in combination with resistance to many other antibiotics, was transferred over 90 per cent of the time, always in association with carbenicillin resistance.

Table 2. Transfer of Antibiotic Resistance from *Esch. coli* Isolated from Chickens.*

Resistance Pattern	No. of Strains Tested	Resistance Transferred
Te	41	Te(4)† 9.7%
Sm	4	Sm(2) 50%
Te, Sm	14	Te(2) 14.3%; TeSm(8) 57%
Te, Su	12	(0) <8%
Te, Sm, Su	10	TeSm(3) 30%
Te, Sm, Am, Cb	15	TeSmAmCb(15) 100%

*Te denotes tetracycline, Sm streptomycin, Su sulfonamides, Am ampicillin, & Cb carbenicillin.

†Figures in parentheses represent no. of strains.

Effect of Eating Eggs from Tet-Fed Chickens on Consumers' Intestinal Flora

Over a two-month period beginning four months after introduction of the tet-feed, three neighbor families (18 family members) consumed eggs daily only from tet-fed chickens whereas two neighbor families (six members) received eggs from non-tet-fed chickens. Boston consumers ate commercial eggs. No effect on intestinal flora was noted from ingestion of eggs from tet-fed chickens: no change or difference in flora was found between the two groups. Eggs carefully washed before distribution were also tested for presence of resistant organisms on the shells; none were found.

Effect of Removal of Tet-Feed from the Farm on the Intestinal Flora of Farm Dwellers

To evaluate the reversibility of the increased number of tetracycline-resistant organisms found in farm dwellers, we recultured stools of 10 of the farm residents twice during the third week of January, 1976, six months after removal of all tet-feed from the farm. Duplicate samples were the same. No detectable tetracycline-resistant organisms (<1 per cent) were found in the intestinal flora of eight out of 10 subjects; one had 5 per cent resistant organisms, and 1 had >80 per cent. These frequencies were much lower than those found before removal of the tet-feed and lower than that found in January of the previous year. They resembled those found in the neighbor population.

Discussion

An increase in resistant intestinal bacteria was found in farm dwellers in contact with tetracycline-fed chickens and the tet-feed. Although this change was seen in the chickens within a week of tetracycline ingestion, it was not obvious in the farm personnel until three to five months after tet-feed was introduced on the farm. Similarly, chickens maintained on tet-free feed inside the barn did not show a change in flora until four to five months after introduction of tet-feed on the farm. The gradual increase in resistance in this non-tet-fed group presumably indicated exposure with time to the antibiotic on the farm. Since the most notable change in human flora was an increase in the number of fecal samples containing almost entirely (>80 per cent) tetracycline-resistant organisms, and since the resistant organisms cultured from the human subjects did not all show the same resistance patterns as those from chickens, we assume that this conversion to resistance resulted from contact with tetracycline in the feed and environment. Transfer of resistant organisms between chickens and man, aided by the selective influence of tetracycline in the environment, could also have contributed.[18]

During the same test period, low numbers of resistant organisms were found in two control groups: neighbors living in the same area and attending the same schools, but with little contact with the animals or feed; and an urban (Boston) group not directly exposed to antibiotics. The fact that an occasional weekly sample in some control persons showed large numbers of tetracycline-resistant organisms suggests unrecognized ingestion of a tetracycline, possibly in food, at some time before the sampling.

Previous studies have reported more frequent drug resistance in bacteria isolated from human beings on farms with livestock given antibiotic feed than from those on farms without animals.[22-24] One report matched antibiotic-resistant patterns of *Esch. coli* from farm families with those from their animals.[25] These studies have recorded such differences many years after multiple drug usage on these farms. Our study now records prospectively a change with time in the intestinal flora of farm dwellers as compared with neighbors in the presence of one antibiotic feed and in the known absence of any other antibiotic usage. The change occurred within four to six months after introduction of the feed to the farm and did not occur in the neighbors. This apparent selection for resistant organisms was reversible after nine months' usage of tet-feed on the farm since it was not present six months after removal of the tet-feed from the farm.

The rapid conversion of animal intestinal flora to drug resistance after animal ingestion of antibiotic-supplemented feed has been described.[13,26,27] Such a finding was seen in our studies with tet-feed. The predominant organism, however, remained *Esch. coli* despite the presence originally of 10 to 20 per cent tetracycline-resistant strains of *P. mirabilis*. This observation suggests that *Esch. coli* have a selective advantage in the intestinal tract over *P. mirabilis*. The different antibiotic-resistance patterns of bacteria from the same chickens fed tetracycline inside and outside the barn suggest an environmental influence, as well, on the intestinal flora. All chickens in a cage showed similar intestinal flora, illustrating a communal reservoir of organisms.

Patients being treated with tetracycline were found to excrete fecal coliforms with multiple resistance patterns.[14] In previous farm studies, multiple resistance patterns were found in bacteria isolated from animals, but the use of many drugs could have led to this finding. We have been able to observe prospectively in this natural farm setting the rapid selection by one drug, tetracycline, for resistance to other unrelated antibiotics. After three to four months' exposure to tetracycline on the farm, chickens and human subjects excreted bacteria that were resistant to other antibiotics, but linked with tetracycline on a plasmid. In the chicken, we had detected low numbers of organisms resistant to sulfonamides, tetracycline and streptomycin before introduction of tet-feed. We assume that these organisms were then selected by tet-feed, but we do not know why the multiply resistant organisms should increase in frequency over those with single tetracycline resistance. The origin of resistance to ampicillin-carbenicillin is also unclear. Furthermore, in the chickens, where alimentation could be controlled, we noted more frequent transfer of the tetracycline-resistant gene as the number of resistance patterns in *Esch. coli* increased.

No change in intestinal flora was seen in persons eating eggs from tet-fed chickens. During the time of this study, no sickness occurred that was associated with resistant organisms. However, the resistant intestinal bacteria represent a reservoir of transferable resistance genes. The tetracycline gene is associated with transfer genes on many plasmids, and, in fact, many resistances were transferred linked with resistance to tetracycline. The possible transfer of these

plasmids from nonpathogenic to pathogenic bacteria or from animal to man must be considered a potential consequence of increased resistant bacteria in the farm environment.

The rise in frequency of resistant organisms in our environment is the obvious result of antibiotic usage. The only means to curtail this trend is to control the indiscriminate use of these drugs. All areas of antibiotic usage deserve critical evaluation. Contrasting views have been expressed regarding the real as opposed to the potential human health hazard of resistant bacteria in animals and the impact of antibiotic-supplemented feeds on this problem.[1,2,7,27,28] The present findings clearly demonstrate, however, that antibiotic-supplemented feed is a factor contributing to the selection of human resistant strains of bacteria. These data speak strongly against the unqualified and unlimited use of drug feeds in animal husbandry and speak for re-evaluation of this form of widespread treatment of animals.

We are indebted to Mary Downing, the Richard Downing family and Sherborn neighbors for co-operation, to David Wilner for help, especially in the initial setting up of this study and raising the chickens, to Dr. Marian Richter, who advised and helped in the bacteriologic studies, to Carol Louik for statistical assistance, and to Pamela Senger for assistance.

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RESEARCH ARTICLE

Staphylococcus aureus CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock

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ABSTRACT **Since its discovery in the early 2000s, methicillin-resistant *Staphylococcus aureus* (MRSA) clonal complex 398 (CC398) has become a rapidly emerging cause of human infections, most often associated with livestock exposure. We applied whole-genome sequence typing to characterize a diverse collection of CC398 isolates ($n = 89$), including MRSA and methicillin-susceptible *S. aureus* (MSSA) from animals and humans spanning 19 countries and four continents. We identified 4,238 single nucleotide polymorphisms (SNPs) among the 89 core genomes. Minimal homoplasy (consistency index = 0.9591) was detected among parsimony-informative SNPs, allowing for the generation of a highly accurate phylogenetic reconstruction of the CC398 clonal lineage. Phylogenetic analyses revealed that MSSA from humans formed the most ancestral clades. The most derived lineages were composed predominantly of livestock-associated MRSA possessing three different staphylococcal cassette chromosome *mec* element (SCC*mec*) types (IV, V, and VII-like) including nine subtypes. The human-associated isolates from the basal clades carried phages encoding human innate immune modulators that were largely missing among the livestock-associated isolates. Our results strongly suggest that livestock-associated MRSA CC398 originated in humans as MSSA. The lineage appears to have undergone a rapid radiation in conjunction with the jump from humans to livestock, where it subsequently acquired tetracycline and methicillin resistance. Further analyses are required to estimate the number of independent genetic events leading to the methicillin-resistant sublineages, but the diversity of SCC*mec* subtypes is suggestive of strong and diverse antimicrobial selection associated with food animal production.**

IMPORTANCE **Modern food animal production is characterized by densely concentrated animals and routine antibiotic use, which may facilitate the emergence of novel antibiotic-resistant zoonotic pathogens. Our findings strongly support the idea that livestock-associated MRSA CC398 originated as MSSA in humans. The jump of CC398 from humans to livestock was accompanied by the loss of phage-carried human virulence genes, which likely attenuated its zoonotic potential, but it was also accompanied by the acquisition of tetracycline and methicillin resistance. Our findings exemplify a bidirectional zoonotic exchange and underscore the potential public health risks of widespread antibiotic use in food animal production.**

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has been described in animals since 1972 (1), but a new lineage, clonal complex 398 (CC398), has emerged among livestock and begun colonizing and infecting humans. Human MRSA infections have been categorized into three groups based on their putative sources: health care-associated MRSA, community-associated MRSA,

and health care-associated MRSA with community onset. A fourth category has recently been added to describe human MRSA cases associated with exposure to livestock (livestock-associated MRSA [LA-MRSA]). Human colonization with LA-MRSA multilocus sequence type 398 (ST398) was first recognized among swine farmers in France and The Netherlands in the early 2000s (2, 3). Since those early reports, ST398 and closely related STs within CC398 have been reported in diverse livestock hosts in many countries around the world (4–8). Human cases of MRSA CC398 have also been increasing rapidly and now account for up to 25% of the total MRSA cases in some parts of The Netherlands (9). Given its rapid emergence and trajectory of increasing importance in humans, the evolutionary history of MRSA CC398 has relevance for the epidemiology of MRSA and global health.

Methicillin-susceptible *S. aureus* (MSSA) CC398 is prevalent among pigs in Europe (10), but the evolution and global dispersal of this group have yet to be clarified. A microarray-based study revealed that the core genomes of 6 CC398 isolates were distinct from those of more than 2,000 *S. aureus* isolates from humans (11), and it has been generally presumed that pigs or other animals are the natural hosts of CC398.

Attempts to better characterize the evolution and epidemiology of CC398 have been hampered by the limited resolution of conventional *S. aureus* typing methods, including multilocus sequence typing (MLST) and *spa* sequence typing. While MLST defines CC398 and is useful for placing CC398 in the context of other *S. aureus* clonal complexes, it is of no use for characterizing variation within the group. *spa* typing has revealed geographic clustering among CC398 isolates in Europe (12, 13); however, the limited number of common *spa* types among CC398 isolates and the potential for homoplasy in the *spa* gene data restrict its phylogenetic utility (14).

Whole-genome sequencing provides a superior genetic fingerprint, which can be used for source tracking and evolutionary studies. Thus far, whole-genome sequencing has been used to study the hospital transmission and spatiotemporal spread of ST239 (15). In this study, we applied whole-genome sequence typing (WGST) to a diverse collection of 89 CC398 isolates to study the origins and evolution of *S. aureus* CC398.

RESULTS

We sequenced the genomes of 88 *S. aureus* CC398 isolates (see Data set S1 in the supplemental material). Among the isolates, we sequenced an average of 2,651,848 bases (standard deviation [SD] = 80,311) at ≥10× coverage. Genomes were sequenced at an average depth of 104.36× (SD = 35.7, using the 2,872,582-base SO385 chromosome as a reference).

Rooting the CC398 tree using ST36 as the outgroup revealed that a cluster of four isolates, characterized by the t899 *spa* type, was the first lineage to diverge from the other CC398 isolates (see Fig. S1 in the supplemental material). Much of the similarity between the t899 cluster and the ST36 isolate was mapped to the same ~123,000-bp region surrounding the *spa* gene. However, this region was incongruent with the phylogenetic signal generated by the rest of the chromosome in the t899 isolates. Comparative genomic analysis with other STs suggested that this region was acquired horizontally from an ST9 donor (see Fig. S2 in the supplemental material). When single nucleotide polymorphisms (SNPs) from this region were excluded from the phylogenetic analysis, the t899 lineage clustered with a more derived clade of European isolates and a clade of human-associated MSSA isolates from France, French Guiana, and the United States was identified as the first divergent CC398 lineage (see Fig. S3 in the supplemental material). This lineage was used to root the final CC398 WGST tree (Fig. 1). With the ST36 genome removed and excluding the SNPs from the 123,000-bp putative horizontally transferred region, we identified 4,238 SNPs, including 1,102 parsimony-informative SNPs with a consistency index (CI) of 0.9591. Among the SNPs, 3,552 were from coding regions (1,071 synonymous and 2,481 nonsynonymous).

The phylogenetic tree presented in Fig. 1 is a highly accurate depiction of the evolutionary relationships among the 89 CC398 strains included in this study (including the reference). The lack of homoplasy among informative SNPs (CI = 0.9591) obviated the need for additional measures of robustness such as bootstrapping (16). The most ancestral lineage (clade I) was composed entirely of MSSA strains from humans in North America, South America, and Europe. In addition, with the exception of one isolate (P23-14_SD4.1), the strains that accounted for the most ancestral lineages within clade II (II group of interest [II-GOI]) were human-associated *S. aureus* from China (including two MRSA strains isolated from Danish adoptees from China). All but one of the livestock-associated strains belonged to clade IIa, which was derived from the human-associated lineages. Clade IIa was composed of several lineages whose evolutionary relationships could not be determined due to poor hierarchical resolution. The lack of resolution was likely due to a rapid radiation following introduction into livestock, as homoplasy was exceedingly rare among the parsimony-informative SNPs. The isolates within clades IIa1 and IIa2 consisted almost entirely of European isolates, while the 10 remaining lineages consisted almost entirely of North American isolates. The IIa1i lineage was dominated by Danish isolates, one-third of which were MSSA, while the IIa1ii lineage consisted largely of MRSA isolates from several European countries, except Denmark. Interestingly, 6 of the 10 smaller lineages included MSSA strains isolated from turkey meat from the United States (IIa-GOI). In this report, we use the term "human associated" for isolates belonging to clade I and clade II-GOI ($n = 19$) and use the term "livestock associated" for isolates belonging to subclade IIa ($n = 70$).

Fifteen different *spa* types were identified among the 89 CC398 isolates, including t011, t034, t108, t567, t571, t899, t1250, t1451, t1793, t2876, t3085, t3625, t5462, t5463, and t5719 (Fig. 1). The two most common *spa* types, t011 and t034, represented 67% of the isolates. While some *spa* types were more common within individual clusters (e.g., t571 was disproportionately common among human-associated isolates), *spa* types were inconsistent with the overall CC398 phylogeny (Fig. 1).

Sixty-one percent (30/49) of the CC398 MRSA isolates harbored staphylococcal cassette chromosome *mec* element (SCC*mec*) subtype Vc (5C2&5) containing the cadmium-zinc resistance gene *czrC*. All of the SCC*mec* Vc (5C2&5) cassettes were present in LA-MRSA strains. Of note, the *czrC* gene was also found in two livestock-associated MSSA isolates. The remaining LA-MRSA isolates carried SCC*mec* (sub)types IVa (2B), IVa (2B&5), IVc (2B), Vb (5C2&5), V*, and V**; a novel VII-like SCC*mec* cassette; and a nontypeable (NT) SCC*mec* cassette. The *mecA* gene was detected in only two human-associated isolates; in both cases, the *mecA* gene was coded within SCC*mec* subtype Vb (5C2&5). The type V* and V** SCC*mec* cassettes contained structurally dif-

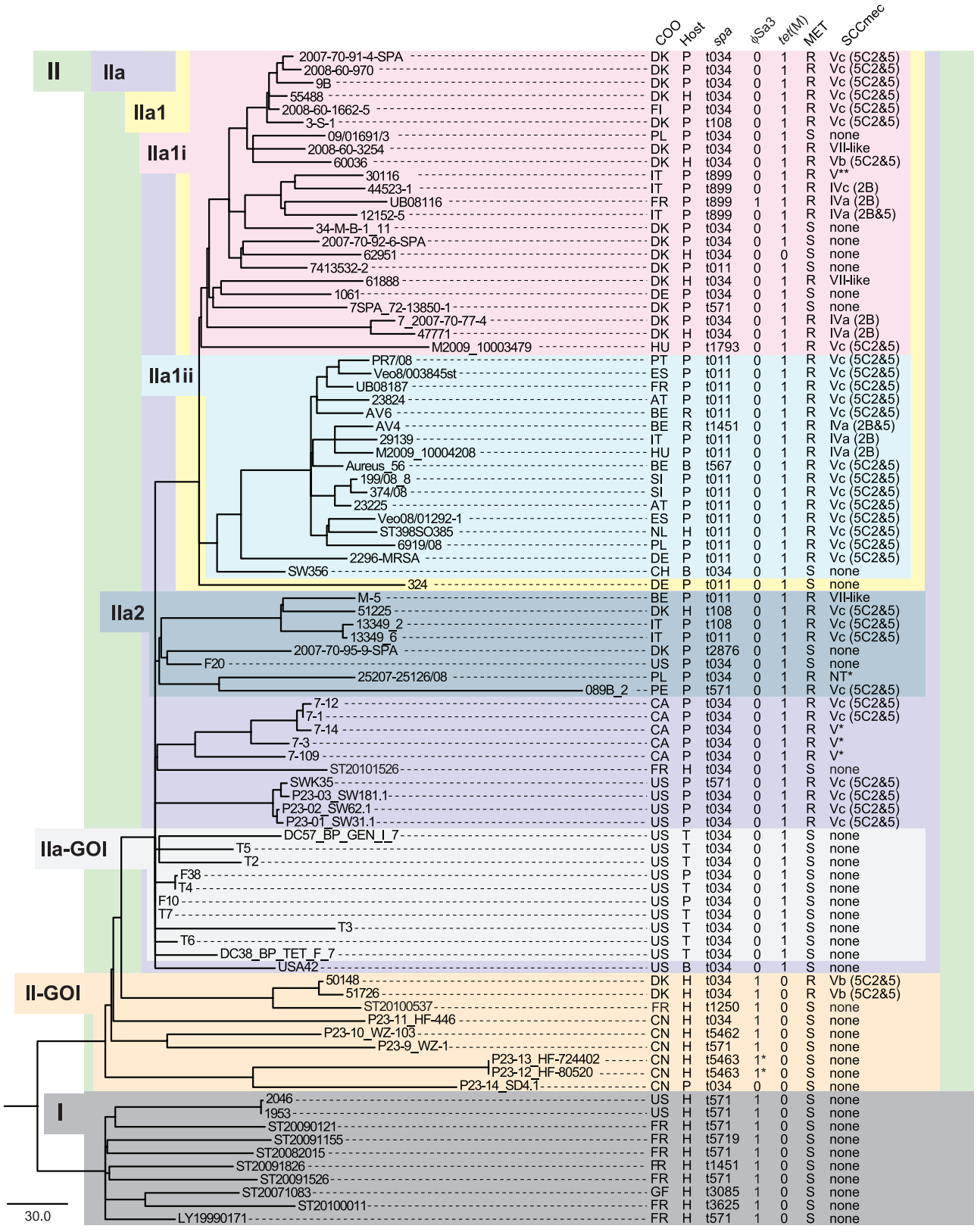

FIG 1 Maximum-parsimony tree of the 89 CC398 isolates (including ST398SO385) based on 4,238 total SNPs, including 1,102 parsimony-informative SNPs with a CI of 0.9591. Clades and groups of importance are labeled in a hierarchical fashion to facilitate description in the text. The tree was rooted with clade I based on an iterative selection process that identified this group as the most ancestral (see Materials and Methods). COO, country of origin; AT, Austria; BE, Belgium; CA, Canada; CH, Switzerland; CN, China; DE, Germany; DK, Denmark; ES, Spain; FI, Finland; FR, France; GF, French Guiana; HU, Hungary; IT, Italy; NL, The Netherlands; PE, Peru; PL, Poland; PT, Portugal; SI, Slovenia; US, United States; P, pig; H, human; R, horse; T, turkey; B, bovine; MET, methicillin susceptibility; R, resistant; S, susceptible.

ferent J1 regions that did not match the J1 regions associated with subtypes a to c. The type VII-like SCC*mec* cassette contained *ccr* type 5 (*ccrC*) and a class C1-like *mec* gene complex element previously identified in SCC*mec* type X (7C1-like) (17). Accordingly, this novel SCC*mec* type was referred to as VII-like (5C1-like) to distinguish it from archetypal SCC*mec* type VII (5C1). The NT cassette contained a class C1-like *mec* gene complex without any previously described *ccr* gene complex. The tetracycline resistance gene *tet*(M) was present in 99% (69/70) of the livestock-associated isolates but absent from the human-associated isolates (Fig. 1; see Data set S1 in the supplemental material).

The prophage integrase gene Sa3int was detected in 29 CC398 isolates. Phylogenetic analysis of the Sa3int sequences showed that they belong to three separate clusters; one clade was typical of φSa3 prophages, one was typical of φAvβ prophages, and the third was suggestive of a novel φSa3 integrase variant (see Fig. S4 in the supplemental material). φSa3 prophages in association with one or more human innate immunomodulatory genes were detected in 95% (18/19) of the human-associated *S. aureus* isolates (Fig. 1; see Data set 1 in the supplemental material). All 18 positive isolates were from human samples, whereas the single isolate lacking φSa3 prophages originated from a pig farm. All 10 isolates belonging to clade I carried *chp* and *scn* (type C φSa3 prophages), whereas 6 of 8 isolates belonging to clade II-GOI carried *sak*, *chp*, and *scn* (type B φSa3 prophages) and 2 isolates carried *scn* only (an IEC type not previously described). In comparison, only 1 of 70 isolates belonging to clade II harbored a φSa3 prophage in association with *sak*, *chp*, and *scn* (type B). Interestingly, 10 livestock-associated isolates belonging to IIa-GOI were largely from turkey meat samples and carried a φAvβ prophage along with the associated genes SAAV_2008 and SAAV_2009 but lacked human innate immunomodulatory genes carried by φSa3 prophages. Sa2int and the *lukF-lukS* genes carried by φSa2 prophages were present in 6 of 19 human-associated isolates. Conversely, all livestock-associated *S. aureus* isolates lacked the *lukF-lukS* genes.

DISCUSSION

Since its discovery, MRSA CC398 has been perceived as a livestock-associated pathogen; however, the WGST-based phylogeny presented here strongly suggests that the CC398 lineage originated in humans as MSSA and then spread to livestock, where it subsequently acquired the SCC*mec* cassette and methicillin resistance. The isolates that formed the most basal clades (I and II-GOI) on the WGST-based phylogenetic trees were almost all human-associated MSSA strains, suggesting that these isolates were the most ancestral of those tested in this study (Fig. 1). Likewise, the clade structure observed in the livestock-dominated IIa clade supports a rapid radiation as CC398 moved from humans to animals (see Fig. S5 in the supplemental material). Thus, livestock-associated CC398 infections in humans may be seen as a reintroduction to the original host.

Epidemiological data suggest that livestock-associated CC398 strains have lower transfer rates, and may be less virulent, in humans than other well-known STs (18). In this study, we showed that the *lukF-lukS* genes encoding Panton-Valentine leukocidin (PVL) were present in only 6 of the 89 genomes, all of which were human associated (see Data set S1 in the supplemental material). Strikingly, we found that all of the human-associated MSSA strains from clade I and clade II-GOI carried φSa3 in association with human innate immunomodulatory genes, whereas φSa3 was identified in only one livestock-associated isolate (see Fig. S6 in the supplemental material). Instead, a φAvβ prophage and the associated genes SAAV_2008 and SAAV_2009 were identified among a group of mainly turkey meat isolates in the livestock-associated clade IIa-GOI. It therefore appears that φSa3 was lost prior to (or early in) the formation of clade II, while φAvβ was introduced into avian MSSA CC398 isolates thereafter (Fig. 1). The human innate immunomodulatory genes carried by φSa3 prophages play crucial roles in human niche adaptation (19, 20), whereas the φAvβ-carried SAAV_2008 and SAAV_2009 genes (encoding a putative ornithine cyclodeaminase and a putative membrane protease of the CAAX family, respectively) belong to the avian-niche-specific accessory gene pool for broiler chicken-associated *S. aureus* ST5 (21). The loss of human-niche-specific genes in livestock-associated isolates, including those from turkeys, may be a result of adaptation to nonhuman hosts. A similar natural history has been reconstructed for broiler chicken-associated *S. aureus* ST5, which appears to have been introduced from humans into the chicken-breeding system, transmitted vertically, and disseminated worldwide (21). The ST5 jump from humans to chickens also appears to have been followed by the acquisition of avian-niche-specific genes (including the SAAV_2008 and SAAV_2009 genes carried by φAvβ prophages) and partial loss of human-niche-specific genes (including human innate immunomodulatory genes carried by φSa3 prophages) (21).

The data presented here strongly suggest that CC398 acquired resistance to methicillin and tetracycline after the introduction to livestock from humans (see Fig. S7a and b in the supplemental material). The tetracycline resistance gene *tet*(M) was nearly universal among livestock-associated CC398 MRSA and MSSA isolates and completely missing from human-associated strains. Consequently, tetracycline use in food animal production is likely to select for livestock-associated *S. aureus* CC398 without differentially selecting for MRSA strains. MRSA can be selected for by a number of broad-spectrum cephalosporins that are used in food animal production in the United States and Europe. Likewise, zinc and other metals are frequently used in animal feed formulations and may coselect for MRSA CC398 strains that carry the *czrC* zinc resistance gene, as suggested previously (22). This hypothesis is supported by our findings that the vast majority of LA-MRSA strains carry SCC*mec* type Vc (5C2&5), which contains the *czrC* gene.

This study demonstrates the potential power of WGST for epidemiological investigations. For example, two of the Danish MRSA isolates came from infants adopted from China. Both isolates were *spa* type t034, which is consistent with the majority of Danish CC398 isolates from pigs and humans; however, WGST showed that the isolates shared a recent common ancestor with a French isolate and that this clade was derived from other clades within II-GOI that were strictly Chinese in origin (Fig. 1, II-GOI). Although the French isolate obscures these results, they are most consistent with a Chinese rather than Danish origin of the isolates. WGST revealed thousands of SNPs among the 89 CC398 strains. These mutations may provide robust phylogenetic signals for future epidemiological and epizootological investigations involving CC398 strains.

spa typing is routinely used for *S. aureus* epidemiology; however, in this study, homoplasy within the *spa* gene led to inconsistencies between the WGST CC398 phylogeny and *spa* types. Some

spa types, such as t571 and t034, were observed in distant clades of the highly accurate WGST phylogenetic tree (Fig. 1). The t899 isolates exemplified the limitations of any single-locus typing method, as the *spa* gene was part of a ~123,000-bp region of DNA acquired from a distantly related *S. aureus* clone. A similar observation was made previously with *S. aureus* ST239, which originated as a hybrid between ST8-like and ST30-like chromosomes (23). Here, reliance on *spa* typing would have incorrectly placed these isolates outside of CC398. Interestingly, the large horizontally acquired region observed among the t899 CC398 strains also carries the SCC*mec* cassette, thus possibly presenting an alternative mechanism for SCC*mec* dissemination among *S. aureus* strains.

In this study, we provide strong evidence that CC398 originated in humans as MSSA and then spread to livestock, where it acquired resistance to methicillin and tetracycline. Genomic analyses presented here, in conjunction with previous epidemiological data, suggest that the jump from humans to animals was followed by a decreased capacity for human colonization, transmission, and virulence, yet livestock-associated CC398 has been linked to an increase in MRSA infections in northern Europe. Further research is required to characterize the full scope of the genetic changes associated with the shift from humans to livestock. Likewise, additional research and surveillance are required to predict the public health impact of MRSA CC398 in the future.

MATERIALS AND METHODS

Bacterial isolates. This study included MRSA ($n = 48$) and MSSA ($n = 40$) CC398 isolates from 19 countries on four continents with strains from humans ($n = 25$) and livestock ($n = 63$, including strains from live animals, meat samples, and environmental contamination) (see Data set S1 in the supplemental material). A previously sequenced ST398 strain, SO385, from The Netherlands was used as the reference and included in all analyses (24).

MLST. MLST was performed as described previously (http://saureus.mlst.net/misc/info.asp) (25). STs were assigned through the MLST database (http://www.mlst.net). The eBURST algorithm v3 was used to assign individual STs to specific CCs (http://eburst.mlst.net).

***spa* typing.** Amplification of the *spa* repeat region was performed using primers *spa* 1113f (5′ AAAGACGATCCTTCGGTGAGC 3′) and *spa* 1514r (5′ CAGCAGTAGTGCCGTTTGCTT 3′) and the conditions described previously (http://www.SeqNet.org). The *spa* types were determined based on the sequencing results using the *spa* plug-in included in the BioNumerics v4.6 software (Applied Math, Sint-Martens-Latem, Belgium).

Genome sequencing. DNA samples were prepared for multiplexed, paired-end sequencing on an Illumina Genome Analyzer IIx (Illumina, Inc., San Diego, CA). For each isolate, 1 to 5 μg DNA in 200 μl was sheared in a 96-well plate with the SonicMAN (part no. SCM1000-3; Matrical BioScience, Spokane, WA) to a size range of 200 to 1,000 bp, with the majority of material at ca. 600 bp, using the following parameters: prechill, 0°C for 75 s; cycles, 20; sonication, 10 s; power, 100%; lid chill, 0°C for 75 s; plate chill, 0°C for 10 s; postchill, 0°C for 75 s. The sheared DNA was purified using the QIAquick PCR Purification kit (catalog no. 28106; Qiagen, Valencia, CA). The enzymatic processing (end repair, phosphorylation, A tailing, and adaptor ligation) of the DNA followed the guidelines described in the Illumina protocol (Preparing Samples for Multiplexed Paired-End Sequencing, catalog no. PE-930-1002, part no.1005361). The enzymes for processing were obtained from New England Biolabs (catalog no. E6000L; New England BioLabs, Ipswich, MA), and the oligonucleotides and adaptors were obtained from Illumina (catalog no. PE-400-1001). After ligation of the adaptors, the DNA was run on a 2% agarose gel for 2 h, after which a gel slice containing 500- to 600-bp fragments of each DNA sample was isolated and purified using the QIAquick Gel Extraction kit (catalog no. 28706; Qiagen, Valencia, CA). Individual libraries were quantified by quantitative PCR on an ABI 7900HT (part no. 4329001; Life Technologies Corporation, Carlsbad, CA) in triplicate at two concentrations, 1:1,000 and 1:2,000, using the Kapa Library Quantification kit (part no. KK4832 or KK4835; Kapa Biosystems, Woburn, MA). Based on the individual library concentrations, equimolar pools of no more than 12 indexed *S. aureus* libraries were prepared at a concentration of at least 1 nM using 10 mM Tris-HCl (pH 8.0)-0.05% Tween 20 as the diluent. To ensure accurate loading onto the flow cell, the same quantification method was used to quantify the final pools. The pooled paired-end libraries were sequenced on an Illumina Genome Analyzer IIx to a read length of at least 76 bp.

Identification of SNPs. Illumina WGS data sets were aligned against the chromosome of the published ST398 reference genome (strain SO385; GenBank accession no. AM990992) (24) using the short-read alignment component of the Burrows-Wheeler Aligner. Each alignment was analyzed for SNPs using SolSNP (http://sourceforge.net/projects/solsnp/). In order to avoid false calls due to sequencing errors, SNP loci were excluded if they did not meet a minimum coverage of 10× and if the variant was present in less than 90% of the base calls for that position. SNP calls were combined for all of the sequenced genomes such that for the locus to be included in the final SNP matrix, it had to be present in all of the genomes. SNPs falling in the duplicated regions on the reference genome were discarded.

Phylogenetic analysis. Phylogenetic trees were generated using the maximum-parsimony method in PAUP v4.0b10. For maximum-parsimony bootstrapping analysis, the analysis was constrained to build a maximum of 1,000 trees (100 replicates, 10 trees each). The root of the tree was determined through an iterative process as follows. A distance matrix and phylogenetic tree was generated comparing the chromosomes of ST398 (GenBank accession no. AM990992), ST36 (GenBank accession no. BX571856), ST8 (GenBank accession no. CP000255), ST1 (GenBank accession no. BA000033), and ST5 (GenBank accession no. BA000018). Through this process, ST36 was determined to be the most closely related non-CC398 STs. ST36 was used as an outgroup to root the CC398 WGST tree and identify the most ancient CC398 bifurcation point. CC398 descendants nearest to this bifurcation point were used to root subsequent trees.

SCC*mec* typing. The presence of *mecA* and SCC*mec* types and subtypes was assessed in all 89 *S. aureus* CC398 isolates. The structural features unique to each of the type 1 to 5 *ccr* gene complexes; class A, B, and C2 *mec* gene complexes; and four J1 subtypes (a to d) of type IV SCC*mec* were determined by a PCR-based multiplex assay described by Kondo et al. (26). Structural features unique to the class C1 and C1-like *mec* gene complexes (17, 27) and the three subtypes of type V SCC*mec* (17) were determined by aligning the Illumina WGS data sets against reference sequences using CLC Genomics Workbench v4.7.2 (CLC bio, Aarhus, Denmark). The following reference sequences were used: *mec* class C1 (GenBank accession no. AB373032); *mec* class C1-like (GenBank accession no. AB505630); and SCC*mec* subtypes Va (5C2) (GenBank accession no. AB121219), Vb (5C2&5) (GenBank accession no. AB462393), and Vc (5C2&5) (GenBank accession no. AB505629).

SCC*mec* nomenclature was applied as proposed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (28). For brevity, the type is indicated by roman numerals and the subtype is identified by a lowercase latin letter. The combination of *ccr* and *mec* gene complexes is indicated by an arabic number and a latin letter, respectively, in parentheses. When a composite of two SCC elements carrying distinct *ccr* gene complexes is identified, this is indicated by an ampersand and an arabic numeral designating the *ccr* type.

Detection of genes associated with antimicrobial resistance and host adaptation. All 89 genomes were analyzed for the presence of the tetracycline resistance gene *tet*(M), the cadmium-zinc resistance gene *czrC*, the φSa3 and φSa2 prophages (identified by Sa3int and Sa2int inte-

grase genes), five genes carried by φSa3 prophages (*sea*, *sep*, *sak*, *chp*, and *scn*), two putative avian-niche-specific genes carried by φAvβ (a φSa3-like prophage) (SAAV_2008 and SAAV_2009), and two PVL genes carried by φSa2 prophages (*lukF-PV* and *lukS-PV*). Local BLASTN searches were performed on *de novo* contigs assembled from the Illumina WGS data sets, as well as a reference assembly, using CLC Genomics Workbench v4.7.2. The presence or absence of genes was determined using thresholds of 90% nucleotide identity, 90% coverage of the query sequence length, and a sequence depth of >10×. The query sequences used were *tet*(M) and *czrC* (GenBank accession no. AM990992); Sa3int, *sea*, *sak*, *chp*, and *scn* (GenBank accession no. NC_009641); *sep* (GenBank accession no. BA000018); SAAV_2008 and SAAV_2009 (GenBank accession no. CP001781); and Sa2int, *lukF-PV*, and *lukS-PV* (GenBank accession no. AB006796).

All *de novo* contigs with BLASTN matches to Sa3int were selected, and the Sa3int genes were retrieved for phylogenetic reconstruction using Sa3int (GenBank accession no. NC_009641) and Avβint (GenBank accession no. CP001781) as reference sequences. Gene sequences were aligned using ClustalW v 2.0 (29), and the trees were generated using the maximum-parsimony method in PAUP v4.0b10.

The φSa3 prophages received letter designations to reflect unique combinations of the five prophage-carried genes that modulate human innate immune responses (*sea*, *sep*, *sak*, *chp*, and *scn*) as described elsewhere (30).

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SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00305-11/-/DCSupplemental.

Data set S1, XLSX file, 0.1 MB.
Figure S1, PDF file, 0.3 MB.
Figure S2, PDF file, 0.7 MB.
Figure S3, PDF file, 0.3 MB.
Figure S4, PDF file, 0.7 MB.
Figure S5, PDF file, 0.2 MB.
Figure S6, PDF file, 0.2 MB.
Figure S7a, PDF file, 0.2 MB.
Figure S7b, PDF file, 0.2 MB.

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ERRATUM

Staphylococcus aureus CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock

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Volume 3, no. 1, doi:10.1128/mBio.00305-11, 2012. The accession numbers for the Illumina sequences generated from the 88 *Staphylococcus aureus* CC398 isolates described in this study are available in the Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/sra) under the following accession numbers: SRX129593 to SRX129632, SRX129682 to SRX129686, SRX129691, SRX129696, SRX129697, SRX129701, SRX129702, SRX129704 to SRX129707, SRX129714, SRX129718, SRX129758, SRX129763, SRX129764, SRX129766, SRX129775, SRX129779, SRX129784, and SRX129816 to SRX129840. Sequences can also be located in the SRA using the following study summary: "*Staphylococcus aureus* CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock."

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Livestock Origin for a Human Pandemic Clone of Community-Associated Methicillin-Resistant *Staphylococcus aureus*

Laura E. Spoor, Paul R. McAdam, Lucy A. Weinert, et al.
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RESEARCH ARTICLE

Livestock Origin for a Human Pandemic Clone of Community-Associated Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT **The importance of livestock as a source of bacterial pathogens with the potential for epidemic spread in human populations is unclear. In recent years, there has been a global increase in community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections of healthy humans, but an understanding of the different evolutionary origins of CA-MRSA clones and the basis for their recent expansion is lacking. Here, using a high-resolution phylogenetic approach, we report the discovery of two emergent clones of human epidemic CA-MRSA which resulted from independent livestock-to-human host jumps by the major bovine *S. aureus* complex, CC97. Of note, one of the new clones was isolated from human infections on four continents, demonstrating its global dissemination since the host jump occurred over 40 years ago. The emergence of both human *S. aureus* clones coincided with the independent acquisition of mobile genetic elements encoding antimicrobial resistance and human-specific mediators of immune evasion, consistent with an important role for these genetic events in the capacity to survive and transmit among human populations. In conclusion, we provide evidence that livestock represent a reservoir for the emergence of new human-pathogenic *S. aureus* clones with the capacity for pandemic spread. These findings have major public health implications highlighting the importance of surveillance for early identification of emergent clones and improved transmission control measures at the human-livestock interface.**

IMPORTANCE **Animals are the major source of new pathogens affecting humans. However, the potential for pathogenic bacteria that originally were found in animals to switch hosts and become widely established in human populations is not clear. Here, we report the discovery of emergent clones of methicillin-resistant *Staphylococcus aureus* (MRSA) that originated in livestock and switched to humans, followed by host-adaptive evolution and epidemic spread in global human populations. Our findings demonstrate that livestock can act as a reservoir for the emergence of new human bacterial clones with potential for pandemic spread, highlighting the potential role of surveillance and biosecurity measures in the agricultural setting for preventing the emergence of new human pathogens.**

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Emerging infections represent a major challenge to human and veterinary medicine. The great majority of new pathogens of humans originate in animal populations, but most are associated with episodic zoonotic infections that do not have the capacity to transmit to other individuals (1). *Staphylococcus aureus* is a major pathogen responsible for considerable human morbidity and mortality on a global scale (2). *S. aureus* is also a leading cause of infections of livestock such as cows and is a major economic burden on the global dairy industry (3). The results of population genetics studies have shown that most strains of *S. aureus* are host specific, indicating a low frequency of cross-species transmission (3). However, recent studies employing multilocus sequence typing (MLST) and whole-genome sequencing (WGS) have identified several *S. aureus* sequence types (ST) that are associated with multiple host species, implying either zoonotic transmission or a recent common ancestor. For example, the poultry-associated sequence type 5 (ST5) and livestock-associated, methicillin-resistant *S. aureus* (LA-MRSA) ST398 clones are descended from bacteria that recently made human-to-animal host jumps (4, 5). Importantly, LA-MRSA ST398 strains have acquired antibiotic resistance due to selective pressures in the pig farming industry and have the capacity to cause severe zoonotic infections of humans in contact with pigs on pig farms (5). However, carriage of LA-MRSA ST398 by pig farmers is transient, and LA-MRSA ST398 does not appear to be readily transmissible between humans, probably due to the loss of expression of proteins associated with the cell wall that are required for human host colonization and transmission (6). Loss of function of superfluous genes is a common feature of bacteria undergoing niche adaptation (7), which is likely to attenuate the capacity to infect the ancestral host

species (6). Recently, Harrison et al. demonstrated that ST130 MRSA isolates with the *mecC* gene have spread from cows to humans resulting in clinical infections (8). However, the potential for animal-specialized strains of *S. aureus* to successfully cross the human species barrier and become epidemic in human populations is unclear. Previously, we reported that the clonal complex 59 (CC59) *S. aureus* clone that is endemic in Taiwan and has spread to other parts of the world may have originated in livestock about 500 years ago, and this has recently been corroborated by Shepheard et al. (9). However, the limited resolution of MLST has precluded rigorous examination of the occurrence of more-recent livestock-to-human host jump events leading to the emergence of new epidemic *S. aureus* clones (10).

S. aureus strains belonging to MLST CC97 are a leading cause of bovine mastitis in Europe, Asia, and North and South America (11, 12). Less commonly, CC97 has also been reported to cause infections of small ruminants, pigs, and humans (11, 13, 14). Importantly, there have been increasing reports of human infections caused by CC97 isolates in Europe, North and South America, Africa, and Asia (13, 15–18). However, the origin of human CC97 strains and their relatedness to livestock-associated CC97 strains are unknown. Here, we investigate the evolutionary history of the CC97 *S. aureus* lineage and identify clones that are epidemic in human populations that evolved through host jumps from cows followed by human host adaptation. Our findings highlight cows as a potential reservoir for the emergence of new clones with the capacity for pandemic spread in humans.

RESULTS AND DISCUSSION

CC97 is an emerging cause of human infections. The earliest report in the literature of a CC97 isolate is the archetypal bovine reference strain of *S. aureus*, Newbould 305 (NCIMB 702892), isolated from a case of mastitis in Canada in 1958 (19), whereas the earliest report of human CC97 isolates relates to strains isolated 38 years later in 1996 (20). However, human CC97 *S. aureus* bacteria have since been identified in at least 35 countries (see Table S2 in the supplemental material). In order to investigate the possibility that infections due to CC97 strains are increasing in prevalence in human populations, we determined the number of CC97-associated strains isolated from MRSA and bacteremia infections in Denmark between 2007 and 2011. Uniquely, in Denmark, submission of all MRSA to the Danish National MRSA Reference Laboratory, Statens Serum Institut, has been mandatory since November 2006, and all MRSA and bacteremia *S. aureus* isolates submitted since 2007 have been genotyped by staphylococcal protein A (*spa*) typing. In Denmark, cases of MRSA caused by CC97-related *spa* types increased from a total of 2 in 2007 to 22 in 2011, equivalent to an 11-fold increase in 5 years. This represents a significant increase in prevalence from 0.3% to 1.7% of total annual MRSA in Denmark since 2007 ($P < 0.01$). Although surveillance systems for other countries are currently inadequate to identify trends in *S. aureus* genotype associated with human infections, a recent study of community-associated *S. aureus* isolated from humans in 16 countries in Europe reported that 3% of community-associated MRSA (CA-MRSA) and 8% of community-associated methicillin-sensitive *S. aureus* (CA-MSSA) were ST97 isolates (21). Taken together, these data suggest that CC97 is an emerging cause of human infections.

Human ST97 *S. aureus* clones originated from bovine-to-human host jumps. In order to investigate the evolutionary history of the CC97 clone, we obtained 220 CC97 *S. aureus* isolates of human, bovine, porcine, and caprine origin, isolated in 18 countries on four continents between 1956 and 2012. For whole-genome sequencing, we selected 43 CC97 *S. aureus* isolates (including 16 MRSA) which broadly represented the breadth of host, geographic, and temporal variation identified among strains reported in the literature, in addition to a single isolate of the closely related ST28 as a human-specific outgroup. The core genome of the 43 CC97 isolates consisted of 2,079,972 bp which included 5,425 high-quality single nucleotide polymorphisms (SNP). Phylogenetic reconstruction of the CC97 lineage was carried out by using the BEAST program (named BEAST for Bayesian evolutionary analysis by sampling trees) (22) (Fig. 1). The consistency index for parsimonious sites predicted using PAUP*4.0b10 (23) indicated a very low predicted frequency of homoplasic events (consistency index [CI] = 0.95), implying an unconflicted phylogenetic signal. Furthermore, a maximum likelihood tree constructed from the same core genome alignment resulted in a largely similar phylogeny with strong bootstrap support at most nodes (see Fig. S1 in the supplemental material). Overall, the high-resolution phylogenetic tree resolves *S. aureus* CC97 into distinct host-associated clades (Fig. 1). Of note, there is considerable genetic diversity among CC97 isolates of livestock origin, which is indicated by numerous deep branches in the phylogenetic tree, compared to human isolates which are restricted to two distinct clades of closely related isolates (Fig. 1). The majority of livestock-associated CC97 isolates lie basal to the human clades, and a continuous-time Markov model with host association as a discrete trait (10, 36) provided strong support that the most recent common ancestor of each human clade was of bovine origin (see Table S3 in the supplemental material). These data indicate that CC97 isolates circulating among human populations are the result of livestock-to-human host jumps which have occurred on at least 2 independent occasions. Human CC97 clade A consists of isolates of both MSSA and MRSA from 12 different countries on four different continents, indicating its global dissemination (Fig. 1). In contrast, human CC97 clade B is represented by a single MSSA isolate from a Danish patient in 1980 and several MRSA bacteria isolated within the last 10 years in the Midlands region of the United Kingdom, indicating a more limited geographic distribution among the strains sampled (13). In order to determine the time frame of the livestock-to-human host jump events, we determined mutation rates for the CC97 lineage, allowing for variation in rates associated with different clades. Initially, an uncorrelated lognormal relaxed molecular clock model was used to determine a mutation rate for the livestock strains only of 1.53×10^{-6} nucleotide substitutions per site per year (95% highest posterior densities [HPDs] of 1.35×10^{-6} to 1.72×10^{-6}). This rate was then fixed for livestock strains, and a local rate clock model was applied to each of the human clades, resulting in estimates of 9.58×10^{-7} (7.80×10^{-7} to 1.15×10^{-6}) for human clade A and 1.29×10^{-6} (1.05×10^{-6} to 1.54×10^{-6}) for human clade B. These data indicate that closely related *S. aureus* clones can have different mutation rates which may reflect variations in selective pressures encountered in different ecological niches. The Bayesian analysis resulted in estimates of host jump events which occurred between 1894 and 1977 for human CC97 clade A and between 1938 and 1966 for human CC97 clade B (Fig. 1; Table S3). In addition, we estimated the date of the most-recent human ancestor (MRCA) of CC97 and the human outgroup ST28 to be AD 784 (BC 325 to

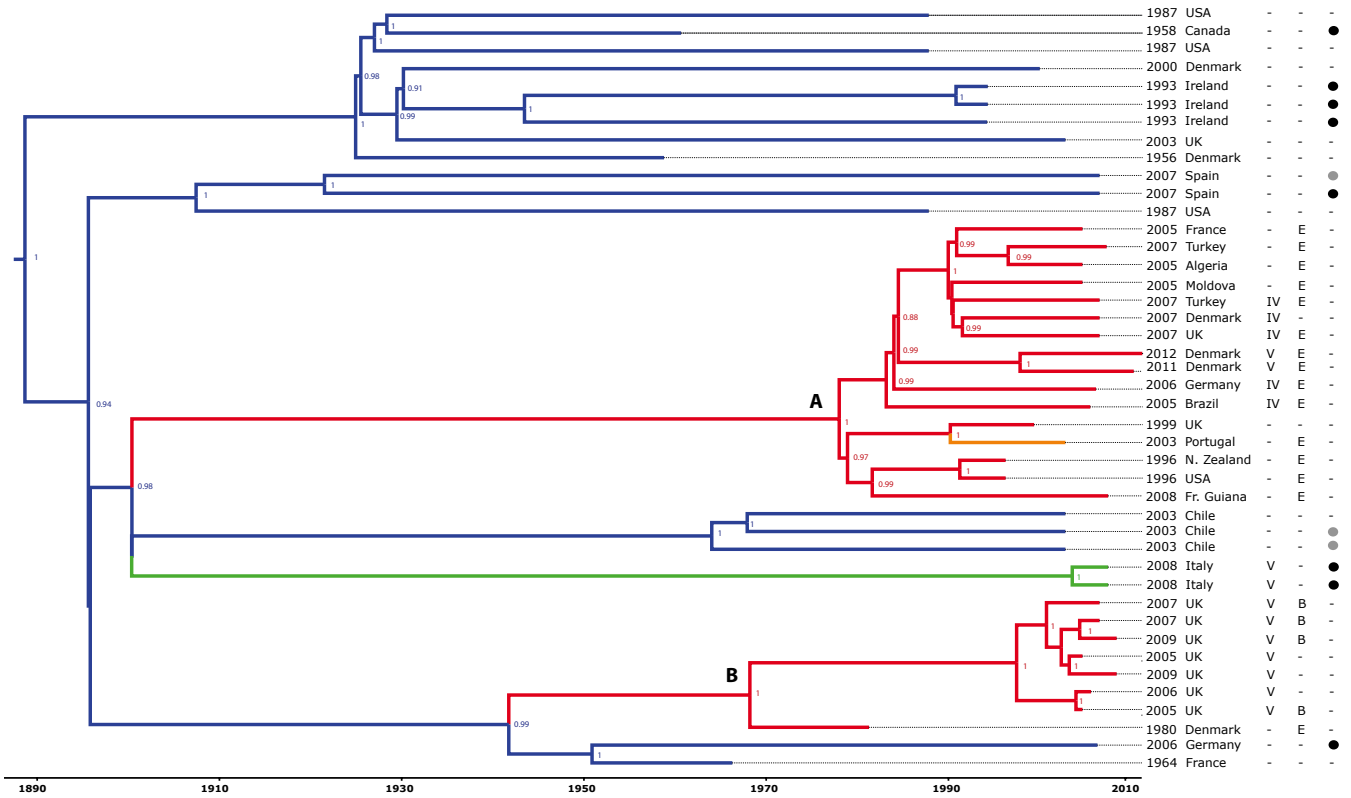

FIG 1 Identification of human epidemic *S. aureus* clones descended from bacteria that made livestock-to-human host jumps. The Bayesian phylogenetic reconstruction of the CC97 lineage is shown. The tree is based on core genome alignment with branches color coded according to the host species association (blue, bovine; green, porcine; orange, caprine; red, human) and date and country of origin of each isolate indicated. The presence or absence (−) of β-toxin phage IEC variants B and E and SCC*mec* type IV or V is indicated by the appropriate letter, and the presence of *S. aureus* pathogenicity island (SaPI)-encoded *vwb* or phage-encoded *lukM/lukF* is denoted by black and gray circles, respectively. The branch lengths are scaled according to the time scale bar (years) and the posterior probability values are indicated at each node. Clades A and B are shown.

AD 1460), which lies within the credibility intervals of the previous estimate of the most recent minimum date for the host jump event using MLST data (Table S3) (10).

The results of previous studies employing MLST have suggested that livestock-associated strains of *S. aureus* originated from human ancestral strains through human-to-animal host jumps leading to host-adapted clones specialized for livestock with occasional host jumps back into humans (10). In particular, the CC59 *S. aureus* clone that is endemic in human populations in Taiwan may have originated in livestock about 500 years ago (10). Here, we provide an example of recently emerged clones of human *S. aureus* that evolved through independent host jumps from livestock. These data demonstrate that livestock represent a potential reservoir of pathogenic bacteria that may cross the species barrier and spread among global human populations.

Methicillin resistance was acquired by human CC97 clones subsequent to the host jumps from cows. Antibiotic susceptibility testing of CC97 isolates revealed that 7 of 17 bovine isolates were sensitive to all antimicrobial agents tested, with a further 6 resistant to only a single agent, demonstrating the low prevalence of antimicrobial resistance among isolates of the leading bovine clone of *S. aureus* (see Fig. S2 in the supplemental material). In contrast, 20 of 23 human isolates were resistant to at least one antimicrobial agent, with some strains resistant to β-lactam antimicrobials, lincosamides, erythromycin, and trimethoprim. Importantly, methicillin resistance is a characteristic of the human CC97 clades, with two distinct staphylococcal cassette chromosome *mec* element (SCC*mec*) types, types IV and V, which are associated with human CC97 clades A and B (Fig. 1). Of note, none of the isolates examined contained the novel *mecC* allele previously found among several bovine MRSA clones responsible for episodes of human zoonotic infection (8). In contrast, all of the bovine *S. aureus* isolates examined in the study are methicillin sensitive, suggesting that resistance was acquired after the host jump from cows to humans, presumably as a result of selective pressures imposed by prescription of antibiotics for treating human infections. Consistent with this, the earliest human isolate identified in clade B (isolated in Denmark in 1980) was sensitive to all antibiotics tested, whereas all other isolates of clade B (isolated since 2005) were resistant to multiple antimicrobial classes (see Fig. S2 in the supplemental material). Recently, it was demonstrated that methicillin and tetracycline resistance was likely acquired by LA-MRSA ST398 strains by antibiotic selective pressures encountered within the pig farming industry (5). Of note, the two ST97 isolates from pigs in the current study were resistant to both tetracycline and methicillin, in addition to ciprofloxacin (Fig. S2). Overall, the antimicrobial susceptibility profiles of the human and pig CC97 isolates demonstrated resistance to a much greater number of antimicrobials than the bovine CC97 *S. aureus* (Fig. S2). These data imply that the dairy industry does not

strongly promote the emergence of antibiotic-resistant *S. aureus*, in spite of the widespread use of antibiotics for treating bovine mastitis. We speculate that this reflects the ability of bovine strains to invade and survive within bovine mammary epithelial cells, a niche which may have a markedly reduced antibiotic selective pressure (24).

The content of MGE among CC97 isolates correlates with host species. Previous studies have highlighted the importance of mobile genetic elements (MGE) in adaptation of *S. aureus* to distinct host species (3). In the current study, of the 20 livestock isolates, 3 and 8 isolates contained MGE encoding the LukM/F leukotoxin and the von Willebrand binding protein (vWbp), respectively, both of which have been demonstrated to have ruminant host-specific activity (3) (Fig. 1). In contrast, none of the human isolates contained the livestock-associated MGE. The family of β-toxin-converting phages (φSa3) associated with human *S. aureus* typically contain an immune evasion cluster (IEC) of genes encoding secreted proteins such as staphylokinase (*sak*), staphylococcal complement inhibitor (*scn*), and chemotaxis inhibitory protein of *S. aureus* (*chp*) which contribute to immune evasion in a human host-specific manner (25). Consistent with previous reports, in the current study, we found that 19 of 23 human isolates and none of 19 bovine or pig isolates contained a φSa3 with an IEC, demonstrating a strong correlation with a human host association (25). Of the 16 human CC97 clade A isolates, 14 had an IEC containing genes *sak* and *scn* (IEC type E), and of the 8 human CC97 clade B isolates, 4 had an IEC containing *sak*, *chp*, and *scn* (IEC type B) and one isolate had an IEC type E (Fig. 1) (25). Of note, the single goat strain that cosegregates with human clade A contains a φSa3 phage which is highly similar to that of the human strains in clade A, implying that it is a human contaminant or the result of a very recent human-to-goat transmission event. In addition, the arginine catabolic mobile element (ACME) is a characteristic of some CA-MRSA clones, including the highly successful USA300 clone which may contribute to enhanced survival during community-associated infections (26). Of note, all seven United Kingdom isolates of the human CC97 clade B contained ACME linked to SCC*mec* type V (13). Taken together, the distribution of MGE among CC97 isolates reveals horizontal gene acquisition events which correlate with adaptation to different host-associated ecological niches.

Concluding comments. The recent global increase in CA-MRSA infections reflects the expansion of an array of *S. aureus* clones with distinct evolutionary histories. Here, we demonstrate that livestock is one potential reservoir of pathogenic bacteria with the capacity to cross the species barrier, undergo host-adaptive evolution, and become established in global human populations. Furthermore, the data suggest that a limited number of genetic events may be sufficient to transform an *S. aureus* strain which has coevolved with bovine hosts over several thousand years into a successful human epidemic lineage.

The importance of hygiene in prevention of hospital transmission of nosocomial pathogens such as MRSA is widely appreciated, and the recent reduction in hospital MRSA infections is likely due in part to improved hygiene measures for controlling transmission (27). Improved biosecurity and hygiene control measures which prevent the spread of bacterial flora between livestock and human hosts may limit opportunities for successful livestock-to-human transmission. Furthermore, regular surveillance of the microbiota in livestock and humans may facilitate the early identification of emergent clones with the capacity to transmit and cause disease among human populations.

MATERIALS AND METHODS

Bacterial isolates. A total of 220 *S. aureus* isolates of CC97 from bovine, human, porcine, and caprine hosts isolated between 1956 and 2012 in 18 different countries on four continents were obtained. For whole-genome sequencing, a total of 43 CC97 strains were selected to represent the breadth of host, clinical, spatial, and temporal variation (see Table S1 in the supplemental material). Strains were grown for 16 h on tryptic soy agar (TSA) at 37°C or in tryptic soy broth (TSB) at 37°C with shaking at 200 rpm. Genomic DNA was isolated using the PurElute bacterial genomic kit (Edge BioSystems, MD), with an amended protocol as previously described (4). MLST was conducted to confirm the sequence type (ST) prior to whole-genome sequencing using methods described previously (28).

Genome sequencing, mapping assembly, and SNP calling. Paired-end Illumina sequencing of bacterial strains was carried out on an Illumina genome analyzer IIx or Miseq, following standard Illumina protocols. Nucleotide distribution and quality scores of raw reads were assessed and filtered for quality using the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html) (genome assembly metrics are provided in Table S4 in the supplemental material). High-quality reads were aligned against the reference genomes of *S. aureus* strains MW2 (accession number NC_003923) or Mu50 (accession number NC_002758.2), variant sites were detected, and consensus sequences were called as described previously (29), with extended base alignment quality (BAQ) computation employed in SAMtools v.0.1.16 (30). The core genome was defined as all nucleotide sites shared by all isolates, and putative recombinant regions were removed after breakpoint detection with the suite of programs included in the Recombination Detection Program v4.13 (RDP) (31). For comparisons of gene content, *de novo* assemblies of short reads was carried out using Velvet v1.0.15 (32) and the VelvetOptimiser.pl script within VelvetOptimiser v-2.1.7 (http://bioinformatics.net.au/software.velvetoptimiser.shtml).

Phylogenetic analysis. Core genome and in-frame protein-coding sequences were extracted from the genome consensus sequences to construct alignments using custom scripts. The maximum likelihood phylogeny was reconstructed using RAxML-7.2.6 (33) implementing a generalized time reversible (GTR) model with gamma correction for rate heterogeneity, and 1,000 bootstrap replicates (see Fig. S1 in the supplemental material). Bayesian phylogenetic analysis was conducted using BEAST v1.7.1 (34) implementing the Hasegawa-Kishino-Yano model of sequence evolution with gamma correction for rate heterogeneity. The rates were calculated by the dated tip method using a three-rate local clock model, with the livestock rate constrained to 1.53×10^{-6} nucleotide substitutions per site per year as determined using an uncorrelated relaxed molecular clock model with a constant coalescent prior (35). For the date of the MRCA with the human outgroup ST28, an in-frame coding sequence (CDS) alignment of CC97 and ST28 sequences was employed which allowed the estimation of mutation rate over all sites and third codon positions only. In each case, the mutation rates were comparable and resulted in similar estimates of the host jump date (Table S3). Ancestral host states were predicted using a model of discrete trait evolution (36), adapting the method by substituting geographic location with host state as described previously (10). The consistency index for parsimony informative sites was determined using PAUP v4.0b10 (23).

Accessory genome analysis. *De novo* contigs and reference assembly sequences were interrogated for specific MGE using BLASTn (37), and MGE sequences were aligned using Mauve v2.3.1 (38). Immune evasion cluster (IEC) type was defined as described previously (25).

Antimicrobial sensitivity testing. All isolates in the study were tested using the Vitek 2 system (AST-P620 card) (bioMérieux United Kingdom Limited, Basingstoke, United Kingdom) with a panel of antimicrobial agents, including cefoxitin, benzylpenicillin, oxacillin, gentamicin, ciprо-

floxacin, inducible clindamycin resistance, erythromycin, clindamycin, linezolid, daptomycin, teicoplanin, vancomycin, tetracycline, tigecycline, nitrofurantoin, fusidic acid, mupirocin, chloramphenicol, rifampicin, and trimethoprim. Results for antibiotic susceptibility were interpreted using standards defined by the Clinical and Laboratory Standards Institute (CLSI) (39).

Statistical analysis. For analysis of the increase in CC97-associated infections in Denmark, the Cochran-Armitage trend test was employed (GraphPad Prism 5.0).

Nucleotide sequence accession numbers. The Illumina sequences generated and used in this study are deposited and available in the Sequence Read Archive (SRA) (http://www.ncbi.nlm.nih.gov/sra) under the study accession number PRJEB1411, located at http://www.ebi.ac.uk/ena/data/view/PRJEB1411. The *S. aureus* isolates are under sample accession numbers ERS212248 to ERS212287 and ERS249844 to ERS249847.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00356-13/-/DCSupplemental.

Figure S1, PDF file, 0.1 MB.
Figure S2, PDF file, 0.2 MB.
Table S1, DOCX file, 0.1 MB.
Table S2, DOCX file, 0.1 MB.
Table S3, DOC file, 0.1 MB.
Table S4, PDF file, 0.2 MB.

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L. E. Spoor and J. R. Fitzgerald designed research. L. E. Spoor, L. A. Weinert, P. R. McAdam, A. R. Larsen, and J. R. Fitzgerald performed research and analyzed data. R. E. Skov, H. Hasman, F. M. Aarestrup, and A. M. Kearns contributed new reagents or analytic tools. L. E. Spoor and J. R. Fitzgerald wrote the paper.

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Original Article

Association Between Antimicrobial Resistance in *Escherichia coli* Isolates from Food Animals and Blood Stream Isolates from Humans in Europe: An Ecological Study

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Abstract

Background: In addition to medical antimicrobial usage, the use of antimicrobials in food animals contributes to the occurrence of resistance among some bacterial species isolated from infections in humans. Recently, several studies have indicated that a large proportion of *Escherichia coli* causing infections in humans, especially those resistant to antimicrobials, have an animal origin.
Methods: We analyzed the correlation between the prevalence of antimicrobial resistance in *E. coli* isolates from blood stream infections in humans and in *E. coli* isolates from poultry, pigs, and cattle between 2005 and 2008 for 11 countries, using available surveillance data. We also assessed the correlation between human antimicrobial usage and the occurrence of resistance in *E. coli* isolates from blood stream infections.
Results: Strong and significant correlations between prevalences of resistance to ampicillin ($r = 0.94$), aminoglycosides ($r = 0.72$), third-generation cephalosporins ($r = 0.76$), and fluoroquinolones ($r = 0.68$) were observed for human and poultry *E. coli* isolates. Similar significant correlations were observed for ampicillin ($r = 0.91$), aminoglycosides ($r = 0.73$), and fluoroquinolone resistance ($r = 0.74$) in pig and human isolates. In cattle isolates, only ampicillin resistance ($r = 0.72$) was significantly correlated to human isolates. When usage of antimicrobials in humans was analyzed with antimicrobial resistance among human isolates, only correlations between fluoroquinolones ($r = 0.90$) and third-generation cephalosporins ($r = 0.75$) were significant.
Conclusions: Resistance in *E. coli* isolates from food animals (especially poultry and pigs) was highly correlated with resistance in isolates from humans. This supports the hypothesis that a large proportion of resistant *E. coli* isolates causing blood stream infections in people may be derived from food sources.

Introduction

The ever increasing development and spread of antimicrobial-resistant bacteria is a major concern, in particular to those antimicrobials that are critically important to human health (WHO, 2007; Collignon *et al*., 2009). In addition to medical antimicrobial usage, veterinary use of antimicrobials is believed to have a significant impact on the increase of the occurrence of resistance among some bacteria species isolated from humans.

Campylobacter and *Salmonella* are well-known causes of human foodborne infections. These pathogens develop resistance in their animal reservoirs, and the resistant strains are transmitted to humans, where they may develop infections that are difficult to treat (Helms *et al*., 2003; Varma *et al*., 2005; Aarestrup *et al*., 2008). Recently, a number of studies have suggested that *Escherichia coli*, especially antimicrobial-resistant strains, might transfer from food animals and cause infections in humans (Johnson *et al*., 2007; Warren *et al*., 2008; Sheldon, 2010). *E. coli* is the most frequent gram-negative rod isolated from blood cultures in clinical settings (EARSS, 2008). Although bloodstream infections represent only a small fraction of all infections caused by *Escherichia coli*, they are often associated with significant mortality (de Kraker *et al*., 2010).

Isolates of *E. coli* are frequently and increasingly resistant to most antibiotics, including ampicillin, quinolones, aminoglycosides, and third-generation cephalosporins (EARSS, 2008). This resistance development complicates the treatment of both

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common community-acquired infections, such as *E. coli* urinary tract infections, and more serious blood stream infections.

E. coli is a part of the normal flora of most animal species, including humans. Therefore, it can be very difficult to determine whether *E. coli* infections are caused by isolates acquired from the established normal flora or from food. Further, there might be a long time from intake of food with a pathogenic *E. coli* isolate and the onset of infection, making it very difficult or impossible to detect food sources using classical epidemiological studies of foodborne outbreaks. With intervention studies it would be very difficult to control and study the food intake of individual humans and follow them until onset of infections.

Studies have shown (1) that multi-resistant *E. coli* can be frequently found in food animals (DANMAP, 2009), (2) widespread carriage of multi-resistant *E. coli* in the community with no healthcare association (Woodford *et al*., 2004; Rogers *et al*., 2011), and (3) indications that most resistant *E. coli* in the bowel of people are derived from food animals (van den Bogaard and Stobberingh, 2000; Johnson *et al*., 2007; Warren *et al*., 2008). To further investigate the potential association between antimicrobial resistance in *E. coli* from food animals (i.e., poultry, pigs, and cattle) and from humans, we took advantage of the fact that several countries have integrated antimicrobial resistance-monitoring programs of animals, food, and humans. Using their data, we analyzed the correlation between the prevalence of antimicrobial resistance detected in *E. coli* isolates from human blood infections with that from poultry, pigs, and cattle. We also assessed the correlation of human antimicrobial usage in 11 European countries with the occurrence of resistance in *E. coli* isolates in blood stream infections.

Materials and Methods

Data collection

E. coli resistance data from human infections. Data on antimicrobial resistance of *E. coli* isolates from humans, between 2005 and 2008, were obtained from the European Antimicrobial Resistance Surveillance System (EARSS) database (available at www.ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/Database.aspx). This comprehensive surveillance system provides data on the prevalence of antimicrobial resistant bacteria in Europe (EARSS, 2008). EARSS collects susceptibility test results of blood stream *E. coli* as aggregated data, where countries report proportions of isolates that were classified as resistant or susceptible to aminoglycosides (gentamicin and/or tobramycin and/or amikacin), aminopenicillins (ampicillin and/or amoxicillin), fluoroquinolones (ciprofloxacin and/or ofloxacin and/or levofloxacin), and third-generation cephalosporins (cefotaxime and/or ceftriaxone and/or ceftazidime). Further information on data collection and reporting can be found at the EARSS Web site. Although guidelines were available, until 2008 there was no harmonization on the interpretation of the susceptibility status of these isolates and countries adopted different breakpoints for the classification of the isolate susceptibility status. Since the available aggregated data did not allow for the re-classification of the isolates according to a common breakpoint, we compared the range of the breakpoints used by countries reporting to EARSS to the EUCAST minimal inhibitory concentration (MIC) Distribution Reference Database (EUCAST).

The comparison between the range of the breakpoints used by countries reporting to EARSS and the EUCAST MIC Distribution Reference Database showed that, assuming that the EARSS data follow the EUCAST distribution, independently of the breakpoints used by the countries (within the given range), there were very few or no isolates expected to show the MIC values between the different used breakpoint range limits. More specifically, the breakpoints used by the assessed countries for ciprofloxacin were either $>1\,\mu g/mL$ or alternatively $>2\,\mu g/mL$. Although we have no information on the MIC distributions in these data, EUCAST ciprofloxacin MIC distribution data show that only 0.4% of the 17,877 isolates from 82 data sources presented $MIC = 2\,\mu g/mL$. This means that it is unlikely that a country using a breakpoint of $>2\,\mu g/mL$ missed any resistant isolates that would be detected by use of a breakpoint $>1\,\mu g/mL$. The same comparison was done for the remaining antimicrobials and all showed similar results, where variation of the adopted breakpoint, within the range of reported breakpoints, had no or minor impact on the resistance prevalences reported by each country.

E. coli resistance data from food animals. Data on antimicrobial resistance on commensal *E. coli* isolates from poultry, pigs, and cattle between 2005 and 2008 were obtained from two published reports on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union (EFSA, 2010a, 2010b). Detailed guidelines on sampling, harmonization, and data reporting performed by the European Food Safety Authority can be found in the published reports. Susceptibilities to ampicillin, fluoroquinolones (ciprofloxacin), aminoglycosides (gentamicin), and third-generation cephalosporins (cefotaxime) reported as MIC were analyzed in this study. Using MIC distributions for these antimicrobials, we applied clinical breakpoints similar to the lower limit of the range of breakpoints adopted to classify the susceptibility status of the human isolates. Breakpoints applied to the resistance data were $>8\,\mu g/mL$ for ampicillin, $>1\,\mu g/mL$ for ciprofloxacin, $>2\,\mu g/mL$ for gentamicin, and $>1\,\mu g/mL$ for cefotaxime. We assumed that all data used from food animals were reliable as all the participating countries managed the quality control acceptance threshold of the proficiency tests organized by the European Union Reference Laboratory.

Antimicrobial usage data. Data on antimicrobial consumption in ambulatory care (non-hospital data) were collected from the European Surveillance of Antimicrobial Consumption (ESAC) database, available at http://app.esac.ua.ac.be/esac_idb/consumption/home.htm. For the same countries for which resistance data were available, data on defined daily doses (DDD)/1000 inhabitants per day of the previous listed antimicrobials, aminoglycosides (J01GB), fluoroquinolones (J01MA), penicillins (J01CA), and third-generation cephalosporins (J01DD), between 2005 and 2008, were collected and the average usage rate in this period was used for the analysis.

Statistical analysis

For each country and antimicrobial, summary data from poultry, pigs, cattle, and human isolates from 2005 to 2008 were analyzed. The analysis was done by antimicrobial and

only data from countries reporting both human and food animal data were used. Spearman correlation coefficients (r) were calculated to assess the correlation between the resistance prevalences reported in food animals (poultry, pigs, and cattle) and human isolates, as well as the correlation between antimicrobial consumption (DDD/1000 inhabitants per day) and *E. coli* resistance prevalence in humans. For each antimicrobial, a regression line was fitted when a regression model assessing the potential impact of the resistance prevalence found in food animals (independent variable) on the resistance prevalence in the human population (dependent variable) showed significance of the predictor. Fisher transformation was used to determine significance of the correlation coefficients and significance was assumed for p-value < 0.05. For the purposes of statistical analysis, the sample size was the number of countries involved.

Results

Between 9 and 11 countries reported resistance data for both human and food animal isolates, depending on the antimicrobial evaluated. The following countries had antimicrobial resistance data available from both animal and human isolates: Austria, Denmark, Finland, France, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and Switzerland. For each antimicrobial, reported resistance prevalences were based on over 100,000 *E. coli* isolates from blood stream infections. Reported resistance prevalences on food animals were based on about 4000 poultry isolates, 4500 pig isolates, and 3500 cattle isolates.

The reported resistance prevalence varied according to the antimicrobial evaluated; resistance to ampicillin was frequently reported in both human and animal isolates, whereas resistance to fluoroquinolones and third-generation cephalosporins showed much lower prevalences. Occurrence of resistance to fluoroquinolones was more common among *E. coli* isolates from humans than from animals, except in Spain, where higher resistances to fluoroquinolones, ampicillin, aminoglycosides, and third-generation cephalosporins were reported for *E. coli* from poultry. Resistance to third-generation cephalosporins was more frequently reported in *E. coli* from humans and poultry, than from pigs and cattle. Wide ranges in the resistance prevalences were reported for all antimicrobials for both human and food animal isolates, illustrating the between-country variation on the prevalences of resistant *E. coli* isolates. The prevalences of resistant isolates, for poultry, pigs, cattle, and human *E. coli* isolates, are shown in Figure 1.

Data on both antimicrobial consumption and resistance in human *E. coli* isolates were available from a total of 10 countries. The average DDD/1000 inhabitants/day by each antimicrobial and country is shown in Figure 2. According to these data, France and Italy presented the highest usage rates of aminoglycosides, ampicillin, and third-generation cephalosporins, among the evaluated countries. Nordic countries and the Netherlands reported the lowest usage rates for third-generation cephalosporins and fluoroquinolones.

Strong and significant correlations between resistance prevalences to ampicillin ($r = 0.94$), fluoroquinolones ($r = 0.68$), aminoglycosides ($r = 0.72$), and third-generation cephalosporins ($r = 0.76$) were detected in *E. coli* isolates from humans and poultry ($p < 0.05$). Similar correlations with ampicillin ($r = 0.91$), aminoglycosides ($r = 0.73$), and fluoroquinolones ($r = 0.74$) were found for pig isolates. In cattle only with ampicillin ($r = 0.72$) was there a significant correlation. All estimated correlations are shown in Table 1. Figures 3 and 4 show regression lines and 95% confidence intervals describing the association between the resistance prevalences in *E. coli* isolates from poultry and humans and from pigs and humans, respectively, where each data point

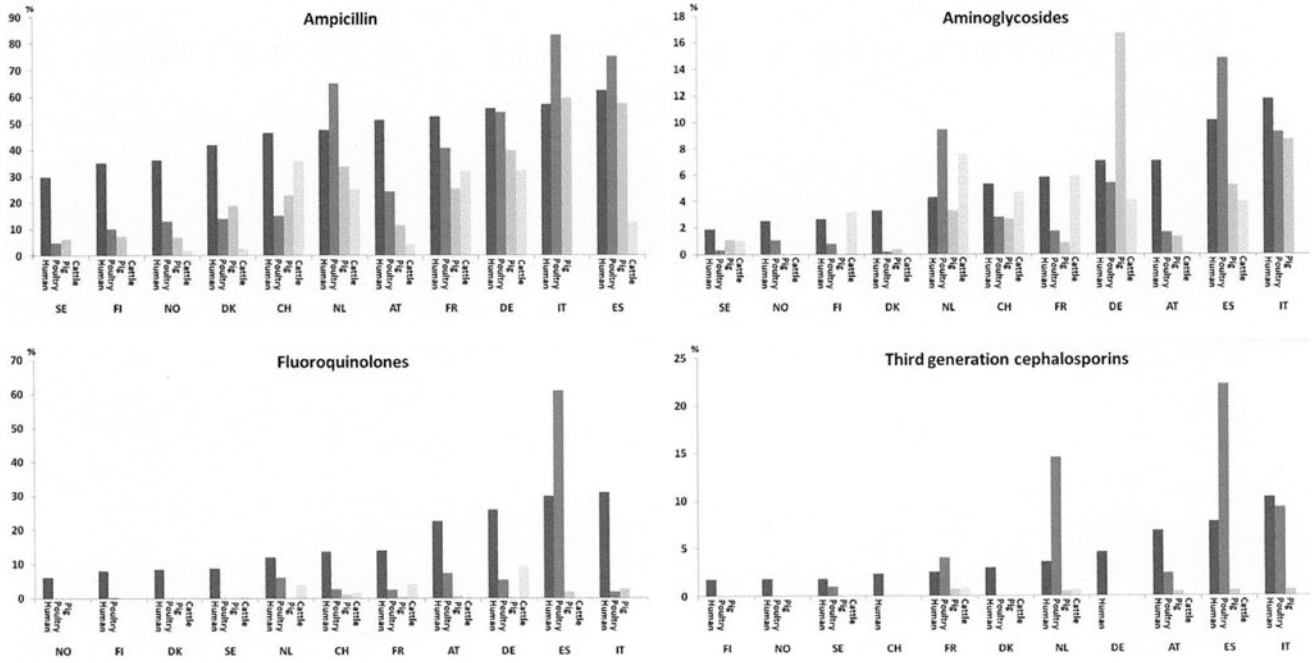

FIG. 1. Prevalence of resistance to selected antimicrobials among *Escherichia coli* isolates from humans, poultry, pigs, and cattle, in European countries between 2005 and 2008.

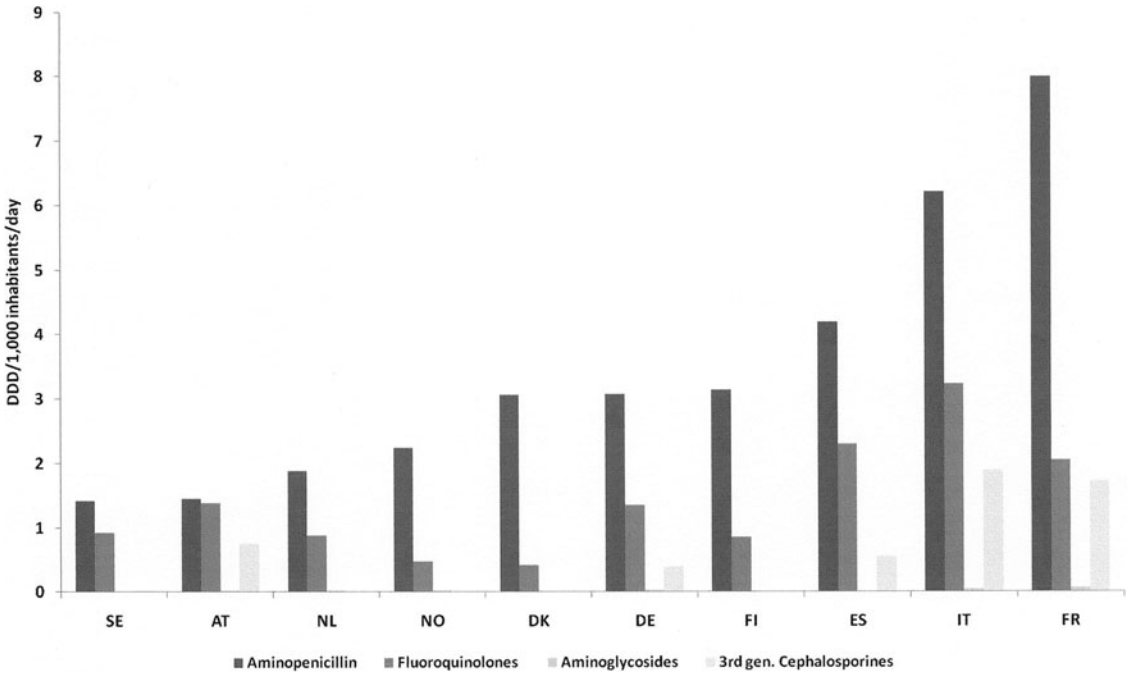

FIG. 2. Average antimicrobial consumption (DDD/1000 inhabitants/day) in ambulatory care, in European countries between 2005 and 2008. DDD, defined daily doses.

represents a country. Regression lines could be fitted for resistance to ampicillin and aminoglycosides in poultry and to ampicillin and fluoroquinolones in pigs, all with $p < 0.05$.

When usage of antimicrobials in humans was examined in comparison to antimicrobial resistance, only the correlations between fluoroquinolones ($r = 0.90$) and third-generation cephalosporins ($r = 0.75$) were significant (Table 1).

Discussion

E. coli is one of the most common bacterial causes of serious infections in people, and increasing resistance, particularly to critically important antimicrobials, is a major concern (Kennedy *et al*., 2008; de Kraker *et al*., 2010). Patients with resistant strains causing blood stream infections had much higher mortality rates as well as higher excess hospital lengths of stay (de Kraker *et al*., 2010). Our results show that there is a strong correlation between the antimicrobial resistance observed in *E. coli* strains from food animals, particularly from poultry and pigs, and the resistance prevalences seen in strains causing blood stream infection in people. Within this scenario, these findings suggest that food animals are a potential source of a substantial proportion of the resistant *E. coli* or the genes encoding for this resistance, which may be taken up by resident *E. coli* flora in cases of life-threatening blood stream infections in people. Our findings are similar to those in the studies of Johnson *et al*. (2006), which suggested that resistant *E. coli* colonizing the intestinal tract of people and then causing infections in people is mainly derived from poultry

Foodborne transmission is thought to be a major route for human acquisition of resistant pathogenic enteric bacteria or potential resistance gene-donor nonpathogenic bacteria (Aarestrup *et al*., 2008). Several studies and types of investigations have demonstrated foodborne transmission of antimicrobial resistance from animals to humans, including epidemiological and outbreak investigations, field studies,

Table 1. Correlation Between Resistance in *Escherichia coli* Isolates from Humans and from Poultry, Pigs, Cattle, and Antimicrobial Consumption in Humans (Ambulatory Care), in European Countries Between 2005 and 2008

	Resistance in Escherichia coli *isolates from humans and from poultry*			*Resistance in* E. coli *isolates from humans and from pigs*			*Resistance in* E. coli *isolates from humans and from cattle*			*Resistance in* E. coli *isolates from humans and antimicrobial consumption*		
Antimicrobial	*Number of countries*	*Correlation* (r)	p-*Value*	*Number of countries*	*Correlation* (r)	p-*Value*	*Number of countries*	*Correlation* (r)	p-*Value*	*Number of countries*	*Correlation* (r)	p-*Value*
Ampicillin	11	0.94	<0.01	11	0.91	<0.01	10	0.72	0.02	10	0.61	0.06
Fluoroquinolones	11	0.68	0.02	10	0.74	0.01	8	0.12	0.77	10	0.90	<0.01
Aminoglycosides	11	0.72	0.01	11	0.73	0.01	10	0.36	0.31	10	0.53	0.12
Third-generation cephalosporins	9	0.76	0.02	9	0.65	0.06	–	–	–	10	0.75	0.01

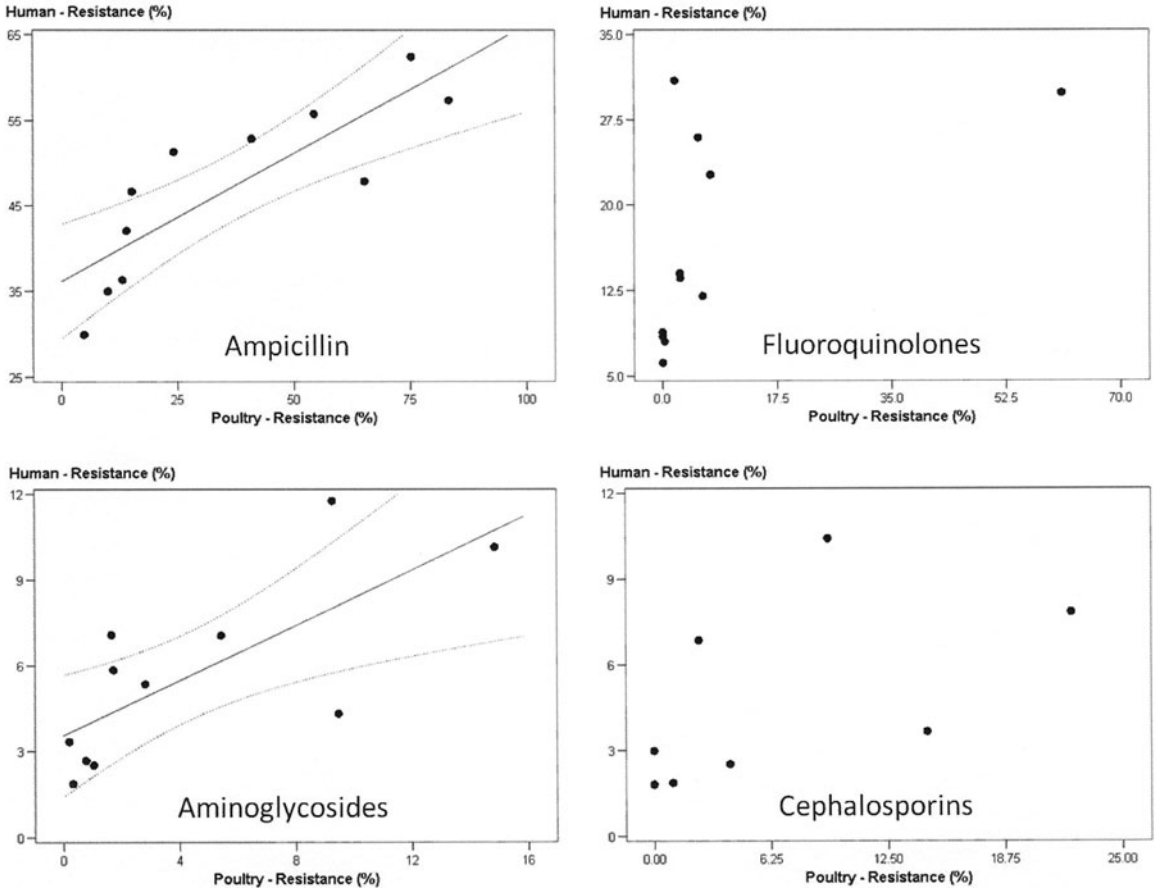

FIG. 3. Association between antimicrobial resistance prevalences in *E. coli* isolates from humans and from poultry, in European countries between 2005 and 2008. Regression line and 95% confidence interval was included when a regression model assessing the potential impact of the resistance prevalence found in poultry on the resistance prevalence in the human population fitted.

case reports, spatial/temporal associations, and molecular typing (Garau, *et al*., 1999; Li *et al*., 2007; Carattoli, 2008). These studies have shown that food animals may be involved in the *E. coli* spread to humans. In this study we only assessed significant correlation between resistance found in *E. coli* from poultry, pigs, and cattle and the resistance in *E. coli* from humans, but other food sources are likely to be relevant in the epidemiology of antimicrobial-resistant *E. coli*.

Our study does not take into account the amount of food imported and consumed in each country. In Denmark, imported poultry has much higher levels of resistant *E. coli* than locally produced poultry (DANMAP, 2009). Food imports may help explain why some countries with lower antimicrobial resistance in food products have higher rates of resistance in human isolates and vice versa. Another limitation of this study is the absence of data on the prevalence of the different *E. coli* phenotypes and resistance genes. Investigations on the phylogenetic distribution and virulence genotypes associated with antimicrobial resistant *E. coli* from humans and poultry products found that antimicrobial-resistant human isolates were similar to poultry isolates (Johnson *et al*., 2007). In Denmark, the prevalence of ESBL-producing bacteria in food animals has increased over the past years, followed by an increasing prevalence of ESBL-producing bacteria in humans (DANMAP, 2007).

Unnecessary (i.e., growth promotion) or excessive use of antimicrobial agents that are considered critically important for humans has been observed and reported in animal husbandry (aquaculture and agriculture) in recent years (McEwen and Fedorka-Cray, 2002; Grave and Wegener, 2006). There is a large body of scientific evidence showing that usage of antimicrobial agents selects for the presence of resistant bacteria in food animals and this poses a risk to human health (WHO, 2007; Aarestrup *et al*., 2008). There are several potential human health consequences of the emergence of antimicrobial resistance in foodborne bacteria, including increased number of infections, increased frequency of treatment failures, reduction in treatment choice after diagnosis, and increased infection severity (Cohen, 1994; Wise *et al*., 1998; de Kraker *et al*., 2010). Therefore, surveillance of resistance among bacteria of animal origin is essential to assess its human health consequences and develop risk management strategies.

The relationships found between these prevalences may be an indication of the transference of resistant bacteria via the food chain, but could also be a consequence of similar within country selective pressures in the discrete reservoirs. While there is already a great amount of data on ambulatory and hospital usage of antimicrobials in most countries, veterinary usage monitoring is still often lacking or vastly incomplete. There are exceptions, with Denmark, the Netherlands,

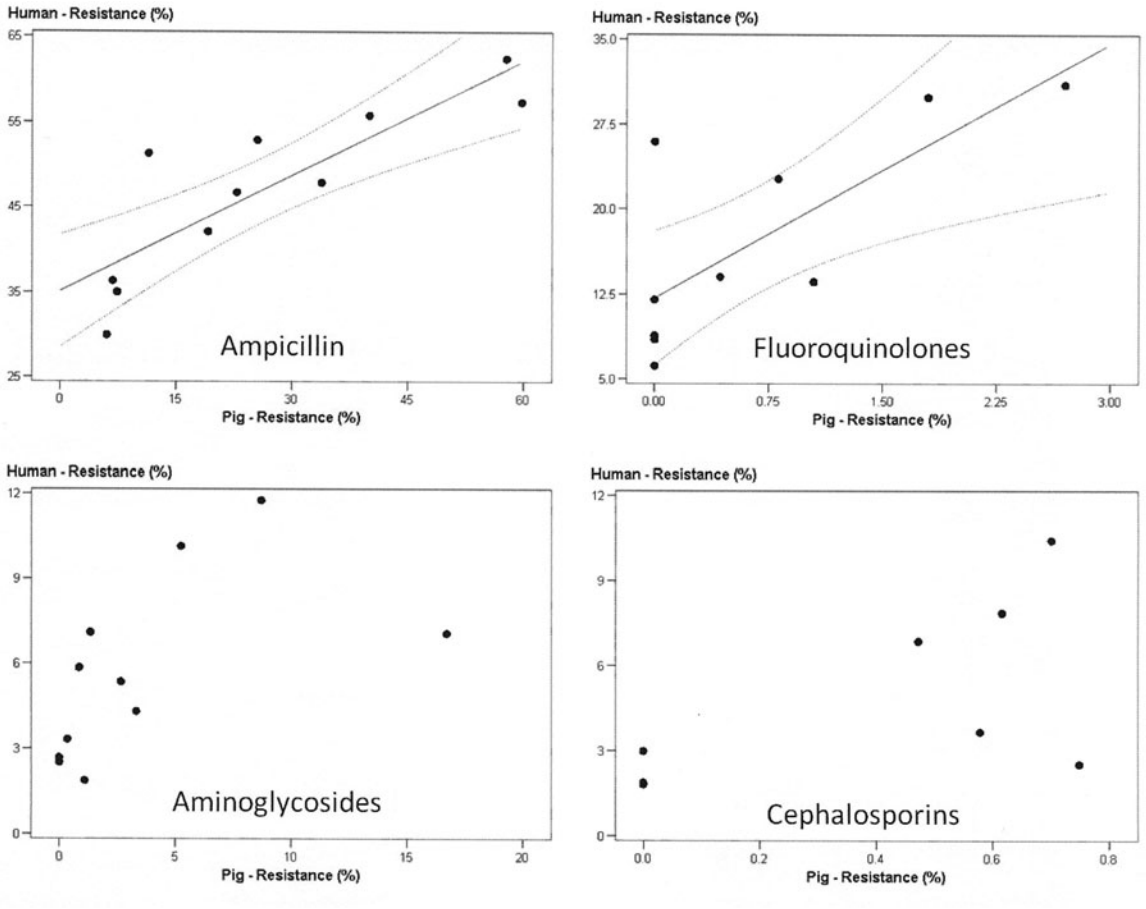

FIG. 4. Association between antimicrobial resistance prevalences in *E. coli* isolates from humans and from pigs, in European countries between 2005 and 2008. Regression line and 95% confidence interval was included when a regression model assessing the potential impact of the resistance prevalence found in pigs on the resistance prevalence in the human population fitted.

Sweden, and Norway being examples of countries with veterinary antimicrobial consumption-monitoring programs established for years. Most of the remaining EU countries are currently obtaining their first estimates on the figures of veterinary antimicrobial sales; so, hopefully, appropriate data will be available soon. A descriptive study assessing the sales of veterinary antibacterial agents between European countries illustrates the fact that current data are relatively sparse and not available in many countries (Grave *et al.*, 2010). When analyzing these data, we could not see any clear correlation between animal and human antimicrobial usage patterns, which supports the hypothesis of foodborne transference of resistant bacteria.

Using the available human consumption data, we also identified significant correlations between usage rates in humans and occurrence of resistance to fluoroquinolones and cephalosporins in *E. coli* isolates from humans. This finding is in line with other studies assessing correlations between antimicrobial usage and occurrence of resistant bacteria (Goossens *et al.*, 2005; van de Sande-Bruinsma *et al.*, 2008). While the association between usage and resistance development has in general been clearly documented, there is still a serious need for large epidemiological studies with participation of several countries. Both EMA (European Medicines Agency) and ESAC are currently working to establish reliable monitoring protocols for human and veterinary antimicrobial consumption in Europe.

In summary, we found a strong correlation between the prevalence of resistance to a number of antimicrobials in *E. coli* isolates from blood stream infections in humans and *E. coli* isolates from poultry and pigs, respectively. These findings exclude antimicrobial usage as the only explanatory variable for the observed resistances in *E. coli* from humans. They suggest that, in addition to the contribution of antimicrobial usage in people, a large proportion of resistant *E. coli* isolates causing blood stream infections in people are likely derived from food animal sources.

Disclosure Statement

No competing financial interests exist.

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Antimicrobial Drug–Resistant *Escherichia coli* from Humans and Poultry Products, Minnesota and Wisconsin, 2002–2004

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The food supply, including poultry products, may transmit antimicrobial drug–resistant *Escherichia coli* to humans. To assess this hypothesis, 931 geographically and temporally matched *E. coli* isolates from human volunteers (hospital inpatients and healthy vegetarians) and commercial poultry products (conventionally raised or raised without antimicrobial drugs) were tested by PCR for phylogenetic group (A, B1, B2, D) and 60 virulence genes associated with extraintestinal pathogenic *E. coli*. Isolates resistant to trimethoprim-sulfamethoxazole, quinolones, and extended-spectrum cephalosporins (n = 331) were compared with drug-susceptible isolates (n = 600) stratified by source. Phylogenetic and virulence markers of drug-susceptible human isolates differed considerably from those of human and poultry isolates. In contrast, drug-resistant human isolates were similar to poultry isolates, and drug-susceptible and drug-resistant poultry isolates were largely indistinguishable. Many drug-resistant human fecal *E. coli* isolates may originate from poultry, whereas drug-resistant poultry-source *E. coli* isolates likely originate from susceptible poultry-source precursors.

Acquired resistance to first-line antimicrobial agents increasingly complicates the management of extraintestinal infections due to *Escherichia coli*, which are a major source of illness, death, and increased healthcare costs (*1–4*). One suspected source of drug-resistant *E. coli* in humans is use of antimicrobial drugs in agriculture. This use presumably selects for drug-resistant *E. coli*, which may be transmitted to humans through the food supply (*5–7*). Supporting this hypothesis is the high prevalence of antimicrobial drug–resistant *E. coli* in retail meat products, especially poultry (*8–11*), and the similar molecular characteristics of fluoroquinolone-resistant *E. coli* from chicken carcasses and from colonized and infected persons in Barcelona, Spain, in contrast to the marked differences between drug-susceptible and drug-resistant source isolates from humans (*12*).

To further assess the poultry-human connection, we used molecular typing to characterize drug-resistant and drug-susceptible *E. coli* isolates from feces of human volunteers or newly hospitalized patients in Minnesota and Wisconsin and from poultry products sold or processed in the same region. Resistance phenotypes of interest include trimethoprim-sulfamethoxazole (TMP-SMZ), quinolones/fluoroquinolones, and extended-spectrum cephalosporins. These agents are used for treatment of human *E. coli* infections. These drugs (or congeners) are also used in poultry production (e.g., each year in the United States an estimated 1.6 billion broiler eggs or chicks receive ceftiofur [*13*]); *E. coli* isolates resistant to these drugs are found in poultry. We examined, according to phylogenetic group distribution and virulence gene profile, whether drug-resistant human isolates more closely resemble susceptible human isolates, which is consistent with acquisition of resistance within humans, or instead resemble poultry isolates, which is consistent with foodborne transmission of poultry-source organisms to humans. We also examined whether poultry-

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source resistant and susceptible isolates are similar, which is consistent with emergence of resistance on farms under selection from agricultural use of antimicrobial drugs.

Methods

Participants and Bacterial Strains

Human fecal samples were collected from 622 adults newly admitted to local hospitals in 4 rural communities in Minnesota (Willmar) or Wisconsin (Eau Claire, La Crosse, and Marshfield) and from 100 healthy self-identified vegetarians in these and nearby communities (*14*). Hospital patients were recruited from June 2002 through May 2003, vegetarians during the first 6 months of 2004. Fecal samples were collected by study personnel by using rectal swabs (hospital patients) or by the participants (vegetarians). To prevent isolation of hospital-acquired flora, inpatients samples were collected within 36 hours of hospital admission. Guidelines of the authors' institutions regarding use of human subjects were followed in this study. The relevant institutional review boards reviewed and approved the protocol. All participants provided informed consent.

A total of 180 retail poultry products (155 chicken and 25 turkey) were sampled (*14*). Conventional brands were purchased systematically from all food markets in the 4 primary study communities from May 2002 through May 2003, with 40 retail items obtained per community (total 160 items). These represented at least 18 plants in 11 states. Twenty samples with labels indicating that the poultry were raised naturally or without antibiotics were purchased in or near the study communities in August 2004. Additionally, 40 freshly slaughtered chicken carcasses from local farmers who raised chickens naturally or without antibiotics were obtained during plant inspections by the Minnesota Department of Agriculture from September 2003 through August 2004. The latter 2 groups of chickens, designated "no antibiotics," were confirmed to have been raised without antibiotics, based on the product label or by contacting the manufacturer or distributor.

Sample Processing

Human fecal samples were suspended and poultry samples and carcasses were massaged in nutrient broth, which was then incubated overnight at 37°C and stored as aliquots at –80°C in glycerol (*14*). Portions of these frozen stocks were transferred to vancomycin-supplemented (20 mg/L) Luria-Bertani broth. After overnight incubation, these broths were plated directly onto modified Mueller-Hinton (MMH) agar (Amyes medium) (*10*) with and without ciprofloxacin (4 mg/L) and (separately) nalidixic acid (32 mg/L), and were then incubated overnight. Samples of these Luria-Bertani broths containing vancomycin were placed in MMH broths supplemented individually with TMP-SMZ (4 mg/L TMP plus 76 mg/mL SMZ), cefoxitin (10 mg/L and 32 mg/L), and ceftazidime (10 mg/L and 32 mg/L). After overnight incubation, these broths were plated onto MMH agar plates supplemented with the corresponding agent (same concentrations) for overnight incubation. Colonies resembling *E. coli* were identified by using the API-20E System (bioMérieux, Marcy-l'Etoile, France).

Susceptibility Testing

At least 1 *E. coli* colony was randomly selected from each MMH agar plate and tested for disk susceptibility to 24 antimicrobial agents by using Clinical Laboratory Standards Institute (CLSI)–recommended methods, interpretive criteria, and reference strains (*15*). For isolates resistant to TMP-SMZ, nalidixic acid, or ciprofloxacin, the MIC was determined by Etest (AB-Biodisk, Sona, Sweden) according to the manufacturer's directions. Isolates from cefoxitin- and ceftazidime-supplemented plates underwent broth dilution MIC determinations with cefotaxime and ceftazidime regardless of disk test results. Isolates were classified as resistant to TMP-SMZ if the TMP MIC was ≥4 mg/L and the SMZ MIC was ≥76 mg/L, to quinolones if the nalidixic acid MIC was ≥32 mg/L, to fluoroquinolones if the ciprofloxacin MIC was ≥4 mg/L, and to extended-spectrum cephalosporins if the MIC to either cefotaxime or ceftazidime was ≥16 mg/L. The latter threshold corresponds with intermediate susceptibility per CLSI criteria and includes isolates with potentially clinically relevant reduced susceptibility. Because of the small number of isolates within each resistance phenotype, isolates were classified as resistant if they met any of these resistance criteria. Isolates that did not meet any of these resistance criteria were classified as susceptible, even though they may have had reduced susceptibility to other drug classes.

From each sample, 1 colony of each resistance phenotype (TMP-SMZ, quinolones, fluoroquinolones, extended-spectrum cephalosporins) and 1 susceptible isolate, as available, were selected. If multiple isolates from a given sample exhibited similar disk diffusion susceptibility profiles, genomic profiles as generated by using random amplified polymorphic DNA (RAPD) analysis were compared in the same gel (*12*). One representative of each unique RAPD genotype (as determined by visual inspection) was arbitrarily selected for further analysis.

Phylogenetic Analysis and Virulence Genotyping

All isolates were categorized as to major *E. coli* phylogenetic group (A, B1, B2, or D) by a multiplex PCR-based assay (*16*) (Table 1). Genes encoding proven or putative virulence factors of extraintestinal pathogenic *E. coli* (ExPEC) were detected in a sequential fashion. All isolates were screened for 5 ExPEC-defining virulence genes and *hlyD* (hemolysin). Isolates were operationally defined as

Table 1. Bacterial traits by source and antimicrobial drug resistance in 931 *Escherichia coli* isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004*

	Prevalence, no. (%)				p value‡		
Trait†	Total (n = 931)	Human, susceptible (n = 460)	Human, resistant (n = 70)	Poultry (n = 401)	HS vs. HR	HS vs. all poultry	HR vs. all poultry
Group A	252 (27)	96 (21)	23 (33)	133 (33)		≤0.001	
Group B1	186 (20)	79 (17)	11 (16)	96 (24)			
Group B2	234 (25)	178 (39)	13 (19)	43 (11)	≤0.001	≤0.001	
Group D	259 (28)	107 (23)	23 (33)	129 (32)		≤0.01	
papA	124 (13)	98 (21)	6 (9)	20 (5)		≤0.001	
papC	163 (18)	100 (22)	10 (14)	53 (13)		≤0.001	
sfa/focDE	69 (7)	65 (14)	2 (3)	2 (0.5)	≤0.01	≤0.001	
afa/draBC	19 (2)	14 (3)	5 (7)	0 (0)		≤0.001	≤0.001
iutA	361 (39)	93 (20)	32 (46)	236 (59)	≤0.001§	≤0.001§	
kpsM II	288 (31)	195 (42)	23 (33)	70 (17)		≤0.001	≤0.01
hlyD	71 (8)	64 (14)	2 (3)	4 (1)	≤0.01	≤0.001	
ExPEC	249 (27)	147 (32)	20 (29)	82 (20)		≤0.001	

*Data are for the total population. Susceptible, susceptible to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins), regardless of other possible drug resistance; resistant, resistant to 1 of the following: trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins).
†Groups A, B1, B2, and D, major *E. coli* phylogenetic groups; *papA* and *papC*, P fimbriae structural subunit and assembly; *sfa/focDE*, S and F1C fimbriae; *afa/draBC*, Dr binding adhesins; *iutA*, aerobactin system; *kpsM* II, group 2 capsule; *hlyD*, α-hemolysin; ExPEC, extraintestinal pathogenic *E. coli* defined by presence of ≥2 of *papA* and/or *papC* (counted as 1), *sfa/focDE*, *afa/draBC*, *iutA*, and *kpsM* II.
‡By Fisher exact test. Values are shown only where p≤0.01. HS, susceptible isolates from humans; HR, resistant isolates from humans. Because drug-resistant and drug-susceptible poultry isolates showed only 1 significant difference (for *iutA*), they were combined into an all-poultry group.
§Negative association.

ExPEC if ≥2 of the following were present: *papA* and/or *papC* (P fimbriae structural subunit and assembly), *sfa/focDE* (S and F1C fimbriae), *afa/draBC* (Dr binding adhesins), *iutA* (aerobactin system), and *kpsM* II (group 2 capsule) (*8*). All ExPEC isolates were then tested for 60 ExPEC-associated virulence genes and alleles thereof. Testing was conducted by using 2 independently prepared lysates of each isolate and established PCR-based methods (*12*,*17*). Isolates from various source groups (e.g., hospital volunteers, conventionally raised poultry) were tested in parallel to avoid cohort effects. The virulence score was the number of virulence genes detected adjusted for multiple detection of the *pap*, *sfa/foc*, and *kps* operons (*12*).

Statistical Methods

The unit of analysis was the individual isolate. Comparisons of proportions were tested by using Fisher exact test (2-tailed). Comparisons of virulence scores were tested by using Mann-Whitney U test (2-tailed exact probability). Principal coordinates analysis (PCA), also known as metric multidimensional scaling, is a multivariate statistical technique used to provide a simpler, low-dimensional graphic summary of the similarity between multiple samples (e.g., isolates) across multiple loci (*18*). New axes for plotting the isolates are derived from a data matrix of estimated dissimilarities between isolates. The first 2 principal coordinates, which account for the most variance, are used to plot the data. The distances between points in the plot represent isolate similarity. The dimensions represented by the (statistically uncorrelated) axes have no intrinsic meaning, i.e., they have no units. Using GenAlEx6 (*19*), we applied PCA to the screening dataset (all isolates) and the extended virulence profile dataset (ExPEC isolates) as a way to collapse the multiple variables for simplified among-group comparisons. For each PCA, results for each isolate from the first 2 PCA axes were used in multiple analysis of variance (MANOVA) to test for among-group differences. These values also were plotted to spatially represent the degree of separation or overlap of isolates on the 2-axis plane. For the ExPEC isolates, pairwise similarity relationships according to extended virulence profiles and phylogenetic group were used to construct a dendrogram according to the unweighted pair group method with arithmetic averages (*20*). The criterion for statistical significance throughout was p≤0.01 to account for multiple comparisons.

Results

Isolation of Drug-Resistant and Drug-Susceptible *E. coli*

Selective processing of 942 human fecal and poultry samples yielded 931 unique *E. coli* isolates, which constituted the study population. Of the 931 isolates, 530 (57%) were from human volunteers and 401 (43%) from poultry products. Of the human isolates, 456 (86%) were from hospital patients and 74 (14%) from vegetarians. Of the poultry isolates, 289 (72%) were from conventionally raised retail poultry and 112 (28%) from poultry raised without antibiotics. The median number of unique *E. coli* isolates

per sample was 1 for human fecal samples and 2 for poultry (range 1–4 for both).

Overall, 331 isolates (70 human, 261 poultry) were classified as resistant on the basis of reduced susceptibility to TMP-SMZ, quinolones/fluoroquinolones, and extended-spectrum cephalosporins. The remaining 600 isolates (460 human, 140 poultry) were susceptible to all these drug classes and were classified as susceptible (regardless of other possible drug resistance). The resistant isolates were distributed by resistance phenotype as follows: TMP-SMZ, 154 (47 human, 107 poultry); quinolones, 115 (26 human, 89 poultry); and extended-spectrum cephalosporins, 114 (14 human, 100 poultry). The 7 fluoroquinolone-resistant isolates (5 human, 2 poultry) were analyzed within the quinolone-resistant group.

Phylogenetic Distribution and Prevalence of ExPEC-defining Markers

The initial screening showed the 931 isolates to be fairly evenly distributed among the 4 major *E. coli* phylogenetic groups (20%–28% per group). However, they had various prevalences (2%–39% each) of the screening ExPEC virulence genes (Table 1). Overall, 27% of the isolates qualified as ExPEC by having ≥2 of the 5 ExPEC-defining markers (Table 1).

For enhanced resolution of similarities and differences, the 243 available ExPEC isolates underwent extended virulence genotyping for 60 ExPEC-associated virulence genes. All but 6 of these traits were detected in ≥1 isolate each, with prevalences ranging from 0.4% to 98% (Table 2).

Prevalence Comparisons

Phylogenetic group distribution and virulence gene prevalence differed considerably according to source (human versus poultry) and resistance status. This finding is shown in Table 1 for all 931 isolates (screening virulence genes only) and in Table 2 for the 243 ExPEC isolates (extended virulence profiles). Drug-resistant and drug-susceptible human isolates were separately compared with the combined group of all poultry isolates (i.e., all susceptible and resistant). We analyzed poultry isolates as a single group because the distribution of traits was similar in drug-resistant and susceptible poultry isolates; i.e., only 1 trait (*iutA*) was significantly associated with resistance among poultry isolates.

Consistent differences in phylogenetic and virulence gene distribution were evident between groups (Tables 1, 2). First, drug-susceptible human isolates differed considerably from drug-resistant human isolates. Second, drug-susceptible human isolates differed from poultry isolates. Third, although human drug-resistant isolates and poultry isolates exhibited some differences, these were considerably fewer and less extreme than those between drug-susceptible human isolates and poultry isolates. Similar results were obtained in subgroup analyses when isolates from hospital patient fecal samples were compared separately with isolates from conventionally raised poultry or when isolates from fecal samples from vegetarians were compared separately with isolates from poultry raised without antibiotics.

PCA

PCA was used to concurrently analyze multiple bacterial characteristics. The first PCA was conducted for the total population (n = 931) with the 7 screening virulence genes plus phylogenetic group. According to a 2 × 2 (source × resistance status) MANOVA of the first 2 axes of the PCA (which accounted for 65% of total variance), all 3 independent variables considered (source, resistance status, and interaction term) showed a p value ≤0.001. Accordingly, pairwise comparisons were made between individual source-resistance groups by 1-factor MANOVA. Susceptible human isolates differed ($p<0.001$) from each of the other 3 groups, whereas the other 3 groups differed marginally from each other. The individual axes supported this conclusion. These axes showed more extreme differences between drug-susceptible human isolates and each of the other 3 groups ($p<0.001$ for 5 of 6 comparisons) than among the other groups ($p>0.01$ for 4 of 6 comparisons).

Next, PCA was conducted for the 243 available ExPEC isolates based on all 60 virulence genes plus phylogenetic group. According to an initial 2 × 2 MANOVA of the results from the first 2 PCA axes (which accounted for 57% of total variance), all 3 independent variables (source, resistance status, and interaction term) showed a p value <0.001. Accordingly, pairwise comparisons were made between individual source-resistance groups by 1-factor MANOVA. Susceptible human isolates differed ($p<0.001$) from each of the other 3 groups, whereas the other 3 groups did not differ significantly from each another. In a plot of the (axis 1–axis 2) plane, drug-susceptible poultry isolates, drug-resistant poultry isolates, and drug-resistant human isolates overlapped and were confined largely to the left half of the grid (negative values on axis 1). In contrast, drug-susceptible human isolates, although overlapping somewhat with these groups, were concentrated principally within the right half of the grid (positive values on axis 1) (Figure 1).

Aggregate Virulence Scores

The various source and resistance groups were also compared for aggregate virulence scores (ExPEC isolates only). According to virulence score distribution, drug-susceptible human isolates (higher scores) segregated widely from the other 3 subgroups (lower scores), which were largely superimposed on each another (Figure 2). Because drug-resistant and drug-susceptible poultry isolates had similar virulence scores, they were combined for statistical

analysis. Drug-susceptible human isolates had the highest scores (median 13.0, range 4.25–20.0). Drug-resistant human and poultry isolates had significantly lower scores that did not differ between humans and poultry (median 9.0, range 6.0–15.25, and median 8.75, range 3.75–15.0, respectively; vs. drug-susceptible human isolates, p<0.001). Similar results were obtained when isolates from hospital patient fecal samples were compared separately with the conventionally raised poultry isolates or when isolates from vegetarian fecal samples were compared separately with isolates from poultry raised without antibiotics (data not shown).

Table 2. Bacterial traits by source and antimicrobial drug resistance in 243 extraintestinal pathogenic *Escherichia coli* (ExPEC) isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004*

	Prevalence, no. (%)				p value¶		
Trait†‡§	Total (n = 243)	Human, susceptible (n = 144)	Human, resistant (n = 20)	Poultry (n = 79)	HS vs. HR	HS vs. all poultry	HR vs. all poultry
Group A	20 (8)	5 (3)	5 (25)	10 (13)	≤0.01#		
Group B1	7 (3)	0	0	7 (9)		≤0.001#	≤0.001#
Group B2	154 (63)	125 (87)	6 (30)	23 (29)		≤0.001	
Group D	62 (26)	14 (10)	9 (45)	39 (49)		≤0.001#	
papA	117 (48)	97 (67)	7 (35)	13 (16)	≤0.01	≤0.001	
F10 allele	38 (16)	32 (10)	5 (25)	1 (1)		≤0.001	≤0.001
F16 allele	12 (5)	5 (3)	5 (25)	2 (3)	≤0.01#		≤0.01
F48 allele	21 (9)	21 (15)	0	0		≤0.001	
papG III	44 (18)	44 (31)	0	0	≤0.01	≤0.001	
sfa/focDE	62 (26)	61 (42)	1 (5)	0	≤0.001	≤0.001	
sfaS	35 (14)	33 (23)	1 (5)	1 (1)		≤0.001	
focG	13 (5)	12 (8)	1 (5)	0		≤0.01	
afa/draBC	15 (6)	11 (8)	4 (20)	0		≤0.01	≤0.001
iha	52 (22)	38 (26)	16 (80)	0	≤0.001#	≤0.001	≤0.001
hra	108 (44)	67 (47)	2 (10)	39 (49)	≤0.001		≤0.01#
cnf1	54 (22)	51 (35)	2 (10)	1 (1)		≤0.001	
hlyD	67 (28)	67 (28)	2 (10)	2 (3)	≤0.01	≤0.001	
hlyF	73 (30)	28 (19)	1 (5)	44 (57)		≤0.001#	≤0.001#
sat	61 (25)	46 (32)	15 (75)	0 (0)	≤0.001#	≤0.001#	≤0.001#
pic	34 (14)	30 (21)	0	4 (5)		≤0.01	
vat	131 (54)	113 (78)	3 (15)	15 (19)	≤0.001	≤0.001	
astA	48 (20)	7 (5)	1 (5)	40 (51)		≤0.001#	≤0.001#
iutA	162 (67)	67 (47)	18 (90)	77 (97)		≤0.001#	
iroN	118 (49)	78 (54)	3 (15)	37 (47)	≤0.001		≤0.01#
fyuA	199 (82)	138 (96)	17 (85)	44 (56)		≤0.001	
kpsM II	215 (89)	137 (95)	16 (80)	62 (78)		≤0.001	
K5 *kpsM*	35 (14)	28 (19)	4 (20)	3 (4)		≤0.001	
iss	69 (28)	23 (16)	2 (10)	44 (56)		≤0.001#	≤0.001#
usp	144 (59)	127 (88)	6 (30)	11 (14)	≤0.001	≤0.001	
H7 *fliC*	52 (21)	52 (36)	0	0	≤0.001	≤0.001	
ompT	184 (76)	131 (91)	9 (50)	40 (51)	≤0.01	≤0.001	
malX	152 (63)	134 (93)	7 (35)	1 (14)	≤0.001	≤0.001	

*Susceptible, susceptible to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins), regardless of other possible drug resistance; resistant, resistant to ≥1 of the following: trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins).

†Traits are shown that showed p≤0.01 for ≥1 comparison each. Groups A, B1, B2, and D, major *E. coli* phylogenetic groups; *papA*, P fimbriae structural subunit with variants F10, F16, and F48; *papG* III, variant P adhesin; *sfa/focDE*, S and F1C fimbriae; *sfaS*, S fimbriae; *focG*, F1C fimbriae; *afa/draBC*, Dr binding adhesins; *iha*, adhesin-siderophore receptor; *hra*, pathogenicity island marker; *cnf1*, cytotoxic necrotizing factor 1; *hlyD*, α-hemolysin; *hlyF*, variant hemolysin; *sat*, secreted autotransporter toxin; *pic*, autotransporter protease; *vat*, vacuolating autotransporter; *astA*, enteroaggregative *E. coli* toxin; *iutA*, aerobactin system; *iroN*, siderophore receptor; *fyuA*, yersiniabactin receptor; *kpsM* II, group 2 capsule; K5 *kpsM*, *kpsM* II variant; *iss*, increased serum survival; *usp*, uropathogenic-specific protein; H7 *fliC*, flagellar variant; *ompT*, outer membrane protease; *malX*, pathogenicity island marker.

‡Traits that did not show p<0.01 but were detected in ≥1 isolate each include the F7–2, F8, F9, F11, F12, F12, F14, and F15 *papA* alleles, *papC* (P fimbriae assembly), *papEF* (P fimbriae tip pilins), *papG* alleles I and II (both internal and flanking sequences), *afaE8* (variant Dr binding adhesin), *gafD* (G fimbriae), F17 fimbriae, *fimH* (type 1 fimbriae), *clpG* (adhesin), *cdtB* (cytolethal distending toxin B), *ireA* (siderophore receptor), *kpsM* III (group 3 capsule), K1 and K2 *kpsM* II variants, *cvaC* (microcin V), *ibeA* (invasion of brain endothelium), and *rfc* (O4 lipopolysaccharide biosynthesis).

§Traits not detected in any isolate include F7–1 and F536 *papA* alleles and K15 *kpsM* II variant.

¶By Fisher exact test. Values are shown only where p≤0.01. HS, susceptible isolates from humans; HR, drug-resistant isolates from humans. Because drug-resistant and drug-susceptible poultry isolates showed no significant differences, they were combined into an all-poultry group.

#Negative association.

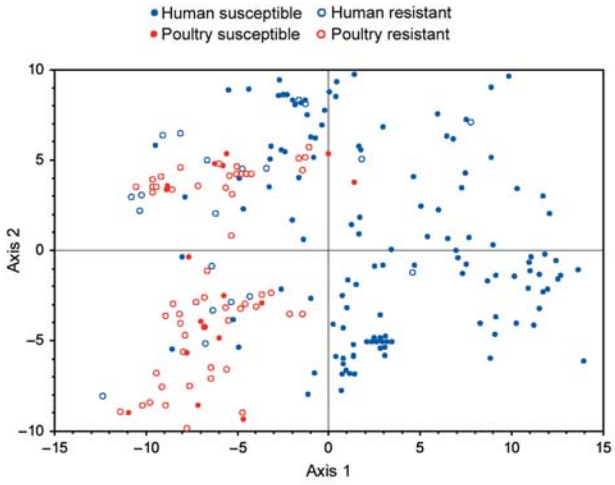

Figure 1. Principal coordinates analysis of distribution of 243 extraintestinal pathogenic *Escherichia coli* isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004, on the axis 1–axis 2 plane. Data include extended virulence genotypes (60 traits) and phylogenetic group (A, B1, B2, D). The axes have no units; they reflect the total score for each isolate derived by summing the isolate's partial score for each variable, which is the product of the loading score assigned to the particular variable for a given axis and the isolate's status for that variable. Axis 1 (positive values to right, negative values to left of central vertical line) accounted for 37% of total variance and showed significant differences between susceptible human isolates versus each of the other groups. Axis 2 (positive values above, negative values below central horizontal line) accounted for 20% of total variance and did not show any significant between-group differences. Resistant, resistant to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins). Susceptible, susceptible to all these agents (regardless of other possible drug resistance).

Dendrogram of Extended Virulence Profiles and Phylogenetic Group

Phylogenetic group and extended virulence profiles among the 243 available ExPEC isolates also were used to construct a similarity dendrogram. The dendrogram showed 3 major clusters, each of which contained 2 prominent subclusters (Figure 3). Isolates were distributed by cluster and subcluster according to source and resistance group; that is, drug-susceptible human isolates accounted for almost all of subclusters 1a, 1b, and 2a. In contrast, drug-resistant human isolates were confined largely to subcluster 3a. Poultry isolates, whether resistant or susceptible, were confined almost entirely to subclusters 2b, 3a, and 3b. Thus, compared with drug-susceptible human isolates, drug-resistant human isolates were significantly more likely to occur within a subcluster, or major cluster, that also contained poultry isolates ($p<0.001$ for each comparison).

The possible effects of nonindependence among multiple isolates acquired from the same sample were assessed by limiting the analysis to a single isolate per sample, keeping a drug-susceptible isolate (if available) and randomly selecting among multiple drug-resistant isolates where required. This resulted in reduced sample sizes of 681 (total population) and 226 (ExPEC population). The analysis results closely mirrored the pattern of significant findings obtained in the full samples.

Discussion

In this study, we analyzed the phylogenetic distribution and virulence genotypes of drug-susceptible and drug-resistant *E. coli* isolates from human volunteers and poultry products in Minnesota and Wisconsin. We found that drug-resistant human isolates, although overlapping somewhat with drug-susceptible human isolates, were more similar overall to poultry isolates than to drug-susceptible human isolates. In contrast, drug-susceptible human isolates differed from poultry isolates. This relationship was observed consistently with diverse analytical approaches and various stratifications of the population. It suggests that many of the drug-resistant human isolates were more likely to have originated in poultry (or a similar nonhuman reservoir) and to have been acquired by humans when these isolates were already drug resistant, than to have emerged de novo in humans by conversion of drug-susceptible human isolates to drug-resistant isolates.

We also found that, regardless of analytical approach and population analyzed, resistant and susceptible poultry isolates were highly similar. This suggests that the resistant poultry isolates likely derived from antimicrobial drug–

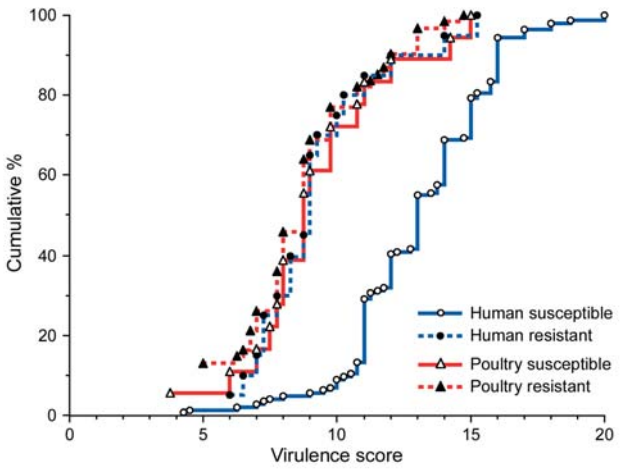

Figure 2. Distribution of virulence factor scores by source and resistance status among 243 extraintestinal pathogenic *Escherichia coli* isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004. Resistant, resistant to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins). Susceptible, susceptible to all these agents (regardless of other possible resistances). The virulence scores of the susceptible human isolates are an average of ≈4 points greater than those of the resistant human isolates or poultry isolates.

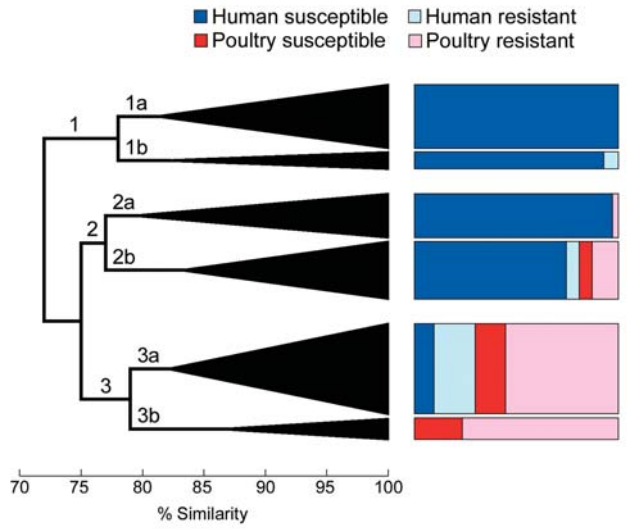

Figure 3. Dendrogram based on extended virulence profiles of 243 extraintestinal pathogenic *Escherichia coli* isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004. The dendrogram (shown here in simplified form) was constructed by using the unweighted pair group method with arithmetic averages based on pairwise similarity relationships according to the aggregate presence or absence of 60 individual virulence genes plus phylogenetic group (A, B1, B2, D). Triangles indicate arborizing subclusters. Major clusters 1, 2, and 3, and subclusters 1a, 1b, 2a, 2b, 3a, and 3b are indicated. Colored boxes to right of dendrogram show the distribution (by source group) of constituent members of each subcluster. Resistant, resistant to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins). Susceptible, susceptible to all these agents.

susceptible, poultry-source *E. coli* by conversion to resistance. This most plausibly would occur within the avian fecal flora under selection pressure from on-farm use of antimicrobial drugs.

Our findings closely resemble those of a recent study of ciprofloxacin-resistant *E. coli* from humans and chickens in the late 1990s in Barcelona, Spain (*12*). These data indicate that these relationships remain valid and are applicable in the United States, to additional resistance phenotypes (specifically quinolones, TMP-SMZ, and extended-spectrum cephalosporins), and to retail poultry products (*12*). Moreover, similar results were obtained with retail poultry products and poultry carcasses from processing plants. This implies that drug-resistant poultry-source *E. coli* isolates originate in the birds, rather than being introduced from some exogenous reservoir later during the packaging and distribution process. This in turn suggests that on-farm practices, including use of antimicrobial agents for growth promotion, metaphylaxis, and therapy (*21*,*22*), may influence characteristics of *E. coli* that contaminate retail poultry products and, seemingly, are then transmitted to humans (*7*).

The greater overall similarity of drug-resistant human isolates to poultry isolates than to drug-susceptible human isolates applied not only to the hospital patient isolates compared with isolates from conventionally raised poultry, but also to the isolates from vegetarians compared with isolates from poultry raised with no antibiotics. This was surprising because the vegetarians ostensibly did not consume poultry and, therefore, should not have been directly exposed to poultry-source *E. coli*. However, this seeming paradox is consistent with the difficulty in confirming poultry consumption (along with most other individual-level exposures) as an epidemiologic risk factor for colonization with drug-resistant *E. coli* isolates among community-dwelling persons ([*23*]; J.R. Johnson, unpub. data). Assuming that the drug-resistant human isolates were derived from poultry, occurrence of poultry-source *E. coli* in both vegetarians and persons with conventional diets suggests that poultry-source drug-resistant *E. coli* may spread extensively through the human population without requiring individual exposure to poultry products. This suggestion would be consistent with evidence that household-level risk factors may be more predictive of colonization with drug-resistant *E. coli* than individual-level risk factors, and that household members often share *E. coli* clones with each another (*23*–*25*). The mechanisms for such diffusion, and methods to block the entry of such strains into the human population and their subsequent spread, need to be defined.

The virulence potential for humans of the present drug-resistant human and poultry *E. coli* isolates, which is related to their direct threat to human health, is unknown. Predictions regarding virulence potential await molecular comparisons with human clinical isolates (*9*,*10*,*12*) and experimental virulence assessment in vivo (*26*,*27*). Nonetheless, the abundance of ExPEC-associated virulence genes in some of these strains is of concern because it suggests a high likelihood of virulence. This would augment any health threat these strains may pose as passive vehicles for drug-resistance genes (*6*,*7*).

Potential limitations of this study warrant comment. Because we did not examine alternative sources for drug-resistant human isolates, we cannot exclude the possibility that other foods (*28*) or nonfood reservoirs (*29*) might yield even closer similarities to drug-resistant human isolates. Whether persons in the study consumed poultry products from the same lots or suppliers as those sampled is not known. Because the study was conducted in Minnesota and Wisconsin in mostly rural communities and with newly hospitalized patients and nonhospitalized vegetarians, generalizability of the results is unknown. We combined several resistance phenotypes because of low frequencies, which may have obscured differences. We also did not assess other molecular characteristics of strains, e.g., pulsed-field gel electrophoresis profiles (*12*), sequence types (*30*),

and resistance elements (*28*). Use of multiple comparisons increased the likelihood of spurious associations (which we addressed by specifying a strict criterion for statistical significance), whereas the small sample size in certain subgroups reduced power for finding true associations.

Strengths of the study include substantial overall sample size, standardized concurrent processing of fecal and poultry samples, close matching of human and poultry samples, extensive molecular typing using virulence-relevant markers, and use of multiple analytical modalities. Additionally, we examined clinically relevant resistance phenotypes.

In summary, our findings suggest that in a contemporary US-based population, many human-source drug-resistant fecal *E. coli* isolates more likely originated in poultry than in humans, whereas drug-resistant poultry isolates likely derive from drug-susceptible poultry isolates. Our data extend this paradigm to clinically relevant agents other than fluoroquinolones, heighten concerns regarding the potential human health risk for antimicrobial drug use in poultry production, and suggest that avoidance of poultry consumption may not reliably provide personal protection.

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WIDESPREAD DISTRIBUTION OF URINARY TRACT INFECTIONS CAUSED BY A MULTIDRUG-RESISTANT *ESCHERICHIA COLI* CLONAL GROUP

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ABSTRACT

Background The management of urinary tract infections is complicated by the increasing prevalence of antibiotic-resistant strains of *Escherichia coli.* We studied the clonal composition of *E. coli* isolates that were resistant to trimethoprim–sulfamethoxazole from women with community-acquired urinary tract infections.

Methods Prospectively collected *E. coli* isolates from women with urinary tract infections in a university community in California were evaluated for antibiotic susceptibility, O:H serotype, DNA fingerprinting, pulsed-field gel electrophoretic pattern, and virulence factors. The prevalence and characteristics of an antibiotic-resistant clone were evaluated in this group of isolates and in those from comparison cohorts in Michigan and Minnesota.

Results Fifty-five of the 255 *E. coli* isolates (22 percent) from the California cohort were resistant to trimethoprim–sulfamethoxazole as well as other antibiotics. There was a common pattern of DNA fingerprinting, suggesting that the isolates belonged to the same clonal group (clonal group A), in 28 of 55 isolates with trimethoprim–sulfamethoxazole resistance (51 percent) and in 2 of 50 randomly selected isolates that were susceptible to trimethoprim–sulfamethoxazole (4 percent, $P<0.001$). In addition, 11 of 29 resistant isolates (38 percent) from the Michigan cohort and 7 of 18 (39 percent) from the Minnesota cohort belonged to clonal group A. Most of the clonal group A isolates were serotype O11:H(nt) or O77:H(nt), with similar patterns of virulence factors, antibiotic susceptibility, and electrophoretic features.

Conclusions In three geographically diverse communities, a single clonal group accounted for nearly half of community-acquired urinary tract infections in women that were caused by *E. coli* strains with resistance to trimethoprim–sulfamethoxazole. The widespread distribution and high prevalence of *E. coli* clonal group A have major public health implications. (N Engl J Med 2001;345:1007-13.)

AN estimated 11 percent of women in the United States report at least one physician-diagnosed urinary tract infection per year, and the lifetime probability that a woman will have a urinary tract infection is 60 percent.[1] The clinical management of urinary tract infection is complicated by the increasing incidence of infections caused by strains of *Escherichia coli* that are resistant to commonly used antimicrobial agents. In recent studies in the United States, the rates of resistance to trimethoprim–sulfamethoxazole among *E. coli* isolates from women with urinary tract infections ranged from 15 to 18 percent.[2-4]

Although urinary tract infection is not usually thought of as a disease associated with community-wide outbreaks, certain multidrug-resistant, uropathogenic lineages of *E. coli* have exhibited epidemic behavior.[5] *E. coli* O15:K52:H1 caused an outbreak of community-acquired cystitis, pyelonephritis, and septicemia in South London in 1987 and 1988[5] and is an endemic cause of urinary tract infection in Barcelona, Spain.[6] This clonal group's distinctive profile of antibiotic resistance contributed to its identification in Europe.[5]

We studied the clonal composition of *E. coli* isolates from women (age range, 17 to 68 years) with uncomplicated, community-acquired urinary tract infections that were resistant to trimethoprim–sulfamethoxazole in order to ascertain whether any clonal group of *E. coli* was disproportionately represented.

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METHODS

Study Cohorts

The study subjects included three cohorts of women (defined as those who were at least 17 years old) with urinary tract infections and a comparison group of healthy women whose stool specimens were analyzed to identify the *E. coli* isolates. The study was approved by the Committee for Protection of Human Subjects of the University of California at Berkeley; informed consent was not obtained, because the study involved neither direct interviews nor chart reviews. The California cohort consisted of women with symptoms of urinary tract infection who were seen at a university health service and were consecutively enrolled in the study between October 11, 1999, and January 31, 2000. A case of *E. coli* urinary tract infection was defined as symptoms suggestive of infection[4] and a culture of a clean-catch urine specimen with more than 10^2 colony-forming units of *E. coli* per milliliter.[7]

The two comparison cohorts were women with uncomplicated cystitis who were seen at university health services in Minnesota and Michigan. For the Minnesota cohort, we analyzed all *E. coli* urinary isolates that were resistant to trimethoprim–sulfamethoxazole and 20 isolates that were susceptible to the combined drugs. The isolates were obtained from students with uncomplicated cystitis who were enrolled in a study between June 1998 and August 1999. For the Michigan cohort, we analyzed a similarly selected sample of urinary isolates from women with acute cystitis who were enrolled in a university-based study between September 1996 and May 1999.

Another comparison cohort consisted of 41 healthy residents at least two years of age (18 male subjects and 23 female subjects) of the university community in California who were free of urinary tract infection. Between August 1998 and November 2000, samples of freshly passed stool obtained from each subject monthly for six months were cultured for *E. coli*.

Isolation of *E. coli*

In California and Minnesota, urine samples were cultured on MacConkey agar. Colonies that were positive for lactose and indole were presumptively identified as *E. coli*. Culture methods for the Michigan cohort have been described previously.[8] One putative *E. coli* colony from each urine culture was arbitrarily selected for further analyses. Five *E. coli* colonies per monthly fecal sample were selected for DNA fingerprinting with a polymerase-chain-reaction (PCR) assay.

Antibiotic Susceptibility Testing

E. coli isolates were screened for susceptibility to trimethoprim–sulfamethoxazole with the use of E-test strips (AB Biodisk, Solna, Sweden) in California, the MicroScan system (Dade Behring, Sacramento, Calif.) in Michigan, and a standard disk-diffusion assay[9] in Minnesota. *E. coli* strain 25922 (from the American Type Culture Collection) was used as the reference strain. Susceptibility to 18 additional antimicrobial agents was assessed for selected isolates by the disk-diffusion method,[10] with the use of standard interpretive criteria.[9] Intermediate susceptibility was interpreted as full susceptibility.

DNA Fingerprinting

For each of the three cohorts of women with urinary tract infections, all isolates that were resistant to trimethoprim–sulfamethoxazole and subgroups of susceptible isolates, selected either randomly (in California and Michigan) or arbitrarily (in Minnesota), were screened with the enterobacterial repetitive intergenic consensus (ERIC2) PCR fingerprinting assay,[11-15] as previously described.[16] Isolates with fingerprints that were indistinguishable on visual inspection were considered to belong to a single clonal group. Pattern A was defined by four predominant bands that were approximately 1145, 1029, 908, and 720 bp; isolates exhibiting this pattern were considered to be members of clonal group A. A pyelonephritogenic isolate CFT073 (O6:K2:H1),[17] provided by Dr. Harry Mobley at the University of Maryland, was used as a reference strain for each ERIC2 PCR run.

Pulsed-Field Gel Electrophoresis

The standardized protocol for molecular subtyping of *E. coli* (O157:H7) by pulsed-field gel electrophoresis (PFGE), as established by the Centers for Disease Control and Prevention,[18] was used to identify a subgroup of the *E. coli* isolates that were indistinguishable by ERIC2 fingerprinting. *Xba*I-digested DNA was electrophoresed in the CHEF DR-II apparatus (Bio-Rad, Hercules, Calif.). Isolates that had indistinguishable PFGE patterns with the use of *Xba*I were reanalyzed with a second enzyme, *Avr*II. The criteria for strain relatedness established by Tenover et al. were used to compare PFGE patterns.[19] The most frequently identified pattern among the California isolates was designated *Xba*I PFGE pattern A.

Virulence Genotyping

Genotypes for 31 putative virulence factors and the 12 known *papA* alleles were determined by multiplex PCR assays, supplemented by dot-blot hybridization, as previously described.[20-22]

Serotyping

Serotyping was performed on *E. coli* isolates at the *E. coli* reference center in University Park, Pennsylvania. Strains that were motile but that did not react with O or H antiserum were classified as nontypable (nt) — O(nt) and H(nt), respectively.

Conjugation Experiments

Selected wild-type isolates that were resistant to trimethoprim–sulfamethoxazole were conjugated with nalidixic acid–resistant, lactose-negative laboratory strain JM109,[23] according to a standard procedure,[24,25] and plated on Luria–Bertani agar[26] containing trimethoprim–sulfamethoxazole (16 and 350 μg per milliliter, respectively) and nalidixic acid (20 μg per milliliter). The putative transconjugants were tested for susceptibility to 18 antimicrobial agents by a disk-diffusion method to identify markers of resistance to additional antimicrobial agents.

Statistical Analysis

Chi-square analysis with the use of generalized estimating equations based on the PROC GENMOD procedure in SAS (version 8.01, SAS Institute, Cary, N.C.) was used to account for clustered sampling in the California cohort, which included women with multiple urinary tract infections. An exchangeable correlation structure was used in the analysis. The chi-square test or Fisher's exact test was also used in analyses in which the data were restricted to the first (primary) episode of urinary tract infection during the study period.

RESULTS

Prevalence of Trimethoprim–Sulfamethoxazole Resistance

A total of 228 women (median age, 22 years) seen at the university health service in California had symptoms suggestive of acute urinary tract infection. A total of 505 consecutive urine samples from these women were cultured, 255 of which yielded more than 10^2 colony-forming units of *E. coli* per milliliter. Twenty-four women had repeated urinary tract infections during the study period: 21 (9 percent) had two infections, and 3 (1 percent) had three infections.

Fifty-five of the 255 *E. coli* isolates (22 percent) were resistant to trimethoprim–sulfamethoxazole (Table 1). All 55 resistant isolates, which were from 47 women, and 50 susceptible isolates, from 49 other women, were selected for further analysis. In the Minnesota cohort, 18 of 82 *E. coli* isolates (22 percent) were resistant to trimethoprim–sulfamethoxazole. All

TABLE 1. RESULTS OF FINGERPRINTING ANALYSIS OF *ESCHERICHIA COLI* ISOLATES OBTAINED FROM WOMEN WITH URINARY TRACT INFECTIONS IN CALIFORNIA, MICHIGAN, AND MINNESOTA.*

VARIABLE	CALIFORNIA	MICHIGAN	MINNESOTA
No. of isolates	255	NA	82
No. of women	228	NA	82
TMP-SMX–resistant isolates — no. (%)			
All isolates	55 (22)	67	18 (22)
Isolates from primary episode	47 (21)	66	—
Clonal pattern A — no./total no. (%)†			
All isolates	28/55 (51)	11/29 (38)‡	7/18 (39)
Isolates from primary episode	23/47 (49)	10/28 (36)	—

*In California and Michigan, some of the women from whom isolates were obtained had recurrent episodes of urinary tract infection. Information for all isolates and for isolates from the primary episode is presented. In Minnesota, only one isolate was obtained from each woman. Fingerprinting was analyzed with the use of the enterobacterial repetitive intergenic consensus polymerase-chain-reaction assay. NA denotes not available, and TMP-SMX trimethoprim–sulfamethoxazole.

†Clonal pattern A was defined by four predominant bands that were approximately 1145, 1029, 908, and 720 bp.

‡A subgroup of 29 TMP-SMX–resistant isolates was selected for analysis of fingerprinting.

18 resistant isolates (from 18 women), plus 20 susceptible isolates (from 20 women), were selected for further analysis. In the Michigan cohort, 29 resistant isolates (from 28 women) and 20 susceptible isolates (from 20 other women) were selected for further analysis (Table 1).

ERIC2 PCR Fingerprinting

In the California cohort, 28 of the 55 *E. coli* isolates that were resistant to trimethoprim–sulfamethoxazole (51 percent) exhibited the same four-band ERIC2 pattern and were therefore identified as belonging to clonal group A (Fig. 1 and Table 1), as compared with only 2 of 50 randomly selected susceptible isolates (4 percent; $P<0.001$, with adjustment for clustered sampling). In the Minnesota cohort, 7 of 18 isolates that were resistant to trimethoprim–sulfamethoxazole exhibited clonal pattern A (39 percent), as compared with only 1 of 20 susceptible isolates (5 percent, $P=0.02$). Likewise, in the Michigan cohort, 11 of 29 isolates that were resistant to trimethoprim–sulfamethoxazole exhibited clonal pattern A (38 percent), as compared with none of the 20 susceptible isolates ($P=0.001$ by Fisher's exact test) (Fig. 1 and Table 1).

ERIC2 PCR was also used to evaluate 925 colonies of *E. coli* isolated from the stool samples obtained from the group of 41 healthy persons. Clonal group A isolates were identified in one or more fecal samples from 5 of the 18 male subjects (28 percent) and 10 of the 23 female subjects (43 percent). Thirteen of the 26 clonal group A isolates (50 percent) were resistant to trimethoprim–sulfamethoxazole (data not shown).

Results of PFGE Analysis

*Xba*I PFGE analysis was performed on 38 clonal group A isolates (23 from the California cohort and a total of 15 from the other two cohorts). Eleven of the California isolates (48 percent) had indistinguishable PFGE findings (designated *Xba*I PFGE pattern A); 7 isolates (30 percent) differed from this pattern by only one to three bands, 4 (17 percent) by four to six bands, and 1 (4 percent) by more than six bands (Fig. 2 and Table 2). *Avr*II PFGE performed on six of the isolates with *Xba*I PFGE pattern A showed that four were indistinguishable, and the other two differed by only two bands. Although none of the isolates from Michigan and Minnesota had patterns that were identical to *Xba*I PFGE pattern A of the isolates from California, two of the eight Michigan isolates and all seven Minnesota isolates had patterns that were moderately or very similar (Table 2).

Antibiotic Susceptibility

Clonal group A isolates from all three cohorts had a significantly higher prevalence of resistance to multiple antibiotics than did the comparison strains (Table 3). A pattern of resistance to six drugs was found in 8 of 19 clonal group A strains (42 percent) but in none of the other strains ($P<0.001$).

Serotyping

Of 41 representative clonal group A isolates from the three cohorts with urinary tract infections, 32 (78 percent) exhibited one of two distinctive serotypes: O11:H(nt) or O77:H(nt). Serotype O11:H(nt) predominated among the California isolates but was rare or nonexistent among the Michigan and Minnesota isolates, whereas serotype O77:H(nt) accounted for large proportions of the Michigan and Minnesota isolates (Table 2). Three of the remaining clonal group A isolates (7 percent of the total) exhibited a unique O antigen, and 6 (15 percent of the total) could not be typed. Of the 13 clonal group A fecal isolates that were serotyped, 9 (69 percent) were O11:H(nt), 2 were other serotypes, and 2 could not be typed.

Virulence Genotyping

Clonal group A was characterized by a distinctive profile of virulence factors that included a complete copy of *pap,* the F16 *papA* allele, *papG* allele II, *iutA, kpsMT* II, and *traT,* and the absence of *sfa/foc, afa/dra, hly, cnf, iroN, iss,* and *malX* (Table 3). This profile, which corresponds with that previously documented for the O15:K52:H1 clonal group,[6,27] was consistent among isolates from all three cohorts, whether or not the strains were resistant to trimethoprim–sulfamethoxazole.

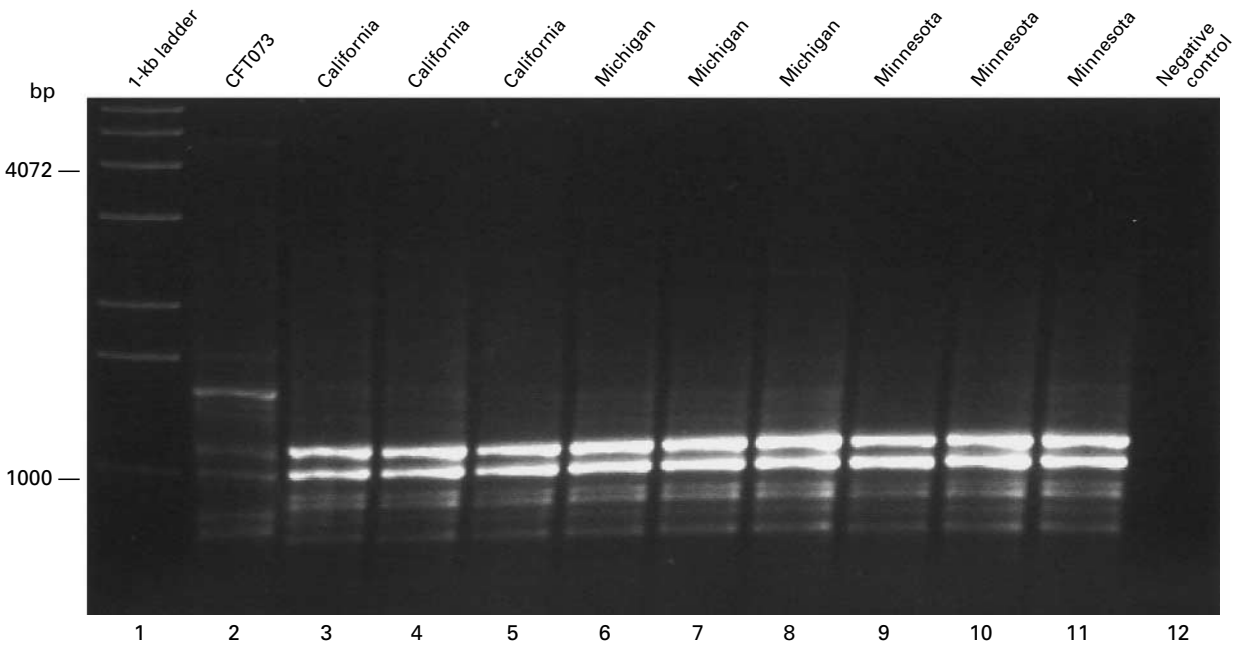

Figure 1. Fingerprint Patterns of *Escherichia coli* Isolates with Resistance to Trimethoprim–Sulfamethoxazole.

Fingerprinting was performed with the use of the enterobacterial repetitive intergenic consensus polymerase-chain-reaction assay. The *E. coli* isolates from California (lanes 3, 4, and 5), Michigan (lanes 6, 7, and 8), and Minnesota (lanes 9, 10, and 11) show the same four-band (two dark and two light) pattern (designated pattern A), indicating that they are all members of clonal group A. Lane 1 shows a 1-kb ladder, lane 2 CFT073 (the reference strain), and lane 12 a negative control.

Conjugation

JM109 transconjugants were derived from five clonal group A isolates in California that were resistant to trimethoprim–sulfamethoxazole. The transconjugants acquired resistance not only to trimethoprim–sulfamethoxazole but also to ampicillin, tetracycline, chloramphenicol, and streptomycin (data not shown).

DISCUSSION

We found that 11 percent of uncomplicated, community-acquired urinary tract infections in women seen during a four-month period at a university health service in California were caused by a single, previously unrecognized clonal group of multidrug-resistant *E. coli*, clonal group A. This clonal group accounted for 51 percent of urinary tract infections caused by *E. coli* strains that were resistant to trimethoprim–sulfamethoxazole at this health center and for high proportions of such isolates from women seen at university health centers in Michigan and Minnesota (38 and 39 percent, respectively). Although a limited number of isolates and locations were surveyed, these data suggest that a single *E. coli* clonal group may have contributed to the recently documented increase in antibiotic resistance among *E. coli* isolates from patients with urinary tract infections in some parts of the United States.[3,4] In the California cohort, if it had not been for this drug-resistant clonal group, the proportion of all primary episodes of urinary tract infections caused by *E. coli* strains that were resistant to trimethoprim–sulfamethoxazole would have been 11 percent instead of 21 percent.

That the *E. coli* isolates with resistance to trimethoprim–sulfamethoxazole represent a phylogenetically distinct clonal group was suggested by their similarities to one another and their differences from other strains with respect to five characteristics: the ERIC2 fingerprinting pattern; O:H serotype, with the unusual serotypes O77:H(nt) or O11:H(nt) predominating among members of clonal group A; PFGE profiles; virulence-factor profiles; and patterns of antibiotic susceptibility. Clonal group A isolates accounted for 11 percent of all urinary tract infections in California and for 9 percent in Minnesota, which represents a substantial prevalence for a single *E. coli* clonal group.[20,27] This finding indicates that clonal group A contributes substantially not only to drug-resistant urinary tract infections but also to urinary tract infections in general.

Clonal group A appears to represent a new lineage of multidrug-resistant, uropathogenic *E. coli* rather than an established virulent clone that has acquired antibiotic resistance. In previous studies of *E. coli* iso-

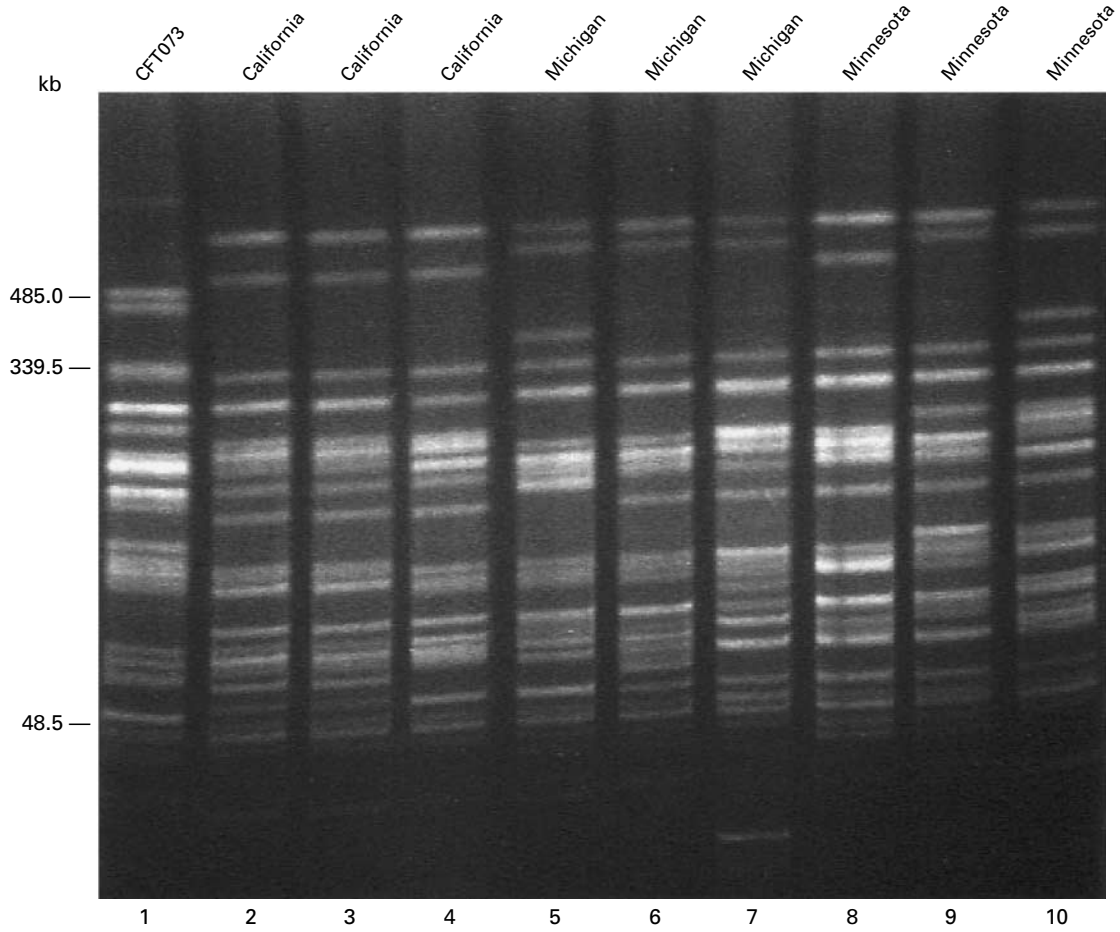

Figure 2. Molecular Subtyping of Clonal Group A Isolates with Resistance to Trimethoprim–Sulfamethoxazole.
Pulsed-field gel electrophoresis with *Xba*I was used for subtyping. Lane 1 shows CFT073 (the reference strain). Clonal group A isolates from California are shown in lanes 2, 3, and 4; those from Michigan in lanes 5, 6, and 7; and those from Minnesota in lanes 8, 9, and 10.

lates from urinary tract infections, O11 and O77 somatic antigens have not been noted, and serogroups O1, O2, O4, O6, O7, O16, O18, O25, and O75 have consistently predominated, accounting for up to 81 percent of isolates.[27-30]

Although urinary tract infection is usually regarded as a sporadic disease caused by organisms from the host's own fecal flora, transmission of *E. coli* between sex partners and household members has been reported.[31,32] Nosocomial outbreaks of *E. coli* pyelonephritis have also been documented.[33] A community-wide outbreak of urinary tract infection due to a single strain occurred in South London in 1986 and 1987.[5] In this outbreak, isolates of *E. coli* O15:K52:H1 that exhibited a distinctive multidrug-resistance phenotype similar to that of the clonal group A isolates in our study were recovered from community-acquired cases of pyelonephritis and bacteremia. This serotype was subsequently identified as a cause of endemic urinary tract infection and bacteremia in other European

TABLE 2. SEROTYPE AND PULSED-FIELD GEL ELECTROPHORETIC (PFGE) PATTERN OF SELECTED CLONAL GROUP A ISOLATES FROM CALIFORNIA, MICHIGAN, AND MINNESOTA.*

SEROTYPE AND PFGE PATTERN	CALIFORNIA	MICHIGAN	MINNESOTA
	no. of isolates (%)		
Serotype	23	11	7
O11:H(nt)	14 (61)	0	1 (14)
O77:H(nt)	6 (26)	8 (73)	3 (43)
Other or nontypable	3 (13)	3 (27)	3 (43)
PFGE pattern	23	8	7
Pattern A	11 (48)	0	0
Pattern that differed from pattern A			
By 1–3 bands	7 (30)	2 (25)	5 (71)
By 4–6 bands	4 (17)	0	2 (29)
By >6 bands	1 (4)	6 (75)	0

*Serotyping was performed on 23 of the 28 isolates in California that were resistant to trimethoprim–sulfamethoxazole and that exhibited pattern A on fingerprinting and on all such isolates in Michigan and Minnesota. Isolates from recurrent episodes of infection are included in the data for California and Michigan. PFGE was performed with *Xba*I-digested DNA; nt denotes nontypable.

TABLE 3. PREVALENCE OF ANTIBIOTIC-RESISTANCE PATTERNS AND VIRULENCE-FACTOR PROFILES OF *ESCHERICHIA COLI* ISOLATES.

ANTIBIOTIC RESISTANCE AND VIRULENCE FACTORS	CLONAL GROUP A ISOLATES	OTHER ISOLATES	P VALUE*
	no. of isolates (%)		
Antibiotic resistance			
Total isolates†	19	21	
TMP-SMZ resistance with or without resistance to other drugs	16 (84)	3 (14)	<0.001
TMP-SMZ and chloramphenicol	12 (63)	1 (5)	<0.001
TMP-SMZ and streptomycin	11 (58)	2 (10)	<0.001
TMP-SMZ and ampicillin	15 (79)	3 (14)	<0.001
TMP-SMZ, ampicillin, streptomycin, and chloramphenicol	9 (47)	1 (5)	0.003
TMP-SMZ, ampicillin, streptomycin, chloramphenicol, and tetracycline	8 (42)	0	<0.001
Virulence factors‡			
Total isolates	25	36	
papA F16 with or without other factors	21 (84)	0	<0.001
papA F16, *traT*	20 (80)	0	<0.001
papA F16, *traT, kpsMT*II, *iutA*	18 (72)	0	<0.001
papA F16, *traT, kpsMT*II, *iutA, fyuA*	16 (64)	0	<0.001
papA F16, *traT, kpsMT*II, *iutA, fyuA, ompT*	16 (64)	0	<0.001
papA F16, *traT, kpsMT*II, *iutA, fyuA, ompT,*	12 (48)	0	<0.001
malX (pathogenicity island) with or without other factors	0	24 (67)	<0.001
malX, hlyA	0	13 (36)	<0.001
malX, hlyA, papA, papG	0	11 (31)	0.002
malX, hlyA, papA, papG, cnfI	0	6 (17)	0.04
malX, hlyA, papA, papG, cnfI, iroN	0	4 (11)	0.13
malX, hlyA, papA, papG, cnfI, iroN, sfa	0	3 (8)	0.26

*Only one isolate per subject was included in these analyses. The P values were therefore calculated with a simple chi-square test or Fisher's exact test.

†Isolates from California, Minnesota, and Michigan were selected for inclusion in the analysis of antibiotic resistance in proportion to the prevalence of resistance to trimethoprim–sulfamethoxazole (TMP-SMZ) among clonal group A isolates as compared with that of other isolates in each cohort. The ratio of TMP-SMZ–resistant to TMP-SMZ–susceptible isolates included in the analysis was 4:1 for clonal group A and 1:6 for other strains in California, 6:2 for clonal group A and 1:6 for other strains in Minnesota, and 6:0 for clonal group A and 1:6 for other strains in Michigan. The patterns of resistance are not mutually exclusive.

‡The distribution of virulence factors was similar among clonal group A isolates from each cohort. All isolates with *papA* allele F16 were also positive for *papAH, papC, papEF,* and *papG* allele II. Independent isolates were selected from each cohort for inclusion in the analysis of virulence factors in order to provide, for clonal group A, up to six TMP-SMZ–resistant isolates and as many TMP-SMZ–susceptible isolates as were available (up to six) and, for non–clonal group A, six TMP-SMZ–resistant and six TMP-SMZ–susceptible isolates. The virulence patterns are not mutually exclusive.

countries[34-36] and, more recently, in the United States.[6] The clonal group A strains exhibit a virulence genotype that is very similar to that of the O15:K52:H1 clonal group, including the F16 *papA* allele, *papG* allele II, *iutA, fyuA, kpsMT* II, and *traT*.[6]

We identified clonal group A strains in fecal samples obtained during the study period from healthy members of the university community in California. The proportion of healthy subjects identified as having fecal colonization with clonal group A on one or more occasions is probably an overestimate of the true prevalence of this infection in the community because of the sampling strategy used. Nevertheless, this analysis does show that clonal group A was circulating in members of the community when the cases of urinary tract infection occurred.

The apparent emergence of clonal group A in three states suggests either the simultaneous expansion in multiple locations of a previously introduced endemic clonal group, possibly as a consequence of increasing antimicrobial selection pressure, or the recent introduction of the clonal group into new environments. The high degree of genetic homogeneity among the California isolates favors the latter hypothesis. One possible explanation for the observed temporal and geographic clustering of a single *E. coli* PFGE type (pattern A) in California is that the strains were spread by one or more contaminated products ingested by community residents, which is similar to the way an enteric pathogen, such as *E. coli* O157:H7, causes community-wide outbreaks after being disseminated by the consumption of contaminated foods.[18,37] If a

large proportion of urinary tract infections caused by drug-resistant strains of *E. coli* in a community were due to the ingestion of widely consumed, contaminated foods, this would constitute a serious and novel public health problem.

Clonal group A isolates were resistant to antibiotics that are commonly used in the outpatient setting to treat urinary tract infection and a wide variety of other infections. Resistance to these agents may persist with the use of any of the antibiotics represented in the resistance phenotype. Additional studies are needed to establish the geographic and temporal distribution of this emerging *E. coli* clonal group and to determine whether it is spread by the ingestion of contaminated foods.

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Comparison of Extraintestinal Pathogenic *Escherichia coli* Strains from Human and Avian Sources Reveals a Mixed Subset Representing Potential Zoonotic Pathogens▽

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Since extraintestinal pathogenic *Escherichia coli* (ExPEC) strains from human and avian hosts encounter similar challenges in establishing infection in extraintestinal locations, they may share similar contents of virulence genes and capacities to cause disease. In the present study, 1,074 ExPEC isolates were classified by phylogenetic group and possession of 67 other traits, including virulence-associated genes and plasmid replicon types. These ExPEC isolates included 452 avian pathogenic *E. coli* strains from avian colibacillosis, 91 neonatal meningitis *E. coli* (NMEC) strains causing human neonatal meningitis, and 531 uropathogenic *E. coli* strains from human urinary tract infections. Cluster analysis of the data revealed that most members of each subpathotype represent a genetically distinct group and have distinguishing characteristics. However, a genotyping cluster containing 108 ExPEC isolates was identified, heavily mixed with regard to subpathotype, in which there was substantial trait overlap. Many of the isolates within this cluster belonged to the O1, O2, or O18 serogroup. Also, 58% belonged to the ST95 multilocus sequence typing group, and over 90% of them were assigned to the B2 phylogenetic group typical of human ExPEC strains. This cluster contained strains with a high number of both chromosome- and plasmid-associated ExPEC genes. Further characterization of this ExPEC subset with zoonotic potential urges future studies exploring the potential for the transmission of certain ExPEC strains between humans and animals. Also, the widespread occurrence of plasmids among NMEC strains and members of the mixed cluster suggests that plasmid-mediated virulence in these pathotypes warrants further attention.

Speculation has long existed regarding a food-borne origin for extraintestinal pathogenic *Escherichia coli* (ExPEC) strains (28, 33, 42) and has spawned recent work investigating *E. coli* contaminants of food and the ExPEC strains of food-producing animals (15, 18, 24, 40). Of particular interest in this regard are avian pathogenic *E. coli* (APEC) strains that cause colibacillosis in poultry (3, 9, 35, 36, 38). Although it has been widely assumed that most APEC strains do not possess zoonotic potential, recent reports have suggested otherwise for certain groups of strains (2, 9, 29, 30, 35, 36), and some researchers have demonstrated that APEC strains and their plasmids may be transmitted to human hosts (27, 38). Recently, APEC isolates have been compared to ExPEC isolates from human urinary tract infections (UTIs) and neonatal meningitis, revealing that these "subpathotypes" have some overlap in serogroups, phylogenetic groups, virulence genotypes, and abilities to cause disease in certain animal models (9, 30, 31, 35, 36). The validity of these observations was sustained by comparison of the first APEC genome sequence with sequenced ExPEC isolates of humans (25), which revealed that few differences existed between the sequenced APEC strain (APEC O1) and human strains. In fact, results of an in silico multilocus sequence typing (MLST) comparison of APEC O1 and all other sequenced *E. coli* genomes showed that APEC O1 belonged to the same sequence type (ST), ST95 (also referred to as ST29), as several well-characterized human ExPEC strains, including uropathogenic *E. coli* (UPEC) strains UTI89 and NU14 and neonatal meningitis *E. coli* (NMEC) strain RS218 (25).

While such data provide compelling evidence that APEC may be linked to human ExPEC, the results should not be overinterpreted to mean that all human ExPEC strains, or even most, are derived from APEC. APEC O1 was chosen for sequencing because it appeared to contain both UPEC- and APEC-like traits, not because it was representative of mainstream APEC (25). Regardless, other reports lend support to the idea that APEC and human ExPEC share chromosomal similarities. For instance, the *ibeA* gene, recognized for its contributions to the invasion of brain microvascular endothelial cells by human NMEC infection, was found significantly more often in APEC strains than in avian commensal strains (9, 10, 31, 34), and when *ibeA* was inactivated in the APEC

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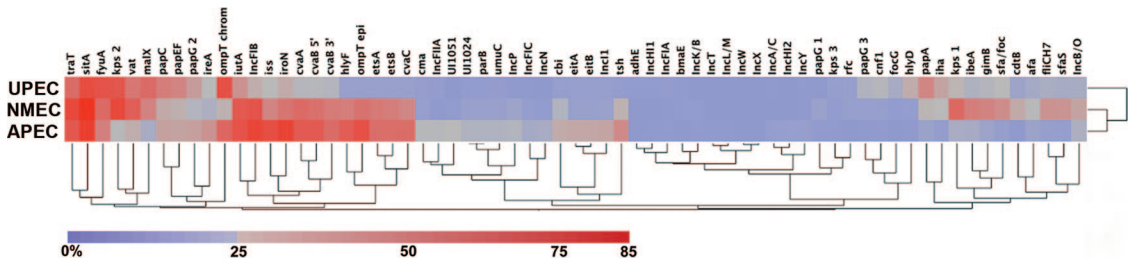

FIG. 1. Two-way clustering of gene prevalence results among the ExPEC subpathotypes. A blue-gray-red heat map was constructed based upon the percentage of each gene examined among each of the subpathotypes. Clustering was performed to illustrate similarities between the prevalence of the genes examined and between the subpathotypes with regard to gene prevalence.

strain BEN 2908, the mutant's ability to invade human brain microvascular endothelial cells and cause avian colibacillosis was significantly reduced compared to the wild type (10). *ibeA* occurs in 14% to 26% of APEC strains (9, 10, 34), and in APEC O1, *ibeA* is found in a chromosomal pathogenicity island (PAI) (25). Such examples of chromosomal virulence attributes occurring in both human and avian ExPEC strains are numerous (25).

In addition to these similarities in chromosomal attributes, similarities may occur between avian and human ExPEC strains in the plasmid-linked genes they possess. Two recent studies provided evidence that the *iss* gene, a marker of ColV virulence plasmids, was present in the majority of both APEC and NMEC populations (9, 20). However, these studies were limited in terms of sample sizes and the number of ColV-associated genes sought. This limitation and a lack of solid phylogenetic linkage between APEC and human ExPEC strains, leaves this a topic of much debate and little proof. Epidemiological studies involving poultry production facilities, their employees, and the consumer would be ideal but are complex and difficult to perform. Rather, we have utilized a genome-based approach to identify similarities and differences between these groups in an effort to provide more substantial evidence that highly related strains coexist in humans and poultry, causing a variety of extraintestinal illnesses. In this study, we performed comprehensive genotyping with large samples of NMEC, UPEC, and APEC strains in an effort to better understand the relationships between the ExPEC subpathotypes.

MATERIALS AND METHODS

Bacterial strains. A total of 1,074 isolates were used in this study, including 531 isolates from cases of human UTIs, 452 *E. coli* isolates implicated in avian colibacillosis, and 91 isolates from cases of human neonatal meningitis. Some of these isolates have been previously described, albeit to a lesser extent (21, 22, 32, 35). APEC isolates were taken from lesion sites of chickens and turkeys raised for meat consumption and laying hens. Lesion sites included the air sacs, liver, pericardium, spleen, reproductive tract, joints, and blood. These birds displayed the typical signs of colibacillosis, including respiratory distress, depression, reduced appetite, reduced mobility, ruffled feathers, and even recent death. APEC isolates came from commercial farms throughout the United States (21, 22, 32, 34). Seventy of the NMEC isolates came from the cerebrospinal fluid of newborns in The Netherlands, isolated from 1989 through 1997 (16). The remaining NMEC isolates were isolated in a similar fashion and over the same time period from patients in the United States. Two hundred of the UPEC isolates came from MeritCare Medical Center in Fargo, North Dakota (36). These isolates were taken from the urine of patients of various ages and sexs affected with uncomplicated UTI. Sixty-seven UPEC isolates came from four hospitals in Seattle, Washington, from the blood cultures of patients with bacteremia arising from a urinary tract source during the 1990s (12, 13, 17, 19). Eleven UPEC isolates are members of the ECOR reference group and were implicated in human cystitis or pyelonephritis (11). One hundred seventy of the UPEC isolates were recovered at multiple locales in the United States during the 1990s, from the urine of pretherapy female patients with uncomplicated acute pyelonephritis (14, 39). Eighty-three UPEC isolates were collected during the 1990s at the University of Minnesota Student Health Center, from the urine of female patients with acute uncomplicated cystitis (14, 39). All organisms were stored at −80°C in brain heart infusion broth (Difco Laboratories, Detroit, MI) with 10% (vol/vol) glycerol, until use.

Phylogenetic typing. Isolates were assigned to phylogenetic groups according to the method described by Clermont et al. (6). Using this method, we assigned isolates to one of four groups (A, B1, B2, or D) based on their possession of two genes (*chuA* and *yjaA*) and a DNA fragment (TSPE4.C2), as determined by PCR. Boiled lysates of overnight cultures were used as a source of template DNA for this study (12). Amplification was performed in a 25-μl reaction mixture as previously described (35).

Multiplex PCR genotyping. Multiplex PCR was performed for the presence or absence of 67 genes/traits. Some of these multiplex panels have been previously described (21–24, 35). Reaction mixtures included positive and negative control organisms. These panels included 50 ExPEC virulence-associated genes and 17 plasmid replicon types. All primers were obtained from Integrated DNA Technologies (Coralville, IA). In all, multiplex panels targeting 67 products were used. PCR was performed as previously described (35). Strains known to possess or lack the genes of interest were examined with each amplification procedure. Reactions were performed twice. An isolate was considered to contain a gene of interest if it produced an amplicon of the expected size.

Amplification of the *svg* gene for identification of the ST95/$B2_1$ strains. A previous study identified the *svg* gene as a distinguishing trait of strains belonging to the ST95/ST29/$B2_1$ subgroups (4). One hundred eight isolates falling into a mixed genotyping cluster (Fig. 1) were assessed for the presence of this gene, as previously described (4). APEC O1 and *E. coli* DH5α were used as positive and negative controls, respectively.

Biostatistics. For APEC, UPEC, and NMEC populations, Fisher's exact test was used to test the null hypothesis of equal gene prevalence rates across the three populations studied. Due to the relatively large number of traits, step-down permutation multiplicity adjustments were used to address the associated inflation of the type I error rate (43). An average linkage cluster analysis was performed based upon the Jaccarddissimilarity coefficient calculated from the presence or absence of all traits examined. This value was used to examine groupings among the isolates from the three populations. The dendrogram resulting from this cluster analysis was combined with a modified heat map (8) to allow visualization of all of the characters used in the analysis in the context of the groups obtained from the cluster analysis. Similarly, average gene prevalence values for each subpathotype were used to construct a two-way clustering diagram. Clustering images and dendrograms were constructed using SAS 9 and JMP 7 (SAS Institute) software.

RESULTS AND DISCUSSION

APEC and NMEC isolates share similarities in plasmid-associated genes but have different chromosomal backgrounds. When examined for the presence of plasmid-carried, ExPEC-associated genes, APEC and NMEC isolates were sim-

TABLE 1. Results of genotyping studies[a]

Gene, strain, or replicon	% of prevalence relative to the total no. of isolates (*n*)			Statistical significance of prevalence			
	UPEC (531)	NMEC (91)	APEC (452)	APEC vs human ExPEC	APEC vs UPEC	APEC vs NMEC	UPEC vs NMEC
traT	67.8	85.6	78.1	+	−	−	−
sitA	83.4	95.6	89.6	−	−	−	−
iutA	48.4	77.8	80.8	++	++	−	+
hlyF	5.6	58.9	75.4	++	++	−	++
etsA	6.0	61.1	67.0	++	++	−	++
etsB	6.0	58.9	66.8	++	++	−	++
ompT epi	5.6	64.4	81.6	++	++	−	++
iss epi	26.6	55.6	82.7	++	++	++	++
iroN	34.8	63.3	87.4	++	++	++	++
cvaA	23.4	68.9	77.4	++	++	−	++
cvaB5′	24.1	65.6	77.4	++	++	−	++
cvaB3′	22.0	61.1	68.1	++	++	−	++
cvaC	5.6	54.4	67.5	++	++	−	++
cmi	3.8	4.4	24.6	++	++	++	−
cba	4.0	21.1	34.3	++	++	−	++
tsh	2.6	31.1	52.7	++	++	+	++
eitA	4.3	5.6	37.2	++	++	++	−
eitB	4.5	5.6	37.2	++	++	++	−
UI1051	0.4	2.2	26.5	++	++	++	−
UI1024	2.4	5.6	19.7	++	++	−	−
parB	2.4	5.6	19.7	++	++	−	−
umuC	3.2	5.6	19.7	++	++	−	−
adhE	2.1	0.0	0.2	−	−	−	−
papA	54.8	28.9	7.5	++	++	++	++
papC	59.7	35.6	40.5	++	++	−	+
papEF	55.4	32.2	39.2	++	++	−	+
papG1	0.6	6.7	1.5	−	−	+	++
papG2	42.9	22.2	40.7	++	−	+	++
papG3	20.2	4.4	0.7	−	++	−	++
kps1	29.2	70.0	15.7	++	++	++	++
kps2	78.5	85.6	25.0	++	++	++	−
kps3	4.0	2.2	1.8	−	−	−	−
malX	68.2	56.7	15.0	++	++	++	−
ireA	26.0	17.8	48.0	++	++	++	−
ibeA	19.2	58.9	14.2	++	−	++	++
gimB	22.6	56.7	8.8	++	++	++	++
vat	62.3	74.4	33.4	++	++	++	−
cnf1	23.4	4.4	1.3	++	++	−	++
fyuA	80.6	68.9	58.2	++	++	−	−
cdtB	8.7	35.6	1.1	++	++	++	++
bmaE	1.3	2.2	0.4	−	−	−	−
sfa/foc	26.4	51.1	4.4	++	++	++	++
hlyD	34.1	3.3	0.9	++	++	−	++
rfc	5.3	4.4	0.4	++	−	−	−
ompT chrom	81.5	31.1	70.4	−	+	++	++
$fliC_{H7}$	16.0	47.8	4.6	++	++	++	++
focG	14.3	2.2	0.0	++	++	−	++
iha	39.2	26.7	3.5	++	++	++	−
afa	12.6	25.6	8.2	+	−	++	+
sfaS	14.1	46.7	4.0	++	++	++	++
IncB/O replicon	14.5	38.9	17.9	−	−	++	++
IncFIC replicon	1.1	3.3	12.4	++	++	−	−
IncA/C replicon	0.6	0.0	3.3	++	+	−	−
IncP replicon	0.8	8.9	21.7	++	++	−	+
IncT replicon	0.0	0.0	0.9	−	−	−	−
IncK/B replicon	0.0	2.2	1.5	−	−	−	−
IncW replicon	0.2	0.0	0.0	−	−	−	−
IncFIIA replicon	3.0	1.1	24.3	++	++	++	−
IncFIA replicon	2.6	1.1	1.5	−	−	−	−
IncFIB replicon	33.5	80.0	86.9	++	++	−	++
IncY replicon	1.7	1.1	4.2	−	−	−	−
IncI1 replicon	4.5	6.7	38.3	++	++	++	−
IncX replicon	0.0	0.0	0.0	−	−	−	−
IncHI1 replicon	1.9	0.0	1.1	−	−	−	−
IncN replicon	0.2	2.2	15.0	++	++	++	−
IncHI2 replicon	0.2	0.0	4.0	++	++	−	−
IncL/M replicon	0.0	0.0	0.7	−	−	−	−
Phylo A	10.5	11.1	36.9	++	++	++	−
Phylo B1	4.5	2.2	15.9	++	++	+	−
Phylo B2	62.7	76.7	17.3	++	++	++	−
Phylo D	22.2	11.1	29.9	++	−	+	−

[a] Values shown for results of genotyping are given in percentages. Two-way comparisons were performed for each gene, strain, or replicon studied between the different groups examined, using Fisher's exact test. For each comparison, a *P* value of <0.05 (+) was considered statistically significant, and a *P* value of <0.01 (++) was also considered statistically significant, while a *P* value of >0.05 (−) was not considered statistically significant. epi, episomal; chrom, chromosomal; Phylo, phylotype.

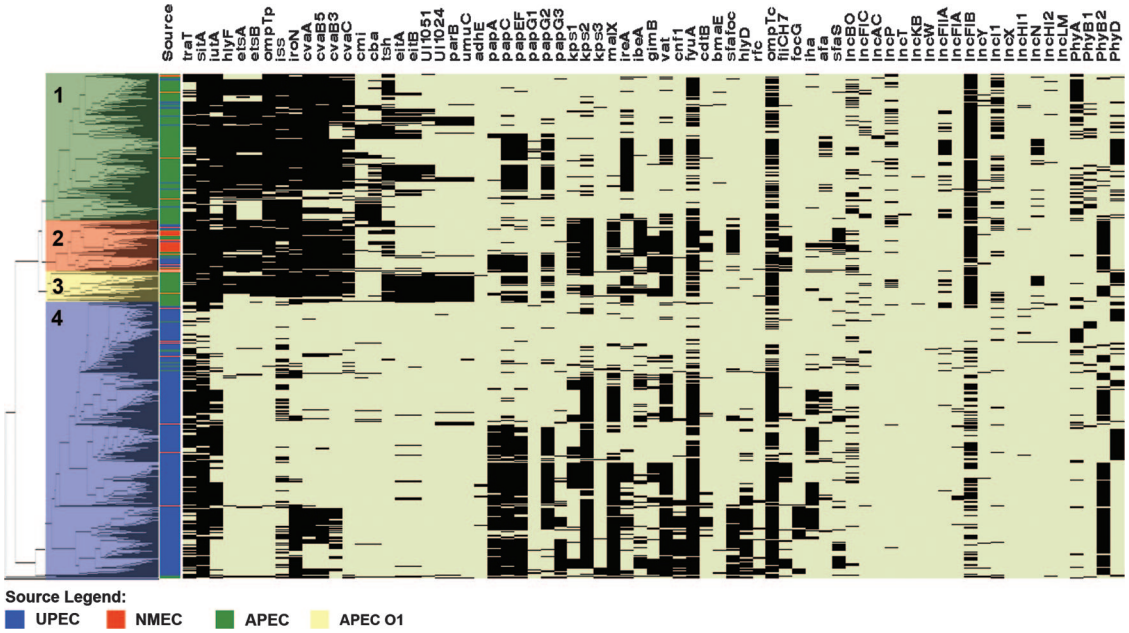

FIG. 2. Results of cluster and discriminant analyses based on the traits examined. From left to right, the dendrogram was constructed based upon the cluster analysis of common traits, and cluster numbers (1 to 4) were discerned using a cutoff based upon overall virulence genotype; the source column indicates the origin of an isolate; the following columns depict individual PCR results for the presence (black) or absence (light green) of plasmid-carried genes, chromosomal genes, plasmid replicons, and phylogenetic type. *ompT*p, episomal *ompT*; *iss*, episomal *iss*; *ompT*c, chromosomal *ompT*.

ilar in their possession of RepFIB and ColV virulence plasmids (Table 1). In particular, APEC and NMEC isolates did not differ significantly ($P > 0.05$) in their possession of most of the genes of the conserved PAI of ColV plasmids, including *sitA*, *iutA*, *hlyF*, *etsAB*, and *ompT* and genes of the ColV operon (21). With regard to plasmid replicon type, both APEC and NMEC isolates had a similarly high prevalence of the IncFIB plasmid replicon, with generally lower occurrences of other replicon types. The FIC, P, and I1 plasmid replicons occurred in a significantly higher proportion of APEC isolates than NMEC isolates. Chromosomal genes possessed by both groups ($P > 0.05$) included some genes of the *pap* operon (26) and *fyuA* of the yersiniabactin operon (41). However, these two groups did exhibit considerable differences in the prevalence of most other chromosomal genes, with NMEC isolates generally possessing them and APEC isolates generally not possessing them. These chromosomal differences were supported by the finding that APEC and NMEC isolates belonged to different phylogenetic groups, with most APEC isolates belonging to groups A (37%) and D (30%) and most NMEC isolates belonging to group B2 (77%). While the phylogenetic typing scheme originally described by Clermont et al. and used here is not the most discriminatory phylogenetic classification method, it has proven effective at rapidly distinguishing between pathogenic and nonpathogenic ExPEC organisms (6, 46). However, caution should be taken when interpreting such results, as more sensitive methods are available for classifying ExPEC isolates by phylogeny, such as MLST. Nevertheless, the rapid phylogenetic typing scheme was useful for the purposes of this study, when combined with virulence genotype.

UPEC isolates have different virulence genotypes than those of both APEC and NMEC. The 531 UPEC isolates examined were significantly different from those of APEC and UPEC in many of the genes studied (Table 1). UPEC isolates possessed the ColV plasmid PAI genes at a significantly lower rate than those of APEC and NMEC, ranging from 5 to 27%. These rates excluded *iutA*, *sitA*, and *iroN*, because these genes can also occur on the UPEC chromosome (37, 44, 45). Chromosomal genes occurring at significantly different rates among the UPEC isolates examined included genes of the *pap* operon, *kps* type 1, *cnf1*, *focG*, *sfa*/*foc*, and IncFIB (compared to APEC and NMEC isolates); *fyuA*, *malX*, *ireA*, *kps* type 2, *vat*, IncFIC, IncP, IncFIIA, IncI1, and IncN (compared to APEC isolates); and *ireA*, *ibeA*, *gimB*, *cdtB*, $fliC_{H7}$, *afa*, chromosomal *ompT*, *sfaS*, and IncB/O (compared to NMEC isolates). Most of the UPEC isolates examined belonged to the B2 and D phylogenetic groups.

APEC strains are different from human ExPEC strains, as a whole. Compared to human ExPEC (UPEC and NMEC) strains, the APEC strains examined were significantly different ($P < 0.01$) in nearly all of the traits examined, with the exception of genes occurring at a high rate among all groups, such as *sitA*, *traT*, chromosomal *ompT*, and those occurring at low rates among all groups, including *adhE*, the *papG* allele 3, the *kps* type 3 capsular synthesis gene, *bmaE*, and several plasmid replicons.

What traits characterize each of the ExPEC subpathotypes? Using two-way clustering, we attempted to characterize the ExPEC subpathotypes examined based upon their possession of genes/traits (Fig. 1). Again, the APEC and NMEC strains appeared to be characterized by the presence of the plasmid-carried PAI of ColV plasmids (21). The UPEC strains examined generally did not contain any of these genes. All three subpathotypes were characterized by the presence of

sitA and *traT*, while only APEC strains were characterized as containing *tsh*.

With regard to chromosome-associated traits, the APEC strains were distinguished from the UPEC and NMEC strains because they lacked most of these genes. The UPEC and NMEC strains were characterized by their possession of genes of the *pap* operon, the *kps* capsular synthesis genes (type 2 for all human ExPEC and type 1 for NMEC), the *malX* PAI marker, *vat*, and their assignment to the B2 phylogenetic group. The NMEC strains also were further characterized by their possession of *ibeA*, *gimB*, and *sfa*/*foc*. All three groups were characterized by their possession of *fyuA*.

Cluster analysis for gene correlations showed close relationships overall between genes of the *pap* operon, *ireA*, and chromosomal *ompT*; between genes of the conserved portion of the ColV PAI; and between several chromosomal PAI-associated genes, including the *kps* type 1 capsular synthesis gene, *ibeA*, *gimB*, *sfa*/*foc*, *cdtB*, and *afa*. Clustering of the subpathotypes UPEC, NMEC, and APEC based upon gene prevalence illustrates that APEC and NMEC strains shared the highest similarities to one another (Fig. 1).

Cluster analysis further defines ExPEC subpathotypes. An additional cluster analysis was performed, grouping isolates together based upon their overall possession or the absence of traits examined. Such an analysis is an excellent supplement to gene prevalence because it allows for a visualization of genetic associations among individual isolates. Four major clusters could be discerned from this analysis (Fig. 2). Clusters 1 and 3 in Fig. 2 contained mostly APEC isolates. Most of the isolates from cluster 1 belonged to the phylogenetic group A, and nearly all of the isolates in cluster 1 contained the genes of the conserved ColV PAI. Some of the isolates within cluster 1 also appeared to contain the *pap* operon, *ireA*, *vat*, chromosomal *ompT*, and *fyuA*. Isolates in this cluster contained the ColB/M operon, the ColV operon, or both. This characteristic could reflect different variants of colicin virulence plasmids that have arisen over time. Isolates from cluster 3 belonged to either the phylogenetic group B2 or D. Isolates in this cluster generally contained the genes of the conserved portion of the ColV PAI, as well as other ColV-associated genes, such as *tsh* and *eitAB*. Cluster 3 isolates generally lacked chromosomal traits.

Cluster 4 (Fig. 2) contained mostly UPEC and some NMEC isolates. Most of the isolates in cluster 4 belonged to the B2 and D phylogenetic groups. These isolates generally lacked genes of the ColV plasmid PAI but contained *traT*, *sitA*, and *iutA*. These isolates also contained the *kps* type 2 capsular synthesis gene, *malX*, *vat*, *fyuA*, and chromosomal *ompT*. Some of the cluster 4 isolates possessed the IncFIB plasmid replicon, but these isolates lacked other known plasmid replicon types. Some of the cluster 4 isolates contained *iroN* and portions of the ColV operon but not other ColV-associated genes. This characteristic could reflect the presence of a chromosomal PAI similar to that of PAI III_{536} of UPEC strain 536 in these isolates (7).

Cluster analysis defines a mixed subset representing B2 strains that also contain a virulence plasmid. Cluster 2 (Fig. 2) contained a mixture of all three ExPEC subpathotypes examined. This cluster contained 108 isolates, including 39 APEC, 50 NMEC, and 19 UPEC isolates (Table 2 and Fig. 3). Nearly all of these isolates appeared to contain the ColV PAI, with the prevalence of these genes within this cluster ranging from 88 to 99% (Table 2). About 25% of these isolates appeared to contain a plasmid variant involving genes of the ColB/M operons and *eitABC*, a putative ABC transporter system (22). Approximately one-third of the isolates from this cluster appeared to possess an intact *pap* operon, and most possessed the *kps*

TABLE 2. Prevalence of genes and/or traits in a mixed genotyping cluster[a]

Gene, strain, or replicon	% of prevalence			
	UPEC	NMEC	APEC	Overall
% of total	8.6	3.6	54.9	10.1
traT	100.0	98.0	98.1	98.1
sitA	100.0	100.0	98.1	98.1
iutA	78.9	98.0	93.5	93.5
hlyF	94.7	94.0	88.9	88.9
etsA	100.0	94.0	90.7	90.7
etsB	94.7	92.0	89.8	89.8
ompT chrom	89.5	96.0	92.6	92.6
iss epi	89.5	80.0	88.9	88.9
iroN	100.0	86.0	90.7	90.7
cvaA	100.0	96.0	98.1	98.1
cvaB5	100.0	96.0	98.1	98.1
cvaB3	94.7	96.0	91.7	91.7
cvaC	89.5	88.0	88.0	88.0
cmi	15.8	2.0	12.0	12.0
cba	21.1	30.0	29.6	29.6
tsh	21.1	44.0	42.6	42.6
eitA	10.5	0.0	11.1	11.1
eitB	10.5	0.0	11.1	11.1
UI1051	5.3	0.0	4.6	4.6
UI1024	0.0	0.0	0.9	0.9
parB	0.0	0.0	0.9	0.9
umuC	0.0	0.0	0.9	0.9
adhE	0.0	0.0	0.0	0.0
papA	73.7	26.0	34.3	34.3
papC	73.7	30.0	40.7	40.7
papEF	84.2	26.0	39.8	39.8
papG1	0.0	0.0	0.0	0.0
papG2	73.7	18.0	37.0	37.0
papG3	5.3	0.0	0.9	0.9
kps1	94.7	90.0	88.9	88.9
kps2	100.0	100.0	98.1	98.1
kps3	0.0	2.0	0.9	0.9
malX	100.0	72.0	78.7	78.7
ireA	63.2	24.0	38.0	38.0
ibeA	31.6	80.0	71.3	71.3
gimB	73.7	78.0	67.6	67.6
vat	73.7	100.0	88.9	88.9
cnf1	0.0	0.0	1.9	1.9
fyuA	100.0	82.0	89.8	89.8
cdtB	5.3	56.0	28.7	28.7
bmaE	0.0	0.0	0.0	0.0
sfafoc	21.1	76.0	50.0	50.0
hlyD	0.0	0.0	0.0	0.0
rfc	0.0	4.0	1.9	1.9
ompT chrom	100.0	40.0	72.2	72.2
$fliC_{H7}$	68.4	72.0	58.3	58.3
focG	0.0	0.0	0.0	0.0
iha	5.3	34.0	16.7	16.7
afa	0.0	40.0	18.5	18.5
sfaS	15.8	56.0	39.8	39.8
IncB/O replicon	10.5	48.0	25.9	25.9
IncFIC replicon	0.0	2.0	2.8	2.8
IncA/C replicon	0.0	0.0	0.0	0.0
IncP replicon	5.3	10.0	13.0	13.0
IncT replicon	0.0	0.0	0.0	0.0
IncK/B replicon	0.0	2.0	0.9	0.9
IncW replicon	0.0	0.0	0.0	0.0
IncFIIA replicon	0.0	0.0	2.8	2.8
IncFIA replicon	0.0	2.0	0.9	0.9
IncFIB replicon	68.4	92.0	84.3	84.3
IncY replicon	5.3	2.0	1.9	1.9
IncI1 replicon	15.8	2.0	20.4	20.4
IncX replicon	0.0	0.0	0.0	0.0
IncHI1 replicon	5.3	0.0	1.9	1.9
IncN replicon	0.0	0.0	0.9	0.9
IncHI2 replicon	0.0	0.0	0.9	0.9
IncL/M replicon	0.0	0.0	1.9	1.9
Phylo A	0.0	2.0	5.6	5.6
Phylo B1	0.0	0.0	1.9	1.9
Phylo B2	100.0	96.0	89.8	89.8
Phylo D	0.0	2.0	2.8	2.8

[a] The cluster shown is that of cluster 2 from Fig. 1. epi, episomal; chrom, chromosomal; Phylo, phylotype.

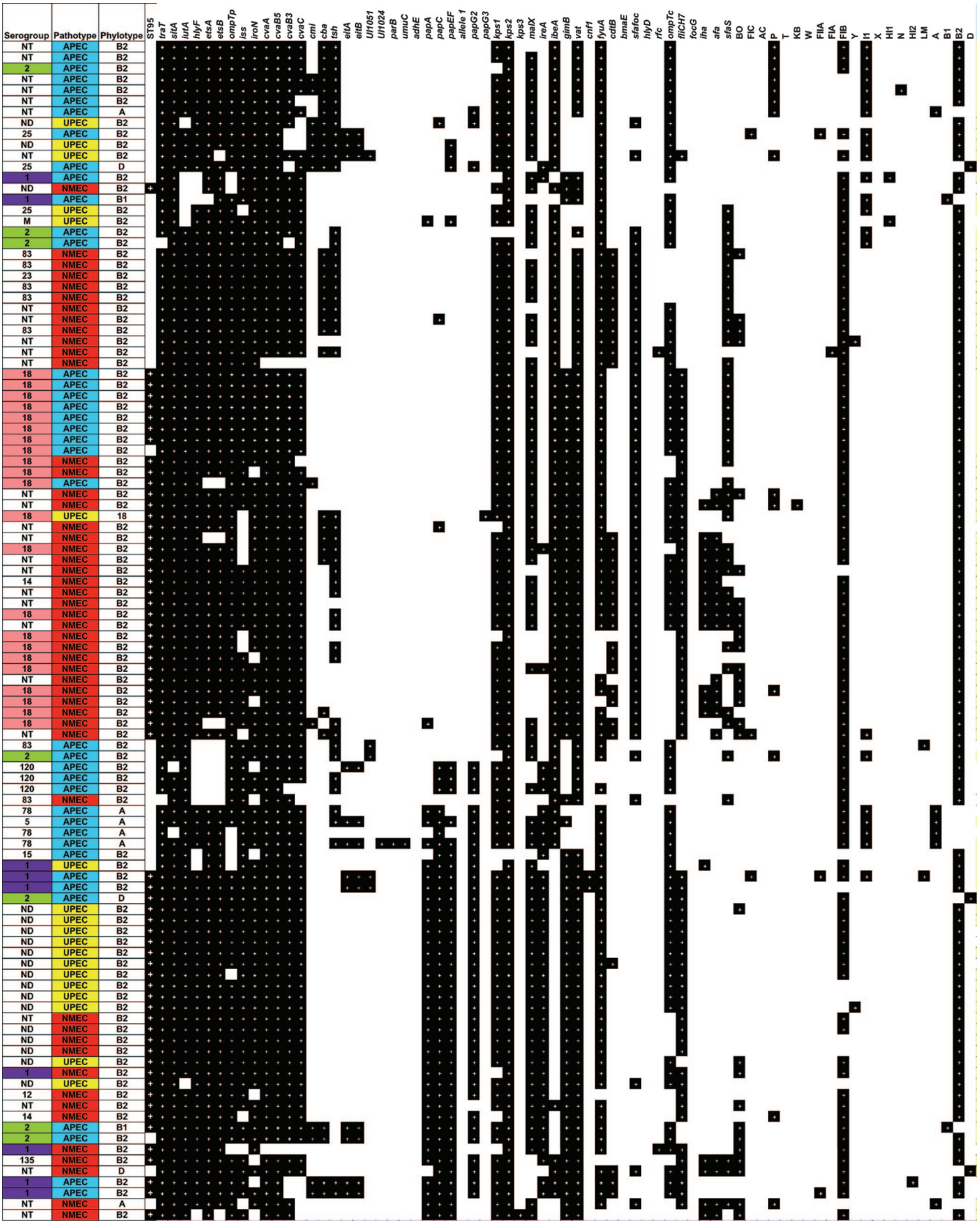

FIG. 3. Overview of isolates belonging to the mixed genotyping cluster. The 108 isolates from the mixed genotyping cluster shown in Fig. 1 are listed in order. For each isolate, the serogroup, pathotype (source), phylotype, and overall genotype are provided. For serogroup O1 (purple), O2 (olive), and O18 (salmon), isolates are colored. Isolates are also colored by source (APEC, sky blue; UPEC, yellow; NMEC, red). For genotype, black squares containing "+" represent a positive PCR, whereas open squares represent a negative PCR.

capsule biosynthesis type 1 or 2. Many of these isolates also contained a wide variety of chromosome-carried ExPEC traits, including *malX*, *ireA*, *ibeA*, *gimB*, *vat*, *fyuA*, *sfa*/*foc*, *ompT*, $fliC_{H7}$, and *sfaS*. Most of these isolates possessed the IncFIB plasmid replicon.

The 108 isolates within this mixed cluster were almost exclusively members of the B2 phylogenetic group (89.8%). Within this genotyping cluster is APEC O1, a strain which has been previously sequenced and analyzed in multiple models of ExPEC infection (25). Like other isolates in this cluster, APEC O1 possesses a ColV-type virulence plasmid with its highly conserved PAI (21). This strain has been shown to cause disease in the 1-day-old chick model of avian colibacillosis and the mouse model of human UTI (T. Johnson, unpublished data) (25). APEC O1 belongs to ST95, a potentially zoonotic sequence type strain, as determined through MLST analysis of housekeeping genes (30, 31). In fact, several recently sequenced or archetypal strains belong to this ST, including UPEC strains UTI89 (5) and NU14, and NMEC strain RS218 (47). These strains all contain a variety of chromosome-carried virulence factors such as those mentioned above. It was recently determined that the *svg* gene appears to be a distinguishing trait of *E. coli* strains belonging to ST95 and the $B2_1$ ribotype (4). When the 108 isolates from the mixed genotyping cluster in this study were analyzed for the presence of *svg*, it was found that 58% of the isolates contained this gene, suggesting their membership within the ST95 group (Fig. 3). Many of the svg^+ isolates belonged to the O1, O2, or O18 serogroup, all of which have been implicated with multiple forms of ExPEC disease. This is in agreement with the work of Achtman and Pluschke (1), who identified the K1 capsule-bearing O1:K1:H7, O2:K1:H7, and O18:K1:H7 strains shown to be closely related by multilocus enzyme electrophoresis. However, the implications and occurrence of ColV plasmids among the $ST95/B2_1$ subgroups have not been previously explored. The results of this study suggest that the acquisition of ColV virulence plasmids by hosts with B2 phylogeny has resulted in strains such as those within the mixed genotyping cluster, with an enhanced ability to cause disease and survive in multiple environments and in the face of multiple pressures. Future work should take unbiased approaches toward determining the prevalence of ColV virulence plasmids among $ST95/B2_1$-positive populations.

Conclusions. This study builds upon previous work involving extensive virulence genotyping of ExPEC populations and provides some insights into the evolution of ExPEC virulence. It is apparent from this study that most APEC, UPEC, and NMEC strains are genetically distinct from one another, and thus, their classification into subpathotypes appears to be justified. Expectedly, APEC strains are characterized by the presence of ColV-like virulence plasmids in strains belonging to the A and D phylogenetic groups. UPEC and NMEC strains are characterized by their possession of chromosome-carried virulence genes, presumably on PAIs, and they belong mostly to the B2 phylogenetic group. Many NMEC strains appear to contain ColV plasmids in addition to this chromosomal background, and cluster analyses suggest that APEC and NMEC strains share many genetic similarities, and, irrespective of host source, nearly 10% of the isolates in this study belong to a genotype cluster representing the most likely zoonotic pathogens. Nearly 50% of the NMEC strains examined belonged to this group, but it also included APEC and UPEC strains. It is evident from this study that the distribution of ColV plasmids is not limited to any particular phylogenetic type, as they are evenly distributed among all four phylotypes. Perhaps, the acquisition of ColV virulence plasmids by B2 strains has provided them with an enhanced ability to cause disease and survive under adverse conditions. If so, such strains thus present a threat to both human and animal health, and further work is required to determine the true zoonotic potential of these strains.

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Ceftiofur Resistance in *Salmonella enterica* Serovar Heidelberg from Chicken Meat and Humans, Canada

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The Canadian Integrated Program for Antimicrobial Resistance Surveillance describes a strong correlation (r = 0.9, p<0.0001) between ceftiofur-resistant *Salmonella enterica* serovar Heidelberg isolated from retail chicken and incidence of ceftiofur-resistant *Salmonella* serovar Heidelberg infections in humans across Canada. In Québec, changes of ceftiofur resistance in chicken *Salmonella* Heidelberg and *Escherichia coli* isolates appear related to changing levels of ceftiofur use in hatcheries during the study period, from highest to lowest levels before and after a voluntary withdrawal, to increasing levels after reintroduction of use (62% to 7% to 20%, and 34% to 6% to 19%, respectively). These events provide evidence that ceftiofur use in chickens results in extended-spectrum cephalosporin resistance in bacteria from chicken and humans. To ensure the continued effectiveness of extended-spectrum cephalosporins for treating serious infections in humans, multidisciplinary efforts are needed to scrutinize and, where appropriate, limit use of ceftiofur in chicken production in Canada.

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Salmonella enterica serovar Heidelberg ranks among the top 3 serovars isolated from persons infected with *Salmonella* in Canada (*1*). It is more frequently reported in North America than in other regions of the world (*2*). Although many *Salmonella* Heidelberg infections result in mild to moderate illness, the bacterium also causes severe illness with complications such as septicemia, myocarditis, extraintestinal infections, and death (3,*4*). *Salmonella* Heidelberg appears more invasive than other gastroenteritis-causing serovars; ≈9% of isolates of this serovar received through the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) during 2003–2005 were recovered from blood samples (*5*). Treatment with antimicrobial agents may be life-saving in the case of invasive infections.

Sources of human *Salmonella* Heidelberg infection include consumption of poultry or eggs and egg-containing products (*6–10*). In Canada, *Salmonella* Heidelberg is commonly isolated from healthy chickens from farm, abattoir, and retail sources (*11*,*12*). It also has been isolated, although less frequently, from ground beef, pork, and turkey meat (*13–15*) and from clinical samples from various animal species (*12*).

Ceftiofur is an extended-spectrum cephalosporin drug approved in Canada for use with numerous label indications in cattle, swine, horses, sheep, turkeys, dogs, and cats. Ceftiofur is also injected in ovo to control *Escherichia coli* omphalitis in broiler chickens; this use is not an approved label indication.

A major public health concern is that use of third-generation cephalosporins, such as ceftiofur, in food animals is leading to resistance to other extended-spectrum cepha-

losporins, such as ceftriaxone and cephamycins (*16*–*20*), a group of antimicrobial agents used to treat a wide variety of human infections. Among other indications, ceftriaxone is the drug of choice for treating severe or invasive salmonellosis in children and pregnant women (*16*,*17*) where fluoroquinolones are not approved and treatment options are limited. Accordingly, third-generation cephalosporins have been classified as Critically Important Antimicrobials in Human Medicine by the World Health Organization (*21*) and as Class 1 Very High Importance in Human Medicine by the Canadian Veterinary Drugs Directorate, Health Canada (*22*).

In Canada, ceftiofur resistance in bacteria from healthy animals or food is mainly reported in chicken *Salmonella* Heidelberg isolates originating from farm, abattoir, and retail samples and in chicken abattoir and retail generic *E. coli* isolates (*11*,*12*). It also is occasionally reported in *Salmonella* isolates from sick animals or in bovine and porcine abattoir or retail *E. coli* isolates but at much lower frequency (*12*).

The objective of this study is to highlight the correlation between ceftiofur-resistant *Salmonella* Heidelberg isolated from retail chicken and the incidence of ceftiofur-resistant *Salmonella* Heidelberg infections in humans across Canada. Public health concerns raised by publication of the CIPARS 2003 annual report, specifically the higher rates of ceftiofur resistance in *Salmonella* Heidelberg isolates from chicken meat than from humans, prompted Québec broiler chicken hatcheries to voluntarily interrupt the extralabel in ovo use of ceftiofur during 2005–2006 (*23*). This study therefore also describes variations in ceftiofur resistance among chicken and human *Salmonella* Heidelberg and chicken *E. coli* strains in that province before, during, and after the voluntary withdrawal.

Materials and Methods

CIPARS is a national program led by the Public Health Agency of Canada (PHAC) dedicated to the preservation of effective antimicrobial drugs for humans and animals through the collection, integration, analysis, and communication of trends in antimicrobial resistance in selected bacterial organisms. Data presented here were collected during 2003–2008 from CIPARS' surveillance of human clinical *Salmonella* isolates and *E. coli* and *Salmonella* isolates from retail chicken. Detailed methods for sample collection, bacterial isolation, antimicrobial resistance testing, and data analysis are described in CIPARS's reports (*12*).

Sample Collection

Human *Salmonella* Isolates

Hospital-based and private clinical laboratories isolated and forwarded human *Salmonella* isolates to their Provincial Public Health Laboratory (PPHL). PPHLs forwarded *Salmonella* isolates to the Enteric Diseases Program, National Microbiology Laboratory (NML), PHAC, for phage type characterization and antimicrobial resistance testing. All isolates (outbreak and nonoutbreak) received passively by the Saskatchewan PPHL were forwarded; the more populated provinces (British Columbia, Ontario, and Québec) forwarded isolates received from days 1–15 of each month. Only 1 isolate per patient was kept for the analysis.

Retail Meat Samples

To use a similar geographic scale as CIPARS surveillance of human clinical *Salmonella* isolates and because we expected a certain level of provincial clustering in food distribution, we designed the study of CIPARS retail surveillance to provide a representative measurement of what consumers from each province were exposed to through ingestion of improperly cooked raw meat or cross-contamination. Randomization and weighted allocation of samples according to demography of the human population ensured that the data generated through retail sampling were representative and reliable at the provincial level. Retail raw chicken samples (most often chicken thigh with skin on) were collected as part of CIPARS retail program that purchases samples weekly (Ontario and Québec) or biweekly (Saskatchewan, British Columbia) from chain, independent, and butcher stores in 15–18 randomly selected census divisions in each participating province. Retail surveillance was initiated in Ontario and Québec in mid-2003 and at the beginning of 2005 in Saskatchewan. Surveillance also was conducted during part of 2007 and all of 2008 in British Columbia.

Microbiologic Analysis

Recovery of Isolates from Retail Chicken Meat

Primary isolations of *E. coli* and *Salmonella* spp. were conducted at the Laboratory for Foodborne Zoonoses, PHAC. Every retail chicken meat sample received was cultivated for *Salmonella*, but only 1 of every 2 samples was systematically selected to be tested for generic *E. coli* isolation. Incubated peptone rinses of chicken meat samples were streaked on eosin-methylene blue agar (Becton Dickinson, Sparks, MD, USA). Presumptive *E. coli* colonies were identified by using the Simmons' citrate and indole tests. Colonies showing negative indole results were identified by using the API 20E (bioMérieux Clinical Diagnostics, Marcy l'Étoile, France). All chicken samples were tested for *Salmonella* with a modified MFLP-75 method of the Compendium of Analytical Methods (*24*). Incubated peptone rinses were injected into modified semisolid Rappaport-Vassiliadis media. Presumptive *E. coli* colonies were injected into triple sugar iron and urea agar slants

and subjected to the indole test. Presumptive *Salmonella* isolates were verified by slide agglutination using PolyA-I and Vi *Salmonella* antiserum (Difco, Becton Dickinson). *Salmonella* isolates were shipped between laboratories on a tryptic soy agar slant by priority courier. No selective media were used to isolate ceftiofur-resistant bacteria.

Serotyping, Phage Typing, and Susceptibility Testing

Human and chicken *Salmonella* isolates were serotyped and phage typed by using published methods (*25*–*28*). MICs were determined by the NML (human isolates) and the Laboratory for Foodborne Zoonoses, PHAC (chicken isolates) by the broth microdilution method (Sensititre Automated Microbiology System; Trek Diagnostic Systems Ltd., Westlake, OH, USA). *Salmonella* and *E. coli* isolates were tested by using the National Antimicrobial Resistance Monitoring System custom susceptibility plate for gram-negative bacteria. The breakpoint used to determine ceftiofur resistance was >4 μg/mL (*29*).

Data Analysis

We analyzed data using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). The yearly proportion of retail chicken samples contaminated with ceftiofur-resistant *Salmonella* Heidelberg (or *E. coli*) and the incidence rate of human infection with ceftiofur-resistant *Salmonella* Heidelberg was calculated as described in CIPARS 2006 annual report (*12*). The Pearson product-moment correlation was used to verify the correlation between ceftiofur-resistant *Salmonella* Heidelberg isolated from retail chicken and human incidence estimates by using the Pearson option in the PROC CORR procedure in SAS. We computed the overall correlation coefficient using data across all provinces under study and computed a specific coefficient for provinces with >5 observations (*30*)

To describe ceftiofur resistance changes by quarter and reduce the noise around the estimate caused by the small number of observations per quarter, we computed a nonweighted rolling average of the prevalence of ceftiofur resistance using data from the current quarter and the previous 2 quarters for chicken *E. coli,* chicken *Salmonella* Heidelberg, and human *Salmonella* Heidelberg isolates from the province of Québec. We tested differences in ceftiofur resistance between years with SAS using χ^2 or Fisher exact tests when appropriate.

Results

Ceftiofur-Resistant *Salmonella* Heidelberg Isolated from Retail Chickens and from Humans

Across Canada, the annual percentage of chicken samples contaminated with ceftiofur-resistant *Salmonella* Heidelberg correlated strongly with the annual incidence of human cases related to this type of isolate (r = 0.91, p<0.0001) (Figure 1). This strongly significant correlation held across time and within different Canadian provinces (Ontario, r = 0.93, p<0.01; Québec, r = 0.89, p = 0.01).

Changes in ceftiofur resistance alone did not explain a number of the temporal changes in exposure (*12*). For example, in Ontario, the decrease in the prevalence of retail chicken contaminated with ceftiofur-resistant *Salmonella* Heidelberg isolates during 2004–2008 (Figure 1) was linked to a decrease in ceftiofur resistance from 58% to 14% (Table) and a decrease in the prevalence of *Salmonella* Heidelberg in chicken from 61% to 15% of all *Salmonella* isolates. In Québec, the decrease in contamination of chicken with ceftiofur-resistant *Salmonella* Heidelberg strains from 2003 to 2004 (Figure 1) was related mainly to a decrease in the prevalence of *Salmonella* Heidelberg (from 71% to 48%) in chicken, whereas the decrease from 2004 to 2006 was attributable mainly to a drop in ceftiofur resistance from 62% to 7% (Table). In British Columbia, the low level of chicken contamination with ceftiofur-resistant *Salmonella* Heidelberg strains resulted mainly from the rarity of *Salmonella* Heidelberg (only 11% of all *Salmonella* in 2007–2008), and low exposure levels in Saskatchewan were related mainly to low ceftiofur resistance among *Salmonella* Heidelberg (Table).

Ceftiofur-Resistant *E. coli* Isolated from Retail Chicken

Retail chicken generally was more frequently contaminated with ceftiofur-resistant commensal *E. coli* than with ceftiofur-resistant *Salmonella* Heidelberg isolates (Figure 1). The proportion of chicken contaminated with ceftiofur-resistant *E. coli* (Figure 1) closely followed changes in ceftiofur resistance (Table) because commensal *E. coli* was recovered from almost all (89%–100%) chicken samples

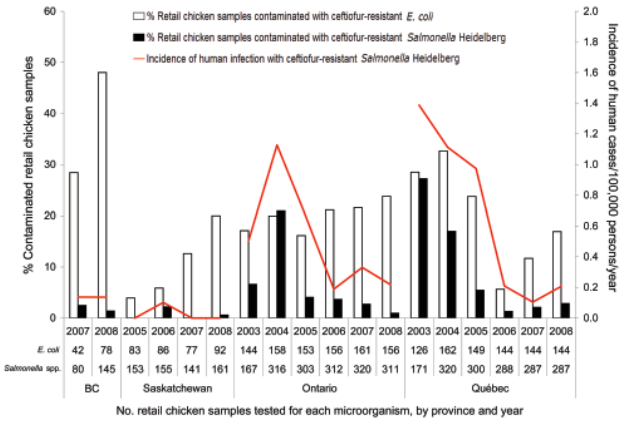

Figure 1. Prevalence of retail chicken contaminated with ceftiofur-resistant *Escherichia coli* and *Salmonella enterica* serovar Heidelberg and incidence of human infections from ceftiofur-resistant *Salmonella* Heidelberg in Canada.

Table. Prevalence of ceftiofur resistance among human and retail chicken *Salmonella* serovar Heidelberg isolates and retail chicken *Escherichia coli* isolates from Canadian provinces surveyed during 2003–2008

Isolate/province	Prevalence of ceftiofur resistance, % (no. resistant isolates/total no. isolates tested)					
	2003	2004	2005	2006	2007	2008
Human clinical *Salmonella* Heidelberg						
Québec	31 (52/167)	36 (42/116)	35 (37/106)	8 (8/96)	6 (4/63)	12 (8/65)
Ontario	18 (31/172)	38 (70/185)	30 (42/140)	10 (12/122)	22 (21/94)	32 (7/22)
Saskatchewan			0 (0/15)	7 (1/14)	0 (0/11)	0 (0/7)
British Columbia					23 (3/13)	19 (3/16)
Chicken retail *Salmonella* Heidelberg						
Québec	65 (13/20)	62 (18/29)	33 (4/12)	7 (1/14)	19 (6/32)	18 (7/38)
Ontario	16 (3/19)	58 (19/33)	27 (3/11)	21 (3/14)	21 (9/42)	14 (3/21)
Saskatchewan			0 (0/5)	13 (1/8)	0 (0/9)	8 (1/12)
British Columbia					50 (2/4)	67 (2/3)
Chicken retail *E. coli*						
Québec	32 (36/111)	34 (54/158)	25 (35/142)	6 (8/135)	13 (17/128)	18 (24/131)
Ontario	18 (24/136)	21 (32/150)	17 (25/145)	22 (34/152)	22 (35/157)	24 (36/150)
Saskatchewan			4 (3/82)	6 (5/85)	13 (10/75)	20 (18/92)
British Columbia					29 (12/42)	49 (34/70)

collected. Exposure to ceftiofur-resistant *E. coli* strains appeared to have increased in recent years in Canada (Figure 1). In 2008, exposure to ceftiofur-resistant *E. coli* strains was highest in British Columbia and lowest in Québec.

Temporal Changes in Ceftiofur Resistance in the Province of Québec, 2003–2008

In 2003–2004, >60% of the chicken *Salmonella* Heidelberg isolates were ceftiofur resistant, and ceftiofur resistance among chicken *E. coli* and human *Salmonella* Heidelberg isolates varied from 30% to 40% (Figure 2). Ceftiofur resistance declined sharply immediately after the first quarter of 2005 among chicken *E. coli* and *Salmonella* Heidelberg isolates, and a similar decline began in the next quarter among human *Salmonella* Heidelberg isolates (Figure 2). This decline steadily continued until the end of 2006. As a result, the prevalence of ceftiofur resistance significantly decreased from 2004 to 2006 among chicken (62% to 7%; p<0.001) and human (36% to 8%; p<0.0001) *Salmonella* Heidelberg isolates and chicken *E. coli* isolates (34% to 6%; p<0.0001 [Table]). Then, from 2006 to 2008, the prevalence of ceftiofur resistance significantly increased among chicken *E. coli* isolates (6% to 18%; p = 0.002), and prevalence of ceftiofur resistance increased, but not significantly, among *Salmonella* Heidelberg from chicken (7% to 18%; p = 0.32) and human (8% to 12%; p = 0.41) isolates (Table).

Discussion

CIPARS data clearly indicate a temporal association between changing levels of contamination of retail chicken with ceftiofur-resistant *Salmonella* Heidelberg strains and incidence of ceftiofur-resistant *Salmonella* Heidelberg infection in humans. This correlation is strong and applies to different regions of Canada. Our observation is consistent with published results from outbreak investigations and case-control studies suggesting that chicken products are a source of human infection with *Salmonella* Heidelberg in Canada (*7*,*8*).

Although humans potentially can become infected with ceftiofur-resistant *Salmonella* Heidelberg from sources other than chicken, chicken appears the most likely source in Canada. Ceftiofur-resistant *Salmonella* Heidelberg has never been reported among CIPARS porcine *Salmonella* of abattoir origin, and it has not been detected among retail pork, abattoir beef, or retail beef, in which *Salmonella* prevalence remains <2% (*12*). Data generated by National Antimicrobial Resistance Monitoring System retail surveillance in the United States indicated that 17% of *Salmonella* Heidelberg

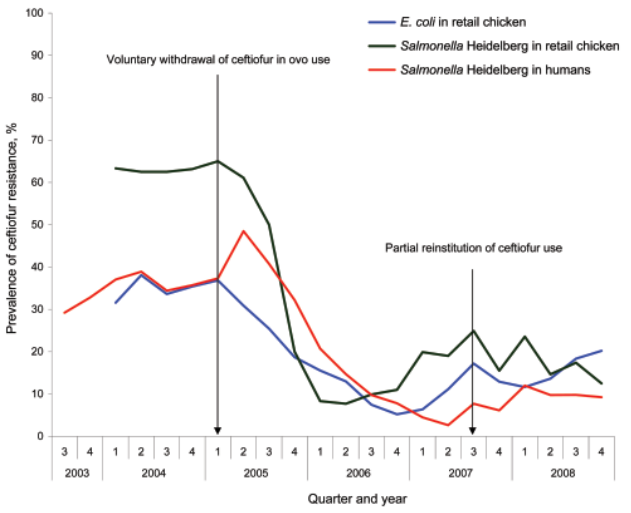

Figure 2. Prevalence of ceftiofur resistance (moving average of the current quarter and the previous 2 quarters) among retail chicken *Escherichia coli*, and retail chicken and human clinical *Salmonella enterica* serovar Heidelberg isolates during 2003–2008 in Québec, Canada.

isolates recovered from ground turkey in 2006 were resistant to ceftiofur (*13*). CIPARS does not conduct ongoing surveillance of retail turkey, and we cannot ignore the possibility that retail turkey could be a source of ceftiofur-resistant *Salmonella* Heidelberg for humans as well. However, turkey consumption in Canada (4.7 kg per capita) was much lower than chicken consumption (33.2 kg per capita) in 2007 (*31*). Lastly, *Salmonella* Heidelberg has been reported in clinical samples from various other animal species in Canada (*12*), and exposure to sick animals could potentially be another source of infection. However, ceftiofur resistance in clinical *Salmonella* Heidelberg isolates remains anecdotal in species other than chicken and turkey (*12*).

Drug use monitoring in chicken is nonexistent in Canada. However, research data indicate a high level of ceftiofur use in Québec hatcheries in 2003–2004, where at least 78% of the lots surveyed in Québec abattoirs (M. Boulianne et al., unpub. data) had received ceftiofur in ovo. During that same period, ceftiofur resistance among retail chicken *Salmonella* Heidelberg isolates were >60%. The rapid and important 82% (*E. coli*) and 89% (*Salmonella* Heidelberg) declines in ceftiofur resistance in Québec retail chicken meat that followed in 2005–2006, as well as in Québec chicken *E. coli* and *Salmonella* isolates collected from passive surveillance of animal clinical isolates conducted by the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) (*32*), is consistent with an effective voluntary withdrawal in 2005 and 2006. In 2007, the Québec broiler industry announced a potential partial reinstitution of ceftiofur use to control omphalitis in young chicks, with the intention of using the drug on a rotational basis and limiting its use to no more than 6 months per year (*32*). Again, CIPARS data from Québec retail chicken sampling in 2007–2008 demonstrating a reemergence of ceftiofur resistance among *E. coli* but at lower levels than in 2003–2004 are consistent with a partial return to ceftiofur use. The simultaneous reduction (and reemergence) in ceftiofur resistance in both retail chicken *E. coli* and *Salmonella* Heidelberg isolates and in clinical chicken *E. coli* and *Salmonella* isolates from MAPAQ surveillance support the hypothesis that the fluctuations in ceftiofur resistance most likely were driven by a common exposure (or reduction of exposure) to ceftiofur in chicken hatcheries, rather than simply being secondary to the natural spread and disappearance of a ceftiofur-resistant clone unrelated to ceftiofur use.

Although Ontario hatcheries had never announced an official withdrawal of ceftiofur use, a drop in ceftiofur resistance also was observed among chicken *Salmonella* Heidelberg isolates in Ontario in 2005. Although some argue that this proves the absence of an association between ceftiofur use and ceftiofur resistance in broiler chicken, movement of hatching eggs, broiler chicks (mostly from Québec to Ontario), and retail chicken meat between these 2 provinces could explain some of the similarities among *Salmonella* Heidelberg isolates in Ontario and Québec (*33*). The withdrawal in Québec might also have led Ontario broiler chicken hatcheries to temporarily decrease their use of ceftiofur in 2005.

In the absence of reliable comprehensive drug use information in the broiler chicken industry, we use resistance in commensal *E. coli* as a surrogate measure of the level of drug use (*34*). The high prevalence of ceftiofur resistance among *E. coli* isolates from British Columbia (almost half of the isolates in 2008 in that province), the increasing prevalence of resistance measured in Saskatchewan, and the 22% ceftiofur resistance among chicken *E. coli* isolates from Ontario when ceftiofur resistance prevalence was at its lowest level in Québec in 2006, indicates that ceftiofur use is unlikely to be restricted to the province of Québec. Lastly, in ovo ceftiofur use has also been reported in US chicken hatcheries (*35*).

Coselection of resistance to cephalosporins by exposure to other antimicrobials or to chemicals in the agricultural environment has been suggested as a confounding factor for the increase in observed resistance. Giles et al. (*36*) report the presence of the *sug*E gene on the same element as the bla_{CMY-2} gene in *Salmonella,* but the capacity of this gene to effectively confer resistance to quaternary ammonium compounds and provide coselection remains uncertain.

The levels of contamination of retail chicken with ceftiofur-resistant *E. coli* represent an additional concern. No selective media for ceftiofur-resistant strains was used, and the level of contamination of retail chicken with ceftiofur-resistant *E. coli* (and *Salmonella* Heidelberg) strains was most likely underestimated. Although this study describes exposure to commensal *E. coli*, such strains occasionally may cause infections in predisposed humans. In addition, the species *E. coli* includes a variety of strains commonly pathogenic for humans, and some strains from the normal flora of animals may carry a variety of virulence determinants that increase their potential for causing disease in humans (*37*). Poppe et al. (*38*) also demonstrated experimentally the acquisition of resistance to extended-spectrum cephalosporins by *Salmonella* serovar Newport from *E. coli* strains by conjugation in poultry intestinal tracts. In addition, molecular characterization of plasmids from field isolates demonstrates that identical bla_{CMY-2} plasmids can be found in both *Salmonella* and *E. coli* from the same chicken (P. Boerlin et al., unpub. data). Because the bla_{CMY-2} gene is horizontally transferable and is frequently observed in ceftiofur-resistant isolates of chicken origin, chicken could potentially be a reservoir of this gene for human pathogens, including *Salmonella* and others.

Except for anecdotal information, little information is available about drugs used by broiler chicken hatcheries and growers in Canada. The absence of on-farm drug use monitoring data prevents us from fully determining the effect of subtle changes in the level of use of ceftiofur (or other drugs) on resistance among bacteria recovered from chickens in Canada. Surveillance data from turkey or other nonsurveyed commodities would be useful to adequately quantify the contribution of each commodity to the overall number of cases related to ceftiofur-resistant *Salmonella* Heidelberg in humans. The impact of disinfectants used by the broiler industry at the farm or processing level on the selection of ceftiofur-resistant strains also needs to be assessed. Lastly, CIPARS is planning a burden-of-illness study to measure the impact of extended-spectrum cephalosporin resistance in *Salmonella* Heidelberg on human health.

Efforts undertaken by Québec chicken hatcheries to voluntarily withdraw use of ceftiofur in 2005–2006 coincided with a markedly reduced prevalence of ceftiofur-resistant *Salmonella* Heidelberg in retail chicken. This drop also effectively reduced the number of ceftiofur-resistant *Salmonella* Heidelberg infections in humans in this province during the same period. This reduction suggests that control of resistance to extended-spectrum cephalosporins is possible by managing ceftiofur use at the hatchery level. The partial reintroduction of ceftiofur use in Québec chicken hatcheries in 2007 with increasing rates of ceftiofur resistance after reintroduction, and indications that ceftiofur is used for the same purpose in other Canadian provinces, is of high concern. An increasing level of exposure to *E. coli* strains carrying horizontally transferable genes conferring resistance to extended-cephalosporins complicates the situation. To ensure the continued effectiveness of extended-spectrum cephalosporins to treat serious human infections, multidisciplinary efforts are needed to scrutinize, and where appropriate, limit use of ceftiofur in Canadian food animal production, particularly in chicken.

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FDA Announces Final Decision About Veterinary Medicine

U.S. Food and Drug Administration (FDA) Commissioner Lester Crawford is announcing the Agency's final decision to no longer allow distribution or use of the antimicrobial drug enrofloxacin for the purpose of treating bacterial infections in poultry. This ruling does not affect other approved uses of the drug. This animal drug belongs to a class of drugs known as fluoroquinolones and is marketed under the name Baytril by Bayer Corporation.

The FDA's Center for Veterinary Medicine (CVM) began proceedings to withdraw use of this animal drug in poultry because of scientific data that showed that the use of enrofloxacin in poultry caused resistance to emerge in *Campylobacter*, a bacterium that causes foodborne illness. Chickens and turkeys normally harbor *Campylobacter* in their digestive tracts without causing poultry to become ill. Enrofloxacin does not completely eliminate *Campylobacter* from the birds' intestinal tracts, and those *Campylobacter* bacteria that survive are resistant to fluoroquinolone drugs. These resistant bacteria multiply in the digestive tracts of poultry and persist and spread through transportation and slaughter, and are found on chicken carcasses in slaughter plants and retail poultry meats.

Campylobacter bacteria are a significant cause of foodborne illness in the U.S. Antimicrobial treatment is recommended for people with severe illness as well as the very young, the elderly, and people with certain medical conditions. Complications of such infections can include reactive arthritis and, more rarely, blood stream infections. Early treatment can mitigate symptoms and may decrease the risk of complications. Fluoroquinolones used in humans are ineffective if used to treat *Campylobacter* infections that are resistant to them. This failure can significantly prolong the duration of the infections and may increase the risk of complications. The proportion of *Campylobacter* infections that are resistant to fluoroquinolones has increased significantly since the use of enrofloxacin in poultry was approved in the U.S.

Bayer Corporation has 60 days from the date of the decision to appeal the withdrawal to a U.S. Court of Appeals. The Final Rule withdrawing approval of the antimicrobial drug enrofloxacin for the purpose of treating bacterial infections in poultry will be effective on September 12, 2005. For the Final Decision please go to www.fda.gov/oc/antimicrobial/baytril.pdf and for the *Federal Register* documents please go to www.fda.gov/ohrms/dockets.

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Food Animals and Antimicrobials: Impacts on Human Health

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Food Animals and Antimicrobials: Impacts on Human Health

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INTRODUCTION

For many decades, antibiotic resistance has been recognized as a global health problem. It has now been escalated by major world health organizations to one of the top health challenges facing the 21st century (40, 65). Some of its causes are widely accepted, for example, the overuse and inappropriate use of antibiotics for nonbacterial infections such as colds and other viral infections and inadequate antibiotic stewardship in the clinical arena (109). But the relationship of drug-resistant bacteria in people to antibiotic use in food animals continues to be debated, particularly in the United States (11, 14, 38, 44, 48, 96, 124).

Many have delved into this question, producing volumes of direct and indirect evidence linking animal use to antibiotic resistance confronting people. Among these are a number of studies which unequivocally support the concern that use of antibiotics in food animals (particularly nontherapeutic use) impacts the health of people on farms and, more distantly, via the food chain (69, 88, 90, 111). While it was hoped by many that the years of experience following the bans on nontherapeutic use of antimicrobials in Europe would clearly signal an end to this practice, arguments continue, largely along the lines of a cost/benefit ratio and perceived deficits in solid scientific evidence. Action in the United States continues to lag far behind that of the European Union, which has chosen to operate proactively based on the "precautionary principle," a guiding tenet of public health. This principle states that "when evidence points toward the potential of an activity to cause significant widespread or irreparable harm to public health or the environment, options for avoiding that harm should be examined and pursued even if the harm is not yet fully understood or proven" (103).

This communication summarizes a large number of studies on the links between antimicrobials used for growth promotion, in particular, as well as other nontherapeutic antimicrobial (NTA) use in animal husbandry and aquaculture, and the emergence of antibiotic-resistant bacteria in humans. The *FAAIR Report* (*Facts about Antibiotics in Animals and the Impact on Resistance*) of the Alliance for the Prudent Use of Antibiotics (APUA) cites areas where antibiotic use can be curtailed and proposes several viable recommendations that could be utilized to reduce the burden of resistance genes created by nontherapeutic antibiotic use in animals (22).

Lastly, we consider whether knowledge gaps exist that need addressing in order to answer persisting questions that fuel the controversy over NTA use in food animals.

ANTIMICROBIAL USE IN ANIMALS: EFFECTS ON ANTIBIOTIC RESISTANCE EMERGENCE

Antimicrobials are delivered to animals for a variety of reasons, including disease treatment, prevention, control, and growth promotion/feed efficiency. Antimicrobial growth promotants (AGPs) were first advocated in the mid-1950s, when it was discovered that small, subtherapeutic quantities of antibiotics such as procaine penicillin and tetracycline (1/10 to 1/100 the amount of a therapeutic dose), delivered to animals in feed, could enhance the feed-to-weight ratio for poultry, swine, and beef cattle (142). For many years, the positive effects of this practice were championed, while the negative consequences went undetected. But microbiologists and infectious disease experts facing antibiotic resistance questioned the possible harm from this use (74, 89, 109, 136). They found that farms using AGPs had more resistant bacteria in the intestinal

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floras of the farm workers and farm animals than in those for similar people and animals on farms not using AGPs. A prospective *in vivo*/*in situ* study in 1975 was performed to evaluate the effect of introducing low-dose in-feed oxytetracycline as an AGP on the intestinal floras of chickens and farm dwellers (111). The results showed not only colonization of the chickens with tetracycline-resistant and other drug-resistant *Escherichia coli* strains but also acquisition of resistance in *E. coli* in the intestinal flora of the farm family. Other studies over the ensuing 3 decades further elucidated the quantitative and qualitative relationships between the practice of in-feed antimicrobials for animals and the mounting problem of hard-to-treat, drug-resistant bacterial infections in humans (83, 162).

Nontherapeutic Agents and Practices

The chief agricultural NTAs, used extensively in the United States and also used in Europe until the 1970s, include drugs that have likewise been employed widely in human medicine. In the absence of complete, unbiased data, this NTA use in the United States is estimated to be equal to (159) or as much as eight times greater than (67, 117) the quantity administered for therapeutic use.

More recently, concerns have arisen over the extensive use of antimicrobials in the burgeoning aquaculture industry, which more than doubled between 1994 and 2004 (36, 84). Eighty to 90 percent of total production occurs in Asia, with 67% occurring in China alone (64). In many parts of the world, fish farming is integrated with sewage or industrial wastewater or with land agriculture, as manure and other agricultural residues are commonly employed in fish feed (123). The overcrowding, unhygienic measures, and other manipulations in this intensive, industrial-scale production act as stressors to the fish and promote an increased use of antibiotic prophylaxis, particularly in the shrimp and carnivorous fish (such as salmon) industries. Moreover, even though the aquaculture use of AGPs in Western Europe and North America has been discontinued, therapeutic treatment of fish generally occurs *en masse* via inclusion in fish food, which results in exposure of the entire body of water to the antibiotic. The broad application of antibiotics in fish food leads to leaching from unconsumed food and feces into the water and pond sediments, where it not only exerts selective pressures on the sediment and water microflora but also can be washed to more distant sites, exposing wild fish and shellfish to trace antimicrobials (36). In this environment, the role of transduction (infection by bacterial phages) is considered highly important in facilitating lateral gene transfer (71). Sorum suggested that, historically, the transfer and emergence of resistance have occurred faster from aquatic bacteria to humans than from terrestrial animal bacteria to humans (141).

In the United States, the total fish industry use of antibiotics was estimated to be 204,000 to 433,000 pounds in the mid-1990s (25) (about 2% of the nonmedical use in cattle, swine, and poultry [117]). In much of the world, however, antibiotics are unregulated and used indiscriminately, and use statistics are rarely collected (25, 157). Although the total quantities of antibiotics employed in aquaculture are estimated to be smaller than those used in land animal husbandry, there is much greater use of antibiotic families that are also used in human medicine (Table 1). In Chile, for example, ~100 metric tons of quinolones are used annually (10-fold greater than the amount used in human medicine), mostly in aquaculture (35). At least 13 different antimicrobials are reportedly used by farmers along the Thai coast (75).

Salmonella and the *Swann Report*

Alarmed by the rise in multidrug-resistant *Salmonella* in the 1960s, the United Kingdom's *Swann Report* of 1969 recognized the possibility that AGPs were contributing largely to the problem of drug-resistant infections (144). It concluded that growth promotion with antibiotics used for human therapy should be banned. The recommendation was implemented first in England and then in other European countries and Canada. The practice continued unchanged, however, in the United States and ultimately also continued in Europe, but with agents that were not used therapeutically in humans. Antibiotics such as bacitracin, avoparcin, bambermycins, virginiamycin, and tylosin gained in popularity as narrower-spectrum substitutes that had a smaller impact on the broad range of gut flora. Unforeseen, however, was the structural relationship between some of these agents and agents used clinically in humans (Table 1). This similarity meant that they shared a single bacterial target and that use of one agent could produce cross-resistance to the other.

Impacts of Nontherapeutic Use

Therapeutics applied properly for the treatment of individual animals tend to control the emergence and propagation of antimicrobial-resistant strains, in large part due to their relatively short-term application and relatively small numbers of animals treated. The resistant strains which may appear are generally diluted out by the return of normal, drug-susceptible commensal competitors (110). In contrast, any extended antibiotic applications, such as the use of AGPs, which are supplied for continuous, low-dose application, select for increasing resistance to the agent. Their use in large numbers of animals, as in concentrated animal feeding operations (CAFOs), augments the "selection density" of the antibiotic, namely, the number (density) of animals producing resistant bacteria. An ecological imbalance results—one that favors emergence and propagation of large numbers of resistance genes (113). The selection is not linked merely to the total amount of antibiotic used in a particular environment but to how many individuals are consuming the drug. Each animal feeding on an antibiotic becomes a "factory" for the production and subsequent dispersion of antibiotic-resistant bacteria. NTA uses are also clearly linked to the propagation of multidrug resistance (MDR), including resistance against drugs that were never used on the farm (10, 52, 59, 60, 92, 107, 111, 132, 141, 153, 154, 164). The chronic use of a single antibiotic selects for resistance to multiple structurally unrelated antibiotics via linkage of genes on plasmids and transposons (111, 143).

Studies on the impact of NTA use on resistance in land food animals have focused primarily on three bacterial genera—*Enterococcus*, *Escherichia*, and *Campylobacter*—and, to a lesser extent, on *Salmonella* and *Clostridium*. All of the above may be members of the normal gut flora (commensals) of food animals

TABLE 1. Antimicrobials used in food animal production[a]

Antibiotic	Purpose	Antimicrobial class	Spectrum of activity	Use in human medicine	Structurally related antibiotic(s)/antibiotic(s) with shared cross-resistance	Comments[c]
Ardacin	Bovine AGP	Glycopeptides	Gram-positive organisms	No	Vancomycin, teicoplanin	Withdrawn from EU in 1997; not licensed in U.S.
Amoxicillin,[b] ampicillin[b]	Aquaculture, oral treatment of swine colibacillosis, treatment of bovine bacterial enteritis and subclinical mastitis	Aminopenicillins	Moderate	Yes	All penicillins	
Avilamycin	AGP for broilers	Orthosomysins	Gram-positive organisms	No	Everninomycin	Withdrawn from EU; not licensed in U.S.
Avoparcin[b]	AGP	Glycopeptides	Gram-positive organisms	No	Vancomycin, teicoplanin	Withdrawn from EU in 1997; not licensed in U.S.
Bacitracin/zinc bacitracin	AGP for poultry, beef cattle, and swine; control of swine dysentery and bacterial enteritis; control of poultry enteritis	Polypeptides	Gram-positive organisms	Yes (zinc bacitracin)	Actinomycin, colistin, polymyxin B	Withdrawn from EU in 1999; available in U.S.; used in Japan
Bambermycin	AGP for poultry, swine, and cattle	Phosphoglycolipids	Gram-positive organisms	No	Vancomycin, teicoplanin	Withdrawn from EU in 2006; available in U.S.
Carbadox	Control of swine dysentery	Quinoxalines	Gram-positive and -negative organisms	No	Other quinoxolines	Withdrawn due to worker toxicity in EU and Canada; available in U.S. and Mexico
Carbomycin[b]	Respiratory disease prevention and treatment in poultry	Macrolides	Gram-positive organisms	No	Other macrolides	
Chloramphenicol	Aquaculture (oral/bath/injection)	Amphenicols	Broad	Yes	All amphenicols	Chloramphenicol approved in U.S. for dogs only
Colistin	Broiler, swine, and cattle feed	Cyclopolypeptides	Gram-negative organisms	Yes	All polymyxins	Used in Japan
Efrotomycin	AGP for swine	Elfamycins	Gram-positive organisms	No	Other elfamycins only	Not marketed
Enrofloxacin[b]	Therapy for bovine and swine respiratory disease, use in aquaculture (oral/bath)	Fluoroquinolones	Broad	No	All quinolones	Banned for use in poultry by FDA in 2005; not approved for aquaculture in U.S.
Erythromycin[b]	Aquaculture (oral/bath/injection); AGP for poultry, cattle, and swine; therapy for poultry respiratory disease and bovine mastitis	Macrolides	Gram-positive organisms	Yes	Oleandomycin and other macrolides and lincosamides	
Florfenicol	Respiratory disease treatment of cattle and swine	Amphenicols	Broad	No	All amphenicols	
Flumequin[b]	Aquaculture (oral)	Fluoroquinolones	Broad	No	Fluoroquinolones and quinolones	Not approved in U.S.
Furazolidone	Aquaculture (oral/bath)	Nitrofurans	Broad	Yes		Banned in U.S. food animals in 2005
Gentamicin[b]	Therapy for swine colibacillosis and dysentery, prevention of early poultry mortality, turkey egg dip	Aminoglycosides	Gram-positive and -negative organisms	Yes	Other aminoglycosides	
Lasalocid	AGP for cattle, poultry, sheep, and rabbits; coccidiosis prevention in poultry and sheep	Ionophores	Gram-positive organisms	No	Not demonstrated	Approved in EU and U.S.
Lincomycin	AGP for chickens and swine; therapy for swine dysentery, pneumonia, chicken necrotic enteritis, and respiratory disease	Lincosamides	Gram-positive organisms	Rare	Erythromycin and other macrolides and lincosamides, clindamycin	Approved in U.S.
Maduramycin	Poultry coccidiostat	Ionophores	Coccidia, Gram-positive organisms	No	Not demonstrated	Approved in EU and U.S.
Monensin	Bovine AGP; prevention/control of coccidiosis in bovines, poultry, and goats	Ionophores	Coccidia, Gram-positive organisms	No	Not demonstrated	Withdrawn from EU as bovine AGP but authorized as poultry coccidiostat

Neomycin[b]	AGP for swine and poultry; treatment/control of swine enteritis and pneumonia; control of mortality from *E. coli* in turkeys, bovines, swine, sheep, and goats; control of respiratory and other poultry diseases; aquaculture (bath)	Aminoglycosides	Gram-positive and -negative organisms	Yes	Gentamicin and other aminoglycosides	Approved in U.S.
Narasin	Poultry feed coccidiostat, prevention of necrotic enteritis in chickens, AGP for cattle	Ionophores	Coccidia, Gram-positive organisms	No	Not demonstrated	Approved in U.S.
Nourseothricin	Swine AGP	Streptothricins	Gram-negative organisms	No	None	Withdrawn from EU; never used in U.S.
Novobiocin	Treatment of staph infections, treatment and control of fowl cholera, treatment of bovine mastitis	Aminocoumarins	Gram-positive organisms	Yes		
Olaquindox	Swine AGP, control of swine dysentery/enteritis	Quinoxalines	Gram-positive and -negative organisms	No	Other quinoxolines	Withdrawn due to worker toxicity in EU and Canada; available in U.S.
Oleandomycin[b]	Poultry and swine AGP	Macrolides	Gram-positive organisms	Yes	Erythromycin and other macrolides	
Ormetoprim	Poultry AGP, prevention of fowl cholera and other infections	Diaminopyrimidines	Broad	No	Trimethoprim	
Oxolinic acid[b]	Aquaculture (oral)	Quinolones	Broad	No	Other quinolones	Not approved in U.S.
Pristinamycin	AGP	Streptogramins	Gram-positive organisms	Yes	Other streptogramins (virginiamycin, quinupristin/dalfopristin)	
Procaine penicillin[b]	AGP in poultry and swine	Beta-lactams	Gram-positive organisms	Yes	Other beta-lactams	Withdrawn in EU; available in U.S.
Roxarsone	AGP for poultry and swine, poultry coccidiostat, treatment of swine dysentery	Arsenicals	Coccidia	No	Other arsenicals	
Salinomycin	Swine AGP, prevention/control of swine dysentery and porcine intestinal adenomatosis, control of *Clostridium perfringens* in growers	Ionophores	Gram-positive organisms	No	Not demonstrated	Withdrawn from EU
Spiramycin[b]	Swine AGP, treatment of bovine mastitis	Macrolides	Gram-positive organisms	Yes	Erythromycin and other macrolides and lincosamides	AGP use withdrawn from EU in 1999; not approved in U.S.
Streptomycin[b]	Aquaculture (bath)	Aminoglycosides	Broad	Yes	All aminoglycosides	
Sulfonamides	Aquaculture (sulfamerazine [oral] and sulfadimethoxine [oral]), swine AGP (sulfamethazine), chicken AGP (sulfadimethoxine)	Sulfonamides	Broad	Yes	All sulfonamides	Sulfamerazine authorized for U.S. aquaculture, but not marketed
Tetracyclines (oxy- and chlor-)[b]	AGP for poultry, swine, and cattle; treatment and control of multiple livestock diseases; aquaculture (oral/bath/injection); control of fish and lobster disease	Tetracylines	Broad	Yes	All tetracyclines	Withdrawn from EU; authorized in U.S.
Tiamulin	Swine AGP; treatment of swine enteritis, dysentery, and pneumonia	Pleuromutilins	Gram-positive organisms, mycoplasmas, spirochetes	No	Tylosin, erythromycin, and other macrolides	Used for disease prevention and treatment in chickens outside the U.S.
Tylosin[b]	Swine AGP, therapeutic treatment of mastitis	Macrolides	Gram-positive organisms	No	Erythromycin and other macrolides and lincosamides	AGP use withdrawn from EU; available in U.S.
Virginiamycin	AGP for broilers	Streptogramins	Gram-positive organisms	Yes	Quinupristin/dalfopristin and other streptogramins	Withdrawn from EU; available in U.S.

[a] Based on data from references 7, 32, 34, 53, 84, 96, 98, 133, and 162.
[b] Highly important in human medicine or belongs to critically important class of human antimicrobials.
[c] EU, European Union.

but possess the potential to become serious human pathogens. The prospective farm study by Levy in 1975 (111) and studies of others in the following decades clearly demonstrated the selective nature of low-dose, nontherapeutic AGPs on both the pathogenic and commensal flora of food animals such as poultry, swine, and cattle (8, 16, 18, 90, 98, 146, 149). Likewise, in the past decade, studies have demonstrated the selective nature of mass treatment with antimicrobials in aquaculture (36, 62, 84). In the latter, studies have focused on *Aeromonas* pathogens of both fish and humans and the subsequent high-frequency transfer of their resistance plasmids to *E. coli* and *Salmonella* (36).

Aarestrup and Carstensen found that resistance derived from use of one NTA (tylosin) was not confined to swine gut bacteria only but could cross species and appear in staphylococci isolated from the skin. While the conversion of gut enterococci to erythromycin (a related human therapeutic) resistance occurred rapidly (within 1 week) the skin-derived resistant organism *Staphylococcus hyicus* appeared more gradually, escalating to a 5-fold increase over 20 days (5).

The finding of bacterial cross-resistance between NTAs used in food animals and human drugs was aptly demonstrated with avoparcin (an AGP) and its close relative vancomycin (an important human therapeutic) when vancomycin-resistant enterococci (VRE) emerged as a serious human pathogen. A connecting link between resistance in animals and humans was revealed when Bates et al. found avoparcin- and vancomycin-coresistant enterococci in pigs and small animals from two separate farms. Ribotyping methods showed that some of the patterns from farms and sewage exactly matched those of *Enterococcus* spp. from the hospital (24). The structures of the two drugs are similar: they are both members of the glycopeptide family (24).

Since that time, numerous studies have examined the impacts of newer NTAs on the floras of animals. The use of tylosin and virginiamycin in Norwegian swine and poultry led to high prevalences of resistance to both these agents in *Enterococcus faecium* (75% to 82% for tylosin and 49 to 70% for virginiamycin) (1). Avilamycin resistance, while significantly associated with avilamycin use, has been observed on both exposed and unexposed farms and was significantly higher in isolates from poultry than in those from swine, despite its use in both these species (4). These findings suggest that other selective agents may be present in the environment or that substances related to avilamycin were not recognized. As described above, not only the drug choice and amount but also the number of animals treated can affect the consequence of its use.

Other findings suggest that complex ecologic and genetic factors may play a role in perpetuating resistance (63). Resistance (particularly to tetracycline, erythromycin, and ampicillin) has been found inherently in some antibiotic-free animals, (10, 45, 93, 130), suggesting that its emergence is related to other factors, such as diet, animal age, specific farm type, cohort variables, and environmental pressures (26). While Alexander et al. found MDR (tetracycline plus ampicillin resistance) in bacteria in control animals, the strains that emerged after AGP use were not related to these (10). In addition, resistance to tetracycline was higher for a grain-based diet than a silage-based one. Costa et al. found non-AGP-related resistances in enterococci, most likely derived from previous flocks, i.e., the farm environment and the feed source appeared to be responsible for the emergence of the unrelated resistances (45). Khachatryan et al. found an MDR phenotype (streptomycin, sulfonamide, and tetracycline [SSuT] resistance phenotype) propagated by oxytetracycline in a feed supplement, but upon removal of the drug, the phenotype appeared to be maintained by some unknown component of the unmedicated feed supplement, possibly one that selects for another gene that is linked to a plasmid bearing the SSuT resistance phenotype (100). The persistence may also relate to the stability of the plasmid in its host and the fact that expression of tetracycline resistance is normally silent until it is induced by tetracycline. Thus, the energy demands exerted on the host by tetracycline resistance are lower. One can conclude that removal of the antibiotic may not lead to rapid loss of the resistant strain or plasmid.

EFFECTS OF BANNING GROWTH PROMOTANTS IN ANIMAL FEEDS IN EUROPE

One of the first bans on AGP use was that imposed on tetracycline by the European Common Market in the mid-1970s (39). Prior to institution of the ban in the Netherlands (1961 to 1974), Van Leeuwen et al. had tracked a rise in tetracycline-resistant *Salmonella* spp. Following the ban, however, they observed a decline in tetracycline resistance in both swine and humans (150).

More than 10 years have passed since the final 1999 European Union ban, during which a plethora of studies from multiple European countries, Canada, and Taiwan have examined antibiotic use and resistance trends subsequent to the removal of key AGP drugs, especially avoparcin, and the consequences on vancomycin resistance in *Enterococcus* (7, 15, 17, 21, 29, 30, 76, 85, 97, 102, 107, 121, 148, 150, 156a). Its structural relationship to and cross-resistance with avoparcin render vancomycin a drug of prime interest for determining the impact of avoparcin in triggering and promoting resistance in human infection.

Avoparcin

In many European countries, the use of avoparcin as a feed additive led to frequent isolation of VRE from farm animals and healthy ambulatory people (3, 18, 102). Since the emergence of the enterococcus as a major MDR pathogen, vancomycin has evolved as a key therapy, often as the drug of last resort. Following the 1995 ban on avoparcin, several investigators reported a decline in animal VRE. In Denmark, frequencies peaked at 73 to 80% and fell to 5 to 6% (7, 18) in poultry. In Italy, VRE prevalence in poultry carcasses and cuts decreased from 14.6% to 8% within 18 months of the 1997 ban (121), and in Hungary, a 4-year study showed not only a decline in prevalence of VRE among slaughtered cattle, swine, and poultry after removal of avoparcin but also a decrease in vancomycin MICs (97). In surveillance studies both before and after the German ban in 1996, Klare et al. showed a high frequency of VRE in 1994, followed by a very low frequency of just 25% of poultry food products in 1999 (102). Similar de-

clines were reported in broiler farms following a ban on avoparcin in Taiwan in 2000 (107).

A dramatic reduction in human carriage of VRE also followed the ban on avoparcin. Parallel surveillances of the gut floras of healthy ambulatory people showed that VRE colonization in Germany declined from 13% in 1994 to 4% in 1998 (102), and in Belgium, it declined from 5.7% in 1996 to ~0.7% in 2001 (68).

Virginiamycin and Other Antibiotics

Increased virginiamycin use in Danish broilers during the mid-1990s correlated with a rise in resistant *E. faecium* prevalence, from 27% to ~70% (7). Following the ban, resistance declined to 34% in 2000. Likewise, in Denmark, the 1998 ban on the use of tylosin in swine resulted in a decline in erythromycin (a structurally related macrolide) resistance, from 66% to 30% (49). Avilamycin use in 1995 and 1996 increased resistance in broiler *E. faecium* strains, from 64% to 77%, while declining applications after 1996 lowered the prevalence to 5% in 2000 (7).

Some of these studies revealed a genetic linkage between bacterial macrolide and glycopeptide resistances in swine, such that neither resistance declined in prevalence until both avoparcin (a glycopeptide) and tylosin (a macrolide) use was limited. With a reduction in tylosin use, the prevalence of glycopeptide-resistant enterococci fell to 6% and macrolide resistance fell from nearly 90% to 47% in *E. faecium* and to 28% in *Enterococcus faecalis* (7). Notably, the first report of transfer of vancomycin resistance from *Enterococcus* to *Staphylococcus aureus* was demonstrated in laboratory mice because of its linkage to macrolide resistance on the same plasmid (119).

One concern voiced following the banning of NTAs was that the incidence of disease in animals would rise and result in a parallel increase in therapeutic use. This has become the subject of some debate. Some countries encountered rises in necrotic enteritis in chickens and colitis in swine soon after the institution of AGP bans (33, 159). In Norway, an abrupt increase in necrotizing enteritis (NE) in poultry broilers was reported following the removal of avoparcin, with a coincident rise in antibiotic therapy. When the ionophore feed additive narasin was approved, NE declined once again (77). It was concluded that the ban on avoparcin consumption produced a negligible effect on the need for antibiotic therapy (76). Likewise, in Switzerland, Arnold et al. reported a postban increase in overall antibiotic quantities used in swine husbandry but observed a stable therapy intensity (prescribed daily dose) (15). By 2003, total animal use of antibiotics in Denmark, Norway, and Sweden had declined by 36%, 45%, and 69%, respectively (76). The most thorough postban analysis of this phenomenon comes from Denmark. In a careful review of swine disease emergence, animal production, and antibiotic use patterns over the years 1992 to 2008, Aarestrup et al. reported no overall deleterious effects from the ban on finishers and weaners in the years 1998 and 2000, respectively. Despite an increase in total therapeutic antibiotic consumption immediately following the ban, no lasting negative effects were detected on mortality rate, average daily weight gain, or animal production (6). Moreover, even if therapeutic use increased, the numbers of animals treated would be reduced compared to those with growth promotion use, so selection density would be decreased (113).

In summary, the in-depth, retrospective analyses in Denmark shed a different perspective on postban concerns over increased therapeutic use. Over time, it appears that the negative after-effects of the ban have waned. As farmers modified their animal husbandry practices to accommodate the loss of banned NTAs, these disease outbreaks became less prominent. Improved immunity and reduced infection rates led to fewer demands for therapeutic antibiotics.

Interestingly, recent studies have shown that the original beneficial aspects observed with AGP use (i.e., weight gain and feed efficiency) appear to have diminished, although the results are mixed and depend upon the kind of animals and type of antibiotic involved. Diarra et al. found no effect on body weight or feed intake in poultry from five different AGPs, and feed efficiency was improved with penicillin only (52). In contrast, Dumonceaux et al. reported a significantly increased body weight (10%) and a 7% increase in feed efficiency with the AGP virginiamycin, but only for the first 15 days (55a). In short, improved farming practices and breeding programs, which may include reduced animal density, better hygiene, targeted therapy, and the use of enzymes, prebiotics, probiotics, and vaccines, appear to have at least partially replaced the beneficial aspects of antibiotic growth promoters (27, 158, 160).

EVIDENCE FOR ANIMAL-TO-HUMAN SPREAD OF ANTIBIOTIC RESISTANCE

Any use of antibiotics will select for drug-resistant bacteria. Among the various uses for antibiotics, low-dose, prolonged courses of antibiotics among food animals create ideal selective pressures for the propagation of resistant strains. Spread of resistance may occur by direct contact or indirectly, through food, water, and animal waste application to farm fields. It can be augmented greatly by the horizontal transfer of genetic elements such as plasmids via bacterial mating (conjugation). We summarize here the evidence for animal-to-human transfer of resistant bacteria on farms using antibiotics for treatment and/or nontherapeutic use.

Resistance Acquisition through Direct Contact with Animals

Farm and slaughterhouse workers, veterinarians, and those in close contact with farm workers are directly at risk of being colonized or infected with resistant bacteria through close contact with colonized or infected animals (Table 2). Although this limited transmission does not initially appear to pose a population-level health threat, occupational workers and their families provide a conduit for the entry of resistance genes into the community and hospital environments, where further spread into pathogens is possible (118, 155).

The majority of studies examining the transmission of antibiotic-resistant bacteria from animals to farm workers document the prevalence of resistance among farmers and their contacts or among farmers before and after the introduction of antibiotics at their workplace. Direct spread of bacteria from animals to people was first reported by Levy et al., who found

TABLE 2. Key evidence for transfer of antibiotic resistance from animals to humans

Transfer type	Species tracked	Animal host(s)	Recipient host(s)	Resistance transferred	Evidence	Reference
Human colonization via direct or indirect animal contact	*E. coli*	U.S. chickens	Animal caretakers, farm family	Tetracycline	Following introduction of tetracycline on a farm, resistant *E. coli* strains with transferable plasmids were found in caretakers' gut floras, with subsequent spread to the farm family	111
	S. aureus, *Streptococcus* spp., *E. coli* and other enterobacteria	French swine	Swine farmers	Erythromycin, penicillins, nalidixic acid, chloramphenicol, tetracycline, streptomycin, cotrimoxazole	Phenotypic antibiotic resistance was significantly higher in the commensal floras (nasal, pharyngeal, and fecal) of swine farmers than in those of nonfarmers	16
	E. coli	U.S. chickens	Poultry workers	Gentamicin	Increase in phenotypic gentamicin resistance in workers through direct contact with chickens receiving gentamicin prophylactically	126
	E. coli	Chinese swine and chickens	Farm workers	Apramycin (not used in human medicine)	Detection of *aac(3)-IV* apramycin resistance gene in humans, with 99.3% homology to that in animal strains	164
	MRSA ST398	Dutch veal calves	Veal farmers	MDR	Human nasal carriage of the *mecA* gene was strongly associated with (i) greater intensity of animal contact and (ii) the number of MRSA-positive animals; animal carriage was related to animal antibiotic treatment	78
Human infection via direct or indirect animal contact	*Salmonella* Newport	Beef cattle (ground beef) receiving chlortetracycline AGP	*Salmonella*-infected patients with diarrhea	Ampicillin, carbenicillin, tetracycline	Direct genetic tracking of resistance plasmid from hamburger meat to infected patients	87
	E. coli	German swine (ill)	Swine farmers, family members, community members, UTI patients	Streptothricin	Identification of transferable resistance plasmids found only in human gut and UTI bacteria when nourseothricin was used as swine AGP	90
	E. coli, *Salmonella enterica* (serovar Typhimurium)	Belgian cattle (ill)	Hospital inpatients	Apramycin, gentamicin	Plasmid-based transfer of *aac(3)-IV* gene bearing resistance to a drug used only in animals (apramycin)	42
	Enterococcus faecium	Danish swine and chickens	Hospital patients with diarrhea	Vancomycin	Clonal spread of *E. faecium* and horizontal transmission of the *vanA* gene cluster (Tn*1546*) found between animals and humans	80
	E. coli	Spanish chickens (slaughtered)	Bacteremic hospital patients	Ciprofloxacin	Multiple molecular and epidemiological typing modalities demonstrated avian source of resistant *E. coli*	95

the same tetracycline-resistant *E. coli* strains in the gut flora of chicken caretakers as in the chickens receiving tetracycline-laced feed (112). The observation extended to the farm family as well and showed an increased frequency of tetracycline-resistant and multidrug-resistant *E. coli* after several months of use of AGP-laden feed. Studies such as this (which examined a variety of antibiotic classes and assorted pathogens) have consistently shown a higher prevalence of resistant gut bacteria among farm workers than in the general public or among workers on farms not using antibiotics (16, 90, 98, 149).

While gentamicin is not approved for growth promotion in the United States, it remains the most commonly used antibiotic in broiler production, being employed for prevention of early poultry mortality (115). A revelatory 2007 study found that the risk for carrying gentamicin-resistant *E. coli* was 32 times higher in poultry workers than in other members of the community: half of all poultry workers were colonized with gentamicin-resistant *E. coli*, while just 3% of nonpoultry workers were colonized. Moreover, the occupationally exposed population was at significantly greater risk for carriage of multidrug-resistant bacteria (126).

New gene-based methods of analysis provide even stronger evidence for the animal origin of bacteria that colonize or infect humans. Homologous relationships between bacterial resistance genes in humans and farm animals have been identified most commonly for food-borne pathogens such as *Escherichia coli* and *Salmonella* (see below) but have also been recorded for various species of *Enterococcus* and for methicillin-resistant *Staphylococcus aureus* (MRSA). Zhang and colleagues found *E. coli* strains resistant to apramycin (an antibiotic used in agriculture but not in human medicine) in a study of Chinese farm workers. All farms in the study that used apramycin as an AGP had workers that carried apramycin resistance genes. The same resistance gene, *aac(3)-IV*, was present in each swine, poultry, and human isolate, with some resistance profiles also matching across species (164). A group of French scientists found the same resistance gene [*aac(3)-IV*] in cow, pig, and human *E. coli* strains that bore resistance to apramycin and gentamicin (42). In another study, similar resistance patterns and genes were detected in *E. faecalis* and *E. faecium* strains from humans, broilers, and swine in Denmark (2). Lee sampled MRSA isolates from cattle, pigs, chickens, and people in Korea and found that 6 of the 15 animal isolates containing *mecA* (the gene responsible for methicillin resistance in *S. aureus*) were identical to human isolates (108).

Antibiotic Resistance Transmission through the Food Chain

Consumers may be exposed to resistant bacteria via contact with or consumption of animal products—a far-reaching and more complex route of transmission. There is undeniable evidence that foods from many different animal sources and in all stages of processing contain abundant quantities of resistant bacteria and their resistance genes. The rise of antibiotic-resistant bacteria among farm animals and consumer meat and fish products has been well documented (36, 108, 122, 162). Demonstrating whether such reservoirs of resistance pose a risk to humans has been more challenging as a consequence of the complex transmission routes between farms and consumers and the frequent transfer of resistance genes among host bacteria. Such correlations are becoming more compelling with the advent of molecular techniques which can demonstrate the same gene (or plasmid) in animal or human strains, even if the isolates are of different species.

For example, Alexander et al. showed that drug-resistant *Escherichia coli* was present on beef carcasses after evisceration and after 24 h in the chiller and in ground beef stored for 1 to 8 days (9). Others isolated ciprofloxacin-resistant *Campylobacter* spp. from 10% to 14% of consumer chicken products (79, 137). MRSA has been reported to be present in 12% of beef, veal, lamb, mutton, pork, turkey, fowl, and game samples purchased in the consumer market in the Netherlands (50), as well as in cattle dairy products in Italy (120). Likewise, extensive antibiotic resistance has been reported for bacteria, including human pathogens, from farmed fish and market shrimp (56, 84, 140).

Some of the antibiotic resistance genes identified in food bacteria have also been identified in humans, providing indirect evidence for transfer by food handling and/or consumption. In 2001, Sorensen et al. confirmed the risk of consuming meat products colonized with resistant bacteria, showing that glycopeptide-resistant *Enterococcus faecium* of animal origin ingested via chicken or pork lasted in human stool for up to 14 days after ingestion (139). Donabedian et al. found overlap in the pulsed-field gel electrophoresis (PFGE) patterns of gentamicin-resistant isolates from humans and pork meat as well as in those of isolates from humans and grocery chicken (55). They identified that when a gene conferring antibiotic resistance was present in food animals, the same gene was present in retail food products from the same species. Most resistant enterococci possessed the same resistance gene, *aac(6′)-Ie-aph(2″)-Ia* (55).

Emergence of Resistance in Human Infections

There is likewise powerful evidence that human consumption of food carrying antibiotic-resistant bacteria has resulted, either directly or indirectly, in acquisition of antibiotic-resistant infections (Table 2). In 1985, scientists in Arizona traced an outbreak of multidrug-resistant *Salmonella enterica* serovar Typhimurium, which included the death of a 72-year-old woman, to consumption of raw milk. Isolates from most patients were identical to the milk isolates, and plasmid analysis showed that all harbored the same resistance plasmid (145). A 1998 *S.* Typhimurium outbreak in Denmark was caused by strains with nalidixic acid resistance and reduced fluoroquinolone susceptibility. PFGE revealed that a unique resistance pattern was common to *Salmonella* strains from all patients, two sampled pork isolates, the swine herds of origin, and the slaughterhouse (118).

Samples from gentamicin-resistant urinary tract infections (UTIs) and fecal *E. coli* isolates from humans and food animal sources in China showed that 84.1% of human samples and 75.5% of animal samples contained the *aaaC2* gene for gentamicin resistance (86). Johnson et al. used PFGE and random amplified polymorphic DNA (RAPD) profiles of fluoroquinolone-resistant *E. coli* strains in human blood and fecal samples and in slaughtered chickens to determine that the two were virtually identical to resistant isolates from geographically linked chickens. Drug-susceptible human *E. coli* strains, how-

ever, were genetically distinct from poultry bacteria, suggesting that the ciprofloxacin-resistant *E. coli* strains in humans were imported from poultry rather than originating from susceptible human *E. coli* (94, 95).

Other reports demonstrate a broader linkage of resistance genes through the farm-to-fork food chain. A resistance-specifying bla_{CMY} gene was found in all resistant isolates of *Salmonella enterica* serotype Newport originating from humans, swine, cattle, and poultry. The host plasmid, which conferred resistance to nine or more antimicrobials, was capable of transmission via conjugation to *E. coli* as well (165). An observed homology between CMY-2 genes in cephalosporin-resistant *E. coli* and *Salmonella* suggested that plasmids conferring resistance had moved between the two bacterial species. The authors found higher rates of CMY-2 in strains from animals than in those from humans, supporting an animal origin for the human pathogen (161). A 2000 study found matching PFGE profiles among vancomycin-resistant *Enterococcus faecium* isolates from hospitalized humans, chickens, and pigs in Denmark. Molecular epidemiology studies have also linked tetracycline resistance genes from *Aeromonas* pathogens in a hospital effluent to *Aeromonas* strains from a fish farm (127). These results support the clonal spread of resistant isolates among different populations (80).

Chronologic studies of the emergence of resistance across the food chain also strongly imply that reservoirs of resistance among animals may lead to increased resistance in consumers of animal food products. Bertrand et al. chronicled the appearance of the extended-spectrum beta-lactamase (ESBL) gene *CTX-M-2* in *Salmonella enterica* in Belgium. This resistance element was identified first in poultry flocks and then in poultry meat and, finally, human isolates (28). A recent Canadian study also noted a strong correlation between ceftiofur-resistant bacteria (the pathogen *Salmonella enterica* serovar Heidelberg and the commensal *E. coli*) from retail chicken and human infections across Canada. The temporary withdrawal of ceftiofur injection from eggs and chicks dramatically reduced resistance in the chicken strains and the human *Salmonella* isolates, but the trend reversed when the antibiotic use was subsequently resumed (57).

In three countries (United States, Spain, and the Netherlands), a close temporal relationship has been documented between the introduction of fluoroquinolone (sarafloxacin and enrofloxacin) therapy in poultry and the emergence of fluoroquinolone-resistant *Campylobacter* in human infections. An 8- to 16-fold increase in resistance frequency was observed—from 0 to 3% prior to introduction to ~10% in the United States and the Netherlands and to ~50% in Spain—within 1 to 3 years of the licensure (61, 128, 137). In the Netherlands, this frequency closely paralleled an increase in resistant isolates from retail poultry products (61), while the U.S. study used molecular subtyping to demonstrate an association between the clinical human isolates and those from retail chicken products (137).

It is now theorized, from molecular and epidemiological tracking, that the resistance determinants found in salmonella outbreaks (strain DT104) in humans and animals in Europe and the United States likely originated in aquaculture farms of the Far East. The transmissible genetic element contains the florfenicol gene (*floR*) and the tetracycline class G gene, both of which were traced to *Vibrio* fish pathogens (*Vibrio damsel* and *Vibrio anguillarum*, respectively). Both drugs are used extensively in aquaculture (36).

In the above examples, the link to nontherapeutic antibiotic use in the farm animals is still circumstantial and largely implied, often because the authors do not report any statistics on farm use of antibiotics. Interpreting these studies is also difficult because of the widespread resistance to some drugs in bacteria of both animals and humans and the ubiquitous nature of resistance genes. Moreover, the same farmer may use antibiotics for both therapeutic and nontherapeutic purposes.

The complexities of the modern food chain make it challenging to perform controlled studies that provide unequivocal evidence for a direct link between antibiotic use in animals and the emergence of antibiotic resistance in food-borne bacteria associated with human disease. While this concrete evidence is limited, a small number of studies have been able to link antibiotic-resistant infection in people with bacteria from antibiotic-treated animals. While not necessarily involving NTAs, these studies substantiate the considerable ease with which bacteria in animals move to people. For example, a multidrug-resistant *Salmonella enterica* strain in a 12-year-old Nebraska boy was traced to his father's calves, which had recently been treated for diarrhea. Isolates from the child and one of the cows were determined to be the same strain of CMY-2-mediated ceftriaxone-resistant *S. enterica* (69). It is now believed that the 1992 multiresistant *Vibrio cholerae* epidemic in Latin America was linked to the acquisition of antibiotic-resistant bacteria arising from heavy antibiotic use in the shrimp industry of Ecuador (13, 156).

By comparing the plasmid profiles of MDR *Salmonella* Newport isolates from human and animal sources, Holmberg et al. provided powerful evidence that salmonella infections in 18 persons from 4 Midwestern states were linked directly to the consumption of hamburger meat from cattle fed subtherapeutic chlortetracycline. A plasmid which bore tetracycline and ampicillin resistance genes was present in the organisms causing serious illness in those persons who ate the hamburger meat and who were also consuming penicillin derivatives for other reasons (87).

One of the most compelling studies to date is still Hummel's tracking of the spread of nourseothricin resistance, reported in 1986. In Germany, nourseothricin (a streptogramin antibiotic) was used solely for growth promotion in swine. Resistance to it was rarely found and was never plasmid mediated. Following 2 years of its use as a growth promotant, however, resistance specified by plasmids appeared in *E. coli*, not only from the treated pigs (33%) but also in manure, river water, food, and the gut floras of farm employees (18%), their family members (17%), and healthy outpatients (16%) and, importantly, in 1% of urinary tract infections (90). Ultimately, the resistance determinant was detected in *Salmonella* and *Shigella* strains isolated from human diarrhea cases (146).

The movement of antibiotic resistance genes and bacteria from food animals and fish to people—both directly and indirectly—is increasingly reported. While nontherapeutic use of antibiotics is not directly implicated in some of these studies, there is concern that pervasive use of antimicrobials in farming and widespread antimicrobial contamination of the environment in general may be indirectly responsible. For instance,

within the past 5 years, MRSA and MDR *Staphylococcus aureus* have been reported in 25 to 50% of swine and veal calves in Europe, Canada, and the United States (51, 78, 101, 114). Graveland et al. noted that this frequency was higher in veal calves fed antibiotics (78). Studies also show that colonization among farmers correlates significantly with MRSA colonization among their livestock (78, 101, 114, 138). In the Netherlands, colonization of swine farmers was found to be more than 760 times greater than that of patients admitted to Dutch hospitals (155). In a study of nasal swabs from veal and veal calf growers, family members, and employees at 102 veal calf farms in the Netherlands, Graveland et al. found that human MRSA sequence type ST398 carriage among the farmers was strongly associated with the degree of animal contact and the frequency of MRSA-colonized animals on the farm. When <20% of calves were carriers, the estimated prevalence in humans was ~1%—similar to that in the general public. With >20% carriage in calves, the prevalence in humans was >10% (78).

Recently, MRSA ST398 has appeared in the community. A Dutch woman without any known risk factors was admitted to a hospital with endocarditis caused by MRSA ST398, suggesting a community reservoir which passed on to people (58). Voss et al. demonstrated animal-to-human and human-to-human transmission of MRSA between a pig and pig farmer, among the farmer's family members, and between a nurse and a patient in the hospital. All isolates had identical random amplified polymorphic DNA profiles (155). Examples of similar MRSA strains among animals and people are mounting (82, 108, 147, 151, 152, 163).

ADDRESSING KNOWLEDGE GAPS: RESERVOIRS OF ANTIBIOTIC RESISTANCE

Historically, considerable attention has been focused on a very small minority of bacterial species that actually cause disease. However, a vast "sea" of seemingly innocuous commensal and environmental bacteria continuously and promiscuously exchange genes, totally unnoticed (116). A staggeringly diverse group of species maintain a large capacity for carrying and mobilizing resistance genes. These bacteria constitute a largely ignored "reservoir" of resistance genes and provide multiple complex pathways by which resistance genes propagated in animals can directly, or more likely indirectly, make their way over time into human pathogens via food, water, and sludge and manure applied as fertilizer. Horizontal (or lateral) gene transfer studies have identified conjugal mating as the most common means of genetic exchange, and there appear to be few barriers that prevent this gene sharing across a multitude of dissimilar genera (104).

While colonic bacteria have received much focused study, water environments such as aquaculture, sludge, freshwater, and wastewaters are prime sites for gene exchange but have been examined minimally for their roles as "mixing pots" and transporters of genes from bacteria of antibiotic-fed animals to humans (116). Aside from the already described impacts of NTA use on bacterial resistance, food animal use of NTAs has broad and far-reaching impacts on these environmental bacteria. It is estimated that 75% to 90% of antibiotics used in food animals are excreted, largely unmetabolized, into the environment (43, 105). Antibiotics or resistant bacteria have been detected in farm dust (81), the air currents inside and emanating from swine feeding operations (41, 72, 129), the groundwater associated with feeding operations (31, 37), and the food crops of soils treated with antibiotic-containing manure (54). This leaching into the environment effectively exposes countless environmental organisms to minute quantities of antibiotic—enough to select bacteria with resistance mutations to promote the emergence and transfer of antibiotic resistance genes among diverse bacterial types (104). The potentially huge impact of all these residual antibiotics on the environmental bacteria that are directly or indirectly in contact with humans has scarcely been examined.

The multiple pathways and intricacies of gene exchange have so far thwarted attempts to qualitatively or quantitatively track the movement of these genes *in vivo*, and thus we are left with minimal direct evidence for linking resistance in animals to that in humans. With extensive gene movement between disparate hosts, it is less likely that the same bacterial hosts will be found in animals and humans and more probable that only the resistance genes themselves will be identifiable in the final pathogens that infect humans. Even these may be altered in their journey through multiple intermediate hosts (161) (Fig. 1). Mounting evidence exists in reports of complex gene "cassettes" which accumulate resistance genes and express multidrug resistance (106, 125).

A few investigators have undertaken the challenging task of developing mathematical models in order to predict the impacts of NTAs on human disease (12, 19, 20, 46, 91, 99, 134, 135). Models can be very useful in attempting to define the types of diverse data sets that are seen in this field. Some explore the entire "farm-to-fork" transmission process, while others tackle only portions of this extremely complex chain or adopt a novel backwards approach which looks first at human infections and then calculates the fraction that are potentially caused by NTA use in animals. Most models are deliberately simplified and admittedly omit many aspects of transmission and persistence. Moreover, current models are frequently based on multiple assumptions and have been challenged on the basis of certain shortcomings, such as limitation to single pathogens only, the determination of lethality while ignoring morbidity, and dependence on estimates of probabilities (19). Chief among these, however, is the lack of a complete understanding of the contribution made by commensals, which may play an important role in augmenting the link between animals and humans. Some models are driven by findings of dissimilar strains in animals and humans and therefore arrive at very low probabilities for a causal link between the two (47). A finding of dissimilar strains, however, overlooks two possibilities. First, it does not exclude the existence of small subpopulations of homologous strains that have gone undetected within the gut floras of animals. These may have been amplified temporarily by antibiotic selection and transferred their mobile genetic elements in multiple complex pathways. Subsequently, they may have declined to nondetectable levels or merely been outcompeted by other variants. Second, it overlooks dissimilarities that evolve as genes and their hosts migrate in very complex ways through the environment. Figure 1 illustrates the difficulties in tracking a resistance gene, since these genes are frequently captured in bacteria of different species or strains

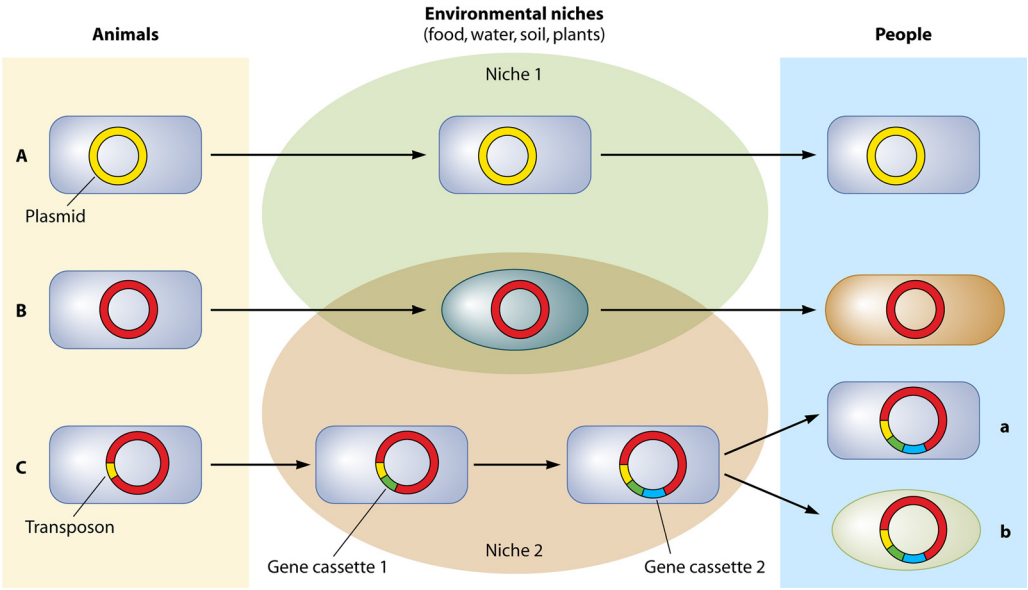

FIG. 1. Several scenarios may present themselves in the genetic transport that occurs as bacteria migrate from animal to human environments. (A) The same host and its indigenous genes in animals are transported unchanged to humans, with a resulting 100% match of the bacterial strain. (B) The genetic structure passes through one or more different hosts, ending in a new host (humans), with a resulting 100% match of DNA. (C) The host and its plasmid-borne genes pass through the environment, picking up gene cassettes en route, with a resulting 100% match for the host only (a) or a low-% match for DNA only (b). In both examples, the plasmid core remains the same.

which no longer resemble the original host. Over time, even the genes themselves may undergo mutations or become entrapped in gene cassettes that alter their genetic landscape. State-of-the-art technology and thoughtful investigation are often necessary to identify and track the actual strains that link animals and humans. These are facets of modeling that have yet to be explored, and obtaining direct evidence for the origins of specific genes can be highly challenging.

In general, the weaknesses of present models lie in their simplicity and the lack of crucial knowledge of microbial loads at each stage of the "farm-to-fork" transmission chain. Many of the available studies that examine links between animals and humans suffer from a failure to examine the antibiotic use practices for the farm animals they investigate. More powerful evidence could have accumulated that would aid in modeling efforts if data on the quantities and uses of farm antibiotics had been reported. These oversights are often due to the lack of registries that record and report the utilization of antibiotics on food animal farms. It is widely advocated that surveillance studies of resistance frequencies at all levels of the transmission chain would aid greatly in reducing our knowledge deficits and would help to inform risk management deliberations (23, 34). A number of localized and international surveillance systems exist for the tracking of human pathogens. In the United States, the National Antimicrobial Resistance Monitoring System (NARMS) has become instrumental in the monitoring of resistance trends in pathogens found in food animals, retail meats, and humans (73). However, at the level of commensals, resistance monitoring is still in its infancy. The Reservoirs of Antibiotic Resistance (ROAR) database (www.roarproject.org) is a fledgling endeavor to promote the accumulation of data that specifically focus on commensal and environmental strains as reservoirs of antibiotic resistance genes. With advances in detection at the genetic level, the potential for tracking the emergence and spread of horizontally transmissible genes is improving rapidly. By capturing geographic, phenotypic, and genotypic data from global isolates from animal, water, plant, and soil sources, the ROAR project documents the abundance, diversity, and distribution of resistance genes and utilizes commensals as "barometers" for the emergence of resistance in human pathogens.

CONCLUSIONS

Data gaps continue to fuel the debate over the use of NTAs in food animals, particularly regarding the contribution and quantitation of commensal reservoirs of resistance to resistance in human disease. Nonetheless, it has been argued reasonably that such deficits in surveillance or indisputable demonstrations of animal-human linkage should not hinder the implementation of a ban on the use of nontherapeutic antibiotics (23). Food animals produce an immense reservoir of resistance genes that can be regulated effectively and thus help to limit the negative impacts propagated by this one source. In the mathematical model of Smith et al., which specifically evaluates opportunistic infections by members of the commensal flora, such as enterococci, it was concluded that restricting antibiotic use in animals is most effective when antibiotic-resistant bacteria remain rare. They suggest that the timing of regulation is critical and that the optimum time for regulating animal antibiotic use is before the resistance problem arises in human medicine (134).

A ban on nontherapeutic antibiotic use not only would help to limit additional damage but also would open up an opportunity for better preservation of future antimicrobials in an era when their efficacy is gravely compromised and few new ones

are in the pipeline. Although the topic has been debated for several decades without definitive action, the FDA has recently made some strides in this direction. Officially, the organization now supports the conclusion that the use of medically important antimicrobials for nontherapeutic use in food animal production does not protect and promote public health (131). Although not binding, a guidance document was released in 2010 that recommended phasing in measures that would limit use of these drugs in animals and ultimately help to reduce the selection pressures that generate antimicrobial resistance (66).

The Danish experience demonstrated that any negative disease effects resulting from the ban of NTAs were short-lived and that altering animal husbandry practices could counter expected increases in disease frequency (6). For aquaculture, also, it has been demonstrated that alternative processes in industry management can be instituted that will reduce antibiotic use without detrimental financial effects (141). Still, it has been argued by some in animal husbandry that the different situation in the United States will result in increased morbidity and mortality, projected to cost $1 billion or more over 10 years. Again, however, the Danish postban evaluation found that costs of production increased by just 1% for swine and were largely negligible for poultry production due to the money saved on antibiotics themselves. Models also showed that Danish swine production decreased by just 1.4% (1.7% for exports), and poultry production actually increased, by 0.4% (0.5% for exports) (158). Such calculations still fail to consider the negative externalities that are added by the burden of antibiotic resistance and the antibiotic residue pollution generated by concentrated animal feeding operations.

Opponents of restriction of NTA use argue that a comprehensive risk assessment is lacking, but such an analysis is impossible without the kind of data that would come out of surveillance systems. Although surveillance systems have been advocated repeatedly (23, 70), such systems are sparse and extremely limited in their scope.

In 2002, working with the accumulated evidence and an assessment of knowledge deficits in the area of animal antibiotic use, the APUA developed a set of guidelines that are still viable today and can be used to guide both policy and research agendas. In summary, APUA recommended that antimicrobials should be used only in the presence of disease, and only when prescribed by a veterinarian; that quantitative data on antimicrobial use in agriculture should be made available; that the ecology of antimicrobial resistance in agriculture should be a research priority and should be considered by regulatory agencies in assessing associated human health risks; and that efforts should be invested in improving and expanding surveillance programs for antimicrobial resistance. Suitable alternatives to NTAs can be implemented, such as vaccination, alterations in herd management, and other changes, such as targeted use of antimicrobials with a more limited dosage and duration so as not to select for resistance to critical human therapeutics (23).

There is no doubt that human misuse and overuse of antibiotics are large contributors to resistance, particularly in relation to bacteria associated with human infection. Interventions in medical settings and the community are clearly needed to preserve the efficacy of antibiotics. Efforts in this area are being pursued by the Centers for Disease Control and Prevention, the Alliance for the Prudent Use of Antibiotics, the American Medical Association, the American Academy of Pediatrics, the Infectious Diseases Society of America, and other professional groups. Still, given the large quantity of antibiotics used in food animals for nontherapeutic reasons, some measure of control over a large segment of antibiotic use and misuse can be gained by establishing guidelines for animals that permit therapeutic use only and by then tracking use and health outcomes.

The current science provides overwhelming evidence that antibiotic use is a powerful selector of resistance that can appear not only at the point of origin but also nearly everywhere else (104). The latter phenomenon occurs because of the enormous ramifications of horizontal gene transfer. A mounting body of evidence shows that antimicrobial use in animals, including the nontherapeutic use of antimicrobials, leads to the propagation and shedding of substantial amounts of antimicrobial-resistant bacteria—both as pathogens, which can directly and indirectly infect humans, and as commensals, which may carry transferable resistance determinants across species borders and reach humans through multiple routes of transfer. These pathways include not only food but also water and sludge and manure applications to food crop soils. Continued nontherapeutic use of antimicrobials in food animals will increase the pool of resistance genes, as well as their density, as bacteria migrate into the environment at large. The lack of species barriers for gene transmission argues that the focus of research efforts should be directed toward the genetic infrastructure and that it is now imperative to take an ecological approach toward addressing the impacts of NTA use on human disease. The study of animal-to-human transmission of antibiotic resistance therefore requires a greater understanding of the genetic interaction and spread that occur in the larger arena of commensal and environmental bacteria.

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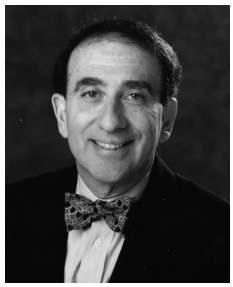
Bonnie Marshall is a Senior Research Associate in the Center for Adaptation Genetics and Drug Resistance in the Department of Microbiology and Molecular Biology at Tufts University School of Medicine in Boston, MA. After obtaining a B.A. in Microbiology at the University of New Hampshire, she did work on herpesviruses at Harvard's New England Regional Primate Research Center and then returned to school to complete a degree in medical technology. In 1977, she joined the laboratory of Dr. Stuart Levy, from which she has published over 23 peer-reviewed publications on the ecology and epidemiology of resistance genes in human and animal clinical and commensal bacteria and environmental strains of water, soils, and plants. Ms. Marshall has also been engaged actively for 30 years with the Alliance for the Prudent Use of Antibiotics, where she is Staff Scientist and serves on the Board of Directors.



Stuart B. Levy is a Board-Certified Internist at Tufts Medical Center, a Professor of Molecular Biology and Microbiology and of Medicine at Tufts University School of Medicine, and Director, Center for Adaptation Genetics and Drug Resistance, also at Tufts University School of Medicine. He received his B.A. degree from Williams College and his M.D. from the University of Pennsylvania. He cofounded and remains active in both The Alliance for the Prudent Use of Antibiotics (1981) and Paratek Pharmaceuticals, Inc. (1996). More than 4 decades of studies on the molecular, genetic, and ecologic bases of drug resistance have led to over 250 peer-reviewed publications, authorship of *The Antibiotic Paradox*, honorary degrees in biology from Wesleyan University (1998) and Des Moines University (2001), ASM's Hoechst-Roussel Award for esteemed research in antimicrobial chemotherapy, and ICS's Hamao Umezawa Memorial Award. Dr. Levy is a Past President of the American Society for Microbiology and a Fellow of the American College of Physicians (ACP), the Infectious Diseases Society of America, the American Academy of Microbiology (AAM), and the American Association for the Advancement of Science. He serves on the National Science Advisory Board for Biosecurity.

Please add Pre-school
From: "Knapp, Shannon" [redacted]
Date: Sun, April 13, 2014 7:20 pm
To: [redacted]

As I read about all of the wonderful work that comes out of your offices, one glaring error continues to haunt me. You refer to K-12 repeatedly. With President Obama's clear commitment to a high quality pre-school experience for all of our children, will you consider changing it to P-12?

Meeting Request from C-FARE Board of Directors for Wednesday, April 30 - The Council on Food, Agricultural and Resource Economics
From: "Caron Gala \(CFARE\)" [redacted]
Date: Tue, April 15, 2014 9:51 am
To: [redacted]
Cc: [redacted]

To: Office of Science and Technology Policy Scheduler
CC: NSTC; PCAST; and Kei Koizumi
From: Council on Food, Agricultural and Resource Economics Business Office
Re: Meeting Request from C-FARE Board of Directors for Wednesday, April 30

Greetings:

Members of the Council on Food, Agricultural and Resource Economics (C-FARE) Executive Committee will be in Washington D.C. on Wednesday, April 30. The C-FARE wishes to build a relationship with the Office of Science and Technology policy in the areas of applied economics related to agricultural science, ecosystem services and health, and food safety and nutrition.

As a result, a small subset of members of the C-FARE Board of Directors wish to meet with the Office of Science and Technology Policy (OSTP) to discuss C-FARE programs, services, as well as some projects that the organization is undertaking. The Members would be delighted have a meeting with individuals who work with the following groups within OSTP:

- National Science and Technology Council (NSTC), Environment, Natural Resources, and Sustainability (CENRS) – specifically the SES: Ecological Services (Subcommittee)
- President's Council of Advisors on Science and Technology (PCAST) and staff involved in the development of the report released last year on the Preparedness of the Agriculture Research Enterprise.

At this time, the members of the Board of Directors are available to meet in the morning or afternoon on Wednesday, April 30th. C-FARE greatly wishes to be most accommodating of your professional's schedules. Thank you in advance for any consideration.

Most kind regards,

Caron

Caron Gala
Council on Food, Agricultural and Resource Economics
C-FARE, Executive Director
[redacted]

The Council on Food, Agricultural, and Resource Economics (C-FARE) is a non-profit organization

based in Washington, DC. C-FARE promotes the work of applied economists and serves as a catalyst for incorporating economic thinking into the analysis of food, agricultural and resource decisions. We serve as a conduit between the academic research and extension community and Washington, DC policymakers and agency personnel, matching expertise to public needs.

[redacted]
www.cfare.org, Join us on Facebook

About the C-FARE Board of Directors

The structure of the board is as follows: C-FARE consists of at least fifteen (15) Directors representing major groups within the (agricultural and applied economics) profession. Directors shall be appointed as follows: three Agricultural and Applied Economics Association (AAEA) members (selected by the AAEA); three National Association of Agricultural Economics Administrators (NAAEA) members, as selected by the NAAEA; other professional societies--one (1) member may be appointed from each society that contributes to the corporation (Southern Agricultural Economics Association); and at-large members to be appointed by the current members of the Board: at-large members are expected to represent strategic areas and skills, and will represent (a) the private sector; (b) non-profit institutions, including research and grant making organizations; (c) other economics professional associations, and (d) additional members of the profession; ex-officio members who are invited to attend Board meetings and assist with C-FARE activities (in many cases these non-voting members are government liaisons).

See the full list of Board Members here: http://www.cfare.org/about

Invitation to C-FARE Event on Agricultural Data on April 29
From: "Caron Gala \(CFARE[redacted]
Date: Tue, April 15, 2014 10:15 am
To: [redacted]
Cc: [redacted]

To: Office of Science and Technology Policy
From: C-FARE Business Office
RE: Invitation to C-FARE Event on Agricultural Data

Greetings, recognizing the ongoing dialogue at the executive level about big data and public data sources, the C-FARE Board of Directors would like to invite your office to this event. Please feel free to contact me if you have any questions regarding the event. Most kind regards, Caron

Caron Gala
Council on Food, Agricultural and Resource Economics
C-FARE, Executive Director
[redacted]
Follow C-FARE on Facebook and Twitter!

Increasing U.S. Agriculture's Competitive Edge:
How Do Public Data and Big Data Fit?
Inaugural Jon Brandt Policy Forum
Council on Food, Agricultural and Resource Economics (C-FARE)

When: Tuesday, April 29, 2014 from 3:30 PM - 5:30 PM*
Where: Room 562 Dirksen Senate Office Building (SD-562)
RSVP:
https://docs.google.com/forms/d/1dBgyKZXIWysFUt3K6kNXTWvX_iomtyY7lDUKdWIRM_I/view

form

American agriculture is facing an information crossroads. Agricultural markets, producer and industry decisions, and public policy that facilitates markets, rely heavily upon public sector data and information. However, the nation's statistical agencies currently face unprecedented pressure to reevaluate their data products. In some cases, agencies have suspended, aggregated, and even eliminated data and statistical series critical for understanding or alleviating market volatility and uncertainties. Simultaneously, technology has enabled growth in big data gathering capabilities. Modern farmers and service providers are harvesting big data and combining it with site specific, farm level information to provide data-driven, prescriptive farming solutions. This dynamic information environment, while exciting, raises a number of challenges. The inaugural forum aims to elucidate a comprehensive understanding of the roles and value of private and public sources of data in facilitating U.S. domestic and international production and markets.

Speakers and Event
JB Penn, Chief Economist at Deere & Company will provide the keynote address to set the stage, discussing the situation, policy environment, and road ahead. A panel of experts will be moderated by **Sara Wyant, President of Agri-Pulse Communications, Inc.**

The panel will include:

- **Mary Bohman**- *Administrator*, USDA Economic Research Service
- **Bob Young**- *Chief Economist & Deputy Executive Director, Public Policy*, American Farm Bureau Federation
- **Mark Harris**- *Chair, Agricultural Statistics Board*, USDA National Agricultural Statistics Service
- **Ted Crosbie**- *Distinguished Science Fellow*, Monsanto
- **Barry Goodwin**- *Distinguished Professor*, North Carolina State University, Ag. and Resource Economics

Panelists will identify ***critical knowledge gaps*** that reflect the agricultural marketplace's contemporary analytical and policy-making needs. These experts will then engage the audience on the value of public information and big data, while addressing public-private issues that will shape the future competitiveness of U.S. agriculture and related industries.

This inaugural forum will build upon the recent C-FARE report, "***From Farm Income to Food Consumption: Valuing USDA Data Products***", developed with support from the USDA Office of the Chief Economist. The forum will also complement the related discourse in articles published in *Choices* magazine.

Hosted by the Council on Food, Agricultural and Resource Economics (C-FARE)
Cosponsored by:

***Networking Reception**
Sponsored by
The Council on Food, Agricultural and Resource Economics (C-FARE)
Tuesday, April 29th, 5:30 PM - 6:30 PM ET
562 Dirksen Senate Office Building
after
'Increasing U.S. Agriculture's Competitive Edge:
How Do Public Data and Big Data Fit?'
Inaugural Jon Brandt Policy Forum

An Emergency PCAST meeting, David Hamburg, and better economic science: Fwd: "From Sweden to Austria, Britain to Italy, nationalist and far-right parties are poised to make record gains next month."

From: "Lloyd Etheredge" [redacted]
Date: Mon, April 21, 2014 1:23 pm
To: "Dr. John Holdren - Science Adviser to President Obama" [redacted]
Cc: "Dr. David Hamburg MD - Former PCAST Member" [redacted]

Dear PCAST Co-Chairs, Vice-Chairs, and Members:

In the light of the Washington Post's forecast of April 13, 2014 **("From Sweden to Austria, Britain to Italy, nationalist and far-right parties are poised to make record gains next month in elections to the European Parliament"**) the enclosed letter requests an emergency meeting of PCAST to recommend initiatives to President Obama for the rapid improvement of economic science. This is important: The US government is not equipped (below your level) to connect all of the dots and solve the problem.

- There is a growing agreement that there are missing variables in economic science.

- For reasons that are discussed in the enclosed correspondence, a briefing by Dr. David Hamburg, a former PCAST member (who was awarded the Presidential Medal of Freedom) and a leader in the prevention of violent conflict and genocide, may help you to make informed decisions about how quickly to move.

The appendices to the enclosed overview of opportunities for rapid scientific learning and economic health (e.g., Vogelstein *et al.* "Cancer Genome Landscapes") are available online at www.policyscience.net at I. A. Dr. Holdren has not yet designated lead responsibilities and budgets to take advantage of these new database opportunities, including international scientific cooperation proposals.

Lloyd Etheredge

Dr. Lloyd S. Etheredge - Director, Government Learning Project
Policy Sciences Center Inc.
[redacted]
URL: www.policyscience.net
[redacted]

[The Policy Sciences Center, Inc. is a public foundation that develops and integrates knowledge and practice to advance human dignity. It was founded by Harold Lasswell, Myres McDougal, and their associates in 1948 in New Haven, CT. Further information about the Policy Sciences Center and its projects, Society, and journal is available at www.policysciences.org.]

THE POLICY SCIENCES CENTER, INC.

Project Director: DR. LLOYD ETHEREDGE
[redacted]

April 21, 2014

Drs. John Holdren (Co-Chair), Eric Lander (Co-Chair), William Press (Vice Chair), and Maxine Savitz (Vice Chair) and Members
President's Council of Advisers on Science and Technology
[redacted] EOB
Washington, DC 20504

Dear PCAST Co-Chairs, Vice-Chairs, and Members:

In light of the Washington Post's forecast of April 13, 2014 **("From Sweden to Austria, Britain to Italy, nationalist and far-right parties are poised to make record gains next month in elections to the European Parliament")** I request that you convene an emergency meeting to recommend initiatives to President Obama for the rapid improvement of economic science.

The equations associated with very high, prolonged, and uncorrected levels of unemployment, especially of adolescent males, predict an angrier and a much more violent political future unless scientists can achieve better insights about economic recovery soon. Forces are building under the surface. Time is not on our side and the US government's fragmented research system and budget allocations are not designed for such a fundamental challenge.

- There is a growing agreement that there are missing variables in economic science. As PCAST members know, there have been exciting discoveries of Big Data analysis methods for biomedical research (e.g., by the Broad Institute; and institutional innovations for rapid learning systems) that can be applied to the discovery of missing variables, new paradigms, and economic health. I am confident that we can do a much better job but the work needs leadership, support by at least one major institution, and the design of a system that requires your purview.

A Briefing by David Hamburg

I suggest that you ask Dr. David Hamburg, a former PCAST member, to brief you. As President of the Carnegie Corporation of NY he organized a brilliant and influential synthesis ofbehavioral science research concerning the forecasting and prevention of the Holocaust and genocide and creating

peaceful futures. If there is to be a future 21st century book like Why England Slept, about the replayed politics of the 1930s, I do not want the retrospective explanation to be that nobody of sufficient stature briefed PCAST about the growing danger or that nobody asked PCAST to design a solution.

I hope that you will quickly give professional advice to President Obama about an upgraded design for a new, rapid learning system. Several steps to take (e.g., when limited scientific models and data systems give unreliable guidance) are straightforward and may require only a formal recommendation to the President by PCAST.[1]

Yours truly,

Lloyd Etheredge

Dr. Lloyd S. Etheredge, Director
Government Learning Project

Cc: David Hamburg

Enclosures:

- LSE, "A Rapid Learning System for G-20 Macroeconomics: From Greenspan to Shiller and Big Data" (unpublished draft, 2014).
- "Young and Jobless Across Europe," The New York Times, November 15, 2013. Graphic
- Anthony Failoa, "A Confederacy of Xenophobes in Europe?" Washington Post, April 13,2014.
- Andrew Higgins, "Right Wing's Surge In Europe Has the Establishment Rattled," The New York Times, November 8, 2013.
- Alan de Bromhead *et aI.,* "Right-Wing Political Extremism in the Great Depression" Unpublished, February 27, 2012.

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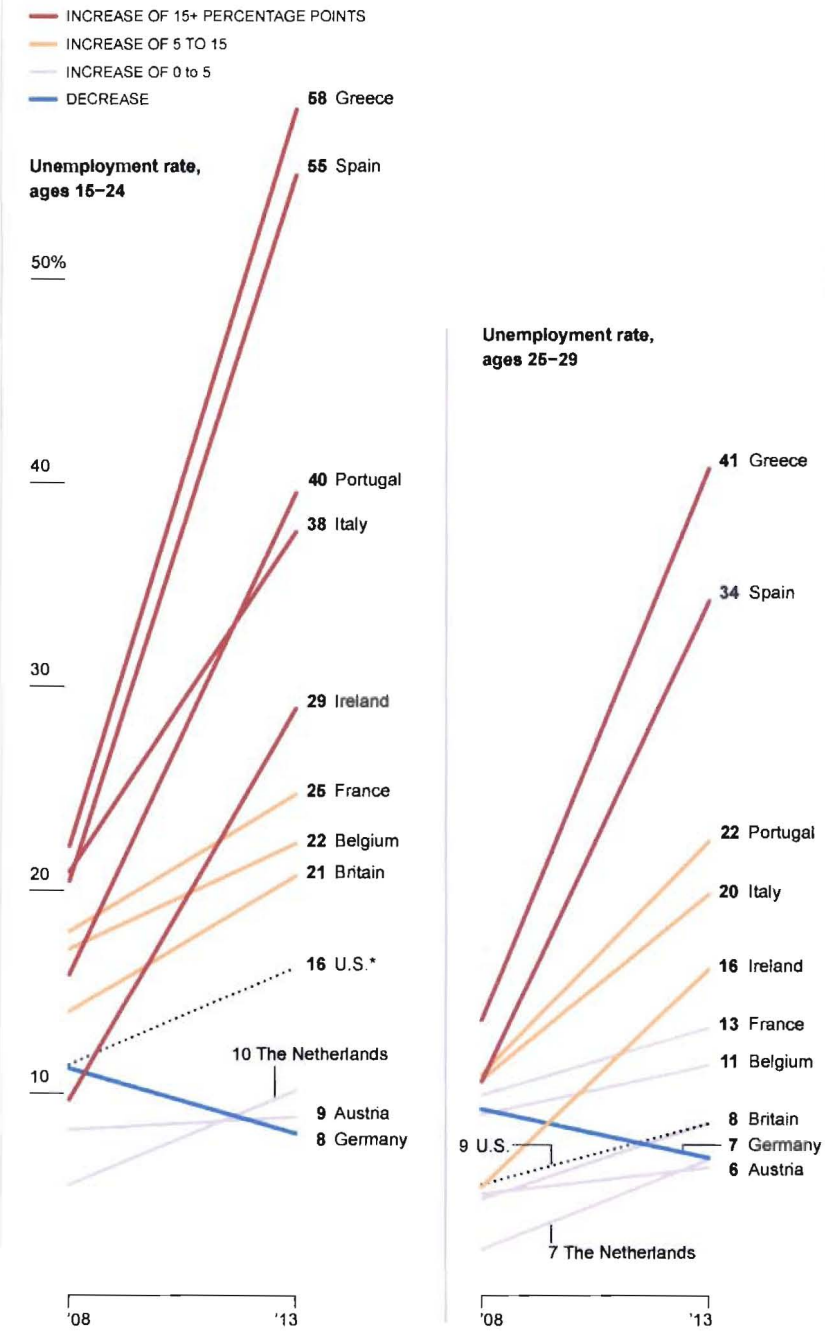
[1] As a contribution to your discussion I enclose a draft proposal, **"A Rapid Learning System for G-20 Macroeconomics: From Greenspan to Shiller and Big Data."** At this point, you might want to design a package of several concurrent approaches and with faster progress than one approach can achieve alone.

Published: November 15, 2013

Young and Jobless Across Europe

NY Times 11/15/13

Change in youth unemployment from the year ended June 2008 to the year ended June 2013.



A Confederacy of Xenophobes in Europe?

By Anthony Failoa. The Washington Post, April 13, 2014.

PARIS — From her nondescript offices in the Paris suburbs, -Marine Le Pen — the blond, hazel-eyed face of France's far right — is leading the charge to build a new alliance of European nationalists, this time by blitzing the ballot box.

A 45-year-old lawyer who wants to halt immigration, Le Pen led France's National Front to historic gains in local elections last month. She did it by destigmatizing the party co-founded by Jean-Marie Le Pen, her 85-year-old father, who once called the Nazi gas chambers a mere -"detail" of history and lost five bids for the French presidency.

In appearances across the country, the younger Le Pen is rolling out a more tempered brand of nationalism that has become a new model across Europe, rejecting her father's overt racism and playing down the party's former links to Nazi collaborators. All the while, she is tapping into the rising economic despair of a nation as well as a backlash against the European Union, the 28-country bloc headquartered in Brussels.

Now she is training her sights on a larger prize. From Sweden to Austria, Britain to Italy, nationalist and far-right parties are poised to make record gains next month in elections for the European Parliament. Rather than see their power diluted, Le Pen is seeking to unite a variety of such parties into an extraordinary coalition of anti-E.U. nationalists.

Together, she said, they would work to turn back the clock on the integration and open borders that have defined post-World War II Europe. "You judge a tree by its fruit," she said last week in her office, a statuette of the Greek goddess of justice resting on a shelf above her. "And the fruits of the E.U. are rotten."

But these are, after all, nationalists, and forging an international alliance of xenophobes is proving to be just as hard as it sounds. On a continent riddled with old grudges and the ghosts of battles past, working together — for some, anyway — means setting aside centuries-old animosities.

Hungary's far-right Jobbik party, for instance, remains locked in a war of words with its counterparts in Romania and Slovakia over Hungarian-speaking regions in those countries that date to the breakup of the Austro-Hungarian Empire. Far-right Italians, meanwhile, are at odds with Austria's Freedom Party over the fate of Alto Adige, a largely German-speaking enclave in northern Italy that has been the site of a political tug of war for years.

But there is also a lingering question about just how much certain parties have truly changed. Indeed, even as Le Pen and her European partners seek to shed their image as far-right extremists, their words have often seemed to undermine that effort.

Le Pen's closest ally, Geert Wilders in the Netherlands, sparked outrage at home last month after fiercely promising his faithful that he would work toward having "fewer Moroccans" in the country. Last week, the Austrian Freedom Party's Andreas Mölzer pulled out of his campaign for reelection to the European Parliament after calling the diverse bloc "a conglomerate of Negroes" whose regulations were worse than Germany's Third Reich.

But unlike her father, who was accused of being anti-Semitic, Le Pen has been accused of espousing Islamophobia — a word she dismissed in an interview as "a creation of the Islamic Republic of Iran."

Yet she has appeared to push the envelope recently, telling French radio that pork-free meals for Muslim and Jewish children would be banned in the cities and towns now controlled by her party. In an interview with The Washington Post, however, she seemed to backtrack, saying that both pork and non-pork meals would be offered in schools.

And although they agree on the fundamental issue of loosening the ties that bind the E.U., the parties remain deeply at odds over a host of issues, including same-sex marriage. The track record for cooperation among members of the far right also bodes ill. Such parties have repeatedly sought to build alliances in the European Parliament, only to see them fall apart because of infighting.

“Nationalists inherently disagree with each other,” said Simon Hix, a professor of comparative politics at the London School of Economics. “They're all like, “My country is the best one in the world,' and then the other one says, ‘No, my country is the best one in the world.' And from there, they all end up fighting.”

But Le Pen insists that this time will be different, that she is gunning for a big win next month. A strong showing by the nationalists, which opinion polls in multiple countries suggest could happen, could effectively put some of the E.U.'s toughest opponents inside its gates.

Once viewed as a paper tiger, the European Parliament, based in Strasbourg, France, has continued to gain power. Even in the best-case scenario for Le Pen, any far-right alliance is unlikely to unseat Europe's mainstream majorities on the center-right and center-left.

But the vote — over four days starting May 22 — could make the far right a stronger force on issues such as immigration legislation and rights of religious minorities. In the name of protecting domestic industries, far-right representatives would seek to bring free trade to a standstill — for example, opposing any attempt to ratify the sweeping E.U.-U.S. free-trade deal that is under negotiation. Analysts say a stronger far right could compel mainstream conservative parties to tow a harder right-wing line.

With France's National Front the likely anchor of any nationalist coalition, it has been up to Le Pen to try to forge a legislative bloc. Success would mean winning at least 25 seats from seven countries. Though almost assured of enough seats, Le Pen appears to be at least one nation shy of the country threshold.

That is partly because of the varying degrees of extremism tolerated by each party. Le Pen dismissed the notion of working with the black-clad ultranationalist members of Greece's Golden Dawn, whom she described as “neo-Nazis.” She also ruled out collaborating with Hungary's Jobbik party, one of whose leaders has called for a government list of Jews in the name of national security.

Meanwhile, one nationalist group, the United Kingdom Independence Party, has refused

to work with her. Like Le Pen, UKIP chief Nigel Farage has sought to position his party as sane moderates who happen to have an anti-E.U., anti-immigration bent. While he touts his party as mainstream, Le Pen's National Front, he insists, is just faking it. "Our view is that whatever Marine Le Pen is trying to do with the Front National, anti-Semitism is still imbedded in that party, and we're not going to work with them now or at any point in the future," Farage told Britain's Telegraph newspaper.

But even her critics concede that Le Pen has determinedly sought to distance herself from her controversial father and has made strides toward steering the party away from explicit racism. In October, the National Front ejected a mayoral candidate, Anne-Sophie Leclere, after she publicly compared France's French Guiana-born justice minister, Christiane Taubira, to a monkey.

In fact, Le Pen is portraying the party as the best ally French Jews could have against a common enemy.

"Not only am I not anti-Semitic, but I have explained to my Jewish compatriots that the movement most able to protect them is the Front National," she said. "For the greatest danger today is the rise of an anti-Semitism in the suburbs, stemming from Muslim fundamentalists."

November 8, 2013. The New York Times.

Right Wing's Surge in Europe Has the Establishment Rattled

By ANDREW HIGGINS

HVIDOVRE, Denmark — As right-wing populists surge across Europe, rattling established political parties with their hostility toward immigration, austerity and the European Union, Mikkel Dencker of the Danish People's Party has found yet another cause to stir public anger: pork meatballs missing from kindergartens.

A member of Denmark's Parliament and, he hopes, mayor of this commuter-belt town west of Copenhagen, Mr. Dencker is furious that some day care centers have removed meatballs, a staple of traditional Danish cuisine, from their cafeterias in deference to Islamic dietary rules. No matter that only a handful of kindergartens have actually done so. The missing meatballs, he said, are an example of how "Denmark is losing its identity" under pressure from outsiders.

The issue has become a headache for Mayor Helle Adelborg, whose center-left Social Democratic Party has controlled the town council since the 1920s but now faces an uphill struggle before municipal elections on Nov. 19. "It is very easy to exploit such themes to get votes," she said. "They take a lot of votes from my party. It is unfair."

It is also Europe's new reality. All over, established political forces are losing ground to politicians whom they scorn as fear-mongering populists. In France, according to a recent opinion poll, the far-right National Front has become the country's most popular party. In other countries — Austria, Britain, Bulgaria, the Czech Republic, Finland and the Netherlands — disruptive upstart groups are on a roll.

This phenomenon alarms not just national leaders but also officials in Brussels who fear that European Parliament elections next May could substantially tip the balance of power toward nationalists and forces intent on halting or reversing integration within the European Union.

"History reminds us that high unemployment and wrong policies like austerity are an extremely poisonous cocktail," said Poul Nyrup Rasmussen, a former Danish prime minister and a Social Democrat. "Populists are always there. In good times it is not easy for them to get votes, but in these bad times all their arguments, the easy solutions of populism and nationalism, are getting new ears and votes."

In some ways, this is Europe's Tea Party moment — a grass-roots insurgency fired by resentment against a political class that many Europeans see as out of touch. The main difference, however, is that Europe's populists want to strengthen, not shrink, government and see the welfare state as an integral part of their national identities.

The trend in Europe does not signal the return of fascist demons from the 1930s, except in Greece, where the neo-Nazi party Golden Dawn has promoted openly racist beliefs, and perhaps in Hungary, where the far-right Jobbik party backs a brand of ethnic nationalism suffused with anti-Semitism.

But the soaring fortunes of groups like the Danish People's Party, which some popularity polls now rank ahead of the Social Democrats, point to a fundamental political shift toward nativist forces fed by a curious mix of right-wing identity politics and left-wing anxieties about the future of the welfare state.

"This is the new normal," said Flemming Rose, the foreign editor at the Danish newspaper Jyllands-Posten. "It is a nightmare for traditional political elites and also for Brussels."

The platform of France's National Front promotes traditional right-wing causes like law and order and tight controls on immigration but reads in parts like a leftist manifesto. It accuses "big bosses" of promoting open borders so they can import cheap labor to drive down wages. It rails against globalization as a threat to French language and culture, and it opposes any rise in the retirement age or cuts in pensions.

Similarly, in the Netherlands, Geert Wilders, the anti-Islam leader of the Party for Freedom, has mixed attacks on immigration with promises to defend welfare entitlements. "He is the only one who says we don't have to cut anything," said Chris Aalberts, a scholar at Erasmus University in Rotterdam and author of a book based on interviews with Mr. Wilders's supporters. "This is a popular message."

Mr. Wilders, who has police protection because of death threats from Muslim extremists, is best known for his attacks on Islam and demands that the Quran be banned. These issues, Mr. Aalberts said, "are not a big vote winner," but they help set him apart from deeply unpopular centrist politicians who talk mainly about budget cuts. The success of populist parties, Mr. Aalberts added, "is more about the collapse of the center than the attractiveness of the alternatives."

Pia Kjaersgaard, the pioneer of a trend now being felt across Europe, set up the Danish People's Party in 1995 and began shaping what critics dismissed as a rabble of misfits and racists into a highly disciplined, effective and even mainstream political force.

Ms. Kjaersgaard, a former social worker who led the party until last year, said she rigorously screened membership lists, weeding out anyone with views that might comfort critics who see her party as extremist. She said she had urged a similar cleansing of the ranks in Sweden's anti-immigration and anti-Brussels movement, the Swedish Democrats, whose early leaders included a former activist in the Nordic Reich Party.

Marine Le Pen, the leader of France's National Front, has embarked on a similar makeover, rebranding her party as a responsible force untainted by the anti-Semitism and homophobia of its previous leader, her father, Jean-Marie Le Pen, who once described Nazi gas chambers as a "detail of history." Ms. Le Pen has endorsed several gay activists as candidates for French municipal elections next March.

But a whiff of extremism still lingers, and the Danish People's Party wants nothing to do with Ms. Le Pen and her followers.

Built on the ruins of a chaotic antitax movement, the Danish People's Party has evolved into a defender of the welfare state, at least for native Danes. It pioneered "welfare chauvinism," a cause now embraced by many of Europe's surging populists, who play on fears that freeloading foreigners are draining pensions and other benefits.

"We always thought the People's Party was a temporary phenomenon, that they would have their time and then go away," said Jens Jonatan Steen, a researcher at Cevea, a policy research group affiliated with the Social Democrats. "But they have come to stay."

"They are politically incorrect and are not accepted by many as part of the mainstream," he added. "But if you have support from 20 percent of the public, you are mainstream."

In a recent meeting in the northern Danish town of Skorping, the new leader of the Danish People's Party, Kristian Thulesen Dahl, criticized Prime Minister Helle

Thorning-Schmidt, of the Social Democrats, whose government is trying to trim the welfare system, and spoke about the need to protect the elderly.

The Danish People's Party and similar political groups, according to Mr. Rasmussen, the former prime minister, benefit from making promises that they do not have to worry about paying for, allowing them to steal welfare policies previously promoted by the left. "This is a new populism that takes on the coat of Social Democratic policies," he said.
I
In Hvidovre, Mr. Dencker, the Danish People's Party mayoral candidate, wants the government in, not out of, people's lives. Beyond pushing authorities to make meatballs mandatory in public institutions, he has attacked proposals to cut housekeeping services for the elderly and criticized the mayor for canceling one of the two Christmas trees the city usually puts up each December. Instead, he says, it should put up five Christmas trees.

Right-wing political extremism in the Great Depression

Alan de Bromhead, Barry Eichengreen, Kevin H O'Rourke, 27 February 2012
[Online at http://www.voxeu.org/article/right-wing-political-extremism-great-depression.
Alan Bromhead is a Ph.D. candidate in Economic and Social History at Oxford where Kevin O;Rourke is Professor of Economic History. Barry Eichengreen is Professor of Economics and Political Science at UC Berkeley.]

The enduring global crisis is giving rise to fears that economic hard times will feed political extremism, as it did in the 1930s. This column suggests that the danger of political polarisation and extremism is greatest in countries with relatively recent histories of democracy, with existing right-wing extremist parties, and with electoral systems that create low hurdles to parliamentary representation of new parties. But above all, it is greatest where depressed economic conditions are allowed to persist.

The impact of the global crisis has been more than just economic.

- In both parliamentary and presidential democracies, governments have been ousted.
- Hard economic times have bred support for nationalist and right-wing political parties, including some that are actively hostile to the prevailing political system.

All this gives rise to fears that economic hard times will feed political extremism, as it did in the 1930s.

Memories of the 1930s inform much contemporary political commentary, just as they have informed recent economic commentary (*eg* Mian *et al* 2010, Giuliano and Spilimbergo 2009). But how exactly did the interwar Depression and economic crisis affect political outcomes and the rise of right-wing anti-system parties? The question has not been systematically studied.

This led us to analyse the elections between WWI and WWII with respect to support for anti-system parties – defined as parties that explicitly advocate the overthrow of a country's political system (de Bromhead *et al* 2012). We focus on right- rather than left-wing anti-system parties since it was right-wing parties that made visible and troubling electoral progress in the 1930s. And it is again right-wing extremist parties that have seemingly made the greatest gains in response to recent economic hard times (Fukayama 2012).

Theories

Explanations for political extremism in this period fall into five broad categories.

- First, support for extremist parties and the instability of democratic systems have been linked to the difficult economic conditions of the interwar years (Frey and Weck 1983, Payne 1996).

A second set of explanations emphasises social differentiation.

- Ethnolinguistic, religious, and class cleavages are fault lines complicating the development of social consensus and hindering the adoption of a concerted response to economic crisis (Gerrits and Wolffram 2005, Luebbert 1987).

This line of argument features prominently in the literature on post-WWI Europe, where new nations were created with little regard for ethnic and religious considerations.

- Third, the legacy of WWI receives considerable attention as a factor shaping the interwar political landscape (Holzer 2002).
- Fourth, certain political and constitutional systems created more scope for anti-system parties to gain influence.

Lijphart (1994), for example, argued that the openness of the political system to new or small parties, whether due to the proportionality of the electoral system or to the effective threshold defined in terms of the share of total votes that a party had to attract in order to win parliamentary representation, was an important determinant of support for extremist parties.

- Finally, an influential tradition associated with Almond and Verba (1989) argues that political culture is an important determinant of the durability of the party system.

The ‘civic culture’ which for these authors is a crucial ingredient of democratic stability is transmitted between generations in the household, in schools, and in the broader society, in part as a result of the exposure of people to the democratic system itself. More recently Persson and Tabellini (2009) have argued that countries with longer histories of democracy accumulate democratic capital, which increases the probability of continuing support for the prevailing party system. These analyses suggest that extremists could have benefitted more from the Depression in countries without a well-developed political tradition and poorly endowed with democratic capital.

Findings

Our data covers 171 elections in 28 countries between 1919 and 1939. While the sample is weighted towards Europe, since interwar elections were disproportionately European, we also include observations for North America, Latin America, Australia, and New Zealand (all elections for which we could obtain information). Anti-system parties are defined, following Sartori (1976), as parties that “would change, if it could, not the government, but the system of government”. Right-wing parties classified as anti-system range from obvious cases like the

NSDAP in Germany to the Arrow Cross in Hungary and the Iron Guard in Romania.

Our major interest is the impact of the Depression on voting patterns and hence how voting shares changed after 1929. Our statistical results (see Annex for details) show that that the Depression was good for fascists. It was especially good for fascists in countries that had not enjoyed democracy before 1914; where fascist parties already had a parliamentary base; in countries on the losing side in WWI; and in countries that experienced boundary changes after 1918.

Since Germany ticks each of these boxes and saw a particularly large increase in the fascist vote, one may ask whether these interaction effects are driven by the German experience alone. The answer is that they are not.

Importantly, it shows that what mattered was not the current growth of the economy but cumulative growth or, more to the point, the depth of the cumulative recession. One year of contraction was not enough to significantly boost extremism, in other words, but a depression that persisted for years was.

The results stand up to the inclusion of control variables, including period dummies, the urbanisation rate, and the effective electoral threshold, and to alternative econometric specifications. In other regressions, we again find that the impact of poor growth was greater in countries where fascists were already represented in parliament and in countries with shorter histories of democracy. Our results are thus consistent with the claim of authors such as Almond and Verba (1989) that political culture mattered, and with the argument of Persson and Tabellini (2009) that countries with a longer history of democracy accumulate social and political capital that increases the probability that they will be able to resist threats to the prevailing political system.

Finally, we find that the electoral success of right-wing anti-system parties was shaped by the structure of the electoral system. A higher minimum share of the vote needed in order for a party to gain parliamentary representation made it more difficult for fringe parties to translate votes into seats and lowered fascist electoral gains.

Conclusions

Our analysis suggests that the danger of political polarisation and extremism is greater in some national circumstances than others. It is greatest in countries:

- With relatively recent histories of democracy,
- With existing right-wing extremist parties, and
- With electoral systems that create low hurdles to parliamentary representation of new parties.

Above all, it is greatest where depressed economic conditions are allowed to persist.

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Annex

Table 1 presents the results of a series of difference-in-difference analyses in which voting shares are regressed on a post-1929 dummy, country characteristics (one per column), and the interaction between these two variables. In all regressions, the post-1929 dummy variable is positive, and it is usually statistically significant suggesting that depression boosted the electoral fortunes of anti-system parties.
Source: see text. Robust standard errors clustered by country in parentheses. ***

Table 1. Determinants of anti-system party vote share, 1919–39

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Country characteristic	Pre-war democracy	Pre-1929 fascist seats	Pre-war agricultural elite	Religious divide	Ethno-linguistic divide	WW1 loser	WW1 boundary changes
Country characteristic	-1.078**	2.213**	0.208	-0.393	-0.626	0.544	1.012*
	(0.523)	(0.959)	(0.536)	(0.522)	(0.472)	(0.646)	(0.494)
Post-1929	10.58**	1.345*	2.717	4.131**	5.698	2.512*	2.251
	(4.718)	(0.770)	(1.668)	(1.609)	(3.405)	(1.277)	(1.655)
Post-1929 * country characteristic	-9.184*	12.30**	5.845	2.582	-1.711	17.78*	6.179
	(4.801)	(5.946)	(5.134)	(5.725)	(3.893)	(8.796)	(4.666)
Constant	1.078**	0.01000	0.440	0.706	0.791*	0.439	-0
	(0.523)	(0.01000)	(0.452)	(0.429)	(0.447)	(0.305)	(6.30e-08)
Observations	159	159	159	159	159	159	159
R-squared	0.299	0.438	0.175	0.119	0.121	0.420	0.207

*p<0.01, ** p<0.05, * p<0.1.
Note: Table 2 shows that the relationship between growth and extremism continues to hold when we estimate fixed effects tobit regressions, using both the Honoré (1992) semi-parametric estimator and the MLE discussed in Greene (2004).

Table 2. Determinants of right-wing anti-system vote share, 1919–39

Period	1 Year	1 Year	2 Years	2 Years	3 Years	3 Years
Method	Semi-Parametric	MLE	Semi-Parametric	MLE	Semi-Parametric	MLE
Growth	-58.79**	-21.72	-63.39	-17.66	-109.6***	-37.08**
	(27.37)	(23.26)	(50.08)	(19.66)	(39.95)	(13.25)
Observations	148	148	136	136	125	125

Source: see text. Fixed effects panel Tobit estimators. Fixed effects not estimated by semi-parametric estimator, and not reported for MLE. *** p<0.01, ** p<0.05, * p<0.1. Marginal effects estimated at means of the independent variables and fixed effects.

Proposal

A Rapid Learning System for G-20 Macroeconomics:

From Greenspan to Shiller and Big Data

by

Lloyd S. Etheredge

March 6, 2014
(Draft)

Proposal

A Rapid Learning System for G-20 Macroeconomics: From Greenspan to Shiller and Big Data

by
Lloyd S. Etheredge[1]

Abstract

There is a growing agreement that there are missing variables in economic science. Robert Shiller (2014) believes that needed progress can be achieved by creating, and then drawing upon, an inclusive behavioral science framework that "accounts for actual human behavior." [2] Independently, Alan Greenspan has started to build this expansion. He draws upon a lifetime of experience, and reflections on the recent economic crisis and recovery, to recommend the behavioral variables that, with appropriate metrics, should be added to the world's data systems and forecasting equations.[3] The purpose of this project is to build upon Greenspan's outline and Shiller's vision and use them as a stimulus for expanded, multi-disciplinary, and inclusive R&D data systems that can be deployed internationally to create a rapid learning system for macroeconomics. [4] [5]

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[1] Lloyd Etheredge is Director, Government Learning Project, at the Policy Sciences Center, Inc., a public foundation; URL: http://www.policyscience.net. Contact: (301)-365-5241 lloyd.etheredge@policyscience.net. This is a draft grant proposal. Comments welcome: Please do not circulate without permission.

[2] Robert Shiller, "The Rationality Debate: Simmering in Stockholm," The New York Times, January 14, 2014.

[3] Alan Greenspan, The Map and the Territory: Risk, Human Nature, and the Future of Forecasting (New York: The Penguin Press, 2013).

[4] The columnist Robert Samuelson reported a disciplinary pessimism about finding new and better policy ideas in current models and data systems at the invitation-only IMF summit last year: Robert J. Samuelson, "The End of Macro Magic," Washington Post, April 21, 2013. Concerning new variables, see also Lawrence Summers, "Lessons Can be Learned from Reinhart-Rogoff Error." Washington Post. May 5, 2013: "In retrospect, it was folly to believe that with data on about 30 countries it was possible to estimate a threshold beyond which debt became dangerous. Even if such a threshold existed, why should it be the same in countries with

The project is timely. Global economic recovery is lagging and established models and data systems have not worked reliably. The addition of new variables (each, likely influenced by several pathways) raises the possibility of a new set of effective policy tools (for example, to restore confidence and accelerate economic recovery). There is exciting and creative thinking among economists that will be captured by the project (i.e., and these upgrade ideas can disappear unless they evolve into metrics and are included in new R&D data systems of the G-20). There are very few problems in the world that cannot be made better by a speedier return to economic health and adding another 1%/year to long-term GDP/capita growth. And in February 2014 the G-20 governments made a public commitment to better results. They promised to *"develop ambitious but realistic policies with the aim to lift our collective GDP by more than 2 percent above the trajectory implied by current policies over the coming five years."* [6] More inclusive economic models and data systems should help to improve economic science and get these results for the G-20 and other nations.

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and without their own currency, with very different financial systems, cultures, degrees of openness and growth experiences?" Summers also recommends surrendering the comfortable dream of "returning to normal" and a world already charted by established equations and data systems: "the presumption that normal economic and policy conditions will return at some point cannot be maintained." ("Economic Stagnation is Not Our Fate - Unless We Let It Be," Washington Post, December 18, 2013). A consulting project for China, with leadership by the Nobelist Michael Spence, concludes that the ideas that must guide China's next phase of growth "step outside well-known economic models" and require tasks of adding metrics and variables into formal models that are "very much on economist's 'to do' list:" Jonathan Schleferfeb, "Nobel Winner's Frank Advice to China's Leadership." The New York Times, February 17, 2014.

[5] The commitment of the policy sciences tradition is to develop inclusive frameworks to guide democratic decision making. See Harold D. Lasswell and Abraham Kaplan, Power and Society: A Framework for Political Inquiry (1950) (New Brunswick, NJ: Transaction Publishers, 2013), reprint with an Introduction by Ronald Brunner; the behavioral sciences made impressive steps toward this goal, even several decades ago: Lloyd S. Etheredge, The Case of the Unreturned Cafeteria Trays (Washington, DC, 1976) and the "Map" (attached as an Appendix to this proposal) and *idem.*, "Wisdom in Public Policy" in Robert Sternberg and Jennifer Jordan (Eds.) A Handbook of Wisdom: Psychological Perspectives (New York: Cambridge University Press, 2005), pp. 257-328; William Ascher, Bringing in the Future: Strategies for Farsightedness and Sustainability in Developing Countries (Chicago: University of Chicago Press, 2009).

[6] Jamie Smyth, "G20 Aims to Add $2 Trillion to Global Economy," Financial Times, February 23, 2014.

Table of Contents

I. Scientific Plan

A Project Director and an Advisory Group will identify specific topics to be addressed in three steps and invite leading researchers to participate in a planning group (N=12-14) for each step. The planning groups will be asked to do justice to the thinking of Greenspan and other theorists. To bring their own creativity to the task. And to assure that the new variables and metrics will, in the spirit of the Michelson-Morley experiment in physics, be politically fair and support the competitive evaluation of variables, pathways, and claims that are civically relevant.

The three steps will be:

1.) Greenspan's List of (known or suspected) missing variables and recommended metrics;

2.) Inclusive Social Science Lists to capture (known or suspected) missing variables and metrics from other theorists and researchers;

3.) Finding Unknown Variables and Organizing Rapid Learning Systems.

- Greenspan is a professional economist and a libertarian. [His mentor and lifelong friend was Ayn Rand (author of Atlas Shrugged, an entry pathway to these policy views for many college students.)] He has taken the unusual step of recommending rapid scientific evaluation of his new economic ideas and these (what others would call) ideological beliefs. He expects these new scientific equations will improve economic forecasts *and,* in the competition of ideas in the political marketplace, prove that libertarians are right.

The Project Director will prepare an initial outline of issues for each planning group, meet with each member for a discussion, prepare a draft paper for a 1 ½ day group meeting, and author a summary report of recommended variables and metrics and next steps from each group. Each of the three Reports will address:

1.) Recommended variables and metrics that are on the shelf and that can be deployed immediately;

2.) Recommended metrics that can become available soon, with additional work;

3.) Important areas where further R&D is needed before metrics can be recommended.

Here (for the three steps, and with three examples for each step) are new variables and clusters of metrics that will be addressed, with my initial commentary about how I believe the discussions of social science advisers will develop and refine the analysis.[7]

A. Greenspan's List

> "We are driven by a whole array of propensities - most prominent, fear, euphoria, and herd behavior."
>
> - Alan Greenspan [8]

Greenspan recommends adding variables to provide a more inclusive account of human nature. Thinking internationally, he also recommends including cultural variables in the new equations because he believes that cultures exert (often) fixed causal forces on economic behavior.

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[7] Greenspan suggests "an apparently inbred upper limit to human IQ" may limit productivity growth in America and other advanced economies to 3%/year (pp. 165-166, 296). The phenomenon is worth investigating and forecasting, but I am skeptical about this explanation.

[8] Also, a capacity for human rationality should be measured in the new equations: The new behavioral variables (fear, euphoria, and herd behavior) can be "broadly subject to reasoned confirmation," *op. cit.*, p. 299. [Including different (and sometimes opposing) logics and mechanisms (like rationality) in different parts of the human brain may seem logically contradictory and unacceptable but an emerging view of human nature, informed by neuroscience, is comfortable with this theoretical upgrade.]

Greenspan adds that "much of animal spirits are heavily tempered by rational oversight. Markets, even in their most euphoric or fear-driven state, do not expect global stock market averages to double or triple overnight, or wheat prices to fall to five cents a barrel" *op. cit.*, p. 35.

1.) Motivation 1 - Fear, Confidence, "Animal Spirits"

> "[T]he world economy is pregnant with multiple equilibria - self-fulfilling outcomes of pessimism or optimism."
>
> - Olivier Blanchard [9]

a.) Fear. An early, simplified mathematics of economics assumed human motivation to be fixed and seeking maximum economic profit, and that knowledge of the world was limited to economic variables (e.g., the prices and other current behavior of markets). Greenspan begins by adding an instinct for survival, risk-aversion, and a hardwired, fast, and compelling response to fear: "fear induces a far greater response than euphoria."[10] [Thus the boom phase of economic crises build across several years while the financial collapses will be sudden panics as these primitive, "fast" brain mechanisms are activated.] [11]

b.) Restoring confidence has emerged as one of the high policy priorities for economic recovery. Greenspan's wider model (discussed below, based on Keynes), includes the genetic endowment of human nature with natural "animal spirits" and a non-rational optimism about the

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[9] Dr. Olivier is Chief Economist at the International Monetary Fund and, for many years, was a member of the MIT Economics Department. Olivier Blanchard, "2011 in Review: Four Hard Truths." Online at http://blog-imfdirect.imf.org/2011/12/21/2011-in-review-four-hard-truths/

[10] *op. cit.*, p. 280.

[11] To psychologists, pain is a physical sensation with specific measurements. Thus, pain-avoidance can be different than risk-avoidance. [Greenspan probably means to include pain-avoidance in his theory: he discusses "the propensity of policy makers to seek the least politically painful solution to a problem . . . We see it everywhere." (p. 224).] The distinction between pain and risk will sometimes be nit-picking, but it helps to distinguish which brain pathways actually might be involved, for whom. While a new breed of Wall Street financiers may instinctively wish to avoid pain, they might be thrilled by the excitement of high risk gambling.

future.[12] [Thus, human nature is on the side of economic health, which will return as soon as we can understand the fear mechanisms and reduce or remove the fear and restore confidence.]

What are the pathways and metrics to model the neuroscience of fear and confidence? The fast "fight/flight" panic mechanism appears, at this point, to be linked to other mechanisms that continue to suppress or inhibit animal spirits and economic confidence. The actual combinations will have different implications for optimal recovery policies.

For example: The conventional remedy of economic pump-priming imagines that 1.) economic reality must be changed and become reliably reassuring (e.g., by reducing interest rates to stimulate investment and by increased government (deficit) spending). As flows of income increase to individuals and businesses, and as they slowly and repeatedly test the waters, confidence gradually is restored, their own spending and/or hiring increases, and the recovery process becomes self-sustaining. Another possibility is 2.) calendar time may be required for healing and recovery, and this might also require outreach steps (recognizing the additional psychological mechanisms involved) for people who have been injured personally or become discouraged. Or 3.) if the fear was activated in the context of a perceived catastrophic failure of trust and/or betrayal by governments and financial institutions who had a moral obligation to be trustworthy, these institutions may be required to restore confidence in themselves and have not done so. [These psychological ideas are consequential: the Federal Reserve systems of the world can spend hundreds of billions of dollars believing that there is a Liquidity Trap and they must keep interest rates low. Yet they will waste the money if the current problem is a Confidence Trap linked to deficient trust in major institutions and guarantors, and a dispiriting anomie.] [13]

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[12] Kahneman agrees with Greenspan and Keynes: "the optimistic bias may well be the most significant of the cognitive biases." Quoted in Greenspan, *op. cit.*, p. 32.

[13] Adding new and direct confidence metrics about governments and political systems is an innovation implied by Olivier Blanchard: "Markets have become more skeptical about the ability of governments to stabilize their public debt" in his "Strong Policy Action - The Essence of Restoring Global Economic Hope" blog, September 20, 2011. Online at http://blog-imfdirect.imf.org/2011/09/20/strong-policy-action-the-essence-of-restoring-global-economic-hope/

Alternatively, there may be 4.) a news-media perpetuation of fear and anger by (for profit) companies (like Fox News) or (with huge campaign contributions) by the Tea Party. [Once, three centrist television networks and sober, professional journalists (NBC, ABC, CBS) conveyed reality to, and constructed reality for, the American people.][14]

A wider set of metrics may allow other confidence-restoring or -building variables, possible brain mechanisms, and policy options to come into focus. For example, leadership-induced confidence: 5.) Experiments by McClelland and Winter found that videos of dramatizing leaders, with speeches rich in achievement images (like President Kennedy), energized people for economic achievement. [Thus: new metrics may show that President Obama and a world of rationalist economists and prosaic politicians are contributing to the current slow rates of economic recovery by the uninspiring public drama that they create.] [15] Or 6.) FDR used yet another set of (*de facto*) psychological theories of fear and brain mechanisms: Declaring that "the only thing we have to fear is fear itself," he presented himself as a confident, cheerful, and even jaunty role model (in a scary and troubled time); and, by using his position as a leader, and new mass communications technology, to *name* emotions he may have created new brain pathways in his listeners that helped them to be self-starting in an internal world that began to bracket fear.[16]

c.) "Animal Spirits." Greenspan's (psychological, political, and economic) theory of

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[14] New capabilities for quantitative analysis of communication flows would provide an interesting cross-national set of metrics. See Robert Harris, The Fear Index (NY: Vintage, 2012). Reprint; Ithiel de Sola Pool, "Content Analysis and the Intelligence Function," reprinted on Lloyd S. Etheredge (Ed.), Humane Politics and Methods of Inquiry: Selected Papers of Ithiel de Sola Pool, vol. 2 (New Brunswick, NJ: Transaction Publications, 2000), chapter 2.

[15] David McClelland and David Winter, Motivating Economic Achievement: Accelerating Economic Development Through Psychological Training (New York: Free Press, 1969).

[16] The G-20 appear to be using, at this point, 7.) a straightforward goal-setting theory of leadership and induced motivation. However the degree of repetition that is needed may be under-estimated. It may be necessary for leaders to communicate goals a hundred times more than they initially believe should be necessary

"animal spirits," borrowed from Keynes, imagines that the success of the capitalist system is the expression of this restless and even joyful human energy and natural optimism (that is not derived from cold, rational calculations), typically channeled into activities with others. Keynes' phrase was used of British students in boarding schools in late Victorian and Edwardian England: the "animal spirits" find natural expression in the freedom of the playing field and, sometimes, in an irreverent, youthful independence and instinct for challenging the rules that enjoined vigilance by headmasters. [17]

Greenspan's theory is a strategic move on a political chessboard. The "animal spirits" of human beings - not the profit-seeking of economic robots programmed for maximum rationality - drive capitalism and economic growth.[18] However these human "animal spirits" are suppressed by regulations and Greenspan's scientific prediction is that the new equations will prove libertarian claims: If we want the capitalist package to work, we should limit government and its regulation and other interference. The *laissez-faire* freedom from regulation that is required for the animal spirits of capitalism to create a better future [and also for the growth of strong, healthy, self-starting entrepreneurs as they move from competition on the playing fields to the

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[17] For further discussion: George Akerlof and Robert Shiller, Animal Spirits: How Human Psychology Drives the Economy, and Why it Matters for Global Capitalism (Princeton, NJ: Princeton University Press, 2009) and Robert Shiller, "Animal Spirits Depend on Trust: The Proposed Stimulus Isn't Enough to Restore Confidence," Wall Street Journal, January 27, 2009.

[18] As a side issue: Greenspan believes that "*To the extent that any human action is at least partially driven by 'spirits,' the material outcomes are less satisfactory in purely economic terms than they would be under the hypothetical presumption that animal spirits did not exist and that human beings' economic behavior was wholly rational.*" Greenspan, *op. cit.*, p. 35. However computer simulations may show Greenspan's view to be untrue: a sociobiology theory might predict that, while irrational over-confidence may increase death rates of individuals or many entrepreneurial firms, this trait could, when there is random variation and changing environments, facilitate adaptation and success of the species. In the study of emerging infectious diseases, for example, millions of individual virus particles may die in the continuing assaults on new antibiotics but, with random variation, the continuing assaults eventually include breakthroughs by resistant mutations and survival and new population growth for the species.

corporate offices - LE] also means that political systems should accept that cycles of boom and bust are an inevitable part of the global capitalist system.

Greenspan's political deductions mandate a careful attention to measurement. There is a distinction between subjectivity (how reality is perceived, interpreted and wired-up in the brain) and Greenspan's almost definitional theory that regulations restrict freedom. There will be abundant challenges for the scientific planning groups to sort out but (to make the points briefly): 1.) actually, the youthful athletic contests on the playing fields of Eton, with their genuine and energetic freedom and competition, also are exquisitely created and affected by rules and regulations, depend upon honest and competent referees and agreed-upon penalties, and the activities are sustained by a moral universe of respect, fairness, and sportsmanship, and norms that distinguish acceptable competitive strategies (e.g., of misdirection) from cheating. Thus, it is not obvious that indexes of financial or environmental regulations *necessarily* will show inhibiting brain/psychological impacts on businessmen that erode their economic motivation and lower the growth rate of GDP. [However, 2.) once the new subjectivity-recognizing metrics are created, Greenspan might be right. In part, the truth depends upon the subjectivity of capitalists - although complaining about government regulations is not compelling evidence that regulations actually do inhibit their economic motivation: some of the most regulated and supervised industries in the world (e.g., the pharmaceutical industry) are the most profitable and innovative.] [19]

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[19] Economists are accustomed to use the hard numbers of conventional economic data. This methodological point about including measures of subjectivity (more easily accepted in other social sciences) is one of the "four hard (*sic*) truths" to improve econometric forecasting recommended in 2011 by Blanchard: "*Perception molds reality.*" *op. cit.* A second-level measurement issue for a planning group, also flagged by Blanchard, is that perceptions can change: "[F]inancial investors are schizophrenic . . . they react positively to news of fiscal consolidation but then react negatively later. . ." *ibid*. A related measurement issue is the contextual principle in behavioral science - i.e., the effect of a variable can depend upon the context in which it occurs. Thus President Kennedy's tax cut may have produced an unusually strong effect on economic growth because it occurred in the frame of his achievement-oriented (N-Ach in the technical language of psychologists) rhetoric and leadership. If so, the Reagan-era

Also, *pace* Greenspan and Keynes, 3.) Social scientists might discover that actual economic motivation can be much greater than the baseline animal spirits of human nature. For example, motivation might be increased by (external) political leadership (see above) or by a non-rational manipulation that, via the visual cortex, activates long-term motivation with vivid images of vast, guaranteed profit. Greenspan's *laissez-faire* utopia of natural, animal spirits may actually achieve only a fraction of what psychologically astute G-20 policies (to design a fully incentivized global capitalist system) could empower capitalism to achieve in the future. [20]

2.) Motivation 2 - The Herd Instinct

"Euphoria will always periodically produce extended bull markets that feed off

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tax cuts would have produced a diminished effect because his Presidential rhetoric was low on N-Ach imagery. For a further discussion: Lloyd S. Etheredge, "President Reagan's Counseling" in Political Psychology, 5:4 (1984), pp. 737-40, online at www.policyscience.net at II. C.

Political combat in the Ayn Rand tradition has used her Objectivist philosophy which (i.e., it is a somewhat closed system) can interpret other people's differing perceptions as a "false consciousness." Greenspan may not readily accept a political philosophy or economic policy based on people's "unthinking" subjective experience of whether they are regulated.

[20] To secure the benefits of new technologies, the American government energized the national capitalist system and built a trans-continental railway system in the 19th century, very quickly, by offering government payments and bonuses (and vivid, high profits) of $16,000, $32,000 or $48,000/mile and assuring land grants, to competing companies who started building westward, and another that started eastward from California.

Similarly, the actual "herd instincts" motivations of Wall Street portrayed in the Academy Award-winning Inside Job and The Wolf of Wall Street appear to have been fueled by vivid images of fabulous profits and cocaine-like drug addiction and pleasure centers in the brain. Greenspan's partly exculpatory theory of human nature notwithstanding, only a very small percentage of self-selecting human beings may actually become involved in high-stakes gambling addictions.

herd behavior, followed by rapid fear-induced deflation of the consequent bubbles."

- Alan Greenspan [21]

"I see no way of removing periodic irrational exuberances without at the same time significantly diminishing the average rate of economic growth and standards of living."[22]

- Alan Greenspan [23]

Greenspan's new "herd instinct" variable moves economic analysis beyond the mathematical assumption that the motivation of human beings is only to maximize selfish economic profits. The herd ("social") instincts have their own aims, expressions and rewards (including contributing to the lives of others).[24] They are expressed in a nonprofit sector of the economy that is capable of astonishing gains in productivity and human benefit (e.g., MOOCs that can make a curriculum equal to the best in the world available to everyone on the planet, without charge) and, also, stunning and baffling inefficiency (e.g., the American health care system). The American media focus on the quarterly performance metrics of the for-profit economy but Greenspan's conceptual and pro-metrics upgrade will engage a planning group to think about

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[21] *op. cit.*, p. 292.

[22] Greenspan predicts that periodic irrational exuberances may grow worse as a result of social media, *op. cit.*, p. 25: "fear and euphoria . . . are contagious processes exaggerated by herding." It is an important prediction, made possible by including the herd instinct set of variables, that should be evaluated for G-20 forecasting.

[23] *op. cit.*, p. 301.

[24] Greenspan includes a propensity to compete in games and for status (p. 26) and power (p. 34).

equivalent quarterly performance metrics for the nonprofit sector.[25]

Adding a "herd instinct" variable also is a strategic move on a political chessboard. Here is the background: The term was introduced (with cross-species examples) by the social psychologist (and neurosurgeon) Wilfred Trotter in 1908.[26] It refers to many human phenomena, including altruism and compassion, standards of fairness, marriage and friendship, the nonprofit sector, all social and mass movement participation - including financial bubbles (and skewing risk-aversion judgments to the mean of a group) - and enlisting in wars, seeking status and power, conformity and followership, the quests for self-esteem, copycat behavior exploited by advertising and marketers, etc. During the 1930s and the Cold War, "herd instinct" became a pejorative term. Alan Greenspan, Ayn Rand, and many allies believed that the herd instinct dangerously drew political supporters to the seductions of collectivism, with the reality of a soul-crushing tyranny (and mistaken economic ideas) of America's mortal enemy, Russia and a global

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[25] I am not sure how far this initial project can go to develop metrics and forecasting equations for the nonprofit sector of the G-20 economies. However, the economics profession and society may benefit in several ways from Greenspan's conceptual upgrade. Typically, doctrinaire economic analysts recommend improving nonprofit institutions by turning them into for-profit hospitals, for-profit public schools, universities with Profit Centers, outsourcing the work of government agencies to the private sector, etc. Greenspan's "herd instinct" variable allows there to be legitimate, different, and important motive instincts that sustain the nonprofit sector and that can used and organized for the common good. (A motivation to maximize economic profit is not required for efficiency: the management consultant Peter Drucker thought that the Girl Scouts of America, with their commitment to "help each girl reach her own highest potential," was better run than Fortune 500 companies.) See also the variables affecting productivity in well-managed public sector and nonprofit institutions identified by the Baldrige awards, www.apqc.org. A discussion of conceptual implications of allowing different motives in models of human nature is Howard Margolis, Selfishness, Altruism and Rationality (Chicago, IL: University of Chicago Press, 1984).

[26] His later popular book influenced the application of scientific method to develop modern advertising and analyze the mass movements of the 1930s, accelerated by the new mass media technologies. W. Trotter, Instincts of the Herd in Peace and War (London: T. F. Unwin, 1916).

Communist movement. In Greenspan's tradition the "herd" (social) instincts also contribute to the well-intentioned, spiritually-eroding, collectivist welfare state (eroding the personality of 47% of Americans, according to the Republican-individualist Presidential candidate, Mitt Romney). The mass psychology of society and human imagination are zero-sum: even when governments enlarge their prominence and hold the high ground as benevolent planners of welfare states (and *de facto* regulators), they restrict and erode the open spaces and zones of freedom that are required for the full development of strong, healthy, self-starting individuals (who become entrepreneurs). [27]

Again, these are moves on a political chessboard and two measurement cautions are in order: a.) Political, educational, social, spiritual, and psychological theorists since Plato's analogy of the Cave and Buddha's teaching of a path to Enlightenment have thought about issues of freedom, liberation, and growth. Many psychologists have researched causal ideas about the growth of healthy, strong, free, responsible, self-starting, enlightened individuals who can become the "entrepreneurs of their own lives" and ethical, civic and business leaders and organizers.[28] Thus, there are likely to be different pathways and coefficients and a package of societal metrics that need to be put on the table; b.) As I indicated above, the Honest Broker scientific refereeing of ideological political arguments requires the measuring of subjectivities: a society with a psychology of "entitlements" *might* be unhealthy, but the appropriate metrics for Sweden may show that "entitlements" are healthy when they are wired-up differently and express and strengthen mutual respect and democracy and provide resources for the genuine personal freedom to grow and prosper. Similarly, constructing a "dependency index" for macroeconomics

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[27] I.e., rather than become victims, or pawns, or the drone employees of others, or people who look to governments and vote for a welfare state.

[28] Etheredge, "Wisdom . . . ," *op. cit.*. E.g., Lawrence Kohlberg, The Philosophy of Moral Development: Moral Stages and the Idea of Justice (San Francisco: Harper and Row, 1981); Jane Loevinger, Ego Development: Conceptions and Theories (San Francisco: Jossey Bass, 1976); see also David Winter, David McClelland, and Abigail Stewart, A New Case for the Liberal Arts (San Francisco: Jossey Bass, 1981).

(as some libertarian think tanks have proposed), equating (almost by definition) the public sources of individual income with an unhealthy, hierarchical, psychological relationship, begs an important measurement question; and c.) Once we see the numbers for a particular culture or subgroup, Greenspan and other libertarians may nevertheless be right.

3.) Culture

> "A specific brand of culture - populism - has been particularly debilitating to economic progress. . . . Capitalism and socialism are specific about the conditions they deem necessary for the creation of wealth and rising standards of living. Populism [for example, in 20th and 21st century Latin America] is not. It is a shout of pain."
>
> "For those economies that seek maximum economic growth, it appears that abstinence and prudence are necessary (although not sufficient) virtues for prosperity."
>
> - Alan Greenspan [29] [30]

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[29] *op. cit.*, pp. 226-227.

[30] Abstinence and prudence are used by Greenspan as economic terms to refer to the percentage of income that is saved and invested for future returns, although there may be other behavioral (e.g., Puritan) correlates that he has in mind.

Concerning other variables, Greenspan writes: "Producing a fully detailed model is beyond the scope of this book. But such a model would include a number of variables reflecting those verities of human nature [or culture - LE] that reveal long-term economic stabilities. Among them are time preference (and interest rates), equity premiums, corporate earnings-price yields, and, since the 19th century, the private savings rate. They reflect the outer limits to fear and euphoria that define the dynamics of the business cycle. For forecasting purposes they can be assumed to continue trendless [unchanged - LE] in the future. . . . In addition there are those stabilities that are not inbred, such as the sum of social benefits and gross domestic savings as a percent of

> "Innovative (thinking outside the box) entrepreneurship and prudence are largely, if not wholly, culturally-driven traits."[31]
>
> - Alan Greenspan [32]

Greenspan recommends cultural characteristics and metrics be included in the new era of 21st century economic forecasting models.[33] His relatively brief and topical discussion includes savings and investment rates (abstinence, forbearance and prudence), cultural differences in entrepreneurial risk-taking, and in the rule of law and corruption.[34] His primary examples are Euro-North countries v. Euro-South countries: Greenspan believes that "becoming more like Germany" (e.g., forbearance, prudence, a work ethic, a commitment to legal economic activity and paying taxes) is (in the abstract) the cultural solution to improve economic forecasts for Greece, Italy, Spain, and Portugal. [35]

Since Greenspan's book went to press there is growing agreement that national and cultural differences must be included in forecasting models. Although these still are, to a degree, a "black

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GDP. Other forecast stabilities include the size of the workforce - those potentially in the workforce have already been born - and average hours worked." p. 292.

[31] China and Japan are cited as cultures that restrict innovation (p. 231).

[32] *op. cit.*, p. 231.

[33] Adherence to the rule of law can be proxied by the share of illegal activity in GDP. Other national/cultural characteristics include social harmony and communications and a functional political system. (p. 231).

[34] Note that there are opposite elements in Greenspan's model of economic growth - prudence (for savings) and risk-taking entrepreneurs.

[35] See also Lewis's observations that include Ireland and Iceland (different peoples with different reasons) that took the cheap credit to the point of disaster: Michael Lewis, Boomerang: Travels in the New Third World (New York: W. W. Norton, 2011).

box," the scientific failure to include them apparently has led to serious policy mistakes during the recent recovery, with (sometimes) opposite national effects of austerity from those that were forecast by economists. [36]

- Again, Greenspan may be right in his list, but there are political implications to these equations and the social science package will need to be robust. For example, a.) Asian cultures with traditions of hierarchy, combined with obligations for moral, benevolent, responsible and competent leadership, may develop a group-based psychology that is a source of competitive economic strength. In Japan, a psychology of dependency within firms (a hated characteristic, in the terms of Ayn Rand or Governor Romney's analysis of American economic performance) may be consistent with a highly competitive global automobile industry; [37] b.) Porter's work on

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[36] Howard Schneider, "An Amazing *Mea Culpa* from the IMF's Chief Economist on Austerity" Washington Post, January 3, 2013 concerning a (still, somewhat mysterious) set of differences that imposed remarkable damage on the Greek recovery and that can change over time. For European recovery, pro-austerity recommendations were based on a forecast of a fiscal multiplier of 0.5 when the actual multiplier sometimes was 1.5, meaning that a dollar reduction in government expenditures actually produced a $1.5 dollar reduction in GDP. Concerning other national/cultural variables that have emerged on the "to do" list to include in forecasting equations, see also Lawrence Summers, "Lessons Can be Learned from Reinhart-Rogoff Error." Washington Post. May 5, 2013 (discussed at footnote 4 above): ". . . [W]hy should it be the same in countries with and without their own currency, with very different financial systems, cultures, degrees of openness and growth experiences?"

[37] Concerning dependency inside a benevolent hierarchy: The allegedly growing American trait cited by Governor Romney as dysfunctional and true of 47% of Americans in a "too generous" welfare state may, as part of a package, be a successful feature of Japanese culture and many of its economic organizations: see Frank Johnson, Dependency and Japanese Socialization: Psychoanalytic and Anthropological Investigations of *Amae* (New York: NYU Press, 1995). The possibilities of cross-cultural learning and of culturally appropriate public policies are explored in Nicolas Berggruen and Nathan Gardels, Intelligent Governance in the 21st Century: A Middle Way Between West and East (New York: Polity, 2012).

international competitiveness suggests a wider set of nation-state metrics.[38]

There are many new cultural and sub-cultural groupings (e.g., c.) the economic behavior and causal dynamics of youth cultures) that might be the units of analysis, especially in countries with high and uncorrected rates of prolonged youth unemployment. Concerning the psychology of lower status individuals and their cultures: There may be d.) a Primate Subordination Syndrome that - even in objectively similar circumstances - reduces motivation, affects stress and endocrine levels and health, inhibits educational achievement, and is pervasively destructive of lower status primates.[39] The comparative neuroscience of lower status cultures may reveal a new universe of unrecognized causes (via the visual cortex and hierarchical imagination) of limitations in human economic potential. e.) The changing (post-deregulation) cultures (supported by changed recruitment and self-recruitment) of Wall Street and the financial world may be critical variables for economic forecasting.[40] f.) There are important (known) sub-cultural differences in

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[38] Michael Porter, Competitive Advantage (New York: Free Press, 1985).

[39] Studies of the Primate Subordination Syndrome may clarify a parallel inhibiting factor in regulations - i.e., if they also are perceived as establishing a status and dominance hierarchy. Subjectivities are important in the measurement of the inhibition of economic motivation by status ranking: Sub-cultures may provide inoculating effects (e.g., strong religious identities with the vividly experienced assurance of love and respect from a Supreme Deity and social support) and perceptions of economic opportunity also may mitigate these effects. See Lloyd S. Etheredge, "Neuropsychology and Rapid Learning Systems About Social Problems," unpublished, January 2010 and October 25, 2012 (online at www.policyscience.net at II. A. For some of the emerging correlates of subjective inequality on health and economic and social participation and (possibly) social problems see Moises Velasquez-Manoff, "Status and Stress," The New York Times, July 27, 2013.

[40] Tom Wolfe, The Bonfire of the Vanities (New York: Picador, 2008), reprint. The new "Masters of the Universe" status psychology may view members of Congress and political leaders (by judging their annual salaries) as (at best) hired middle management. The global political manipulation and exploitation of tax laws and regulations reflect a subjective change. In the 1960s most American businessmen felt poorly informed about the world beyond the water's edge

the motivation for economic achievement, and problems of structural discrimination and limited economic opportunities for different groups, that effect economic performance. (Euro-South and other cultures that discriminate against women or that limit access to good schools and higher education for their youth (to cite obvious examples) may inhibit their own economic growth). [41]

B.) Inclusive Social Science Lists

In Step 2 a planning group will reach out to include known (or suspected) R&D variables and metrics from other economists and disciplines. These ideas, like Greenspan's, are at risk of disappearing unless they evolve into metrics and their contribution can be evaluated by inclusion in R&D data systems.[42] At this point, we can measure almost any variable once we agree what they are.

4.) Behavioral Economics and Neuroscience

Researchers in behavioral economics often complain (rightly) that they are constrained to use small N experimental studies and do not yet have national data systems to allow the relevance of

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and were hesitant to become involved in political lobbying: Raymond Bauer, Ithiel de Sola Pool, and Lewis Dexter, American Business and Public Policy: The Politics of Foreign Trade (New York: Atherton, 1963). For the historic evolution of accounting and legal departments (from "just pay what we owe") into major profit centers with global strategic plans and lobbying see, for example, David Kocieniewski, "GE's Strategies Let It Avoid Taxes Altogether," The New York Times, March 24, 2011.

[41] Max Weber, The Protestant Ethic and the Spirit of Capitalism (New York: Penguin Classic, 2002) reprint; David McClelland, Human Motivation (New York: Cambridge University Press, 1988), Charles Murray, Human Accomplishment (New York: Harper Collins, 2009), and the work of Dean Keith Simonton.

[42] For a range of emerging diagnoses about missing variables see the IMF Rethinking Macro Policy II: First Steps and Early Lessons conference of April 2013, with papers online: http://www.imf.org/external/np/seminars/eng/2013/macro2/

their discoveries to be evaluated. This planning project will be their chance. [43] [44] [45]

Among other theorists, David Brooks has started to map a universe of fresh thinking about social and economic policy based on neuroscience discoveries. There is a new Society for Neuroeconomics (neuroeconomics.org) and emerging doctoral programs in neuroeconomics, and neurobiology and social science, whose members might suggest metrics for panel studies with genetics and brain data.[46] Full genomic mapping has fallen to $1,000 per individual and is heading toward $100 per individual: already genetic data (with some behavioral, social, and environmental data and electronic health records) are available in research databases (e.g., N=500,000 for the www.rpgeh.kaiser.org project).

An exciting challenge for this fourth task is to evaluate the possibility of genetic diversity in

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[43] Daniel Kahneman, Thinking, Fast and Slow (New York: Farrar, Straus, and Giroux, 2011). The growth of behavioral economics with support from the Sloan and Russell Sage Foundations is addressed in Floris Heukelom, Behavioral Economics: A History (New York: Cambridge University Press, 2014).

[44] This step also will include metrics to explain and forecast innovation rates. Greenspan believes that it is the role of the financial sector to assemble and channel needed funds. However, a wider list of metrics is needed: innovation systems are much wider than a financial system. See Robert D. Atkinson and Stephen J. Ezell, Innovation Economics: The Race for Global Advantage (New Haven: Yale University Press, 2012).

[45] Olivier Blanchard posits a new behavioral variable: "adjustment fatigue . . .which is leading to maybe less reforms than would be desirable." Transcript of a Press Briefing World Economic Outlook, October 8, 2013. International Monetary Fund, online.

[46] Concerning new funding, metrics and data systems: James Gorman, "The Brain's Inner Language," The New York Times, February 24, 2014. Investments include the EU's decade-long $1 billion Human Brain Project and the Obama Administration's $100 million startup.

the most important aspects of human nature relevant to economic behavior.[47] Only a very small number of people participate in creating financial booms and catastrophes and they may be atypical.

5.) Human and Social Capital

At the beginning of the 21st century most of the world has decided that market capitalism is the best engine for the future. There is a powerful and reciprocal relationship between the human and social capital of a society and the performance and outcomes (intended and unintended) of the economic system.

a.) Education. Especially in an emerging age of information technology and skills, investments in human beings are probably the most powerful contributions to economic growth. An exciting cluster of measurements can help to understand new, transformative opportunities for MOOCs and global education. We can bring a curriculum, equal to the best in the world, to the desktops of everybody in the world, virtually without charge. There is much experimentation to be done, and many additional investments required to turn online resources into a truly powerful education.[48] The second planning group will be asked to address the question: What should we measure?

STEM education has been proposed as a global metric, but one of the best areas for R&D research may be the psychological package of attainments that allows individuals to flourish as

[47] Greenspan believes that human nature is homogenous with respect to the major characteristics affecting economic behavior and performance. However high IQ is an exception: higher IQ increases capacities for abstraction and forethought, self-control, and delayed gratification, and thereby supports successful entrepreneurship and capitalism.

[48] U.S. President's Council of Advisers on Science and Technology (PCAST), Memorandum to President Obama concerning economic mobility, higher education, and MOOCs. December 2013. Online at www.whitehouse.gov.

"entrepreneurs" in their own lives and in the freer and more individualist societies implied by the system of market capitalism.[49] [50] A neuroscience snapshot of this larger "future-imagining-and-realization" or "taking responsibility for projects" cluster might include developing: 1.) capacities to be self-starting; and 2.) to create clear goals in which there is a genuine personal stake and that call forth commitment; 3.) to relate to aspects of realities as socially- and personally- created and changeable; 4.) learning how to identify or create alternatives; and 5.) how to decide upon and develop plans of action, assemble resources and enroll people and support (sometimes, including coaching); 6.) new brain mechanisms linking together abstraction, foresight and self-management (to achieve goals); 7.) a growing capacity to persevere (for short periods in doing elementary school assignments to several years when writing a Ph. D. thesis or book, and, then, even decades; 8.) growing cognitive capacities to manage integrated complexity and live and work with uncertainties and open-ended lines of thinking; 9.) capacities to persevere through a possible roller coaster of emotions along a path; 10.) to be self-reflective and able to think honestly and with integrity about what is working or not working; 11.) to be responsible about outcomes and breakdowns; and 12.) bring self-initiated projects to completion at a level of excellence.

In many areas of the world, formal educational systems (K-12 and college- even formal business schools) are not focused on doing the best job that they can to support this cluster and the future health that they imply for the world's economic systems. STEM education may support this growth, but it is a narrow idea and, in the wrong hands, any content-specific curriculum and testing program can become the use of authority and peer pressure to motivate

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[49] This educational cluster also will work for nonprofit institutions. The achievement/competitive drive for market capitalism is a separate psychological dimension: see McClelland and Winter, *op. cit.*

[50] The sociology of the G-20 education system and its relationship to G-20 economics involves a much wider set of issues. Mass production technologies may only have required mass production classrooms, with the goal of producing socialized students who were certified as willing to sit at desks for long periods and perform tasks assigned by authority, to reasonable standards, even if these were boring. Unless there are the right G-20 measurements, STEM education also can develop in this model.

behavior and produce diligent and mechanistic equation-solving or memorization. In truth, thoughtful measurement will be required from a planning group because the "being the entrepreneur and organizer of your own future" cluster might grow in many ways and from different sources: learning how to write academic papers and plan research, how to go step by step in your head to solve an algebra or geometry problem, practicing and achieving excellence in a competitive sport or playing the cello, or being a leader in student government, or (perhaps) an evolution of MOOCs, capstone projects, and new ways of teaching.[51]

b.) Moral breakdowns of institutions (including moral betrayal) may slow economic recovery, even if the issues are not discussed in public. David Brooks writes: "Moreover, it is harder to accept that psychological factors like uncertainty and anxiety really are a mirage . . . It has been harder to dismiss morality as a phantom concern, too. Maybe in a nation of [economic] robots the government can run a policy that offends the morality of the citizenry, but not in a nation of human beings."[52]

c.) The possibility that we are destroying social capital (without being fully aware of the

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[51] Comparative metrics for the performance of educational systems may be revealing: Greenspan's (Euro-South) cultural critique of Italy, Greece, Spain and Portugal suggests that serious limitations of their authority-oriented and conventional K-12 educational systems also may exist. Public policy research in the US has found that first-rate schools (and graduation from high school) works. Good K-12 schools is emerging as one of the best societal investments for the economic and personal success of individuals. See Ron Haskins and Isabel Sawhill, Creating an Opportunity Society (Washington, DC: Brookings, 2009).

[52] David Brooks, "The Two Cultures," The New York Times, November 15, 2010. Online. See also Robert Fogel's AEA Presidential Address "Catching Up with the Economy," American Economic Review, 89:1 March, 1999), pp. 1-21 about "commodities that lack material form" and his The Fourth Great Awakening and the Future of Egalitarianism (Chicago: University of Chicago Press, 2000). Anomie in the former Soviet Union (a case that Greenspan does not discuss) is a striking example of perceived moral breakdowns that illustrates their devastating effects on many aspects of life.

process) is raised by Charles Murray and other writers.[53] 1.) High divorce rates and single parent families may (especially without compensating investments) be a bad idea for children with long term costs to themselves and to society. 2.) There may be a vital degree of now-eroding social capital, and trust, that depend upon the experience of people that good values and hard work and social responsibility are appreciated and rewarded. Politicians across the US political spectrum now run for office and address a perception that "playing by the rules" is not working in America. 3.) In Europe, astonishing and uncorrected rates of youth unemployment are accompanied by a politically dangerous and demoralizing public discussion of "a lost generation" that ultimately may not accept its fate.[54] 4.) The expectation (and reality) of social mobility may be part of social capital: variables associated with social mobility, across regions in the US and abroad, are likely to be revealing of partial blockage in causal pathways for economic health.[55] 5.) The Spence *et al.* consultation process addressing Chinese economic growth predicts that greater inequality in society is corrosive and becomes dysfunctional: inequality creates different interests and erodes a political process; it also fuels political combat and redirects energies that could be used more productively.[56]

- d.)The Psychological Economy. The broader agenda for the planning group will be to

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[53] Charles Murray, Coming Apart: The State of White America, 1960-2010. (New York: Crown Forum, 2012).

[54] For forecasting equations illustrating potential destabilizing effects of prolonged high unemployment for different groups, see Alan de Bromhead *et al.*, "Right-wing Political Extremism in the Great Depression." Unpublished working paper online at www.voxeu.org.

[55] See the geographic variations in social mobility within the US and new variables identified by by Raj Chetty and his associates: http://www.equality-of-opportunity.org/

[56] Edwin Lim, Ian Porter, Paul Romer, and Michael Spence, "Medium and Long Term Development and Transformation of the Chinese Economy: A Synthesis Report." March 2011 (online at www.cairncrossfund.org): "If not addressed such disparities risk fueling greater social conflict and instability," p. 69. See also their lesson for new models: "social policymaking must be tightly integrated with economic policymaking," *op. cit.*, p. 71.

advance the sensitive and respectful measurement of the "psychological economy" - which I define (following Fogel) as all aspects of the economy - its input and functioning, and its "commodity" outputs and effects - that lack a material form. Coming from the metrics and the limited concerns of national income accounting (and independent and dependent variables defined by accountants and the tax code) and the physical realities of nation-state, steel plant economies, these new metrics will help us to grasp a changing world of complex, sometimes interdependent, systems and subsystems whose outputs shape the quality of our lives and the material-form economy.[57] One of the leading edge questions (likely to be flagged as "important, but needing further research before metrics can be recommended") is a refined understanding of the social recognition and status economy and the production systems that societies link to what Greenspan calls the "herd' (social) instincts. Competition for recognition and status can be as important as competition for economic rewards. And institutions and societies create status scarcities and competitions that function as motivators. (Arguably, one of the most important reforms that Margaret Thatcher brought to the UK was to make it socially acceptable for higher status people to become successful entrepreneurs.)

6.) Political Variables

> "Shadow banking is a form of financial intermediation whose funding is not supported by the traditional banking safety nets . . . the shadow banking system remained slightly more than half the size of the regular banking system throughout the 2002 to 2011 period . . . In the United States alone, shadow banking constituted $23 trillion in assets at the end of 2011, by far the largest constituent of the global network of

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[57] See, *inter alia*, Lasswell and Kaplan, *op. cit*.; Robert E. Lane's pioneering The Market Experience (New York: Cambridge University Press, 1991) and The Loss of Happiness in Market Democracies (New Haven: Yale University Press, 2001); Robert Putnam, Bowling Alone: The Collapse and Revival of American Community (New York: Simon and Schuster, 2001); Fogel, "Catching Up" and The Fourth . . ., *op. cit.*

nonbank credit intermediaries."

-Alan Greenspan [58]

"Institutional flaws are best prevented, because they are hard to fix. Once an institutional structure is in place, people quickly acquire a vested interest in its preservation. The flawed structure then becomes surprisingly resistant to reform, as the US health-care system clearly demonstrates."

- Lim, Porter, Romer and Spence [59]

"Our highest priority going forward is to fix our broken political system."

- Alan Greenspan [60]

Every societal goal has a production function: most desired outcomes can be produced several ways and by different mixes of inputs. In turn, politically, each input mix may allocate new economic income, status, power and control differently, to different beneficiaries and constituencies. To improve scientific knowledge and (with a genuine Honest Broker intent) to build political support, step six also will seek input from a full range of think tanks, activists, and others to expand upon Greenspan's list.

Greenspan has a wide range of personal observations about dysfunctional political systems, ranging from a theory that an *angst* caused by American political schism and conflict is reducing long-term business investments and slowing recovery, to genuine puzzlement about why Washington leaders cannot sit down (as they did in earlier days) for drinks after hours and reach

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[58] *op. cit.*, pp. 40-41.

[59] Edwin Lim, Ian Porter, Paul Romer, and Michael Spence, "Medium and Long Term Development and Transformation of the Chinese Economy: A Synthesis Report." March 2011 (online at www.cairncrossfund.org), p. 71. Discussed in footnote 4.

[60] *op. cit.*, p. 302.

compromises.[61] This initial project cannot do full justice to the range of these conceptual, theoretic, and measurement issues, which have extensive literatures in several social science disciplines. However, several key political system issues can be reviewed by the second planning group.

a.) Is "Human Nature" a Political Misdirection?

Social scientists will instinctively ask whether Greenspan's ("it's human nature!") ideas are a political misdirection and *mea culpa* that focus attention away from the real (political) variables that should be included in the world's macroeconomic prediction equations. Yes, the Tulip Mania of the 1630s and many of the financial bubbles and panics of history may have been produced by primitive emotions and people who stumbled through history to a catastrophe and did not understand the eventual behavior of the system and their fate.[62] However, the world's "shadow" banking systems [whose size is indicated by Greenspan in the quotation at the beginning of this section] and their international lobbying expenditures and political largesse did not arise by accident.[63] And the first edition of Kindleberger's Manias, Panics, and Crashes: A History of Financial Crises (now in its sixth edition) was published in 1978.[64] Of course Kindleberger did not intend to write a handbook, but subgroups in generations of financial analysts, by now, may have gone to school on the amounts of money that they can make if they

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[61] Greenspan has a list of observations about how the American political system (and the Latin American political systems, and the Chinese political system, and the Euro-South political systems, etc.) are dysfunctional. Some of the problems might benefit from a greater degree of agreement in economic science: Greenspan may be right that Latin American Populism is a "shout of pain" and can be shown to lack a coherent and effective economic theory.

[62] However see CW and AJKD, "Was Tulipmania Irrational?" The Economist, October 13, 2013.

[63] See also Sebastian Mallaby, More Money Than God: Hedge Funds and the Making of a New Elite (New York: Penguin Press, 2011).

[64] (New York: Basic Books, 1978).

activate asset bubbles, and then manipulate the irrationalities and deceive the trust of others and, thus, outsmart the system. In the recent crisis, brilliant hedge fund managers hyped asset bubbles, falsified or obscured credit ratings, and also bought insurance (e.g., through AIG) to cover themselves when the asset bubbles finally burst. *Pace* Greenspan, perhaps the instincts that society has to blame, or worry about, are not homogenous endowments of animal spirits or shared herd (social) instincts of people drawn into the irrational exuberance of competitive games, but brilliantly rational, realistic, strategic (gratification-deferring) predators at the atypical upper tail of statistical distributions? In a phrase of the psychologist William James, "the beaked and taloned predators," with an absence of social instincts?

b.) Politics can be the continuation of economic competition by another name. The news media can draw audiences by creating a drama that implies, ultimately, that governments are in charge of societies. However some businessmen do not live inside this media-created drama. The possibility that some wealthy entrepreneurs might relate to national government and politics as dependent variables (to be manipulated and managed) is a possibility that may be especially important to explore for G-20 nations since assertive (and, ultimately, poorly regulated) actors in a subset of G-20 countries may collude and act across national boundaries. There is indirect evidence to suggest that growing asymmetries of brainpower and concentrations of wealth are deployed against (penetrated) political systems to induce deregulation and achieve other benefits. Specifically, the world had, from the late 1970s through 2003 (according to IMF data) 117 crises of banking systems in 93 countries in which much or all of the capital of the system was exhausted. In Martin Wolf's assessment of these cases, the banking industries developed strategies of privatizing their gains during the upside of financial bubbles, then secured government bailouts from taxpayers as losses during the crisis phase became large enough to wipe-out remaining bank equity and destroy the economy. In 27 of the earlier crises, taxpayers were stuck with added public debt equal to, or greater than, 10% of GDP, often much more. When similar, highly strategic people continue to win ["privatize the gains"], with a similar *modus operandi,* the better, new forecasting models might be based on the classic dynamics of predator-prey

ecosystems described by the Lotka-Volterra equations.[65] If this theory proves to be correct, and the G-20 nations want to improve economic forecasting, the best question suggested by upgraded social science forecasting could be: "What are *they* [the alpha predators] planning next?" And every chief of state might ask that the best (public) economic forecasting models be accompanied by secret reports and forecasts from his intelligence agencies based on massive penetration of the national and global financial sectors and especially the "shadow" sector.

By contrast with Greenspan, it is interesting to consider the perspective of David Stockman, a former OMB Director for President Reagan who later made a fortune on Wall Street. In Stockman's view, the major players always are trying to outsmart each other - and the same instincts are directed against governments as on the economic playing fields; in his analysis, few governments, including the American government, can play in this new game and win.[66]

C. Finding Unknown Variables and Organizing Rapid Learning Systems

"We are confronted with . . . 'unknown unknowns' . . . "
- Olivier Blanchard [67]

The third planning step will develop methods to find unknown variables and causal relationships and organize rapid learning systems.

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[65] Martin Wolf, Fixing Global Finance (Baltimore, MD: Johns Hopkins University Press, 2008), pp. 32-33. Lloyd S. Etheredge, "Predator-Prey Models: Forecasting a Global Financial System with Asymmetries of Brainpower and Money," Memorandum # 17 for the Fischhoff (NRC) Committee on Behavioral and Social Science Research to Improve Intelligence Analysis for National Security. Online at www.policyscience.net at II. D.

[66] David Stockman, The Great Deformation: The Corruption of Capitalism in America (New York: Public Affairs, 2013).

[67] David Wessel, "Olivier Blanchard's Five Lessons for Economists from the Financial Crisis," Wall Street Journal, April 1, 2013.

The model for Step 3 will be the new rapid learning systems of international biomedical research that use "Everything Included," large N, curated databases partly underwritten at public expense. Until recently, cancers were classified by their site of occurrence (e.g., breast cancer, lung cancer). Now, with "Everything Included" databases (100,000++ variables per patient, and tens of millions of patients and their genetic information and electronic health records being linked in international networks), new machine learning algorithms have established themselves as a disruptive, breakthrough technology. They brilliantly help human researchers to replace old paradigms more quickly than traditional systems of single investigator awards. An investigator is not limited to imagine (ahead of time) the specific hypothesis to be tested and, then, fated to discover unknown variables only by accident.

With this powerful investment in new scientific technology, the biomedical world is changing. It now appears that there may be 10 or more different types of cancer that appear in the breast or the lung (etc.), each with its own complex causal pathway (linked to the genetics of the specific individual). Each type has its own universe of newly emerging treatment possibilities and the exciting future that humanity is facing is a new *precision* medicine also tied to genetic and other unique characteristics of each patient. [68]

Discovering unknown variables and relationships is becoming an automated science. This could be G-20 macroeconomics!

For this third planning group I think that the challenges to develop "Everything Included" research strategies are: What constitutes Everything? (For example, when you include psychology and neuroscience and when the social sciences do not yet have the equivalent of the periodic table and the human genome?) How fast do we want to learn? And what G-20 priorities to recommend? It is an open-ended question, and the powerful machine-learning Big Data, paradigm-busting methods may be sensitive to initial omissions of variables or error rates in

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[68] B. Vogelstein *et al.*, "Cancer Genome Landscapes," Science, March 29, 2013, pp. 1546 - 1558, attached to this proposal.

metrics.

7.) Big Data and Private Sector Partnerships

The seventh planning project will map how the startup of "Everything Included" R&D economic data systems can be linked together in international partnerships with the private sector. A useful initial global project might be data mining and rapid, cumulative learning concerning consumer/household behavior and marketing.[69] Just as 11,000 individual Walmart store managers in 27 countries are currently expected to run three to five experiments each week, so a R&D consortium of interested global corporations could be linked with leading business schools in rapid learning systems.

For example, it might be easy for these partnerships to organize large N, randomized cross-cultural experiments of advertising and marketing for all demographic groups and all nations and cultures.[70] Companies like Mastercard or Google would have incentives to contribute data to an initial R&D data system, since discoveries of how their data can be combined with other data make the business case for why their future data should be purchased to improve economic models and forecasting, worldwide.[71]

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[69] See Liran Einav and Jonathan Levin, "The Data Revolution and Economic Analysis." Unpublished manuscript, May 1, 2013 prepared for the NBER Innovation Policy and the Economy conference.

[70] Concerning steep cost reductions by designing collaborative global rapid learning systems, see Michael S. Lauer and Ralph D'Agostino, "The Randomized Registry Trial - The Next Disruptive Technology in Clinical Practice?" The New England Journal of Medicine, October 24, 2013, pp. 1579 - 1581.

[71] Academic social science might benefit from this project. American social psychologists typically have used their undergraduates as experimental subjects, and there are sparse discussions in standard textbooks about how human beings in other cultures might behave differently than American undergraduates in the late 20th and early 21st centuries. A discovery by American

8.) New Methods

> "How reliable are these tools? . . . They work but they don't work great. People and institutions find ways around them."
>
> - Olivier Blanchard [72]

The third planning group also will consider recommendations for faster and better learning cycles in the US and G-20 nations. For example:

a.) Data collection and analysis should be faster and supplemented by new methods to estimate coefficients. Traditional forecasting uses quarterly time series data and regression equations, but this clearly is too slow and unable to detect changing coefficients in a timely fashion. A new universe of real-time sampling and monitoring will be useful: Walmart has global data on sales, by store and product, online within 24 hours and the global banking system clears most of the transactions of the world economy within several days. Soon, it could be possible to monitor economic behavior and track the effects of economic policies in real time.

b.) To work through, and master, the integrated complexity that economic science must face requires new methods for modeling and display. These are large, living, complex and (sometimes) adaptive systems composed of large, living, complex and (sometimes) adaptive subsystems that may be loosely or tightly coupled or even partly inconsistent with each other. The biomedi-

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Express [informal communication] that social media effects (e.g., knowledge of a friend's purchase) have 3- to 5- times greater impact to influence purchasing decisions of Egyptian teenagers (compared to American teenagers) may stimulate thinking for a wider universe of new and informative discoveries about cross-cultural social psychology.

[72] Wessel, *op. cit.*. Blanchard's five lessons emphasize the need for analytic tools with much more "plumbing" detail: "We do macro on the assumption that we can look at aggregates in some way and then just have them interact in simple models. I still think that's the way to go, but [experience] shows the limits of that approach. When it comes to the financial system, it's very clear that the details of the plumbing matter."

cal world has been evolving new and sophisticated computer simulation models of the human body (beginning at the molecular level) - with extensions to medical practice decisions, public health and government policy - that will be worth evaluating for their applications to macroeconomics and G-20 forecasting.[73]

c.) Cross-walking past economic policy mistakes and forecasting errors in G-20 countries may be useful: A new, meta-learning strategy in biomedical research is to analyze the eventual discovery of lethal side-effects of approved drugs and to calculate how much larger new rapid-learning data systems should become if we want to catch such types of mistakes in the future in three months, or six months, or two years (etc.).[74]

d.) Panel studies are another useful innovation, especially to achieve the "Everything Included" vision for R&D. [Traditionally, economists have correlated independent and dependent variables (defined by accountants or tax laws) and told (without an independent examination of the mechanisms) a rational choice, profit-maximizing story to explain the links. Now, with alternative explanations and pathways, panel studies can, using multiple methods, provide much more information, and in depth, to compare different theories.] Especially with compensation, many people might be willing participants. In addition to formal guarantees of privacy, the panel membership could be limited to several years and, thereby, reduce concerns about broader invasions of privacy. There are multiple groups of actors in economies, and a diverse range of these panels are likely to be recommended by the planning group.

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[73] For example David Eddy's Archimedes Project originally developed for Kaiser Permanente: http://archimedesmodel.com/ . The model and its mathematical methods recently have been acquired for international medical practice, pharmaceutical research, and public health advising by STG, a global venture capital company.

[74] See Larry Norton's overview of the wider rapid learning system for cancer, https://www.ecri.org/Video/2013_TA_Conf/4-Session-1-Norton.mov. See Blanchard (2011) *op. cit.*

e.) Computer-assisted content analysis (discussed in footnote 14) may help to understand public moods and the emotional component of recovery processes.

f.) Empirically-defined variables (rather than accounting-defined variables) might be useful experiments. Greenspan's forecasting ideas place great weight on the (alleged) very high rate of consumption (and low savings) in American households, but most families may view many of their expenditures differently, as investments contributing long-term benefits to their lives and the lives of their children.

g.) To libertarians, except for the contributions of a minimal government (e.g., national defense), most public sector expenditures can be just political shell games and "theft" (transfer payments). Greenspan does not use this term: However a current lack of analysis methods skews his analysis into a story of how the financial sector, securing private savings, plays the leading role in the economy by assembling and allocating funds for the new investments that increase productivity and the possibility of higher standards of living. Yet all sectors (including governments) actually make investments. The broader measurement challenge for forecasting equations is to measure what investments are good investments, not who makes them. Whether society is "investing enough" cannot be calculated, as Greenspan does, by the percentage of the average household income that is saved: the public sector investments (paid through taxes or deficits) also must be measured and evaluated.

h.) Weighted scenarios and game-theoretic methods (even war games) may be useful to forecast the emerging national and global financial systems with asymmetries of wealth and brainpower. In testimony to draft laws and regulations, some economists already systematically analyze loopholes and vulnerabilities and forecast how these will be exploited.[75]

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[75] Charles Calomoris and Alan Meltzer, "How Dodd-Frank Doubles-Down on 'Too Big to Fail,'" Wall Street Journal, February 12, 2014. See also Sheila Blair's answer to the questions: "Can regulators ever be as nimble as the regulatees?" and "Given the cat and mouse game between regulators and regulatees, do we have to live with regulatory uncertainty?" In her "Everything the IMF Wanted to Know About Monetary Regulation and Wasn't Afraid to Ask"

9.) Rapid Learning Systems

> "With a century and half of clear, detailed information on crisis after crisis, the burning question is not How did this happen? but How did we ignore that long history, and think that we had solved the problems with the business cycle?"
>
> - Joseph Stiglitz [76]

An evolving design of a global rapid learning system for macroeconomics needs a self-reflective theory of itself - and metrics. The practical realities of the system, and the speed of its learning cycles in the G-20 (and beyond), will depend upon the evolving design of a complex (sometimes) adaptive system composed of complex (sometimes adaptive) subsystems. Once, the focus of philosophers was to discover how a single individual could become wise: Today, we recognize wider problems, especially in democratic systems: How, in Stiglitz's terms (in the quotation above) do we get *other* people (and systems) to listen and to remember?[77]

Creating a rapid learning system also will depend upon recognizing that it is in the self-interest of each G-20 nation, in a world of globalizing economies, that other nations (and private sector decision makers) adopt realistic and evidence-based policies and that everybody prospers. Upon a news media that support the system. And upon funding, honesty and reliability, and institutional homes, and much else. What are the variables to measure, the theories to test, who are the allies, where is the funding, what are the disruptive technologies to deploy?

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online at http://www.imf.org/external/np/seminars/eng/2013/macro2/pdf/sb.pdf

[76] Joseph Stiglitz, "The Lessons of the North Atlantic Crisis for Economic Theory and Policy," http://blog-imfdirect.imf.org/2013/05/03/the-lessons-of-the-north-atlantic-crisis-for-economic-theory-and-policy/

[77] See also Etheredge, "Wisdom . . ." *op. cit.*; Ascher, *op cit.*

II. Work Plan – May 1, 2014 – June 30, 2017

The project will organize an Advisory Committee to develop initial plans. Next, it will complete three steps in three years (each step taking about a year, with three areas of focus). Each step will have a planning group (N=12-14 members, with a degree of overlap) and will produce a Report.

Each planning group will produce a report (i.e., the grant will deliver three Reports) to the sponsors with recommendations of variables and metrics to produce a state-of-the-art international rapid learning system for macroeconomics. Each of the three Reports will address: 1.) Recommended variables and metrics that are on the shelf and that can be deployed immediately; 2.) Recommended metrics that can become available soon, with additional work; 3.) Important areas where further R&D is needed before metrics can be recommended.[78]

The budget supports a full time Principal Investigator with part-time assistance and expenses. Expenses include honoraria and travel for Advisory Committee and planning group members, initial discussions between the PI and each working group member, and a 1 ½ day meeting of each planning group.

The Advisory Committee (five members) will be the joint responsibility of the PI and [the home institution for the project]. The project is envisioned as part of a long term research program at [the home institution] devoted to achieving a rapid learning (international) system for macroeconomics. [The home institution] may seek additional funds for Fellowships, research to analyze new data, and additional, concurrent conferences and lecture series.

[78] This initial project will focus on macroeconomics of the G-20 system. Additional data systems and better forecasting equations may benefit all countries.

III. Budget and Budget Narrative (to be added)

IV. Attachments

Bert Vogelstein *et al.*, "Cancer Genome Landscapes," Science, March 29, 2013, pp. 1546 - 1558.

Lloyd S. Etheredge, The Case of the Unreturned Cafeteria Trays (Washington, DC: American Political Science Association, 1976).

Lloyd S. Etheredge, Brief Biography and *Curriculum Vitae.*

Undergraduate Questionnaire on Reproductive Cloning
From: "michael oals" [redacted]
Date: Wed, April 23, 2014 8:23 pm
To: [redacted]

Dear Sir or Madam,

To begin let me introduce myself my name is Michael Anthony Oals II I am a undergraduate student at Delaware technical community college the purpose of this email is to interview you so to say, on a topic that is a philosophical moral and scientific/ ethical dilemma that sooner or later we will have to either fight against or accept the topic I am speaking of is Reproductive Cloning.

1. If a soul is a gift from god bestowed upon one when in the womb then does that mean a human created from science is a proverbial clock just mirroring the original or is it a person in its own right because it is a human in the technical regards albeit born in an artificial way and because it does have thoughts and feelings even if those thoughts and feelings are another's isn't it possible that it will form its own personality different from the original based upon nature and nurture?
2. Since the soul is part of us does that mean that all our cells formed together collectively make up the proverbial soul and if so wont that mean a embryonic baby is human.
3. What are your views on the topic of cloning?
4. Is cloning humanity's next step or is it a lash out at god?
5. If reproductive cloning produced viable to term samples and are introduced into society what in your view would the world/people do would they accept them as humans or destroy them as monsters?
6. Would it be right to allow reproductive cloning as a source of "replacement parts" for those who are terminally ill?
7. Immortality something we all seek would be basically achieved with reproductive cloning what would this do to society to our world
8. In a military aspect reproductive cloning would produce an infinite amount of soldiers would this justify allowing it?

Thank you,

Michael Anthony Oals II

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Michael Oals