

Antibiotic Susceptibility Patterns and Beta–Lactamase Production of Animal and Human Isolates of *Campylobacter* in Lagos, Nigeria

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Forty-three stool samples and rectal swabs were collected from diarrhoeic and 20 non-diarrhoeic patients under 5 years of age at various general hospitals in Lagos. A total of 110 faecal samples from animals (101 from chickens and 9 from pigs) were obtained from Mitchel farm, Agege and Oshodi – Isolo Local Government farms at Ejigbo. *Campylobacter* species were isolated from 6 (13.9%) of 43 children with gastroenteritis and none from 20 asymptomatic subjects. Forty-nine (48.5%) isolates from the hundred and one faecal specimen were isolated from chicken, while 3 (33.33%) out of 9 were from pigs. *Campylobacter jejuni* was the most prevalent accounting for 79% of the total isolates and *C. coli* accounted for 25%. All the human strains were gentamicin sensitive, while streptomycin and cloxacillin were resistant. Erythromycin had a high activity (83.3%) on human strains and only 59.6% activity on animal strains. About ten percent (9.6%) of the strains produced beta-lactamase.

Introduction

Campylobacter species has been recognized as an animal pathogen since 1913 by McFadyean and Stockman, and the organism has been involved in infection, abortion, enteritis in cattle, sheep, swine, birds and other domestic animals (Adetosoye and Adeniran, 1987; Adegbola *et al.* 1991). In some cases, they live as commensals in these animals (Firehammer, 1965). The organism has also been recognized in human infection and since 1972 when the first isolates were made from human faeces by Dekeyser *et al.* (1972). The organism has frequently been isolated from cases of gastroenteritis in humans especially in Nigeria (Low *et al.*, 1981; Olusanya *et al.*, 1983; Coker and Dosunmu – Ogunbi, 1984, 1985). Antibiotic susceptibility patterns and beta-lactamase production of campylobacters vary from one country to another. This paper reports the results on the antibiotic susceptibility and beta-lactamase production of human and animal isolates of *Campylobacter* in Lagos.

Materials and Methods

Sample collection

Stool samples and rectal swabs were collected from diarrhoeal and non-diarrhoeal patients at Isolo General Hospital, Oshodi-Isolo local Government, Health Centre Oshodi and Olikoye Ransome Kuti Children Emergency Centre at the Lagos University Teaching Hospital (LUTH), Idi-Araba. Faecal samples from animals were obtained from Mitchel Farms, Agege and Oshodi-Isolo local Government farms at Ejigbo.

Isolation

Stool samples were suspended in Mueller-Hinton broth and rectal swabs were inoculated onto Butzler-type medium (Coker and Dosunmu-Ogunbi, 1984). Plates were incubated in candle extinction jar to provide an atmosphere of reduced oxygen at 42°C for 72 h. Suspected colonies were subcultured on Butzler-type medium and blood agar and incubated at 42 °C in candle jar and also

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in ordinary air. Colonies were confirmed as *Campylobacter* on the basis of colonial morphology (typically grey, flat, glossy and effuse with a tendency to spread along the direction of tracts of the inoculating wire), inability to grow in air, growth at 42 °C, positive oxidase and catalase reactions and Gram negative reaction according to Cowan (1993). Briefly, for the catalase test, a small amount of grown material on a glass rod was immersed in 3% hydrogen peroxide and visualized for bubbles as a positive result for catalase production.

Oxidase test was carried out by soaking a strip of filter paper in freshly prepared 1% solution of tetramethyl-*p*-phenylenediamine dihydrochloride. This is at once used by rubbing a speck of culture on it with a platinum loop. A positive reaction is indicated by an intense deep purple hue, appearing within 5 – 10 s, a delayed positive reaction by colouration in 10 – 60 s, and a negative reaction by absence of colouration or colouration later than 60 s.

Antibiotic susceptibility testing

The disc diffusion method of Bauer *et al.* (1966) was employed in this study. The following antibiotic discs were used, ampicillin (µg), chloramphenicol (10 µg), cloxacillin (5 µg), erythromycin (10 µg), gentamicin (10 µg), streptomycin (25 µg), tetracycline (50 µg) and penicillin (2 i. u.). The control organism used was *Campylobacter jejuni* NCTC 11168.

Biotyping of isolates

The method of Lior (1984) was employed. This involved an extended biotyping scheme using hippurate hydrolysis, rapid hydrogen sulphide production and DNase activity. This separates *C. jejuni* into four biotypes and *C. coli* into two biotypes.

Hippurate hydrolysis

100 µl of 10% sodium hippurate was distributed evenly in each well (1 well/culture). A lid was placed on the tray and incubated at 37 °C for 4h. After 4 h, one drop of 1:1 mixture of acetone and butanol, in 0.2% ninhydrin was added to each well and incubated for ten minutes and read. A positive

control strain of *C. jejuni* 11168 and a negative control of *C. coli* NCTC 11366 was included. A positive test gave deep purple colour, indicating the presence of glycine which resulted from hydrolysis of hippurate, while a negative result was colourless. *Campylobacter jejuni* are generally hippurate positive, while *C. coli* are hippurate negative.

Rapid hydrogen sulphide production

This required a semi-solid medium that was freshly prepared every 2 weeks. The basal component of the medium (Albimi brucella broth, prepared by adding sodium citrate in 0.1 g/100 ml of broth to BBL brucella broth). Growth from a 24 to 48 h culture was rolled onto bead, gently suspended in the upper third of tube of semi-solid medium and incubated for 2 h in a 37 °C water bath. A positive test showed black discolouration around bacterial pellet, while a negative reaction was colourless.

DNase testing

The DNA and agar were dissolved by boiling. On cooling, O-toluidine blue (final concentration 0.0075 g w/v) was added and the media (15 ml) distributed in petridishes. A large loopful of growth from a 24 h culture plate was spread over a 3 mm diameter on DNA agar. Incubation was aerobically at 37 °C for 24 h. A zone of clearing around the inoculum indicates a positive result. This test detects preformed enzyme, no growth occurs on the nutrient deficient medium.

Detection of beta-lactamase

The starch paper method of Odugbemi *et al.* (1977) was employed. Briefly, strips of starch paper (Basildon Bond Paper) 7 cm by 4 cm were soaked for 10 minutes in the solution of benzylpenicillin containing 100 mg/ml and then spread smoothly in a petri-dish. Each strip of filter paper was used to test the strains including the standard *Staphylococcus aureus* with a fine bacteriological loop, colonies of *C. jejuni* were collected from the surface of the blood agar culture plate and transferred to surface of the test paper and spread over an area of 2–3 mm. The plates were incubated at 37 °C for 30 minutes after which the paper was

flooded with iodine solution. This caused the paper to turn uniformly black within about 30 seconds. Penicillinase producing strains were detected by the decolouration of blue-black surrounding the organism.

Results and Discussion

In this study, 52 (47.3%) strains of *Campylobacter* were isolated from one hundred and ten specimens from animals and 6 (13.9%) strains from 43 specimens (stool and rectal swabs) of children with gastrointestinal disorder and none from 20 control children without diarrhoea. All children examined were below the age of 5 years. A total of 110 faecal specimens were obtained from domestic animals (101 from chicken and 9 from pigs). The results from this study showed that 49 (48.5%) *Campylobacter* isolates were obtained from chicken, while 3 (33.3%) were from pigs (Table I). These results showed that *C. jejuni* was more common in chicken accounting for 39 (75%) of 52 isolates, while *C. coli* predominates in pigs accounting for 3 (100%) of 3 isolates. This result correlates with the findings of Skirrow (1982) and that of Adegbola *et al.* (1991), the latter reported a preponderance of *C. jejuni* in chickens accounting for 59 (67.8%) out of 87 isolates and *C. coli* predominated in pigs accounting for 11 (91.7%) of 12 isolates. The results from population correlates with various studies in Europe, North America and Australia where the isolation rate is between 5% and 11% (Butzler *et al.*, 1973; Skirrow, 1977). In

Africa, studies showed an isolation rate of 0.6% – 16.5% (Demol and Bosman, 1978; Billingham, 1981; Chowdbury and Mahgub, 1981; Coker and Adefeso, 1994). The isolation rate of 13.9% in this study also correlates with that of Coker and Adefeso (1994), who reported an isolation rate of 16.5% in children under two years. Higher isolation rate in African children compared with European children has been attributed to poor standard of hygiene, lack of sanitation and close proximity to animals.

Five (83.3%) of the isolates belong to *Campylobacter jejuni* biotypes I and II, while only I (16.7%) belong to *C. coli*. Of the forty-nine isolates from chicken, 28 (57.1%) were of *C. jejuni* biotype I, II (22.4%) were of biotype II and 10 (20.4%) belong to *C. coli*. All the isolates from pigs belong to *C. coli* (Table II). *Campylobacter jejuni* biotype I was the most prevalent in this study. This report confirmed similar results obtained by Alabi *et al.* (1986) who reported 52.5% of *C. jejuni* biotype I and 28.7% of biotype II. Coker *et al.* (1989) also observed 41% of *C. jejuni* biotype I, 36% of *C. jejuni* biotype II, 1 and 14% of *C. coli*.

Table III. Antibiotic sensitivity pattern of the isolates.

Antibiotics	No. of human strains sensitive (n=6)(%)	No. of animal strains sensitive (n=52)(%)
Ampicillin	4 (66.7)	42 (80.8)
Chloramphenicol	4 (66.7)	45 (86.5)
Cloxacillin	0 (0)	2 (3.8)
Erythromycin	5 (83.3)	31 (58.6)
Gentamicin	6 (100)	50 (96.1)
Penicillin	5 (83.3)	10 (19.2)
Streptomycin	0 (0)	13 (25)
Tetracycline	4 (66.7)	38 (72.3)

Table I. Isolation of *Campylobacter* from different sources.

Specimen	No. examined	No. positive	percentage positive
Human	43	6	13.93
Chicken	101	49	48.5
Pigs	9	3	33.3
Total	153	58	37.9

Table II. Biotype distribution of *Campylobacter* isolates.

Biotype	Human n=6	Chicken n=49	Pigs n=3
<i>C. jejuni</i> biotype I	4(66.7%)	28(57.1%)	0
<i>C. jejuni</i> biotype II	1(16.7%)	11(22.4%)	0
<i>C. coli</i>	1(16.7%)	10(20.4%)	3(100%)

The antibiotic sensitivity pattern showed that all the human strains (100%) and almost all the animal strains (96.1%) were sensitive to gentamicin. All the human strains were resistant to streptomycin and cloxacillin. Erythromycin had a high activity (83.3%) on human strains and only 59.6% activity on animal strains. High antibacterial activity was also shown by tetracycline, ampicillin and chloramphenicol on both human and animal strains (Table III). This confirmed the results obtained elsewhere (Frelend *et al.*, 1984; Goosens *et al.*, 1989). Agbonlahor *et al.* (1987) obtained a 100% sensitivity rate with gentamicin. This study confirmed this finding. A resistance rate of 100%

human strains were observed with streptomycin, while a resistance rate of the animal isolates was 75%. This correlates with the findings of Coker and Adefeso (1994) who reported a resistance rate of 83.3% with streptomycin. Thirty-one (59.6%) out of 52 strains that were resistant to erythromycin were from chicken and pigs. Erythromycin resistant isolates have previously been reported

(Vanhoof, 1980). None of the isolates from humans were beta-lactamase positive, while 5 (9.6%) out of 52 strains from animals produced beta-lactamase. This result is slightly higher than an earlier report by Coker *et al.* (1988) who reported a rate of 6.4% and Smith *et al.* (1997) who reported 3% rate.

- Adegbola R. A., Akinkuade F. O., Alabi S. A., Coker A. O. and Odugbemi T. (1991), Prevalence, biotypes and serogroups of *Campylobacter jejuni* and *C. coli* in domesticated animals in Nigeria. *Trop. Vet.* **9**, 43 – 45.
- Adetosoye A. I. and Adeniran M. O. A. (1987), *Campylobacter* enteritis in animals in Ile-Ife, Oyo State, Nigeria. *Rev. Elev. Med. et Pays Trop.* **40**, 39 – 40.
- Agbonlahor D. E., Coker A. O. and Odugbemi T. (1987), Antimicrobial susceptibility of diarrhoeagenic *C. jejuni* and *Y. enterocolitica* isolated in Nigeria. *Cent. Afr. J. Med.* **33**, 149 – 153.
- Alabi S. A., Coker A. O., Dosumu-Ogunbi O. and Odugbemi T. (1986), Biotype and serogroup distribution of *Campylobacter* isolates from children in Nigeria. *J. Clin. Microbiol.* **24**, 856 – 858.
- Bauer A. W., Kirby W. M. D., Sherris J. C. and Turck M. (1966), Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**, 493 – 496.
- Billingham J. D. (1981), *Campylobacter* enteritis in the Gambia. *Trans. Roy. Soc. Trop. Med. Hyg.* **75**, 641.
- Butzler J. P., Dekeyser P., Detrain M. and Dehaam F. (1973), Related vibrios in stools. *J. Paediatr.* **83**, 493 – 495.
- Chowdhury M. N. H. and Mahgaub E. (1981), Gastroenteritis due to *Campylobacter jejuni* in Riyadh, Saudi Arabia. *Trans. Roy. Soc. Trop. Med. Hyg.* **75**, 359 – 361.
- Coker A. O. and Dosunmu-Ogunbi O. (1984), Isolation of *Campylobacter jejuni* in Lagos, Nigeria. A new bacterial agent of diarrhoea. *E. Afr. Med. J.* **61**, 52 – 55.
- Coker A. O. and Dosunmu-Ogunbi O. (1985), Gastroenteritis due to *Campylobacter jejuni* in Lagos, Nigeria. *C. Afr. J. Med.* **31**, 72 – 74.
- Coker A. O., Olukoya D. K. and Odugbemi Tolu (1988), Antibiotic susceptibility patterns and beta-lactamase production of strains of *Campylobacter* species isolated in Nigeria. Proceedings of the 4th International Workshop on *Campylobacter* infections, Goteborg, Sweden (Eds Kaijser, B. and Falsen), E. Published by Goterna, Kunglav, Sweden. pp 153 – 154.
- Coker A. O., Olaiya B., Obi C. L. and Alabi S. A. (1989), Characterization and antibiotic sensitivity of *Campylobacter jejuni* and *Campylobacter coli* isolated from children at the Lagos University Teaching Hospital (LUTH) Lagos, Nigeria. *J. Trop. Med. Hyg.* **92**, 104 – 107.
- Coker A. O. and Adefeso A. O. (1994), The changing patterns of *Campylobacter jejuni* / *coli* in Lagos, Nigeria after ten years. *E. Afr. Med. J.* **71**, 437 – 440.
- Dekeyser P., Gessuin-Detrain M., Butzler J. P. and Sternon J. (1972), Acute enteritis due to related *Vibrio*: first positive stool culture. *J. Infect. Dis.* **125**, 390 – 392.
- Demol P. and Bosmans E. (1978), *Campylobacter* enteritis in Central Africa (letter). *Lancet* **1**, 604.
- Firehammer B. D. (1965), The isolation of vibrios from ovine faeces. *Cornell Veterenary* **55**, 482 – 494.
- Freland C., Prost L. and Prost A. (1984), Les infections humaines a *Campylobacter fetus*. *Semaine des Hopitaux* **60**, 2416 – 2425.
- Goossens H., Coignau H., Vlaes L. and Butzler J. P. (1989), *In vitro* evaluation of antibiotic combination against *Campylobacter fetus*. *J. Antimicrob. Chemother.* **24**, 195 – 201.
- Low D. C., Lawande R. V. and Hall C. (1981), *Campylobacter* enteritis in Northern, Nigeria. (Letter). *Trans. Roy. Soc. Trop. Med. Hyg.* **75**, 757.
- Mcfadyean F. and Stockman S. (1913), Final report of the Departmental Committee appointed by the Board of Agriculture and Fisheries to inquire into epizootic abortion part III Abortion in Sheep London: His Majesty's Stationary offices, London.
- Odugbemi T. O., Hafiz S. and McEntegart M. G. (1977), Penicillinase producing *Neisseria gonorrhoea*: detection by starch paper technique. *Br. Med. J.* **2**, 500.
- Olusanya O., Adebayo J. O. and Williams B. (1983), *Campylobacter jejuni* as a bacterial cause of diarrhoea in Ile-Ife, Nigeria. *J. Hyg.* **91**, 77 – 80.
- Skirrow M. B. (1977), *Campylobacter* enteritis, a new disease. *Br. Med. J.* **11**, 9 – 11.
- Skirrow M. B. (1982), *Campylobacter* enteritis – The first five years. *J. Hyg.* **89**, 175 – 184.
- Smith S. I., Coker A. O. and Olukoya D. K. (1997), The changing patterns of β -lactamase activity amongst *Campylobacter jejuni* and *C. coli* in Lagos, Nigeria. *Biomedical Lett.* **56**, 179 – 181.
- Vanhoof R., Cordia B., Dierick R., Geignall B. and Butzler J. P. (1980), Bacteriostatic and bactericidal activities of antimicrobial agents against *Campylobacter* subsp *jejuni*. *Antimicrob. Agents Chemother.* **18**, 118 – 121.