

Antibiotic susceptibility pattern of strains of *Campylobacter coli* isolated in Osogbo, Nigeria

*OC Adekunle¹, AO Coker², DO Kolawole³

¹Department of Medical Microbiology & Parasitology, Ladoko Akintola University of Technology, Osogbo, Nigeria.

²Department of Medical Microbiology & Parasitology, University of Lagos, Lagos, Nigeria.

³Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

*Corresponding Author: toyintoro@yahoo.com
P.O. Box 14066, U.I. Ibadan, Oyo State, Nigeria.

Abstract

Gastroenteritis due to *Campylobacter* species has just been established in Osogbo. There is a need to establish the antibiotic sensitivity pattern of isolates of *Campylobacter*. The study was done to determine the antibiotic susceptibility patterns and *Campylobacter coli* isolate obtained from stool specimens from children with diarrhea in Osogbo. The strains of *Campylobacter coli* were isolated from 602 children between 0 and 36 months of age. The antimicrobial susceptibility test was carried out. All isolates tested were sensitive to erythromycin, gentamycin, tetracycline, ciprofloxacin whereas none was sensitive to cotrimoxazole.

Keywords: *Campylobacter*; Gastroenteritis; Antibiotics; Antimicrobial; Susceptibility.

Introduction

Campylobacter is the most frequently reported cause of acute inflammatory gastroenteritis in developed countries and major cause of intestinal disease in children less than 2 years of age in developing countries (Alfred et al., 2006). A collective name for infections diseases caused by members of these bacteria is called campylobacteriosis (Coker et al., 2002). Campylobacteriosis is a self-limited disease, and antimicrobial therapy is not generally indicated in most cases. However, treatment can decrease the duration and the severity of illness if it is initiated early in the course of infection.

Antibiotics have a role in reducing the symptoms, shortening the span of illness and controlling the transmission in the community (Oberhelman and Taylor, 2000). Erythromycin, tetracycline and quinolones have all been recommended in different clinical settings to treat campylobacter gastroenteritis (Vanhoof et al., 1978). Fluoroquinolones also shorten the diarrhea and severity of symptoms caused by *C. jejuni* (Goodman et al., 2000). Since the late 1980's resistance in campylobacter isolates to fluoroquinolones has been increasing especially in Europe (Smith et al., 1999). A relationship has been found between the use of fluoroquinolones in animals and increase in fluoroquinolones resistant campylobacter infection in humans (Odugbemi et al., 1977). The antibiotic susceptibility patterns of campylobacters seem to vary widely from country to country and from place to place (Coker and Adefeso, 1995). Gastroenteritis due to *Campylobacter* species has just been established in Osogbo (unpublished). This paper reports the results of studies on the antibiotic susceptibility

patterns and beta-lactamase production of *Campylobacter coli* isolated from stool samples of children in Osogbo.

Materials and Methods

Subjects were patients between age 0 and 36 months who presented with diarrhea at pediatric unit of General Hospital Asubiaro, Lautech Teaching Hospital and Jaleyemi Hospital, all in Osogbo. A total of 602 patients were diagnosed with diarrhea. *Campylobacter jejuni* NCTC11168 (Natural Collection for Types and Culture) were used as control in this study. Three isolates of *Campylobacter coli* were obtained and were tested for their susceptibility to antimicrobial agents. All the strains were isolated using Butzler-type medium and biotyped by the Lior typing scheme.

Beta-lactamase production

It is generally known that organisms that produce beta-lactamase are resistant to certain antibiotics. This test was carried out to detect any beta-lactamase production which may confer ability to resist antibiotics on these isolates. Beta-lactamase production by *Campylobacter* isolates was detected by starch paper technique (Coker and Adefeso, 1995). Strips of starch paper were soaked for 10 minutes in a solution containing 10⁵mg/ml benzyl penicillin and then spread smoothly in a petri dish.

Colonies of *Campylobacter* were transferred to the surface of test paper and incubated at 37°C for 30 minutes. The paper was later flooded with iodine solution. Discoloration of the blue-black colony surrounding the organisms with the widening

of the white-halo was interpreted as positive for beta-lactamase production.

Antimicrobial susceptibility testing

This was done according to method described by Bauer et al., 1966. The Kirby Bauer disc diffusion method for in-vitro susceptibility testing was employed in this study. Five colonies of each strain of *Campylobacter coli* were suspended in a sterile bijoux bottle containing 5mls of Mueller – Hinton and incubated overnight at 37°C. The overnight both cultures were diluted to 10⁷ colony forming units per ml. Sterile cotton wool swabs were inserted into the standardized inoculums, drained of and then used to inoculate well dried – Mueller – Hinton agar plates. The following antibiotic discs (Ampicillin 25kg, Streptomycin 25kg, Erythromycin 0kg, Contrinoxazole 25kg, Nitrofurantiorin 200kg, Ciprofloxacin 25kg, Tetracycline 25kg, Nalidix acid 30kg, Gentamycin 10kg, Ceftriazone 30kg, were placed on to the inoculated agar plates. All plates were incubated in candle extinction jar at 37°C for 48 hours.

The diameters of zones of inhibition were measured to the nearest millimeter. The zones of inhibition of the test strains when comparable with the zones of inhibition of control organism were interpreted as sensitive, while those showing no zones of inhibition or narrow zones of inhibition than those of

sensitive control organisms were interpreted as resistant.

Statistical analysis

To determine the significance of differences in susceptibility, the SPSS software (version 10.1) was used. The differences were considered significant when the p-value was less than 0.05. 100% (3/3) of the strains were resistant to contrimaxazole; 66% (2/3) were resistant to ampicillin and nalidixic acid; 33% (1/3) were resistant to streptomycin and ceftriazone. The susceptibility of the strains to ciprofloxacin, erythromycin, tetracycline, gentamycin and nitrofurantoin was 100% (3/3).

Results

The susceptibility pattern of *Campylobacter coli* is shown in Table 1. Three isolates of *Campylobacter coli* were sensitive to ciprofloxacin, Erythromycin, Tetracycline, Gentamycin, and Nitrofurantion and none was sensitive to Contrimoxazole (Table 1). The minimum inhibitory concentrations of the different antibiotics used are shown in table 2. Table 3 showed the antibiotic susceptibility of the control strain i.e. NCTC11168 used in this study. The control was susceptible to all the antibiotics used except the contrimoxazole. Assessment of possible production of beta-lactamase showed that none of the isolates produced beta-lactamase (Table 4).

Table 1: Antibiotic susceptibility of *Campylobacter coli* isolated in Osogbo, Nigeria.

<u>Antibiotics</u>	<u>Number of sensitive strains</u>	<u>Number of resistant strains</u>
Ampicillin (25µg)	1	2
Streptomycin (10µg)	2	1
Ciprofloxacin (5µg)	3	0
Nalidixic acid (30µg)	1	2
Erythromycin (10µg)	3	0
Tetracycline (10µg)	3	0
Gentamycin	3	0
Contrimoxazole (25µg)	0	3
Nitrofurantion (200µg)	3	0
Ceftriazone (30µg)	2	1

Table 2: Minimum Inhibitory Concentration (MIC) values observed.

<u>Antibiotics</u>	<u>MIC</u>
Ampicillin	0.2µg/ml
Streptomycin	4µg/ml
Ciprofloxacin	0.025µg/ml
Nalidixic acid	4µg/ml
Erythromycin	2µg/ml
Tetracycline	0.3µg/ml
Gentamycin	0.25µg/ml
Nitrofurantion	5µg/ml
Ceftriazone	6µg/ml

Control
Campylobacter jejuni NCTC11168 (i.e. National Collection for Types and Control 11168) was used

as the control. The control displayed the same susceptibility and resistance as the ones we isolated. This is shown in table 3.

Table 3: Antibiotic susceptibility of the control (NCTC11168) used in this study.

Antibiotics	Number of sensitive strains	Number of resistant strains
Ampicillin (25µg)	+	-
Streptomycin (10µg)	+	-
Ciprofloxacin (5µg)	+	-
Nalidixic acid (30µg)	+	-
Erythromycin (10µg)	+	-
Tetracycline (10µg)	+	-
Gentamycin	+	-
Contrimoxazole (25µg)	-	+
Nitrofurantion (200µg)	+	-
Ceftriaxole (30µg)	+	-

Result of beta-lactamase production test
 None of the strains produced beta-lactamase.

Discussion

Antimicrobial treatment can shorten the duration and severity of illness if initiated early in the course of infection; however, antibiotic susceptibility studies are very necessary because variation occurs from country to country and from place to place. This therefore necessitated this present study. The result of this study showed that the *Campylobacter coli* isolated were highly susceptible to most commonly available antibiotics except contrimoxazole. Erythromycin and ciprofloxacin which are drugs of choice for campylobacter infections are still very useful in this environment (Coker and Adefeso, 1995). Studies in Europe have shown *C. jejuni* to be sensitive in-vitro to antibiotics such as tetracycline, erythromycin, gentamycin, chloramphenicol and cloxacillin while many strains are resistant to ampicillin, penicillin and metronidazole (Jawetz, 2002). A study conducted in Lagos showed that most species are sensitive to gentamycin, nalidixic acid and tetracycline and they are found to be resistant to erythromycin and ampicillin (Coker et al., 1988).

The rate of resistance to these drugs in developing countries is higher compared to that of developed countries. This is due to the use of these drugs for infections other than gastroenteritis and self medication which are often the causes of resistance in developing countries. In developed countries, the use of these antibiotics in animal feeds and also traveling to developing countries are causes of resistance to antibiotics treatment. The increase in erythromycin resistance in developed countries is often low and stable at

approximately 1% to 2%; this is not true for some areas in developing countries like Lagos, Nigeria. In 1984, 82% of campylobacter strains from Lagos, Nigeria were sensitive to erythromycin and 10 years later i.e. 1994, 20.8% were sensitive (Coker et al., 1988). Furthermore, resistance to macrolide and azithromycin by campylobacter isolates was found to be between 7% and 15% in 1994 and 1995 in Thailand (Hoge et al., 1998). *C. jejuni* is generally susceptible to erythromycin with resistance rates of less than 5% (Reina et al., 1983; Sjogren et al., 1987). Rates of erythromycin resistance in *C. coli* vary considerably, sometimes up to 80% of strains showing resistance in some studies (Huang et al., 1992; Lachance et al., 1993). All these reports necessitated studies on antibiotics susceptibility from time to time and from place to place as this will help in management of these diseases. For instance, Hoge et al. (1998) reported an increase in ciprofloxacin resistance among campylobacter species from zero in 1991 to 84% in 1995 in Thailand.

Resistance to fluoroquinolone and macrolide had been reported and the number of macrolide and fluoroquinolone-resistant isolates from humans is influenced by several factors. These factors include veterinary use of macrolides (approved for use as antimicrobial growth promoters or as therapeutic drugs) and fluoroquinolones (only approved as therapeutic drugs) at a given location (Aarestrup et al., 1997); association with recent or current antimicrobial treatment of patients; origin of isolates (whether from blood or sterile sites, children or adults, inpatients or

outpatients); travel and sampling strategy, susceptibility testing procedure (Engberg, 1999). Furthermore, Ellis-Pegler et al. (1995) reported that fluoroquinolone resistance developed in 18% to 28% of patients in their prospective trial. Development of resistance has been reported within 24 hours of treatment, however, prolonged therapy e.g. in immunosuppressed patients is also a risk factor (Tee and Mijch, 1998).

In this study, none of the campylobacter isolates produced beta-lactamase which may confer ability to be resistant to antibiotics on them. It has been reported that *C. jejuni* and *C. coli* may also produce beta-lactamase that appears to be active against amoxicillin, ampicillin and ticarcillin. This enzyme has been reported to be inhibited by clavulanic acid but not by sulbactam or tazobactam (Lachance et al., 1991). The resistance to cotrimoxazole is 100% in this study. This is in agreement with the findings of Dupont et al. (1987) who reported the inactivity of cotrimoxazole against campylobacter species. Coker et al. (1988) also reported that none of the isolates of campylobacter tested in Lagos was sensitive to cotrimoxazole. However, following the results of this study, it can be suggested that in Osogbo, Nigeria, erythromycin, tetracycline, gentamycin and ciprofloxacin can be used to treat gastroenteritis caused by campylobacter species.

References

Aarestrup FM, Nielson EM, Madsen M, Engberg J, 1997. Antimicrobial susceptibility patterns of thermophilic campylobacter species from humans, pigs, cattle, and broilers in Denmark. *Antimicrobial Agents and Chemotherapy*, 41: 2244-2250. <http://aac.asm.org/cgi/content/abstract/41/10/2244>

Coker AO, Adefeso AO, 1995. The changing pattern of *Campylobacter jejuni/coli* in Lagos, Nigeria after 10 years. *East African Medical Journal*, 71: 437-440.

Coker AO, Olukoya DK, Odegbemi T, 1988. Antibiotics susceptibility patterns and beta-lactamase production of strains of campylobacter species isolated in Nigeria. *Proceedings of the 4th International Workshop on Campylobacter Infections*, Goteborg, held in Sweden. Edited by Kaijser B and Failisen E. Published by Gothenburg Kungaly, Sweden 1988, pp 153-154.

Dupont HI, Ericsson CD, Robison A, Johnson PC, 1987. Current problems in antimicrobial therapy for bacterial enteric infection. *American Journal of Medicine*, 82: 324 – 328.

Ellis-Pegler RB, Hyman LK, Ingram RJ, McCarthy MA, 1995. Placebo controlled evaluation of lomefloxacin in the treatment of bacterial diarrhea in the community. *Journal of Antimicrobial Chemotherapy*, 36: 259-263.

Engberg J, Harestrup FM, Taylor DE, Gerner-Smidt P, Nachmakin I, 2007. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli* resistance and trends isolated in humans. *Emerging Infectious Diseases*, 7: 24-34. <http://www.cdc.gov/eid/content/13/7/pdfs/vol13no07.pdf>

Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P, 1998. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. *Clinical Infectious Diseases*, 26: 341-345.

Huang MB, Baker CN, Banerjee S, Tenover FC, 1992. Accuracy of the E-test for determining antimicrobial susceptibilities of staphylococci, enterococci, *Campylobacter jejuni*, and gram-negative bacteria resistant to antimicrobial agents. *Journal of Clinical Microbiology*, 30: 3243-3248. <http://jcm.asm.org/cgi/content/abstract/30/12/3243>

Lachance N, Gaudreau C, Lamothe F, Lariviera LA, 1991. Role of the beta-lactamase of *Campylobacter jejuni* in resistance to beta-lactamase agents. *Antimicrobial Agents and Chemotherapy*, 35: 813-818. <http://aac.asm.org/cgi/content/abstract/35/5/813>

Lachance N, Gaudreau C, Lamothe F, Turgelon F, 1993. Susceptibilities of beta-lactamase positive and negative strains of *Campylobacter coli* to B-lactam agents. *Antimicrobial Agents and Chemotherapy*, 37: 1174-1176. <http://aac.asm.org/cgi/reprint/37/5/1174>

Reina J, Borrell N, Serra A, 1995. Emergence of resistance to erythromycin and fluoroquinolone in thermotolerant campylobacter strains isolated from faeces between 1987 and 1991. *European Journal of Clinical Microbiology and Infectious Diseases*, 11: 1163-1166.

Sjogren E, Kaijser B, Werner M, 1992. Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* isolated in Sweden: a 10-year follow-up report. *Antimicrobial Agents and Chemotherapy*, 36: 2847-2849. <http://aac.asm.org/cgi/content/abstract/36/12/2847>

Tee N, Mijch A, 1998. *Campylobacter jejuni* bacteremia in human immunodeficiency virus (HIV)-infected and non-HIV-infected patients: comparison of clinical features and review. *Clinical Infectious Diseases*, 26: 91-96. <http://www.journals.uchicago.edu/doi/pdf/10.1086/517748>