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Plasmid Replicon Typing of Commensal and Pathogenic *Escherichia coli* Isolates[▽]

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Despite the critical role of plasmids in horizontal gene transfer, few studies have characterized plasmid relatedness among different bacterial populations. Recently, a multiplex PCR replicon typing protocol was developed for classification of plasmids occurring in members of the *Enterobacteriaceae*. Here, a simplified version of this replicon typing procedure which requires only three multiplex panels to identify 18 plasmid replicons is described. This method was used to screen 1,015 *Escherichia coli* isolates of avian, human, and poultry meat origin for plasmid replicon types. Additionally, the isolates were assessed for their content of several colicin-associated genes. Overall, a high degree of plasmid variability was observed, with 221 different profiles occurring among the 1,015 isolates examined. IncFIB plasmids were the most common type identified, regardless of the source type of *E. coli*. IncFIB plasmids occurred significantly more often in avian pathogenic *E. coli* (APEC) and retail poultry *E. coli* (RPEC) than in uropathogenic *E. coli* (UPEC) and avian and human fecal commensal *E. coli* isolates (AFEC and HFEC, respectively). APEC and RPEC were also significantly more likely than UPEC, HFEC, and AFEC to possess the colicin-associated genes *cvaC*, *cbi*, and/or *cma* in conjunction with one or more plasmid replicons. The results suggest that *E. coli* isolates contaminating retail poultry are notably similar to APEC with regard to plasmid profiles, with both generally containing multiple plasmid replicon types in conjunction with colicin-related genes. In contrast, UPEC and human and avian commensal *E. coli* isolates generally lack the plasmid replicons and colicin-related genes seen in APEC and RPEC, suggesting limited dissemination of such plasmids among these bacterial populations.

Naturally occurring bacterial plasmids are extremely diverse, self-replicating extrachromosomal elements that encode a variety of traits, including antibiotic and heavy metal resistance, virulence, and environmental adaptability and persistence (10, 14–18). Plasmids are also important agents of horizontal gene transfer (HGT), playing a major role in bacterial adaptation to environmental change (32). Additionally, plasmids contribute to genome plasticity by their carriage of mobile genetic elements, such as insertion sequences and transposons, that can interact with the bacterial chromosome and contribute to homologous or nonhomologous recombination (10).

Due to their role in HGT, especially with regard to the emergence and dissemination of antimicrobial resistance (10, 12), much attention has been paid to the identification and classification of bacterial plasmids. Plasmids have historically been classified according to their incompatibility with other plasmids, a property that is related to their replication (5). Incompatibility (Inc) typing is based on the fact that two plasmids sharing common replication and partitioning elements are unable to proliferate stably in the same cell line (5). Since Inc typing is based on replication factors, the terms Inc and Rep to describe plasmid types have been used interchangeably (3, 5, 10). Currently, there are 26 known Inc groups occurring among the *Enterobacteriaceae*, a number which has remained static for several years (5, 10). Classification of plasmids into Inc groups is desirable because specific plasmid types have been associated with virulence and/or antimicrobial resistance (1, 2, 4, 11, 12, 14–18, 22, 23, 35, 36).

Unfortunately, physical Inc testing is tedious, particularly when applied to large bacterial populations. In 1988, Couturier and coworkers (5) developed a hybridization method for the comprehensive typing of bacterial plasmids according to replicon type. From this study, a bank of Rep probes corresponding to 19 different Inc groups in the *Enterobacteriaceae* was developed (5). Although this procedure represented a significant advance in plasmid typing, the method was time-consuming, labor-intensive, and incompatible with current high-throughput approaches. Recognizing a need for a streamlined procedure, Carattoli et al. (3) demonstrated that a PCR-based replicon typing protocol could be used to detect 18 plasmid replicons frequently found among the *Enterobacteriaceae*. Although their procedure is greatly simplified, it still requires several steps, including five multiplex and three simplex PCR procedures. Therefore, it would be tedious and costly to apply this procedure to large collections of isolates.

Extraintestinal pathogenic *E. coli* (ExPEC) is a pathotype causing a wide variety of diseases of humans and animals. This group has been found to commonly possess plasmids associated with virulence and antimicrobial resistance (6, 14–18, 26,

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TABLE 1. Bacterial strains used in this study

Group	Source	Location(s)	Dates of isolation
APEC	Colibacillosis lesion sites	Multiple poultry operations in Georgia, Minnesota, California, Nebraska	1994–2004
AFEC	Feces of healthy birds	Multiple poultry operations in Minnesota and North Dakota	1994–1998
RPEC	Retail poultry	Maryland, Georgia, and Oregon retail grocers	1998–2000
UPEC	Human UTI	North Dakota hospital	2002–2003
HFEC	Feces of healthy humans	Healthy volunteers in Iowa, Minnesota, and North Dakota	1995–2004

34). This is particularly true within the commercial poultry environment, where ExPEC isolates causing colibacillosis—referred to as avian pathogenic *E. coli* [APEC]—have abundant plasmids (7, 14–18). In fact, we have recently reported that ColV and ColBM virulence plasmids are a defining trait of the APEC pathotype (16, 17, 26). These plasmids have been shown to belong to the IncFIB incompatibility group and contain a highly conserved region harboring the FIB replicon, the ColV and/or ColBM operons, and several known virulence genes and iron acquisition and transport operons (16, 17). Additionally, these and other plasmids encoding multiple drug resistances have been isolated from APEC and uropathogenic *E. coli* (UPEC) (14–18).

Recent work has also focused on the possibility of APEC as a food-borne source of virulent clones with the capacity to cause human extraintestinal diseases, such as urinary tract infections (UTIs) and neonatal meningitis (27, 28). However, while some ExPEC isolates of humans and birds are similar, most appear to have distinct differences (27). Therefore, a more likely scenario would be that APEC serves as a reservoir of plasmid-linked virulence and/or resistance genes for other ExPEC isolates via contaminated poultry meat. However, definitive statements about this cannot be made without a better

TABLE 2. Primers used in plasmid replicon typing studies

Replicon	Primer		Annealing temp (°C)	Amplicon size (bp)
	Direction	Sequence (5′ to 3′)		
Panel 1				
B/O	F	GCGGTCCGGAAAGCCAGAAAAC	60	159
	R	TCTGCGTTCCGCCAAGTTCGA		
FIC	F	GTGAACTGGCAGATGAGGAAGG	60	262
	R	TTCTCCTCGTCGCCAAACTAGAT		
A/C	F	GAGAACCAAAGACAAAGACCTGGA	60	465
	R	ACGACAAACCTGAATTGCCTCCTT		
P	F	CTATGGCCCTGCAAACGCGCCAGAAA	60	534
	R	TCACGCGCCAGGGCGCAGCC		
T	F	TTGGCCTGTTTGTGCCTAAACCAT	60	750
	R	CGTTGATTACACTTAGCTTTGGAC		
Panel 2				
K/B	F	GCGGTCCGGAAAGCCAGAAAAC	60	160
	R	TCTTTCACGAGCCCGCCAAA		
W	F	CCTAAGAACAACAAAGCCCCCG	60	242
	R	GGTGCGCGGCATAGAACCGT		
FIIA	F	CTGTCGTAAGCTGATGGC	60	270
	R	CTCTGCCACAAACTTCAGC		
FIA	F	CCATGCTGGTTCTAGAGAAGGTG	60	462
	R	GTATATCCTTACTGGCTTCCGCAG		
FIB	F	GGAGTTCTGACACACGATTTTCTG	60	702
	R	CTCCCGTCGCTTCAGGGCATT		
Y	F	AATTCAAACAACACTGTGCAGCCTG	60	765
	R	GCGAGAATGGACGATTACAAAACTTT		
Panel 3				
I1	F	CGAAAGCCGGACGGCAGAA	60	139
	R	TCGTCGTTCCGCCAAGTTCGT		
Frep	F	TGATCGTTTAAGGAATTTTG	60	270
	R	GAAGATCAGTCACACCATCC		
X	F	AACCTTAGAGGCTATTTAAGTTGCTGAT	60	376
	R	TGAGAGTCAATTTTTATCTCATGTTTTAGC		
HI1	F	GGAGCGATGGATTACTTCAGTAC	60	471
	R	TGCCGTTTCACCTCGTGAGTA		
N	F	GTCTAACGAGCTTACCGAAG	60	559
	R	GTTTCAACTCTGCCAAGTTC		
HI2	F	TTTCTCCTGAGTCACCTGTTAACAC	60	644
	R	GGCTCACTACCGTTGTCATCCT		
L/M	F	GGATGAAAACTATCAGCATCTGAAG	60	785
	R	CTGCAGGGGCGATTCTTTAGG		

understanding of the plasmid content of *E. coli* within these environments. Thus, the aim of this study was to employ a simplified replicon typing procedure to determine the plasmid replicon profiles of 1,015 *E. coli* isolates. These isolates, which included commensal and pathogenic *E. coli* isolates obtained from avian and human hosts and isolates from retail poultry meat, were analyzed to determine if these groups share similar plasmid profiles.

MATERIALS AND METHODS

Bacterial strains and plasmids. Isolates used for the plasmid replicon typing studies were obtained from a variety of sources within the United States (Table 1). Of the 1,015 isolates in this study, 422 originated from sites of infection in birds clinically diagnosed with colibacillosis (APEC), and 92 were commensal strains obtained from fecal swabs of apparently healthy chickens and turkeys (avian fecal *E. coli* [AFEC]). These originated from a variety of locations, including California, Georgia, Minnesota, Nebraska, North Dakota, and Iowa (26, 27). Two hundred strains were isolated from retail chicken breasts (retail poultry *E. coli* [RPEC]) obtained from three FoodNet laboratories (Maryland, Georgia, and Oregon) participating in the National Antimicrobial Resistance Monitoring System (http://www.fda.gov/cvm/narms_pg.html). One hundred one strains were isolated from the feces of healthy, antibiotic-free humans (human fecal *E. coli* [HFEC]) from Iowa, Minnesota, and North Dakota, and 200 isolates originated from cases of human UTI (UPEC) from a hospital in North Dakota (27).

Positive controls used in the replicon typing procedure were kindly provided by Alessandra Carattoli (Istituto Superiore di Sanità, Rome, Italy). These were originally created within the laboratory of Werner K. Maas and have been described elsewhere (3, 5). *E. coli* DH5α was used as a negative control for all of the genes studied (29). Additionally APEC O1, known to possess plasmids containing the IncFIB and IncHI2 replicons and to harbor *cma* and *cbi* (17, 18), and APEC O2, known to possess plasmids containing the IncFIB and IncFIIA replicons and to harbor *cvaC* (15, 16), were used as positive wild-type control strains. All bacterial strains were stored promptly at −70°C in brain heart infusion broth (Difco Laboratories, Detroit, MI) with 10% glycerol until use (30).

Multiplex PCR for plasmid replicon typing and colicin-associated genes. *E. coli* isolates were examined for the presence of 18 plasmid replicons using three multiplex panels (Table 2). Isolates were also surveyed for their possession of *cvaC*, the structural gene of the ColV operon, *cma*, the colicin M activity gene, and *cbi*, the colicin B immunity gene, as previously described (16, 17). All primers, annealing temperatures, and expected amplicon sizes are listed in Table 2. Primers were obtained from Integrated DNA Technologies (Coralville, IA). Template DNA for PCR was prepared using a boiling lysis procedure as previously described (13). Targeted genes were amplified in a multiplex panel using a simplified version of the multiplex PCR technique described by Carattoli et al. (3). That is, the eight panels previously used for PCR were reduced to three (Table 2). PCR was performed using AmpliTaq polymerase Gold (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Conditions used for PCR were as follows: 5 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 60°C, and 90 s at 72°C; and a final extension of 5 min at 72°C. Amplicons were visualized on 1.5% Tris-acetate-EDTA agarose gels alongside a 1-kb ladder (Promega Corp.), and if an amplicon of the expected size was observed, then an isolate was considered positive for that particular gene. To validate the multiplex panels, amplicons from the positive controls were excised from agarose, purified, and bidirectionally sequenced using their respective primers. The amplicon sequences were compared to the National Center for Biotechnology Information database using BLAST.

Biostatistics. Two-way frequency tables of source population (AFEC, APEC, RPEC, HFEC, and UPEC) versus trait (plasmid replicon type, *cvaC*, *cma*, and *cbi*) were generated to allow comparisons of the frequency of occurrence of the plasmid replicons and colicin-associated genes across populations (33). Because there were a number of traits being assessed simultaneously in this study, a resampling-based multiplicity adjustment to the Fisher's exact test results was applied using the MULTTEST procedure in SAS (38). Comparisons of the proportions of isolates from systemic disease and cellulitis for each plasmid replicon were also made using Fisher's exact test with a resampling-based multiplicity adjustment to control type I error (38). Utilizing the principle of small multiples in graphical display (37), a color-coded matrix icon was created for each trait to summarize all possible pairwise comparisons among the five populations in two ways.

TABLE 3. Occurrence of plasmid replicons and plasmid-linked genes among various *E. coli* isolates

Trait	% of isolates possessing trait				
	AFEC (n = 92)	APEC (n = 422)	RPEC (n = 200)	HFEC (n = 101)	UPEC (n = 200)
Replicons					
B/O	4.4	19.2	1.5	19.8	24.0
FIC	7.6	12.1	1.5	4.0	2.0
A/C	0.0	3.1	3.0	1.0	0.0
P	4.4	21.8	15.0	0.0	2.0
T	0.0	0.0	0.0	0.0	0.0
K/B	0.0	1.0	3.0	0.0	0.0
W	0.0	0.0	0.5	0.0	0.5
FIIA	8.7	23.7	3.0	1.0	5.0
FIA	9.8	1.4	0.0	3.0	1.5
FIB	51.1	87.2	86.0	45.5	56.0
Y	1.1	4.3	5.5	6.0	3.5
I1	17.4	41.0	34.5	6.9	6.5
Frep	67.0	90.0	92.5	53.5	72.5
X	0.0	0.0	1.5	0.0	0.0
HI1	0.0	1.2	3.0	4.0	2.0
N	10.9	16.1	0.0	0.0	0.0
HI2	3.3	4.3	0.0	0.0	0.0
L/M	0.0	0.7	3.0	0.0	0.0
Genes					
cvaC	10.9	68.0	36.0	3.0	7.5
cma	8.7	24.0	24.0	4.0	3.0
cbi	14.1	33.4	36.5	4.0	4.5

Plasmid profiles were obtained by concatenating the presence or absence of each trait in a prespecified order into a character string variable using SAS. A one-way frequency table of this new variable allowed easy assessment of the most commonly occurring profiles. A two-way frequency table of this plasmid profile variable versus source population enabled comparisons of unique plasmid profiles across the populations (38).

Average linkage cluster analysis based upon Jaccard distances calculated from the presence or absence of the various virulence factors among the isolates was used to look for groups or clusters of isolates among the five populations. A composite graphic combining the dendrogram from this cluster analysis, color-coded bands for each source population, and a modified heat map illustrating the presence or absence of each trait for each isolate was generated to allow assessment of all data elements for all isolates simultaneously (27).

RESULTS

Validation of the simplified plasmid replicon typing procedure. The replicon typing procedure used in this study was a modified version of that described by Carattoli et al. (3). Changes in this procedure included (i) a reduction in the number of overall PCRs per isolate from eight to three; (ii) use of a boiling lysis preparation rather than a commercial genomic DNA purification kit; and (iii) use of a universal cycling procedure for all three panels. These changes resulted in an overall decrease in the time, materials, and costs to type these isolates. To validate the simplified procedure, we tested the three multiplex panels against the following: known positive controls, containing cloned plasmid replicons; the negative control *E. coli* DH5α, containing no plasmid replicons; and APEC strains O1 and O2, containing multiple plasmids which were previously sequenced and analyzed (5, 14–18). The cloned replicons produced bands of expected sizes for their respective replicon types. Also as expected, APEC strains O1 and O2 were positive for the IncFIB and Frep amplicons, and

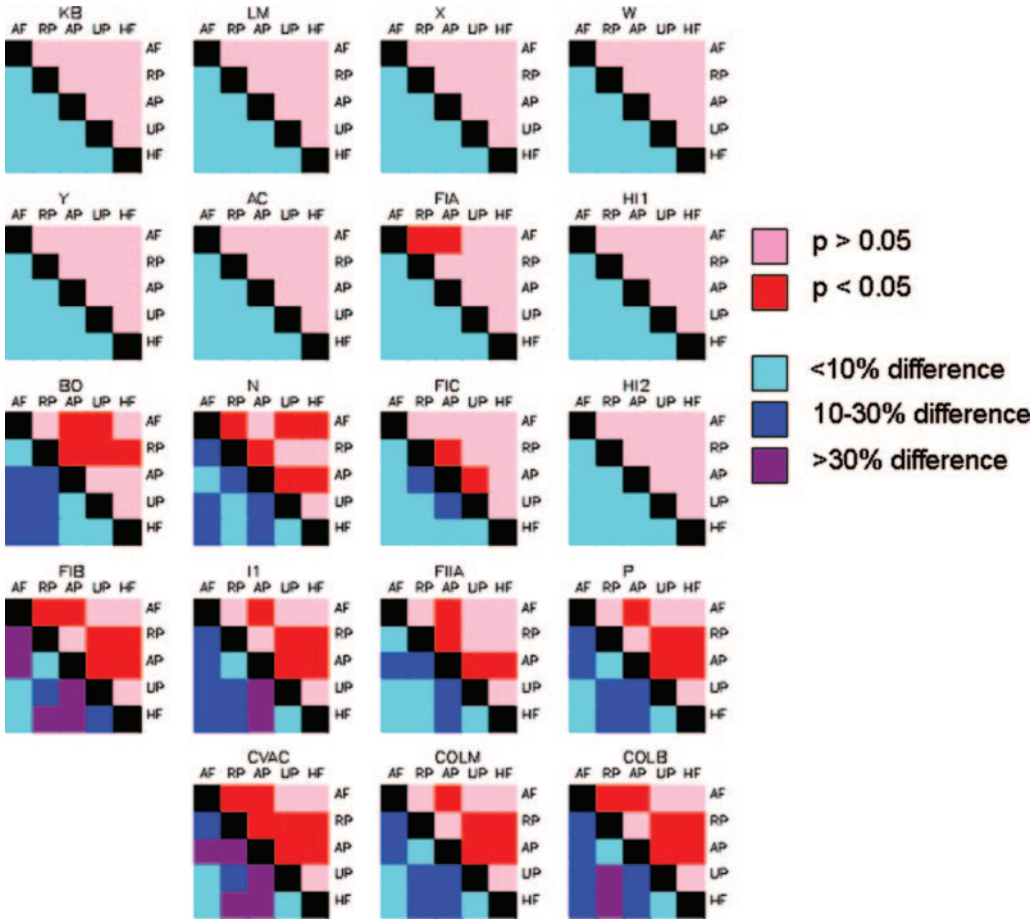

FIG. 1. Small multiple-matrix icons for each of the traits examined in this study. Each icon has 25 cells (5 rows by 5 columns). The order of groups within each icon was chosen to represent data from the commensal isolates on the edges of each matrix icon (far top and bottom and far left and far right), with the results for the pathogens (APEC and UPEC) in the center. Cells in the lower triangular area of each icon (i.e., below the black boxes) color code the absolute differences between pairs of populations, with cyan (light blue) indicating a difference of less than 10% in the incidence rates between two populations. Blue cells identify differences of between 10 and 30%, while purple cells show populations that differ by more than 30%. Cells in the upper triangular area of each icon (i.e., above the black boxes) code the results of the multiplicity-adjusted Fisher's exact tests. Pink means that the difference in the incidence rates between two populations was not significant, whereas red cells identify populations that show a difference in the incidence rates using a type I error rate of 5%. Cells on the diagonal, which would compare populations to themselves, are colored black to indicate that these comparisons are not meaningful. T and Frep results were omitted to reduce redundancy.

APEC O1 was also positive for the IncHI2 plasmid. No amplicons were observed in the negative control, *E. coli* DH5α. Sequencing and BLAST analysis verified that amplicons of expected sizes were specific for their respective replicons.

Prevalence of plasmid replicons and colicin-associated genes among APEC, UPEC, RPEC, HFEC, and AFEC. A total of 1,015 *E. coli* isolates were examined for the presence of the 18 plasmid replicon types using the simplified three-panel multiplex PCR protocol. The most commonly occurring amplicons in each of the five groups examined were Frep and IncFIB (Table 3). IncFIB was the most common replicon type found among APEC isolates, followed by IncI1, IncFIIA, IncP, IncB/O, IncN, and IncFIC. The IncFIB plasmid replicon was also the most common type occurring among AFEC isolates, followed by IncI1 and IncN. The *E. coli* isolates from retail poultry (RPEC) closely mirrored APEC with regard to the presence of IncFIB and IncI1 plasmid replicons (see the figures). Among the UPEC, IncFIB, and IncB/O plasmid replicons were most often identified, with other replicon types being much less frequently observed. Results for the human commensal isolates examined (HFEC) mirrored the UPEC results.

Matrix icons were used to better visualize differences among populations for the various traits examined (Fig. 1). These icons tell the viewer if the incidences of replicon types for the groups being compared are statistically different from one another (red or pink in the top half) and also how great the differences are between the groups (light blue, dark blue, or pink in the bottom half). In general, most of the significant differences observed were found among the IncN, IncFIB, IncI1, IncFIIA, and IncP replicons and in the distribution of *cvaC*, *cbi*, and *cma* genes. For these replicons, significant differences in prevalence were usually seen between APEC or RPEC and UPEC, HFEC, or AFEC, indicating distinct differences between these groups in terms of plasmid profiles. *cvaC*, *cbi*, and *cma*—genes of the ColV, ColB, and ColM colicin

TABLE 4. Plasmid replicons among APEC isolates from lesions associated with cellulitis or systemic disease

Replicon or gene	% of isolates with trait		*Z* value	*P* value[a]
	Cellulitis (n = 77)	Systemic disease (n = 292)		
B/O	6.5	21.2	2.98	0.0028
FIC	29.9	9.2	4.70	<0.0001
A/C	13.0	1.7	4.46	<0.0001
P	15.6	23.6	1.52	NS
T	0.0	0.0		NS
K/B	0.0	2.2	1.37	NS
W	0.0	0.0		NS
FIIA	31.2	25.3	1.03	NS
FIA	0.0	1.4	1.03	NS
FIB	92.2	85.6	3.03	0.0024
Y	3.9	4.1	0.08	NS
I1	63.6	31.8	5.10	<0.0001
Frep	90.9	91.8	0.24	NS
X	0.0	0.0		NS
HI1	0.0	1.4	0.05	NS
N	7.8	15.4	1.72	NS
HI2	9.1	2.4	2.73	0.0062
L/M	0.0	1.0	0.89	NS
cvaC	50.6	69.5	3.10	0.0019
cbi	46.8	33.2	2.20	0.0278
cma	36.4	25.0	1.99	0.0466

[a] *P* values are from resampling-based multiplicity-adjusted Fisher's exact tests for the null hypothesis that the percentages for each replicon are equal between the cellulitis and systemic isolates (33). NS, not statistically significant.

operons, respectively (16, 17)—were included to better discriminate among the different types of IncFIB-containing isolates. The *cvaC* gene occurred significantly more often among APEC isolates than all other *E. coli* isolates examined (Table 3 and Fig. 1). With the exception of APEC, *cvaC* occurred significantly more often in the RPEC isolates than it did in all the other groups. *cma* and *cbi* also occurred significantly more often among APEC and RPEC isolates than in the other groups of *E. coli* isolates (Table 3 and Fig. 1).

Prevalence of plasmid replicons and colicin-associated genes among APEC isolates associated with cellulitis or systemic disease. Because of expected differences in genotype between APEC isolates causing systemic disease and those causing cellulitis, these groups were also compared for plasmid replicon types and colicin-associated genes. Significant differences in plasmid replicon content were observed between APEC isolates recovered from systemic disease and those from cases of cellulitis (Table 4). In particular, the IncA/C, IncFIB, IncFIC, IncI1, and IncHI2 replicons occurred significantly more often among cellulitis strains, whereas the IncB/O replicon was observed significantly more often among APEC strains recovered from systemic disease. Additionally, *cvaC*, *cbi*, and *cma* prevalences were significantly different between groups, with *cvaC* occurring more often among the systemic disease isolates and *cbi* and *cma* occurring more often among the cellulitis isolates. The IncT, IncW, and IncX replicons were not detected in any of these isolates.

Most frequently identified plasmid profiles among *E. coli* isolates. Overall, a high degree of variability in plasmid profiles was observed among the 1,015 *E. coli* isolates, with 221 different combinations identified. Only 13% of the *E. coli* isolates failed to yield any of the tested replicons or colicin-associated genes (Table 5). Among the replicon profiles identified, IncFIB occurring alone was the most common. Several other common replicon combinations involving IncFIB were found: IncFIB plus *cvaC*, IncFIB plus IncI1 plus *cvaC*, and IncFIB plus IncI1. Among APEC isolates, the most common replicon profiles identified were IncFIB plus *cvaC* and IncFIB plus *cvaC* plus IncI1, and among RPEC isolates, the most common profile was IncFIB plus *cvaC*. The IncFIB replicon was also the most commonly identified type among HFEC and UPEC isolates tested, although these populations did not tend to contain colicin-associated genes or additional replicon types (Table 5).

A two-way clustering heat map was produced to better illustrate the similarities and differences in the occurrence of plasmid replicon profiles between the groups examined (Fig. 2). This map used trait prevalence to cluster groups (*y* axis) and traits (*x* axis) based upon their similarities in trait prevalence. From this analysis, APEC and RPEC were found to be more closely related to each other, in terms of plasmid trait profile, than they were to UPEC, HFEC, and AFEC. Also, *cvaC* (ColV), *cbi* (ColB), *cma* (ColM), IncFIB, IncI1, and IncP clus-

TABLE 5. Most frequently occurring plasmid profiles among different groups of *E. coli* isolates

Plasmid profile (overall %)	No. (%) with profile				
	UPEC	RPEC	APEC	HFEC	AFEC
None (12.6)	53 (26.5)	13 (6.5)	6 (1.3)	30 (29.7)	26 (25)
FIB (12.1)	63 (31.5)	19 (9.5)	5 (1.1)	30 (29.7)	6 (5.8)
FIB + *cvaC* (6.5)	3 (1.5)	29 (14.5)	29 (6.4)	2 (2.0)	3 (1.9)
FIB + I1 + *cvaC* (3.8)	2 (1.0)	8 (4)	29 (6.4)	0 (0.0)	0 (0.0)
FIB + I1 (3.2)	2 (1.0)	12 (6.0)	12 (2.7)	1 (1.0)	5 (4.8)
FIB + B/O (2.6)	17 (8.5)	0 (0.0)	1 (0.2)	6 (5.9)	2 (1.9)
FIB + *cbi* + *cma* (2.5)	0 (0.0)	17 (8.5)	6 (1.3)	1 (1.0)	1 (1.0)
B/O (2.1)	14 (7.0)	0 (0.0)	1 (0.2)	6 (5.9)	0 (0.0)
FIB + I1 + P + *cvaC* (2.0)	0 (0.0)	7 (3.5)	12 (2.7)	0 (0.0)	1 (1.0)
FIB + FIIA + P + N + *cvaC* (1.6)	0 (0.0)	0 (0.0)	16 (3.5)	0 (0.0)	0 (0.0)
cvaC (1.5)	0 (0.0)	0 (0.0)	12 (2.7)	0 (0.0)	3 (2.9)
FIB + I1 + *cbi* + *cma* (1.4)	0 (0.0)	9 (4.5)	5 (1.1)	0 (0.0)	0 (0.0)
FIB + N + *cvaC* (1.2)	0 (0.0)	0 (0.0)	12 (2.7)	0 (0.0)	0 (0.0)
FIB + FIIA (1.1)	5 (2.5)	1 (0.5)	4 (0.9)	1 (1.0)	0 (0.0)
FIB + *cvaC* + *cbi* + *cma* (1.1)	1 (0.5)	6 (3.0)	4 (0.9)	0 (0.0)	0 (0.0)
I1 (1.1)	0 (0.0)	4 (2.0)	2 (0.4)	2 (2.0)	3 (2.9)

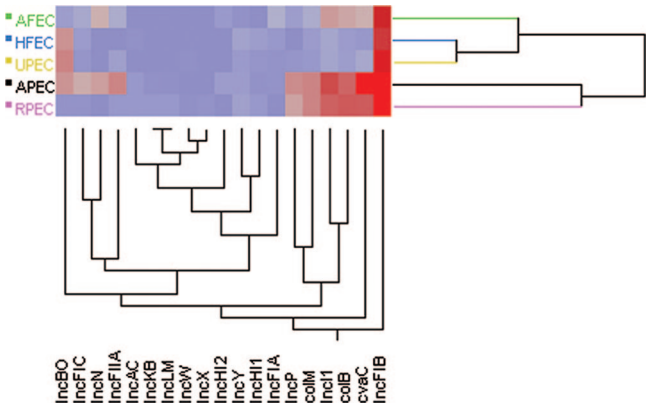

FIG. 2. Two-way clustering heat map illustrating relationships between different groups (*y* axis) with regard to traits examined (*x* axis). Colors within the heat map range from light blue (least prevalent) to dark red (most prevalent), illustrating the prevalence of a particular trait within a particular group. Clustering analysis was performed based upon the colors generated (using JMP in the SAS environment). T and Frep results were excluded to reduce redundancy.

tered together based upon their similarities in occurrence among the five groups examined.

Additionally, an average linkage cluster analysis was performed on the data in an effort to better discern patterns of replicon types among the different *E. coli* groups. This analysis grouped organisms according to plasmid/gene profile and allowed an illustration of an isolate's source relative to its profile. These results are combined with a heat map display of the presence or absence of each gene to help understand the basis of the clusters (Fig. 3). The isolates clustered into five distinct groups, illustrated in the column immediately to the right of the dendrogram. One cluster (light blue) contained most of the UPEC, HFEC, and AFEC but also a mix of isolates from other sources. Isolates of this cluster generally contained an F-type plasmid but lacked other plasmid types and/or colicin-associated genes. The next two clusters (gold and red) contained mostly APEC and RPEC, which were characterized by possession of an F-type plasmid, colicin-associated genes, and/or additional plasmid replicons. The fourth cluster (dark blue) contained mostly UPEC, HFEC, and AFEC and generally lacked the traits examined. The fifth cluster (purple) contained almost exclusively APEC and RPEC, and these isolates possessed an F-type plasmid replicon type along with colicin-associated genes and additional plasmid replicons.

DISCUSSION

The purpose of this study was to create and use a simplified plasmid replicon typing procedure to examine five populations of *E. coli* from avian hosts, human hosts, and retail poultry meat. These populations included APEC, human UPEC, AFEC, HFEC, and RPEC. This study focused on the plasmid profiles for each of the five groups to determine if there were overlaps in profiles among *E. coli* isolates originating from poultry, poultry products, or humans.

This study revealed that APEC and RPEC isolates shared similar plasmid profiles. That is, these groups could be distinguished from UPEC, HFEC, and AFEC based upon their possession of multiple plasmid replicons (i.e., replicons in addition to IncFIB) and the presence of one or more of the three

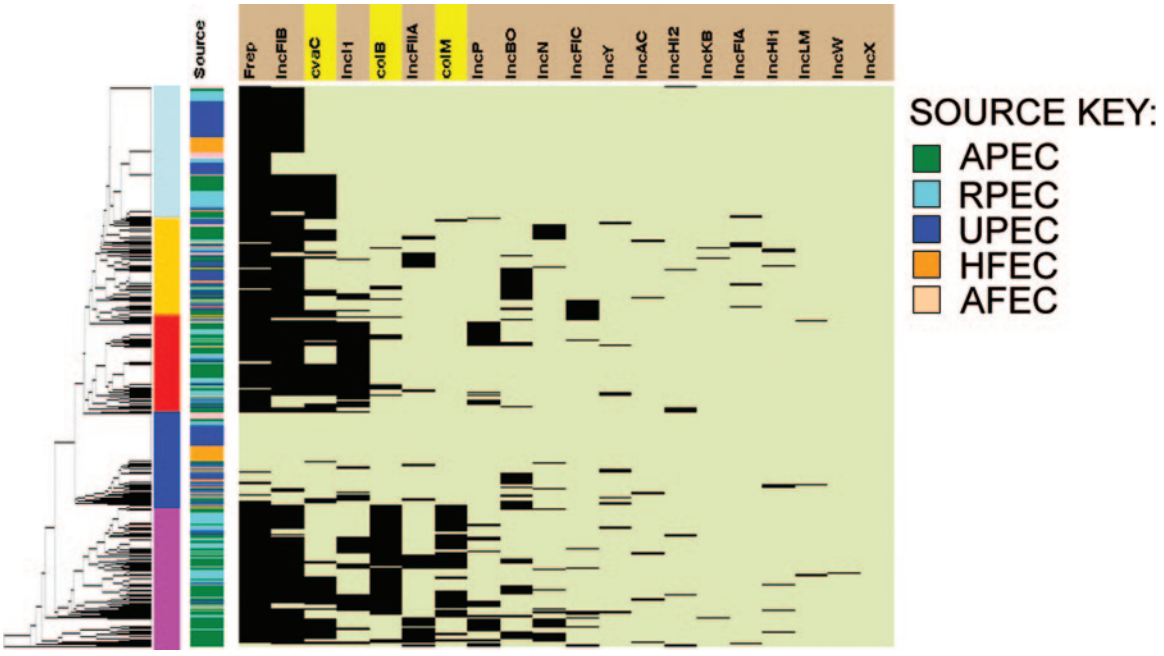

FIG. 3. Results of cluster analyses, based on 16 replicon types (tan) and colicin-related genes (yellow). IncT was omitted because it was absent from all populations. The leftmost portion is the dendrogram resulting from the average linkage cluster analysis based on Jaccard distances. Just to the right of the dendrogram is column 1, which identifies clusters (colored light blue, gold, red, blue, and purple) created based upon trait profiling results. Column 3 identifies isolate sources. Columns 4 to 23 show the replicon and genotyping results for each isolate tested. Each column in this group shows the results for a single trait. Black, the trait is present; light green, the trait is absent.

colicin-related genes examined (Fig. 1 to 3). While definitive statements cannot be made about these observations without further study, the results presented here show that *E. coli* isolates contaminating retail poultry are more similar to APEC than AFEC in terms of their plasmid profiles. This finding would appear to be counterintuitive, as one might expect that the primary source of RPEC is the feces of apparently healthy poultry at slaughter (8). Further studies will be needed to confirm our observation, but the results presented here are intriguing and warrant further attention.

Recent attention has been given to the notion that APEC might be a food-borne source of ExPEC causing human UTIs or act as a reservoir of plasmid-linked virulence and resistance genes for human UPEC. However, the plasmid replicon profiles of the APEC and UPEC strains examined here were very different from one another. In fact, the plasmid profiles of UPEC showed more similarities to those of commensals than they did to APEC profiles. These findings are in congruence with a previous study comparing APEC and UPEC (27), which showed that there were significant differences in the distribution of plasmid-associated traits between the two groups of isolates. Thus, if APEC isolates are involved in human UTIs, either as a causative agent or as a reservoir of plasmid-linked resistance and virulence traits for UPEC, they are unlikely to be involved in many of these infections.

APEC plasmids have received recent attention for their role in virulence and antimicrobial resistance. Indeed, a conserved portion of APEC virulence plasmids may be the defining trait of the pathotype, and the virulence and antimicrobial resistance genes associated with these plasmids are widespread among APEC isolates (16, 17, 26, 39). Since it appears that virulence and R plasmids commonly cotransfer during conjugation, as is the case with several well-studied APEC isolates (14–18, 31), the impact that these plasmids may have on the proliferation of multidrug-resistant, highly virulent strains in the environment cannot be overemphasized. Results presented in this study suggest that this may be the case, as the most commonly occurring plasmid profiles among APEC and RPEC involve the presence of multiple plasmid replicons which have been associated with both virulence and multidrug resistance (1, 2, 4, 11, 12, 14–18, 22, 23, 35, 36).

There has also been recent interest in commensals as a source of antimicrobial resistance genes for pathogenic strains. The transfer of R plasmids from animal to human *E. coli* strains is well-documented (20, 21, 24, 25). Our results indicate that commensal *E. coli* isolates from humans and poultry birds do harbor plasmid types which have been shown to carry mobile genetic elements encoding drug resistance, albeit at a low frequency compared to that of APEC and RPEC. Therefore, the results of this study lend credibility to the idea that commensal *E. coli* isolates are reservoirs of plasmids harboring genes for multidrug resistance and/or virulence.

Previously, it has been difficult to characterize large populations of *E. coli* strains by their plasmid replicon types. However, in the present study, a simplified multiplex PCR protocol for replicon typing was validated and used to characterize over 1,000 *E. coli* isolates, which are representative of the types and numbers of plasmids they possess. However, pitfalls in this screening method do exist. While the presence of a plasmid replicon in an isolate likely indicates the presence of a particular plasmid type, it is possible that such a replicon type might have integrated into the bacterial chromosome or exist on cointegrate plasmids with multiple replicons (9, 19). However, BLAST analysis of the plasmid replicons sought in this study suggests that while this phenomenon does occur, it is rare. Another potential pitfall with this technique is that unknown plasmid replicons will be missed with this screening procedure. This is also a possibility, as observed with the control strain APEC O1, which possesses four plasmids, of which only two are typeable. Nevertheless, the total number of Inc groups appears to have plateaued in recent years, indicating that there may be a finite number of plasmid types in the environment (5). Still, the best method to ultimately verify the presence of a particular plasmid type is still gentle isolation and careful visualization. However, the techniques presented here are an extremely useful way to analyze large populations for the potential presence of plasmids.

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