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Full Length Research Paper

A survey of Enterobacteriaceae in hospital and community acquired infections among adults in a tertiary health institution in Southwestern Nigeria

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Hospital and community acquired infections, continue to be a threat to public health, causing morbidities and mortalities. This survey was carried out to determine the prevalence of Enterobacteriaceae in nosocomial and community acquired infections among adults in LAUTECH Teaching Hospital in Osogbo southwestern Nigeria. Two hundred and forty isolates from General Out Patient Department (GOPD) and two hundred and forty isolates from different wards (Surgical, Medical, Gyneacological, Pediatric, Burn Unit and Ear, Nose and Throat wards) of the hospital were collected. The bacterial strains were isolated from Cerebrospinal fluid (CSF), Urine, Pus, Ear swab, Blood, Sputum and Pleural fluid. The isolates were identified on the basis of standard microbiological and biochemical techniques as describe by Cowan and Steel. The incidence of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae* and *Citrobacter freundii* was studied according to their distribution among different wards and nosocomial patients, specimens and age groups of patients. Five genera belonging to the family of bacteria Enterobacteriaceae were isolated from 240 community acquired infections and hospital acquired infections in different wards according to different age groups in this study. *E. coli* were most frequent in all the specimens with 49.2 and 47.5% for urine samples in community acquired and nosocomial infection respectively. Nosocomial infections are common with *E. coli* and *K. pneumoniae* causing a significant proportion of these community acquired infections.

Key words: Enterobacteriacae, hospital acquired, community, tertiary health institution.

INTRODUCTION

Hospital and community acquired infections constitutes serious public health problem throughout the world causing morbidity and mortality. Hospital acquired infections are generally described as infection acquired during hospital care or stay which was not present or incubating at the time of admission. This is in contrast to community-acquired infections which are acquired anywhere other than in a healthcare facility, in settings such as schools, exercise facilities, or any place you come in contact with other people or with surfaces that have been contaminated (Somwant et al., 2007; Coffin and Zaoutis, 2008).

Several studies have however, reported a prevalence of hospital acquired infections between 5 and 10% (Somwant et al., 2007; Olawale et al., 2011; Pittet et al., 2008). *Escherichia, Klebsiella, Enterobacter, Serratia, Proteus* and *Citrobacter*, genera are obligatory and opportunistic pathogens responsible for infections ranging from urinary tract, surgical wounds and lower respiratory tract infections (Mouton et al., 2001) among hospital acquired infections. Many species of these

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general are members of the normal intestinal flora. *Escherichia coli* are the most common isolates reported from many hospital laboratories (Bello et al., 2005).

Bacteremia is caused by *Klebsiella, Enterobacter,* and *Serratia* species and they are also frequently involved in infections with respiratory tract procedures, such as tracheostomy and manipulations using contaminated inhalation therapy equipments (Bello et al., 2005). Other organisms that are occasionally encountered in Urinary tract infections are *Klebsiella, Enterobacter* and *Serratia* species (Shah et al., 2002). *E. coli* causes approximately 86% of urothrocystitis, about 8% of chronic bacterial prostatitis and up to 90% acute pyelonephritis (Guentzel, 1995). Proteus species frequently cause infections of the urinary tract, surgical wounds and lower respiratory tract (Alli et al., 1998). *Proteus mirabilis* is believed to be the most common cause of infection – related kidney stones, which is one of the most serious complications of recurrent bacteriuria (Alli et al., 1998). *Citrobacter freundii* and *C. diversus* have been isolated predominantly as super infecting agents from urinary and respiratory tract infections. *Citrobacter* septicaemia may occur in patients with multiple predisposing factors; *Citrobacter* species also cause meningitis, septicemia and pulmonary infection in neonates and young children (Shah et al., 2002).

In hospital acquired pneumonias, bacteria such as *Pseudomonas aeruginosa*, *Enterobacter*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens* and *Proteus* species are the most frequently isolated pathogens causing nosocomial infections. Pathogenesis can be caused by aspiration or inhalation of aerosolized particles containing the bacteria. Colonization of the gram negative bacteria in the pharynx, increased gastric pH and contaminated equipment are the primary source of pathogenesis.

In community acquired infections, the major cause of urinary tract infections is *E. coli* (Guentzel, 1995). It also account for majority of cases of prostatitis and pyelonephritis. *Klebsiella, Proteus* and *Enterobacter* species are also other common urinary tract pathogens (Alli et al., 1998). *K. pneumoniae* accounts for a small percentage of pneumonia cases, however, extensive damage produced by the organism results in high case fatality rates over 80% of untreated cases.

Disturbance or eradication of the normal intestinal and body flora generally by antibiotic therapy may allow resistant nosocomial strains to overgrow (Alli et al., 1998). Nosocomial strains progressively colonize the intestine, pharynx or other organs with increase length of mobility and hospital stay. This may result into an increase risk of infections (Shah et al., 2002). The bacteria responsible for many common out patient infections too have developed resistant strains, which are posing new obstacles to effective therapy (Butler et al., 2001). The major sites of nosocomial infections, in order of decreasing frequency are the urinary tract, surgical sites, pneumonias (lung infection), and blood stream. The most incriminated premier nosocomial pathogen is *E. coli* (Guentzel, 1995).

Nosocomial infections are a serious threat to all hospitalized patients and in particular to those who require endotracheal intubation and mechanical ventilation. Accurate diagnosis of pneumonia and correct identification of pathogens of great importance and should be achieved as quickly as possible to avoid prolonged hospitalization and increased risk of mortality (Shah et al., 2002). With better understanding and treatment of nosocomial infections, the risks of contracting nosocomial infections associated with these pathogens can be reduced to the nearest minimum if not eradicated. It is against this background that this study was designed to determine and analyze the prevalence of Enterobacteriaceae in relation to community and hospital acquired infections in wards, specimens and age of patients in LAUTECH Teaching Hospital Osogbo in Southwestern Nigeria.

MATERIALS AND METHODS

Study location

This survey was carried out between July 2009 and June 2010 at the Medical Microbiology and Parasitology Department of LAUTECH Teaching Hospital Osogbo, Osun State, Nigeria, to find out the incidence of Enterobacteriaceae in community acquired and nosocomial infections.

Selection of cases

Some of the known risk factors for these hospital and acquired community infections include older age, long period of hospital stay, having invasive or manipulative procedures carried out, subjects staying in wards with traditionally high prevalence of nosocomial infections, long period on immunosuppressive drugs, immunosuppressive conditions, irrational use of antibiotics, improper hospital waste disposal and the absence of hospital implementation policy on infection control. This could form the basis for comparism of case selections. Two hundred and forty samples were collected from patients attending general outpatient department and another set of two hundred and forty bacterial samples were obtained from patients hospitalized in different wards of LAUTECH Teaching Hospital Osogbo. The nosocomial isolates were isolated from patients who had a minimum of 7 days in the hospital as described by Shah et al. (2002) prior to sample collection and the subjects must be free of infection at the time of hospital admission. Various samples collected from the patients like Blood, Cerebrospinal fluid (CSF), pus, sputum, urine, pleural fluid and peritoneal fluid were cultured for the presence of bacteria belonging to the family Enterobacteriaeae according to Shah et al. (2002).

Reagents and cultural media

Blood agar base, Cystein Lactose Electrolytes Deficiency (CLED) agar and Mac Conkey's agar obtained from Oxoid Ltd. Basing stoke, Hamphire, England. Triple sugar iron agar, peptone water, motility, indole and gas (H_2S) test medium, citrate test medium and urease test medium were obtained from Difco Laboratories, Detroit, Michigan, USA.

Table 1. Distribution of Nosocomial isolates in various specimens.

Specimen \ Isolates	*Escherichia coli*	*Klebsiella pneumoniae*	*Enterobacter cloacae*	*Proteus mirabilis*	*Proteus vulgaris*	*Citrobacter freundii*	Total
CSF	03	04	00	00	00	00	07
Blood	07	06	02	02	00	00	17
Urine	76	30	8	12	10	08	144
Pus and other fluids	32	20	10	04	04	02	72
Total	118	60	20	18	14	10	240

Culture of specimen

All the samples were inoculated on blood agar and Mac Conkey's agar except urine samples which were inoculated on CLED agar. Inoculated plates were in incubated at 37℃ in ambie nt air for 16 – 24 h as described by Cowan and Steel (1970).

Identification of Isolates

After overnight incubation, the culture plates were examined for growth. Identification was performed macroscopically and microscopically by using the standard microbiological and biochemical techniques (Cowan and Steel, 1970; Shal et al., 2002). These criteria were used in pathogens identification.

Sensitivity testing of isolates

All bacterial isolated were characterized by using the standard methods described by Pekarski (1989). Stokes disc diffusion agar method was employed for Antibiogram of isolates obtained. The antibiotics used include augumentin (30 µg), peflacine (30 µg), ceftriaxone (10 µg), cephalexin (10 µg), cotrimoxazole (25 µg), amoxicillin (25 µg), tetracycline (30 µg), nalidlic acid (30 µg) gentamycin (10 µg), erythromycin (5 µg) and nitrofurantoin (300 µg).

Data analysis

Data was analyzed using the EPI Info software to generate frequency tables. The χ^2 (Chi-Square) test was used to determine significant relationship between relevant categorical variables at P≤0.05.

RESULTS

Distribution of 240 nosocomial isolates in various specimens was presented in Table 1. Urine samples accounted for 144 (60%) isolates, 72 (30%) isolates were obtained from pus and other aspirates, 17 (7.1%) from blood and the remaining 07 (2.9%) were obtained from Cerebrospinal fluid. *E. coli* was the most prevalent isolate 118 (49.2%) which was statistically significant (p<0.05) compared to others. This is closely followed by *K. pneumoniae* 60 (25.0%), 20 (8.3%) *Enterobacter cloacae* 20 (8.3%), *Proteus mirabilis* 18 (7.5%) *Proteus valgaris* 14 (5.8%) and *Citrobacter freundii* 10 (4.2%).

From Table 2, *E. coli* is the most prevalent organism in all the age groups, age group 60 years and above yielded highest number of isolates , 35 out of 118 (29.7%). The age group 18-40 years had least number of isolates with 28 out of 118 (23.7%). The least commonly isolated organism is *C. freundii* with 10 (4.2%) isolates and age group 50 – 60 accounted for highest number of these that is, 4 out of 10 (40%). Age group 50 – 60 years had 71 of 240 (29.6%) isolates while age group 40 – 50 years had 46 of 240 (19.2%) isolates as the least.

Table 4, shows, the distribution of Community acquired isolates in various species according to age. *E. coli* remained to be the lead compared to other organisms in the table. Age group 60 and above had highest isolates 36 out of 126 (28.6%) while the least isolates were recorded in age group 40 – 50 years, 26 out of 126 (20.6%). There was no statically significant difference in this prevalence (p > 0.05) and any observed difference was due to mere chance. The least recorded isolate is *C. freundii* with 9 isolates out of 240. Age group 60 and above had highest prevalence of 4 out of 9 (44.4%) while age group 18 – 40 years and 40 – 50 years had 1 out of 9 (11.11%) each as the least prevalence rate in this study.

From Table 3, a total of two hundred and forty isolates of Enterobacteriaceae were equally collected from out patient department during the study period. The highest number of isolates were obtained from urine which is 194 out of 240 (47.5%), pus 42 of 240 (17.5%). 38 (15.8%) from ear swab, 19 (7.9%) from high vaginal swab, 13 (5.4%) from blood and only 6 (2.5%) isolates from cerebrospinal fluid been the least.

Among these isolates *E. coli* was the most abundant 130 (54.2%), followed by *K. pneumoniae* 56 (23.3%). 18 (7.5%) isolates of *E. cloacae* (7.5%), 16 *P. mirabilis*, (6.7%), 11 *P. vulgaris* (4.6%) and 9 isolates of *C. fruendii* (3.8%) were obtained from out door isolates. Among these 130 isolates, *E. coli* was most prevalent in urine 62 out of 130 (47.7%), followed by 25 from pus (19.2%), 16 from ear swab (12.3%), 9 (6.9%) from high vaginal swab (HVS) (6.9%), 10 from blood (7.7%), 5 from cerebrospinal fluid (CSF) (3.8%), and 3 from sputum (2.3%).

Among these 240 isolates, 41 isolates were *K. pneumoniae*. This was most prevalent in urine 28, 7 are from pus, 8 from ear swab, 5 from HVS, 4 from sputum, 3 from blood and only one from CSF. Urine samples have

Table 2. Distribution of Nosocomial Isolates in various species according to age group.

Age group / Isolates	18 – 40	40 – 50	50 – 60	60 and above	Total
E. coli	28	25	30	35	118
K. pneumonia	13	10	20	17	60
E. cloacae	04	05	06	05	20
P. mirabilis	05	03	06	04	18
P. vulgaris	03	02	05	04	14
Cit. freundii	02	01	04	03	10
Total	55	46	71	68	240

E: *Escherichia*; K: *Klebsiella*; Ent: *Enterobacter* P: *Proteus*; Cit: *Citrobacter*. P<0.05.

Table 3. Distribution of community acquired isolates in various specimens.

Specimen	*Escherichia coli*	*Klebsiella pneumoniae*	*Enterobacter cloacae*	*Proteus mirabilis*	*Proteus vulgaris*	*Citrobacter freundii*	Total
Urine	62	28	10	08	01	05	114
Pus	25	07	03	03	02	02	42
Hus	09	05	00	03	01	01	19
Stool	00	00	00	00	00	00	00
Sputum	03	04	01	00	00	00	08
Ear swab	16	08	04	02	07	01	38
Blood	10	03	00	00	00	00	13
CSF	05	01	00	00	00	00	06
Total	130	56	18	16	11	09	240

E. coli: *Escherichia coli*; K.p: *Klebsiella pneumoniae*; Ent. cl: *Enterobacter cloacae*; P.mir: *Proteus mirabilis*; P. vul.: *Proteus vulgaris*; C. fr.: *Citrobacter fruendii*. P<0.05.

Table 4. Distribution of community acquired Isolates in various species according to age group.

Age group / Organism	18 – 40	40 – 50	50 – 60	60 and above	Total
E. coli	30	26	32	38	126
K. pneumonia	12	09	17	20	58
Ent. cloacae	03	04	06	05	18
P. *mirabilis*	03	04	04	06	17
P. *vulgaris*	03	02	04	03	12
Cit. *freundii*	01	01	03	04	09
Total	52	46	66	76	240

E: *Escherichia*; K: *Klebsiella*; Ent: *Enterobacter* P: *Proteus*; Cit: *Citrobacter*. P<0.05.

highest number of isolates in the five genera isolated except for *P. vulgaris* which showed highest prevalence in pus. *E. coli* is the overall highest isolate in frequency with 130 (54.2%) while *P. vulgaris* has the least with 11 (4.6%) as shown in Table 3.

Antibiogram of bacteria isolates as shown in Table 5 revealed that ceftriaxone, peflacine, and ofloxacin are most effective against most of the pathogens isolated in this study. Gentamycin showed moderate to high activities while the pathogens were resistant to tetracycline, nalidixic acid and erythromycin.

DISCUSSION

Five genera belonging to the family of bacteria Entero-

Table 5. Antibiotic susceptibility pattern of baterial isolates.

Antibiotic	Percentage sensitivity					
	E. coli	*K. pneumonia*	*Ent. cloacae*	*P. mirabilis*	*P. vulgaris*	*Cit. freundii*
Ofloxacine	100	85	86	100	100	100
Peflacine	100	72	85	100	100	100
Ceftriaxone	100	100	100	100	100	100
Gentamycin	80	81	76	60	80	80
Amoxycillin	18	40	98	75	18	18
Cephalexin	98	97	100	100	98	98
Cotrimoxazone	0	20	62	0	0	0
Tetracycline	0	0	18	7	0	0
Nalidixic acid	0	0	10	0	0	0
Nitrofurantoin	25	0	50	80		
Erythromycin	0	0	42	0		

bacteriaceae were isolated from 240 each of hospital acquired infections and community acquired infections in different wards according to different age groups in a tertiary health hospital in south western Nigeria.

The bacteria were isolated from urine, pus, blood, high vaginal swab, CSF, sputum and ear swabs. It was shown that *E. coli* were most frequent in all the specimens with 49.2 and 47.5% for urine samples in community acquired and nosocomial infection respectively. The age group 60 years and above in both cases of nosocomial and community acquired infections recorded *E. coli* as most abundant organism. This result varied with work of Shah et al. (2002) that recorded *E. coli* as highest in the age group 50-60 years. In accordance with another work (Mouton et al., 2001), who recorded *E. coli* in 65 years and above as cause of both nosocomial and community acquired infections.

In the period 1988-1993, after *E. coli*, *Klebsiella* was the leading cause of Gram-negative bacteraemia, from 6-7% in the late 1980s to 12-13% in more recent years. Urinary tract infection was the underlying source of 58% of community-acquired *Klebsiella bacteraemia* as against 28% of hospital-acquired *Klebsiella bacteraemia* (Butler et al., 2001). There are many of these infections in the community that are different from those reported in studies on *K. pnemoniae* bacteremia from referral centers (Haddy et al., 1989). Urinary tract infections (25.6%) were the main types of infection in the cancer patients in oncology intensive care unit according to Velasco et al. (1997). The most common organisms isolated were from Entrobacteriaceae (29.7%). This increased incidence of Enterobacteriaceae in urine could be due to the indwelling urinary catherter, and central vainous catheters used for patients. *Klebsiella* spp 9.1% and *P. mirabilis* 4.9% were responsible for the risk of nosocomial bacteria transmission during ultrasound scanning in LAUTECH Teaching Hospital Osogbo in a survey in 2005 (Bello et al., 2005).

In medical, surgical and intensive care wards of Swiss University hospital, surgical site infections were most prevalent (30% of all nosocomial), followed by urinary tract infections according to a survey in May 1996 (Harbarth et al., 1999). The most frequently isolated organism were Enterobacteriaceae (Harbarth et al., 1999). Nosocomial infections are considered as a heavy burden on health services. A total of 240 isolates were obtained from subjects belonging to different age groups. Most of the nosocomial isolates were obtained from age group 50-60 years with (29.6%) while most of the community acquired isolates were from age group 60 years and above with (31.7%).

E. *coli* was most prevalent organism (24.6%) in the age groups 50-60 for nosocomial infection and 60 and above community acquired infection respectively (15.8%). In the current study, 130 out of 240 isolates were *E. coli* 62 of 130 (54.2%). *E. coli* were obtained from urine in cases of community acquired infections accounting for (47.6%) in all age groups. It was observed by Alli et al. (1998) that 30% of all blood stream infections were found in patients over 50 years and that 65% of these were cause by gram-negative organisms, these results are similar to the findings of the present study. Prevention of infections, particularly those that are hospital acquired, is difficult and may be impossible. Sewage treatment, water purification, proper hygiene, and other control methods for enteric pathogens will reduce the incidence of *E. coli* and other entero-pathogens of Enterobacteriaceae. However, these control measures are rarely available in less developed regions of the world like Nigeria. All hospital staff both Clinical and non Clinical can do much to reduce nosocomial infections through identification and control of predisposing factors, education and training of hospital personnel, and adequate microbial surveillance. Observance of standard procedures and use of aseptic conditions for all medical interventions will go a long way in control of hospital acquired infection.

This study of infection rates provides specific surveillance data for further inter hospital comparisons

and also to assess the influence of invasive medical interventions, thus allowing for the implementation of preventable measures to control infections. Education of staff regarding pathogenesis, diagnosis, and appropriate intervention needed for nosocomial infections is essential to its control and prevention (Shah et al., 2002). Also essential is the evaluation and alteration if needed, of policies and procedures to better provide control and prevention of nosocomial infections.

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