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Original Research Article

THE ROLE OF ENTEROBACTER SPECIES IN CHILDHOOD DIARRHEA IN NSUKKA METROPOLIS

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ABSTRACT

Outbreaks of diarrhea are still a serious problem among children. A cross sectional study was carried out to investigate the role of *Enterobacter* species in childhood diarrhea in Nsukka metropolis. Stool samples were collected from 200 participants comprising of 100 hospital diarrhea patients and 100 non-diarrhea apparently healthy individuals. Stool culture, biological characterization and antimicrobial susceptibility tests were carried out on. Of the hundred diarrhea patient, 28 (28.0%), 15 males and 13 females were positive for *Enterobacter*species, while 10 (10%) of the 100 non-diarrhea control subjects (6 males and 4 females), were also positive for *Enterobacter* species. The difference, however, was not statistically significant ($p>0.05$). Specie specifications showed 12(42.9%) and 6(60.0%) *Enterobacter cloacae*, 10(35.7%), and 3(30.0%) *Enterobacteraerogenes*, 4(14.3%), and 0(0.0%) *Enterobacterasburiae*, (7.14%), and 1(10.0%) *Enterobacterhormaechie*2, for diarrhea and non-diarrhea subjects respectively. In both study groups, the age group 0 to 2 years recorded the highest incidence 18(64.29%) and 6(60.0%) in diarrhea and non-diarrhea participants respectively. Statistically, the different was also not significant ($p> 0.05$). The result of the antibiogram showed that *Enterobacter*were mostly resistant to Gentamycin, Tetracycline, Amoxicillin, Nalidixic acid, Nitrofurantoin, Chloramphenicol and Cotrimoxazole but sensitive to Ofloxacin, Ciprofloxacin and Augmentin. This study suggests that *Enterobacter*species play roles in diarrhea episodes in Nsukka, hence, the need for further studies to delineate the importance of this organism in diarrhea cases and factors predisposing to it in Nigeria.

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INTRODUCTION

Enterobacter species are responsible for many nosocomial infections, and less commonly community acquired infections, including urinary tract infections (UTI), respiratory infections, soft tissue infections, and many others [1]. Some species of *Enterobacter* are microflora of the mammalian gastrointestinal tracts and are also present in human skin surfaces, water, food, soil, and sewage [2]. *Enterobacter* has become increasingly resistant to many antibiotics. The presence of *B*-lactamases in *Enterobacter* species is the primary mechanism of antibiotic resistant [3].

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It has been discovered that enterotoxigenic strains of *Enterobacter* have potential to cause food born-illness [4, 5]. *Enterobacter* species havebeen implicated as a possible agent of diarrhea and gastroenteritis. They produce enterotoxin, which perhaps explains why the experimental ingestion of *Enterobacter* in food causes diarrhea. [6]. In the work of Ali and colleagues [7], *Enterobacter* species alongside other eneterobactriaecae were isolated from fecal specimen of patients with diarrhea, 19 of which were from children under 5 years. Again, in a prospective work of Franklin [8], *Enterobacter* species were isolated from 5 infants aged 3 to 18 months. *Enterobacter* infections mainly occur in substantially compromised patient: the very old, the very young, especially those with severe underlying diseases such as neoplasm or immune suppression including human with immunodeficiency virus [9, 2].Their infections in digestive tract typically start when the organism invades the mucus tissues that line the digestive tract as pre-existing organisms in the stomach and intestine, or as newly transmitted from contaminated food and water [10]. Although the prevalence of *Enterobacter* species have been reported in diarrhea, there is paucity of information on the association of *Enterobacter* species in diarrhea entities in this part of the world. This work therefore aimed to determination the role of *Enterobacter*species in cases of diarrhea amongst children aged 0 to 12 years in Nsukka metropolis.

MATERIALS AND METHODS

Specimen: One hundred (100) diarrheal and 100 non-diarrheal stool were used

Media: MacConkey agar (MCA) blood agar base; sulphideindole motility agar (SIM) Simon Citrate Agar, Peptone water, Muller Hinton and Muller Broth Hinton Agarall from oxoid (Cambridge UK).

Ethical Approval and Considerations

Ethical approval was obtained from the ethical committee of Bishop Shanahan Hospital, Nsukka, with reference number: BSH/EC/0103/25.

Sample Collection

Two hundred (200) stool samples from children 0-12 years were collected from patients accessing medical treatment in Bishop Shanahan Hospital and General Hospital Nsukka. The consent of the parents of the participants was sought and they were counseled on the nature of the study and its importance.

Isolation of *Enterobacter* Species

Each specimen was inoculated unto freshly prepared blood agar and MacConkey agar and incubated overnight at 37^{o}C for 18-24 hours. The lactose fermenters and colonies having the characteristics morphology of *Enterobacter* were selected for identification.

Identification of *Enterobacter* Species

Gram stained slides of the isolates were under microscope to establish Gram reaction and cellular morphology typical of *Enterobacter*species. The isolates were subsequently identified using the following biochemical methods: SulphideIndole Motility test, Simmon citrate test, methyl red test, Voges-proskauer test and sugar fermentation tests.

Antibiotics Susceptibility Testing

This was done using modified Kirby Bauer technique of disc diffusion method, according to the Clinical and Laboratory Standard Institute (CLSI), 2024 [11]. The isolates were prepared in Mueller-Hinton broth and adjusted to 0.5 McFarland turbidity standard. Commercially prepared antibiotic discs (Abek Biological Ltd, Liverpool, United Kingdom) containing: Ofloxacin (5µg), Augmentin (20µg), Ciprofloxacin (5µg), Tetracycline (30µg), Gentamycin (10µg), Amoxicillin (10µg), Nalidixic acid (30µg), Nitrofurantoin ((300µg), Chloramphenicol (30µg) and Cotrimoxazole (25µg) were used.

Data Analysis

Results were analyzed in terms of percentage using Statistica Package and Solution Set (SPSS) version 21

RESULTS

Of the 100 diarrhea samples, 28(28.0%) were positive for *Enterobacter* species, comprising *Enterobacter cloacae* 12(42.9%), *Enterobacter aerogenes* 10(35.7%), *Enterobacter asburiae* 4(14.3%) and *Enterobacter hormaechie* 2(7.1%).Hundred (100) non-diarrhea (control) stool specimens yielded 10 *Enterobacter* species, comprising *E. cloacae* 6(60.0%), *E. aerogenes* 3 (30.0%), and *E. hormaechie* 1(10.0%) (Table 1).

Table I: Biochemical characteristics of *Enterobacter isolates*.

	No. of isolates													
Organisms	D	ND	Total	MR	VP	M	LAC	GLU	CIT	SUC	IND	MAN	H_2S	DUL
Enterobacter cloacae	12(42.9%)	6(60.0%)	18(64.3%)	-	+	+	+	AG	+	+	-	+	-	+
Enterobacter acrogens	10(35.7%)	3(30.0%)	13(46.4%)	-	+	+	+	AG	+	+	-	+	-	-
Enterobacter asburiae	4(14.3%)	0(0.0%)	4(14.2%)	+	-	-	+	A	+	+	-	+	-	-
Enterobacter hormaechie	2(7.1%)	1(10.0%)	3(10.0%)	-	+	+	+	AG	+	+	-	+	-	-

Key:MR = Methyl red, MAN = Mannitol, H_2S = Hydrogen sulphide, AG = Acid + Gas,VP = Voges-Proskauer, CIT = Citrate, GLU = Glucose, A = Acid, M = Motility, SUC= Sucrose, D = Diarrhea, DUL = Dulcitol, LAC = Lactose, IND = Indole, ND = Non-diarrhea.

Table 2 and 3 show age and sex distributions and percentage positive cases for *Enterobacter* species. Of the 48 male and 52 females among the diarrhea group, 15(31.3%) males and 13(25.0%) females were positive for *Enterobacter* species while for the non-diarrhea group, a total of 10(10.0%) were positive for *Enterobacter*species comprising 6(14.3%) males and 4(6.9%) females. For age wise distribution, the age group 0-2 years has the highest positive isolates 18(34.0%) and 6(15.0%) for diarrhea and non-diarrhea groups respectively. Despite the difference in the sex age-wise distributions between diarrhea group and non-diarrhea group, the differences between them is not statistically significant (p> 0.05).

Table 2: Age and sex distribution and percentage prevalence among diarrhea patients

				Percentage Positive Cases for *Enterobacter* Species		
Age group (years)	**Males**	**Females**	**Total**	**Males**	**Females**	**Total**
0-2	28	25	53	11(39.3%)	7(28.0%)	18(34.0%)
3-5	9	12	21	2(22.2%)	2(16.0%)	4(19.0%)
6-8	7	9	16	1(14.3%)	2(22.2%)	3(18.8%)
9-11	2	4	6	1(50.0%)	1(25.0%)	2(33.3%)
12	2	2	4	0(0.0%)	1(50.0%)	1(25.0%)
Total	48	52	100	15(31.3%)	13(25.0%)	28(28.0%)

Table 3: Age and sex distribution and percentage positive cases among control subject (non-diarrhea).

				Percentage Positive Cases for *Enterobacter* Specie		
Age Group (years)	**Males**	**Females**	**Total**	**Males**	**Females**	**Total**
0-2	22	18	40	4(18.22%)	2(11.1%)	6(15.0%)
3-5	8	16	24	1(12.5%)	1(6.3%)	2(8.3%)
6-8	7	5	12	0(0.0%)	1(20.0%)	1(8.3%)
9-11	3	8	11	1(33.3%)	0(0.0%)	1(9.1%)
12	2	11	13	0(0.0%)	0(0.0%)	0(0.0%)
Total	42	58	100	6(14.3%)	4(6.9%)	10(10.0%)

Enterobacter isolates were more sensitive to ofloxacin (63.2%) Augmentin (47.4%) and ciprofloxacin (44.7%) than other antibiotics used:gentamycin (21.1%) tetracycline (15.8%), Nalidixic acid (13.2%), amoxicillin (10.58%) chloramphenicol (10.5%) and cotrimoxazole (10.5%) (Table 4).

Table 4: Percentage Susceptibility of 38 Isolates of *Enterobacter* to Different Antimicrobials.

Antimicrobial agent	Susceptible	Intermediate	Resistant
Ofloxacin	N = 24(63.2%)	N = 10(26.3%)	N = 4(10.5%)
Augmentin	N = 18(47.4%)	N = 13(34.2%)	N = 7(18.4%)
Ciprofloxacin	N = 17(44.7%)	N = 10(26.3%)	N = 11(28.9%)
Gentamycin	N = 8(21.1%)	N = 18(47.4%)	N = 12(31.6%)
Tetracycline	N = 6(15.8%)	N = 15(39.5%)	N = 17(44.7%)
Amoxicillin	N = 4(10.5%)	N = 18(47.7%)	N = 16(42.1%)
Nalidixic acid	N = 5(13.2%)	N = 20(52.6%)	N = 13(34.2%)
Nitrofurantoin	N = 6(15.8%)	N = 26(68.4%)	N = 6(15.8%)
Chloramphenicol	N = 4(10.5%)	N = 10(26.3%)	N = 24(63.2%)
Cotrimoxazole	N = 4(10.5%)	N = 12(31.6%)	N = 22(57.9%)

N = number of *Enterobacter*, N in (%) are results.

DISCUSSION

Enterobacter infections are becoming more common in our environment and require urgent attention. These pathogens are frequently associated with a multidrug-resistance phenotype, mainly due to their adaptation to hospital environment and the ability of the organism to easily acquire numerous mobile genetic element harboring resistance and virulence genes, including efflux pump mechanism, which make their treatment difficult. [3, 12]. In this study, 28% positive *Enterobacter*isolates were recovered from the diarrhea patients while 10.0% were isolates from the control group (non-diarrhea) (Tables 2 and 3). These positive isolates in the control group may be due to carrier condition or as normal flora of the gastrointestinal tract. This agrees with Gorbach [8] who isolated 41 *Enterobacter* species, with 29 (70.7%) from diarrhea cases and 12 (29.3%) from normal stool. The high prevalence among the diarrhea patients is a strong indication that *Enterobacter* plays a vital role in diarrhea cases. This is in line with the report on *Enterobacter* infection with respect to childhood diarrhea (6). Specie-specific prevalence showed that *Enterobacter cloacae* has the highest frequency 12 (42.9%) and 6 (35.7%) followed by *Enterobacter aerogenes* 10 (60.0%) and 3 (30.0%) for diarrhea and non-diarrhea groups respectively (Table I). This is in line with the reports by Mezzatesta *et al.,* [13], who isolated mainly *E.cloacae* and *E. aerogenes.* There is also a disparity in the prevalence between male and female subjects, which is in contrast with the report of Hiba *et al.*[14] but in line with the report[15] that males are more prolifically affected in the pediatrics population. However, from this study, one cannot conclusively say that age plays a significant role in the pathogenesis of *Enterobacter* species since it did not embrace people of all ages. The highest incidence recorded in the age group 0-2 years that are immunologically immature, seems to support a previous report [10] that *Enterobacter* infection is more in the immunologically compromised. The effect of climate and environmental factors in the role of *Enterobacter* as an aetiologic agent of diarrhea did not receive adequate attention but Munuswamy [17] reported that infections due to *Enterobacter* are mainly found in environment where infection control is poor as is typified by the average Nigerian hospital and community environments. From the result of antimicrobial susceptibility, *Enterobacte r*encountered in this study were strongly sensitive to Ofloxacin, ciprofloxacin and Augmentin. The organisms were resistant to amoxicillin, chloramphenicol, tetracycline and cotrimoxozole. This rhymes with other reports that *Enterobacter* species are resistant to and readily develop resistance to most routinely used drugs which is due mainly to the production of beta-lactamase enzyme [13].

CONCLUSION

Enterobacter species have been implicated as a possible agent of diarrhea and gastroenteritis. This work has succeeded in isolating *Enterobacter* species from diarrhea cases amongst children aged 0-12 years particularly linking *Enterobacter* species with diarrhea diseases in Nsukka. Though from this work it's not statistically significant when compared with the control group, there is a pointer heralding a possible increase in

significance of *Enterobacter*in this part of the world, especially with our very poor and insanitary conditions and indiscriminate drug abuse. It is therefore solicited that diagnostic laboratories include the search for *Enterobacter* species as an offending organism in diarrhea diseases especially in childhood. There is also a need for the public to be enlightened on proper hygiene, other control measures and effect of indiscriminate use of antibiotics. Educational programs for hospital personals regarding risk reduction for transmission of *Enterobacter*and other nosocomial pathogens is also very necessary.

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AUTHORS' CONTRIBUTION

CCB designed the work, NWE carried out the work under the supervision of CCB and, JNE drafted the original manuscript. MCA and JOO provided technical assistance and helped review the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest whatsoever

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