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Prevalence and antimicrobial resistance of *Campylobacter* species isolated from the avian eggs

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ABSTRACT

Campylobacter is one of the most important food borne pathogens that cause bacterial gastroenteritis worldwide. The most commonly isolated species in humans are *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli*. The emergence of antimicrobial resistance in Campylobacter spp. has been a growing public health concern globally. Information about antimicrobial resistance of *Campylobacter* at different levels of production is important for the development of control strategies for this pathogen. This study was conducted to determine the prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from different eggs from different avian species in Iran. A total of 440 egg samples were collected from different avian and analyzed for the presence of *Campylobacter* spp. in eggshell and eggs content under sterile conditions using *Campylobacter* selective agar base and the species were identified by biochemical tests. The suspected colonies were subjected to PCR assay for final confirmation as *Campylobacter* spp., and identification of *C. jejuni* or *Campylobacter coli*. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method using Mueller Hinton agar. *Campylobacters* were detected in a total of 7 out of 100 (7%) eggshell of chicken samples and in 3 out of 60 (5%) eggshell of duck samples. In addition, *Campylobacter* spp. were also detected in 3.3%, 2.5%, 4.2%, 5% and 3.8 of the eggshell of goose, ostrich, partridge, quail and turkey samples, respectively. The overall prevalence rate of *Campylobacter* species from different avian eggs was found to be 7.7% (34/440). Among different avian egg samples, *Campylobacter jejuni* was more frequently isolated 28 (n = 28, 6.3%) than *C. coli* 6 (n = 6, 1.3%). In addition, the prevalence of *C. jejuni* was highest in summer and lowest in autumn. In this study *Campylobacter* spp. showed significant difference in resistance pattern with tetracycline and ciprofloxacin but gentamicin resistance was not found in both *C. coli* and *C. jejuni* isolates. Therefore, gentamicin is safe and effective drugs for the treatment of human campylobacteriosis if avian egg is considered as the source of infection.

1. Introduction

Campylobacter is the leading cause of bacterial zoonotic gastroenteritis in both developing and developed countries (Raissy, Khamesipour, Rahimi, & Khodadoostan, 2014; WHO, 2000). More than 95% of human infections are due to *Campylobacter jejuni* or *Campylobacter coli* (Butzler, 2004) but other species like *Campylobacter fetus*, also involved in human disease (Lastovica & Skirrow, 2000, chap. 5). Human disease is characterized by diarrhea, fever, and abdominal cramping (Allos, 2001) and the dangerous Guillain-Barré syndrome can be in rare cases a result of campylobacteriosis (CDC, 2010).

Poor hygiene and sanitation and close proximity to animals in developing countries all contribute easy and frequent acquisition of any enteric pathogen including *Campylobacter* spp. and this is responsible for sporadic cases (Martin, 1999). Different animals have been found to be reservoirs of *C. jejuni* such as birds and rodents (Luetchefed, Blaser, Reller, & Wang, 1980). Chicken and turkey products are considered the most common sources of *Campylobacter* (Zhao et al., 2010). Birds are an important reservoir of *Campylobacter* due to their high body temperature which

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provides an optimum growth temperature for these thermotolerant species (Hald et al., 2015). The intestine of poultry are easily colonized with *C. jejuni*, a one-day-old chick can be colonized with as few as 35 organisms (Alber, Far uque, Faraque, Sack, & Mahalanabis, 1999; Hilton, Mortiboy, Banks, & Penn, 1997). In a farm, the spread of *Campylobacter* occurs through contaminated food and water as well as feces (Denis, Refregier-Petton, Laisney, Ermel, & Salvat, 2001).

Human campylobacteriosis is generally self-limiting, although in severe cases it requires antimicrobial therapy. Erythromycin and ciprofloxacin are often the drugs of choice (McDermott et al., 2004). Fluoroquinolones such as ciprofloxacin have been used for first-line treatment of bacterial gastroenteritis in the absence of a microbiological diagnosis (Allos, 2001).

The incidence of human *Campylobacter* infections is increasing worldwide along with the proportion of isolates resistant to antibiotics (Moore et al., 2006). Antimicrobial resistance of *Campylobacter* spp. to fluoroquinolones, which are generally used for the empiric treatment of bacterial gastroenteritis, has increased during the past two decades, mainly as a result of the approval of this group of antimicrobials for the use in food producing animals (Han, Lestari, Pu, & Ge, 2009; Nelson & Harris, 2006; Ommi, Hemmatinezhad, Hafshejani, & Khamesipour, 2016). Resistance to erythromycin (Taylor, Ng, & Lior, 1985; Yan & Taylor, 1991) or tetracycline in *Campylobacter* spp. (Tenover, Williams, Gordon, Nolan, & Plorde, 1985) has been reported earlier in up to 15% of strains, and resistance to the newer quinolones has recently been reported (Endtz, Ruis, van Klingesen, Jansen, van der Reyden, & Monton 1991; Gootz & Martin, 1991). Information about antimicrobial resistance of *Campylobacter* at different levels of production is important for the development of control strategies for this pathogen. Therefore, continued concern about antimicrobial resistance is warranted, as are studies of the activity of new agents used for treatment of diarrheal diseases (Taylor & Courvalin, 1988).

While surveys of chicken meat and products, and its association with food borne pathogens are widely available in Iran, such information on other avian eggs is scarce. Therefore, the objective of this study was to determine the prevalence and antimicrobial susceptibility pattern of *Campylobacter* species isolated from the avian eggs in Iran.

2. Materials and methods

2.1. Sample collection

Eggs samples from chicken (n = 100), duck (n = 60), goose (n = 60), ostrich (n = 40), partridge (n = 48), quail (n = 80), and turkey (n = 52) were collected randomly from different outlets in Isfahan province over a period of 12 months from September 2014 to October 2015. All of the egg samples were purchased from the shopping malls and supermarkets of various parts of Iran. All egg samples were produced for routine daily consumption. A total of 440 eggs were analyzed for the presence of *Campylobacter* in eggshell and eggs content (egg white and egg yolk). All samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and transported to the laboratory on crushed ice. The samples were kept in a refrigerator at 4 °C until testing within about 4 h.

2.2. Bacteriological methods

Samples were processed immediately upon arrival using aseptic techniques. A swab technique was used to sample the shell surface of the intact eggs. Sterile cotton swabs dipped in sterile buffered peptone water were used to swab the entire surface area of the eggshell. At first, the swab samples were directly taken from the surface of each egg. Then, surfaces were sterilized by immersion in 70% alcohol for 2 min, air dried in a sterile chamber for 10 min, then cracked with a sterile knife. At second, swab samples from the egg white and yolk were taken from each egg. Five milliliters of each egg's white and each egg yolk and in the cases of eggshell, swab samples taken from 5 cm^2 of eggs were inoculated into 45 ml of Preston enrichment broth base containing *Campylobacter* selective supplement IV (HiMedia Laboratories, Mumbai, India) and 5% (vol/vol) defibrinated sheep blood at 37 °C. After inoculation at 42 °C for 24 h in a microaerophilic condition (85% N_2, 10% CO_2 and 5% O_2), 0.1 ml of the enrichment broth was then streaked onto *Campylobacter* selective agar base (HiMedia Laboratories, Mumbai, India) supplemented with an antibiotic supplement for the selective isolation of *Campylobacter* species (HiMedia Laboratories, Mumbai, India) and 5% (vol/vol) defibrinated sheep blood and incubated at 42 °C for 48 h under the same conditions. One presumptive *Campylobacter* colony from each selective agar plate was subjected to biochemical tests. For identification, we used standard microbiological and biochemical procedures including Gram staining, production of catalase, oxidase, hippurate hydrolysis, urease activity, indoxyl acetate hydrolysis, and susceptibility to cephalotin (Rahimi & Ameri, 2011). The suspected colonies were subjected to PCR assay for final confirmation as *Campylobacter* spp., and identification of *Campylobacter jejuni* (*C. jejuni*) or *C. coli*.

2.3. DNA extraction and PCR amplification

All of the *Campylobacter* colonies were another time subcultured on Preston enrichment broth base containing *Campylobacter* selective supplement IV (HiMedia Laboratories, Mumbai, India) and 5% (vol/vol) defibrinated sheep blood at 37 °C. Genomic DNA was extracted from the colonies growth on Preston's broth using a Genomic DNA purification kit (Fermentas, GmbH, Germ any, K0512) according to the manufacturer's protocol. We used the polymerase chain reaction (PCR) procedures in this study which Rahimi et al. described previously (Rahimi, Momtaz, Ameri, Ghasemian-Safaei, & Ali-Kasemi, 2010). In this protocol, three genes were selected for the identification of the *Campylobacter* spp., *C. jejuni* and *C. coli*. They were the 16S rRNA gene, the mapA gene, and the ceuE gene, respectively (Rahimi et al., 2010). The sequences of the primers used for gene amplification are presented in Table 1.

Amplification reactions were performed in a 30 ml mixture. It was containing 0.6 U Taq polymerase (Fermentas, GmbH, Germany), 100 μ moL l^{-1} of each dNTP, 0.11 μ moL $l^{-1}$of MD16S1 and MD16S2 primers. It was also containing 0.42 μ moL $l^{-1}$of MDmapAl, MDmapA2, COL3 and MDCOL2 primers in the Fermentas buffer (Fermentas, GmbH, Germany). Amplification reactions were carried out using a DNA thermal cycler (Master Cycle Gradiant, Eppendrof, Germany). They were one cycle of 10 min at 95 °C, 35 cycles each consisting of 30 s at 95 °C. Also, they were 1 min and 30 s at 59 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. The amplification generated 857 bp, 589 bp, and 462 bp DNA fragments corresponding to the *Campylobacter* species, *C. jejuni* and *C. coli*, respectively. We used *C. coli* (ATCC 33559) and *C. jejuni* (ATCC 33560) as the positive controls and sterile distilled water as the negative control. Amplified the PCR products were electrophoresed in 1.5% agarose gel and a 100-bp DNA ladder was used as a size marker. After staining with ethidium bromide, the gel was visualized with UV gel documentation apparatus.

2.4. Antimicrobial susceptibility testing

One strain from each *Campylobacter*-positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing