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Campylobacter coli: prevalence and antimicrobial resistance in antimicrobial-free (ABF) swine production systems

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***Objectives*: To determine the prevalence and antimicrobial resistance of *Campylobacter* species in swine reared in the intensive and extensive antimicrobial-free (ABF) production systems at farm and slaughter. In the ABF system, antimicrobials are neither used for growth promotion nor therapeutic purposes.**

***Methods*: Swine faecal and carcass swabs were collected from 10 groups of pigs (five each from intensive and extensive ABF farms) at the finishing farm and the slaughter plant. A total of 292 pigs at farm (extensive 118; intensive 174) and 254 carcass swabs (extensive 134; intensive 120) were collected during the study. *Campylobacter* species were isolated under microaerobic conditions and confirmed by biochemical testing. Up to three presumptive *Campylobacter* colonies per positive pig/carcass were further characterized. Speciation was done by PCR, targeting *ceuE* and *hipO* genes for *Campylobacter coli* and *Campylobacter jejuni*, respectively. The isolates were tested for their antimicrobial resistance profile using the agar dilution method against six antimicrobials.**

***Results*: A total of 526 *Campylobacter* isolates were cultured from 292 pigs and 254 carcasses sampled. All the isolates were found to be *C. coli*. Overall prevalence of *C. coli* was 55.8% on farm (55% extensive and 56.3% intensive) and 26% at slaughter (32.8% extensive and 18.3% intensive). There was no significant difference in *C. coli* between the intensive and extensive systems on the finishing farms ($P = 0.83$). At post-chill stage, *C. coli* were isolated only from the extensively reared ABF pigs. Antimicrobial resistance against ciprofloxacin (MIC > 4 mg/L) was found at the farm level in both the intensive- and extensive-reared groups. The erythromycin/nalidixic acid/tetracycline resistance pattern (3%) was the most common pattern in multidrug-resistant *C. coli*.**

***Conclusions*: This study highlights the high prevalence of diverse and antimicrobial-resistant *C. coli* in the ABF production systems of swine. This is the first study reporting the isolation of ciprofloxacin-resistant strains from ABF pigs in the USA and warrants concern.**

Keywords: pig, *Campylobacter* species, antibiotic resistance, antimicrobial-free production

Introduction

Campylobacter is an important food-borne pathogen and is responsible for causing ~2.4 million illnesses and 150 deaths in the USA annually.[1] Although an estimated 95% of the infections in humans are attributed to *Campylobacter jejuni*, the importance of *Campylobacter coli* is being recognized due to its ability to show increased resistance to a greater number of antimicrobials.[2,3] Pigs and the production and processing environment at the farm and slaughter have been shown to be suitable for *C. coli* with many studies reporting them as the primary reservoir of this pathogen.[4]

Studies on pigs reared in the conventional system of production where antimicrobials are used regularly for therapeutic and growth promotion have reported the prevalence of antimicrobial-resistant strains of *C. coli*.[4,5] Studies of antimicrobial-free (ABF) production systems have been done before in the poultry industry in Europe.[6] However, no study has been reported on swine reared in the ABF system in the United States. Under the ABF system of pig production, no antimicrobials were either added in the feed for growth promotion nor given for therapeutic purposes. Any pig that was treated for any infection was immediately removed from the herd. The primary objectives of the study were to

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determine the prevalence and antimicrobial susceptibility of *Campylobacter* isolates from the extensive (outdoor) and intensive (indoor) ABF production systems on-farm and at slaughter.

Materials and methods

Production systems and sample collection

In the extensive ABF pig production system, pigs were reared in open fields in a barricaded area and had free access to the environment including soil and water. Under the intensive system, pigs were reared in confined barns with concrete slatted floors. A total of five finishing farms were sampled from each system over a period of 2 years from 2002 to 2004. At every farm visit, we sampled 30 pigs and collected ~10 g of fresh faecal samples per rectum with gloved hands. Carcass samples were collected using sterile swabs soaked in 10 mL of buffered peptone water (Becton Dickinson, NJ, USA). Ten individual carcass samples were collected at each of the three processing stages: pre-evisceration, post-evisceration and post-chill. Carcass samples were collected by swabbing at the jowl, belly and the ham region. Pigs from the two ABF systems were slaughtered at two different slaughter plants. The intensively reared pigs were slaughtered in a slaughter plant with a blast chiller system for cooling the carcass in 2 h with a temperature of –30°C. The extensive pigs were slaughtered in a smaller plant using overnight chilling to cool the carcass (1–4°C for ~18 h). The study protocol has been approved by the Institutional Animal Care and Use Committee.

Campylobacter isolation and speciation

Campylobacter isolation from the samples was done by directly plating a loopful of the sample onto campy-cefex selective plates and incubating under microaerobic conditions (CO_2: 10%, O_2: 5% and N_2: 85%) at 42°C for 48 h. Up to three presumptive *Campylobacter* colonies per positive sample were selected for further analysis. Biochemical confirmation of colonies was done using the catalase (3% H_2O_2) and the oxidase tests (tetramethyl-*p*-phenylenediamine) (Becton Dickinson, NJ, USA).

PCR detection of *C. coli* and *C. jejuni* was done using species-specific primers. The *ceuE* and the *hipO* gene were used for detection of *C. coli* and *C. jejuni*.[7] The forward and reverse primers for *ceuE* gene amplification were CC2, 5′-GATTTTATTATTTGTAGCAGCG-3′ and CC3, 5′-TCCATGCCCTAAGACTTAACG-3′ and for the *hipO* gene amplification were Hip1A, 5′-ATGATGGCTTCTTCGGATAG-3′ and Hip2B, 5′-GCTCCTATGCTTACAACTGC-3′, respectively. PCR thermocycling conditions were the same as described previously.[7]

Antimicrobial susceptibility testing

The agar dilution method was used to determine the susceptibility to six antimicrobials according to the NCCLS guidelines for MIC determination for Enterobacteriaceae.[8] The antimicrobials (with their concentration ranges and breakpoints for resistance) were: chloramphenicol (0.25–128 mg/L, 32 mg/L); ciprofloxacin (0.008–4 mg/L, 4 mg/L); erythromycin (0.06–32 mg/L, 8 mg/L); gentamicin (0.06–32 mg/L, 16 mg/L); nalidixic acid (0.25–128 mg/L, 32 mg/L); and tetracycline (0.06–32 mg/L, 16 mg/L). *C. jejuni* ATCC 33560 was used as the quality control (QC) organism for this test.

Statistical analysis

Campylobacter prevalence and frequency were compared using the χ^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.

Results

Campylobacter prevalence

Campylobacter was isolated from all the farms and slaughter plants. The overall *Campylobacter* prevalence at the farm and slaughter level was 55.8% and 26%, respectively. Up to three isolates per positive sample (pig or carcass) were further analysed. All the 526 *Campylobacter* isolates in this study, including 366 from farm and 160 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 ($P = 0.83$). However, a significantly higher proportion of *C. coli* was found at the pre-evisceration stage of processing extensively reared ABF pigs ($P < 0.001$). In both of the ABF systems, there was an increase in *C. coli* prevalence at post-evisceration followed by a significant decrease at the post-chill stage ($P < 0.002$). On comparing the two ABF systems at the post-chill stage, we observed *C. coli* only from the carcasses of extensively reared ABF pigs.

Antimicrobial resistance: frequency and patterns

We detected resistance to all of the six antimicrobials tested. Overall, isolates exhibited the highest frequency of resistance against tetracycline (48.6%) and erythromycin (39.7%) (Table 1). A significantly higher proportion of *C. coli* isolates from the intensive system were resistant to the above two antimicrobials at the finishing farms ($P < 0.001$). Resistance against ciprofloxacin (MIC > 4 mg/L; 0.5%) 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 0.2% and 1.9% of the total isolates, respectively.

Fourteen different resistance patterns were observed. A total of 188 isolates (35.3%) were pan-susceptible (Table 2). A significantly higher proportion of isolates from extensive production units was found to be pan-susceptible ($P < 0.001$). The most common resistance pattern, erythromycin/tetracycline, was exhibited in *C. coli* isolates more commonly from the intensive systems both at farm and slaughter ($P < 0.001$). We observed six multidrug-resistant (MDR) (resistance to three or more antimicrobials) patterns in 23 (4.4%) of the isolates, erythromycin/nalidixic acid/tetracycline (3%) being the predominant pattern. Except for one isolate with chloramphenicol/erythromycin/nalidixic acid/tetracycline resistance and another with chloramphenicol/erythromycin/tetracycline resistance isolated at the post-evisceration step, none of the isolates at the slaughter level was MDR showing phenotypic diversity between isolates from the farm and slaughter.

Discussion

The high prevalence of *C. coli* in pigs at the finishing farms seen in this study is similar to that reported in many previous studies with prevalence ranging from 50.4% to 94%.[4,5] Heuer *et al.*[6] compared *Campylobacter* prevalence between organic outdoor and conventional poultry flocks and reported higher prevalence in the outdoor flocks. High prevalence in extensively reared pigs could be attributed to horizontal transmission via the open environment where the pigs have unrestricted access to the soil and water. *C. coli* has been shown to be present in the environment in both soil and water.[9] *C. coli* was isolated at the post-chill stage only from the carcasses belonging to the extensively reared system and could

Table 1. Comparison of antimicrobial resistance frequency among the *C. coli* isolates from intensive and extensive reared ABF pigs at different stages of production

			Number of isolates resistant to antimicrobial (%)					
Production stage	ABF system	Isolates tested	chloramphenicol	ciprofloxacin	erythromycin	gentamicin	nalidixic acid	tetracycline
Finishing farm	extensive	162	1 (0.6)	2 (1.2)	32 (19.7)[1]	–	5 (3)	62 (38.2)[2]
	intensive	204	7 (3.4)	1 (0.5)	113 (55.3)[1]	–	22 (10.7)	120 (58.8)[2]
Slaughter								
pre-evisceration	extensive	47	–	–	11 (23.49)[3]	–	–	15 (31.9)
	intensive	6	–	–	1 (16.6)	–	–	3 (50)
post-evisceration	extensive	52	1 (1.9)	–	22 (42.3)[3]	1 (1.9)	–	15 (28.8)
	intensive	43	1 (2.3)	–	27 (62.7)	–	1 (2.3)	38 (88.3)
post-chill	extensive	12	–	–	3 (25)	–	–	3 (25)
	intensive	–	–	–	–	–	–	–
Total isolates		526	10 (1.9)	3 (0.5)	209 (39.7)	1 (0.2)	28 (5.3)	256 (48.6)

ABF, antimicrobial free. For each antimicrobial, figures sharing common digits in the superscripts were significantly different at $P < 0.05$ (χ^2 test and Fisher's exact two-tailed test).

Table 2. *C. coli* antimicrobial resistance patterns: comparison between the intensive and the extensive ABF production system at the farm and slaughter

	Production stage							
	finishing farm[b]		slaughter[c]					
			pre-evisceration		post-evisceration		post-chill	
Resistance pattern[a]	extensive	intensive	extensive	intensive	extensive	intensive	extensive	intensive
Pan-susceptible	84 (51.8)	46 (22.5)	26 (54.2)	2 (40)	22 (41.5)	1 (2.3)	7 (58.3)	–
CHL/ERY	1 (0.6)	2 (1)	–	–	–	–	–	–
ERY/TET	15 (9.2)	61 (30)	4 (8.3)	1 (20)	7 (13.2)	25 (59.5)	1 (8.3)	–
NAL/TET	1 (0.6)	7 (3.4)	–	–	–	–	–	–
CHL/ERY/TET	–	2 (1)	–	–	–	–	–	–
CIP/NAL/TET	2 (1.2)	–	–	–	–	–	–	–
ERY/NAL/TET	–	16 (7.8)	–	–	–	–	–	–
CIP/ERY/NAL/TET	–	1 (0.5)	–	–	–	–	–	–
ERY/NAL	–	2 (1)	–	–	–	–	–	–
CHL/ERY/NAL/TET	–	–	–	–	–	1 (2.3)	–	–
CHL/ERY/GEN	–	–	–	–	1 (1.8)	–	–	–
Total isolates	162	204	48	5	53	42	12	–

Results are shown as numbers of isolates, with percentage resistance given in parentheses.
[a]CHL, chloramphenicol; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; NAL, nalidixic acid; TET, tetracycline.
[b]Number of *C. coli* isolates at the farm level: 162 (extensive ABF) and 204 (intensive ABF).
[c]Number of *C. coli* isolates tested at the slaughter level: 113 (extensive ABF) and 47 (intensive ABF).

be due to the slaughterhouse effect as there was no blast chilling system in the plant where the extensive herds were slaughtered.

Even though neither tetracycline nor macrolides were used as growth promoters in ABF herds, a significant proportion of isolates from the slaughterhouse, including post-chill samples, showed resistance against tetracycline and erythromycin. A high proportion of *Campylobacter* isolates showing resistance to these two antimicrobials has been reported before in pigs that were reared in the conventional production systems.[3,5] None of the slaughterhouses where the samples were collected were dedicated for ABF herds. Thus, the likelihood of cross-contamination at lairage and processing remains a possibility. Resistance against ciprofloxacin was also detected at the farm level in the ABF production systems. Resistance against ciprofloxacin and chloramphenicol in *C. coli* is striking since both the antimicrobials are not licensed for use in any system of pig production in the USA. High resistance against ciprofloxacin has been reported in 100% of *C. coli* from pigs.[3] The erythromycin/nalidixic acid/tetracycline resistance pattern was the most common MDR pattern in our study and has also been reported by Payot *et al.*[5] to be the most common MDR pattern

in their study. It should be noted that none of these antimicrobials (tetracycline and macrolides) or related classes of antimicrobials were used in any of the swine farms in this study.

In conclusion, this study highlights the prevalence of antimicrobial-resistant *C. coli* from both the extensive- and the intensive-type ABF production system. MDR ciprofloxacin-resistant *C. coli* isolates from swine is alarming since this antimicrobial is used in the treatment of severe invasive cases of campylobacteriosis. This study also indicates the possible role played by environmental factors in the dissemination of antimicrobial-resistant *C. coli* strains.

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