Comparison of Multilocus Sequence Analysis and Virulence Genotyping of *Escherichia coli* from Live Birds, Retail Poultry Meat, and Human Extraintestinal Infection

Author(s): Jessica L. Danzeisen , Yvonne Wannemuehler , Lisa K. Nolan , and Timothy J. Johnson
Source: Avian Diseases, 57(1):104-108. 2013.
Published By: American Association of Avian Pathologists
DOI: http://dx.doi.org/10.1637/10218-042812-ResNote.1
URL: http://www.bioone.org/doi/full/10.1637/10218-042812-ResNote.1

AVIAN DISEASES 57:104–108, 2013

Research Note—

Comparison of Multilocus Sequence Analysis and Virulence Genotyping of *Escherichia coli* from Live Birds, Retail Poultry Meat, and Human Extraintestinal Infection

Jessica L. Danzeisen,[A] Yvonne Wannemuehler,[B] Lisa K. Nolan,[B] and Timothy J. Johnson[ABC]

[A]Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN 55108
[B]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011

Received 5 May 2012; Accepted 31 August 2012; Published ahead of print 6 September 2012

SUMMARY. To examine the correlations between virulence genotyping and multilocus sequence analysis of *Escherichia coli* from poultry and humans, 88 isolates were examined. The isolates were selected from a population of over 1000 based on their assignment to nine different virulence genotyping clusters. Clustering based on multilocus sequence analysis mostly correlated with virulence genotyping, although multilocus sequence analysis demonstrated higher discriminatory ability and greater reliability related to inferred phylogenetic relationships. No distinct patterns in host source were observed using inferred phylogeny through multilocus sequence analysis, indicating that human, avian, and retail meat isolates are diverse, and some belong to multiple shared clonal complexes. Clonal complexes with host source overlap included ST95 and ST23 and additional novel groups, underscoring the diversity of avian pathogenic *E. coli* and the potential importance of these novel groups as avian and zoonotic pathogens.

RESUMEN. *Nota de Investigación*—Comparación entre el análisis de secuencias multilocus y la genotipificación por virulencia de cepas de *Escherichia coli* obtenidas de aves vivas, de expendios de venta de carne de aves al menudeo y de infecciones extraintestinales en humanos.

Para examinar las correlaciones entre la genotipificación por virulencia y el análisis de secuencias multilocus de cepas de *Escherichia coli* obtenidas de aves de corral y de humanos, se examinaron 88 aislamientos. Los aislados fueron seleccionados de una población de más de 1000 con base en su asignación en nueve grupos diferentes de genotipos de virulencia. El agrupamiento basado en el análisis de secuencias multilocus mostró correlación principalmente con la genotipificación por virulencia, aunque el análisis de secuencias multilocus demostró poseer mayor capacidad discriminatoria y una mayor confiabilidad de acuerdo con las relaciones filogenéticas inferidas. No se observaron patrones distintos con relación a los hospederos mediante inferencias basadas en la filogenia por el análisis de secuencias multilocus, lo que indica que los aislamientos de humanos, de aves y de expendios de venta de carne son diversos, y algunos pertenecen a complejos clonales múltiples compartidos. Los complejos clonales con hospederos de origen comunes incluyeron los grupos ST95 y ST23 y los grupos adicionales nuevos subrayan la diversidad de cepas patógenas de *E. coli* y la importancia potencial de estos nuevos grupos como patógenos aviares y zoonóticos.

Key words: avian, *Escherichia coli*, colibacillosis, extraintestinal

Abbreviations: AFEC = avian fecal *Escherichia coli*; APEC = avian pathogenic *Escherichia coli*; CGEC = crop/gizzard *Escherichia coli*; ExPEC = extraintestinal pathogenic *Escherichia coli*; MLST = multilocus sequence typing; NMEC = neonatal meningitis-associated *Escherichia coli*; RPEC = retail poultry *Escherichia coli*; ST = sequence type; UPEC = uropathogenic *Escherichia coli*

Extraintestinal pathogenic *Escherichia coli*, or ExPEC, causes a number of diseases in animals and humans, including urinary tract infection, neonatal meningitis, and sepsis (4,22,23,24). In poultry, a subset of ExPEC referred to as avian pathogenic *E. coli* (APEC) has long been associated with a group of diseases collectively known as colibacillosis. Extensive virulence genotyping of ExPEC has been performed, revealing that the ExPEC subpathotypes can be defined to some extent by their possession of subsets of virulence genes (5,6,8,10,13,20,21). In APEC, these genes are primarily associated with a plasmid type known as ColV (14,15). In human ExPEC, several virulence genes contained on chromosomal pathogenicity islands have been associated with their pathogenesis (11). Despite the fact that most APEC and human ExPEC strains differ from one another, a small proportion of strains has been identified that are highly similar in their overall virulence genotype profiles (6,16).

It is assumed that the source of virulent human ExPEC clones that are responsible for disease originate from the host's fecal flora, but there is no consensus as to how these clones come to inhabit the fecal flora. Based on the identification of APEC and human ExPEC with strong genomic similarities, some work suggests that a potential source of ExPEC clones that colonize human hosts is contaminated poultry meat (7,9,13). This concept is also based upon observations that some avian pathogenic *E. coli*, or APEC, strains are capable of causing disease in models mimicking human ExPEC infection (16,26). These findings have particularly focused on strains belonging to the ST95 clonal complex, concluding that they are most likely to be involved in zoonotic transfer of ExPEC from poultry to humans. Examples of potentially zoonotic ExPEC belonging to the ST95 clonal complex include avian pathogenic *E. coli* (APEC) O1:K1:H7 strain APEC O1 (12), uropathogenic *E. coli* (UPEC) O18:K1:H7 strain UTI89 (3), and neonatal meningitis-associated *E. coli* (NMEC) O18:K1:H7 strain IHE3034 (17). Additionally, many APEC and some human sepsis strains belong to the O78 serogroup, and these strains might also possess zoonotic potential. However, the genetic relationships of avian source and human ExPEC outside of these groups have not been extensively examined using phylogenetic typing techniques, although virulence genotyping has demonstrated some similarities. Thus, the purpose of this study was to examine avian source *E. coli* and human ExPEC

[C]Corresponding author. Mailing address: 1971 Commonwealth Avenue, 205 Veterinary Science, St. Paul, MN 55108; e-mail: joh04207@umn.edu

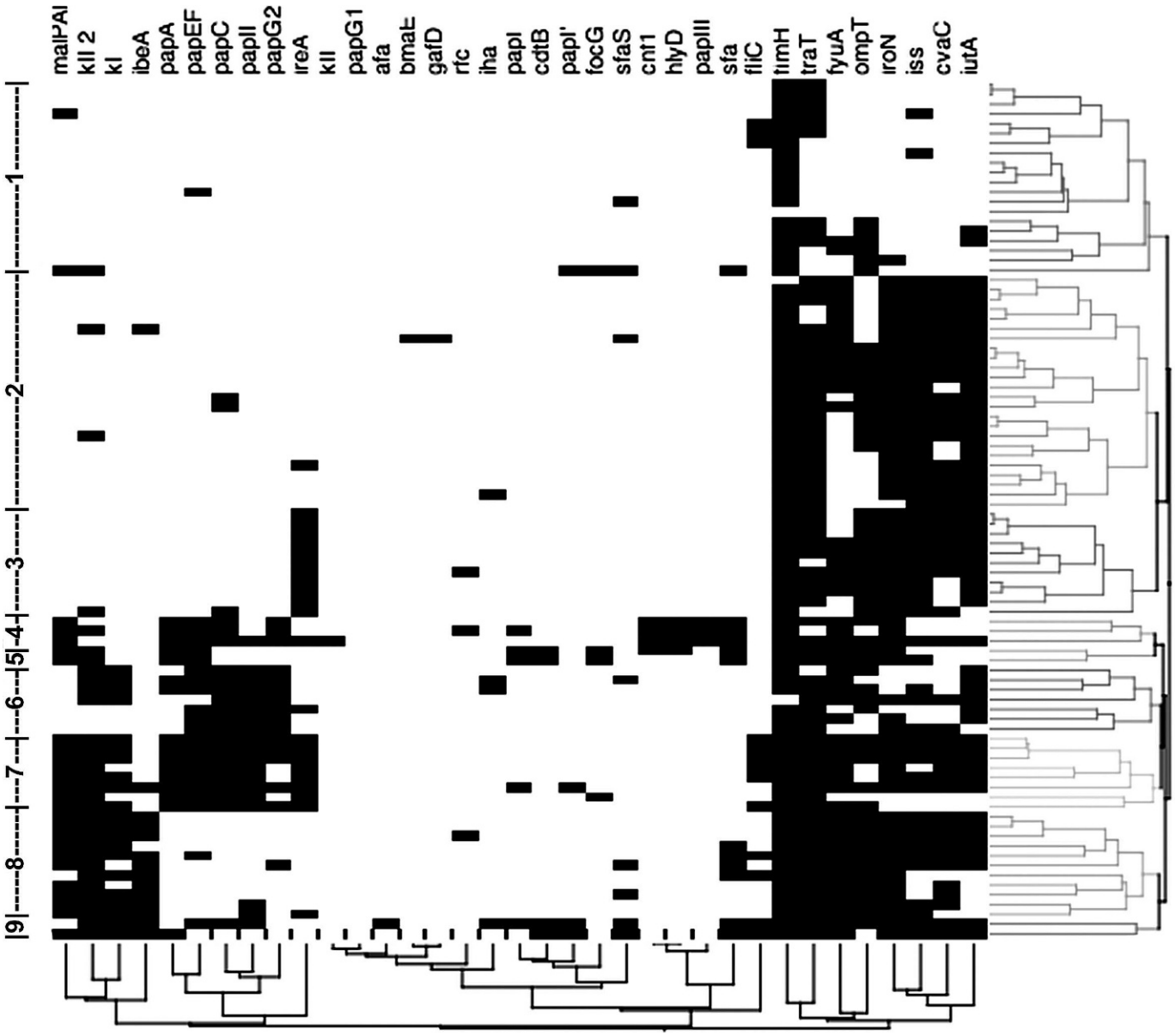

Fig. 1. Two-way hierarchical clustering of virulence genotyping results from the 88 isolates used in this study. Genotyping was previously performed for 35 genetic loci including ExPEC virulence genes (16,20,21). Clustering was performed using Ward's method. Established genotyping clusters are depicted to the left of the clustering image. The ordering of isolates is different in Fig. 1 and Fig. 2.

from multiple virulence genotyping clusters for phylogenetic relationships using multilocus sequence analysis.

MATERIALS AND METHODS

Bacterial isolates. In total, 88 isolates were used in this study, including 23 APEC isolates from visceral lesions of commercial birds with colibacillosis, 12 isolates from cloacal swabs of healthy commercial birds (designated avian fecal *E. coli* or AFEC), 13 isolates from the crops or gizzards of healthy commercial birds (designated crop/gizzard *E. coli* or CGEC), 14 isolates from retail poultry meat (designated retail poultry *E. coli* or RPEC), 12 NMEC isolates from human neonatal meningitis, and 14 UPEC isolates from human urinary tract infection. Isolates were selected based upon their distribution within previously established virulence genotyping clusters (13,16) to examine isolates with similar genotype profiles from multiple avian and human sources. The phylogenetic groups, serogroups, and plasmid carriage of these isolates were previously reported (13,16). Two-way hierarchical clustering was performed here based upon these traits using JMP 9.0 (SAS Institute, Cary, NC) to establish genotyping clusters of the selected isolates. In total, isolates from multiple sources within nine different genotyping clusters were studied (Fig. 1). Isolates representing different host sources (avian or human) and locations (lesions in APEC, urine in UPEC, cerebrospinal fluid in NMEC, crops and gizzards from healthy birds, cloacal swabs from healthy birds, and retail chicken breasts) were selected for analysis.

Multilocus sequence analysis. Multilocus sequence typing (MLST) was performed using the Achtman scheme, which is the most widely used MLST scheme for *E. coli* (28). Portions of the following gene sequences were amplified following this protocol: *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*. The *aspC* gene was also analyzed using previously designed primers (19). Sequence allele types were generated using the MLST database (http://mlst.ucc.ie/mlst/dbs/Ecoli) for all gene sequences except *aspC*. The sequencing data for all eight gene sequences were edited and trimmed using Seqman (Lasergene, Madison, WI). The sequencing data generated here were deposited in GenBank under

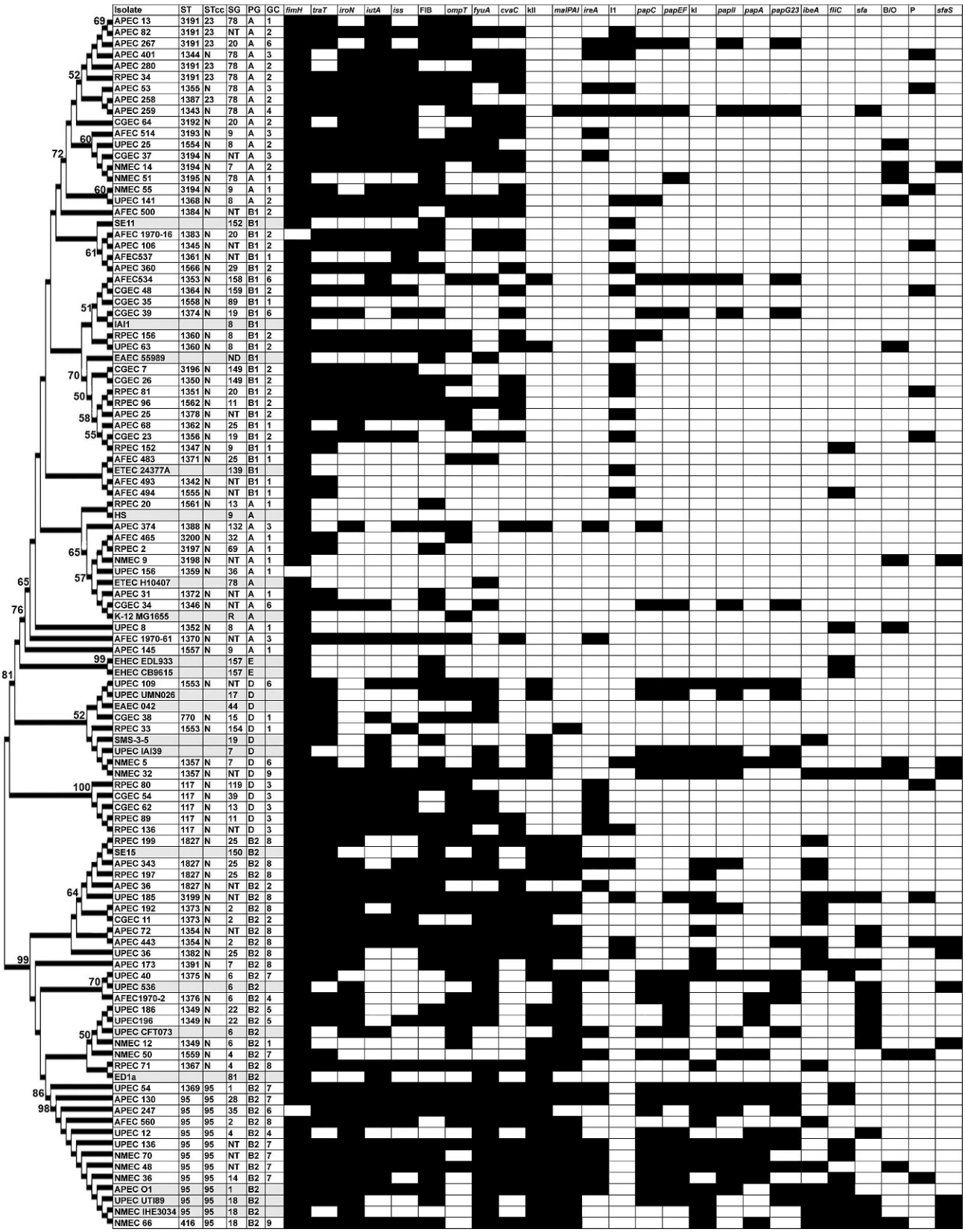

Fig. 2. Dendrogram depicting inferred phylogeny of 88 isolates and reference *E. coli* genomes constructed using MEGA5 based upon eight sequenced housekeeping genes. See Materials and Methods section for a description of the parameters used for dendrogram construction. Isolates are abbreviated as follows: avian pathogenic *E. coli* = APEC; retail poultry *E. coli* = RPEC; crop/gizzard *E. coli* = CGEC; avian cloacal *E. coli* = AFEC; uropathogenic *E. coli* = UPEC; neonatal meningitis *E. coli* = NMEC. Reference genomes are also included and are shaded gray where virulence genotyping was not performed. Bootstrap confidence values greater than 50% are listed at major nodes. Data to the right of the dendrogram depict ST type according to the Achtman database (ST), ST clonal complex (STcc; ''N'' indicates No STcc), isolate, serogroup (SG), phylogenetic group (PG), genotyping cluster from Fig. 1 (GC), and presence (black) or absence (white) of sought virulence genes and plasmid replicons.

accession numbers JF892729–JF893432. Sequences were concatenated for each isolate and aligned using ClustalW in MEGA5 (25). The evolutionary history was inferred using the maximum likelihood method based on the Tamura-Nei model. In total, 4095 positions were used in the final data set.

RESULTS AND DISCUSSION

Eighty-eight isolates belonging to avian and human sources were selected from a population of more than 1000 isolates based upon their previously established virulence genotyping profiles (13). Based upon this analysis, nine major genotyping clusters were identified. We selected approximately equal numbers of isolates from each isolate source belonging to each of the nine genotyping clusters. Since some genotyping clusters were overrepresented in our previous study, we selected numbers from each genotyping cluster roughly proportional to total isolate representation. We then reconstructed the genotyping diagram using only the isolates in this study, and this resulted in nine genotyping clusters that were slightly different due to differences in the genes analyzed and clustering criteria (Fig. 1). Multilocus sequence was performed, and an alignment was used to construct a dendrogram illustrating the relationships of the 88 isolates relative to one another and to sequenced reference *E. coli* strains (Fig. 2). As expected, the isolates clustered broadly by *E. coli* phylogenetic group. When multilocus sequence analysis was compared to virulence genotyping, the results generally agreed, although some outliers were observed. This is most likely explained by the fact that many of the virulence genes previously sought are contained on ColV plasmids, which have the propensity to be transferred via horizontal gene transfer and are found throughout all *E. coli* phylogenetic groups. Isolates containing greater numbers of previously sought virulence genes were found within the B2 phylogeny, which is also expected, since these virulence genes are typical of human ExPEC strains, which are mainly members of the B2 phylogenetic group. Avian-source isolates were found throughout the dendrogram, including within the phylogenetic group B2 that typifies human ExPEC (20). Several APEC, avian cloacal *E. coli*, crop/gizzard *E. coli*, and retail poultry *E. coli* strains were identified among this group. Similarly, NMEC and UPEC were mostly found within the phylogenetic group B2 but were also scattered throughout the dendrogram.

Previous work has suggested that B2 isolates belonging to the ST95 MLST complex, which includes *E. coli* belonging to the serogroups O1 and O18, are likely zoonotic pathogens capable of causing disease in both poultry and humans (6,16,18). These data support this, since some APEC and avian cloacal *E. coli* were identified as members of the ST95 group using MLST. However, it is evident here that other clonal complexes might play a role in the zoonotic transmission of human ExPEC via contaminated poultry meat. Previous comparisons have identified similarities between avian-source and human ExPEC with regard to virulence gene content, and genotyping approaches such as MLST have demonstrated that certain clonal complexes, such as ST10, ST95, ST23, ST117, ST131, and ST746, contain *E. coli* from both avian and human sources (1,2,6,16,20,27). In our analysis, isolates belonging to clonal complex ST23 (top clusters in Fig. 2) were also identified that contained avian cloacal *E. coli*, crop/gizzard *E. coli*, *E. coli* from retail poultry, UPEC, and NMEC. In addition, several isolates not belonging to any previously identified sequence types (STs) were observed containing isolates from both avian and human sources. While this study was limited in terms of numbers of isolates from each source group, the results suggest that other clonal complexes exist containing avian-source isolates that might possess zoonotic potential. The relationship between ST and specific virulence genotypes tested here, however, was unclear. This is likely due to the fact that many of the genes sought were plasmid-encoded, and others were components of horizontally acquired genomic islands. Further analysis of specific STs for an expanded repertoire of virulence genes would be required to fully understand correlations between genotype and these STs.

The hypothesis that retail poultry serves as a foodborne vehicle for human ExPEC has been previously suggested (20). If this hypothesis is correct, then ExPEC with zoonotic capability must occur on retail poultry meat. Multilocus sequence analysis of the isolates in this study also demonstrated that many of them belonged to dendrogram clusters containing both APEC and human ExPEC. Taken together, these data support previous findings in that they verify the presence of *E. coli* strains belonging to these clonal complexes within the normal flora of the bird and on retail poultry meat. Furthermore, while a great deal of diversity exists among avian commensal *E. coli* and ExPEC, overlap exists with regard to clonal complexes that contain avian-source and human ExPEC. These clonal complexes with source overlap include ST95 and ST23, but also include novel clonal groups with source overlap. Thus, the results of this study further confirm that APEC-contaminated poultry is a potential source of ExPEC important in human disease. This work also confirms the fact that those isolates causing disease in poultry are extremely diverse, likely reflecting the complex nature of colibacillosis. We conclude that future efforts to examine the zoonotic potential of APEC should include isolates outside of the ST95 and ST23 clonal complexes, as they may also contain avian-source *E. coli* and human ExPEC with pathogenic and zoonotic potential.

REFERENCES

1. Adiri, R. S., U. Gophna, and E. Z. Ron. Multilocus sequence typing (MLST) of *Escherichia coli* O78 strains. FEMS Microbiol. Lett. 222: 199–203. 2003.

2. Bergeron, C. R., C. Prussing, P. Boerlin, D. Daignault, L. Dutil, R. J. Reid-Smith, G. G. Zhanel, and A. R. Manges. Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. Emerg. Infect. Dis. 18:415–421. 2012.

3. Chen, S. L., C. S. Hung, J. Xu, C. S. Reigstad, V. Magrini, A. Sabo, D. Blasiar, T. Bieri, R. R. Meyer, P. Ozersky, J. R. Armstrong, R. S. Fulton, J. P. Latreille, J. Spieth, T. M. Hooton, E. R. Mardis, S. J. Hultgren, and J. I. Gordon. Identification of genes subject to positive selection in uropathogenic strains of *Escherichia coli*: a comparative genomics approach. Proc. Nat. Acad. Sci. U. S. A. 103:5977–5982. 2006.

4. Dho-Moulin, M., and J. M. Fairbrother. Avian pathogenic *Escherichia coli* (APEC). Vet. Res. 30:299–316. 1999.

5. Ewers, C., E. M. Antao, I. Diehl, H. C. Philipp, and L. H. Wieler. Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic *Escherichia coli* strains with zoonotic potential. Appl. Environ. Microbiol. 75:184–192. 2009.

6. Ewers, C., G. Li, H. Wilking, S. Kiessling, K. Alt, E. M. Antao, C. Laturnus, I. Diehl, S. Glodde, T. Homeier, U. Bohnke, H. Steinruck, H. C. Philipp, and L. H. Wieler. Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: how closely related are they? Int. J. Med. Microbiol. 297:163–176. 2007.

7. Jakobsen, L., A. Kurbasic, L. Skjot-Rasmussen, K. Ejrnaes, L. J. Porsbo, K. Pedersen, L. B. Jensen, H. D. Emborg, Y. Agerso, K. E. Olsen, F. M. Aarestrup, N. Frimodt-Moller, and A. M. Hammerum. *Escherichia coli* isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection. Foodborne Pathog. Dis. 7:537–547. 2010.

8. Johnson, J. R., M. A. Kuskowski, A. Gajewski, S. Soto, J. P. Horcajada, M. T. Jimenez de Anta, and J. Vila. Extended virulence genotypes and phylogenetic background of *Escherichia coli* isolates from patients with cystitis, pyelonephritis, or prostatitis. J. Infect. Dis. 191:46–50. 2005.

9. Johnson, J. R., J. S. McCabe, D. G. White, B. Johnston, M. A. Kuskowski, and P. McDermott. Molecular analysis of *Escherichia coli* from retail meats (2002–2004) from the United States National Antimicrobial Resistance Monitoring System. Clin. Infect. Dis. 49:195–201. 2009.

10. Johnson, J. R., E. Oswald, T. T. O'Bryan, M. A. Kuskowski, and L. Spanjaard. Phylogenetic distribution of virulence-associated genes among *Escherichia coli* isolates associated with neonatal bacterial meningitis in the Netherlands. J. Infect. Dis. 185:774–784. 2002.

11. Johnson, J. R., and T. A. Russo. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. Int. J. Med. Microbiol. 295:383–404. 2005.

12. Johnson, T. J., S. Kariyawasam, Y. Wannemuehler, P. Mangiamele, S. J. Johnson, C. Doetkott, J. A. Skyberg, A. M. Lynne, J. R. Johnson, and L. K. Nolan. The genome sequence of avian pathogenic *Escherichia coli* strain O1:K1:H7 shares strong similarities with human extraintestinal pathogenic *E. coli* genomes. J. Bacteriol. 189:3228–3236. 2007.

13. Johnson, T. J., C. M. Logue, Y. Wannemuehler, S. Kariyawasam, C. Doetkott, C. DebRoy, D. G. White, and L. K. Nolan. Examination of the source and extended virulence genotypes of *Escherichia coli* contaminating retail poultry meat. Foodborne Pathog. Dis. 6:657–667. 2009.

14. Johnson, T. J., K. E. Siek, S. J. Johnson, and L. K. Nolan. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. J. Bacteriol. 188:745–758. 2006.

15. Johnson, T. J., Y. Wannemuehler, C. Doetkott, S. J. Johnson, S. C. Rosenberger, and L. K. Nolan. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J. Clin. Microbiol. 46:3987–3996. 2008.

16. Johnson, T. J., Y. Wannemuehler, S. J. Johnson, A. L. Stell, C. Doetkott, J. R. Johnson, K. S. Kim, L. Spanjaard, and L. K. Nolan. Comparison of extraintestinal pathogenic *Escherichia coli* strains from human and avian sources reveals a mixed subset representing potential zoonotic pathogens. Appl. Environ. Microbiol. 74:7043–7050. 2008.

17. Moriel, D. G., I. Bertoldi, A. Spagnuolo, S. Marchi, R. Rosini, B. Nesta, I. Pastorello, V. A. Corea, G. Torricelli, E. Cartocci, S. Savino, M. Scarselli, U. Dobrindt, J. Hacker, H. Tettelin, L. J. Tallon, S. Sullivan, L. H. Wieler, C. Ewers, D. Pickard, G. Dougan, M. R. Fontana, R. Rappuoli, M. Pizza, and L. Serino. Identification of protective and broadly conserved vaccine antigens from the genome of extraintestinal pathogenic *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A. 107:9072–9077. 2010.

18. Moulin-Schouleur, M., C. Schouler, P. Tailliez, M. R. Kao, A. Bree, P. Germon, E. Oswald, J. Mainil, M. Blanco, and J. Blanco. Common virulence factors and genetic relationships between O18:K1:H7 *Escherichia coli* isolates of human and avian origin. J. Clin. Microbiol. 44:3484–3492. 2006.

19. Reid, S., C. Herbelin, A. Bumbaugh, R. Selander, and T. Whittam. Parallel evolution of virulence in pathogenic *Escherichia coli*. Nature 406:64–67. 2000.

20. Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, M. K. Fakhr, and L. K. Nolan. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology 151:2097–2110. 2005.

21. Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, and L. K. Nolan. Characterizing the APEC pathotype. Vet. Res. 36:241–256. 2005.

22. Russo, T. A., and J. R. Johnson. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J. Infect. Dis. 181:1753–1754. 2000.

23. Saif, Y. M., and A. M. Fadly. Diseases of poultry. Blackwell, Ames, IA. 2008.

24. Smith, J. L., P. M. Fratamico, and N. W. Gunther. Extraintestinal pathogenic *Escherichia coli*. Foodborne Pathog. Dis. 4:134–163. 2007.

25. Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731–2739. 2011.

26. Tivendale, K. A., C. M. Logue, S. Kariyawasam, D. Jordan, A. Hussein, G. Li, Y. Wannemuehler, and L. K. Nolan. Avian-pathogenic *Escherichia coli* strains are similar to neonatal meningitis *E. coli* strains and are able to cause meningitis in the rat model of human disease. Infect. Immun. 78:3412–3419. 2010.

27. Vincent, C., P. Boerlin, D. Daignault, C. M. Dozois, L. Dutil, C. Galanakis, R. J. Reid-Smith, P. P. Tellier, P. A. Tellis, K. Ziebell, and A. R. Manges. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerg. Infect. Dis. 16:88–95. 2010.

28. Wirth, T., D. Falush, R. Lan, F. Colles, P. Mensa, L. H. Wieler, H. Karch, P. R. Reeves, M. C. Maiden, H. Ochman, and M. Achtman. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol. Microbiol. 60:1136–1151. 2006.

ACKNOWLEDGMENTS

This project was funded through the College of Veterinary Medicine at Iowa State University and the College of Veterinary Medicine at the University of Minnesota. Bioinformatic analysis was performed using tools available from the Minnesota Supercomputing Institute.