Ribotype Characterization and Antimicrobial Susceptibility Profiles of Campylobacter coli Isolates from Swine.

Sheffield CL, Hume ME, Droleskey RE, Harvey RB, Bischoff KM.

USDA, ARS, Southern Plains Agriculture Research Center, Food and Feed Safety Research Unit, 2881 F&B Road, College Station, TX 77845, USA.

ABSTRACT

Sheffield CL, Hume ME, Droleskey RE, Harvey RB, Bischoff KM. Characterization and Antimicrobial Susceptibility Profiles of Campylobacter coli Isolates from Swine. Online Journal of Veterinary Research, 7:52-58, 2003. The genetic relatedness and antimicrobial susceptibility profiles of 104 Campylobacter coli isolates from feces of 3 sows and rectal swabs of 18 piglets housed in the same facility, were determined using ribotyping and disc diffusion respectively. C. coli isolates segregated into 20 ribogroups and exhibited 22 antibiotic susceptibility profiles. No discernible pattern of ribogroup relatedness was observed between sows and offspring, or littermates. Results suggest a high level of both ribotypic and antimicrobial susceptibility diversity within C. coli isolates from both related and non-related pigs housed in a single facility.

KEY WORDS: Campylobacter, Swine, RiboPrinter®, Ribotype, Antimicrobial Sensitivity

INTRODUCTION
Foodborne diseases in the United States are the cause of an estimated 81 million illnesses per year, (CDC, 2001; Mead et al 1999). It is estimated that 2.5 million of these illnesses can be attributed to Campylobacter spp. (Fitzgerald et al 2001a; Fitzgerald et al 2001b; Hume et al 2002; Zhao et al 2001). In 2000, Campylobacteriosis had the highest incidence of diagnosis per 100,000 persons of any foodborne illness in the United States. FoodNet (CDC-USDA-FDA, Foodborne Disease Active Surveillance Network) reported that over the first five years of operation, Campylobacter was the most frequently diagnosed pathogen associated with foodborne illness (CDC 2001). Campylobacter spp. have been isolated from a wide variety of sources including cattle, poultry, swine, sheep, raw meats, raw milk, seafood, and cooked meats (Fitzgerald et al 2001b; Harvey et al 1999; Hume et al 2002; Nielsen et al 2000; Wassenaar and Newell DG 2000; Zhao et al 2001).

Traditional phenotypic methods of identifying Campylobacter isolates include serotyping, biotyping, and phage typing (De Boer et al 2000; Desai et al 2001; Duim et al 1999; Fitzgerald et al 2001a; Hume et al 2002; Nielsen et al 2000; Wassenaar and Newell DG 2000). Each of these methods have intrinsic limitations including: considerable time and technical expertise needed to maintain reagents (Fitzgerald et al 2001a; Wassenaar and Newell DG 2000); cross-reactivity between antigens (Desai et al 2001; Fitzgerald et al 2001a); occurrence of untypeable isolates (De Boer et al 2000; Duim et al 1999; Engberg et al 2001; WasFitzgerald et al 2001a; Wassenaar and Newell DG 2000); test instability (Hume et al 2002); restricted availability of specific serotyping reagents (De Boer et al 2000; Desai et al 2001; Duim et al 1999); and limited discrimination (De Boer et al 2000; Desai et al 2001; Duim et al 1999; Fitzgerald et al 2001b; Nielsen et al 2000).


Antimicrobial resistance among foodborne pathogens is a problem that has increased over the last 40 years (Aquino et al 2002; Swartz NM 2002; Teuber M 1999). Antimicrobial resistance in foodborne pathogens (e.g., Campylobacter, Salmonella and some strains of Escherichia coli) represents a public health risk on several levels (Aarestrup and Wegener HC 1999; Aquino et al 2002; McEwen and Fedorka-Cray P 2002; Swartz NM 2002; Teuber M 1999; Travers and Barza M 2002; White et al 2002). Antimicrobial resistance can lead to increased virulence of the strain, possible complication of the initial choice of antimicrobial treatment (Travers and Barza M 2002), and increased treatment failure (Aarestrup and Wegener HC 1999). A recent study suggests that annually, antimicrobial resistance results in an additional 17,668 Campylobacter infections, leading to ninety-five hospitalizations (Barza and Travers K 2002). Recent studies revealed that C. coli, which is primarily isolated from swine, exhibits both higher levels of antimicrobial resistance and
resistance to a wider variety of antimicrobial agents than other Campylobacter spp (Aarestrup et al 1997; Saenz et al 2000).

Here we report the ribotypic diversity and antimicrobial susceptibility profiles of Campylobacter isolates from sows and piglets housed in a single farrowing barn.

---

**MATERIALS AND METHODS**

**Sample Collection**: Sows were Yorkshire X American Landrace. Piglets were the offspring of Yorkshire X American Landrace sows and either Duroc or Hampshire boars. All animals were maintained at a commercial farrow-to-finish operation, in a single open-sided unscreened farrowing barn consisting of covered pens on a sloped concrete floor. Each pen was equipped with an automatic feeder and nipple water. Waste was washed from the pens daily with water; the waste was flushed into sloped gutters outside the pens. The practice of feeding antimicrobial agents as growth promoters was not used in this operation during the previous 7 years. Piglets remained on-sow until weaned at 21 days of age. No animals in this study had an obvious disease condition or were being given antimicrobial agents.

Campylobacter isolates were collected from sows and piglets when the piglets were weaned at 21 day of age. Feces (0.5 g) from each sow was mixed into 10 ml of Campylobacter enrichment broth (Hume et al 2002) and shaken vigorously. Rectal swabs from piglets were placed in individual tubes containing 0.9 ml of sterile phosphate buffered saline (PBS; pH 7.2), transported to the laboratory, and mixed into 10 ml of Campylobacter enrichment broth. All samples were incubated microaerobically (10% carbon dioxide, 5% oxygen, and 85% nitrogen) at 42°C for 24 hours. Ten microliters from each enriched culture was streaked onto Campy-Cefex agar plates and incubated for 48 hours. Five colonies from each plate were transferred individually to fresh Campy-Cefex agar plates and incubated for 24 hours. Colonies picked from these plates were suspended in sterile PBS and brought to a final concentration equal to a McFarland standard number 6. A 200-μl aliquot of this material was spread onto Tryptic soy agar plates containing 5% sheep blood (BBL, Cockeysville, MD) and incubated at 42°C for 24 hours. The bacterial lawn was suspended in 4 ml of sterile PBS and mixed with sterile glycerol (20% v/v) for storage at -70°C. Campylobacter spp. isolates were identified by growth characteristics, colony morphology, and PCR (primers supplied by Integrated DNA Technologies, Inc. Coralville, IA).

**Ribotype Analysis**: The RiboPrinter® microbial characterization system (Qualicon, Inc.; Wilmington, DE) characterizes the 5, 16, and 23 sRNA and flanking regions of a bacterial sample using specified restriction enzymes. The resulting ribosomal RNA pattern (RiboPrint® pattern) is automatically added to the existing database. The system then characterizes and/or identifies the bacterium by comparing the RiboPrint® pattern to reference patterns in both the standard and custom libraries. Characterization (inclusion in a ribogroup) requires that the ribopattern be a 90% or greater match to an existing ribopattern. Characterization is based on both size and intensity of the bands within the pattern. The ribogroup profile used for characterization is a dynamic profile. Each time a new pattern is characterized as a specific ribogroup, the composite pattern is altered slightly, as the new data is incorporated into the profile. Identification requires that the ribopattern be an 85%
or greater match to an existing static ribopattern located in the standard or the custom libraries to be labeled as that specific bacterium.

Campylobacter isolates were prepared for ribotyping by culturing on Campy-Cefex agar plates and incubated for 48 hours to allow a bacterial lawn to form. Bacterial lawns were collected while the isolates were in log phase growth. Bacteria were suspended in a neutral pH buffer (Qualicon), heated at 90°C for 10 minutes, combined with two additional lytic enzymes (Qualicon) and analyzed according to manufacturer’s instructions using the restriction enzyme PstI.

**Dendrograms:** Using Molecular Analysis Fingerprinting Software, version 1.6 (Bio-Rad Laboratories, Hercules, CA) dendograms of the ribogroups were determined, based on Unweighted Pair Group Method using Arithmetic averages (UPGMA) analysis and the Dice similarity coefficient.

**Antimicrobial Resistance Profiles:** Using a modification of the method outlined by Fallacara, et al (2001), isolates were screened for resistance to amikacin (30 μg/ml), ampicillin (10 μg/ml), ciprofloxacin (5 μg/ml), clindamycin (2 μg/ml), erythromycin (15 μg/ml), gentamycin (10 μg/ml), lincomycin (2 μg/ml), neomycin (30 μg/ml), tetracycline (30 μg/ml), and tobramycin (10 μg/ml) (BD biosciences, Sparks, MD). Briefly, an inoculum equivalent to a 0.5 McFarland turbidity standard was streaked over the entire surface of the Diagnostic Sensitivity Testing agar (Oxoid, Ogdensburg, NY) plates, using a sterile cotton-tipped swab. This resulted in the formation of a uniform lawn of bacteria over the surface of each plate. Antibiotic disks were distributed over the plates with no more than eight disks per plate. Plates were incubated microaerobically for 48 hours at 42°C. Zone diameters surrounding each antibiotic disk were measured and breakpoints determined as described by Fallacara et al (2001).

---

**RESULTS**

One hundred and four isolates from Campylobacter-positive animals were identified by PCR as *C. coli*. Twenty ribogroups were characterized by automated ribotyping following PstI digestion (Table 1).

**Table 1.** Ribogroups Characterized by PstI Digestion of *C. coli* Isolates From Sows and Piglets.

<table>
<thead>
<tr>
<th>Animal Type &amp; Number</th>
<th>Ribogroup (number of isolates)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sow 6</td>
<td>C(2), P(1), Q(1), R(1)</td>
</tr>
<tr>
<td>Piglet 779</td>
<td>B(4), F(1)</td>
</tr>
<tr>
<td>Piglet 780</td>
<td>B(1), N(3), O(1)</td>
</tr>
<tr>
<td>Sow 8</td>
<td>B(5)</td>
</tr>
<tr>
<td>Piglet 790</td>
<td>C(5)</td>
</tr>
</tbody>
</table>
In 43% of the animals all isolates were represented by one ribogroup. In 24% of the animals, isolates segregated into two ribogroups. The remaining 33% of the animals exhibited isolates representing three or more ribogroups. No link was observed between the Campylobacter ribogroups isolated from the sows (6; 8; and 9) and their offspring (779, 780; 790; and 791, 793, 795).

Seventy-nine percent of the isolates were resolved into one of five ribogroups. The three most predominant ribogroups were A, B, and C representing 14%, 22%, and 33% of the isolates, respectively (Table 1). Five ribogroups were isolated from multiples animals, with ribogroups A, B, C, D, and G representing 3, 7, 11, 2, and 3 animals, respectively.

Ribogroups analyzed by Unweighted Pair Group Method using Arithmetic averages (UPGMA) revealed four main clusters of 76-85% similarity containing 19 ribogroups (Figure 1).

**Figure 1.** Ribogroup patterns and dendogram from PstI digestion of Campylobacter coli isolates from sows and piglets.
One ribogroup (G) was less than 60% similar to the four main clusters. The biggest cluster (1) contained 6 ribogroups, with a range of 76-97% similarity. Two clusters (2, 4) contained 4 ribogroups each, with a range of 76-88 % and 85-93% similarity, respectively. Cluster (3) contained 5 ribogroups, with a range of 82-92% similarity. In clusters with similarity greater than 85%, only ribogroup (B) was found in animals from the same litter.

No single susceptibility profile was associated with any ribogroup which contained three or more isolates. All isolates evaluated, exhibited resistance to both lincomycin and tobramycin, but were sensitive to erythromycin. Resistance to neomycin, ampicillin, tetracycline, gentamycin, amikacin, and clindamycin were exhibited by 96, 91, 80, 44, 10, and 6% of the isolates respectively. Sensitivity to ciprofloxacin, amikacin, tetracycline, clindamycin, and ampicillin were exhibited by 91, 84, 75, 13, and 2% of the isolates respectively. An intermediate response to gentamycin, clindamycin, ciprofloxacin, tetracycline, and neomycin were exhibited by 56, 19, 9, 7, and 4% of the isolates respectively. A moderately sensitive response to ampicillin, and amikacin were exhibited by 7 and 6% of the isolates respectively.
DISCUSSION

Campylobacters are widespread among animals used for food production, often resulting in the contamination of meat products. As an outcome of this diverse contamination, campylobacteriosis is the most common foodborne bacterial diseases with worldwide social and economic consequences (Petersen and Neilsen EM 2001). Thus, it is increasingly important to be able to distinguish between Campylobacter strains and subtypes in order to trace routes of transmission and environmental diffusion. While numerous molecular typing methods exist, an ideal method has yet to be developed. Such a method would be easy to perform, cost-effective, relatively rapid, amenable to statistical analysis and automation, useful for all strains, reproducible, and characterized by a good balance between increased discriminatory power and applicability (De Cesare et al 2001b). Ribotyping has proven useful for subtyping a wide variety of bacteria including Campylobacter spp., E. coli, Listeria monocytogenes, and Clostridium perfringens (Kilic et al 2002). Further, in previous studies the RiboPrinter® has been a valuable tool for ribotyping bacterial strains (Brisse and Verhoef J 2001; De Cesare et al 2011a; Hollis et al 1999; Quale et al 2001).

In this study, 20 ribogroups were characterized within the 104 Campylobacter isolates from three sows and 18 piglets all housed in a common farrowing barn. This marked genetic diversity among the Campylobacter isolates is not unexpected (Weijtens et al 1999), and has been observed in poultry (Hanninen et al 2001; Heuer et al 2001; Nielsen et al 2000; Petersen and Nielsen EM 2001). The presence of multiple Campylobacter ribogroups within a single farrowing barn may be attributed to the introduction of exogenous bacteria via water, contaminated materials, farm personnel, insects, or other wild and domestic animals, and sows that are added to the farrowing barn. Further, part of the ribogroup multiplicity may be ascribed to random and spontaneous genomic modifications within existing Campylobacter ribotypes.

One sow and six piglets within this study exhibited only a single Campylobacter ribogroup. This may be an artifact due to the intermittent shedding of Campylobacter. Intermittent shedding has been previously reported in pigs (Hume et al 2002; Weijtens et al 1999), poultry (Hume et al 2002; Jeffrey et al 2000; White et al 2002) and cattle (Hoar et al 2001). The ribogroup(s) isolated from each animal represent a snapshot of the range of strains being shed at the time of sampling. Among the animals sampled, there was no evidence of shared ribogroups between a sow and her offspring. Piglets 791, 793 and 795 were the only littermates to share a ribogroup (G). However, these animals exhibited other non-related ribogroups (B, D, E, I). Ribogroup (C) was the most widely distributed occurring in 11 of the 21 animals.

Ninety-eight Campylobacter isolates exhibited a total of 22 unique antibiotic resistance profiles. We did not detect any relationship between the distribution of antibiotic resistance profiles and ribogroups. Further, sensitivity to erythromycin and resistance to tobramycin and lincomycin spanned all ribogroups.

In conclusion, one hundred and four Campylobacter isolates from three sows and 18 piglets all housed in one farrowing barn exhibited 20 ribogroups based on a single sampling timepoint. The results of this study highlight the need to expand the number of isolates collected from each animal and to collect samples over a more
prolonged time frame. This expanded sampling would provide a more complete picture of the dynamics of the Campylobacter population within each facility of the farrow-to-finish swine operation.

REFERENCES


