Campylobacter Antimicrobial Drug Resistance among Humans in Ilorin, Nigeria.

S. O. Samuel[1], A. O. Aboderin[2], T. A. T. Salami[3], E. A. Omonigbehin[4] and A. O. Coker[5]

SUMMARY

Background: Though *Campylobacter* enteritis is a self-limiting disease, antimicrobial agents are recommended for extraintestinal infections and for treating immunocompromised persons. Erythromycin and ciprofloxacin are drugs of choice. The rate of resistance to these drugs is increasing in both developed and developing countries thus compromising their use in therapy. Continued surveillance of resistance pattern is necessary to guide rational use of antimicrobial agents in therapy when such are indicated.

Objective: This study aims at determining the resistance of *Campylobacter jejuni/coli* to common antimicrobial agents.

Methods: *Campylobacter jejuni/coli* recovered from cases of childhood diarrhoea in Ilorin, Nigeria were studied. All the organisms were isolated by standard techniques using the Butzler-type medium and they were preserved in 15% glycerol cryopreserve medium. The in-vitro antibiotic susceptibility testing for all organisms was performed by employing the Kirby-Bauer disc diffusion method. Production of beta-lactamase by the isolates was determined by the starch paper technique.

Results: High level resistance to cotrimoxazole, ceftriaxone, and ampicillin were 96%, 84% and 68% respectively. On the other hand none of the isolates had any evidence of resistance to erythromycin and ciprofloxacin. Only one of the 25 isolates tested produced beta-lactamase.

Conclusion: *Campylobacter jejuni/coli* are susceptible to firstline dugs, erythromycin and ciprofloxacin. However high level resistance to other agents portends a possibility of transfer of acquired resistance and the need for continuous surveillance of resistance pattern.

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From: [1] Department of Medical Microbiology and Parasitology, College of Medicine, Ambrose Alli University, Ekpoma, Nigeria. [2] Department of Medical Microbiology and Parasitology, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. [3]Department of Medicine, College of Medicine, Ambrose Alli University, Ekpoma, Nigeria. [4] National Institute of Medical Research Yaba, Lagos, Nigeria.[5] Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria.

Correspondence: **S. O. Samuel,** Department of Medical Microbiology and Parasitology, College of Medicine,Ambrose Alli University, Ekpoma, Nigeria.E-mail: olowosamuel2003@yahoo.com

INTRODUCTION

Campylobacter jejuni and related organisms are important human pathogens causing acute human enterocolitis[1]. Despite their wide occurrence, *Campylobacter* species were not recognized as a cause of diarrhoea in humans until 1957[2] and their impact in terms of sheer numbers of human infections emerged only in the past 25 years[3]. *Campylobacter* is now the most commonly reported bacterial cause of gastrointestinal infection in the United States, England and Wales, and Australia[4]. Reports from Africa have also shown that these organisms are highly prevalent enteric pathogens especially in children[5]. Species are microaerophilic gram-negative bacilli. The organisms can colonize the intestinal tract of many animals including poultry, cattle and swine without causing illness, and is transmitted via contaminated food and water[6]. Ingestion of *C. jejuni* can cause diarrhoea associated with abdominal pain and vomiting. The disease is self-limiting, lasting 3-5 days, but in some cases it can persist for 2 weeks or longer, whereas bacteraemia has occasionally been reported. Post-infection complications associated with a preceding *C. jejuni* infection are rare, and consist mainly of neurological immunopathologcal disorders, such as Gullain-Barre syndrome[7].

Most *Campylobacter* enteric infections being self-limiting do not require antimicrobial drug treatment. However antimicrobial drug therapy is justified for severe and long-lasting infections or in immunocompromised patients[8]. When indicated, a macrolide such as erythromycin is the antimicrobial of choice. Ampicillin, co-amoxiclav and fluoroquinolones are recommended alternative therapies[9]. Resistance of *Campylobacter* to antimicrobial agents has increased substantially during the past 2 decades and has become a matter of concern in severe human infections[8].

Recently we gave the first known report of *Campylobacter jejuni/coli* from Ilorin, Nigeria [10]. In the present article we describe the antimicrobial resistance pattern as well as beta-lactamase production in the local strains.

MATERIALS AND METHODS

Twenty five isolates of *Campylobacter jejuni/coli* from children aged 0-36 months, with diarrhoea were tested for their susceptibility to antimicrobial agents. All organisms were isolated using Butzler-type medium [11]. They were identified as *Campylobacter jejuni/coli* by being spiral, gram-negative, motile, oxidase positive, catalase positive, growing at 37^{0}C and 42^{0}C but not at 25^{0}C. The isolates were stored in a 15% glycerol

cryopreserve medium containing ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP) (Oxoid Ltd. London, U.K) and kept frozen until the susceptibility testing was done.

Antimicrobial Susceptibility Testing

The in-vitro antibiotic susceptibility testing for all the 25 isolates was performed by employing the Kirby-Bauer disc diffusion method[12]. Five colonies of each strain of *Campylobacter* isolate were suspended in a sterile bijou bottle containing 5mls of Mueller-Hinton broth (Oxoid Ltd. London, U.K) and incubated overnight at 37^{O}C. The overnight broth cultures were diluted to 10^{6} colony forming units per ml. Sterile cotton wool swabs were inserted into the standardized inoculum, drained off and then used to inoculate well dried Mueller-Hinton agar plate. The following antibiotic discs: ampicillin 25µg, ciprofloxacin 25µg, gentamicin 10µg, erythromycin 10µg, nalidixic acid 30µg, nitrofurantoin 200µg, ceftriaxone 30µg, colistin 25µg, streptomycin 25µg and tetracycline 25µg, were placed on to the inoculated agar plates. All plates were incubated in candle extinction jars at 42^{0}C for 48hours. The diameters of zones of inhibition were measured to the nearest millimeter using a ruler. The zones of inhibition of the test strains when compared with the zones of inhibition of control organisms were interpreted as sensitive while those showing no zones of inhibition or narrower zones of inhibition than those of sensitive control organisms were interpreted as resistant. The control organism was *Campylobacter jejuni* 11168.

Detection of Beta-lactamase

Testing for beta-lactamase production by the isolates was performed by the starch paper technique[13]. Strips of starch paper were soaked for 10 minutes in a solution of Benzyl penicillin containing 10^{5}mg/ml and then spread smoothly in a Petri-dish. With a fine bacteriological loop (2mm diameter) colonies of bacteria collected from the surface of the culture plate were transferred to the surface of the paper and spread over area of 2-3mm. The inocula were placed at least 1.5cm apart. Plates were incubated at 37^{0} C for 30minutes after which the papers were flooded with iodine solution (Gram's iodine diluted 1 in 2). The iodine solution turned the paper uniformly black within 30seconds. Beta-lactamase producing strains were detected by the decoloration of the blue black colour, surrounding the organisms with the widening of the white halo in the cause of the ensuring five minutes, while the surface of the inocula remained whitish. Penicillinase– negative strains of *C. jejuni* did not produce any decoloration of the surrounding area. *Staphylococcus aureus* ATCC 29523 was used as a positive control.

RESULTS

The susceptibility pattern of *Campylobacter jejuni/coli* is shown in Table 1. There was high level resistance to cotrimoxazole, ceftriaxone, and ampicillin being 96%, 84% and 68% respectively. None of the isolates had any evidence of acquired or natural resistance to erythromycin and ciprofloxacin. The other antibiotics tested were intermediate in resistance rates between the two extremes of resistance. The enzyme beta-lactamase was produced by only one of all the 25 isolates.

Table 2 shows Resistance antibiogram of *Campylobacter jejuni/coli*. All the 25 (100%) isolates were found to be resistant to one or more drugs (Table 2). A total of eleven distinct antibiograms were encountered and the patterns varied from resistance to a single antimicrobial agent to that of seven. Most frequently encountered antibiograms were cotimoxazole-ampicillin-ceftriaxone and cotrimoxazole-ceftriaxone. Multiple resistance (resistance to at least two antibiotics) was the rule for all isolates except for just one that was resistant to cotrimoxazole only.

Table1: Rates of Susceptibility of 25 *Campylobacter jejuni/coli* to selected antibiotics

Antibiotic	Resistant strains Number (%)
Cotrimoxazole (25µg)	24 (96)
Ceftriaxone (30µg)	21 (84)
Ampicillin (25µg)	17 (68)
Nalidixic acid (30µg)	6 (24)
Colistin (25µg)	4 (16)
Streptomycin (25µg)	3 (12)
Tetracycline (25µg)	3 (12)
Nitrofurantoin (200µg)	2 (8)
Gentamicin (10µg)	1 (4)
Erythromycin (10µg)	0 (0)

Table 2 – Resistance antibiogram of *Campylobacter jejuni/coli*

Resistance pattern resistant	Resistance antibiogram	No of strains (%)
i	COT	1(4)
ii	COT, CEF,	8(32)
iii	COT, AMP, CEF	9(36)
iv	COT, AMP, CEF, NIT	4(16)
v	AMP, NAL, CEF, TET GEN	1(4)
vi	COT, AMP, NAL, COL, STR, TET	1 (4)
vii	COT, AMP, NAL, NIT, CEF, COL, STR	1 (4)

COT = Cotrimoxazole, AMP= Ampicillin, CEF = Ceftriaxone, NIT = Nitrofurantoin, NAL = Nalidixic acid, TET = Tetracycline,COL = Colistin, GEN= Gentamicin, STR = Streptomycin.

DISCUSSION

The susceptibility pattern of *Campylobacter jejuni/coli* studied showed a high level of resistance to cotrimoxazole (96%) and ampicillin (68%). These are first line drugs used in the empiric treatment of diarrhoeal and other childhood illnesses. High level resistance to co-trimoxazole by *Campylobacter jejuni/coli* and other enteric pathogens has been reported elsewhere[14,15]. There is no doubt that there is a widespread use and misuse of these agents within the communities and hospital settings. There is an obvious need to reconsider the continued use of these agents for empiric treatment of enteric infections.

All the 25 isolates studied were susceptible to erythromycin and ciprofloxacin. These drugs are the first- and second-choice antimicrobial agents for the treatment of *C. jejuni* enterocolitis[16]. These findings are interesting in the context of widespread and increasing resistance of *Campylobacter jejuni/coli* to macrolides and fluoroquinolones in different geographic

regions or countries[8]. This is especially relevant in countries where fluoroquinolones are used for prophylactic treatment or growth promoting in poultry. There is a need for continued surveillance of resistance patterns in the local isolates as there are now large-scale poultry farms in Nigeria. The high rate of resistance to ceftriaxone (84%) is disturbing because microorganisms have potential for transfer of resistance across species or genus borders. Transfer of ceftriaxone resistance to other enteric gram-negative bacilli portends grave consequences in the management of gram-negative sepsis.

Twenty-four (96%) of *Campylobacter jejuni/coli* in our study are multiresistant. So far, the multiresistance of *C. jejuni* has not led to severe consequences, since Campylobacter infections are often self-limiting and require no antimicrobial treatment. However, therapy is needed in severe cases of enteritis, in invasive infections and in patients who are immunocompromised. Efforts should therefore be made to preserve the current active and useful drugs for the treatment of *C. jejuni* infections and containment of multidrug resistance in enteric bacterial infections.

One (4%) of the isolates produced beta-lactamase. This is similar to reports on beta-lactamase production from strains isolated from Ile-Ife, south-west, Nigeria[14] where none of the strains demonstrated beta-lactamase activity. However reports from Lagos, also south-west, Nigeria showed evidence of beta-lactamase activity ranging from 6.4% (17) to 12.5%[18]. The high level resistance to ampicillin in our study and the relative absence of beta-lactamase activity suggests that the mechanism of resistance may not be due to drug inactivation by hydrolysis of the beta-lactam ring and that there might be no benefit in the use of potentiated beta-lactam drugs such as amoxicillin-clavulanic acid in the treatment of resistant strains.

The antimicrobial treatment for C*ampylobacter* and other enteric bacterial infections is often initiated before the results of antimicrobial susceptibility testing are available if at all (as many laboratories do not routinely isolate the organisms); therefore, a general knowledge of the expected susceptibility is a prerequisite to starting treatment with an appropriate antimicrobial agent. Erythromycin, ciprofloxacin and gentamicin demonstrated effectiveness in the treatment of infections due to *Campylobacter jejuni/coli* and should be considered in the treatment of *Campylobacter* enteritis in this geographic region. Continued surveillance is needed to monitor changes in the resistance trends and the efficacy of current drugs.

REFERENCES

1. Skirrow M. *Campylobacter* enteritis; a new disease. *Br Med J.* 1977; **2**: 9 – 11.
2. King E. O. Human infection with *Vibrio fetus* and a closely related *Vibrio*. *J Infect Dis* 1957; **101**: 119 – 128.
3. Allos B. M. *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin Infect Dis* 2001; **32**: 1201 – 1206.
4. Altekruse S., Stern N., Fields P. *Campylobacter jejuni* – an emerging foodborne pathogen. *Emerg Infect Dis*. 1999; **5**: 28 – 35.
5. Coker A. O., Isokpehi R. D., Thomas B. N., Amisu K. O., Obi C. L. Human campylobacteriosis in developing countries. *Emerg Infect Dis*. 2002; **8(3):** 237 – 243.
6. Centres for Disease Control. *Campylobacter* isolates in the United States, 1982–1986. *MMWR* 1988; **SS-2**, 1.
7. Speed B., Kaldor J., Cavanogh P. Gullain-Barre syndrome associated with *Campylobacter jejuni* enteritis. *J Infect* 1984; **8**: 85 – 86.
8. Gallay A., Prouzet-Mauleon V., Kempf I., Lehours P., Labadi L., Camou C. *et al*. *Campylobacter* Antimicrobial Drug Resistance among Humans, Broiler Chickens, and Pigs, France. *Emerg Infect Dis* 2007; **13(2):** 259 – 266.
9. Nachamkin I., Blaser M. J. *Campylobacter* 2nd ed. Washington: ASM Press 2000.
10. Samuel S. O., Aboderin A. O., Akanbi II A. A., Adegboro B., Smith S. I. and Coker A. O. *Campylobacter* enteritis in Ilorin, Nigeria. *E Afri Med J* 2006; **83(7):** 63 – 69.
11. Coker A. O., Dosumu-Ogunbi O. Isolation of *Campylobacter jejuni* in Lagos, Nigeria. A "new" bacterial agent of diarrhea *E. Afri. Med. J*. 1984; **61**: 52 – 55.
12. Bauer A.W., Kirby W.M.M., Sherris J.C., Turk M. Susceptibility testing by standardised single disc method. *Amer. J. Clin. Path.* 1966; **45:** 493 – 496.
13. Odugbemi T. O., Hafiz S., McEntegart M. G. Penicillinase-producing *Neisseria gonorrhoeae*: detection by starch paper technique. *Br Med J.* 1977; **2(6085):** 500.
14. Aboderin A. O., Oyelese A. O., Coker A. O. Antibiotic susceptibility pattern and beta-lactamase production of strains of *Campylobacter* species isolated in Ile-Ife, Nigeria. *Nigerian Journal of Health and Biomedical Sciences.* 2004; **3(2):** 65 – 68.
15. Dupont H. I., Ericsson C. D., Robinson A., Johnson P. C. Current problems in antimicrobial therapy for bacterial enteric infection. *Am J Med.* 1987; **82(4A):** 324 – 328.
16. Engberg J., Aarestrup F. M., Taylor D. E., Gerner-Smidt P., Nachamkin I. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. *Emerg. Infect. Dis.* 2001; **7:** 24 – 34.
17. Coker A. O., Olukoya D. K., Odugbemi T. Antibiotic susceptibility patterns and beta – lactamase production of strains of campylobacter species isolated in Nigeria. Proceeding of the 4th international workshop on *Campylobacter* infections. Goteborg, Sweden. Edited by B, KaijserB. And FalsenE, Published by Gotherma, Kungal Y, Sweden, 1988. pp.153 – 154.
18. Smith S. I., Sansa I. I., Coker A. O. Antibiotic susceptibility pattern and beta–lactamase production of animal and human isolates of *Campylobacter* in Lagos, Nigeria. *Z Naturforsch.* 1999; **54**: 583 – 586.