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Short communication

Antimicrobial resistance of 114 porcine isolates of *Campylobacter coli*

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Abstract

Campylobacter species were isolated from 24 pig farms in 10 different regions of Korea, and were assayed with regard to their susceptibility to eight antimicrobial agents. A total of 114 *Campylobacter* isolates from 572 intestinal samples were all identified as *C. coli* via both classical methods and molecular methods, including 16S rDNA sequence analysis and polymerase chain reactions (PCR) using specific primer sets for the hippuricase gene and the aspartokinase gene, designed to differentiate *C. coli* from *C. jejuni*. Minimal inhibitory concentrations of seven antimicrobial agents were determined via agar dilution: the MIC_{90}s were 64 μg/ml for ampicillin, 8 μg/ml for chloramphenicol, 64 μg/ml for ciprofloxacin, 16 μg/ml for enrofloxacin, ≥128 μg/ml for erythromycin, ≥128 μg/ml for gentamicin, and ≥128 μg/ml for tetracycline. The proportion of isolates resistant to each antimicrobial agent was as follows: 28.9% for ampicillin, 2.6% for chloramphenicol, 84.2% for ciprofloxacin, 83.3% for enrofloxacin, 46.5% for erythromycin, 20.2% for gentamicin, and 56.1% for tetracycline. All 114 isolates were found to be resistant to at least one antimicrobial agent, and 61 isolates (53.5%) were found to be multi-drug resistant (resistant to more than three antimicrobial agents in different classes).

Keywords: *Campylobacter coli*; Swine; Antimicrobial resistance

1. Introduction

The zoonoses which occur most frequently in the industrialized world today include food-borne infections induced by bacteria enzootic to food animals. Most notable among these bacterial species are *Salmonella*, *Campylobacter*, *Yersinia*, *Listeria*, and enterohaemorrhagic *Escherichia coli* species. In recent years, human campylobacteriosis has shown a dramatic increase in industrialized countries, and currently represents one of the principal causes of bacterial food-borne disease (Taylor and Blaser, 1991; Tam et al., 2003). *Campylobacter* infections tend to be self limiting, and do not normally require treatment. However, in immunocompromised patients, such infections can result in bacteremia. *Campylobacter* bacteremia can have a fairly high mortality rate (>30%). Fatalities due to treatment failures have also been reported (Pasternack, 2002).

Food animals are considered to be the primary reservoirs of the *Campylobacter* species which induce infections in humans (WHO, 1997; Harvey et al., 1999). *Campylobacter jejuni* appears to predominate among cattle and broiler chickens, but is only found at a low frequency among pigs, in which *C. coli* predominates (Cabrita et al., 1992; Aarestrup and Wegener, 1999).

An increase in *Campylobacter* resistance, especially to fluoroquinolones and erythromycin, as well as to other antimicrobial agents, has also been observed (Aarestrup and Engberg, 2001; Bae et al., 2005; Moore et al., 2006). As the potential exists for the transmission of antimicrobial-resistant animal isolates to humans (Engberg et al., 2001; Angulo et al., 2004; Kim et al., 2006), the presence of antimicrobial-resistant isolates in the food chain has raised concerns that the treatment of human infections will be compromised (Bodhidatta et al., 2002). The development of resistance in zoonotic bacteria constitutes a public health risk, principally as the result of an increased risk of treatment failures (FDA, 2001; Helms et al., 2005). In addition, development of resistance, notably via the acquisition of transmissible genetic elements (Velazquez et al., 1995), may affect other bacterial properties, most notably the ability to colonize an animal host or to persist within a farm or food-processing environment.

In order to gather information regarding the antimicrobial resistance of *Campylobacter* in animals, *Campylobacter* were

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isolated from swine, and the antimicrobial resistance of the isolates was investigated.

2. Materials and methods

2.1. Isolation and identification of Campylobacter

During a four-month period from July to October 2004, samples were collected from 24 pig farms located in 10 different regions in the Kyung-gi and Chungcheong Provinces of Korea. Intestinal samples ($n=572$) were obtained at the abattoir immediately after slaughter, and were immediately transported to the laboratory in insulated containers.

The samples were washed twice in sterile phosphate buffered saline (PBS, pH 7.0) in order to remove intestinal contents. The washed intestinal membranes were then smeared onto *Brucella* solid media (BBL, Sparks, MD, USA) supplemented with 10% horse serum, amphotericin B (2.5 μg/ml; Fungizone, Sigma, St. Louis, MO, USA), Skirrow's supplement (polymyxin B, 2.5 IU/ml; vancomycin, 10 μg/ml; trimethoprim, 5 μg/ml) and campy blood-free selective medium (CCDA, Acumedia, Baltimore, MA, USA); samples were then incubated for 48 h under microaerophilic conditions at 42 °C. Catalase-positive, oxidase-positive colonies with the characteristic translucent morphology of *Campylobacter* were isolated and microscopically examined after Gram staining. After the identification of each isolate via 16S rRNA gene sequencing, one isolate per sample was stored at −70 °C for further examination.

2.2. Identification of Campylobacter by PCRs of 16S rRNA, hippuricase, and aspartokinase genes

PCRs of 16S rRNA, hippuricase, and aspartokinase genes were performed for species identification. PCR was performed with *cah* primers (cah-1, 5′-AAT ACA TGC AAG TCG AAC GA-3′; cah-2, 5′-TTA ACC CAA CAT CTC ACG AC-3′) as designed by others (Marshall et al., 1999) to amplify a 1004-bp fragment within the coding region of the 16S rRNA gene in *Campylobacter*, *Arcobacter* and *Helicobacter* species. Primers (hip-1, 5′-ATG ATG GCT TCT TCG GAT AG-3′; hip-2, 5′-GCT CCT ATG CTT ACA ACT GC-3′) designed by Hani and Chan (1995) were used to amplify a portion of the hippuricase gene found only in *C. jejuni*. Primers (CC18F, 5′-GGT ATG ATT TCT ACA AAG CGA G-3′; CC519R, 5′-ATA AAA GAC TAT CGT CGC GTG-3′) designed by Linton et al. (1997) were used to amplify an aspartokinase gene in *C. coli*. Template DNA was prepared by cetyltrimethylammonium bromide (CTAB) method (Honore-Bouakline et al., 2003) and PCR amplifications were performed as described in previous papers. *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 were included as controls.

For sequencing, DNA fragment was purified using the QIAquick Gel extraction Kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's and sequenced using an ABI 3100 automated sequencer. DNA sequences, using the online BLAST algorithm at the National Center for Biotechnology Information web server (www.ncbi.nlm.nih.gov) were compared.

2.3. Minimal inhibitory concentration (MIC)

MICs were assayed on Muller–Hinton agar plates (Beckton Dickinson, MD, USA) containing 5% sheep blood, via standard agar dilution, in accordance with the recommendations of the Clinical Laboratory Standards Institute (CLSI; NCCLS, 2005). The isolates were grown microaerobically for 48 hours on Brucella agar containing Skirrow's supplement or Trypticase soy agar containing 5% sheep blood (BBL) at 42 °C. *Campylobacter jejuni* ATCC 33560 and *C. coli* ATCC 33559 were included in each batch of the agar dilution tests, and CLSI approved MIC quality control limits for these strains were used for the control of agar dilution performance.

MICs were assayed using erythromycin, ciprofloxacin (Korea Research Institute of Chemical Technology), enrofloxacin (Dr Ehrenstofer, GmbH, Germany), ampicillin, chloramphenicol (Fluka, Buchs, Switzerland), gentamicin, and tetracycline at a variety of concentrations ranging from 0.5 μg/ml to 128 μg/ml. All chemicals used in this study were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated. The MIC interpretive standards of *Staphylococcus* spp. and veterinary pathogens (NCCLS, 1999) were employed as breakpoints for *Campylobacter* resistance for erythromycin and enrofloxacin, respectively. For breakpoints of other antimicrobial agents, MIC interpretive standards for members of the Enterobacteriaceae family were utilized (NCCLS, 2005).

3. Results and discussion

3.1. Isolation of Campylobacter

During a four-month period, 572 samples of porcine intestine were collected from 24 pig farms located in 10 different regions in Kyung-gi and Chungcheong Provinces where more than half of Korean population lives and a total of 114 *Campylobacter* isolates (20.1%) were acquired. When the isolates were identified via classical methods including the catalase test, oxidase test, and Gram staining, as well as molecular techniques including 16S rRNA gene sequencing, and PCRs with specific primers for the hippuricase gene present in *C. jejuni*, and aspartokinase gene present in *C. coli*, all of these isolates were identified as *C. coli*.

3.2. MIC of C. coli

MICs of ampicillin, chloramphenicol, ciprofloxacin, enrofloxacin, erythromycin, gentamicin, and tetracycline for *C. coli* are shown in Table 1. MICs of erythromycin, gentamicin, and tetracycline fell into two separate groups. For example, erythromycin-resistant isolates showed MICs of higher than 64 μg/ml whereas erythromycin-susceptible isolates had MICs of less than 16 μg/ml, and there were no isolates with MICs between 64 μg/ml and 16 μg/ml. The MIC_{50}s of ciprofloxacin, enrofloxacin, and tetracycline were higher than the limit for resistance criteria, whereas the MIC_{90}s of every antimicrobial agent except chloramphenicol were found to be higher than the limits for resistance criteria. In this study, the proportion of