Population Structure and Molecular Epidemiology of Campylobacter coli Isolates of Porcine Origin from Different Geographic Regions and Production Systems

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Abstract
The genotypic diversity of C. coli isolates recovered from pigs from different geographic regions and production systems were investigated by multilocus sequence typing (MLST) method. A total of 99 C. coli isolates, 50 from Ohio and 49 from Wisconsin, representing the different production systems (conventional and antibiotic free) and resistance patterns (pan-susceptible, Ery/Tet‡ and others) were analyzed. Fifty different sequence types (ST) were identified by sequencing the seven housekeeping genes (aspA, glnA, glkA, glyA, pgm, tkt, and uncA). Seven of them were new STs (ST-3813, 3814, 3815, 3816, 3817, 3818 and 3819) identified for the first time. Of these, three resulted from new allele sequence at tkt and uncA loci and the remaining resulted from new combinations of the previously described alleles. All STs belonged to one clonal complex with a founder genotype of ST-828. This clonal complex consists STs that were previously identified from human campylobacteriosis cases. The founder genotype (ST-828) was the second most common (n=5) in the study, next to ST-854 (n=10). Other predominant STs identified were ST-1096 (n=5) and ST-1100 (n=5). The total number of MLST alleles per locus varied between three for aspA and nine for tkt. The most common allele at each MLST locus was aspA33, glnA39, glkA30, glyA82, pgm104, tkt35 and uncA17. Nineteen STs comprising 23 isolates were identified solely from Ohio, 15 STs comprising 20 isolates were identified only from Wisconsin and the remaining 16 STs comprising 56 C. coli isolates were shared in both geographic regions. In addition, similar resistance pattern was represented by multiple STs. The C. coli population I_A was 0.46 indicating some degree of recombination and the rate of recombination was higher among Ohio isolates (I_A = 0.38) than Wisconsin (I_A = 0.57). Furthermore, the I_A was higher in Ohio conventional C. coli strains (0.1). The weak clonal structure of strains demonstrate the usefulness of MLST for investigation of the epidemiology of C. coli and its potential for classifying clones based on geographic origins.

Introduction
Campylobacter species are one of the leading causes of bacterial foodborne infection (7). C. jejuni is responsible for up to 90% of human campylobacteriosis cases, and the majority of the remaining cases are believed to be caused by C. coli. A wide array of molecular typing methods, including pulsed-field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP), have been used to study the epidemiology and population structure of Campylobacter isolates (1, 2). However, limitations of these methods for routine surveillance of Campylobacter infections and identification of the sources of sporadic cases of campylobacteriosis have been reported (4). In addition, the Campylobacter genome is known to be hypervariable (10). Multilocus sequence typing is a newly developed typing method and has emerged as an important tool to study weak clonal populations, sporadic cases and global epidemiology (2). An MLST scheme for C. coli was developed in 2005 (2, 9) and showed its advantage to study molecular epidemiology and population structure.

There are very limited studies that have assessed the genotypic diversity of C. coli recovered from different production systems and geographic regions. Therefore, the objectives of this study were to assess the population structure and molecular epidemiology of C. coli isolates recovered from different production systems, geographic regions, and showing various resistance phenotypes.

Material and methods
Selection of C. coli strains
A total of 99 C. coli isolates recovered from different production systems and geographic regions and showing different antimicrobial resistance patterns were selected. The primary selection criteria was
based on equal representation of the two geographic regions (Ohio and Wisconsin) and production systems (ABF and conventional). ABF was defined as farms that do not use any antimicrobials post-weaning and those that use antimicrobials for therapeutic purposes were required to remove the pig(s) to a separate barn. Conventional farms were those that use antimicrobials routinely for prophylactic and therapeutic purposes. The composition of selected isolates is presented in Table 1.

Amplification and sequencing of the housekeeping genes

Selected *Campylobacter coli* isolates were cultured on Mueller-Hinton (MH) agar under microaerophilic conditions at 42°C for 48 hours. A thick suspension of *Campylobacter* cells was made in 1.5 ml of molecular biology grade water and genomic DNA was purified using the Qiagen DNeasy tissue kit (Qiagen, Valencia, CA). Polymerase chain reactions (PCR) of the seven housekeeping genes were performed using primers described previously by Dingle et al. (2). The PCR amplicons were purified using the QIAquick 96 PCR purification kit (Qiagen, Valencia, CA) and sequenced using the GenomeLab™ Dye Terminator Cycle Sequencing (DTCS) quick start Kit (Beckman Coulter, Fullerton, CA).

Assignment of allele numbers, sequence types and clonal complexes

The allelic profiles, the sequence types (STs) and clonal complexes were generated by querying the *C. jejuni*/*C. coli* MLST database (http://pubmlst.org/campylobacter/). The ST was assigned to each isolate on the basis of the combination of the seven alleles. A newly described allelic profile and sequence type were deposited in the *Campylobacter* MLST database.

Data analysis

The assignment of ST data into clonal complexes was performed using the program eBURST (Based Upon Related Sequence Types) with a group definition of identical alleles at five of the seven loci with at least one other ST in the group. Linkage analysis was performed by calculating index of association ($I_A$) using the program START2 (Sequence Type Analysis and Recombinational Tests).

Results

Multi-Locus Sequence Typing (MLST) of *C. coli* isolates

A total of 99 *C. coli* isolates recovered from two production systems (ABF and conventional) and two geographic regions were typed using MLST. Seven housekeeping genes were sequenced and a total of 50 different sequence types (STs) were identified. Seven ST were identified for the first time. Of these, three resulted from new allele sequences and the remainder resulted from new combinations of the previously described alleles. The most common ST in this study was ST 854 (n=10). The total number of MLST alleles per locus varied between three for *aspA* and nine for *tkt*. The number of MLST alleles described for *glnA*, *gltA*, *pgm* and *unc* were four, whereas the number of alleles in *glaA* locus was six. For each MLST locus, there was one predominant allele (*aspA*33, *glnA*39, *gltA*30, *glaA*82, *pgm*104, *tkt*35 and *uncA*17).

Population structure (clonal complex) and molecular epidemiology

Based on the clonal complex definition criteria set above, we found one clonal complex with founder genotype ST 828 where all the STs were included. We observed seven sub-clones, which were represented by multiple STs. Sub-clone 828 was the most frequently represented with 28 isolates, 10 of which were ST-854 and the other 5 were ST-828. ST-3816 and 3819 (two of the newly identified STs) were classified under sub-clone 828. The second most represented sub-clone was 1096 with 20 *C. coli* strains. The newly identified ST, ST-3817, was categorized in this sub-clone. The index of association ($I_A$) for the complete collection of the *C. coli* strains was 0.46, indicating the *C. coli* population is recombining to some degree. The rate of recombination was higher in Ohio (0.38) than Wisconsin (0.57). Higher recombination rate was observed among Ohio conventional *C. coli* strains (0.1).

Of the 50 STs identified in this study, 19 STs comprising 23 strains were identified solely from Ohio, 15 STs comprising 20 strains were identified only from Wisconsin and the remaining 16 STs comprising 56 *C. coli* strains were observed in both regions. A majority of the sequence types were specific to a production system. Seventeen STs (34%) were found only in ABF production system; nineteen STs (36%) only from conventional production systems and the remaining fourteen STs (28%) were shared between the two production systems. All resistance patterns were represented by multiple STs across geographic regions and production systems.
Discussion

*Campylobacter* is one of the leading causes of foodborne gastroenteritis worldwide. Pigs play a significant role in *C. coli* epidemiology as their intestinal tract and environment are commonly colonized by *C. coli* (3). The goal of this study was to examine the genetic diversity of *C. coli* strains recovered from different geographic regions, production systems, and resistance patterns. We identified 50 different sequence types (ST), indicating that there are diverse STs circulating in pigs. Previous investigations conducted with *C. coli* isolates from different hosts, including pigs, showed a substantial level of diversity in alleles and STs (8, 11). In this study, seven new STs were identified for the first time. These new STs were recovered from different geographic regions and production systems. More isolates with these alleles and STs are required to determine their distribution in pig environments or whether they are introduced from other sources.

The most common ST in our study was ST-854. Previous studies conducted in US also indicated the presence of ST-854 in pig samples (8, 11). However, there are studies carried out in Europe that suggested ST-854 had host preference to chickens (2, 5). This variation in the distribution of ST-854 might be due to differences in geographic location. Interestingly, some of the STs reported in this study, including ST-828, ST-827, ST-899, and ST-1591 were previously identified from human stool samples with gastroenteritis cases in Europe (2, 5, 6). However, there was no epidemiological link between the STs associated with human clinical cases and our STs. Nevertheless, it indicates the potential of pigs as asymptomatic carriers for the STs that have been previously implicated for human gastroenteritis. The finding of identical STs from multiple sources suggests that many different transmission routes may play a role in the epidemiology of *C. coli* (8).

In the current study, the overall *C. coli* population *I* was 0.46, indicating some degree of recombination. There were some STs that were identified only in one geographic region or production system, however, the number of strains represented by such ST were low. In addition, several identical STs were identified from both geographic regions and it is difficult to determine the association between region and ST, if there is any, as the majority of the STs are represented by a very small number of strains (<5 strains). Our finding was in agreement with Miller et al. (8) where several groups of *C. coli* strains with identical STs were recovered from different geographic locations and time periods. In this study, the same resistance patterns were represented by multiple STs, indicating antibiogram typing of *C. coli* may not be a good indicator of the relatedness of the strains.

Conclusion

Our study showed the presence of diverse *C. coli* STs circulating in pigs with weak clonal structure. As a result, MLST can be an important tool to investigate the molecular epidemiology of *C. coli* and helps for classifying clones based on geographic origins. Yet, the relation between STs and geographic regions, production systems, and resistance profiles needs to be further investigated by including larger numbers of *C. coli* strains.

References


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<th>Region (n)</th>
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Table 1 Composition of C. coli isolates selected for multilocus sequence typing
ABF – antimicrobial free farm, Conv – conventional farm, Ery/Tet+ – resistant to erythromycin and tetracycline plus any other antimicrobials, Pansusceptible – susceptible to the tested antimicrobials

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