Evaluation of First Line Agents' Resistance Amongst Campylobacter Isolates Using MIC Breakpoints at Aga Khan University Clinical Laboratory

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Abstract

Objective
Campylobacter is one of the most common causes of gastroenteritis especially in Pakistan, with an increasing drug resistance. Usually erythromycin and ciprofloxacin are considered the first line agents however, over a period of time, resistance against these antimicrobials has been observed in various regions of the world. Growing erythromycin resistance is of concern since these strains mostly turn out to be multi-drug resistant and there are very few options available to fight against them. The aim of the study is to evaluate frequency of erythromycin and ciprofloxacin resistance in *Campylobacter* species.

Material and methods
A total of 107 isolates were isolated from stool as well as blood samples from Jan 2014 to March 2016 and identified by conventional tests. Minimum inhibitory concentration (MIC) of erythromycin and ciprofloxacin was performed by using agar dilution method on 5% defibrinated sheep blood Mueller-Hinton agar plates as per CLSI recommendation. *C. jejuni* ATCC® 33560 is used as control for agar dilution testing.

Results
A total of 107 isolates were collected over the period of 2014 to 2016. Out of 107, 14.02% of the isolates were from blood samples while 885.98% were collected from stool. Seventy seven (77%) were *C. jejuni* while the rest were labeled as *Campylobacter spp*. Erythromycin resistance was 14%, ciprofloxacin resistance 93%. Of the 15 erythromycin strains, 11 were *C. jejuni* while 4 were *Campylobacter spp.* All 15 erythromycin resistant isolates were 100% resistance to ciprofloxacin.

Conclusion
There has been significant rise in resistance to first line agents in *Campylobacter* resulting in difficulty in identifying the better treatment option for Campylobacter and demanding the newer treatment options.

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Key words
Campylobacter species, erythromycin, ciprofloxacin resistance

Introduction
Campylobacter is one of the most common cause of gastroenteritis in humans worldwide, especially in developing countries such as Pakistan where it is one of the leading cause of gastroenteritis.[1] Though *Campylobacter* gastroenteritis is self-limiting, antimicrobial therapy may be required in severe cases of enterocolitis, immune-compromised population, pregnant females and extreme of ages.[2] The mortality rate associated with invasive *Campylobacter* is estimated at 24 deaths per 10,000 culture confirmed cases or 200 deaths per year in the United States.[3] Neurological and rheumatological sequelae are also serious post infection complications.[2-4]

Increasing drug resistance is presenting a challenge in the treatment of Campylobacteriosis. Erythromycin, a macrolide, and ciprofloxacin, a fluoroquinolone, are considered drugs of choice for its treatment. However, resistance against these antimicrobials has been observed worldwide.[5,6] A study from Pakistan reported 47.5% quinolone resistance in their isolates.[7] Similar quinolone-resistant pattern is also reported for *Campylobacter* isolates of North America (29%), Africa (41%), Europe (80%), Thailand (80%) and India (86.66%).[6,8,9] Likewise, in a report from Pakistan, 2.9% of *Campylobacter* isolates were resistant to erythromycin.[7] Growing erythromycin resistance is of concern since these strains mostly turn out to be multi-drug resistant.[8]

The aim of this study is to evaluate frequency of erythromycin and ciprofloxacin resistance in *Campylobacter* species.

Material and Methods
A total of 107 isolates were isolated from stool as well as blood samples from Jan 2014 to March 2016 at Pathology and clinical laboratory Laboratory Aga Khan University hospital (AKU). Samples at AKU laboratory are processed according to the American Society of Microbiology (ASM) guidelines. After initial isolation, identification is performed through conventional tests such as gram stain, catalase, oxidase and hippurate hydrolysis. Minimum inhibitory concentration (MIC) is performed by using agar dilution method on 5% defibrinated

sheep blood Mueller-Hinton agar plates as per CLSI recommendations. The MIC breakpoints used for resistance are those recommended by the Clinical and Laboratory Standards Institute (CLSI). Isolates were considered susceptible (S) intermediate (I) and resistance (R) based on these CLSI criteria (Table 1). The antimicrobials tested are erythromycin and ciprofloxacin (Sigma). *C. jejuni* ATCC® 33560 is used as control for agar dilution testing.

Data Analysis

The data was coded and analyzed by using Microsoft® Excel 2010 software. Frequency and percentage of the categorical variables i.e. gender, source of specimen (stool and blood), results of antimicrobial susceptibility (susceptible and resistant) for two agents (erythromycin and ciprofloxacin) were calculated

Results

There were a total of 107 isolates of Campylobacter between Jan 2014 to march 2016 at AKU Laboratory. Out of 107, 14.02% (n=15) of the isolates were from blood, and 85.98% (n=92) were from stool samples. Seventy seven (77%) were *C. jejuni* while the rest were labeled as *Campylobacter spp.* Erythromycin resistance was 14% (15/107), ciprofloxacin resistance 93.4% (100/107). Of the 15 erythromycin strains, 11 were *C. jejuni* while 4 were *Campylobacter spp* as shown in Table 1. All 15 erythromycin resistant isolates were also resistant to ciprofloxacin.

Table 1.

Antimicrobial agents	Break points (µg/ml)			No. % of resistant strains n = 107		Significance of difference in resistance rates
	S	I	R	C. jejuni	C. spp	(*P* value)*
Erythromycin	4	8	16	11	4	0.735
Ciprofloxacin	1	2	4	73	20	0.082

**P* value was calculated using Fisher's exact test.

Discussion

Campylobacteriosis is a food borne, self-limiting disease. Antimicrobials are given in cases of immune-compromised, pregnant females and extreme of ages. Quinolones and macrolides have been considered first line agents but rise in resistance in *Campylobacter* against these antibiotics has been attributed to use of antimicrobials in veterinary medicine. India have reported 86% to 97% of resistance to quinolones while another study from china reported up to 99% resistance in *Campylobacter coli* from animal origin.[9,11,12] In 2010, less than half of the isolates in a study from Pakistan were resistant to quinolones, but in our study almost all were resistant, thus demonstrating a significant rise in resistance within 5 years.[7]

As a result of increasing quinolone resistance in *Campylobacter*, macrolides are now considered the treatment of choice. Our center reported 2.9% erythromycin resistance in 2007, with this study demonstrating a sharp increase. A study from Iran reported 77.7%of erythromycin resistance whereas a report from India showed 22.2% resistance to macrolides in 2013.[13,9] Resistance has been seen to vary with regard to the species of *Campylobacter*.[8] But In our study, as well as a 2013 study from India, there were similar rates of resistance in *C. Jejuni* and other species.[9]

Previously, some studies have suggested that macrolide resistant *Campylobacter* strains are uniformly multidrug resistant (resistance to 3 or more drugs classes) and treatment is challenging.[8] In our study only quinolone was tested and all our erythromycin resistant isolates were resistant to ciprofloxacin also. This raises concerns regarding treatment of these multidrug resistant *Campylobacter*. Effective results have been reported with carbapenems with excellent in vitro susceptibilities.[8] However, further studies are needed regarding in vitro susceptibilities of erythromycin resistant *Campylobacter* to other antimicrobial agents.

In conclusion, there has been a rising trend in resistance of *Campylobacter* to erythromycin and ciprofloxacin. Further studies are required to evaluate new treatment options.

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