Prevalence and Antibiotic Profile of *Enterobacter species* isolated from Children with Diarrhea in Abeokuta, Ogun State, Nigeria

[1, 2]Akingbade OA, [1]Olasunkanmi OI, [3]Akinjinmi AA, [4]Okerentugba PO, [5]Onajobi BI, [4]Okonko IO

[1]Department of Microbiology, Federal Medical Centre, Idi Aba, Abeokuta, Nigeria
[2]Department of Microbiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
E-mail: a.olusola@yahoo.co.uk, olusola.akingbade@yahoo.co.uk, 08063529234
E-mail: olasunkanmitayo@gmail.com, 08062392362
[3]Department of Chemical Pathology, Federal Medical Centre, Idi Aba, Abeokuta, Nigeria
E-mail: tundeuluv@yahoo.com, 08060706263
[4]Medical Microbiology Unit, Department of Microbiology, University of Port Harcourt,
P.M.B. 5323, Choba, East-West Road, Port Harcourt, Rivers State, Nigeria;
Tel: +2348035380891; E-Mail: mac2finney@yahoo.com, iheanyi.okonko@uniport.edu.ng
[5]Department of Biological Sciences, Al-Hikmah University, P.M.B. 1601, Adewole Ilorin, Kwara State, Nigeria.

Abstract: A total of one hundred and fifty diarrhoea faecal samples were collected from children presenting with diarrhoea. The biochemically identified *Enterobacter sp* isolates were subjected to the antibiotic sensitivity testing. The antibiotic sensitivity was carried out using Kirby-Bauer diffusion method. Of the 150 faecal samples collected, 19(12.6%) yielded growth of *Enterobacter sp*. Five (14.7%) of the *Enterobacter sp* were detected among patients within age ≤ 1 - 3 years while 14(12.1%) were recovered from children within age ≤ 4 - 5 years old. *Enterobacter sp* recorded high resistance to ampicillin 84.2%, cloxacillin (78.9%), cefotaxime (73.7%), cotrimoxazole and ceftazidime (63.2%) respectively and were susceptible to cefixime chloramphenicol and streptomycin. The high level of antimicrobial resistance observed in this study raises a broader discussion about the indiscriminate use or misuse of antibiotics and the risks of empirical antibiotic therapy in children of a very young age. In conclusion, regulating the use of antimicrobial may be necessary to reduce the resistance to drugs. Government also, should encourage the development of new vaccines to help reduce the incidence of emerging diarrheal diseases.

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1. Introduction

Diarrhoea and gastroenteritis comes the second position among top ten diseases admitted to hospital in the world (Bista *et al*., 1993). 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 investigations 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 than 315,000 deaths of preschool age children are recorded annually as a result of diarrhoea disease (Babaniyi, 1991; Alabi *et al*., 1998). The genus *Enterobacter* is more specifically a nosocomial opportunistic pathogen and is sought out to be one of the many key causes for extra intestinal infections next to *E. coli*. They are most frequently found in the gastrointestinal tract and are studied in clinical sites in stool samples (Atlas and Richard, 1996).

Enterobacter aerogenes as well as others in its genus are known to be resistant to antibiotics, especially *E. aerogenes* and *E. cloacae*. *Enterobacter aerogenes* causes disease in humans through inadvertent bacteria transfer in hospital settings. Selection of enteric bacteria like *E. aerogenes* is opportunistic and only infects those who already have suppressed host immunity defenses. Infants, the elderly, and those who are in the terminal stages of other disease or are immunosuppressed are prime candidates for such infections (Janda *et al*, 2006). Additionally, *E. aerogenes* as well as other enteric bacteria, is known to have drug-resistant characteristics. There has been some success in dealing with infections through antibiotics however; the fast development of multidrug resistance has become an increasingly growing problem (Sankaran, and Neeraja, 2000).

Enterobacter species produce type 1 or type 3 mannose sensitive hemagglutinins (MSHA) and rarely produce mannnose-resistant hemagglutinins. Additionally, production of a variety of siderophores

by enterobacters is also commonly seen. *E. cloacae* generate the hydroxyamate siderophore aerobactin, which is commonly used with microbial species that cause invasion disease. Additionally, several toxins have been found to be produced by *Enterobacter* species. Usually these toxins are described to having single strains or are limited in the number of isolates (Janda *et al*, 2006).

Treatment for *E. aerogenes* is difficult due to the highly resistant nature of the species. *Enterobacter aerogenes* has shown to display multidrug resistance due largely to mutations that encode porins (protein channels) and membrane efflux pumps that pump out antibiotics before they can harm the organism. These have been shown to be non-specific which accounts for their multiple drug resistance. Structurally unrelated molecules such as B-lactam antibiotics, quinolones, tetracyclines, and chloramphenicol are all kept at bay (Chevalier et al, 2004). In this study, we investigated the prevalence and antibiotic profile of *Enterobacter sp* isolated among children with diarrhea in Abeokuta, Nigeria.

2. MATERIALS AND METHODS

2.1. Study Population

The study population was diarrhea patients aged ≤ 5 years old attending the Out Patient Department (OPD) of the Sacred Heart Hospital, Lantoro Abeokuta, Ogun State, Nigeria. This study was approved by the Ethical committee of the hospital.

2.2. Sample Collection

A total of one hundred and fifty diarrhea faecal samples were collected from the patients. The diarrhoeal faecal samples were collected into sterile, transparent, wide mouthed bottles. The name, age and sex of the patients were properly labeled on the universal bottles.

2.3. Processing of Specimens

The specimens were processed according to the guidelines provided by Cheesbrough (2004) for the laboratory diagnosis of enteric pathogens.

2.4. Culture

The faecal samples were inoculated aerobically on sterile MacConkey agar and Eosin Methylene Blue Agar plates and incubated aerobically at 37^{o}C for 24 hours. Isolated pure cultures of bacteria were subjected to various morphological and biochemical tests.

2.5. Antimicrobial sensitivity testing

Commercially available antimicrobial discs (Abtek Biological Ltd UK) were used to determine the drug sensitivity and resistance pattern of the isolates. A number of 11 different antibiotics with different disc concentration such as Gentamycin (Gen), Erythromycin (Ery), Ampicillin (Amp), Cefixime (Cf), Cotrimoxazole (Cot), Tetracycline (Tet), Streptomycin (Str), Cloxacillin (Cxc), Cefotaxime (Cft), Ceftazidime (Caz) and Chloramphenicol (Chl) were used in this study. The antimicrobial sensitivity test of each isolate was carried out as described by the Kirby – Bauer disc diffusion method (Bauer *et al*; 1966) as recommended by the National Committee for Clinical Laboratory Standards. The turbidity of the bacterial suspensions was compared with 0.5Macfarland's barium sulfate standard solution. The standardized bacterial suspension was then swabbed and inoculated on to Muller Hinton Agar (Lab M Limited, UK) using sterile cotton swabs and left to dry for 10minutes, before placing the antimicrobial sensitivity discs. Antibiotic impregnated discs of 8mm diameter were used for the test. After incubation, the diameter of the zone of inhibition were measured and compared with zone diameter interpretative chart (CLSI 2007) to determine the sensitivity of the isolates to antibiotics.

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3. Results

Out of the 150 faecal samples collected from children presenting with diarrhea, 19(12.6%) yielded growth of *Enterobacter sp.* Table 1 showed prevalence of *Enterobacter sp* in relation to sex. In table 2, Five (14.7%) of the *Enterobacter sp* detected was found among patients within age ≤ 1 – 3 years while 14(12.1%) were recovered from patients within age ≤ 4 - 5 years. The susceptibility studies showed that *Enterobacter sp* were susceptible to cefixime (63.2%), chloramphenicol and streptomycin (57.9%), high resistance was recorded to ampicillin (84.2%), cloxacillin (78.9%), cefuroxime (73.7%), cotrimoxazole and ceftazidime (63.2%) respectively (Table 3).

Table 1: Prevalence of *Enterobacter sp* in relation to sex

Sex	No. of faecal samples tested	No. / (%) positive *Enterobacter sp*
Females	76	12(15.8%)
Males	74	7(9.5%)
Total	150	19(12.6%)

Table 2: Prevalence of *Enterobacter sp* in relation to age

Age group	No. of faecal samples tested	No. / (%) positive for *Enterobacter sp*
≤1 – 3	34	5(14.7)
≤ 4 -5	116	14(12.1)
Total	150	19(12.6%)

Table 3: *In - vitro* susceptibility patterns of *Enterobacter sp* isolates from diarrheoa faecal samples

No.	Amp	Cxc	Caz	Cft	Cxm	Gen	Str	Tet	Chl	Cot	Ery
S	3(15.8)	4(21.1)	7(36.8)	12(63.2)	5(26.3)	10(52.6)	11(57.9)	10(52.6)	11(57.9)	7(36.8)	10(52.6)
R	16(84.2)	15(78.9)	12(63.2)	7(36.8)	14(73.7)	9(47.4)	8(42.1)	9(47.4)	8(42.1)	12(63.2)	9(47.4)

Gen = Gentamycin, Amp = Ampicillin, Cf = Cefixime, Cot = Cotrimoxazole, Tet = Tetracycline, Str = Streptomycin, Cxc = Cloxacillin, Amx = Amoxicillin, Cft = Cefotaxime, Caz = Ceftazidime, Er = Erythromycin, S – Sensitive, R – Resistant

4. DISCUSSION

The study showed that 12.6% of children with diarrhoea had *Enterobacter sp* infection similarly; many researchers have also isolated *Enterobacter* species from faecal samples of children and adults (Jung *et al,* 2008 and Motayo *et al*, 2013). *Enterobacter sp* has not been included among the important bacteria that cause diarrhoea infections. It's presence in 12.6% of the diarrhoea cases in this study showed that the specie should not be over looked. Bahal *et al.*, (2001) reported that the most important cause of acute watery diarrhea in young children in Nigeria include *rotavirus*, enterotoxigenic *Escherichia coli, Shigella, Campylobacter jejuni* and *Cryptosporidia, Vibrio cholerae, Salmonella* and enteropathogenic *Escherichia*. But studies have shown that most strains of Enterobacter express an aerobactin-mediated iron uptake system, commonly associated with extra-intestinal human bacterial pathogens. Some strains produce a haemolysin resembling the α-haemolysin produced by strains of *E. coli*. Additionally, an outer membrane protein, OmpX, may be a pathogenic factor for *Enterobacter sp.* This particular protein appears to reduce the production of porins on the gram-negative bacteria, leading to decreased sensitivity to β-lactam antibiotics and therefore might play a role in cell invasion of the host Greenwood *et al* (2002).

Enterobacter sp generate hydroxyamate siderophore aerobactin, which is common with microbial species that cause invasion disease (Greenwood *et al,* 2002). The antibiogram from this study revealed that *Enterobacter sp* was resistant to 84.2% of the ampicillin and had 78.9%, 63.2% and 57.9% resistant to cloxacillin, cotrimoxazole and streptomycin respectively, similar research has shown that *Enterobacter sp* are resistant to ampicillin (Bailey and Scott, 1974).

According to Greenwood *et al* (2002) the treatment of *Enterobacter sp* is difficult due to high resistant nature of the species. *Enterobacter sp* had high resistant to cefuroxime and ceftazidime in this study, this is in agreement to a research conducted by Greenwood *et al* (2002) who also reported high resistant to cephalosporins. The resistant to penicillin class (such as ampicillin and cloxacillin) and cephalosporins class (such as ceftazidime and cefuroxime) in this study might be because of the production of chromosomal beta-lactamase with cephalosprinase activity. According to Greenwood *et al* (2002) *Enterobacter sp* possess an inducible chromosomal cephalosporinase, allowing for the rapid development of resistance during treatment or therapy.

The resistant of *Enterobacter sp* to two of the third generation cephalosporins class (ceftazidime and cefotaxime) in this study is worrisome because third generation cephalosporins have wide antimicrobial activity spectrum and fewer adverse effects and considered by many as the best drugs for the empirical treatment of severe acute infectious diarrhea in children (Leibovitz *et al.*, 2000).

Cephalosporins are under the class of B-Lactam antibiotics and this type of antibiotics work by inhibiting bacterial cell wall synthesis. B-Lactams covalently binds to and inactivates transpeptidase enzymes, which are responsible for cross-linking the amino acid that forms the peptidoglycan layer of the cell wall. Since the transpeptidase enzyme is now inactivated by the antibiotic, and the cell wall can no longer form cross linkages, the cell will eventually burst and lyse, thus killing the bacteria. Bacteria have developed different types of resistance to the antibiotics. For example, the enzyme B-lactamase serves to hydrolyze and break the B-Lactam rings of antibiotics and therefore nullifies the antibiotics effects but research as shown that the B-Lactamase of

Enterobacter sp plays a different role. They do not hydrolyze the B-Lactam rings instead *Enterobacter sp* resistance to cephalosporins arises from mutation due to constitutive production of B-Lactamases. Some sort of mutation caused the prevention of the binding of the drug to the enzyme.

Tetracycline, chloramphenicol, gentamycin and streptomycin were sensitive to *Enterobacter sp* in this study this is opposed to the resistant reported by Greenwood *et al* (2002) to tetracycline, chloramphenicol, streptomycin and gentamycin. According to Hogenauer *et al* (1998) in spite of the low cost and broad antimicrobial spectrum of tetracycline, the use of tetracycline in pediatric patients is limited by permanent dental discoloration in children younger than eight years of age. The result from this study draws attention to the importance of notifying diarrheal disease. The high level of antimicrobial resistance observed in this study raises a broader discussion about the indiscriminate use or misuse of antibiotics and the risks of empirical antibiotic therapy in children of a very young age. In conclusion, regulating the use of antimicrobial may be necessary to reduce the resistance to drugs. Government also, should encourage the development of new vaccines to help reduce the incidence of emerging diarrheal diseases.

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