**Phenotypic heterogeneity of *Campylobacter coli* isolated from conventional and antimicrobial free swine at farm and slaughter**

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**Abstract**

The aim of this study was to determine the phenotypic and genotypic diversity of multiple *Campylobacter coli* isolates (n = 3) present in the same pig fecal and carcass samples at farm and slaughter, respectively. We isolated 1459 *C. coli* (1110 on farm and 349 from slaughter) from 908 pigs and 757 carcasses and characterized them for their antimicrobial susceptibility profile to a panel of six antimicrobials using the agar dilution method. A subset of 40 isolates representing 10 pigs and eight carcass samples were further genotyped by multi-locus sequence typing (MLST). Phenotypic diversity of *C. coli* isolates at the four fold minimum inhibitory concentration (MIC) levels within the same sample was detected in 39% (n = 192) pigs and 40.2% (n = 58) carcass swabs with no significant difference between the two sources ($P$ = 0.721). Phenotypic heterogeneity based on the resistance patterns was observed in 32.5% (n = 162) of the farm samples and in 30.5% (n = 44) carcass swabs at slaughter ($P$ = 0.64). Genotypic diversity based on MLST was detected in the 40 isolates which were represented by 22 sequence types (ST). In conclusion, we detected multiple *C. coli* subtypes from individual pig or carcass samples. Our study clearly signifies the importance of testing multiple colonies to make appropriate and valid conclusions in epidemiological based studies.

**Introduction**

Recent data from the US indicates that *Campylobacter* was responsible for 5,825 cases with an annual incidence of 12.68 per 100,000 individuals (MMWR, 2009). *C. coli* is an important species of *Campylobacter* that has been reported to exhibit high frequency of antimicrobial resistance and more potent virulence gene profile than *C. jejuni* (Thakur et al., 2009). Studies conducted on *Campylobacter* in pigs and broilers have revealed the importance to characterizing multiple isolates from the individual host to have a better understanding of pathogen diversity (Weijtens et al., 1999; Cesare et al., 2008). However, no study has been conducted in pigs to determine both the phenotypic and genotypic diversity of *C. coli* in pigs and to determine whether diversity on farm is the same or different at slaughter. The aim of this study was to investigate whether pigs were co-colonized with different strains of *C. coli*, and if yes, to determine whether this phenotypic diversity was also replicated at the genotypic level. We analyzed the MIC levels and antimicrobial resistance patterns at the phenotypic level and the utilized MLST data at the genotypic level for this purpose.

**Material and Methods**

A total of 1459 *C. coli* (1110 on farm and 349 from slaughter) isolated from 908 pigs and 757 carcasses were characterized at the phenotypic and genotypic levels (Thakur et al., 2006). Three presumptive *Campylobacter* colonies from each sample (fecal/carcass sample) were characterized. The agar dilution method was used for determining the MIC of the isolates against a panel of six antimicrobials as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2006). The list of antimicrobials with their abbreviations, range of concentrations (mg/l) and breakpoint levels (mg/l) used are: chloramphenicol (Chl; 0.25-128; 32), ciprofloxacin (Cip; 0.008-4; 4), erythromycin (Ery; 0.06-32; 8), gentamicin (Gen; 0.06-32; 8), nalidixic acid (Nal; 0.25-128; 32) and tetracycline (Tet; 0.06-32; 16).

A total of 40 *C. coli* isolates from 10 pigs and eight carcasses were selected systematically representing the different antimicrobial resistance profiles and temporal and spatial attributes and

genotyped by MLST (Dingle et al., 2005). Isolates with six or more shared alleles at each locus were considered as members of the same clonal complex or lineage. The frequency of antimicrobial resistance patterns and MIC of *C. coli* isolates between the farm and slaughter level were compared using the $\chi^2$ test (Minitab Inc. PA, USA) and Fisher's exact two-tailed test wherever applicable. A value of $P < 0.05$ was considered statistically significant. Phylogenetic analysis and determination of variable sites in the unique alleles was done using the MEGA software version 4.2.

**Results**

We compared the distribution of antimicrobial resistant *C. coli* isolates at the farm and slaughter. A total of three isolates per positive samples were selected randomly and further tested for their resistance to six antimicrobials. *C. coli* isolates exhibited highest frequency of resistance against tetracycline (66.2%) followed by erythromycin (53.6%). Resistance against ciprofloxacin was detected in 17 (1.5%) isolates from on-farm specimens only. We observed 11 different MDR patterns with resistance to three or more antimicrobials in 79 (5.4%) of the isolates with $Ery^R$-$Nal^R$-$Tet^R$ (2.7%) being the predominant one. Heterogeneity at the phenotypic level taking four-fold differences in the MIC was observed at the farm and slaughter levels. Overall, *C. coli* heterogeneity based on the MIC level was observed in 38.2% (n = 192) in pigs sampled on farm at the four fold dilution difference (Table 1). At slaughter, heterogeneity was detected in 40.2% (n = 58) of the carcass swabbed with no significant difference with isolates from the farm level (P = 0.721). The overall heterogeneity based on the resistance patterns was observed in 32.5% (n = 162) of the samples from farms and in 30.5% (n = 44) carcass swabs (Table 1).

Analyzing the MLST data on the subset of 40 *C. coli* isolates that represented 10 pigs and eight carcasses revealed a total of 22 STs. Overall, we detected phenotypic and genotypic diversity within multiple isolates picked up from a single pig at farm or carcass at slaughter. Among the isolates representing a single pig or carcass, a total of 11 isolates representing two pigs and three carcasses had a different ST but the same resistance pattern. A total of three pigs representing six isolates had different genotypic and phenotypic profiles. Using the eBURST software, we detected 3 groups within the cluster of 40 isolates that were represented by 22 STs (Figure 1). Group one was the largest including 23 isolates and represented by 10 STs. This group consisted of six STs that were unique to slaughter and the remaining four were unique to the farm level. A total of seven STs were designated as singletons and not assigned any group.

**Discussion**

In this study we tested three colonies of *Campylobacter* from a positive sample to get a better understanding of its diversity (heterogeneity) in pigs at the phenotypic and genotypic levels. Phenotypic heterogeneity among the *C. coli* isolates was observed at both the four fold MIC level and antimicrobial resistance pattern levels. In recent studies, strain variation in *Campylobacter* within a host has also been reported from broiler ceca and carcasses (Hunter et al., 2009; Cesare et al., 2008). *C. coli* isolates in our study exhibited highest frequency of resistance to $Tet^R$ and $Ery^R$ which is similar to previous reports (Delsol et al., 2004; Payot et al., 2004). Phenotypic heterogeneity detected in our study indicates the presence of multiple strains of *C. coli* strains in swine and necessitates the testing of multiple isolates from one positive host. This has important implications, especially in scenarios where administration of a particular antimicrobial may not result in appropriate response to the therapy primarily due to infection with multiple strains exhibiting unique resistance profiles.

In the present study, we report the result of genotyping 40 *C. coli* isolates that represented 10 pigs and eight carcasses by MLST. The presence of unique STs within at farm or slaughter indicates that specific STs could have adapted to unique environments. None of the STs representing the 23 isolates from the farm were detected at slaughter. This indicates that multiple *C. coli* genotypes were grouped together in different clusters and that unique STs are associated with the processing (slaughter) environment. This could potentially indicate that the pigs get exposed to different *Campylobacter* strains in the slaughter environment by cross contamination through a number of sources including contact with pigs from different farms, during transportation or while resting in the lairage. Similar studies conducted in *C. jejuni* and *C. coli* have reported the association of ST complexes to specific niche (Thakur et al., 2006; Miller et al., 2006).

The results of our study highlight the high genotypic diversity of antimicrobial resistant *C. coli* in the swine production systems. The genotypic diversity of *C. coli* and the detection of unique STs in different production phase could provide these isolate a selective advantage over other bacterial pathogens for rapidly evolving and dominating a particular environment. The absence of shared genotype in isolates from the sow, its respective piglets and the littermates previously highlights the extent of diversity of this pathogen (McCarthy et al., 2007). Similar observations were made when we analyzed the STs with respect to the antimicrobial resistance patterns. Barring a few STs that were restricted to specific resistance patterns (ST-1413 was associated with isolates with CipR-EryR-$Nal^R$-$Tet^R$ pattern); most of the STs were found to be associated with various resistance patterns again highlighting the diverse genetic makeup of this species. All the STs that were members of the three different clusters in our study were unique and no overlapping STs were detected. This clearly indicates the predilection and preference of particular *C. coli* genotype dependent on either the source (pig or carcass) and origin (farm or slaughter).

**Conclusion**

In conclusion, the results of our study clearly show the presence of multiple *C. coli* isolates within the same pig or carcass. This result signifies the importance of testing more than one *Campylobacter* colony from a host. The presence of multiple strains of *C. coli* in the same host may have important implications which can range from identifying the exchange of genetic material, detecting transmission sources and helping in outbreak investigations.

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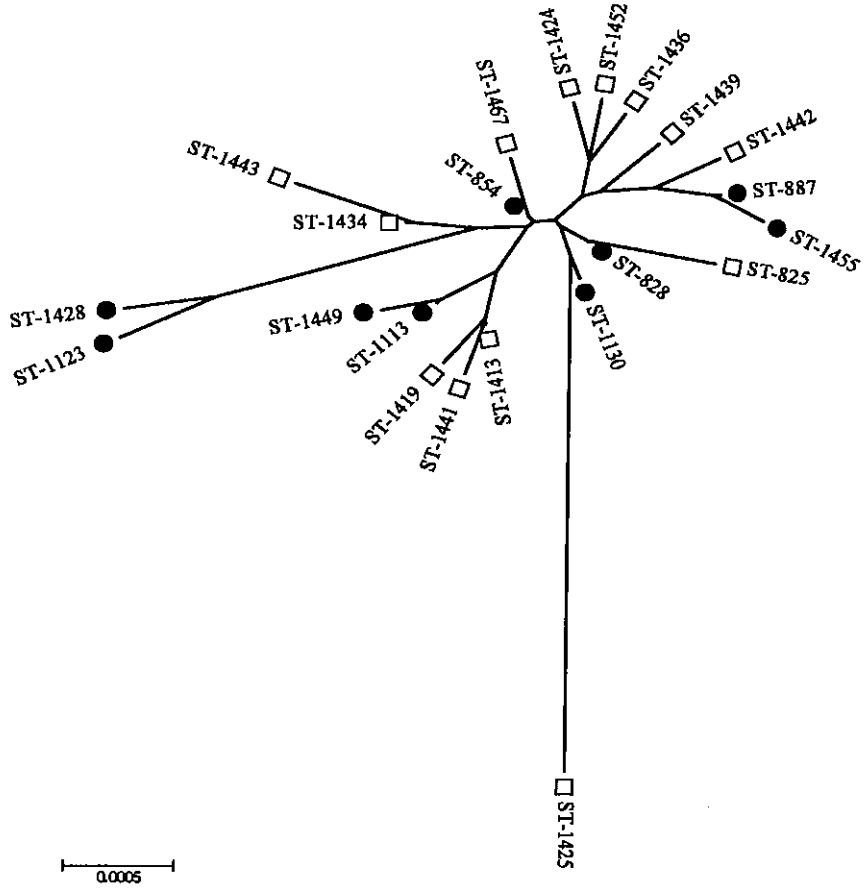
Table 1. Phenotypic heterogeneity of *C. coli* isolates at MIC (4-fold dilution) and antimicrobial resistance patterns at farm and slaughter

| Sampling Stage | Pig/Carcass Tested | Pigs/Carcass Positive n (%)[a] | Heterogenicity n (%)[b] MIC (4-Fold difference)[c] | Antimicrobial Resistance pattern[d] |
|---|---|---|---|---|
| Farm | 908 | 497 (54.7) | 192 (38.6) | 162 (32.5) |
| Slaughter | 757 | 144 (19) | 58 (40.2) | 44 (30.5) |
| Total | 1665 | 641 (38.4) | 250 (39) | 206 (32) |

[a] Represents the total number (%) of pigs or carcass sampled at farm and slaughter, respectively.
[b] Represents the total number (%) of *C. coli* isolates exhibiting phenotypic heterogeneity
[c] Total number (%) of isolates from the same sample exhibiting phenotypic heterogeneity at the four fold MIC level, [d] Total number (%) of isolates from the same sample exhibiting phenotypic heterogeneity at the antimicrobial resistance pattern level

Figure 1 Radial neighbor-joining tree representing the 22 unique sequence types (ST) identified among the 40 *Campylobacter coli* isolated from swine at farm and slaughter




Legend
- ● STs detected at Slaughter
- □ STs detected on Farm