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Associations Between Multidrug Resistance, Plasmid Content, and Virulence Potential Among Extraintestinal Pathogenic and Commensal *Escherichia coli* from Humans and Poultry

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Abstract

The emergence of plasmid-mediated multidrug resistance (MDR) among enteric bacteria presents a serious challenge to the treatment of bacterial infections in humans and animals. Recent studies suggest that avian *Escherichia coli* commonly possess the ability to resist multiple antimicrobial agents, and might serve as reservoirs of MDR for human extraintestinal pathogenic *Escherichia coli* (ExPEC) and commensal *E. coli* populations. We determined antimicrobial susceptibility profiles for 2202 human and avian *E. coli* isolates, then sought for associations among resistance profile, plasmid content, virulence factor profile, and phylogenetic group. Avian-source isolates harbored greater proportions of MDR than their human counterparts, and avian ExPEC had higher proportions of MDR than did avian commensal *E. coli*. MDR was significantly associated with possession of the IncA/C, IncP1-α, IncF, and IncI1 plasmid types. Overall, inferred virulence potential did not correlate with drug susceptibility phenotype. However, certain virulence genes were positively associated with MDR, including *ireA*, *ibeA*, *fyuA*, *cvaC*, *iss*, *iutA*, *iha*, and *afa*. According to the total dataset, isolates segregated significantly according to host species and clinical status, thus suggesting that avian and human ExPEC and commensal *E. coli* represent four distinct populations with limited overlap. These findings suggest that in extraintestinal *E. coli*, MDR is most commonly associated with plasmids, and that these plasmids are frequently found among avian-source *E. coli* from poultry production systems.

Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) have received considerable attention because of their complex nature and ability to cause a variety of important extraintestinal diseases in humans and animals (Johnson and Russo, 2002). Several subpathotypes of ExPEC have also been described, based on host source, specific disease syndrome, and virulence genotype. These include uropathogenic *E. coli* (UPEC) causing urinary tract infection (UTI), neonatal meningitis-associated *E. coli* (NMEC) causing meningitis of the newborn, and avian pathogenic *E. coli* (APEC) causing colibacillosis in poultry (Kaper, 2005). These diseases are costly to the human health care system and poultry industries, and cause considerable morbidity and mortality. Thus, the control of these diseases is an important area of focus.

It has been shown that ExPEC commonly possess large, transmissible plasmids encoding multidrug resistance (MDR) (Johnson and Nolan, 2009). By comparison, less is known about the prevalence of such plasmids in commensal *E. coli*. Further, the scope of horizontal gene transfer in relation to the dissemination of MDR in *E. coli* in the fecal and vaginal flora

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of healthy humans and animals is not known. Since ExPEC that cause clinical disease are thought to emerge from the fecal microbiota of healthy hosts, it is plausible that some commensal intestinal *E. coli* could also harbor large, transmissible plasmids conferring a multidrug-resistant phenotype. Notably, subsets of avian *E. coli* may represent a zoonotic threat via the consumption of contaminated poultry meat (Ewers *et al.*, 2007; Johnson *et al.*, 2007a, 2008, 2009a; Smith *et al.*, 2007b). However, it is unclear whether these subsets also represent a threat with regard to the dissemination of MDR to human bacterial populations, which would be more likely if MDR in avian strains is encoded on mobile genetic elements.

To address these important knowledge gaps, we assessed 2202 previously characterized, disease-associated ExPEC and commensal *E. coli* from healthy human and avian hosts for their antimicrobial susceptibilities. Our goal was to combine these data with existing information to determine the associations among antimicrobial susceptibility, plasmid content, and virulence potential, in relation to host species and clinical origin.

Materials and Methods

Bacterial strains

The 2202 study isolates originated from a variety of sources, isolated between 1990 and 2005 (Table 1) (Obata-Yasuoka *et al.*, 2002; Johnson *et al.*, 2002a, 2002b, 2008; Rodriguez-Siek *et al.*, 2005a, 2005b). All these isolates have been previously characterized for their virulence gene content and phylogenetic group membership, and some have been previously characterized for plasmid content (Johnson *et al.*, 2007c). All isolates were stored frozen at −80°C in Brain Heart Infusion Broth (Difco Laboratories) with 10% glycerol and had undergone limited passage since their initial isolation in an attempt to ensure their genetic stability.

Antimicrobial susceptibility

All *E. coli* isolates were examined for their antimicrobial susceptibilities by using the National Antimicrobial Resistance Monitoring System panels CMV5CNCD (some APEC isolates) and CMV1AGNF (remaining isolates) by Trek Diagnostics according to Food and Drug Administration, United States Department of Agriculture, and Clinical Laboratory Standards Institute recommendations (Clinical and Laboratory Standards Institute, 2010). A 96-well microtiter plate was used to test the susceptibility of strains to the following 14 antimicrobials (drug name abbreviation; breakpoint used): amikacin (AMI; ≥64 μg/mL), amoxicillin/clavulanic acid (AUG; ≥32/16 μg/mL), ampicillin (AMP; ≥32 μg/mL), cefoxitin (FOX; ≥32 μg/mL), ceftiofur (TIO; ≥8 μg/mL), ceftriaxone (AXO; ≥4 μg/mL), chloramphenicol (CHL; ≥32 μg/mL), ciprofloxacin (CIP; ≥4 μg/mL), gentamicin (GEN; ≥16 μg/mL), kanamycin (KAN; ≥64 μg/mL), nalidixic acid (NAL; ≥32 μg/mL), streptomycin (STR; ≥64 μg/mL), trimethoprim/sulfamethoxazole (SXT; ≥4/76 μg/mL), and tetracycline (TET; ≥16 μg/mL). Inoculation of panels was carried out according to the manufacturer's instructions. CLSI-specified control strains of *E. coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* were used to validate each batch of plates. Strains displaying resistance to ≥3 classes of antimicrobial agents tested were defined as exhibiting MDR.

Plasmid replicon and resistance gene typing

Isolates were also examined for the presence of plasmid replicon types by using multiplex polymerase chain reaction (PCR), as previously described (Carattoli *et al.*, 2005; Johnson *et al.*, 2007c). Additionally, selected isolates were examined for the presence of class 1 integron-associated genes using primers designed in this study (Table 2). PCR was performed as previously described (38). Amplicons were visualized on 2% TAE agarose gels alongside appropriate size standards (Minnesota Molecular, Inc.). Reactions were performed twice, and, if a discrepancy was identified, they were repeated again.

Virulence gene and phylogenetic typing

For all isolates, multiplex PCR-based genotyping for 32 ExPEC-associated virulence factor-encoding genes (VFs) was performed as previously described (Johnson and Stell, 2000; Rodriguez-Siek *et al.*, 2005b). Some of these data are previously described (Rodriguez-Siek *et al.*, 2005a, 2005b; Johnson *et al.*, 2007a, 2008). Determination of major *E. coli* phylogenetic group (A, B1, B2, and D) was done according to the interpretive approach described by Clermont *et al.* (2000).

Statistical methods

Comparisons of proportions were tested by using Fisher's exact test (two-tailed) or Chi-squared distributions (Snedecor

Table 1. *Escherichia coli* Strains Used in This Study

Group	N	*Source*	*Dates of isolation*	*Country*
APEC	909	Lesions of commercial broilers and turkeys with colibacillosis	1990–2005	United States
Avian fecal *Escherichia coli*	422	Feces of healthy commercial broilers and turkeys	1990–2004	United States
UPEC	559	Urine of human patients with bacteriuria (with or without symptoms)	1995–2003	United States
NMEC	70	Cerebrospinal fluid isolates from human neonates with meningitis	1989–1997	The Netherlands
Human fecal *E. coli*	156	Rectal swabs of healthy humans	1995–2004	United States
Human vaginal *E. coli*	86	Vaginal swabs from healthy women	1999–2001	Japan

APEC, avian pathogenic *E. coli;* UPEC, uropathogenic *E. coli;* NMEC, neonatal meningitis-associated *E. coli.*

Table 2. Novel Primers Used in Polymerase Chain Reaction Studies

Primer	*Gene*	*Description*	*Sequence (5′ to 3′)*	T_{Anneal} (°C)	*Predicted amplicon size (bp)*
QAC F	*qacEΔ1*	Quaternary ammonium compound resistance gene	GCCCCTTCCGCCGTTGTCATAATC	63	250
QAC R			CGGCCTCCGCAGCGACTTCC		
SULI F	*sulI*	Sulfonamide resistance gene	CGCCGCTCTTAGACGCCCTGTCC	63	405
SULI R			CAACGGTGGCGCCCAAGAAGGAT		
INT F	*intI1*	Integrase gene for class 1 integrons	CACTCCGGCACCGCCAACTTTC	63	490
INT R			GAACGGGCATGCGGATCAGTGAG		
MERA F	*merA*	Mercury resistance gene	GATCCGCGCCGCCCATATCGCCCATCTG	63	250
MERA R			CACGCGCTCGCCGCCGTCGTTGAGTTG		
TETA F	*tetA*	Tetracycline resistance gene	CGGGGCGACTGGGGCGGTAGC	63	372
TETA R			CAAAGCGCGGCCGGCACCTGTC		

and Cochran, 1989; Westfall, 1999) using SAS. Hierarchical two-way clustering, which clusters data based on overall traits on both the X and Y axis, was performed on the raw MIC values and visualized by using JMP for a graphical display of all characters used, in the context of the groups obtained from the cluster analysis (Johnson *et al.*, 2008). Overall similarity relationships among the individual isolates with regard to VF profiles and phylogenetic group were assessed by using principal coordinates analysis (PCoA), a multivariate technique related to correspondence analysis enabling plotting of the major patterns within a dataset (Peakall and Smouse, 2006). By means of Genalex6 (Peakall and Smouse, 2006), PCoA was applied to the entire dataset. Each axis in PCoA represents a unique weighted composite of all the individual variables in the dataset. Individual isolates were assigned values on each axis on the basis of study variables and each variable's weighting factor on the particular axis. These values (for pairwise combinations of the first three axes) were plotted as a series of Cartesian grids, to show the distribution of the individual isolates (and their respective source groups) in two-dimensional space. They were also used in multivariate analysis of variance (MANOVA) to determine whether the comparison groups differed significantly according to the first three PCoA axes. If the initial multivariate ANOVA identified a significant overall difference, then univariate ANOVA was used to test pairwise comparisons of individual groups according to each PCoA axis, with use of a Bonferroni correction for multiple *post-hoc* comparisons as appropriate.

Results

ExPEC and commensal E. coli *differ in their antimicrobial susceptibilities and plasmid replicon possession*

The 2202 total *E. coli* isolates were examined for 67 traits, including susceptibility to 14 antimicrobial agents, possession of 17 plasmid types, possession of 32 ExPEC virulence genes, and *E. coli* phylogenetic group membership. The goal of this work was to identify associations between MDR, plasmid replicon content, and virulence genotype. Compared with avian commensal *E. coli* ($n=422$), APEC isolates ($n=909$) exhibited a significantly greater prevalence of resistance ($p<0.05$) to AMP, GEN, KAN, STR, SXT, and TET (Table 3), and a significantly higher prevalence of the IncB/O, IncP1-α, IncFIIA, IncFIB, IncN, and IncHI2 replicons. Similarly, compared with human fecal *E. coli* ($n=156$), UPEC ($n=559$) exhibited a significantly greater prevalence of resistance to AMP, CHL, KAN, STR, SXT, and TET, and NMEC ($n=70$) had a significantly higher prevalence of resistance to STR, and a significantly higher prevalence of the IncB/O, IncP1-α, and IncFIB plasmid replicons. Likewise, compared with the human vaginal *E. coli*, NMEC had a significantly higher possession of the IncB/O, IncP1-α, and IncFIB plasmid replicons. When analyzed by phylogenetic group, the group B2 isolates had a lower prevalence of antimicrobial resistance, whereas the group A and B1 isolates tended to have a higher prevalence of resistance (Table 3). The B2 isolates also had a lower prevalence of some plasmid replicon types, including IncP1-α and IncI1 (Table 3).

Overall, 37.4% of isolates were susceptible to all antimicrobial agents tested, with most of the pan-susceptible isolates belonging to the UPEC group (Fig. 1). Among the remaining 62.6% of isolates, 20 distinct resistance profiles shared by 15 or more isolates were identified (Fig. 2). Among these MDR isolates, two profiles were identified with ≥8 resistances: AMP-AUG-CHL-FOX-GEN-STR-TET-TIO ($n=41$) and AMP-AUG-CHL-FOX-GEN-KAN-STR-TET-TIO ($n=34$); these occurred only among avian-source isolates. Overall, MDR (≥3 resistances) was most prevalent among APEC (34.9%) and AFEC (31.3%) isolates, and was less prevalent among UPEC (19.5%), NMEC (11.4%), and human commensal isolates (10.3%).

Associations between antimicrobial susceptibility and plasmid replicon type

Comparisons of drug-resistant and drug-susceptible isolates according to plasmid replicon content showed that several plasmid types occurred in a significantly higher proportion of resistant isolates ($p<0.05$) than of their susceptible counterparts (Table 4). Chi-squared distributions were also used to identify significant associations between plasmid replicon type and resistance phenotype (Table 5). Using both approaches, several replicon types were strongly associated with MDR. Replicons associated with the greatest numbers of resistance markers included IncA/C (resistance to AUG, AMP, AXO, CHL, CIP, FOX, GEN, KAN, STR, SXT, TET, and TIO), IncP1-α (GEN, KAN, NAL, STR, and TET), IncI1 (AMP, AUG, AXO, GEN, KAN, NAL, STR, TET, and TIO), and IncFIB (AUG, AXO, GEN, KAN, STR, and TIO).

Table 3. Prevalence of Antimicrobial Resistance and Plasmid Replicon Types Among 2202 *Escherichia coli* Isolates from Humans and Poultry

		Prevalence of trait within each group (column percent)									
Trait		*Avian*		*Human*				*Phylogenetic group*			
Category	*Specific trait*	*APEC* (n=*909*)	*Avian fecal* (n=*422*)	*UPEC* (n=*559*)	*NMEC* (n=*70*)	*Human fecal* (n=*156*)	*Human vaginal* (n=*86*)	*A* (n=*641*)	*B1* (n=*310*)	*B2* (n=*753*)	*D* (n=*498*)
Resistance	AMI	0	0	0	0	0	0	0	0	0	0
	AUG	19	13.0	0.9	4.3	2.6	0	19.5	16.8	3.6	7.2
	AMP	34.4	26.5[a]	36.5[b]	25.7[c]	20.5[d]	43	38.4	31.9	30.5	28.3
	FOX	15.6	12.6	0.0	0	1.3	0	17.2	11	2.7	6.6
	TIO	11.9	10.7	0	0	0	0	13.1	10	1.5	5.4
	AXO	13.6	12.6	0	0	0	0	15.1	10.0	2.9	5.4
	CHL	9.5	9.2	9.8[b]	4.3[c]	3.2[d]	8.1	11.9	7.7	6.1	9.8
	CIP	1	0.2	0.4	0	0	0	1.1	0.6	0.1	0.4
	GEN	25	17.1[a]	1.3	0	0	1.2	18.7	23.2	5.8	14.3
	KAN	24.6	18.1[a]	8.2[b]	1.4	1.3	2.3	21.5	18.7	11.3	14.1
	NAL	4	4.3	1.6	0	0	3.5	3.4	5.5	1.7	2.8
	STR	52.5	44.5[a]	21.1[b]	22.9[b]	7.1[d]	30.2	47.6	43.5	24.3	42.8
	SXT	11.1	7.3[a]	16.5[b]	7.1	8.3	9.3	9.8	10.0	10.6	15.3
	TET	35.8	49.1[a]	22.4[b]	15.7	13.5	17.4	40.7	38.1	19.1	36.3
Replicon	B/O	14	4.3[a]	14.5	48.6[b,c]	14.1	7	16.2	13.5	10.9	12
	FIC	6.8	5	1.1	4.3	2.6	1.2	5.1	4.5	2.9	5.6
	A/C	6.7	4	0.9	0	0	0	8.6	3.2	1.6	1.2
	P	19.4	8.1[a]	0.7	11.4[b,c]	1.3	2.3	14.2	15.2	3.1	13.1
	T	0.4	0	0	0	0	0	0.2	0	0	0.6
	K/B	1.4	0.9	0	2.9	0.6	0	0.8	1.6	0.7	1
	W	0	0	0.2	0	0	1.2	0	0	0	0.4
	FIIA	12.8	4.7[a]	3	1.4	1.3	0	5.1	9	2.7	15.1
	FIA	2.1	5.5[a]	2.5	1.4	3.2	0	3.4	2.6	2.7	2.4
	FIB	84.8	32.7[a]	32.9[b,c]	85.7[b,c]	44.9[d]	66.3	60.4	51	56.3	62.4
	Y	3.7	1.9	2	1.4	4.5	4.7	3.3	5.8	1.5	3
	I1	35.9	32.2	4.3	5.7	7.1	5.8	35.1	36.8	9.6	19.1
	X	0.3	0.5	0	0	0	1.2	0.5	0.3	0.3	0
	HI1	3.7	7.3	2.5	0	1.3	0	2.5	3.9	3.5	5.4
	N	7.8	3.8[a]	0.2	0	0	0	2	1.3	1.2	12.4
	HI2	3	0.9[a]	0.2	0	0	0	2.2	1.9	0.4	1.8
	L/M	2.8	4.5	0	0	0.6	0	0.8	3.5	2.1	2.6

[a]Indicates significantly different from APEC ($p<0.05$).
[b]Indicates UPEC or NMEC significantly different from human fecal *E. coli* ($p<0.05$).
[c]Indicates UPEC or NMEC significantly different from human vaginal *E. coli* ($p<0.05$).
[d]Indicates human fecal *E. coli* significantly different from human vaginal *E. coli* ($p<0.05$).
AMI, amikacin; AUG, amoxicillin/clavulanic acid; AMP, ampicillin; FOX, cefoxitin; TIO, ceftiofur; AXO, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; and TET, tetracycline; Remaining traits are plasmid replicon types.

Associations of virulence gene content with antimicrobial susceptibility and plasmid replicon type

Among human isolates, significant associations of VF presence with individual resistance phenotype included those of *kII*, *pap*, *ibeA*, *fyuA*, *iutA*, *traT*, *iha*, and *afa* individually with AMP resistance; and of *ibeA*, *bmaE*, *iutA*, *gafD*, and *afa* individually with TET resistance (Supplementary Table S1; Supplementary Data are available online at www.liebertonline.com/fpd). Among avian isolates, significant associations included those of *cvaC*, *iss*, *iutA*, and *traT* individually with resistance to FOX, GEN, KAN, STR, and TIO, individually.

Similar analyses were performed to identify associations of plasmid replicon type with VFs (Supplementary Table S1). Among human isolates, highly significant associations included those of the IncFIB plasmid type with *kI*, *kII*, *malPAI*, *ibeA*, *fyuA*, *cvaC*, *iss*, *iutA*, *traT*, and *fliC* individually, and of the IncB/O plasmid type with *cvaC* and *iss* individually. Among avian isolates, significant associations included those of the IncFIB plasmid type with *kI*, *ireA*, *ibeA*, *fyuA*, *cvaC*, *iss*, *iutA*, and *traT* individually; of the IncN plasmid type with *pap*, *ompT*, *ireA*, *fyuA*, *cvaC*, *iss*, and *iutA* individually; and of several other plasmid types (i.e., IncB/O, IncA/C, IncP, IncFIIA, and IncI1) with *cvaC*, *iss*, and *iutA* individually.

Class 1 integron possession is associated with Tn21, Tn10, and multiple plasmid replicon types

Due to the previously established association of *E. coli* MDR with class 1 integrons, a subset of 1244 isolates were also examined for class 1 integron genes (*intI1*, *sulI*, and *qacEΔ1*), and *merA* and *tetA*, which are components of Tn*21* and Tn*10*,

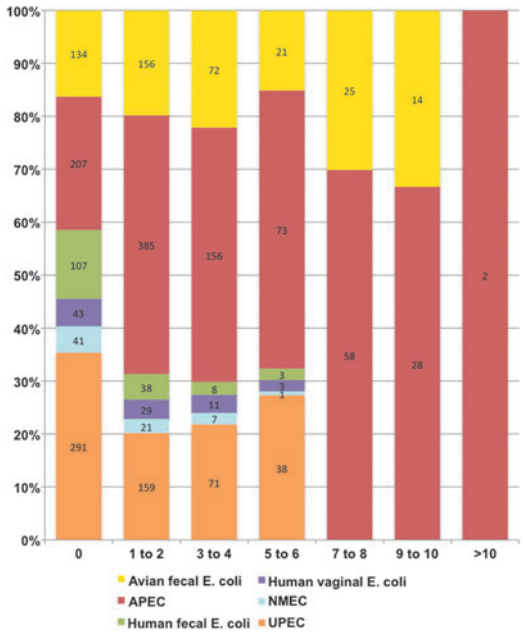

FIG. 1. Proportions (%) of isolate sources (y axis) relative to number of resistance phenotypes possessed (x axis). Each bar depicts the total source distribution of isolates relative to number of phenotypic resistances possessed.

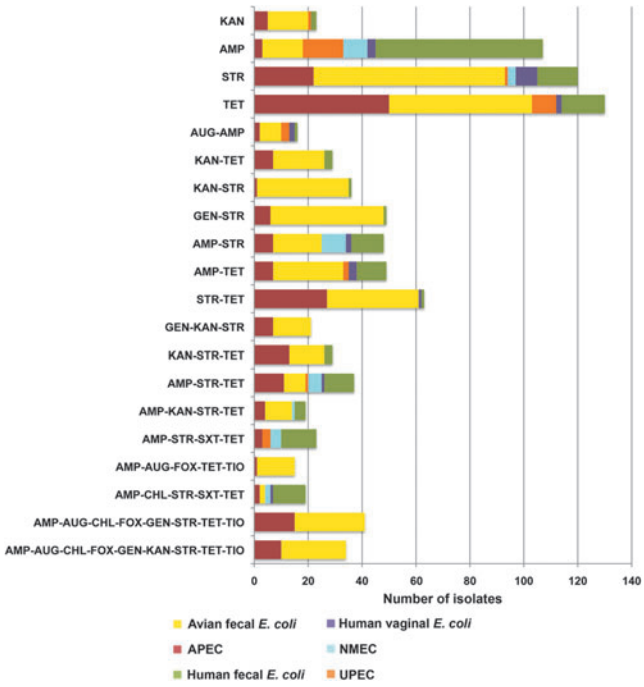

FIG. 2. Most prevalent antimicrobial resistance profiles among the 2202 *Escherichia coli* study isolates. The X-axis depicts the number of isolates possessing a given profile, using a stacked-bar presentation. Profiles with more than fifteen isolates were included.

respectively (Liebert *et al.*, 1999). The three class 1 integron genes were jointly present in 344 (27.7%) of the isolates examined, presumptively defining the presence of class 1 integrons (Table 6). Of the presumptive class 1 integron-containing isolates, 55.2% contained *merA* and 66.6% contained *tetA*, values significantly greater than for the remaining isolates (2.2 and 13.0%, respectively; $p < 0.001$). Plasmid replicon types significantly associated with class 1 integron positivity included IncA/C, IncP1-α, IncFIB, and IncI1. The only pathotype significantly associated with class 1 integron positivity was APEC, which comprised 89% of the isolates positive for class 1 integron genes (vs. 59.7% of class 1 integron-negative isolates; $p < 0.0001$).

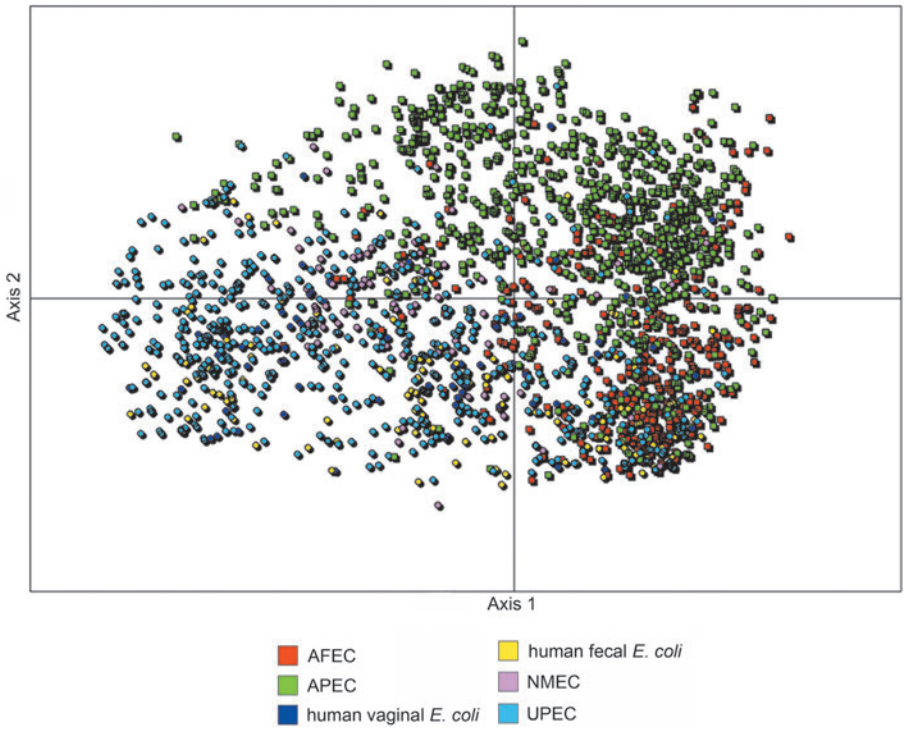

FIG. 3. Principal coordinates analysis of 2202 human and avian *Escherichia coli* isolates based on 67 traits, grouped by source of isolation. The analysis included antimicrobial susceptibility, plasmid replicon content, phylogenetic group, and virulence gene content. Data shown are the axis 1-versus-axis 2 plot. The axis 1-versus-axis 3 and axis 2-versus-axis 3 plots yielded similar findings (not shown).

Table 4. Prevalence (%) of Plasmid Replicon Types Among Antimicrobial-Resistant (R) and Antimicrobial-Susceptible (S) *Escherichia coli* Isolates for Each Antimicrobial Agent Tested

	AUG[a]		*AMP*		*FOX*		*TIO*		*AXO*		*CHL*		*CIP*		*GEN*		*KAN*		*NAL*		*STR*		*SXT*		*TET*	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
B/O	14.2	12.9	14.1	12.6	10.2	13.4	9.2	13.4	8.5	13.5	7.7	13.6	0.0	13.2	7.8	13.9	15.7	12.6	10.6	13.2	12.2	13.6	10.4	13.4	10.7	14.2
FIC	4.2	4.4	3.4	4.9	4.6	4.4	1.3	4.6	1.7	4.6	2.6	4.6	0.0	4.4	8.8	3.7	4.0	4.5	0.0	4.5	**6.2**[b]	**3.3**	6.4	4.1	1.6	5.7
A/C	**28.8**	**0.7**	**9.9**	**0.8**	**34.0**	**0.8**	**40.5**	**1.0**	**35.6**	**1.0**	**33.0**	**0.9**	**25.0**	**3.7**	**20.5**	**1.1**	**7.4**	**3.1**	6.1	3.7	**8.4**	**1.0**	**7.2**	**3.3**	**8.8**	**1.4**
P	13.3	9.9	10.8	10.0	13.7	9.9	13.1	10.1	9.0	10.4	9.8	10.3	16.7	10.2	**32.6**	**6.6**	**13.7**	**9.6**	**19.7**	**10.0**	**15.0**	**7.4**	11.6	10.1	**16.1**	**7.5**
T	0.0	0.2	0.0	0.3	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	1.0	0.1	0.0	0.2	0.0	0.2	0.5	0.0	0.0	0.2	0.0	0.3
K/B	**2.9**	**0.7**	1.3	0.7	3.6	0.6	**4.6**	**0.6**	4.0	0.6	0.5	0.9	0.0	0.9	0.3	1.0	0.9	0.9	0.0	0.9	0.5	1.2	0.4	1.0	1.1	0.8
W	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1
FIIA	3.8	7.5	4.7	8.2	2.0	7.6	0.7	7.6	1.7	7.6	1.0	7.7	**25.0**	**7.0**	**11.7**	**6.3**	9.1	6.7	4.5	7.2	7.7	6.7	**11.2**	**6.6**	2.1	9.4
FIA	2.5	2.9	3.5	2.5	3.6	2.7	2.0	2.9	2.8	2.8	5.7	2.5	**16.7**	**2.7**	2.0	3.0	**6.3**	**2.2**	**9.1**	**2.6**	3.8	2.2	6.0	2.4	**4.3**	**2.1**
FIB	**76.3**	**55.9**	59.8	57.4	78.7	56.1	**79.7**	**56.5**	**79.7**	**56.3**	61.3	57.9	66.7	58.1	**73.9**	**55.6**	**67.2**	**56.4**	66.7	57.9	**65.9**	**53.4**	56.4	58.3	57.0	58.7
Y	2.5	3.0	3.2	2.8	2.5	3.0	2.0	3.0	2.8	3.0	1.5	3.1	0.0	3.0	2.3	3.1	2.8	3.0	0.0	3.0	2.5	3.2	2.4	3.0	1.8	3.5
I1	**40.4**	**20.8**	**26.0**	**21.5**	**37.1**	**21.6**	**34.6**	**22.1**	**32.8**	**22.1**	17.5	23.5	16.7	23.0	**50.5**	**18.5**	**37.3**	**20.3**	**36.4**	**22.6**	**33.9**	**16.3**	26.0	22.6	**29.0**	**20.2**
X	0.0	0.3	0.1	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3	1.1	0.1	1.5	0.2	0.0	0.4	0.0	0.3	0.4	0.2
HI1	4.2	3.6	4.5	3.3	4.1	3.6	5.9	3.5	6.2	3.5	5.7	3.5	**16.7**	**3.6**	5.2	3.4	3.4	3.7	4.5	3.7	4.7	3.1	3.6	3.7	**7.4**	**1.9**
N	1.3	4.3	1.8	5.1	2.5	4.1	1.3	4.2	2.3	4.2	1.0	4.3	0.0	4.0	1.6	4.4	3.1	4.2	4.5	4.0	4.3	3.8	1.6	4.3	1.3	5.3
HI2	2.5	1.3	1.5	1.4	2.5	1.3	0.7	1.5	1.1	1.5	0.5	1.5	8.3	1.4	2.3	1.3	2.0	1.4	1.5	1.5	**2.6**	**0.7**	1.6	1.4	1.1	1.6
L/M	2.1	2.0	2.2	2.0	1.5	2.1	1.3	2.1	2.3	2.0	0.5	2.2	**16.7**	**2.0**	1.6	2.1	0.9	2.3	3.0	2.0	2.5	1.8	1.2	2.1	**4.3**	**1.0**

[a]AMI was not included, because no resistant isolates were identified.
[b]Bold numbers indicate significant differences in prevalence ($p < 0.05$) by using Fisher's Exact Test.

Table 5. Chi-Squared Distributions Between Plasmid Replicon Type and Resistance Phenotype on 2202 Isolates

	AMI	*AUG*	*AMP*	*FOX*	*TIO*	*AXO*	*CHL*	*CIP*	*GEN*	*KAN*	*NAL*	*STR*	*SXT*	*TET*
B/O							+[a]		+					+
FIC									+			+		++
A/C		++	++	++	++	++	++	+	++	+		++	+	++
P									++	+	+	++		++
T									+			+		
K/B														
W														
FIIA		+	+	+	+		++	+	+				+	++
FIA							+	+		++	+	+		+
FIB		++		++	++	+			++	+		++		
Y														+
I1		++	+		+	+			++	++	+	++		++
X										+				
HI1														++
N		+	+				+		+				+	++
HI2												+		
L/M								+						++

[a]Indicates a positive association between traits ("+" = $p < 0.05$; "++" = $p < 0.0001$).

Principle Coordinates Analysis to compare source groups

For an integrated analysis, PCoA was used to collapse the entire dataset into a small number of derived variables (i.e., principal coordinates or axes). The PCoA showed a separation of isolates based on source of isolation (Fig. 3), in which avian-source isolates were usually separated from human-source isolates. The overall ANOVA was highly significant ($p < 0.01$), although only 18% of the variance in the dataset was explained by between-population differences, whereas 82% was explained by within-population differences. In pairwise comparisons between individual source groups according to their values on individual PCoA axes using MANOVA, all groups were significantly different ($p < 0.05$) from one another on one or more axes (Supplementary Table S2).

Discussion

The goal of this study was to assess and compare avian and human ExPEC and commensal *E. coli* for their antimicrobial susceptibility, and to identify correlations between antimicrobial resistance phenotype, plasmid replicon possession, virulence factor possession, and *E. coli* phylogenetic group membership. Multiple studies have examined the antimicrobial susceptibilities of ExPEC and commensal *E. coli*, including *E. coli* isolated from commercial poultry, the commercial farm environment, retail poultry meat, human UTI, and the fecal flora of healthy humans and animals (Schroeder *et al.*, 2003; Yang *et al.*, 2004; Johnson *et al.*, 2005a, 2005b; Zhao *et al.*, 2005; Miles *et al.*, 2006; Diarrassouba *et al.*, 2007; Wallmann *et al.*, 2007; Khaitsa *et al.*, 2008; Ozawa *et al.*, 2008; Ahmed *et al.*, 2009; Bonnet *et al.*, 2009; Cook *et al.*, 2009). However, fewer studies have explored the correlations between antimicrobial resistance and genetic traits, with contrasting results regarding the correlations between antimicrobial resistance phenotype, phylogenetic distribution, and virulence gene content (Johnson *et al.*, 2009b).

Our results showed that MDR and the plasmids and mobile elements encoding for MDR are widespread among avian-source *E. coli*, irrespective of the clinical status of their host of isolation, while being only sporadically found among human-source *E. coli* isolates. When we compared VFs and resistance phenotype, certain highly significant correlations were observed. These included correlations between CHL resistance and possession of *iha* and *pap*; AMP resistance and possession of *afa*, *iha*, and *iutA*; and SXT resistance and possession of *afa* and *iutA*. Precedent exists for the association between VF and MDR. For example, it has been previously shown that MDR is common in bovine isolates carrying the afimbrial AfaE-VIII adhesin (Girardeau *et al.*, 2003). In addition to these positive associations, though, a greater number of individual VFs were negatively associated with resistance phenotypes, including genes such as *kI*, *kII*, *papACEFG*, *ompT*, *fyuA*, *sfa*, *fliC*, and *cdtB*, and resistances including FOX, GEN, STR, TET, and TIO. The nature of these negative associations remains unclear.

A notable finding was the strong positive association of avian-source isolates harboring APEC-associated VFs (*cvaC*, *iss*, *iutA*, and *traT*), and a number of plasmid types (IncB/O,

Table 6. Plasmid Replicons Associated with Class 1 Integron-Positive Isolates

	Prevalence of trait, column %		
Trait	*Integron-positive*[a] (n = *344*)	*Integron-negative*[a] (n = *900*)	p-*value*
merA	55.2	2.2	<0.0001
tetA	66.6	13.0	<0.0001
A/C	15.1	0.9	<0.0001
P	42.2	4.6	<0.0001
FIB	83.7	73.4	<0.0001
I1	50.9	19.0	<0.0001
Avian fecal	4.9	13.1	<0.0001
APEC	89.0	59.7	<0.0001
NMEC	2.9	6.6	0.012
UPEC	3.2	20.7	<0.0001

[a]Integron-positive isolates were defined as those possessing *intI1*, *qacEΔ1*, and *sul1*. Only the traits significantly differing ($p < 0.05$) between integron-positive and integron-negative groups are shown.

IncA/C, IncP1-α, IncFIIA, IncFIB, IncI1, and IncN), with certain resistance phenotypes (FOX, GEN, KAN, STR, TET, and TIO). These APEC VFs typically are encoded on IncFIB/IncFIIA plasmids known as ColV plasmids (Johnson *et al.*, 2006a, 2006b). Although some ColV plasmids have been shown to contain resistance modules, they seem to be rare among ColV plasmids and typically involve Tn*10*-like elements encoding a limited number of resistances (Mellata *et al.*, 2009; Fricke *et al.*, 2009a). However, APEC strains also commonly possess ColV virulence plasmids with co-transferring R plasmids (Johnson *et al.*, 2005c, 2006c, 2007c). The results of our replicon typing suggest that co-transferring ColV and MDR-encoding plasmids are widely prevalent among APEC isolates. Seemingly, then, the presence of an F plasmid in an avian *E. coli* strain might enhance its ability to acquire and disseminate other MDR-encoding plasmids, such as IncA/C, IncI1, and IncP1-α plasmids. Certainly, though, the complexities of the poultry production environment could also drive the selection of multidrug-resistant APEC, as multiple selective pressures exist (Singer and Hofacre, 2006). The mechanisms driving the emergence of co-transferring ColV plasmids and MDR-encoding plasmids need to be further investigated.

Regarding the commonality (or lack thereof) between human and avian-source *E. coli*, definitive conclusions are limited by our inclusion of isolates that differ temporally, geographically, and by source of isolation. Nonetheless, our results, as exemplified by the PCoA, suggest that although some overlap exists between isolates from poultry and humans, overall relatively few human-source isolates resemble the pool of avian-source isolates. Certainly, the human ExPEC isolates generally lacked MDR, whereas the APEC isolates had a high occurrence of MDR, and the two groups have previously been shown to differ in their VF content (Johnson *et al.*, 2004, 2005b, 2007a, 2008, 2009a). However, within-group variation was extensive, and some of the multidrug-resistant avian-source isolates fell within the human-source clusters, and vice versa. This is supportive of previous work suggesting that certain subsets of ExPEC are capable of zoonotic transfer (Johnson *et al.*, 2007a, 2008). Thus, although the potential for zoonotic transmission of multidrug resistant avian-source clones to humans probably does exist (Johnson *et al.*, 2007b; Manges *et al.*, 2007; Price *et al.*, 2007; Jakobsen *et al.*, 2010), the actual frequency of such transmission relative to the entire human and avian ExPEC populations might be relatively low.

The main MDR-associated plasmid types in this study were IncA/C, IncP1-α, IncF, and IncI1. IncA/C plasmids have received extensive recent attention because of their emergence in human clinical and production animal settings, broad host range, and ability to encode for extended-spectrum β-lactamases (ESBLs) (Welch *et al.*, 2007; Fricke *et al.*, 2009b; Call *et al.*, 2010; Suzuki *et al.*, 2010; Veldman *et al.*, 2010). The IncA/C plasmids identified were exclusive to avian-source isolates and were associated with resistance to 12 or more of the antibiotics tested. IncI1 and IncF plasmids have also been associated with ESBL genes and MDR (Garcia-Fernandez *et al.*, 2008; Marcade *et al.*, 2009; Woodford *et al.*, 2009; Smet *et al.*, 2010; Sampei *et al.*, 2010), and IncP1-α plasmids include the "Birmingham" plasmids known for their broad host range, promiscuity, and carriage of resistance genes (Thomas and Smith, 1987). Although MDR-encoding IncI1, IncF, and IncP1-α plasmids were identified among human-source *E. coli* isolates, their prevalence was low compared with that of avian-source isolates. This again suggests that the transfer of these elements, or of *E. coli* clones possessing these plasmids, from poultry to humans might be rare. However, these isolates were derived from multiple geographical sources, and these populations are rapidly changing with regard to plasmid possession and resulting MDR phenotypes. This underscores the need for continued monitoring for these mobile genetic elements.

Conclusions

Although disagreement exists regarding to what extent multidrug-resistant, poultry-associated strains have emerged and are persisting due to antimicrobial usage in the poultry production environment (Smith *et al.*, 2007a), it is evident that MDR is now widespread in *E. coli* of poultry origin and is associated with conjugative plasmids. It should be acknowledged that there is bias in the dataset analyzed here with regard to geographical and temporal origins, thus limiting our ability to draw conclusions with regard to antimicrobial resistance phenotypes between the groups analyzed. However, this did not hamper our observations regarding the strong correlations between resistance phenotype and plasmid content. It is essential that future efforts address the risk of transfer of such plasmids from food animal bacterial populations to humans, and the underlying biological mechanisms enabling the dissemination and persistence of these plasmids among bacterial populations.

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Disclosure Statement

No competing financial interests exist.

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