The Veterinary Journal 211 (2016) 100–103

Contents lists available at ScienceDirect

The Veterinary Journal

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

Short Communication

Antibiotic resistance and polymorphism in the quinolone resistance-determining region of *Campylobacter* spp. isolated from 1-day-old ducklings

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ARTICLE INFO

Article history:
Accepted 22 September 2015

Keywords:
Campylobacter spp
Ducklings
Antibiotic resistance
tet(O)
gyrA

ABSTRACT

Thirty-three isolates of *Campylobacter coli* and three isolates of *Campylobacter jejuni* were recovered from 150 1-day-old ducklings. All isolates were sensitive to chloramphenicol and amikacin, but resistant to sulfamethoxazole-trimethoprim (SXT) by the disc diffusion method. Most isolates were susceptible to tetracycline and erythromycin, but resistant to ofloxacin and ciprofloxacin. Of the 33 *C. coli* isolates, nine were positive for the tetracycline resistance gene *tet(O)*, although only two of these were resistant to tetracycline in the disc diffusion test. None of the isolates possessed mutations in the quinolone resistance-determining region (*QRDR*) of the *gyrA* gene infrequently linked to FQ-resistance. The finding indicated that ducklings may be a source of antibiotic resistant *Campylobacter* spp. with potential poultry and public health hazard.

Ducks are frequently infected by *Campylobacter coli* and *Campylobacter jejuni* without showing clinical signs and may serve as a carrier of *Campylobacter* spp. to other animals (Colles et al., 2011; Carrique-Mas et al., 2014). Hatcheries are a potential source of infection for 1-day-old chicks through environmental contamination, but it is uncertain whether vertical transmission occurs through the egg (Zhang, 2008).

Erythromycin, fluoroquinolones (FQ), gentamicin and tetracycline are effective treatments for disease due to *Campylobacter* spp., but careful use of antibiotics is required to reduce the risk of emergence of resistant strains, which could be transmitted to humans (Gupta et al., 2004; Zhang, 2008). The resistance of *Campylobacter* spp. to tetracycline is commonly associated with the presence of the *tet*(O) gene, which could be transferred from resistant strains to sensitive strains (Avrain et al., 2004). Likewise, mutations in the quinolone resistance-determining region (*QRDR*) of *gyrA*, especially T86I, have been linked to the resistance of *Campylobacter* spp. to FQ with incidence of hypermutable phenotype in *Campylobacter* resistant strains to FQ (Bachoual et al., 2001; Luo et al., 2003). The aim of this study was to provide information on antibiotic resistance of *Campylobacter* spp. in 1-day-old ducklings in Egypt.

A total of 150 faecal meconium samples (~1 g each) were collected in 9 mL Bolton broth (Oxoid) from 1-day-old commercial meat ducklings (e.g. Muscovy, Mallard). All samples were submitted to the Reference Laboratory for Veterinary Quality Control on Poultry Production, Giza, for routine examination in 2011 and 2012. Isolation and biochemical identification of *Campylobacter* spp. were performed according to ISO 10272-1[1] using blood-free selective media (CCD agar and Karmali agar; Oxoid). Resistance against ampicillin, tetracycline, erythromycin, ciprofloxacin, ofloxacin, sulfamethoxazole-trimethoprim (SXT), gentamicin, amikacin and chloramphenicol (Oxoid) was determined using the disc diffusion method test conducted following the recommendations of the Clinical and Laboratory Standards Institute.[2]

DNA was extracted from bacterial isolates from positive samples using the QIAamp DNA Mini kit (Qiagen). Generic PCR assays were used to amplify partial fragments of different genes, namely, *ceuE* for *C. coli*, *mapA* for *C. jejuni*, *tetO* for tetracycline resistance gene and *gyrA* for FQ resistance gene of *Campylobacter* spp. using specific oligonucleotide primers (Metabion) (see Appendix: Supplementary Table S1). *C. jejuni* WHO C 10-1 and *C. coli* WHO C 10-2 were used as positive controls. The 25 µL PCR reaction contained 12.5 µL of Emerald Amp Max PCR Master Mix (Takara), with 1 µL containing

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[1] ISO 10272-1, 2006. Microbiology of food and animal feeding stuffs: Horizontal method for the detection of thermotolerant *Campylobacter*. International Standards Organization, Geneva.

[2] CLSI, 2009. Performance standards for antimicrobial disk susceptibility tests: Approval Standard, Tenth Ed. Performance Standards for Antimicrobial Susceptibility Test; M02-A10 and M100-S20.

http://dx.doi.org/10.1016/j.tvjl.2015.09.020
1090-0233/

	AzEg2	AzEg3	AzEg9	AzEg8	AzEg7	AzEg4	AzEg11	AzEg1	AzEg12	AzEg5	AzEg6	AzEg10
AzEg2	ID	100	98.2	98.9	98.7	99.4	98.7	97.9	98.7	79.2	78.7	78.9
AzEg3	100	ID	98.2	98.9	98.7	99.4	98.7	97.9	98.7	79.2	78.7	78.9
AzEg9	97.6	97.6	ID	98.7	98.7	97.6	98.9	97.1	97.9	79.4	78.9	79.2
AzEg8	98.4	98.4	98.4	ID	98.9	98.4	99.2	97.4	98.2	79.2	78.7	78.9
AzEg7	97.6	97.6	97.6	98.4	ID	98.2	99.2	97.9	98.7	79.4	78.9	79.2
AzEg4	99.2	99.2	96.9	97.6	96.9	ID	98.2	98.2	99.2	78.9	78.4	79.4
AzEg11	98.4	98.4	98.4	99.2	98.4	97.6	ID	98.2	98.4	79.4	78.9	79.2
AzEg1	96.9	96.9	95.3	96.1	96.9	96.9	96.9	ID	98.9	79.2	78.7	79.2
AzEg12	97.6	97.6	96.9	96.9	97.6	98.4	96.9	97.6	ID	79.2	78.7	79.7
AzEg5	80.7	80.7	80.7	80.7	81.5	80.7	80.7	80.7	81.5	ID	97.4	98.7
AzEg6	78.4	78.4	78.4	78.4	79.2	78.4	78.4	78.4	79.2	95.3	ID	97.6
AzEg10	80	80	80	80	80.7	80.7	80	80	81.5	97.6	96.1	ID

Fig. 1. Homology of the *gyrA* gene from selected *Campylobacter* spp. isolates ($^{\text{nucleotide}}/_{\text{amino acid}}$).

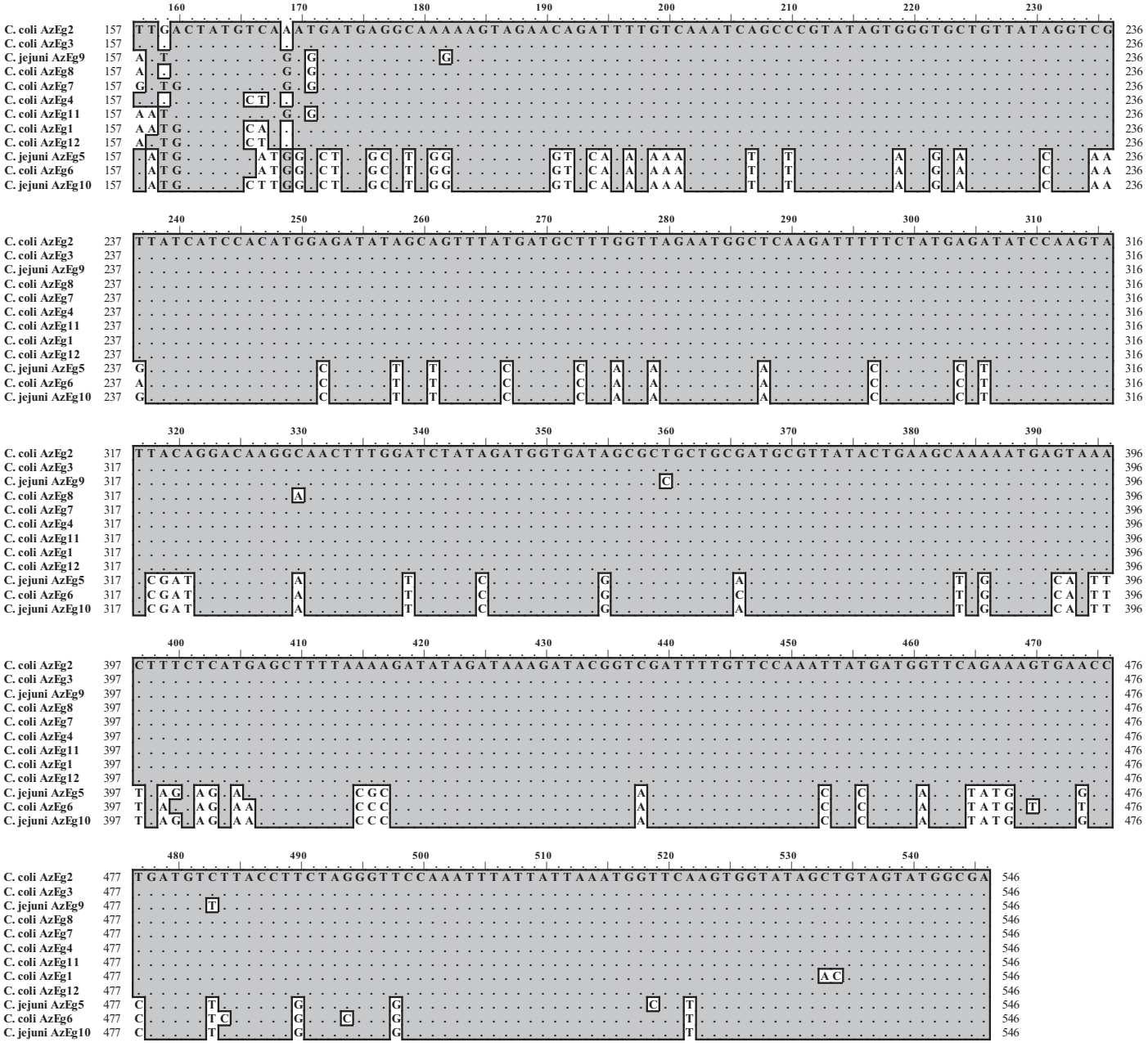

Fig. 2. Nucleotide sequence alignment of 390 nucleotides of the *gyrA* gene from *Campylobacter* spp. isolated from 1-day-old ducklings in Egypt. Nucleotides correspond to positions 157–546 of the full *gyrA* gene of the reference strain.