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Species shift and multidrug resistance of *Campylobacter* from chicken and swine, China, 2008–14

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Objectives: The objective of this study was to investigate the prevalence and antimicrobial resistance of *Campylobacter* isolated from broiler chickens and swine during 2008–14.

Methods: *Campylobacter* isolates were collected from samples of intestinal content and excreta from broiler chickens and swine from slaughter houses as well as conventional farms in five Chinese provinces during 2008–14. The agar dilution method was used to determine the susceptibility of *Campylobacter* isolates to seven antimicrobial agents. The χ^2 test and Fisher's exact test were used to perform the statistical analysis.

Results: In total, 989 *Campylobacter jejuni* and 1991 *Campylobacter coli* were isolated from 10 535 samples. MIC results revealed a high prevalence of multidrug resistance among these *Campylobacter* isolates. In addition, we observed an apparent shift of the dominant species from *C. jejuni* to *C. coli* in chickens and this species shift coincided with an increased prevalence of macrolide-resistant *C. coli*. It is worth noting that almost 100% of the *C. jejuni* and *C. coli* isolates were resistant to fluoroquinolones.

Conclusions: The high prevalence of fluoroquinolone and macrolide resistance in *Campylobacter* suggests that these two clinically important antibiotic classes may no longer be suitable for the treatment of human campylobacteriosis in China. Thus, enhanced surveillance and control efforts are needed to reduce antimicrobial resistance in this group of major foodborne pathogens.

Introduction

Campylobacter was frequently reported as the leading cause of bacterial-derived foodborne illnesses worldwide.[1,2] As a zoonotic pathogen, *Campylobacter* has a broad animal reservoir, and it infects humans via contaminated food, water or milk.[1,3] Poultry and pigs are two primary reservoirs of *Campylobacter*.[4] Antimicrobial agents have been used for decades in food-producing animals to control, prevent and treat infections and to promote growth.[5] *Campylobacter* spp. isolated from livestock, poultry and the environment become increasingly resistant to multiple antimicrobials.[6–8] The emergence and rapid spread of antimicrobial resistance among *Campylobacter* strains pose an enormous threat to food safety and human health.[8]

In China, there has not been a national surveillance system that maintains detailed data on the general use of antimicrobial agents and the occurrence of antimicrobial resistance in food animals, foods and humans. Longitudinal data are not available about the prevalence and antimicrobial resistance of *Campylobacter*. Considering that pork and chicken are the most consumed meats in China, we investigated the prevalence and antimicrobial resistance of *Campylobacter* from broiler chickens and swine during 2008–14.

Materials and methods

Isolation and identification of Campylobacter

Campylobacter isolates were collected from samples of intestinal content and excreta from broiler chickens and swine from slaughter houses as well as conventional farms in five provinces during 2008–14 (no samples were collected in 2010 and 2011). These provinces, including Guangdong, Ningxia, Shandong, Shanghai and Henan, are geographically separated areas, which represent the major food animal production provinces in China for broiler chickens and swine (Figure 1). The detailed protocols of strain isolation and species identification were described previously.[9]

Antimicrobial susceptibility testing

The agar dilution method as described by the CLSI (2008)[10] was used to determine the susceptibility of *Campylobacter* isolates to seven

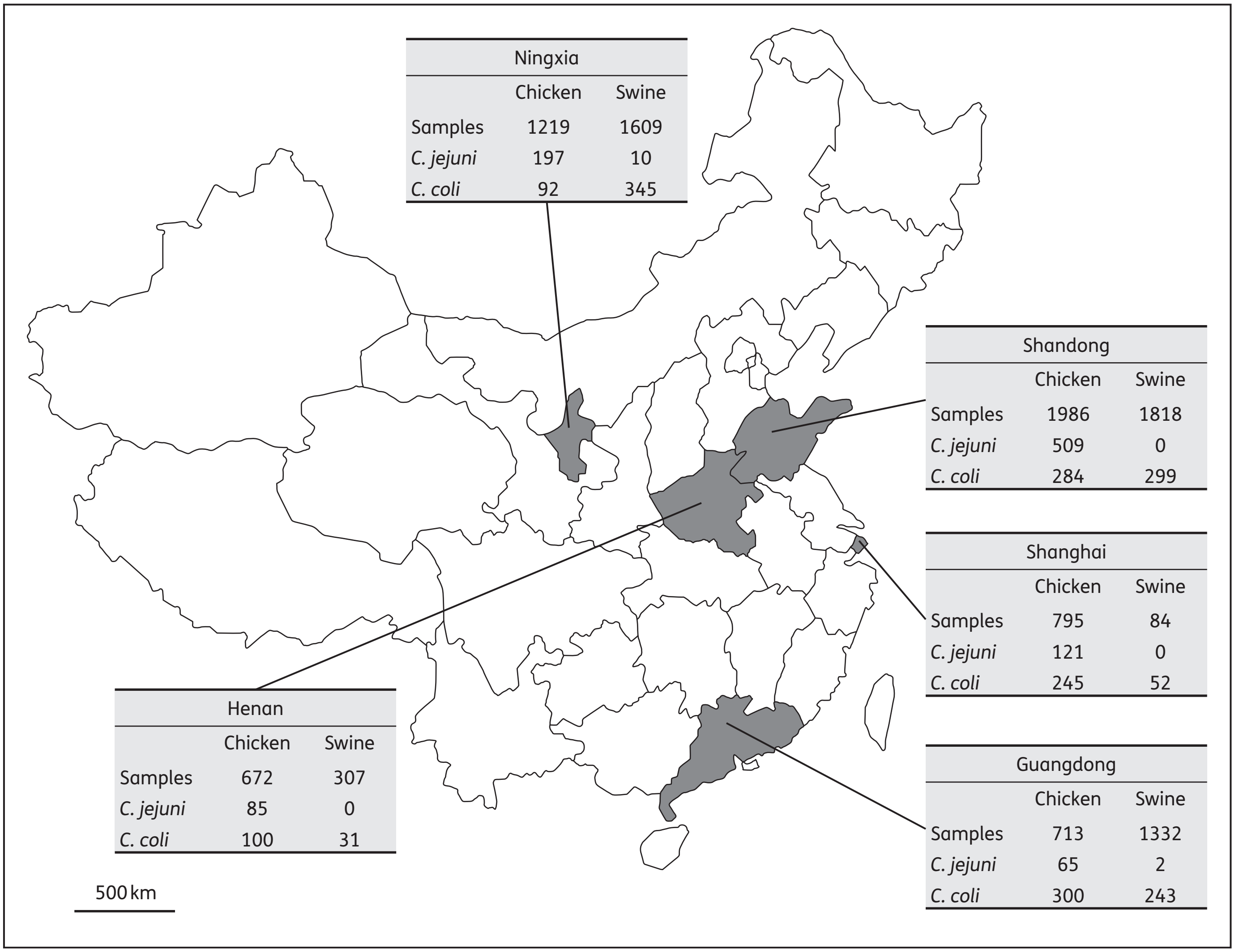

Figure 1. Geographical distribution of sample collection areas in China during 2008–14. Each label shows the province, sample size and the number of obtained *Campylobacter* isolates.

antimicrobial agents (kanamycin, gentamicin, erythromycin, ciprofloxacin, tetracycline, clindamycin and florfenicol). The MIC ranges of antimicrobial agents and the resistance breakpoints of all antimicrobial agents are summarized in Table S1 (available as Supplementary data at *JAC* Online) according to the CLSI (2008, 2010) and *NARMS (National Antimicrobial Resistance Monitoring System) Retail Meat Annual Report 2010*.[10–12] *Campylobacter jejuni* ATCC 33560 and *Escherichia coli* ATCC 25922 were used as quality-control strains. A *Campylobacter* isolate resistant to three or more classes of antimicrobial agents was defined as an MDR isolate.[13]

Statistical analysis

The χ^2 test and Fisher's exact test were used to perform the statistical analysis.

Results and discussion

Of the total 10535 samples collected during the period of investigation, 5385 (51.1%) samples were caeca content of chickens and 5150 samples (48.9%) were swine faeces. In total, 977 *C. jejuni* and 1021 *Campylobacter coli* were isolated from chickens, and 12 *C. jejuni* and 970 *C. coli* were isolated from swine (Table S2). Overall, the isolation rates of *Campylobacter* in chicken-sourced samples ranged from 32.7% to 44.2% during 2008–14, while the isolation rates of swine-sourced samples for *Campylobacter* ranged from 13.4% to 26.1%.

During this period, we noticed a shift of species compositions in the chicken isolates. The percentage of *C. jejuni* isolates from chicken had been shifted significantly, from 75.6% (208/275) in 2008 and 78.7% (233/296) in 2009 to 37.9% (119/314) in 2012, 42.8% (263/614) in 2013 and 29.9% (154/515) in 2014. The χ^2 test revealed a significant linear trend among the ordered years from 2008 to 2014 ($P<0.0001$). During the same period, the percentage of *C. coli* increased from 19.3% (53/275) in 2008 and 21.3% (63/296) in 2009 to 62.1% (195/314) in 2012, 56.8% (349/614) in 2013 and 70.1% (361/515) in 2014 ($P<0.0001$) (Table S2). This finding indicated that *C. coli* had become the dominant *Campylobacter* species in chickens. This finding is interesting, as *C. jejuni* is known to be the dominant species in chickens.[7,14,15] In contrast, the composition of *Campylobacter* species in swine was stable during the period, with *C. coli* as the dominant species accounting for 98.8% (970/982) of the total swine isolates (Table S2).

Overall, the prevalence of antibiotic resistance is high among the isolates from both chicken and swine. Because of the small number of *C. jejuni* isolates of swine origin ($n=12$) and the failure of recovery for another 6 *C. jejuni* and 1 *C. coli* isolates from the laboratory stock, these 19 isolates were excluded from the analysis of antimicrobial resistance. In total, 463 (47.7%) of the 971 (chicken=971; swine=0) tested *C. jejuni* isolates and 1614 (81.1%) of the 1990 (chicken=1020; swine=970) tested *C. coli* isolates were resistant to three or more classes of antimicrobial agents and, thus, were considered MDR.[13] The overall multidrug resistance rates of *C. jejuni* of chicken origin decreased during 2008–14. Specifically, the rate of multidrug resistance in *C. jejuni* of chicken origin decreased from 93.1% (188/202) in 2008 to 51.5% (120/233) in 2009, 63.9% (76/119) in 2012, 18.3% (48/263) in 2013 and 20.1% (31/154) in 2014 ($P<0.0001$) (Figure 2a). The *C. coli* of chicken origin maintained the high multidrug resistance level over the investigated period, which did not show a significant linear trend of multidrug resistance during 2008–14 ($P=0.4713$) (Figure 2b). In contrast, the multidrug resistance of *C. coli* from swine origin showed an increasing trend in the investigation period. The percentage of MDR *C. coli* isolates from swine were from 72.6% (69/95) in 2008 to 67.5% (154/228) in 2009, 68.7% (322/469) in 2012, 78.3% (72/92) in 2013 and 93.0% (80/86) in 2014 ($P=0.0004$). In addition, there was an increasing trend in the percentage of isolates resistant to five or more antimicrobial agents, which were 34.7% (33/95) in 2008, 23.2% (53/228) in 2009, 31.3% (147/469), 41.3% (38/92) in 2013 and 65.1% (56/86) in 2014 ($P<0.0001$) (Figure 2c).

Resistance to fluoroquinolone and tetracycline was highly prevalent among the *C. jejuni* and *C. coli* isolates. Specifically, 99.4% (965/971) of *C. jejuni* isolates of chicken origin, 99.2% (1012/1020) of *C. coli* isolates of chicken origin and 97.0% (941/970) of *C. coli* isolates of swine origin were resistant to ciprofloxacin. Likewise, 95.6% (928/971) of *C. jejuni* isolates of chicken origin, 97.5% (995/1020) of *C. coli* isolates of chicken origin and 91.1% (884/970) of *C. coli* isolates of swine origin were resistant to tetracycline.

Macrolide resistance in *C. coli* is much more prevalent than that in *C. jejuni*. Specifically, 10.5% (102/971) of *C. jejuni* isolates of chicken origin, 73.2% (747/1020) of *C. coli* isolates of chicken origin and 54.0% (524/970) of *C. coli* isolates of swine origin were resistant to erythromycin. We compared the macrolide resistance of *C. coli* and *C. jejuni* isolated between 2008–09 and 2012–14 using Fisher's exact test. The macrolide-resistant *C. jejuni* isolates of chicken origin reduced from 13.3% (58/435) in 2008–09 to 8.2% (44/536) in 2012–14 ($P=0.0114$). However, the resistant *C. coli* isolates of chicken origin increased from 48.7% (56/115) in 2008–09 to 76.4% (691/905) in 2012–14 ($P<0.0001$). In China, several types of macrolides, including tylosin, tilmicosin, erythromycin, kitasamycin and tulathromycin, are widely and increasingly used for poultry and livestock production. This change in the prevalence of macrolide resistance coincided with the species shift from *C. jejuni* to *C. coli* in chickens, suggesting that increased macrolide selection pressure in chicken production might have contributed to the species shift. Macrolide-resistant *C. coli* isolates of swine origin increased from 44.0% (142/323) in 2008–09 to 59.0% (382/647) in 2012–14 ($P<0.0001$), probably due to the increased use of macrolide antibiotics in swine production during the period.

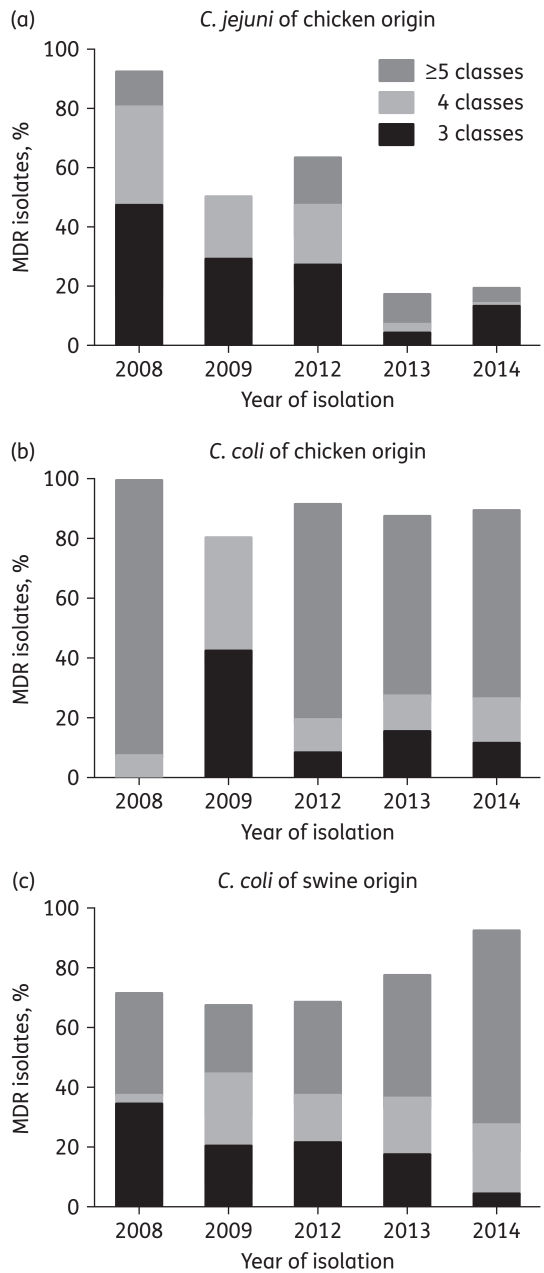

Figure 2. Multidrug resistance among *C. jejuni* and *C. coli* isolated from chicken and swine populations during 2008–14. (a) Multidrug resistance among *C. jejuni* of chicken origin. χ^2 test showed a significant decreased trend ($P<0.0001$). (b) Multidrug resistance among *C. coli* isolates of chicken origin. The χ^2 test showed no significant linear trend ($P=0.4713$). (c) Multidrug resistance among *C. coli* of swine origin. The χ^2 test showed a significant increased trend ($P=0.0004$).

In conclusion, this study revealed that *Campylobacter* is prevalent in both chicken and swine populations in China and that their isolates are highly resistant to multiple antibiotics. Overall, the prevalence of MDR isolates of *C. coli* is much higher than that of *C. jejuni*. Thus, *C. coli* may have better adaptation and survivability under antimicrobial selection pressure. This finding may explain why *C. coli* replaced *C. jejuni* as the dominant *Campylobacter* species in chicken populations in the reported period. The high prevalence of fluoroquinolone and macrolide resistance in *Campylobacter* suggests that these two clinically important antibiotic classes may no longer be suitable for the treatment of human campylobacteriosis in China, compromising their utility for clinical treatment. Thus, enhanced surveillance and control efforts are needed to reduce antimicrobial resistance in this group of major foodborne pathogens.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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