PREVALENCE, ANTIMICROBIAL RESISTANCE AND GENOTYPIC DIVERSITY OF CAMPYLOBACTER COLI ISOLATED FROM ANTIMICROBIAL FREE SWINE PRODUCTION SYSTEMS

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Abstract A cross-sectional study was conducted to determine the prevalence, antimicrobial resistance profile and genotypic diversity of Campylobacter in swine herds. Overall, Campylobacter coli were the only species isolated from the study with a prevalence of 55.8% and 27.9% at farm and slaughter respectively. C. coli prevalence between the two ABF systems was not significantly different at farm (P=0.83). Highest frequency of resistance was seen against tetracycline (48.3%) and erythromycin (40.6%). We detected significantly higher proportion of C. coli isolates from the intensive system resistant to the above two antimicrobials at the finishing farms (P<0.001). Resistance against the fluoroquinolone ciprofloxacin was detected in isolates from farm and in both types of ABF herds (n=3). We observed 11 different multi drug resistant (MDR) patterns in 25 isolates (4.6%) with Ery Nal Tet (2.9%) being the predominant one. PFGE analysis revealed C. coli to be genotypically diverse with clustering of isolates from both the production systems together in different groups. The study highlighted the common occurrence of antimicrobial resistance in ABF herds irrespective of whether the herds were managed in extensive or intensive units.

Introduction Campylobacter is an important foodborne pathogen and is responsible for causing 2.4 million illnesses and 150 deaths in the United States every year (Mead et al., 1999). Although the majority of the infections in humans are attributed to Campylobacter jejuni, the importance of C. coli is being recognized due to its ability to show increased resistance to more number of antimicrobials (Tam et al., 2003). Pigs and their environment at farm and slaughter have been shown to be suitable for C. coli with many studies reporting them as the primary reservoirs of this pathogen (Harvey et al., 1999). Several studies on pigs reared in the conventional system of production where antimicrobials are used regularly for therapeutic and growth promotion purposes have reported the prevalence of antimicrobial resistant strains of C. coli (Payot et al., 2004; Looveren et al., 2004). No study has been done in case of pigs reared in ABF system where no antimicrobials are used for any of the above purposes. The primary objectives of the study, therefore, were to determine the prevalence, antimicrobial susceptibility and genetic diversity or similarity of Campylobacter isolates from the two ABF production systems, namely the extensive (outdoor) and the intensive (indoor) system at production (on-farm) and processing (at slaughter) and understand their phenotypic and genotypic diversity.

Materials and Methods Swine fecal and carcass swabs were collected from 10 groups of pigs (five each from intensive and extensive ABF farms) at the farm and the slaughter plant. Campylobacter isolation from the fecal sample was done by directly plated loopful of the sample onto campy-cefex selective plates and incubated under microaerobic conditions (CO₂: 10%, O₂: 5% and N₂: 85%) at 42°C for 48 hours. For isolation of the pathogen from the carcass swabs, the swabs were first soaked in 30 ml of Bolton broth (Oxoid, Hampshire, UK) and incubated under microaerobic conditions for 48 hours. After two days of incubation, approximately 10 µl of the enriched liquid was streaked onto campy-cefex plates and further incubated for 48 hours similar to the fecal sample processing. Speciation was done by PCR targeting ceuE and hipO genes for C. coli and C. jejuni respectively. The isolates were tested for their resistance profile using the agar dilution method against six antimicrobials. The list of antimicrobial with their abbreviations and range of concentrations used is: chloramphenicol (Ch; 0.25-128 mg/liter), ciprofloxacin (Cip; 0.008-4 mg/liter), erythromycin (Ery; 0.06-32 mg/liter), gentamicin (Gen; 0.06-32 mg/liter), nalidixic acid (Nal; 0.25-128 mg/liter) and tetracycline (Tet; 0.06-32 mg/liter). Genotyping of 55 representative isolates from farm and slaughter was done by PFGE.
Results Overall, *Campylobacter* prevalence at the farm and slaughter level was 55.8% and 27.9% respectively. All the 542 *Campylobacter* isolates in this study including 372 from farm and 170 from slaughter were *C. coli*. There was no significant difference in *C. coli* prevalence at the farm level for the extensive (55%) and intensive (56.3%) rearing systems as shown in (Figure 1, P=0.83). In both the ABF systems, there was an increase in *C. coli* prevalence at post-evisceration followed by a significant decrease at the post-chill stage (P<0.002). We observed significantly higher proportion of *C. coli* at the pre-evisceration stage of processing extensive reared ABF pigs (P<0.001). Within individual system, we did not observe any difference in prevalence at the post-chill stage between the USDA method and the single swipe method of carcass swabbing (Figure 1). However, on comparing the two ABF systems at the post-chill stage, we observed *C. coli* only from the carcasses of extensively reared ABF pigs.

We detected resistance to all the six antimicrobials tested. Overall, isolates exhibited highest frequency of resistance against tetracycline (48.3%) and erythromycin (40.6%) (Table1). A total of 195 isolates (36%) were pansusceptible. On comparing the resistance profile of isolates from the extensive and intensive ABF systems, we detected significantly higher proportion of *C. coli* isolates from the intensive system resistant to the above two antimicrobials at the finishing farms (P<0.001). Higher proportions of *C. coli* isolates from the ABF system at the post-evisceration step were resistant to tetracycline and erythromycin than at the pre-evisceration step (P<0.05). Resistance against the fluoroquinolone ciprofloxacin was detected in isolates from on-farm specimens and in both types of ABF herds (n=3). Gentamicin and chloramphenicol resistant isolates were rare and observed in 0.1 and 1.8% of the total isolates. A total of 15 different resistance patterns were observed exhibited by with resistance being shown to either single antimicrobial or to a combination of them. On comparing the two systems, significantly higher proportion of isolates from extensive were found to be pansusceptible than those from the intensive farms (P<0.001).

The most common resistance pattern observed in this study was resistance to erythromycin and tetracycline, Ery Tet. This pattern was significantly higher exhibited in *C. coli* isolates from the intensive system both at farm and slaughter (P<0.001). We observed 11 different multi drug resistant (MDR) patterns in 25 (4.6%) of the isolates with Ery Nal Tet (2.9%) being the predominant one. Except for one isolate with Ch Ery Nal Tet and another with Ch Ery Gen resistance pattern isolated at the post-evisceration step, none of the isolates at the slaughter level were MDR.

*C. coli* isolates (55) representing both the production types and all the stages of production at farm and slaughter were genotyped by PFGE to determine the diversity or clonality of the isolates at the farm and slaughter levels. Using 70% genetic similarity cut off level as an arbitrary index of similarity among the isolates, we observed a total of 8 groups. Overall, clonal type 4 and clonal type 7 were the major groups encompassing 31 out of the 55 *C. coli* isolates. Clonal type 4 was the largest group with 17 isolates followed by type 7 with 14 isolates. Clonal types 6 and 8 were composed of isolates from the Intensive farms only. For the remaining, isolates from the two ABF systems both from the farm and slaughter were grouped together in different clusters.

Discussion High prevalence of *C. coli* in pigs at the finishing farms seen in this study is similar to reported in many studies with prevalence ranging from 50.4% to 94% (Payot et al., 2004, Looveren et al., 2004). All the *Campylobacter* isolates in this study were *C. coli*. *C. coli* has been known to be mostly prevalent in pigs with a prevalence ranging up to 100% (Saenz et al., 2000). Similar study comparing two ABF systems have been conducted in poultry where *Campylobacter* prevalence was compared between the organic outdoor and the conventional poultry flocks. The authors in this study reported a prevalence of 91% and 4.5% in the outdoor reared organic poultry flock and 86.2% and 10.3% for the conventional flock for *C. jejuni* and *C. coli*, respectively (Heuer et al., 2001). Increase in the prevalence in ABF pigs could be attributed to various management conditions. High prevalence in extensively reared nursery pigs could be attributed to horizontal transmission via the open environment where the pigs have unrestricted access to various environmental risk factors. *C. coli* have shown to be present in the environment in both soil and water (Leatherbarrow et al., 2004). It is possible that these pigs had high prevalence of *C. coli* due to access to water in the open.

Higher prevalence at the post-evisceration than the pre-evisceration step of the slaughter plant could be a result of various manipulations including evisceration etc. that can contribute to cross contamination of the carcass. Between the two ABF type systems, *C. coli* could be isolated at the post-chill stage only from the carcasses belonging to the extensively reared system. This
could be due to the slaughter house effect with the extensively reared ABF pigs being slaughtered in the plant that employs the 24 hour freezing method as opposed to the blast-chilling system practiced in the plant that slaughtered the intensively-reared ones. The blast-chiller system chills the carcasses at -35°C temperature for two hours before it is kept in a regular chiller unit. *Campylobacter* has been shown to be highly susceptible to oxygen, cold and drying conditions (Korsak et al., 1998).

Even though neither tetracycline nor macrolides were used as growth promotants in any of the ABF herds, significant number of isolates from slaughterhouse, including post-chill samples, showed resistance against tetracycline and erythromycin. This may be attributed to in-plant cross contamination of *Campylobacter* between different pig herds. This is especially likely since none of the slaughter houses where the samples were collected were dedicated for ABF herds. Thus, the likelihood of cross contamination at lairage and processing could be high. Ciprofloxacin resistant observed here is important since fluoroquinolones are not licensed for use even in the conventional pig production system. Similarly, resistance seen against chloramphenicol (1.8%) is striking since this antimicrobial has not been used in any system of pig production in the US for the last two decades. This highlights the possible role played by environmental factors like soil and water in the spread of these resistant strains. Ery Tet was the most common resistance pattern since the isolates were resistant to these two antimicrobials more than any other in this study. Ery Nal Tet resistance pattern was also reported by to be the most common MDR pattern in another study (Payot et al., 2004).

Analysis of the PFGE dendrogram revealed *C. coli* to be genetically diverse with clustering of isolates from both the production systems together in different groups. 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 (Weijtens et al., 1999). The presence of different banding patterns in isolates sampled from the same farm on the same day highlighted the high level of genetic diversity among the *C. coli* isolates. We conclude that *C. coli* present in swine farms consists of genetically diverse clones.

**Conclusions** This study highlights the prevalence of antimicrobial resistant *C. coli* from both the extensive and the intensive type ABF production system at farm and slaughter. High prevalence of *C. coli* observed at the farm level provides a strong potential for introduction of the pathogen at slaughter. The presence of antimicrobial resistant *C. coli* at the farm and slaughter is concerning and points to other sources for acquiring these strains in the absence of antimicrobial use. This is the first report of isolation of fluoroquinolone resistant *C. coli* from pigs in the United States and among ABF pigs.

![Figure 1. C. coli prevalence at the farm and slaughter level in the two ABF production systems. Abbreviations on the X-axis: F-Ext (Extensive Finishing, n=118); F-Int (Intensive Finishing, n=174); PRE-Ext (Extensive Pre-Evisceration, n=38); PRE-Int (Intensive Pre-Evisceration, n=40); POST-Ext (Extensive Post-Evisceration, n=48); POST-Int (Intensive Post-Evisceration, n=40); CUS-Ext (Extensive Post-Chill USDA, n=48); CUS-Int (Intensive Post-Chill USDA, n=40); C-Ext (Extensive Post-Chill Single Swipe, n=48) and C-Int (Intensive Post-Chill Single Swipe, n=39). *Bars sharing common superscripts were significantly different at P < 0.05 (chi-square test)
REFERENCES


<table>
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<tr>
<th>Production Stage</th>
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<th>Isolates Tested</th>
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<th>Erythromycin</th>
<th>Gentamicin</th>
<th>Nalidixic Acid</th>
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<td>1 (0.6)</td>
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<td>1 (0.5)</td>
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<td>27 (13)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (16.7)</td>
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<td>3 (0.5)</td>
<td>220 (40.6)</td>
<td>1 (0.2)</td>
<td>58 (6.1)</td>
<td>262 (48.3)</td>
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Table 1. Comparison of antimicrobial resistance frequency among the C.coli isolates from Intensive and Extensive reared ABF pigs at different stages of production. ABF: Antimicrobial Free Farms

*Isolates with percentage in the brackets. For each antimicrobial, figures sharing common alphabet superscripts were not significantly different (P > 0.05) while figures sharing common digits in the superscripts were significantly different at P < 0.05 (chi-square test)