ORIGINAL ARTICLE

Prevalence and Antibiotic Resistance Patterns of *Campylobacter jejuni* and *Campylobacter coli* from Children with Diarrhoea at University of Maiduguri Teaching Hospital Maiduguri, Nigeria

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ABSTRACT

Background: Campylobacter species have been recognised as a leading cause of acute bacterial gastroenteritis in humans. Owing to the high prevalence of resistance, antibiotics are losing effectiveness in clinical treatment of human campylobacteriosis. **Objective:** This study was conducted to determine the prevalence and antibiotic resistance pattern of Campylobacter. **Methods:** Stool specimen was suspended in Campylobacter enrichment broth and incubated at 42^0 C for 48 hours in microaerophilic conditions. Campylobacter agar containing cefoperazone and vancomycin was inoculated with 5µl of the enrichment broth and incubated at 42^0 C for 48 h as above. *Campylobacter jejuni* and *C. coli* were identified using conventional bacteriological methods and confirmed with molecular techniques. Sensitivity testing was performed using disc diffusion method. **Results:** Out of the 250 stool samples from children with diarrhoea tested, 37 (14.8.%) was positive for Campylobacter species. Of the 100 samples from non-diarrhoea children examined, two (2%) was positive for Campylobacter species. *Campylobacter coli* (56.8%) was more frequently isolated compared to *C. jejuni* (43.2%). Most of the isolates were from children aged 19 – 24 months. Males (17.6 %) were more affected than females (11.8%). Antibiotic sensitivity testing results revealed high resistance rates among the isolates to erythromycin, ciprofloxacin, nalidixic acid, tetracycline, gentamicin, streptomycin, cotrimoxazole and azithromycin and no resistance to chloramphenicol. Multidrug resistance (resistance to > 3 antibiotics) was observed in 53.8% of C. coli and 46.2% of C. *jejuni* isolates. **Conclusion:** The findings of this study underscore the significance of Campylobacter infections as a major public health concern in paediatric populations in Maiduguri.

Keywords: Campylobacter, prevalence, Antibiotic resistance, diarrhoea, Maiduguri

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Introduction

Campylobacter is widely recognized as a significant cause of enterocolitis in humans globally. [1, 2,3] Campylobacter enteritis is essentially a foodborne disease with ingestion of raw meat, often reported as a source of human infection.[4,5,6] Drinking unpasteurized milk or contaminated water as well as contact with animals have been linked to Campylobacter disease.[4,7]*Campylobacter jejuni* and *C. coli* have been the most frequently reported pathogenic Campylobacter species associated with human enterocolitis.[3,7,8] Other species of Campylobacter including, *C. lari*, and *C. upsaliensis* have also been described to cause disease in humans.[6]

Campylobacter infection manifests as diarrhoea, fever, headache and vomiting.[3,9] *Campylobacter jejuni* has also been occasionally associated with extraintestinal diseases including, Guillain–Barrê syndrome, reactive arthritis, and irritable bowel syndrome.[8,10] Although, Campylobacter enteritis is typically a childhood disease, the organism has also been association with disease in adults having underlying medical conditions like, HIV/AIDS, immunological disorders and cancers.[4]

Recent studies showed an increasing trend of antibiotic resistance among Campylobacter isolates,[11] which is believed to be a result of the spread of resistant Campylobacter through the food chain.[5,9] This observation indicates a serious public health issue, since it limits treatment options for Campylobacter diseases. There is a dearth of information on prevalence and antibiotic sensitivity of human Campylobacter strains in Nigeria. This study aimed to investigate the prevalence and antibiotic resistance patterns of Campylobacter species in human enteric infections in Maiduguri, Northeastern Nigeria.

Methods

The Study area

The study was carried out at the University Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria. The facility is a tertiary health institution that offers clinical, teaching and laboratory services to the majority of States in Northeastern Nigeria. However, most of the patients that attend the paediatric unit of the hospital are from Maiduguri and other towns within the State. Maiduguri is the capital city of Borno State, located in the Northeastern region of Nigeria in West Africa. Maiduguri is situated in the semi-arid Sahel region on Latitude 11.85^{o}N, longitude 13.15^{o}E.

The climate of Maiduguri is hot and dry with two distinct seasons (wet and dry). The population of the city is approximately 1.2 million people according to 2020 population estimate.[12] The inhabitants of Maiduguri engage mainly in farming, trading and livestock herding. This description provides context for the current study.

Study design

This was a cross-sectional study conducted at University of Maiduguri Teaching hospital, Maiduguri between October, 2019 and December, 2020.

Study population

The subjects were children aged 0-36 months old who presented with diarrhoea ($\geq$ loose stools in 24 hours) and non-diarrhoea children (controls) attending outpatient clinic at UMTH.

Inclusion Criteria

Children aged 0-36 months presenting with diarrhoea ($\geq$ 3 loose stools in 24 hours) and children with no symptoms of gastrointestinal infection within 14 days, whose parents/guardians agreed to participate in the study.

Exclusion Criteria

Children with chronic diarrhoea, underlying medical conditions like HIV/AIDS or cancer, recent antibiotic treatment (within 2 weeks), children whose parent/guardian declined to participate in the study.

Sample Size determination

The sample size was calculated applying the formula $n= Z^2 p(1-p)/E^2$ where n is the required sample size, Z is the Z-score corresponding to the desired confidence level (1.96 for 95% CI), p is expected prevalence, and E is the level of precision.[6] Campylobacter prevalence rate of 20% was used.[6] With the known prevalence, the minimum sample size was calculated as $n= 1.96^2 \times 0.2x (1-0.2) / 0.05^2 =$ 245 and adjusted to 250 to help maintain statistical significance.

Recruitment Strategy

Convenience sampling method was used to enroll 350 children aged 0-36 months, comprising 250 children presenting with diarrhoea ($\geq$ 3 loose stools in 24 hours) and 100 asymptomatic children (control) from the out-patient paediatric unit at UMTH over a period of 12-months aiming to recruit 30 children per month.

Isolation and identification of Campylobacter

Campylobacter detection and isolation were based on procedures described by Grove-white et al.[13] One gram of stool sample was suspended in 9 mL Campylobacter enrichment broth (LAB M Ltd, UK) containing cefoperazone, vancomycin, trimethoprim and natamycin (LAB M Ltd, UK), and incubated at 42^{o}C for 48 h under microaerophilic atmosphere (12% CO_2, 3% H_2, 11% O_2, 74% N_2) provided with Campbag kit (BioMerieux, France). Campylobacter selective agar medium (IDG Ltd, UK) incorporated with cefoperazone and vancomycin was inoculated with 5µl of the broth culture and incubated at 42^{o}C for 60 – 72 h under microaerophilic condition as above and examined at intervals of 24 h, 48 h and 72 hours. From the positive plates, 2-3 colonies with Campylobacter features were selected and sub-cultured on Columbia

Blood agar (Oxoid, UK) and then incubated at 42°C for 24 h under microaerophilic conditions. Presumptive Campylobacter isolates were Gram-stained and biochemical tests such as oxidase and catalase production tests as well as dryspot (Oxoid, UK) agglutination test were performed for confirmation of isolates as Campylobacter.

Multiplex polymerase chain (mPCR) reaction for suspected Campylobacter

Deoxyribonucleic acid extraction of *Campylobacter jejuni* and *C. coli* was done as previously described.[14,15] The hippurate hydrolase (hip) genes of *C. jejuni* and aspartate (asp) genes of *C. coli* were detected by multiplex polymerase chain reaction using the primers: Forward 5′ GAA-GAG-GGT-TTG-GGT-GGT-G-3′ and Reverse 5′ RAG-CGC-TTC-GCA-TAA-CTT-G-3′ for *C. jejuni* and primers: Forward 5′ATA-AAA-GAC-TAT-CGT-CGC-GTG-3′and Reverse 5′GGT-ATG-ATT-TCT-ACA-AAG-CGA-G-3′ for *C. coli.* A 20µL reaction which contained 2 µl of each template DNA and 1 µl of each primer of 10pmol/µl (Bioneer, Korea) in 14µl deionized water was used. The mPCR reaction was performed in a thermocycler (Eppendorf AG, Hamburg, Germany) with initial denaturation at 96°C for 3 minutes annealing at 55°C for 30 seconds and extension at 72°C for 5 minutes. The mPCR amplicons were electrophoresed in 1.5% agarose gel (Bioneers, Korea), stained with ethidium bromide and visualised under ultraviolet (UV) light.

Antibiotic susceptibility testing

Antibiotic susceptibility testing of the isolates was carried out using agar disc diffusion method.[16] Ten different commonly used antibiotics (Oxoid, UK) including, ampicillin (25 µg), tetracycline (25µg), chloramphenicol (30µg), erythromycin (15µg), azithromycin (10 µg), gentamicin (10 µg), streptomycin (25µg), nalidixic acid (30 µg), cotrimoxazole (25 µg) and ciprofloxacin (25 µg) were used. In the test, a single Campylobacter colony picked from Columbia blood agar (Oxoid, UK) was suspended in 5 ml of saline and adjusted to McFarland 0.5.[16,17] The suspension was inoculated onto Mueller-Hinton agar (Oxoid, UK) supplemented with 10% sheep blood using sterile cotton swab. Antibiotic discs were placed on inoculated agar plates and incubated along with Campygen gas packet (BioMiereux, France) to provide microaerophilic conditions at 42°C for 24 hours. Susceptibility of the isolates to tested antibiotics was determined by measuring zone of inhibition around each disc and categorised as sensitive or resistance following the interpretative charts of the Clinical and Laboratory Standards Institute.[17] *Escherichia coli* (ATCC, 25922) sensitive to all antibiotics included in the study was used as control strain to evaluate the performance of culture media and susceptibility of antibiotic discs.

Susceptibility tests for nalidixic acid and cephalothin were performed on the isolates as recommended by Clinical and Laboratory Standards Institute.[17] Campylobacter isolates that were sensitive to nalidixic acid and resistant to cephalothin were considered as *C. jejuni* and *C. coli,* whereas isolates resistant to nalidixic acid and cephalothin were regarded as other *Campylobacter species*.[18]

Ethical consideration

The study was approved by Ethical and Research Committee of UMTH and informed consent was obtained from the parents/guardians of the children

Results

Thirty-seven (14.8%) of the two hundred and fifty specimens from children with diarrhoea were positive for *Campylobacter species*. Two (2%) of the one hundred samples from non-diarrhoea children were positive for *Campylobacter species* (Table 1). The age distribution of patients with *Campylobacter enteritis* is shown in Table 2. Most of the positive cultures (6/26; 67.2%) were from children aged 19-24 months. Campylobacter prevalence rate in males was higher compared to females (17.6% vs. 11.8), Table 3. C. coli was the dominant *Campylobacter species* isolated (56.8%).

The antibiotic resistance patterns of tested Campylobacter isolates are presented in Table 4. The isolates were highly resistant to ampicillin (75.7%), Tetracycline (81.1%), erythromycin (56.8%), azithromycin (48.6%), nalidixic acid (59.5%), ciprofloxacin (54.1%), cotrimoxazole (48.8%) streptomycin (37.8%) and gentamicin (32.4%), no isolate was found resistant to chloramphenicol. The antibiotic resistance patterns showed that 4 (10.8%) of the isolates were resistant to one antibiotic, 10 (27.0%) isolates were resistant to two antibiotics and 23 (62.2%) isolates were multidrug resistance (resistance to 3 antibiotics). There was no substantial difference observed in the prevalence of multidrug resistance between *Campylobacter jejuni* (43.2%) and *Campylobacter coli* (56.8%) isolates.

Table 1: **Distribution of faecal cultures**

Study population	No. Examined		Total	No. positive (%)
	Male	Female		
Patients with diarrhoea	131	119	250	37 (14.8)
Controls (Patients without gastrointestinal symptoms)	55	45	100	2 (2.0)

Table 2: **Age distribution of patients with *Campylobacter enteritis***

Age (months)	No examined	No. positive (%)		
		Campylobacter spp	*C. jejuni*	*C. coli*
0-6	54	5 (9.3)	2 (4.0)	3 (60.0)
7-12	52	8 (15.4)	3 (37.5)	5 (62.5)
13-18	66	13 (19.6)	7 (53.8)	6 (46.2)
19-24	26	6 (23.1)	2 (33.3)	4 (66.7)
25-30	25	2 (8.0)	1 (50.0)	1 (50.0)
31-36	27	3 (11.1)	1 (33.3)	2 (66.7)
Total	250	37 (14.8)	16 (43.2)	21 (56.8)

Table 3. **Sex distribution of children with Campylobacter enteritis**

Sex	No examined	No. positive	% Positive
Male	131	23	17.6
Female	119	14	11.8
Total	250	37	14.8

Table 4: **Resistance rate of the isolated *Campylobacter* strains to antibiotics**

Antibiotics	*C. jejuni* (n=16)	*C. coli* (n=21)	Total (n=37)
Ampicillin	11(68.8)	17 (80.9)	28 (75.7)
Tetracycline	12 (75.0)	18 (85.7)	30 (81.1)
Chloramphenicol	0 (0.0)	0 (0.0)	0 (0.0)
Erythromycin	9 (56.3)	12 (57.1)	21 (56.8)
Azithromycin	7 (43.8)	11(52.4)	18 (48.6)
Streptomycin	6 (37.5)	8 (38.1)	14 (37.8)
Gentamicin	5 (31.3)	7 (33.3)	12 (32.4)
Nalidixic Acid	9 (56.3)	13 (61.9)	22 (59.5)
Ciprofloxacin	8 (50.0)	12 (57.1)	20 (54.1)
Cotrimoxazole	6 (37.5)	12 (57.1)	18 (48.6)
Resistance to one antibiotic	2 (12.5)	2 (12.5)	4 (10.8)
Resistance to two antibiotics	4 (25.0)	6 (28.6)	10 (27.0)
Resistance to 3 or more antibiotics	7 (43.8)	16 (76.2)	23 (62.2)

Discussion

The overall prevalence of Campylobacter species in children with diarrhoea was 14.8.0% in this study, this is similar to the findings of previous studies conducted in Nigeria, [1,2,9] but differs from the prevalence rate reported in Mozambique (1.7%),[8] the United States (3.5%) and the United Kingdom (3.5%) respectively.[6] The highest Campylobacter prevalence rate (23.1%) was observed in children aged 19 – 24 months in this study. This finding agrees with earlier studies conducted in Nigeria but disagrees with the reports from developed countries where the infection is common in the adult population.[4,6] This finding strengthens the observation that the epidemiology of Campylobacter infections differs between developed and developing countries and that infection occurs very early in developing countries.[1,5] In this study, Campylobacter was more prevalent in children with diarrhoea (14.8%) compared to children without diarrhoea (2%), suggesting a strong association between Campylobacter infection and diarrhoeal disease.[1,19] The isolation rate of Campylobacter was higher in males (17.6%) than females (11.8%) in the our study. This finding concurs with the observation of higher infection rate in males compared to females in Northcentral Nigeria (13% vs. 5.6%).[19] The disparity in frequency of infection in males compared to females was linked to hormonal and immunological variations between gender.[4,5,20] *Campylobacter coli* (56.8%) was more prevalent than *Campylobacter jejuni* (43.2%) in our study. This finding is comparable with studies that identified *Campylobacter coli* as a leading cause of gastroenteritis in African children.[1,5]

A comparative analysis of antibiotic resistance patterns in Campylobacter species revealed variations across studies. Consequently, the resistance rate to azithromycin (48.6%) observed in our study was lower than reported in a similar study from South Africa (64.4%).[21] A previous study conducted in Nigeria reported that resistance rate to gentamicin and streptomycin was 4% vs.12% respectively,[7] which disagrees with the high gentamicin and streptomycin resistance rates (32.4% vs.37.8%) observed in our study. The discrepancy in resistance rates between our study and the prior study can be attributed to evolution of Campylobacter strains and resistance patterns over time. Karikari et al., found Campylobacter species resistant to fluoroquinolones in Ghana.[22] This result correlates with elevated resistance to nalidixic acid and ciprofloxacin (59.5% vs. 54.1%) described in this study, but exceeded those reported from the United States (40.4%)[6] and Europe (35.1%).[5,6] The prevalence of ampicillin resistance (75.7%) in this study is comparable with the rate reported in South Africa (65%)[21] and Madagascar (85%).[7] Tetracycline resistance (81.1%) in the present study was comparable to reports in Ghana (85.7%),[22,] but lower than the reports from Europe (55.6%).[3] Cotrimoxazole resistance (48.6%) observed in our study was incomparable to rate reported in India. (68.4%).[15] No chloramphenicol-resistant Campylobacter species was detected in our study. This finding is in agreement with the observation of a Nigerian study, which found *Campylobacter jejuni* isolates highly susceptible to chloramphenicol (92.0%).[18] In contrast to the findings of this study, a survey from South Africa found moderate resistance to chloramphenicol among Campylobacter species isolated from clinical specimens (32.5%).[21] Multidrug resistant *Campylobacter jejuni* and *Campylobacter coli* isolates were detected in 43.8% and 56.8% of our samples respectively, with resistance to $\geq$3 antibiotics. This is incomparable to reports from India (78.9%),[23] but similar to reports from Madagascar (48.7%).[7] In our study, Multidrug resistance was more prevalent in *C. coli* compared to *C. jejuni* isolates. This observation collaborates with reports of increasing trend of multidrug resistance in *C. coli* compared to *C. jejuni* in developing countries.[6,11,18,23] Multidrug resistances in Campylobacter strains causing human infection poses significant challenges for treatment and public health.

Conclusion

The present study reveals a high prevalence of *Campylobacter enteritis* among children aged 19-24 months in Maiduguri, with an alarming antibiotic resistance rate. It is important that diagnostic laboratories in the study area routinely test for Campylobacter infection in stool samples.

Limitation of the study

The drawback of this study was the small sample size, which may not be a true representative of the larger population. Future studies should comprise large number of children from multicenter to better understand Campylobacter epidemiology and antibiotic resistance in paediatric patients in the study area.

Authors Contributions

AD conceived the study and did sample collection with MYS and ASB. AD and MYS did the isolation and sensitivity testing of Campylobacter. AD, JJA and MYS prepared the manuscript and analysed data. JAA revised manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declared no conflicts of interest.

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