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Research Article

Frequency of Isolation of *Enterobacter* Species from a Variety of Clinical Specimens in a Teaching Hospital in Nigeria

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Abstract

Purpose: *To determine the frequency of occurrence of Enterobacter species and their antibiogram from clinical specimens of blood, cerebrospinal fluid, urine and wound obtained from University of Benin Teaching Hospital, Benin City, Nigeria.*
Methods: *Specimens were obtained from patients who were seen at the various units of the hospital during the period January 2008 to June 2010. The total number of specimens was 6632, and were obtained from 1678 adult males, 2010 adult females and 2944 children. The specimens were collected prior to commencement of antibiotic therapy, and cultured immediately using standard bacteriological methods. Growths were identified by colonial morphology and characteristics, and biochemical reactions. Antimicrobial sensitivity test was performed according to Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) recommendation. The control organism was a sensitive strain of Eschrichia coli (NCTC 10418)*
R***esults:*** *Two species of Enterobacter, namely, E. aerogenes (104; 97.2 %) and E. sakazakii (3; 2.8 %) were isolated from the four types of clinical specimens, accounting for 1.6 % of all the samples. Sensitivity to antibacterials was as follows: ceftazidime (55.0 %), ofloxacin (53.3 %) and amoxicillin clavulanate (48.3 %). They were strongly resistant to the other antibiotics used in the study, especially the cephalosporins. There was no significant difference in infection rate among the age groups ($p > 0.05$). However, there was significant difference ($p < 0.05$) between isolates from cerebrospinal fluid, on the one hand, and those from wound, urine and blood, on the other hand.*
Conclusion: *The rate of isolation of Enterobacter species in the health facility was low. Remarkable drug resistance of the organisms make them clinically significant pathogens.*

Keywords: *β-Lactam antibiotics, Opportunistic infections, Bacterial resistance, Enterobacter species.*

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INTRODUCTION

Enterobacter aerogenes, *E. Cloacae* and *E. sakazakii* are commonly encountered *Enterobacter* spp. in most clinical specimens. These three species are differentiated by urease test and pigment production. *E. cloacae* is urease positive while the other two are negative. *E. sakazakii* produces yellow pigment which differentiates it from the other two species [1].

These organisms are Gram-negative capsulated bacilli and in the Petri dish, form round mucoid colonies; optimum growth temperature is 37 ^{0}C. *Enterobacter* species are similar to *Klebsiella* species with large round mucoid colonies, but can be differentiated by a few tests such as motility and urease tests [2]. They are facultatively anaerobic, catalase positive, citrate positive, indole negative and oxidase negative. They ferment glucose and lactose with the production of acid, and are sucrose positive [2].

The organisms are distributed in water, soil, sewage, dairy products and vegetables, are part of the commensal enteric flora and usually are not pathogenic. However, some strains are known to produce shig-like toxin [3]. *Enterobacter* species have also been associated with nosocomial infections and a variety of opportunistic infections involving the urinary and respiratory tracts, and cutaneous wounds. *E. sakazakii* which is a pigmented strain of *E. cloacae* has been encountered in several cases of meningitis, bacteremia and sepsis [4]. It has also been associated with outbreak of necrotizing enterocolitis associated with the strain in powdered milk formula, and fatality rate is as high as 75 %. [5,6]. These enterobacter organisms cause significant morbidity and mortality. They can also cause community acquired infections resulting in endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis and ophthalmic infections [7]. Risk factors for nosocomial infections include hospitalization for more than 2 weeks, invasive procedures and presence of central venous catheter, hands of hospital personnel and hospital equipments. Enterobacter species has an outer membrane that contains among other things lipopolysaccharides from which Lipid-A plays a major role in sepsis. Lipid-A, which is an endotoxin, is the major stimulus for the release of cytokines which are the mediators of systemic inflammation and its complications [8] *Enterobacter* species are resistant to most antibiotics, especially the cephalosporins. Their resistance to β-lactam antibiotics, chloramphenicol, quinolones and tetracyclines have been well documented [9]. Reports abound in the literature on the increasing resistance of *Enterobacter* species to the penicillins and all generations of cephalosporins, and their emergence in clinical specimens.

The paucity of information on the clinical significance of the *Enterobacter* species in Edo State south-central Nigeria, necessitated this study, the aim of which was to determine the frequency of isolation of Enterobacter species and their antibiogram from clinical specimens obtained from University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.

EXPERIMENTAL

Materials

Chocolate and blood agars, McConky agar, nutrient agar (oxoid no cm 2), Cistern lactose electrolyte deficient (CLED) agar, brain heart infusion broth and thioglycolate broth were all obtained from Oxoid, UK.

Study setting

The study, which was prospective and cross-sectional, was carried out at the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The hospital has a bed complement of six hundred (600) and an equipped microbiology laboratory that provides laboratory services to the neighbouring states of Ondo, Delta, Kogi and Anambra.

Sampling method

The samples used in the study were obtained consecutively from patients who were seen at the various facilities in the hospital during the period January 2008 to June 2010. The patient population was 6632, comprising 1678 (25.30 %) adult males, 2010 (30.32 %) adult females and 2944 (44.38 %) children. Only one sample per patient was obtained. The clinical specimens consisted of wound, blood, cerebrospinal fluid and urine.

The number of wound specimens was 2960 (44.63 %) made up of 1040 (35.1 %) from adult males, 920 (31.1 %) from adult females and 1000 (33.8 %) from children. Urine samples numbered 1840 (27.74 %) comprising 610 (33.2 %) from adult males, 960 (52.2 %) from adult females and 270 (16.7 %) from children. Blood samples numbered 328 (4.95 %) made up of 18 (5.5 %) from adult males, 109 (33.2 %) from adult females and 201 (61.3 %) from children. The total number of cerebrospinal fluid samples was 1504 (22.7 %) consisting of 10 (0.7 %) from adult males, 21(1.4 %) from adult females and 1473 (97.9 %) from children.

Culture of isolates

Wounds

Wound swab was cultured on blood and MacConkey agars. The specimens were aseptically inoculated on the media and streaked to obtain distinct colonies [10]. After inoculating on media, a grease-free slide was smeared for Gram staining and microscopy. Two blood agar plates inoculated with specimen were incubated - one aerobically and the other anaerobically. The anaerobic incubation was done using a gas pact in an anaerobic jar. The pact was cut on one side to introduce 10 ml of water and placed in a jar along with the plates for anaerobic incubation. The jar was closed and was not opened until 24 h later.

Blood

Blood samples were aseptically collected from patients, 1 ml of which was aseptically introduced into nutrient and thioglycollate broths in bottles and incubated at body temperature (37 ^{o}C). The bottles were examined daily for growth (which manifests as turbidity or gas bubbles). Where growth was detected, the nutrient broth was subcultured on chocolate, blood and MacConkey agars and incubated aerobically while thioglycollate broth was subcultured on blood agar and incubated anaerobically as described above. Following subculture, Gram staining and microscopy were carried out.

Cerebrospinal fluid

Cerebrospinal fluid (0.5 ml) was obtained through a spinal tap and cultured immediately on chocolate, blood and MacConkey agar plates. Gram staining and microscopy were carried out. The culture on chocolate plate was incubated in 5 - 10% carbon dioxide environment for 24 h at 37 ^{o}C.

Urine

Urine was obtained in a 20 ml sterilin container and after thorough mixing, a wire loop (30 morse gauge) with a chamber of 3.26 mm circumference was used to streak the mixed urine on blood, MacConkey andCLED agars, and incubated aerobically for 24 h. All the plates were incubated at a temperature of 37 ^{o}C. Isolates were identified based on colonial morphology, motility, Gram staining, oxidase and biochemical reactions using the protocol of Cowan and Steel [2,11].

Susceptibility studies

Susceptibility of isolates was determined by the agar diffusion method, using a modification of Kirby-Bauer disc diffusion technique [12]. The antibiotic discs (Abtek Biologicals Ltd, Liverpool, England) were: ceftazidime 30 µg, ofloxacin 5 µg, amoxicillin clavulanate 30 µg, gentamycin 10 µg,

ceftriazone 30 µg, cefuroxime 30 µg, cefotaxime 30 µg, cloxacillin 10 µg, furadantoin 300 µg, and erythromycin 10 µg.

Isolates from overnight culture were used for the sensitivity test. A colony of the isolate was picked with a straight sterile wire, inoculated into sterile 5 ml peptone water, shaken to dissolve it and poured on a sterile nutrient agar plate. The inoculated agar plate was swayed gently to ensure that the whole agar surface was covered. The plate was drained to remove excess fluid. The antibiotic discs were placed on the agar surface, leaving a space of 25mm between them, and pressed slightly to ensure sufficient contact with the agar surface. The plates were then incubated at 37 ^{o}C for 24 h. The inhibition zone diameter (IZD) was measured, and IZD < 5 mm was taken as resistance while IZD > 5 mm was considered sensitive.

Statistical analysis

Gen Stat software (release 8.1) was used to analyze the data obtained. Differences in the distribution of isolates among the age groups as well as among the clinical specimen types were determined while statistically significant differences were computed Student's t-test and analysis of variance (ANOVA). Differences between specimen types (p <0.05) was determined using Duncan multiple range test.

RESULTS

The specimens (6632) yielded 107 (1.6%) isolates of enterobacter species.There were 104 *Enterobacter aerogenes* and 3 *Enterobacter sakazakii* spp. The distribution of organisms among the age groups are shown in Table 1. There was no significant difference in the distribution of isolates among the age groups (p < 0.05). However, as Table 2 shows, there was a significant difference between isolates from cerebrospinal fluid and those from other specimens (p < 0.05) except for the isolates from blood specimens.

The susceptibility of the *Enterobacter* isolates to various antibiotics is shown in Tables 4 and 5.

Based on the antibiogram, *Enterobacter aerogenes* was moderately sensitive to ceftazidime, ofloxacin and amoxicillin clavulanate while showing strong resistance to the other drugs. *Enterobacter sakazakii*, on the other hand, exhibited higher susceptibility to the antibiotics except erythromycin, cloxacillin and oxacillin. *Enterobacter aerogenes*, which was isolated from urine, demonstrated good susceptibility to nitrofuradantoin but was moderately susceptible to ceftazidime and ofloxacin.

Table 1: Distribution of isolates from specimens among adult males, females and children

Specimen	Distribution of isolates				Frequency (%)
	Total	Adult male	Female adult	Children	
Wound	45	8	12	25	0.71
Urine	44	15	22	7	0.61
Blood	17	2	3	12	0.25
CSF*	1	Nil	Nil	1	0.01
Total	*107*	*25 (0.37%)*	*37 (0.56%)*	*45 (0.68%)*	*1.58*

**Cerebrospinal fluid based on the total number of samples*

Table 2: Isolates in all specimens and their frequency

Isolates	Number	Frequency (%)
Proteus rettgerri	383	5.77
Staphylococcus aureus	1564	23.58
Klebsiella pneumoniae	638	9.65
Escherichia coli	782	11.79
Pseudomonas aeruginosa	686	10.33
Alcaligenes faecali	628	9.47
Providencia stuartii	293	4.41
Proteus mirabilis	498	5.51
Proteus vulgaris	438	6.60
Proteus morganii	194	2.93
Candida albicans	283	4.27

Table 3: Distribution of *Enterobacter* spp among the specimens

Specimen	Distribution of isolates			Frequency (%)
	Total	*E. aerogenes*	*E. sakazakii*	
Wound	45	45	Nil	0.71
Urine	44	44	Nil	0.61
Blood	17	14	3	0.25
CSF*	1	1	Nil	0.01
Total	*107*	*104*	*3*	*1.58*

**Cerebrospinal fluid*

Table 4: Antibiogram of *E. aeroges* and *E sakazakii* in wound, blood and cerebrospinal fluid specimens (percentages in parentheses)

Organism	Caz	Ofx	Aug	CN	CXM	CRO	CTX	E	CL	OXC	OB
E aerogenes	33 (55)	32 (53.3)	29 (48.3)	14 (23.3)	10 (16.6)	13 (21.6)	3 (5.0)	nil	3 (5.0)	3 (5.0)	4 (6.6)
E. sakazakii	2 (66.6)	2 (66.6)	2 (66.6)	2 (66.6)	2 (66.6)	2 (66.6)	2 (66.6)	nil	2 (66.6)	1 (33.3)	1 (33.3)

Caz = ceftazidime; Ofx = ofloxacin; Aug = amoxicillin clavulanate; CN = gentamicin; CXM = cefuroxime; CRO = ceftriaxone; CTX = cefotazime; E = erythromycin; CL = cephalezime; OXC = oxacillin; OB = cloxacillin

Table 5: Antibiogram of *E. aerogenes* isolated from urine specimens (percentages in parentheses)

Organism	Total	CAZ	OFX	AUG	CN	CXM	CRO	CTX	E	F	OB
E. aerogenes	44	24 (54.5)	19 (43.1)	15 (34.1)	11 (25)	4 (9.1)	5 (11.4)	6 (13.6)	2 (4.55)	35 (79.5)	Nil

Caz = ceftazidime; Ofx = ofloxacin; Aug = amoxicillin clavulanate; CN = gentamicin;
CXM = cefuroxime; CRO = ceftriaxone; CTX = cefotazime; E = erythromycin; CL = cephalezime; OXC = oxacillin; OB = cloxacillin

DISCUSSION

The study shows a very low distribution of *Enterobacter* species in University of Benin Teaching Hospital. The existence of many enterobacter species in clinical samples has been reported [7]. The present study found mainly *E. aerogenes* and an insignificant number of *Enterobacter sakazakii*. Other reports in the literature claimed the presence of *E. cloacae, E. taylorae, E. aerogenes* and *E sakazakii* in clinical specimens [13]. *E aerogenes*, which was the predominant isolate in our study, is commonly encountered in clinical specimens. It is known to be associated with a variety of opportunistic infections [3]. It is associated with infections involving the urinary and respiratrory tracts, and cutaneous wounds, and occasionally causes sepsis and meningitis. *E. sakazakii* which was scantily encountered in the study has been reported in several cases of neonatal meningitis and sepsis, and has a high fatality rate [4]. Thus, the organism can be highly virulent [5,6]. Recent reports have linked *E. sakazakii* infection in neonates with contaminated powdered milk formulas [14].

The epidemiology of *Enterobacter* infection as seen from this study appear to place children at relatively higher risk of infection, followed by adult females, with adult males least predisposed to infection. However, the differences were not statistically significant ($p > 0.05$). This mean that all age groups and gender types are equally predisposed to infection. Children are, however have been known to be most susceptible to any infection as a result of habits, exposure and lower immunity. *Enterobacter* infection is largely associated with decreased immunity. Those heavily affected are the immunocompromised as well as factors such as increased length of stay in the hospital; increased length of stay in the intensive unit; those on urinary catheter and long term antibiotic administration [15]. The distribution of enterobacter species in the clinical specimens indicated a significant difference among the specimen types. There was statistically no difference in the distribution of isolates in the other specimen categories. Thus cerebrospinal fluid (CSF) is the least likely to be infected *Enterbacter* spp. This may be due to the fact that organisms do not easily gain access to the cerebrospinal fluid as a result of some sort of barrier.

The antibiogram showed that *Enterobacter aerogenes* exhibited high resistance to the antibiotics used in the study; these are also the antibiotics commonly used in these setting for the treatment of infections. The observed resistance is agrees with literature reports which claimed that *Enterobacter* species are reisistant to most antibiotics. The resistance of this species to β-lactam antibiotics, chloranphenicol, quinolones and tetracycline is well documented in scientific literature [9]. There are numerous reports in the literature on the increasing resistance of *Enterobacter* species to penicillins and all generations of cephalosporins and their emergence in clinical specimens [9].

The antibiogram of *Enterobacter sakazakii* showed the organism to be more susceptible to most antibiotics, except oxacillin and cloxacillin *Enterobacter aerogenes* was the only species of *Enterobacter* that was isolated from urine specimens and they exhibited a slightly different antibiogram. They were less susceptible than those from wound, blood and cerebrospinal fluid. However, the high susceptibility to furadantoin is an indication that the drug could be the drug of choice for any Enterobacter urinary tract infection. They were moderately susceptible to ceftaxidime and ofloxacin. The high resistance to other drugs in the antibiogram should be a cause for concern.

Pathology issues

The *Enterobacter* species, like other members of *Enterobacteriaceae* family contain an outer membrane which contains, among others, lipopolysaccharides of which lipid-A plays a major role in sepsis. Lipid-A,

which is an endotoxin, is the major stimulus for the release of cytokines which are the mediators of systemic inflammation and its complications [8]. In the present study, ceftaxidime (a cephalosporin) and ofloxacin (a quinolone) exhibited some moderate efficacy and thus could be effective in the management of *Enterobacter* infection. This observation contradicts some literature claims which reported that *Enterobacter* spp are resistant to these drugs [9].

The high resistance of *Enterobacter* organisms is associated with the production of extended spectrum β-lactamase which confers on them resistance to many antibiotics. β-Lactamases are the major defense systems of Gram-negative bacteria [16]. Since β-lactam antibiotics came into clinical use, β-lactamase evolved with them [17]. It is very likely that *Enterobacter* species acquired their resistance to drugs through the production of this enzyme, β-lactamase. Worst still, enterobacter species together with some members of Enterobacteriaceae carry a gene for chromosomally encoded β-lactamase. Unlike the plasmid-mediated gene, the enzyme is not usually expressed in the uninducible form. They become induced by antibiotics, body fluids and some amino acids. It is a concern that organisms such as *Enterobacter aerogenes* harboring genes for inducible β-lactamase may show false susceptibility if tested in the uninduced state[16]. This may explain the causes of some treatment failures as the organism develops resistance during therapy. For this reason, management of any enterobacter infection should be done with caution.

Risk factors for enterobacter infections include not only long hospitalization and presence of indwelling catheters since infections have also been traced to equipment, contaminated objects and hands of hospital personnel. The risk factors for extended spectrum beta lactamase (ESBL)-producing organisms are little different from the risk factors of other nosocomial infections [18]. In outbreak situations, successful intervention has usually involved the institution of barrier precautions in the form of hand washing, as well wearing of cloves and gowns. Presently, there are no β-lactam antibiotics in development that can treat infections caused by organisms producing some of the new β-lactamases. The available agents should be used judiciously and effective control measures should be implemented in situations of outbreak to prevent further spread of the pathogens.

CONCLUSION

The frequency of isolation of *Enterobacter* species from clinical specimens obtained from University of Benin Teaching Hospital, Nigeria was low. Virulence and remarkable drug resistance of the organisms make them clinically significant pathogens.

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