COMPARATIVE GENOTYPING OF CAMPYLOBACTER COLI USING MULTILOCUS SEQUENCE TYING AND PULSED FIELD GEL ELECTROPHORESIS

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Abstract A total of 100 Campylobacter coli isolates from swine reared in conventional and antimicrobial free (ABF) farms were genotyped using multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE) to evaluate their discriminatory power, throughput and index of association to determine the genotypic similarity and clonal structure of the isolates. MLST had a higher discriminatory power of 0.936 than 0.889 for PFGE and higher throughput. Isolates were clustered into 26 groups by MLST compared to 11 by PFGE. Out of the 65 sequence types (ST) observed in the whole population, 50 STs here have reported for the first time. Majority of the STs were found specific to either the farm (n=38) or the slaughter (n=22). We observed ST-1413 associated only with isolates from the ABF system exhibiting the CIP ERY NAL TET resistance pattern. The high genetic diversity and the weak clonal structure of the C. coli population among swine as observed by using these two methods was further highlighted by the index of association (I_A value) of 0.293.

Introduction Foodborne pathogens result in approximately 76 million illnesses in the US every year1. Campylobacter is the most common bacterial etiological agent causing gastroenteritis annually in 2.5 million patients in the US (Mead et al., 1999). Campylobacter jejuni in humans is considered to be the most important Campylobacter species causing 95% of the foodborne infection cases. Poultry has been recognized as the primary reservoir of C. jejuni while pigs are mostly implicated as reservoirs of C. coli (Harvey et al., 1999). It is important to use typing methods that have high discriminatory power to identify and differentiate sources of this pathogen in animals. MLST is a method that is based on indexing the genetic variation seen in housekeeping genes (Dingle et al., 2001) and has recently been standardized for C. coli (Dingle et al., 2005). PFGE is another genotyping method that has been used over the years for investigating C. jejuni outbreaks and genotyping C. coli (Sails et al., 2003). Although this method is highly discriminatory, interlaboratory comparisons are little difficult due to complex protocols and accessibility to equipment and software for analyzing the patterns. No study has been done comparing the diversity of C. coli in swine at farm and slaughter using both MLST and PFGE. The utility of MLST and PFGE methods as tools for understanding the epidemiology of this pathogen in the swine environment is important to determine. Therefore, in this study, we used these two methods for genotyping 100 phenotypically diverse C. coli isolated from swine and compare their discriminatory power, throughput and group associations.

Materials and Methods A total of 100 strains were randomly selected from 1472 C. coli isolates that were isolated as a part of a cross-sectional study conducted on swine farms and slaughter plants (JFP Submitted). The selected strains were from the conventional and ABF production systems representative of the processing stages at farm and slaughter and the resistance patterns observed during the entire study. Antimicrobial susceptibility testing was done using agar dilution method. MLST of the seven housekeeping genes (aspA, glnA, gltA, glyA, pgm, tkt and uncA) for C. coli was done following the previously described method (Dingle et al., 2005). The allele profile for individual C. coli isolates was deposited on the MLST website (www.mlst.net) and appropriate sequence type (ST) was assigned from the Campylobacter database. PFGE was done following the rapid protocol for Campylobacter (Ribot et al., 2001). Analysis of PFGE data was performed using Bionumerics software (Applied Maths, Kortrijk, Belgium). Simpson's index of diversity was used to compare the discriminatory power of the two genotyping systems used in this study (Gaston). The index of association (I_A) using the START program was used to calculate to assess the clonal structure of the population (Smith et al., 1993).

Results Assignment of the allele frequencies and STs was done using the Campylobacter MLST database (www.campylobacter.mlst.net). A total of 65 STs were generated from sequence typing of 100 C. coli isolates with 47 STs occurring singly and ST 1413 being the most common seen in 7 isolates from the carcass of ABF pigs. Fifty new STs were assigned for the first time after sub-
mitting the information to the mlst database with 24 STs originating from the ABF isolates. Within individual production systems, we observed STs that were found specific to the processing stages either at the farm or slaughter (Table 1). The three most predominant STs occurring in the database included ST-1413 (7 isolates), ST-854 (6 isolates) and ST-1123 (5 isolates) representing 18% of the isolates. Multiple STs were generated with the majority of the STs specific either to the farm or the slaughter stage. Thirty-eight out of the 65 STs were found to be specific to the farm and another 22 were found only at the slaughter stage. Based on the MLST dendrogram generated, we observed a total of 26 clusters with cluster 9 being the largest (n=18) (Table 2). Clusters 2, 5, 6, 19 and 21 comprised of isolates from the farm while clusters 7, 8 and 24 included slaughter isolates only. The $I_A$ for the whole population was 0.293 indicating a weak clonal structure. However, the $I_A$ values for the ABF and the conventional population was 0.279 and 0.535 respectively indicating a slightly more clonal structure of C. coli isolated from the conventional system. MLST method had a discriminatory index of 0.936.

For the PFGE analysis, the Smal digested genome of C. coli resulted in the generation of an average 6-10 bands. Using 70% similarity as the cut off, a total of 11 clusters were observed with cluster 1 (17 isolates), 11 (16 isolates) and 3 (15 isolates) being the major ones comprising 48% of the total isolates. Three clusters had isolates grouped together based on their resistance pattern. These included cluster 4 (n=4; TET), cluster 6 (n=3, TET) and cluster 10 (n=6, CIP ERY NAL TET). Isolates from cluster 6 and 10 were epidemiologically related being isolated from the same slaughter and farm trips. However, the overall population exhibited considerable genotypic diversity. The PFGE method had a discriminatory index of 0.889. Overall, MLST was able to discriminate better between the C. coli isolates from the conventional and the ABF system.

**Discussion**

There are a numerous studies that have reported the weak clonal population structure and the hypervariable genome of *Campylobacter* (Dingle *et al.*, 2001; Wassenaar *et al.*, 2000). This makes the choice of using a genotyping method for determining the source of an outbreak or comparing isolates from different sources more complex and difficult to interpret. Our results were consistent with other studies where both these methods have been shown to differentiate between closely related strains of *Campylobacter* (Sails *et al.*, 2003). The distribution of specific STs among the isolates at different stages of production and with particular resistance patterns indicated that certain STs were adapted to specific stage or production. Similar results have been reported by other studies where specific clones of *C. jejuni* have been found associated with particular niches (Colles, 2003).

The results observed in our study can be attributed to the different management conditions of these two systems or rearing pigs in North Carolina. The modern pig production system in this area uses a pyramid system where pigs from same breeding unit are moved into distinct farrowing units. This closed pyramid system may help control dissemination of infectious agents across production systems and also contain persistent strains within a production system. However, specific STs observed within a production system were detected at different levels depending on the production phase. Isolates from the farm and slaughter were clustered together in different groups and we could not detect any correlation between the banding pattern, the resistance pattern and the source of the isolates (feces or carcass). Our results are corroborated by previous studies that indicate the existence of multiple *Campylobacter* genotypes in pigs housed together (Hume, 2002). The possible reason for this genetic variability is the hypervariable genome of this pathogen which results due to the spontaneous intramolecular genomic recombinations (On, 1998; Wassenaar, 2000). We observed clusters with isolates that were not related either temporally or spatially indicating significant genetic diversity or presence of multiple reservoirs of this pathogen.

We found MLST to have a better discriminatory power and throughput than PFGE. MLST was able to further discriminate between the clusters defined by PFGE. For example, two groups of isolates representing the nursery, finishing and slaughter plant levels for the same farm, that is, related temporally and spatially and placed in the same cluster by PFGE, were further discriminated by MLST. Many studies have reported the better discriminatory power of PFGE compared to MLST when used for typing *C. jejuni*. MLST has been found to be as discriminatory as PFGE for distinguishing between temporally related isolates and the epidemic causing isolates in different outbreaks caused by *C. jejuni* (Sails *et al.*, 2003). The ability of MLST to have the same value for epidemiological typing as that of PFGE, AFLP and ribotyping has been demonstrated before (Duim *et al.*, 2003). The overall *C. coli* population had a weaker clonal structure ($I_A=0.293$) when compared to the $I_A$ value of 0.57 for *C. jejuni*. We found the *C. coli* population isolated from the conventional production system to be slightly more clonal than that of the ABF system based on
their IC₅₀ values. This could be attributed to the presence of different lineage of this species circulating in these production systems. Our results differ from other studies that have reported *C. coli* to be less diverse than *C. jejuni* by using MLST and AFLP (Dingle et al., 2005, Duim et al., 1999). However, it is possible that these isolates may not be the true representative of the *C. coli* population existing in these systems at farm and slaughter.

**Conclusions** This study highlights the high genotypic diversity of antimicrobial resistant *C. coli* in the swine population. *C. coli* isolated from the conventional farms had a slightly stronger clonal structure than those from the ABF system. We found MLST to have better discriminatory power and throughput than PFGE. MLST has the potential to be used for studying the molecular epidemiology of *Campylobacter* at national or global level due to the discriminatory power, reproducibility and ease with which data can be exchanged between different laboratories via the internet. PFGE on the other hand gave us good discrimination but not as well as MLST.

**References**


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<tr>
<th>Production Type</th>
<th>Processing Stage</th>
<th># Strains</th>
<th># STs (%Diversity)</th>
<th># New STs (%)</th>
<th># Unique STs (%)</th>
<th>PFGE Type, (n)</th>
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<tr>
<td><strong>ABF (N=50)</strong></td>
<td>Nursery</td>
<td>9</td>
<td>8 (89)</td>
<td>4 (50)</td>
<td>4 (50)</td>
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<td></td>
<td>Finishing</td>
<td>27</td>
<td>20 (74)</td>
<td>11 (55)</td>
<td>11 (55)</td>
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<td>5 (100)</td>
<td>1 (60)</td>
<td>1 (60)</td>
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<tr>
<td></td>
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<td>6 (86)</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td>1, 5, 8 (3)</td>
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<td>1 (50)</td>
<td>1 (50)</td>
<td>8 (1)</td>
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<td>7 (88)</td>
<td>6 (75)</td>
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<tr>
<td></td>
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<td>21</td>
<td>18 (86)</td>
<td>12 (67)</td>
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<td>1 (100)</td>
<td>1 (100)</td>
<td>2 (1)</td>
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Table 1. Total number of *C. coli* isolates under each production system including the MLST STs and PFGE groups.
Figure 1. PFGE dendrogram showing % similarity between the 100 C. coli isolates from the ABF system.