Antimicrobial Susceptibility of Thermophilic Campylobacter Isolates from Diverse Origin

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(Received 21.08.2015; Accepted 12.12.2015)

ABSTRACT

Campylobacteriosis is a leading cause of human gastroenteritis and is recognized as one of the most important foodborne zoonosis. The occurrence of increasingly resistant strains of Campylobacter to the clinically important antibiotics is a concern. The present study was undertaken with the aim of determining the antibiogram pattern of 30 Campylobacter isolates comprising 24 C. jejuni (Chicken meat and skin – 6, quail caecum – 8, dog faeces – 2, chicken caecum – 6, pig faeces – 2) and 6 C. coli (chicken caecum – 3 and one each from dog faeces, pig faeces and quail caecum) isolates. The study revealed that Campylobacter isolates were resistant against several antibiotics including tetracycline, cephalosporins, co-trimoxazole and carbenicillin. Resistance was also detected against ciprofloxacin and nalidixic acid, while most isolates were susceptible against erythromycin. The prevalence of resistance among isolates from diverse origin may pose threat to human and animals.

Keywords: Antibiotic resistance, Campylobacter, C. jejuni, C. coli

Introduction

Campylobacteriosis is a leading cause of human gastroenteritis and is recognized as one of the most important foodborne zoonosis in both the developed as well as developing countries. The thermophilic campylobacters, especially Campylobacter jejuni and Campylobacter coli account for 95% of these infections (Ruiz-Palacios, 2007). Global estimates of the burden of diarrhoeal illnesses reveal that Campylobacter is responsible for 7.5 million disability-adjusted life years (DALYs) or 8.4% of the total burden of diarrheal diseases, and ranks fourth among identified pathogens after rotavirus (18.7 million DALY) and typhoid fever (12.2 million DALY) and cryptosporidiosis (8.3 million DALY) (Murray et al., 2012).

Strains of Campylobacter spp. are widely distributed among food animals. The natural habitat of these bacteria is the intestine of birds and warm-blooded animals (Adekunle et al., 2009). Antimicrobial agents have been critical in fighting microbial infections. The extensive use of antibiotics in food animals and poultry has resulted in the emergence and dissemination of antimicrobial resistant bacteria. The development and spread of resistance in zoonotic bacteria, like Campylobacter with its reservoirs in healthy food animals like poultry, pigs and cattle, has become a global public health problem (EFSA, 2008). Antimicrobial resistance has been reported in Campylobacter spp. worldwide, and the situation seems to be rapidly deteriorating in developing countries too, owing to uncontrolled and widespread use of antibiotics. The self-limiting nature of campylobacteriosis does not warrant treatment with antibiotics, except in serious cases wherein macrolides are normally considered as the drug of choice, with fluoroquinolones as alternate. The occurrence of increasingly resistant strains of Campylobacter to the clinically important antibiotics is a concern. The present study was carried out to investigate the resistance pattern of C. jejuni and C. coli isolates against multiple classes of antibiotics.

Materials and Methods

A total of 30 Campylobacter isolates comprising 24 C. jejuni (Chicken meat and skin – 6, quail caecum – 8, dog faeces – 2, chicken caecum – 6, pig faeces – 2) and 6 C. coli (chicken caecum – 3 and one each from dog faeces, pig faeces and quail caecum) were subjected to antibiotic susceptibility studies. The disk diffusion method described by Bauer et al. (1966) was used to determine the antibiotic susceptibility pattern of the isolates against 22 antibiotics (HiMedia, India). The antibiotics used...
along with their concentration and classes have been listed in Table 1. The pure colonies from blood agar plates were suspended in 0.1% buffered peptone water and turbidity of the suspension adjusted to 1 McFarland standard (\(3.0 \times 10^8\) cells/ml). One hundred microlitre of the suspension was spread on Mueller Hinton agar No. 2 (Titan, India) supplemented with 5% defibrinated sheep blood and antimicrobial disks were placed on the surface of the agar plates using sterile tweezers followed by incubation at 42°C for 24-48 h under microaerophilic atmosphere (5% CO\(_2\)).

**Results and Discussion**

There is growing scientific evidence that the use of antibiotics in food animals leads to the development of resistant pathogenic bacteria that can reach humans through the food chain (van Looveren et al., 2001).

A total of 30 isolates were subjected to antibiotic sensitivity testing against 22 antibiotics belonging to 7 classes. The results have been represented graphically in Fig. 1 and 2 for *C. jejuni* and *C. coli*, respectively.

The antibiotic profiling studies of *C. jejuni* and *C. coli* isolates in the present study revealed 100% sensitivity to the fluoroquinolone class of antibiotics. However, among *C. jejuni*, 33.3% of the isolates were resistant to ciprofloxacin and 37.5% to nalidixic acid. Similarly, 50% and 33.3% *C. coli* isolates were resistant against ciprofloxacin and nalidixic acid, respectively. Ge et al. (2002) and Luangtongkum et al. (2007) reported a similar finding in isolates from raw meats and poultry intestine, respectively. Rahimi and Ameri (2011) reported a similar finding in meats from various poultry species. In contrast, Jain et al. (2005) reported 71.4% resistance to ciprofloxacin. There are several reports around the world describing fluoroquinolone-resistant *Campylobacter* infections in humans worldwide (Engberg et al., 2001; Gupta et al., 2004). Oberhelman and Taylor (2000) observed the resistance to erythromycin ranging from 0-16% in developing countries with *C. coli* strains being more resistant than *C. jejuni*.

A low resistance was reported against erythromycin in the present study, with only a single isolate of *C. jejuni* (3.3%) found resistant. These findings are in agreement with Prasad et al. (1994) who reported 1.3% resistance and Ewnetu and Mihret (2010) who reported 12.5% resistance. Elango et al. (2010) reported 7.7% resistance against erythromycin in milk isolates. A high rate of tetracycline resistance was observed in this study with 95.8% *C. jejuni* and 83.3% *C. coli* isolates found resistant. Ge et al. (2002), Luber et al. (2003) and Senok et al. (2007) reported similar findings with high resistance to tetracycline among the poultry isolates. Bester and Essack (2008) reported high resistance to tetracycline in a study on broiler and layer isolates with 98.2% and 100% resistance, respectively.

![Fig. 1. Antibiogram of *C. jejuni* isolates](image-url)
Table 1. List of antibiotics

<table>
<thead>
<tr>
<th>Class</th>
<th>Antibiotics used and concentration (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroquinolones</td>
<td>Ciprofloxacin (10), moxifloxacin (5), ofloxacin (5), sparfloxacin (5), levofloxacin (5), norfloxacin (10), gatifloxacin (5), nalidixic acid (30)</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Tetracycline (30)</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Ceftazidime (30), cefotaxime (30), ceftriaxone (30), cephalothin (30)</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Erythromycin (15)</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>Co-trimoxazole (25)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Amikacin (10), kanamycin (30), streptomycin (10), gentamicin (10), tobramycin (10)</td>
</tr>
<tr>
<td>Penicillins</td>
<td>Ampicillin (10), carbenicillin (100)</td>
</tr>
</tbody>
</table>

The isolates in the present study were resistant to the third generation cephalosporins with 83.3%, 91.7%, and 100% resistance recorded against ceftazidime, cefotaxime and cephalothin, respectively amongst C. jejuni isolates. All the C. coli isolates were resistant to cephalothin. These observations are in agreement with that of Hamidian et al. (2011). The resistance against cephalothin was on the higher side in agreement with findings of Yang et al. (2008) and Rajagunalan et al. (2012). Also, high degree of resistance was seen to co-trimoxazole as reported by Rajagunalan et al. (2012).

Nearly all the isolates showed 100% sensitivity to aminoglycoside class of antibiotics which support the findings of Hamidian et al. (2011) who reported similar findings in human isolates. Low resistance to aminoglycosides was also reported by Elango et al. (2010). Ewnetu and Mihret (2010) observed 25.5% resistance against streptomycin among human and chicken isolates, similar to our findings.

It has been reported that Campylobacter spp. produce β-lactamases that confer resistance to the β-lactam drugs like carbenicillin. Among the penicillins, 79.2% C. jejuni isolates were resistant against carbenicillin, whereas only 16.7% isolates were resistant to ampicillin. The C. coli isolates were 100% resistant to carbenicillin. Jonker and Picard (2010) have reported 95.4% resistance against carbenicillin.

The increase in resistance to commonly used antibiotics coupled with multidrug resistance poses

![Fig. 2. Antibiogram of C. coli isolates](image-url)
major risk for treatment failure. The number of quinolone-resistant and macrolide-resistant Campylobacter strains causing human infections has increased markedly in the past decade. Resistance to both macrolides and fluoroquinolones must be considered highly undesirable in this organism, as the two classes are generally advocated as first- and second-line drugs for antimicrobial treatment of Campylobacter enteritis. Detection of resistant isolates for commonly used antimicrobials may pose a threat to humans and chickens by limiting therapeutic options. The prudent use of antibiotics in food-producing animals is the need of the hour. The prolonging of useful life of antimicrobial drugs can be done by developing new drugs, and utilization of other infection control measures such as use of prebiotics/probiotics to reduce the bacterial load in proven reservoirs, timely detection of the infection and search of suitable vaccines, etc.

Acknowledgements

The authors are thankful to the Director, ICAR-Indian Veterinary Research Institute for supporting this research and providing facilities. The present work was carried out under the ICAR funded project ‘Outreach Programme on Zoonotic Diseases’.

References


