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ROLE OF *CAMPYLOBACTER JEJUNI/COLI* IN DIARRHOEA IN ILE-IFE, NIGERIA

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ABSTRACT

Background: ***Campylobacter jejuni/coli* are well established causative agents of diarrhoea. In Nigeria, gastroenteritis due to *C. jejuni* was first reported in northern part of the country in 1981 and the South-Western part in Ile-Ife in 1983.**

Objective: **To re-examine the role of *C. jejuni* as an agent of diarrhoea after more than a decade at Ile-Ife, Nigeria and to determine the biological characteristics of local strains.**

Design: **A prospective case control study.**

Setting: **Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile-Ife, Nigeria.**

Subjects: **Three hundred and three children with diarrhoea and 100 children with other medical conditions other than diarrhoea were randomly selected.**

Main Outcome Measures: **Isolation of *C. jejuni/coli* from stool samples collected from the patients and controls. Biological characteristics of the isolates.**

Results: **Fifty eight (19.1%) and 6% of the patients and controls, had the organism respectively. *Campylobacter coli* accounts for 53.3% of isolates. All the isolates were susceptible to erythromycin and there was no evidence of ß-lactomase production.**

Conclusion: ***Campylobacter jejuni* is an important diarrhoea agent in our environment and should be considered strongly in children with diarrhoea. Complete characterization of local, strains is necessary.**

INTRODUCTION

Campylobacter jejuni/coli are well established causative agents of diarrhoea in Nigeria. Gastroenteritis due to *C. jejuni* was first reported in the northern part of the country in 1981(1) and the South-Western part in Ile-Ife in 1983(2). In 1984, the most comprehensive report on *C. jejuni* as an agent of diarrhoea particularly in children was given by Coker and Dosunmu Ogunbi(3,4). Since then, the isolation rate of *Campylobacter spp.* from cases of diarrhoea in the country has increased and has led to a series of careful biological studies on the local strains(5-8).

It is necessary to re-examine the role of *C. jejuni* as an agent of diarrhoea after fifteen years of its first isolation in Ile-Ife, Nigeria. It will be informative to compare current isolation rate with that of 1983 and to determine the biological characteristics of the local strains.

MATERIALS AND METHODS

Subjects: Stool samples and rectal swabs were collected from a total of 303 patients with diarrhoea and 100 without diarrhoea for the past two weeks (as controls). All the subjects and controls were randomly selected from patients attending Urban Comprehensive Health Centres: Childrens Emergency Room and Children Ward II of Ife State Hospital and finally Primary Health Centre, Enuwa all at Ile-Ife. Samples were collected over a period of nine months from April to December 1997. Diarrhoea was defined as passage of three or more loose and watery stools per day. All the subjects and controls were aged between 0 and 36 months.

Ethical Consideration: Though a non-invasive study, informed consent was obtained from each of the mothers of the children. The study was approved by the Research and Ethical Committee of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife.

Methods: Rectal swabs and stool samples were cultured directly on Butzler-type medium(3). These were incubated at 37°C in candle extinction jars for 72 hours. In positive specimens characteristic effuse colonies were formed. These colonies were gram stained and showed spiral gram-negative bacteria. Isolates were considered to be *Campylobacter jejuni/coli* if they were motile, oxidase positive, catalase positive grew at 37°C and 42°C but not at 25°C.

All isolates were stored in a medium containing ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP) and kept frozen. The isolates were later characterized in the Nigerian institute for Medical Research (NIMR), Lagos, Nigeria. They were characterized by antibiotics susceptibility testing, beta-lactamase production and biotyping.

The disc methods for *in-vitro* susceptibility testing of Bauer *et al.*(9) was employed. Five colonies of each strain of *Campylobacter* isolates were suspended in a sterile bijou bottle

containing 5mls of Mueller-Hinton broth Oxoid Ltd., London UK and incubated overnight at 37°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 (cotrimoxazole 25µg, 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 37°C for 48 hours. The diameter of zones of inhibition were measured to the nearest millimeter using a ruler. The zones of inhibition of the test strains when comparable with the zones of inhibition of control organism were interpreted as resistant. The control organism was *Campylobacter jejuni* NCTC 11168. All isolates were tested for evidence of beta-lactamase activity by the starch paper technique(10). *Staphylococcus aureus*-ATCC 25293 was used as a positive control. Isolates were biotyped using the Lior typing schemes(11).

Limitation of the study: The greatest limitation of the study lies in the fastidious and microaerophilic nature of the organism. In the process of transporting the isolates to Lagos many of them failed to subculture. As a result only thirty isolates were further characterised in the study.

RESULTS

Fifty eight (19.1 %) of the three hundred and three samples from children with diarrhoea were positive for *Campylobacter* species. Six (6%) of the one hundred samples from non-diarrhoea children were positive for *Campylobacter* species.

Table 1

Results of Faecal Cultures

Study Population	Number of Children Examined			No. Positive (%)
Study Population	M	F	Total	
Patients with diarrhoea	183	120	303	58(19.1)
Control (patients without gastrointestinal symptoms)	63	37	100	6(6)

Table 2

Age Distribution of patients with Campylobacter Enteritis

Age (months)	Children with diarrhoea		% positive
0-6	83	7	12.1
7-12	119	33	56.9
13-18	64	11	19.0
19-24	24	5	8.6
25-30	5	1	1.7
31-36	6	1	4.7
Total	303	58	100

Table 2 shows the age distribution of children with diarrhoea. Most of the positive isolates were from children below the age of two years. Sex distribution of the *Campylobacter* isolates is shown in Table 3. There is an isolation rate of 13.5% in males compared to 5.6% in females.

Table 3

Distribution of C. jejuni/coli by sex

Sex	Children With Diarrhoea		
	Number examined	Number Positive	% Positive
Male	183	41	13.5
Female	120	17	5.6
Total	303	58	19.1

The antibiotic susceptibility pattern of the isolates is shown in Table 4. None of the isolates produced beta lactamase. Biotype distribution of the thirty isolates showed that 14(46.6%) were C. *jejuni* while 16(53.3%) were *C. Coli.*

Table 4

Susceptibility rate of the isolated strains to antibiotics

Antibiotic	Number of sensitive strains	% of sensitive strains
Cotrimoxazole (25µg)	1	3.3
Ampicillin (25µg)	28	93.3
Nalidixic acid (30µg)	29	96.7
Nitrofuradantoin (200µg)	29	96.7
Cefriaxone (30µg)	29	96.7
Colistin (25µg)	29	96.7
Streptomycin (25µg)	29	96.7
Tetracycline (25µg)	30	100.0
Erythromycin (10µg)	30	100.0
Gentamicin (10µg)	30	100.0
Ciprofloxacin (25µg)	30	100.0

Table 5

Biotype distribution of isolates

Biotypes	Number Positive	Percentage Positive
C. jejuni		
Biotype I	10	33.3
Biotype II	4	13.3
C. coli		
Biotype I	16	53.3
Total	30	99.9

DISCUSSION

Results of this study have shown that *Campylobacter jejuni/coli* is a common diarrhoeagenic agent among children. Isolation rate of 19.1% is the highest reported in Nigeria. Previous rates have ranged

from 5.2% (3) through 11%(6) to 16.5%(12).

This high isolation rate compares favourably with what was obtained in other developing countries like Gambia(13), South Africa(14) and Thailand(15). Isolation rate in control children without diarrhoea was 6%. The difference between this and 19.1% in subject group was statistically significant (P<0.01) lending credence that *Campylobacter jejuni/coli* is an agent of diarrhoea.

Fifty six (96.6%) of all isolates were obtained from children below the age of two years. This is similar to earlier reports in the country and other developing countries(7,12,14). The findings strengthens the observation that the epidemiology of infection is quite different in the developed and developing countries and that infection occurs very early in the developing countries.

The infection was more common in males (13%) than in females (5.6%). This finding is in agreement with the work of Coker and Adefeso(12) where infection in males was 11.8% and 4.8% in females. in fact, it is similar to an earlier work(4). However, a slight male predominance 1.7:1 was shown in a British survey(16).

In this study all strains were susceptible to tetracycline, erythromycin, gentamicin and ciprofloxacin while 96.7% of the isolates were resistant to cotrimoxazole. The high rate of resistance is due to abuse of the antibiotic in our infections, ear infections and patients with gastro intestinal symptoms also use the drug. Moreover, cotrimoxazole is a drug commonly used for children especially for gastroenteritis that has not resolved after a few days. Antimicriobial susceptibility testing did not reveal resistance to erythromycin. This agrees with another report elsewhere(17) where no resistance to erythromycin was revealed. However, it contrasts with increasing erythromycin resistance from 0%(7) through 18%(6) and then 79.2% reported in Lagos, Nigeria(12).

None of the thirty strains were beta-lactamase producers. Beta-lactamase producers however, have been reported in Lagos, Nigeria(12,18,19).

Further identification of the isolates showed that 46.6% were *C. jejuni* while 53.3% were *C. coli*. This has not been the experience in Lagos, Nigeria where the predominant species have been *C. jejuni,* over the years(6,12,20). However, in many developing countries such as Central African Republic, Chile and Hong Kong, *C. coli* accounts for a higher proportion of infections than elsewhere. Although *C. coli* is particularly associated with pigs, this association is not exclusive, and in some areas *C. coli* is commonly found in chickens(21).

In conclusion, the isolation rate of (19.1%) is the highest reported in Nigeria. *Campylobacter coli* accounts for 53.3% of all isolates, and erythromycin the drug of choice for *campylobacter enteritis* is still very useful in this environment. It is recommended that the pattern identified in this study be confirmed in a larger number of isolates in the future. In addition further characterization like serotying, plasmid and genomic analysis be included.

REFERENCES

1. Low, D.C., Lawande, R.V. and Hall, C. *Campylobacter enteritis* in Northern Nigeria (Letter). *Trans. Roy. Soc. Trop. Med Hyg.* 1981; **75**:757.
2. Olusanya, C, Adebayo, L.C. and Williams, B. *Campylobacter jejuni* as a bacterial agent of diarrhoea in Ile-Ife, *Nigeria. J Hyg.* 1983; **91**:77-80
3 Coker, A.O., and Dosunmu-Ogunbi, O. Isolation of *Campylobacter jejuni* in Lagos, Nigeria A "new" bacterial agent of diarrhoea. *East Afr. Med J.* 1984; **61**:52-55
4. Coker, A. O. Studies on local strains of *Campylobacter jejuni* in Lagos, Nigeria, PhD. thesis submitted to the University of Lagos, Nigeria, 1986.
5. Coker, A.O. and Dosunmu-Ogunbi, O. Biotype among *Campylobacter jejuni* strains isolated in Lagos. *Int. J. Microbiol.* 1983; **1**:77-99.
6. Coker, A.O., Olaiya, E., Obi, C.L. and Alabi, S.A. 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.* 1989; **92**:104-107.
7. Coker, A.O., and Dosunmu-Ogunbi, O. Gastroenteritis due to *Campylobacter jejuni* in Lagos, Nigeria *Centr. Afr. J. Med.* 1985; **31**:72-74.
8. Obi, C. L. and Coker, A.O. Production of antisera against *Campylobacter* species in Lagos, Nigeria. *Centr. Afri. J. Med.* 1988; **34**:139-141.
9. Bauer, A.W, Kirby, W.M.M., Sherris, J.C. and Turk, M. Susceptibility testing by standardised single disc method: *Am. J. Clin. Path.* 1966; **45**:493-496.
10. Odugbemi, T.O., Hafiz, S. and McEntegart, M.D. Penicillinase producing *Neisseria gonorrhoae* detection by starch paper technique. *Brit. Med. J* 1977; **2**:500.
11. Lior, H. New extended biotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* and *Campylobacter laridis. J. Clin. Microbiol.* 1984; **20**:636-640.
12 Coker, A.O. and Adefeso, A.O. The changing pattern of *Campylobacter jejuni* in Lagos, Nigeria after ten years. *East Afr. Med. J.* 1994; **71**:437-440.
13. Billingham, L.D. *Campylobacter enteritis* in the Gambia. *Trans. Roy. Soc. Trop. Med. Hyg.* 1981; **75**:641-644.
14. Bokkenheuser, V.D., Richardson, N.J., Bryner, J.B., Roux, D.J., Schutte, A.B. Koorhof, H.J., Freiman, I. and Hartman, F. Detection of enteric Camyplobacteriosis in children. *J. Clin. Microbiol.* 1979; **9**:227-232.
15. Taylor, D.N. and Echeverria, P. The influence of immunity and strain characteristics on the epidemiology of campylobacteriosis. *J. Clin. Microbiol.* 1988; **26**:863-868.
16. Skirrow, M.B. A demographic Survey *of Campylobacter, Salmonella* and *Shigella* infections in England. *Epidemiol. Infect.* 1987; **99**:647-657.
17 Cabrita, J., Pires, I., Vlaes, L., Coignau, H., Levy, J., Goessens, R. Gonacalves, A.P., de mol P. and Butzier J.P. *Campylobacter enteritis* in Portugal: epidemiological features and biological markers. *Euro. J. Epidemiol.* 1992; **9**:22-26.
18. Coker, A.O., Olukoya, D.K. and Tolu, O. 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, Edited by B. Kaijser, and E. Falsen, *Published by Gotherma, Kungaly, Sweden*. Pp. 153, 1988.

19. Smith, S.I, Coker, A.O. and Olukoya, D.K. The changing patterns of ß-lactamase activity amongst *Campylobacter Jejuni* and *C. coli* in Lagos, Nigeria *Biomedical Lett.* 1997; **56:**179-181.
20 Alabi, S.A., Coker, A.O. Dosunmu-Ogunbi, O. and Tolu, O. Biotype and Sero-group distribution of *Campylobacter Jejuni* isolates from Children in Nigeria. *J. Clin. Microbiol.* 1986; **24:**856-858.
21. Nachamkin, I., Blaser, M.J. and Tompkins, L.S (Eds.) *Campylobacter jejuni*-current status and future trends. Published by American Society for Microbiology, 1325 Massachussetts Ave. N.W. Washington DC 2005. 1992; 3-8.

CORRECTION

In an article published in our March issue, "Antibiotic sensitivities of common bacterial pathogens in urinary tract infection at Gondar hospital, Ethiopia" Volume 79, pages 140-142, one of the authors name F. Moges was incorrectly printed as A.F. Moges. We apologise for this inadvertent error.