Enteric Bacteria Pathogens Associated With Diarrhoea of Children in the Federal Capital Territory Abuja, Nigeria

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ABSTRACT: A survey to determine the significance of bacterial species as possible pathogenic microorganism that cause diarrhoea was carried out in the Federal Capital Territory Abuja, Nigeria. The prevalence, age- specificity, seasonality and locality relative to their association with diarrhoea in children younger than five years of age were determined. Stool specimen from 404 children younger than five years of age were collected randomly from five hospitals in Abuja and assessed for microbiological profile of enteric pathogens. Antimicrobial susceptibility tests were performed on all identified relevant isolates using the Kirby-Bauer disc diffusion method. The prevalence of bacteria associated diarrhoea ranged from 18.8 to 22.4% among hospitals located in the Municipality, whereas hospitals located in the satellite settlements it ranged from 18.1to 20.9%. The prevalence of infectious diarrhoea was age specific being highest at the age of 7-12 months and lowest at the age of 37-48 months. There was variation in the month - wise distribution of bacteria associated diarrhoea being high in the months of August and November. We found that *Escherichia coli* was the most frequently isolated bacteria in all age groups(62.8%) and significantly associated with diarrhoea at the age of 13-24 months ($P < 0.05$). However, the isolation rate of *Staphylococcus aureus* and *Klebsiella species* in this study depict these bacteria as veritable aetiological pathogen of infectious childhood diarrhoea. The disk diffusion testing for the antibiotic susceptibility illustrates a generally increased resistance to Amoxycillin, Amoxycilin-clavulanic acid and Cephalexin by all bacteria strain tested. The strains of *Salmonella species* showed substantial resistance to Amoxycillin, Amoxycilin-clavulanic acid, Cephalexin and Cefuroxime. Periodic laboratory - based survey for bacteria pathogens associated with diarrhoea of children should be emphasized to clarify their epidemiological significance and facilitate effective prevention and control. [New York Science Journal 2009;2(7):62-69]. (ISSN: 1554-0200).

Key words: epidemiological, children, diarrhoea, pathogenic, susceptibility

1. Introduction

Diarrhoea disease is one of the leading causes of illness in young children in developing countries (Parashar et al., 2003). The public health significance of diarrhoeal disease cannot be overemphasised. Diarrhoea diseases are the cause of almost three million deaths annually mainly among children younger than five years of age (Seung-Hak et al., 2006).Although extensive investigation of diarrhoea have not been reported, the diarrhoea – specific mortality in children younger than five years of age in Africa has been estimated at about 106 per 1000(Olowe et al., 2003).Available reports in Nigeria indicate that more 315,000 deaths of preschool age children are recorded annually as a result of diarrhoea disease(Babaniyi,1991;Alabi et al., 1998). The main aetiology of the diarrhoea is related to a wide range of bacteria, enteroparasites and viruses (Vargas et al., 2004). The contribution of the various pathogens to diarrhoea may differ substantially between regions depending on local meteorological, geographic, and socio-economic conditions (Reither et al., 2007). Underlying reasons for the spread of diarrhoeal diseases are found in poor hygiene and sanitation, limited access to safe drinking water as well as in inadequate education of health care providers and recipients (Curtis et al., 2000; Thapar and Sanderson, 2004).A knowledge of the pathogens associated with diarrhoea is pertinent in not only allowing the optimum use of available interventions but will also direct efforts aimed at developing specific therapies and preventive vaccines. Unfortunately, due to limited resources the microbiological diagnoses of diarrhoea are not done easily in many settings in Nigeria. The aim of the present study was to determine the spectrum of bacteria enteropathogens causing childhood diarrhoea in Municipal and

satellite setting in the Federal Capital Territory Abuja, Nigeria.

2. Materials and Methods

Study design

Four hundred and four children younger than five years of age who had diarrhoea both out patients and those who were admitted were randomly selected from five hospital (University of Abuja Teaching Hospital,National Hospital Abuja, Maitama District Hospital, Mpape Health Centre and Nya-nya General Hospital) in the Federal Capital Territory Abuja, Nigeria. Informed consent was obtained from parents and/or close relatives. The study was reviewed and approvals were obtained from the ethical committees of University of Abuja Teaching Hospital Gwagwalada, Abuja; National Hospital Abuja and Bwari Area council Health Department. A single faecal sample was collected from each child with diarrhoea on presenting at the hospital. The relevant clinical data were recorded in a proforma. Faeces of children with the presence of blood and mucous were excluded from the study.

Laboratory Analysis

The faecal samples were examined with the naked eye for consistency, colour and atypical components such as mucous, blood and parasites. By the same token fresh and concentrated(by the formalin-ether technique) samples were examined by light microscopy for the presence of leucocytes, red blood cells, parasitic ova and cysts and by the modified Ziehl-Neelsen (Kinyoun's) acid fast stain for *Cryptosporidium* protozoan *parvum* (Henriken and Pohlenz,1981).

The faecal samples were cultured on differential and selective media for bacteria cultivation in order to isolate bacteria enteropathogens. Selenite F broth, Alkaline peptone water, MacConkey, Eosin methylene blue agar, Xylose lysine desoxycholate agar(XLD),Thiosulphate citrate bile salt sucrose(TCBS) and Campylobacter blood-free selective agar were used for the isolation of bacteria pathogens. The bacterial isolates were identified by standard biochemical tests. Identification was confirmed with the API 2OE system (BioMereiux).Serogrouping and serotyping was performed by slide agglutination with polyvalent and monovalent sera obtained from SIFIN, Institut fur Immunpraparate, und Nahrmedien, GmbH Berlin. (Lennette et al., 1996).

The faecal samples were screened for the presence of Rotavirus antigen by Enzyme immnuno assay (Rota-Strip,Coris Bioconcept,Belgium).Faecal suspension were made in vials using Tris-EDTA buffered saline containing 0.1% Sodium Nitrate (NaN_3) and incubated thereafter it was used to impregnate nitrocellulose membrane strips sensitized with mouse monoclonal antibody directed against human Rotavirus Group A VP6 antigens. The impregnated strips were incubated to allow for reaction and the strips were examined for the development of red colour bands which were interpreted according to the manufacturer's instructions. Specimens with equivocal results and those positive by EIA were tested again by Latex agglutination (Slidex Rotakit2; BioMerieux, Marcy 1'Etoile, France).

Antibiotic susceptibility of the four most isolated species namely *Escherichia coli, Klebsiella species*,*Staphylococcus aureus* and *Salmonella species* tested for their susceptibility to some antibiotics(Nalidixic acid, Ciprofloxacin, Cephalexin, Cefuroxime, Amoxycillin, Ceftriaxone and Amoxycilin-clavulanic acid) by modified disc diffusion technique Kirby-Bauer according to the National Committee for Clinical Laboratory Standards(NCCLS,1997). The strains were characterized as susceptible, intermediately resistant or resistant using the OSIRIS software version 3x (Bio-Rad Laboratories Hercules, CA)

Results

Four hundred and four children younger than five years old with acute diarrhoea were included in the study, out of which 184(45.5%) were male and 220(54.5%) were female.

Table 1 shows the distribution of diarrhoeal children by age. It shows that diarrhoea is statistically associated with age and majority of cases occurring in children between 7months and 2 years of age.

Table 1: Age distribution of diarrhoeal children in the Federal Capital Territory, Abuja, 200

AGE GROUP (MONTHS)	NUMBER	PERCENT (%)
0-6	62	15.3
7-12	145	36.0
13-24	117	29.0
25-36	32	7.9
37-48	20	4.9
49-60	28	6.9
Total	404	100.0

x^2=17.075, Pvalue=0.004, df =5

Bacteriological analysis showed that at least one bacterial isolate were recovered from 277 out of the 404 stool specimens; 174(62.8%) were strains of *Escherichia coli* others are *Salmonella typhi* 9 (3.2%), *Staphylococcus aureus* 16(5.8%) and other Enterobacteriacea. Data about parasites and Rotavirus are not shown (Table2).

Table 2: Distribution of Bacterial Organisms Isolated from diarrhoeal children in the Federal Capital Territory, Abuja, 2008

SPECIES	NUMBER of Isolates	PERCENTAGES (%) **Per Total No. of Bacteria Isolated**	**Per Total No. of Samples Analysed**
Escherichia coli	174	**62.8**	43.1
Salmonella typhi	9	3.2	2.2
Klebsiella pneumoniae	24	8.7	5.9
Staphylococcus aureus	16	5.8	4.0
Enterobacter cloacae	5	1.8	1.2
Pseudomonas species	11	4.0	2.7
Morganella morganii	4	1.4	1.0
Proteus species	34	12.3	8.4
TOTAL	**277**	**100.0**	**68.5**

X^2=72.246, Pvalue=0.000, df =7

Table 3 shows the hospital and locality wise distribution of stool samples and the bacterial organisms isolated from diarrhoeal children from all the centres used for this study in the Federal Capital Territory, Abuja. The overall percentage of diarrhoea associated with enteric bacteria was 68.5% and ranged from 18.8% to 22.4% among stool samples from centre located in the Municipality, while in the centres in the satellite settlements it ranged from 18.1% to 20.9. There was no clustering of cases locality wise or relative to time. Only 17.9% of the total diarrhoeal children were in-patients of the various health centres.

Table 3: Hospital and locality distribution of bacterial organisms isolated from diarrhoeal children in the Federal Capital Territory, Abuja, 2008.(NHA-National hospital Abuja,MDH-Maitama District Hospital, UATH- University of Abuja Teaching Hospital, NGH-Nya-nya General Hospital, MHC-Mpape Health Centre)

Location	Name of hospital	No. of stools	No. of isolated bacteria	(%) of isolated bacteria
Municipality	NHA	80	62	22.4
	MDH	82	52	18.8
Satellite	UATH	81	55	19.8
	NGH	79	50	18.1
	MHC	82	58	20.9
Total		**404**	**277**	**100.0**

X^2=58.227, Pvalue=0.009, df =12

The isolation rates of bacterial species are as indicated in Figure 1. Figure 1**A** shows monthly isolation rate of strains of *Escherichia coli* .The monthly isolation rate of strains with specific pathogenic pattern shows that infectious diarrhoea is high in the months August and November as depicted by Figure 1**B**.The isolation rate of *Staphylococcus aureus* and *Klebsiella species* are indicated by Figure 1**B** portray these bacteria as veritable aetiological pathogens of infectious diarrhoea.

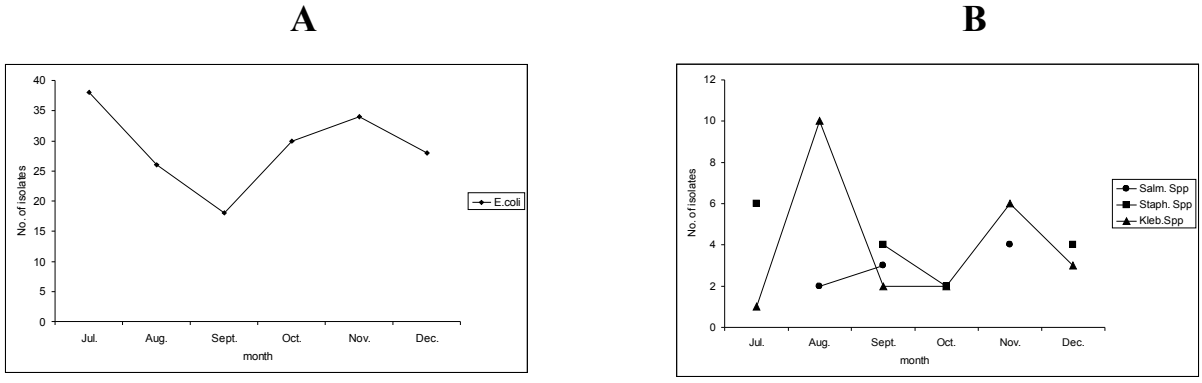
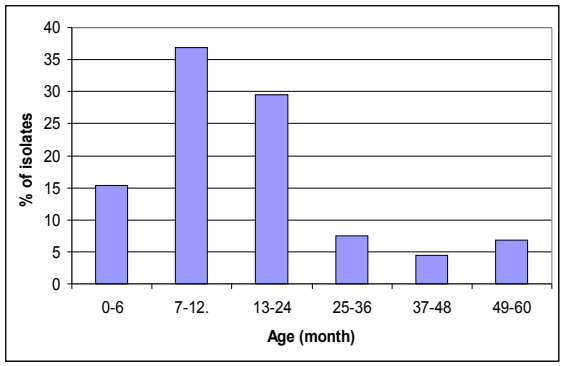


Figure 1: Monthly Isolation rate of bacteria organism: isolation rate of *Escherichia coli* (**A**); (*Salmonella species,Staphylococuus aureus and Klebsiella species)* (**B**)from diarrhoeal children in the Federal Capital Territory, Abuja, 2008

The age specific association of bacterial isolates are shown by Figure 2. The youngest child with diarrhoea was 3weeks old and within the youngest age group of 0-6months. The peak age (7-12 months) of enteropathogen detection depicts age specific association with infectious diarrhoea. Bacterial isolation age – wise diminished between the ages of 25-36 months and 49-60 months being lowest at the age of 37-48 months (Figure 2**A**). The isolation of strains of *Escherichia coli* was highest at the age of 13-24 months and lowest at the age of 37-48 months (Figure 2B). The result of the disk diffusion testing for the antibiotic susceptibility (data not shown) of the 174 strains of *Escherichia coli,* 9 strains of *Salmonella species*,16 strains of *Staphylococcus aureus*, and 24 strains of *Klebsiella species* showed a generally increasing resistance to Amoxycillin, Amoxycillin-clavulanic acid and Cephalexin by all strain tested. The strains of *Staphylococcus aureus* tested were 37.5% resistant to Nalidixic acid and Cefuroxime respectively, 31.3% were Ciprofloxacin – resistant, 87.5% were resistant to Amoxycillin.

A **B**



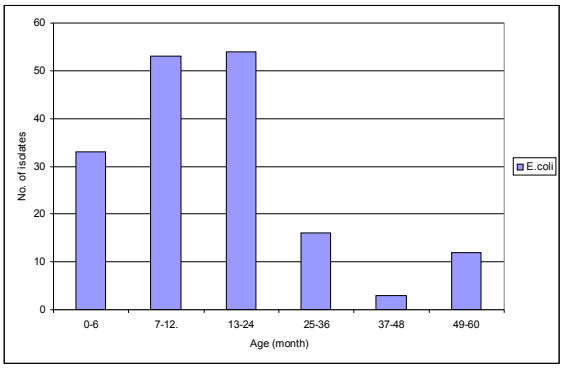
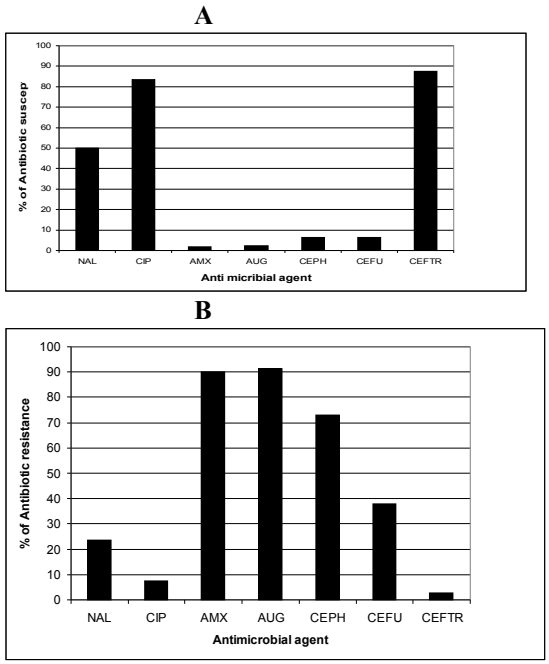


Figure 2: Age distribution of total bacteria isolate **A**; *Escherichia coli* (**B**) from diarrhoeal children in the Federal Capital Territory, Abuja, 2008.



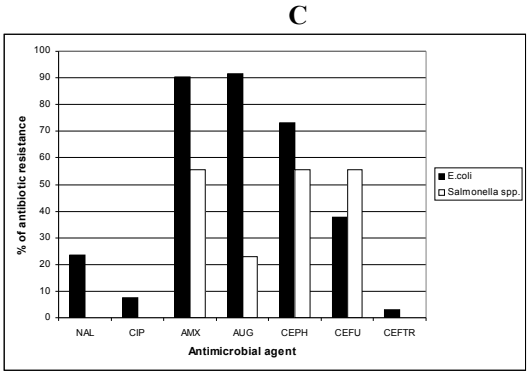


Figure 3: Antibiotic susceptibility pattern of *Escherichia coli* from diarrhoeal children in the Federal Capital Territory Abuja, 2008.
Nal:Nalidixicacid;
Cip: Ciprofloxacin;**Amx:**Amoxycillin;
Aug: Amoxycillin-clavulanic acid;
Ceph: Cephalexin;**Cefu:**Cefuroxime;
Ceftr: Ceftriaxone

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The strains of *Staphylococcus aureus* were equally 100% resistant to Amoxycilin-clavulanic acid and Cephalexin. Of the *Escherichia coli* strains, 23.6% were resistant to Nalidixic acid, 91.4% were Amoxycillin-clavulanic acid - resistant, 90% were resistant to Amoxycillin,73% were Cephalexin - resistant, and 37.9% were resistant to Cefuroxime(Figure3 A and B).The strains of *Salmonella species* showed 55.6% resistance to Amoxycillin, Amoxycilin-clavulanic acid, Cephalexin and Cefuroxime respectively. The antibiotic susceptibility pattern of strains *Escherichia coli* and strains of *Salmonella species* as compared in Figure 3C are distinct

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4. Discussion

Acute diarrhoea due to bacteria infections is an important cause of morbidity and mortality in infants and young children in most developing countries including Nigeria (Adegunloye, 2005). Clarification of the enteropathogens involved in diarrhoeal disease in the country is an essential step towards the implementation of effective primary health care activities against the disease (Olowe et al., 2003). In this study our result shows that eight bacterial species (*Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus Enterobacter cloacae*, *Pseudomonas species, Morganella morganii and Proteus species)* were isolated from diarrhoeal children. The prevalence of cases of diarrhoea in Abuja, Nigeria with a potential bacterial pathogen detected was 65.8% of all patients screened. This though in contrast to a recent report of 83.1% from similar study in Abakaliki, south – eastern Nigeria (Ogbu et al., 2008) is consistent with the reports of 63.3%-71.83% in Tanzania and 50-60% in other developing countries (Vargas et al., 2004). The variation in prevalence between the two Nigerian cities might be attributed to differences in infrastructural and socioeconomic indices. The percentage of diarrhoea cases from which bacteria was isolated ranged from 18.8% to 22.4% among stool samples from centres located in the Municipality and 18.1% to 20.9% from the centres in the satellite settlements. The difference between the Municipal and satellites centres shown by our data is similar to the report of a previous surveillance study in Korea (Seung-Hak et al., 2006).

Although there are geographical difference in the spectrum of bacteria incriminated in childhood diarrhoea, *Escherichia coli* and *Klebsiella* species were isolated at a relatively high rate. Statistical analysis showed that *Escherichia coli was* significantly associated with diarrhoea in children younger than 3 years (P<0.05). There appear to be conflicting reports about the association of *Salmonella* species with diarrhoea (Seung-Hak et al., 2006). Conversely, the occurrence of *Salmonella* species in this study is in conformity with the findings from Abakaliki, south –eastern Nigeria (Ogbu et al., 2008), Sao Paulo Brazil (Ethelberg et al.,2006) , Bissau, Guinea Bissau(Valetiner-Branth et al.,2003) and Hong Kong (Nelson et al., 2004). In addition, our report of *Salmonella* species (2.2%) is more inclined to former reports from similar studies in Bangladesh (Albert et al., 1999) and Korea (Seung-Hak et al., 2006). Bacterial isolation age – wise diminished between the ages of 25-36 months and is in consonance with past reports from Brazil,Denmark and Turkey(Olesen et al., 2005, Diniz-Santos et al., 2005 and Karadag et al., 2005). The seasonal variation in the incidence of the bacterial designated by monthly isolation rate in this study agrees with previous reports (Al-gallas et al., 2007).

We evaluated the antibiotic susceptibility of the strains of *Escherichia coli,* of *Salmonella species*, and *Staphylococcus aureus.* Our data depicts a prevalence of antimicrobial resistance by these strains to the β-lactam class of antibiotics (Amoxycillin, Amoxycillin-clavulanic acid, and Cephalexin) which are frequently used empirically for the treatment of diarrhoea. However, all the strains had varying percentage susceptibility to Nalidixic acid and Ciprofloxacin. This means that these antibiotics are fairly effective in the treatment of diarrhoea due to these pathogens but the use of Ciprofloxacin in young children has grave risks. It is noteworthy that the arbitrary empirical use of antibiotics might be responsible for the emerging resistance pattern as shown by this study.

This study determined the significance and the association of the strains of *Escherichia coli* as a predominant enteropathogen causing diarrhoea in children younger than 5 years in Abuja, Nigeria. We shall subsequently seek to further clarify this association with the respective diarrhoegenic *E.coli* categories by investigating their virulence properties.

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