International Journal of Food Microbiology 146 (2011) 94–98

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

# Antimicrobial resistance in *Campylobacter coli* isolated from pigs in two provinces of China

Shang-Shang Qin [a,1], Cong-Ming Wu [a,1], Yang Wang [a], Byeonghwa Jeon [b], Zhang-Qi Shen [c], Yu Wang [a], Qijing Zhang [c,*], Jian-Zhong Shen [a,**]

[a] National Center for Veterinary Drug Safety Evaluation, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China
[b] Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Canada PE C1A4P3
[c] Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA

ARTICLE INFO

*Article history:*
Received 15 October 2010
Received in revised form 24 January 2011
Accepted 25 January 2011

*Keywords:*
Pig
*Campylobacter coli*
Antimicrobial resistance

ABSTRACT

The aim of this study was to determine the prevalence, antimicrobial resistance and molecular epidemiology of *Campylobacter coli* isolated from swine in China. A total of 190 *C. coli* isolates obtained from two slaughter houses and ten conventional pig farms in Shandong (SD, n = 95) and Ningxia (NX, n = 95) provinces were tested for their susceptibility to 14 antimicrobials. A high prevalence (>95%) of ciprofloxacin and tetracycline-resistant strains was observed in both SD and NX. The erythromycin and clindamycin resistance rates of *C. coli* from NX (ERY: 54.7% CLI: 43.2%) were higher than those from SD (ERY: 37.9%, CLI: 35.8%). A significant difference ($P<0.05$) was observed in erythromycin resistance rate, but not ($P>0.05$) in clindamycin resistance rate. while the resistance rates of ampicillin and kanamycin in NX (AMP: 34.7%, KAN: 43.2%) were significantly lower ($P<0.05$) than those in SD (AMP: 51.6%, KAN: 71.6%). None of the tested isolates were resistant to phenicols. The majority of the isolates from both provinces (SD: 80% and NX: 73.7%) showed multi-drug resistance profiles. The point mutations of A2075G in the 23S rRNA and C257T in the *gyrA* gene were detected in 98% (87/89) of macrolide resistant isolates and all ciprofloxacin resistant isolates, respectively. In addition, all tetracycline-resistant isolates harbored the *tet (O)* gene. The high prevalence of antimicrobial resistance in *C. coli* strains derived from pigs in China was observed and was likely due to the extensive use of various antimicrobials. Prudent use of antimicrobial agents on farms should be further emphasized to control the dissemination of antimicrobial resistant *C. coli*.



## 1. Introduction

*Campylobacter* is one of the most common food-borne pathogens causing bacterial diarrhea in humans. *Campylobacter jejuni* and *Campylobacter coli* are the main species responsible for most *Campylobacter* infections (Alfredson and Korolik, 2007). The majority of the infections caused by *Campylobacter* are self-limiting, however, antimicrobial treatments are required for *Campylobacter* infections causing appendicitis, bacteremia, or other severe complications (Luber et al., 2003). As enteric organisms, *Campylobacter* spp. are exposed to antimicrobial agents that are widely used to control, treat and prevent diseases in food producing animals. Under the selection pressures of antimicrobials, antibiotic resistant *Campylobacter* emerges and can be transmitted to humans through the food chain, which potentially compromises the efficacy of antimicrobial treatment of human infections (Desmonts et al., 2004; Piddock et al., 2008).

As an important *Campylobacter* species associated with food-borne diseases, *C. coli* isolates show higher levels of resistance than *C. jejuni* isolates (Bywater et al., 2004; Chen et al., 2010; Van Looveren et al., 2001). Pigs were considered the primary reservoir of *C. coli* (Harvey et al., 1999; Thakur and Gebreyes, 2005a,b), and multiple studies have reported the prevalence of antimicrobial resistant *C. coli* from pigs in both developed countries and developing countries (Bywater et al., 2004; Ishihara et al., 2006; Padungtod et al., 2006; Payot et al., 2004a,b; Pezzotti et al., 2003; Sáenz et al., 2000; Schuppers et al., 2005; Shin and Lee, 2007; Thakur and Gebreyes, 2005a,b; Varela et al., 2007). However, no data have been reported on the prevalence, antimicrobial susceptibility profile and antimicrobial resistance mechanism of *C. coli* isolates from pigs in China. In this study, we analyzed 190 *C. coli* isolates collected from swine slaughter houses and conventional pig farms in Shandong (East China) and Ningxia (West China) provinces.

## 2. Materials and methods

### 2.1. Origin and identification of Campylobacter isolates

A total of 1143 samples were collected from ten conventional pig farms and two pig slaughter houses in Shandong (SD) (six conventional

* Correspondence to: Q. Zhang, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA. Tel.: +1 515 294 2038; fax: +1 515 294 8500.
** Correspondence to: J.-Z. Shen, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China. Tel.: +86 10 62732803; fax: +86 10 62731032.
*E-mail addresses:* zhang123@iastate.edu (Q. Zhang), sjz@cau.edu.cn (J.-Z. Shen).
[1] The first two authors contributed equally to this work.

0168-1605/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2011.01.035

pig farms and one pig slaughter house) and Ningxia (NX) (four conventional pig farms and one pig slaughter house) provinces during a period from November 2008 to June 2009 (Table 1).

The fresh feces (farm origin) and intestinal contents (slaughter plant origin) were placed on ice and transported to the laboratory within 5 h of collection and cultured for *Campylobacter* species. Samples (loopful, approximately 10 μl) were plated directly onto *Campylobacter* Selective Agar (Base) (Oxoid Lte., Basingstoke, England) containing 5% fresh sterile defibrinated sheep blood and *Campylobacter* supplement III (Sigma-Aldrich, St. Louis, MO, USA), (Chen et al., 2010; Thakur and Gebreyes, 2005a,b) and then incubated under microaerobic conditions ($CO_2$:10%, $O_2$:5%, $N_2$:85%) at 42 °C for 48 h. Presumptive *Campylobacter* colonies were selected for further identification by using API-Campy (BioMerieux, Marcy l'Etoile, France) kits and PCR strategies as previously described (Keramas et al., 2003; Linton et al., 1997). All the isolates were stored in 20% glycerol-Mueller–Hinton broth at −80 °C until further required for use.

### 2.2. Antibiotic susceptibility testing

The standard agar dilution method as described by Clinical and Laboratory Standards Institute (CLSI, 2008) was used to determine the Minimal inhibitory concentration (MIC) of *Campylobacter* to 14 antibiotic agents including nalidixic acid, ciprofloxacin, enrofloxacin, levofloxacin, erythromycin, azithromycin, tetracycline, doxycycline, gentamicin, kanamycin, ampicillin, clindamycin, chloramphenicol and florfenicol. All the antimicrobial agents except nalidixic acid (Sigma) were obtained from the China Institute of Veterinary Drug Control (Beijing, China). The MIC ranges of antimicrobial agents and the resistance breakpoints for all antimicrobial agents are summarized in Table 2. *C. jejuni* ATCC33560 and *C. coli* ATCC33559 were used as quality control strains. A *C. coli* isolate resistant to three or more classes of antimicrobials was defined as a multi-drug resistant isolate.

### 2.3. Detection of resistance determinants

According to the MIC results, antimicrobial resistant isolates were selected to analyze the genetic determinants associated with quinolone, macrolide, or tetracycline resistance. Mismatch amplification mutation assay (MAMA) - PCR was employed to detect the mutations of A2074C and A2075G in 23S rRNA gene responsible for macrolide resistance (Alonso et al., 2005) and the C257T (Thr-86-Ile) mutation in the quinolone resistance determining region (QRDR) associated with high-level quinolone resistance (Zirnstein et al., 2000). A 505 bp *gyrA* QRDR region and a 697 bp 23s rRNA region containing the resistance associated mutations were amplified and sequenced (Alonso et al., 2005; Zirnstein et al., 2000). The negative and positive controls for MAMA PCR were designed according to the sequencing results. In addition, the *tet(O)* gene was examined by PCR in all tetracycline-resistant *C. coli* strains. The primers *tet(O)*-F:5′-AGTTTCTGCAAAGGATGGCAT-3′ and *tet(O)*-R:5′-GATTGACCTTCAGGCGTTGAT-3′ were designed from the conserved regions of *tet(O)* gene in *Campylobacter* spp. The PCR mixture contained 25 μl *Premix Taq™* (ea. 0.4 mM dNTP Mixture, 4 mM $Mg^{2+}$, and 1.5 U of *Ex Taq™* DNA polymerase, TaKaRa), 0.5 μM of each forward and reverse primer (1 μl each) and 1 μl of DNA template (ca. 100 ng of genomic DNA) prepared by the boiling method, and water was added for a final PCR mixture of 50 μl. PCR was performed on a veriti 96 well Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with the following cycling condition: Initial activation at 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 58 °C for 35 s, and 72 °C extension for 45 s, and a final extension at 72 °C for 8 min.

### 2.4. Statistical analysis

Prevalence and frequency of antimicrobial resistance profiles of *C. coli* isolates obtained between SD and NX were compared by using the chi-square test at a P significance level of 0.05.

## 3. Results and discussion

### 3.1. Campylobacter prevalence

A total of 192 (16.8%) *Campylobacter* isolates including 190 *C. coli* and 2 *C. jejuni* strains were obtained from 1143 collected samples after identification. The overall isolation rate of *C. coli* was 16.6%. Prevalence of *C. coli* in SD (13.4%) and NX (21.4%) were significantly different ($P<0.01$), and the isolation rates varied greatly from region to region, ranging from 8.6 to 24.6%.(Table 1) Overall, more than 98.9% of our isolates were *C. coli*, which was consistent with previous findings that pigs mainly harbor *C. coli* (Thakur and Gebreyes, 2005a,b).

### 3.2. MIC and resistance determinants of C. coli

The distribution of MIC values at which 50% and 90% of *C. coli* growth was inhibited is summarized in Table 2. The resistance to ciprofloxacin (SD: 99%, NX: 95.8%) and tetracycline (SD: 99%, NX: 95.8%) was high among the 190 *C. coli* isolates. Isolates obtained from SD ($n=95$) exhibited significantly higher resistance rates against levofloxacin (91.6%) than isolates from NX ($n=95$) (60%) ($P<0.01$).

The frequency of ciprofloxacin and tetracycline-resistant *C. coli* was over 95% from both provinces evaluated, which was only similar to that of Spain (CIP: 100% TET:94.4%) (Sáenz et al., 2000), but higher than that of Canada (CIP: 2.4% TET:63.7%) (Varela et al., 2007), Korea (CIP: 83.3% TET:56.1%) (Shin and Lee, 2007), Thailand (CIP: 86% TET:81%) (Ekkapobyotin et al., 2008), Italy (CIP: 36.2% TET:76.6%) (Pezzotti et al., 2003), Sweden (CIP: 21.1% TET: 1.9%) (Bywater et al., 2004) and Switzerland (CIP: 26.1% TET:9.4%) (Schuppers et al., 2005).

Fluoroquinolones are widely used for treatment and disease control in the pig production in China. It has been documented that fluoroquinolone-resistant mutants can develop rapidly during treatment

**Table 1**
The sources and numbers of *Campylobacter* strains isolated from different regions of China.

| Region | Source of isolates | Number of farms or slaughter | Number of samples | Number of *Campylobacter* | | Total isolates for each region |
|---|---|---|---|---|---|---|
| | | | | *C. coli*[a] | *C .jejuni* | |
| Laiwu | Conventional pig farm | 1 | 103 | 18(17.5) | 0 | 18(17.5) |
| Jinan | Conventional pig farm | 1 | 105 | 9(8.6) | 0 | 9(8.6) |
| Zhucheng | Conventional pig farm | 4 | 400 | 47(11.8) | 0 | 68(13.5) |
| | pig slaughter house | 1 | 102 | 21(20.6) | 0 | |
| Shandong (SD) | | 7 | 710 | 95(13.4) | 0 | 95(13.4) |
| Yinchuan | pig slaughter house | 1 | 268 | 66(24.6) | 2(0.7) | 68(25.4) |
| Lingwu | Conventional pig farm | 3 | 123 | 23(18.7) | 0 | 23(18.7) |
| Zhongwei | Conventional pig farm | 1 | 42 | 6(14.3) | 0 | 6(14.3) |
| | Ningxia (NX) | 5 | 443 | 95(21.4) | 2(0.4) | 97(21.9) |
| | Total number (%) | 12 | 1143 | 190(16.6) | | |

[a] Numbers in parentheses indicate the percentages.

**Table 2**
Distribution of minimum inhibitory concentration (MIC; μg/ml) for antimicrobials in *C. coli* isolated from Shandong and Ningxia provinces in China.[a]

| Antimicrobials | Test range (μg/ml)[b] | Origin of isolates[c] | Distribution ( No. of isolates) of MIC (μg/ml) | | | | | | | | | | | | | $MIC_{50}/MIC_{90}$ (μg/ml) | No.(%)of resistance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ≤0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | >128 | | |
| Levofloxacin | 0.06–128 | SD | 0 | 0 | 1 | 0 | 0 | 0 | 7 | **26** | 48 | 13 | 0 | 0 | – | 16/32 | 87(91.6) |
| | | NX | 1 | 0 | 0 | 1 | 1 | 5 | 30 | **54** | 3 | 0 | 0 | 0 | – | 8/8 | 57(60) |
| Ciprofloxacin | 0.06–128 | SD | 0 | 0 | 1 | 0 | 0 | 0 | **5** | 18 | 42 | 22 | 7 | 0 | – | 16/32 | 94(99) |
| | | NX | 1 | 0 | 1 | 0 | 1 | 1 | **4** | 21 | 35 | 29 | 2 | 0 | – | 16/32 | 91(95.8) |
| Nalidixic acid | 0.5–256 | SD | – | – | – | 0 | 0 | 0 | 0 | 0 | 1 | 9 | **23** | 48 | 14 | 128/>128 | 85(89.5) |
| | | NX | – | – | – | 0 | 1 | 0 | 0 | 0 | 0 | 18 | **55** | 17 | 4 | 64/128 | 76(80) |
| Enrofloxacin | 0.06–128 | SD | 0 | 1 | 0 | 0 | 0 | 3 | **22** | 49 | 11 | 2 | 7 | 0 | – | 8/16 | 91(95.8) |
| | | NX | 0 | 0 | 0 | 2 | 1 | 4 | **21** | 58 | 9 | 0 | 0 | 0 | – | 8/8 | 88(92.6) |
| Erythromycin | 0.06–256 | SD | 0 | 0 | 0 | 13 | 21 | 13 | 8 | 4 | 0 | **0** | 0 | 7 | 29 | 4/>128 | 36(37.9) |
| | | NX | 0 | 0 | 0 | 17 | 17 | 8 | 0 | 0 | 1 | **1** | 3 | 12 | 36 | 128/>128 | 52(54.7) |
| Azithromycin | 0.03–256 | SD | 27 | 27 | 4 | 2 | 1 | 0 | 0 | **1** | 0 | 1 | 7 | 24 | 1 | 0.125/128 | 34(35.8) |
| | | NX | 3 | 24 | 14 | 1 | 1 | 0 | 0 | **0** | 0 | 0 | 9 | 28 | 15 | 64/>128 | 52(54.7) |
| Ampicillin | 0.25–256 | SD | – | – | 0 | 0 | 1 | 1 | 9 | 15 | 20 | **14** | 2 | 15 | 18 | 32/>128 | 49(51.6) |
| | | NX | – | – | 0 | 3 | 1 | 3 | 15 | 28 | 12 | **7** | 2 | 20 | 4 | 8/128 | 33(34.7) |
| Gentamicin | 0.06–256 | SD | 0 | 0 | 0 | 13 | 25 | 33 | 2 | **0** | 1 | 1 | 17 | 3 | 0 | 2/64 | 22(23.2) |
| | | NX | 0 | 1 | 0 | 11 | 56 | 4 | 0 | **1** | 0 | 2 | 7 | 13 | 0 | 1/128 | 23(24.2) |
| Kanamycin | 0.06–256 | SD | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 9 | 10 | 0 | **0** | 45 | 23 | 128/>128 | 68(71.6) |
| | | NX | 0 | 0 | 0 | 0 | 0 | 4 | 13 | 28 | 9 | 0 | **0** | 1 | 40 | 16/>128 | 41(43.2) |
| Clindamycin | 0.06–128 | SD | 0 | 0 | 5 | 7 | 24 | 18 | 7 | **6** | 11 | 13 | 4 | 0 | – | 2/32 | 34(35.8) |
| | | NX | 5 | 6 | 11 | 15 | 4 | 0 | 13 | **16** | 13 | 10 | 2 | 0 | – | 4/32 | 41(43.2) |
| Chloramphenicol | 0.125–64 | SD | – | 0 | 0 | 0 | 2 | 24 | 52 | 16 | 1 | **0** | 0 | – | – | 4/8 | 0 |
| | | NX | – | 0 | 0 | 0 | 12 | 55 | 20 | 3 | 5 | **0** | 0 | – | – | 2/4 | 0 |
| Florfenicol | 0.125–64 | SD | – | 0 | 0 | 4 | 47 | 41 | 3 | **0** | 0 | 0 | 0 | – | – | 1/2 | 0 |
| | | NX | – | 0 | 16 | 7 | 38 | 30 | 4 | **0** | 0 | 0 | 0 | – | – | 1/2 | 0 |
| Tetracycline | 0.06–256 | SD | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | **0** | 2 | 13 | 45 | 34 | 128/>128 | 94(99) |
| | | NX | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | **3** | 7 | 16 | 40 | 25 | 128/>128 | 91(95.8) |
| Doxycycline | 0.06–256 | SD | 0 | 0 | 0 | 0 | 1 | 0 | 0 | **0** | 12 | 53 | 29 | 0 | 0 | 32/64 | 94(99) |
| | | NX | 0 | 0 | 1 | 0 | 0 | 3 | 5 | **19** | 36 | 29 | 2 | 0 | 0 | 16/32 | 86(90.5) |

[a]Boldface numbers indicate breakpoints for antimicrobial resistance. MIC breakpoints for *Campylobacter* for ciprofloxacin, nalidixic acid, erythromycin, azithromycin, clindamycin, tetracycline, doxycycline, gentamicin, chloramphenicol, florfenicol were described by the NARMS Annual Report 2005. The breakpoints for enteric bacteria for ampicillin and kanamycin were also from the NARMS Annual Report 2005. MIC breakpoints for *Enterobacteriaceae* for levofloxacin and enrofloxacin were recommended by CLSI (2008).
[b]Test ranges were based on the approved CLSI (2008) standards for *Campylobacter*.
[c] SD: isolates were collected in Shandong (SD) (n = 95); NX: isolates were collected in Ningxia (NX) (n = 95).

and lead to the emergence of fluoroquinolone-resistant *Campylobacter* (Luangtongkum et al., 2009). In addition, fluoroquinolone-resistant clones could persist stably for long periods in the absence of antimicrobial selection pressure and may outcompete susceptible clones (Luangtongkum et al., 2009), which was another possible reason that a high fluoroquinolone resistance rate was observed among *C. coli* isolated from pigs in both provinces in this study. The point mutation C257T (Thr-86-Ile) in QRDR of the *gyrA* gene was considered the main mechanism for high-level resistance to fluoroquinolone in *Campylobacter* (Piddock et al., 2003). This mutation was found in all of the 185 ciprofloxacin resistant isolates (SD: n = 94 and NX: n = 91) with the MICs ranging from 4 to 64 μg/ml in our study. Furthermore, 20 *C. coli* isolates were randomly chosen from the ciprofloxacin resistant isolates for sequencing the QRDR region. The sequencing results showed that no other amino acid mutations like Asp-90, Ala-70, and Pro-104 (Payot et al., 2006; Piddock et al., 2003) in QRDR region linked to ciprofloxacin resistance were detected except for the Thr-86-Ile change. Only two silence mutations in QRDR region were detected at position 157 (serine; AGC replaced by AGT, detected in all sequenced isolates) and position 99 (Phenylalanine; TTT replaced by TTC, detected in half of the sequenced isolates).

Tetracyclines were commonly used as feed additives in conventional pig farms in China, and tetracycline resistance is usually associated with the *tet(O)* gene located either on chromosome or on transmissible plasmids in both *C. coli* and *C. jejuni* (Pratt and Korolik, 2005). All of the 185 tetracycline-resistant isolates (SD: n = 94 and NX: n = 91) with MICs ranging from 32 to ≥128 μg/ml harbored the *tet(O)* gene as determined by PCR. This gene was highly prevalent among the tetracycline-resistant isolates examined in this study, suggesting that the high resistance rates to tetracycline was due to the presence of the *tet(O)* gene in the isolates.

The prevalence of macrolide resistance was significantly higher in NX (55%) than in SD (38%) ($P<0.05$). The frequency of erythromycin resistant *C. coli* observed in this study (SD: 38%, NX: 55%) was lower than that found in Canada and Spain (Sáenz et al., 2000; Varela et al., 2007), but comparable with the findings reported in other countries (e.g. US, Italy, Belgium, Japan and Korea) (Ishihara et al., 2006; Pezzotti et al., 2003; Shin and Lee, 2007; Thakur and Gebreyes, 2005a,b; Van Looveren et al., 2001). In addition, most of the erythromycin resistant isolates (100% in SD and 94% in NX) in this study demonstrated high-level resistance to erythromycin (MIC ≥ 128 μg/ml). The point mutation A2075G in 23S rRNA was associated with high-level (MIC ≥ 128 μg/ml) erythromycin resistance and mutation A2074T/C was responsible for low-level erythromycin resistance in *Campylobacter* (Payot et al., 2006). No A2074C mutations were detected among the 89 erythromycin resistant *C. coli* isolates, however, most of the erythromycin resistant *C. coli* isolates (87 out of 89 isolates; NX: n = 53, SD: n = 36) except ZC113 and YC18 harbored A2075G mutations in their 23S rRNA gene as determined by using MAMA PCR. Sequencing of the two A2075G negative isolates revealed no mutations in their 23S rRNA gene, which is consistent with the results of MAMA PCR. In addition, no mutations were detected in the *rplD* and *rplV* genes encoding L4 and L22 proteins in the two strains (data not shown). Further investigations are required to study whether the CmeABC efflux pump (Cagliero et al., 2006) or other unknown mechanisms contribute to the high-level resistance to macrolide in the two isolates. The resistance rate of clindamycin (36% in SD and 43% in NX) in the *C. coli* strains was similar to that of erythromycin (38% in SD and 55% in NX), which could be explained by cross-resistance between erythromycin and clindamycin in *Campylobacter* (Varela et al., 2007).

All 190 *C. coli* isolates were susceptible to chloramphenicol and florfenicol. Both $MIC_{50}$ and $MIC_{90}$ of the two antimicrobials were lower than their breakpoints of MIC. The resistance rates for gentamicin were similar in both provinces (SD: 23.2% and NX: 24.2%), which showed relative low level compared with other antimicrobials except phenicols in our test.

Isolates obtained in SD (n = 95) exhibited significantly higher resistance rates against kanamycin (71.6%) than isolates from NX (n = 95) (43.2%) ($P<0.01$), and similar observation was found in ampicillin resistant *C. coli*. (SD: 51.6%, NX: 34.7%) ($P<0.05$). The difference of antimicrobial agents used for treatment in conventional swine production practice in different provinces may have contributed to the difference resistance rates between the two provinces. We observed that all gentamicin resistant isolates showed resistance to kanamycin. Furthermore, all of the kanamycin resistant *C. coli* isolates were resistant to tetracycline. The coexistence of the kanamycin resistant *aphA-3* gene and tetracycline-resistant *tet(O)* gene on the same plasmids may explain the association of the antibiotic resistance (Gibreel et al., 2004). The genetic basis for ampicillin resistance was not investigated in this study and the potential implication of β-lactamase genes in the *C. coli* strains needs further investigation.

### 3.3. Multi-drug resistance profile

A high proportion (146 out of 190) of the *C. coli* (76.8%) isolates was multi-drug resistant strains (MDRS), which displayed as many as 19 resistance patterns. Different multi-drug resistance patterns (19 in total) of *C. coli* isolates from SD and NX were summarized in Table 3. In general, the frequency of MDRS observed in SD (80%) and NX (73.7%) were not significantly different ($P>0.05$). The rate of MDRS in this study was much higher than those reported from Korea (56.1%) (Shin and Lee, 2007), Canada (29.7%) (Varela et al., 2007), France (37%) (Payot et al., 2004a,b), and the UK (3.8%) (Randall et al., 2003). The predominant resistance pattern of the isolates from SD was quinolone–kanamycin–tetracycline (68/76 MDRS; 89.5%), while quinolone–macrolide–tetracycline (50/70 MDRS; 71.4%) was the predominant pattern among MDRS from NX. The difference in major multi-drug resistance patterns between two provinces might be due to different preferences with regard to the use of antimicrobials in each province. In this study, 45.3% of the MDRS showed the quinolone–macrolide–tetracycline (QMT) resistance pattern, which was the most common pattern reported previously (Payot et al., 2004a,b; Thakur and Gebreyes, 2005a,b). Acquired resistance to multiple antimicrobials was associated with over expression of multi-drug resistant efflux pumps or possession of multiple resistance determinants (Quinn et al., 2007). Payot et al. (2004a,b) described overexpression of CmeB in a number of *C. coli* MDRS from pigs, but the expression level of *cmeABC* in the isolates obtained in this study was not determined and remains to be examined in future work.

**Table 3**
Multi-drug resistance patterns of the *C. coli* strains isolated from Shandong and Ningxia provinces in China.

| Antimicrobial resistance pattern[a] | No.(%) of multi-drug resistance strains | |
|---|---|---|
| | SD (n = 95)[b] | NX (n = 95)[b] |
| QAT | 7(7.4) | 6(6.3) |
| **QKT** | **15(15.8)** [c] | 2(2.1) |
| QMC | 0 | 2(2.1) |
| QMT | 0 | 5(5.3) |
| QGKT | 7(7.4) | 3(3.2) |
| QAKT | 6(6.3) | 2(2.1) |
| **QMCT** | 1(1.1) | **10(10.5)**[c] |
| QMKT | 1(1.1) | 4(4.2) |
| QMAT | 0 | 1(1.1) |
| QAGKT | 5(5.3) | 3(3.2) |
| QMKCT | 2(2.1) | 5(5.3) |
| QMAKT | 1(1.1) | 2(2.1) |
| QMACT | 0 | 5(5.3) |
| QGKCT | 0 | 1(1.1) |
| QMGKT | 0 | 1(1.1) |
| AGKCT | 0 | 1(1.1) |
| **QMAKCT** | **21(22.1)**[c] | 3(3.2) |
| QMGKCT | 3(3.2) | 4(4.2) |
| **QMAGKCT** | 7(7.4) | **10(10.5)**[c] |
| Total | 76(80) | 70(73.7) |

[a] Abbreviation of antimicrobial agent: Q, quinolones (nalidixic acid, ciprofloxacin, enrofloxacin and levofloxacin); M, macrolides (erythromycin and gentamicin); A, ampicillin; K, kanamycin; G, gentamicin; C, clindamycin; T, tetracycline.
[b] Numbers in parentheses indicate the percentages.
[c] Boldface indicates prevalence pattern in different provinces.

In conclusion, our study represents the first report on the high prevalence of antimicrobial resistant *C. coli* isolated from pigs in China. Notably, many of the isolates are resistant to multiple antimicrobial agents with high MIC values. The high prevalence of antimicrobial resistance in the *C. coli* isolates suggests a high antibiotic selection pressure in the swine production system. Thus, prudent measures should be implemented to reduce the emergence, transmission and persistence of antimicrobial resistant *C. coli*.

## Acknowledgements

This study was supported by the grant from the Program for Chang Jiang Scholars and the Innovative Research Team at the University of China (No. IRT0866), and the Exclusive Research Found for Public Welfare from Ministry of Agriculture of People's Republic of China (No. 200903055).

We thank Dr. Yu-Qing Liu (The Shandong Academy of Agricultural Sciences) and Dr. Gui-Qin Wang (Ningxia University) for kind help in the sample collection.

## References

Alfredson, D.A., Korolik, V., 2007. Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli*. FEMS Microbiology Letters 277, 123–132.

Alonso, R., Mateo, E., Churruca, E., Martinez, I., Girbau, C., Fernández-Astorga, A., 2005. MAMA-PCR assay for the detection of point mutations associated with high-level erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli* strains. Journal of Microbiological Methods 63, 99–103.

Bywater, R., Deluyker, H., Deroover, E., de Jong, A., Marion, H., McConville, M., Rowan, T., Shryock, T., Shuster, D., Thomas, V., Vallé, M., Walters, J., 2004. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. Journal of Antimicrobial Chemotherapy 54, 744–754.

Cagliero, C., Mouline, C., Cloeckaert, A., Payot, S., 2006. Synergy between efflux pump CmeABC and modifications in ribosomal proteins L4 and L22 in conferring macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. Antimicrobial Agents and Chemotherapy 50, 3893–3896.

Chen, X., Naren, G.-W., Wu, C.-M., Wang, Y., Dai, L., Xia LN, L.P., Zhang, Q., Shen, J.Z., 2010. Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. Veterinary Microbiology 144, 133–139.

Clinical and Laboratory Standards Institute, 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Informational Supplement. CLSI Document M31-A3. Clinical and Laboratory Standards Institute, Wayne,PA.

Desmonts, M.H., Dufour-Gesbert, F., Avrain, L., Kempf, I., 2004. Antimacrobial resistance in *Campylobacter* strains isolated from French broilers before and after antimicrobial growth promoter bans. Journal of Antimicrobial Chemotherapy 54, 1025–1030.

Ekkapobyotin, C., Padungtod, P., Chuanchuen, R., 2008. Antimicrobial resistance of *Campylobacter coli* isolates from swine. International Journal of Food Microbiology 128, 325–328.

Gibreel, A., Sköld, O., Taylor, D.E., 2004. Characterization of plasmid-mediated aphA-3 kanamycin resistance in *Campylobacter jejuni*. Microbial Drug Resistance 10, 98–105.

Harvey, R.B., Young, C.R., Ziprin, R.L., Hume, M.E., Genovese, K.J., Anderson, R.C., Droleskey, R.E., Stanker, L.H., Nisbet, D.J., 1999. Prevalence of *Campylobacter* spp. isolated from the intestinal tract of pigs raised in an integrated swine production system. Journal of the American Veterinary Medical Association 215, 1601–1604.

Ishihara, K., Yamamoto, T., Satake, S., Takayama, S., Kubota, S., Negishi, H., Kojima, A., Asai, T., Sawada, T., Takahashi, T., Tamura, Y., 2006. Comparison of *Campylobacter* isolated from humans and food-producing animals in Japan. Journal of Applied Microbiology 100, 153–160.

Keramas, G., Bang, D.D., Lund, M., Madsen, M., Rasmussen, S.E., Bunkenborg, H., Telleman, P., Christensen, C.B.V., 2003. Development of a sensitive DNA microarray suitable for rapid detection of *Campylobacter* spp. Molecular and Cellular Probes 17, 187–196.

Linton, D., Lawson, A.J., Owen, R.J., Stanley, J., 1997. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. Journal of Clinical Microbiology 35, 2568–2572.

Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C.M., Zhang, Q., 2009. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiology 4, 189–200.

Luber, P., Wagner, J., Hahn, H., Bartelt, E., 2003. Antimicrobial Resistance in *Campylobacter jejuni* and *Campylobacter coli* Strains Isolated in 1991 and 2001–2002 from poultry and humans in Berlin, Germany. Antimicrobial Agents and Chemotherapy 47, 3825–3830.

Padungtod, P., Kaneene, J.B., Hanson, R., Morita, Y., Boonmar, S., 2006. Antimicrobial resistance in *Campylobacter* isolated from food animals and humans in northern Thailand. FEMS Immunology and Medical Microbiology 47, 217–225.

Payot, S., Avrain, L., Magras, C., Praud, K., Cloeckaert, A., Chaslus-Dancla, E., 2004a. Relative contribution of target gene mutation and efflux to fluoroquinolone and erythromycin resistance, in French poultry and pig isolates of *Campylobacter coli*. International Journal of Antimicrobial Agents 23, 468–472.

Payot, S., Bolla, J.M., Corcoran, D., Fanning, S., Mégraud, F., Zhang, Q., 2006. Mechanisms of fluoroquinolone and macrolide resistance in *Campylobacter* spp. Microbes and Infection 8, 1967–1971.

Payot, S., Dridi, S., Laroche, M., Federighi, M., Magras, C., 2004b. Prevalence and antimicrobial resistance of *Campylobacter coli* isolated from fattening pigs in France. Veterinary Microbiology 101, 91–99.

Pezzotti, G., Serafin, A., Luzzi, I., Mioni, R., Milan, M., Perin, R., 2003. Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. International Journal of Food Microbiology 82, 281–287.

Piddock, L.J.V., Griggs, D., Johnson, M.M., Ricci, V., Elviss, N.C., Williams, L.K., Jørgensen, F., Chisholm, S.A., Lawson, A.J., Swift, C., Humphrey, T.J., Owen, R.J., 2008. Persistence of *Campylobacter* species, strain types, antibiotic resistance and mechanisms of tetracycline resistance in poultry flocks treated with chlortetracycline. Journal of Antimicrobial Chemotherapy 62, 303–315.

Piddock, L.J.V., Ricci, V., Pumbwe, L., Everett, M.J., Griggs, D.J., 2003. Fluoroquinolone resistance in *Campylobacter* species from man and animals: detection of mutations in topoisomerase genes. Journal of Antimicrobial Chemotherapy 51, 19–26.

Pratt, A., Korolik, V., 2005. Tetracycline resistance of Australian *Campylobacter jejuni* and *Campylobacter coli* isolates. Journal of Antimicrobial Chemotherapy 55, 452–460.

Quinn, T., Bolla, J.-M., Pagès, J.-M., Fanning, S., 2007. Antibiotic-resistant *Campylobacter*: could efflux pump inhibitors control infection? Journal of Antimicrobial Chemotherapy 59, 1230–1236.

Randall, L.P., Ridley, A.M., Cooles, S.W., Sharma, M., Sayers, A.R., Pumbwe, L., Newell, D.G., Piddock, L.J.V., Woodward, M.J., 2003. Prevalence of multiple antibiotic resistance in 443 *Campylobacter* spp. isolated from humans and animals. Journal of Antimicrobial Chemotherapy 52, 507–510.

Sáenz, Y., Zarazaga, M., Lantero, M., 2000. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997–1998. Antimicrobial Agents and Chemotherapy 44, 267–271.

Schuppers, M.E., Stephan, R., Ledergerber, U., Danuser, J., Bissig-Choisat, B., Stärk, K.D.C., Regula, G., 2005. Clinical herd health, farm management and antimicrobial resistance in *Campylobacter coli* on finishing pig farms in Switzerland. Preventive Veterinary Medicine 69, 189–202.

Shin, E., Lee, Y., 2007. Antimicrobial resistance of 114 porcine isolates of *Campylobacter coli*. International Journal of Food Microbiology 118, 223–227.

Thakur, S., Gebreyes, W.A., 2005a. Prevalence and antimicrobial resistance of *Campylobacter* in antimicrobial-free and conventional pig production systems. Journal of Food Protection 68, 2402–2410.

Thakur, S., Gebreyes, W.A., 2005b. *Campylobacter coli* in swine production: antimicrobial resistance mechanisms and molecular epidemiology. Journal of Clinical Microbiology 43, 5705–5714.

Van Looveren, M., Daube, G., De Zutter, L., Dumont, J.M., Lammens, C., Wijdooghe, M., Vandamme, P., Jouret, M., Cornelis, M., Goossens, H., 2001. Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. Journal of Antimicrobial Chemotherapy 48, 235–240.

Varela, N.P., Friendship, R., Dewey, C., 2007. Prevalence of resistance to 11 antimicrobials among *Campylobacter coli* isolated from pigs on 80 grower–finisher farms in Ontario. Canadian Journal of Veterinary Research 71, 189–194.

Zirnstein, G., Helsel, L., Li, Y., Swaminathan, B., Besser, J., 2000. Characterization of gyrA mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. FEMS Microbiology Letters 190, 1–7.