

## Molecular Epidemiologic Investigation of *Campylobacter coli* in Swine Production Systems, Using Multilocus Sequence Typing

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**Multilocus sequence typing of 151 *Campylobacter coli* isolates from swine reared in conventional ( $n = 74$ ) and antimicrobial-free ( $n = 77$ ) production systems revealed high genotypic diversity. Sequence type (ST) 1413 was predominant and observed among ciprofloxacin-resistant strains. We identified a *C. coli* ST 828 clonal complex consisting of isolates from both production systems.**

*Campylobacter* is an important food-borne pathogen. Preliminary data estimated by FoodNet for diseases caused by enteric pathogens for the year 2004 show *Campylobacter* infection to have an incidence of 12.9 cases per 100,000 persons, second only to *Salmonella* (1). Various genotypic approaches have been used for outbreak investigations, source tracking, and determining the distribution of *Campylobacter* in humans, animals, and the environment (2, 3, 4). However, the *Campylobacter* genome has been shown to be hypervariable, which limits the use of methods like pulsed-field gel electrophoresis, *flaA* restriction fragment length polymorphism, and serotyping. It is therefore important to use a typing method that is based on indexing variation seen in the more conserved region of the genome, such as multilocus sequence typing (MLST) (7, 19, 23).

There is a paucity of information on the molecular epidemiology of *Campylobacter coli*, a species commonly identified in swine. The main objective of this study was to use MLST for determining the genotypic diversity and presence of clonal complexes and their association with antimicrobial resistance patterns in *C. coli* in swine in two distinct production systems. Swine reared in the conventional system were exposed to antimicrobials either for growth promotion or for therapeutic purposes. Under the antimicrobial-free (ABF) system, antimicrobials were not used for any purpose and animals exposed to antimicrobials due to sickness were removed from the group. We included 151 *C. coli* isolates, including 77 isolates from the ABF system (50 from the farm environment and 27 from the slaughter environment) and 74 from the conventional production system (46 from the farm environment and 28 from the slaughter environment) that were selected from a larger collection of 1,459 *C. coli* isolates (22). Selection of isolates was conducted systematically, representing the farm, slaughter, and antimicrobial resistance profiles and the total number of iso-

lates recovered at each stage. This is represented by the higher number of isolates selected for genotyping from the farm environment than at slaughter. We used random-number-generator software to select among isolates with the same resistance profiles and isolated from the same farm and slaughter stages. Overall, the 151 *C. coli* isolates selected for typing using MLST satisfied the preset criteria for selection. Antimicrobial susceptibility testing against six antimicrobials was done using the agar dilution method as recommended by the Clinical Laboratory Standards Institute (CLSI) (18). MLST of the seven housekeeping genes (*aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt*, and *uncA*), sequence type (ST) generation, and analysis were done by following a method described previously (5, 6). Isolates with six or more shared alleles at each locus were considered members of the same clonal complex. The degree of clonality was determined using the index of association and phylogenetic analysis as shown previously (11, 12). A minimum spanning tree was created using Bionumerics software version 4.0 (Applied Maths, Kortrijk, Belgium). We used the ClustalW software (available at <http://www.ebi.ac.uk/clustalw>) to perform the sequence alignments.

A total of 33 different alleles were observed, and 51 (68.9%) out of 74 STs detected in this study are reported here for the first time. We detected 51 STs (31 from the farm environment and 20 from the slaughter environment) in the ABF and 49 STs (30 from the farm environment, 19 from the slaughter environment) in the conventional system. Overall, isolates that were temporally and spatially related were further differentiated based on STs, implying a higher discriminatory power of MLST than that of the phenotypic approaches. For example, erythromycin-resistant isolates 690 and 699 (conventional), isolated at the same sampling visit at slaughter, were associated with STs 1421 and 1443, respectively. We also found STs that were unique and observed only in a specific production system or processing stage, showing evidence of niche adaptation. For instance, ST 1413 ( $n = 9$ ) was observed only in isolates from the conventionally reared pigs. Similarly, ST 1428 was isolated only at slaughter at different sampling visits and not in the farm environment. Cases of ST showing

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TABLE 1. ST 828 lineage, sequence types, and resistance patterns observed in *C. coli* from swine in the farm environment and at slaughter

ST	Allele no. for:							No. of isolates with ST	Production system(s) <sup>a</sup>	Resistance profile <sup>b</sup>
	<i>aspA</i>	<i>ghnA</i>	<i>gltA</i>	<i>glyA</i>	<i>pgm</i>	<i>tkt</i>	<i>uncA</i>			
825	33	39	30	82	113	47	17	4	ABF (F), conv (F)	E, T, ChET
828	33	39	30	82	104	43	17	2	ABF (S)	Pansusceptible, T
854	33	38	30	82	104	43	17	7	ABF (F), conv (F, S)	E, T, ET
887	33	38	30	82	104	85	68	4	Conv (S)	T, ChET, ChEGNaT
890	33	38	30	82	104	35	36	5	ABF (F, S), conv (F, S)	T, ET, NaT, ChET
1055	33	39	30	82	104	47	17	1	ABF (F)	CENaT
1056	33	39	30	82	104	43	36	2	ABF (S), conv (F)	E, ET
1058	33	39	30	82	104	35	17	1	ABF (S)	Pansusceptible
1059	33	153	30	82	104	35	17	1	Conv (F)	ET
1068	33	39	30	78	104	43	17	2	ABF (F), conv (S)	NAL, E
1096	33	38	30	82	104	35	17	9	ABF (F, S), conv (F, S)	E, ET, ChET, ChEG, CENaT, ENaT
1102	33	38	30	82	152	173	68	1	ABF (F)	E
1112	33	39	30	82	104	43	68	7	ABF (F, S), conv (F)	Pansusceptible, E, T, ET, NaT, ChET
1113	33	38	30	78	104	35	17	1	ABF (S)	T
1123	53	38	44	82	118	35	36	6	ABF (F), conv (S)	Pansusceptible, T, ET, ChENaT
1130	33	38	30	82	104	43	68	2	ABF (F), conv (S)	T, ET
1134	33	38	30	82	104	173	68	5	ABF (F), conv (F)	Pansusceptible, NaT, ChET
1142	33	153	30	82	104	43	36	1	Conv (S)	T
1177	33	38	30	82	104	85	17	3	ABF (F), conv (F)	ENa
1185	33	38	44	82	104	35	36	1	ABF (S)	ET
1200	53	38	30	82	118	35	36	1	ABF (F)	T
1413	33	38	30	82	104	117	17	9	Conv (F)	T, ChET, CENaT
1414	33	39	37	82	104	43	68	2	ABF (F)	Pansusceptible, ET
1415	33	39	47	82	104	43	36	1	ABF (S)	E
1417	33	39	44	82	104	35	36	3	Conv (F, S)	E, ET
1419	33	38	46	82	104	117	17	1	Conv (F)	CENaT
1421	33	38	44	82	104	117	36	1	Conv (F)	E
1422	33	38	46	82	104	173	17	1	Conv (F)	ET
1423	33	39	46	82	104	47	17	1	Conv (F)	ET
1424	33	39	132	82	104	44	17	1	ABF (F)	ChNaT
1426	53	38	30	81	118	85	36	1	ABF (F)	Pansusceptible
1428	53	38	30	81	118	43	36	4	Conv (S)	E, ET
1429	33	39	44	82	189	35	36	1	Conv (F)	ET
1436	33	39	44	82	104	44	17	1	ABF (F)	T
1437	33	39	46	82	104	43	68	1	ABF (F)	ENaT
1438	33	153	30	82	104	44	17	3	ABF (F), conv (F, S)	ET, CNaT
1439	33	38	30	82	104	173	17	2	Conv (F)	ENaT
1441	33	38	37	82	104	117	17	1	Conv (F)	CENaT
1442	33	38	132	82	104	85	17	1	Conv (F)	ET
1444	33	39	30	82	104	171	17	1	ABF (F)	E
1445	33	39	30	82	104	85	17	4	ABF (S), conv (F)	Pansusceptible, T, ET
1446	33	38	30	82	118	35	17	2	ABF (F)	ET
1449	33	38	37	78	104	35	17	1	ABF (S)	T
1450	53	39	44	82	104	35	36	4	ABF (S)	Pansusceptible, Na, T, ET
1452	33	39	46	82	104	44	17	2	ABF (F)	Pansusceptible, CNaT
1455	33	38	37	82	104	85	68	1	Conv (S)	T
1463	33	153	30	82	104	85	17	1	ABF (F)	NaT
1464	33	39	30	82	104	117	17	1	Conv (F)	EGT
1465	33	39	30	82	113	117	17	1	Conv (F)	EGT

<sup>a</sup> ABF (F), antimicrobial-free production system (finishing farm); ABF (S), antimicrobial-free production system (slaughter); conv (F), conventional production system (finishing farm); conv (S), conventional production system (slaughter).

<sup>b</sup> Ch, chloramphenicol; C, ciprofloxacin; E, erythromycin; G, gentamicin; Na, nalidixic acid; T, tetracycline.

niche adaptation have been reported for swine and cattle (5, 17).

The higher frequency of unique STs observed in the slaughter environment than in the farm environment indicates that pigs can be exposed to new strains in the harvesting process. This also implies that not all strains detected at slaughter originated from the farm environment, and other factors, such as cross-contamination during trucking and in holding pens, remain a concern. It is possible that the different management and environmental factors may create selective pressure that

enables specific STs to persist in a diverse environment. However, it is important to point out that although we tested three isolates per sample, it is possible that we may have missed sampling these genotypes in the farm environment. We observed different MLST profiles even for isolates that had the same antimicrobial resistance profile and were from the same sample. In addition, we also observed a larger number of STs at the farm level, which could be attributed to many reasons. Among the conventionally reared pigs, one potential reason could be the commingling of pigs from different farms at the

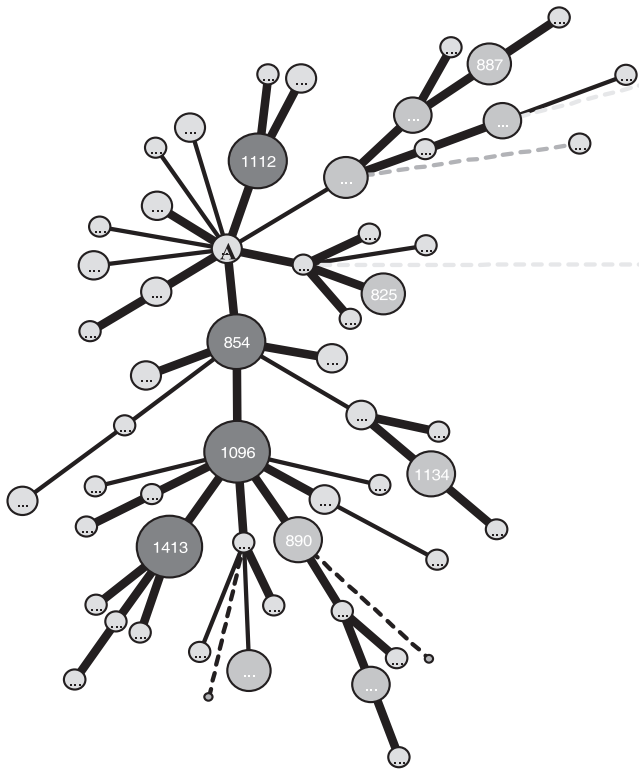


FIG. 1. Minimum spanning tree constructed using the STs from 151 *C. coli* isolates from the two production systems. The ST 828 complex shown is the putative founder of the major clonal complex. The sizes of the circles are proportional to the numbers of isolates with the indicated STs. The ST 828 complex is represented by the letter A.

finishing farms. In the case of the ABF pigs, in addition to commingling, horizontal transmission via exposure to the environment could be another reason why the pigs were infected with this pathogen. Horizontal transmission of *Campylobacter* from environmental sources has been suggested to be an important route for the spread of infection in food animals (8, 10, 13).

The high genotypic diversity of the *C. coli* population was reflected by the low index-of-association value for the overall population (0.37) and also at the individual production level, ranging from 0.34 for the ABF system to 0.62 for the conventional system. High divergence was observed in the alleles, including those with ST 1415 (47 nucleotide changes), when all seven housekeeping gene sequences were concatenated and compared to other STs displaying extreme sequence divergence. This could be an indication of a recombinational event, which in turn contributes to the weak clonality of the population (15). Hume et al. reported the absence of shared genotypes from isolates from sows, their respective piglets, and their littermates, highlighting the extent of the diversity of this pathogen (9). Similar observations were made when we analyzed the STs with respect to the resistance patterns. Barring a few STs that were restricted to specific resistance patterns, most of the STs were found to be associated with different resistance patterns, again highlighting the diverse genetic makeup of this species (Table 1). However, it is important to highlight that the multidrug-resistant *C. coli* isolates that were

resistant to ciprofloxacin from the conventional farms were associated only with ST 1413. This ST is reported here for the first time and could represent an ST associated with ciprofloxacin-resistant *C. coli* strains.

Phylogenetic comparison of *C. coli* isolates from the two production systems revealed that the two populations of *C. coli* had no fixed differences between them and had 67 shared mutations, indicating similarity at the sequence level. A minimum spanning tree constructed using the 74 STs revealed close clustering of isolates irrespective of their origin (Fig. 1). A single clonal complex (ST 828 complex) which included STs ( $n = 53$ ) representing isolates from both of the production systems was identified (Table 1 and Fig. 1). The identification of clonal complexes in *C. jejuni* and their use as an epidemiological unit have tremendously helped in comparing isolates from different parts of the world (2, 4, 5, 14, 20, 21). Miller et al. recently reported a *C. coli* clonal complex comprising isolates from diverse sources, including humans, swine, chickens, sheep, a marmoset, and manure (16). The potential progenitor strain ST 828 has been reported for humans, swine, and cattle from different parts of the world (5, 17). This indicates that ST 828 is an important ST that has potential to infect both humans and animals and is an important finding from a food-borne-disease perspective. We acknowledge that typing of more isolates, including isolates from diverse sources, will help in better defining this apparent lineage.

In conclusion, MLST of *C. coli* isolates from swine highlighted the overall weak clonal population and the diverse genetic makeup of this species. However, the high proportion of STs being shared and the close clustering of STs in the two production systems provide evidence that these genotypically diverse *C. coli* strains are commonly shared between pigs reared in different production systems. This finding might potentially explain the reason for the high prevalence of antimicrobial-resistant *C. coli* in the ABF production system (22). Given the potential of pig isolates to cause disease in humans (14), we recommend testing of more *C. coli* isolates from diverse geographic sources and time points to better define the clonal relationships and lineages.

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