PREVALENCE OF HOSPITAL-ACQUIRED ENTEROCOCCI INFECTIONS IN TWO PRIMARY-CARE HOSPITALS IN OSOGBO, SOUTHWESTERN NIGERIA

Olawale, Kafayat Olayinka; Fadiora, Solomon Olufemi and *Taiwo, Samuel Sunday

Department of Medical Microbiology/Parasitology and 2Surgery, College of Health Sciences, Ladoke Akintola University of Technology, PMB 4400, Osogbo, Nigeria

E-mail: samtaiwo2003@yahoo.com

Abstract

Enterococci are opportunistic bacteria that become pathogenic when they colonize niches where they are not normally found. Of recent, they have become major cause of nosocomial infections, especially of the bloodstream, urinary tract and surgical sites. The aim of this study is to determine the point-prevalence rate of human enterococci infections among hospitalized patients in Osogbo, Nigeria. The study was conducted between January and June 2009 in two primary-care hospitals in Osogbo and involved a total of 118 patients who developed clinical evidence of infection at least 48 hours after hospital admission. Appropriate clinical samples were collected from the patients after an informed consent and cultured for isolation/biochemical identification of Enterococcus species at the Bacteriology Laboratory of Ladoke Akintola University of Technology, Osogbo using standard microbiological methods. There were 525 hospital admissions within the time frame of the study of which 118 (22.5%) developed hospital acquired infection (HAI); 58 (49.2%) of which cultured positive for bacterial pathogens. Enterococci were isolated from infective focus in 7 patients, giving a prevalence rate of hospital-acquired enterococci infection of 5.9%. Two species of Enterococcus were identified; Enterococcus faecalis from urinary tract infection (UTI) and surgical site infection (SSI) of 6 (85.7%) patients and Enterococcus faecium from UTI in 1 (14.3%) patient. Other bacteria recovered from other infective foci were Klebsiella spp 31.0%, Pseudomonas spp 20.7%, Staphylococcus aureus 17.2%, Escherichia coli 12.1%, Staphylococcus epidermidis 3.4%, Streptococcus pneumoniae 1.7% and Serratia spp 1.7%. All the enterococci isolates were multiply antibiotic resistant, and 42.9% were vancomycin-resistant enterococci (VRE) with the VRE strains showing resistance to wider range of antibiotics than the vancomycin-sensitive strains. Other Gram-positive and Gram negative bacterial isolates also demonstrated multiple resistance to all commonly available antibiotics in this community except E. coli and Pseudomonas spp which were relatively sensitive to ciprofloxacin and ceftazidime. This limited study demonstrated a high prevalence rate of multiple antibiotic resistant enterococci infections among hospitalized patients in this environment. There is need for systematic surveillance of hospitals for enterococci infections; prudent use and rational prescription of antibiotics and stringent measures to reduce the prevalence rate by health education on infection control measures such as isolation, cleaning, disinfection and sterilization.

Keywords: Nosocomial, Prevalence, Enterococcus, Vancomycin-Resistance, Primary Care

Introduction

Enterococci are hardy, facultatively anaerobic Gram positive cocci in pairs or short chains that can grow and survive in many environments (Murray, 1990). They are part of normal flora of intestine of humans and animals but may be responsible for serious infections. Of the over 20 Enterococcus species (Facklam et al, 2002), 2 species are particularly pathogenic to man; Enterococcus faecalis causes 85-90% of enterococci infections while Enterococcus faecium causes 5-10% (Lewis & Zervos, 1990; Gordon et al, 1992; Patterson et al, 1995). Other Enterococcus species known to cause human infections include E. avium, E. gallinarium, E. casseliflavus, E. dirans, E. raffinosus and E. mundtii

Enterococci are among the most frequent causes of nosocomial infection, particularly in intensive care units (ICU) where they are selected by therapy with cephalosporin and other antibiotics to which they are resistant. They are transmitted from person to person primarily on the hand of hospital personnel, some of whom may carry the organism in their gastrointestinal tracts. Occasionally, enterococci are transmitted on medical devices. In patients, the most common sites of enterococci infections are the urinary tract, wound and biliary tract along with other species of bacteria where it may be difficult to define the pathogenic role of the enterococci (Murray, 1990). In neonates, meningitis and bacteraemia may occur and endocarditis may occur in adults. Enterococci infection is equally distributed between sexes (Gordon et al, 1992), although urinary tract infections are more common in healthy women than men and in elderly patients due to high incidence of urinary instrumentation.
In the routine microbiology laboratory, enterococci are characterized by their morphologic appearance on Gram stain and on culture, and are distinguished from the non-group D streptococci by their ability to survive in the presence of 40% bile, ability to hydrolyze aesculin, growth in 6.5% NaCl and a positive pyrrolidonylarylamidase test (Facklam et al, 2002). The treatment of enterococci infection is usually problematic because they are usually resistant to β-lactam antibiotics and aminoglycosides, though synergistic action of a combination of these drugs may be effective. The glycopeptide, vancomycin was the drug of choice but resistance to this drug is now on the increase. Newer antibiotics, such as the combination of quinupristin and dalfopristin are currently used to treat vancomycin resistant enterococci infection (Arias & Murray, 2008).

In Nigeria, the role of enterococci in clinical infections has not been appreciated hence reports of enterococci infections are very few. A previous study in Ilorin (Taiwo et al, 2002) reported 2.8% of 642 bacteria wound isolates to be Streptococcus (Enterococcus) faecalis. Another study in Lagos reported that 11% of 35 vancomycin susceptible E. faecalis isolates obtained from different clinical specimens exhibited high-level resistance (HLR) to gentamicin (MIC ≥ 2,000 μg/ml) and 32% exhibited HLR to streptomycin (Iregbu et al, 2002). In recent times in the teaching hospital in Osogbo, we have isolated E. faecalis from blood stream and wound infections (Taiwo et al, 2008; Fadiora et al, 2009) amongst other pathogens, however, the prevalence and magnitude of enterococci infections in this environment is largely unknown. The objectives of this study are; to determine the prevalence of hospital acquired enterococci infection and the common Enterococcus species in primary-care hospitals in Osogbo, Nigeria and to determine their susceptibility profile to commonly prescribed antibiotics. This information is to serve as a pilot data for more extensive molecular epidemiology of enterococci infections that will be necessary for the formulation of control policy in this environment.

Materials and Method

Study area

This research is a descriptive cross sectional study carried out over a 6 month period (January to June 2009) in two primary-care hospitals and the bacteriology laboratory of the Ladoke Akintola University of Technology, Osogbo Southwestern Nigeria. The two hospitals offer general medical and surgical as well as gynaecologic and obstetric services, and were selected because of their relatively high patient patronage and consent of their hospital management board.

Subjects

The subjects were patients who developed clinical evidence of infections at least 48 hours after hospital admission. One hundred and eighteen patients were studied. Informed consent was obtained from each subject and the ethical approval of the two hospital management was obtained before the conduct of the study. Demographic and clinical data were collected from each patient into a designed form.

Laboratory procedure

Appropriate clinical specimens (blood, urine, wound swabs, sputum and stool) were collected from each subject as applicable and transported to the bacteriology laboratory for processing, aerobic cultures and isolation on Sheep Blood agar/other appropriate culture media, and biochemical identification of enterococci and other bacterial pathogens according to recommended techniques and procedures (Cheesbrough, 2000).

Isolation/identification of enterococci

Enterococci were identified on Sheep Blood agar plate as non-haemolytic 0.5-1mm size streptococci-like colonies; on MacConkey agar as small dark-red magenta colonies and on CLED agar as small yellow colonies from fermentation of lactose (Cheesbrough, 2000). The colonies were confirmed as enterococci with Gram stain positivity, negative catalase test, positive bile-aesculin (bile insolubility) test, growth in 6.5% NaCl broth and as Enterococcus species by specific sugar (glucose, lactose, mannitol, sorbitol and arabinose) fermentation reactions (Facklam & Collins, 1989; Facklam et al, 1989).

Antibiotic susceptibility test

The susceptibility of each enterococci isolate to oxacillin and vancomycin was determined using Clinical and Laboratory Standards Institute (CLSI) disk diffusion method (CLSI, 2007) on Mueller-Hinton agar (supplemented with 2% NaCl) with 1μg oxacillin and 30μg vancomycin disks and incubating at 35°C for 24 hours. Oxacillin zone diameter (ZD) of inhibition ≥14mm defined oxacillin susceptibility in enterococci while vancomycin ZD ≥17mm defined vancomycin susceptibility (CLSI, 2007).

Susceptibility of each isolate to other antibiotics (ampicillin 10μg, erythromycin 15μg, gentamicin 10μg, cotrimoxazole 25μg, tetracycline 10μg, ceftazidime 30μg and ciprofloxacin 5μg) was performed using the disk diffusion method of Bauer et al (1966). ZD for susceptibility to these antibiotics in enterococci were; ampicillin ≥17mm, erythromycin ≥23mm, gentamicin ≥15mm, cotrimoxazole ≥16mm, tetracycline ≥19mm, ceftazidime ≥18mm and ciprofloxacin ≥21mm (CLSI, 2007). Staphylococcus aureus ATCC 25923 serve as negative and E. faecalis ATCC 51299 as positive control strains.
Data entry and Statistical analysis

All data (demographic and clinical) were entered into Window Vista 2007 laptop computer with GraphPad statistical software. Frequency tables were generated and relationship between variables tested with Chi square or Fisher’s Exact test with significant value set at \( P<0.05 \).

RESULTS

Over the 6 months period of study, there were 525 hospital admissions in the two primary-care hospitals in Osogbo, Nigeria. A total of 118 patients who developed clinical evidence of infection 48 hours after hospitalization were enrolled into the study. The hospital-acquired infection (HAI) rate in the two hospitals was 22.5%. Table 2 show the age and sex distribution of the eligible patients. The age group 20-29years constitute the largest proportion (42.4%) followed by age group 30-39 years (23.7%) and others as shown in Table 2. Table 3 shows the prevalence of enterococci infection of 5.9% (7 of 118) among the patients, with surgical site and urinary tract infections being the most prevalent with 18.8% and 2.6% rates respectively.

Of the 118 patients with clinical infection, 58 (49.2%) were cultured positive for bacterial pathogens (with one microbial pathogen isolated in each case) while 60 (50.8%) were bacteriologically sterile. Enterococci were isolated in 7 (5.9%) patients and 2 species were identified using the sugar fermentation test described by Facklam & Collins (1989); E. faecalis in 6 and E. faecium in 1 (Table 3). Other non-enterococcal bacteria recovered were Staphylococcus aureus 10 (17.2%), Staphylococcus epidermidis 2 (3.4%), Streptococcus pneumoniae 1 (1.7%), Klebsiella spp 18 (31.0%), Serratia spp 1 (1.7%), Escherichia coli 7 (12.1%) and Pseudomonas spp 12 (20.7%). The six E. faecalis were isolated from cases of SSI (5) and UTI (1) while the only E. faecium was isolated from a case of UTI (Table 4).

Table 5 shows the resistant pattern of the Enterococcus species. All isolates were resistant to ampicillin and oxacillin while 3 (42.9%) including the only E. faecium were resistant to vancomycin (VRE). The isolates also showed multi-drug resistant patterns with the VRE showing resistant to wider range of antibiotics (7 antibiotics) than the vancomycin sensitive strains (4-5 antibiotics).

Table 6 shows the susceptibility profile of other Gram-positive pathogens isolated. Most of the isolates were resistant to commonly used antibiotics in this environment. Table 7 shows antibiotic susceptibility of Gram-negative pathogens isolated in the study. Most of the isolates were also resistant to all antibiotics except E. coli which was sensitive to ciprofloxacin (85.7%) and Pseudomonas spp which was sensitive to ceftazidime (100%) and ciprofloxacin (58.3%).

| Age Group(Years) | Male | Female | Total (%) |
|------------------|------|--------|-----------|
| ≤ 10             | 5    | 6      | 11 (9.3)  |
| 10-19            | 18   | 32     | 50 (42.4) |
| 20-29            | 9    | 19     | 28 (23.7) |
| 30-39            | 10   | 6      | 16 (13.6) |
| 40-49            | 2    | 4      | 6 (5.1)   |
| 50-59            | 1    | 1      | 2 (1.7)   |
| 60-69            | 3    | 0      | 3 (2.5)   |
| 70-79            | 0    | 1      | 1 (0.8)   |
| 80-89            | 1    | 0      | 1 (0.8)   |
| 90-99            | -    | -      | -         |
| > 100            | -    | -      | -         |
| Total            | 49 (41.5) | 69 (58.5) | 118 (100) |

| Clinical diseases | Specimen | Number of patients (%) | Enterococci isolates (%) |
|------------------|----------|------------------------|--------------------------|
| Gastro-enteritis | Stool    | 18 (15.3)              | -                        |
| Blood stream infection | Blood | 20 (16.9)              | -                        |
| Respiratory tract infection | Sputum | 9 (7.6)              | -                        |
| Surgical site infection | Swab/biopsy | 32 (27.1) | 6                        |
| Urinary tract infection | Urine | 39 (33.1)              | 1                        |
| Total            | 118 (100) | 7 (5.9)                |                          |
Table 3: Enterococci species identified by sugar fermentation reactions in two primary-care hospitals in Osogbo

| Species          | No of strain | Glucose | Lactose | Mannitol | Sorbitol | Arabinose |
|------------------|--------------|---------|---------|----------|----------|----------|
| E. faecalis      | 6            | + (100) | + (100) | + (100)  | + (100)  | - (0)    |
| E. faecium       | 1            | - (0)   | + (100) | + (100)  | - (0)    | + (100)  |

Table 4: Bacterial isolates from hospital acquired infections in two primary-care hospitals in Osogbo, Nigeria

| Isolate           | Number | Percentage |
|-------------------|--------|------------|
| Enterococcus faecalis | 6      | 10.4       |
| Enterococcus faecium           | 1      | 1.7        |
| Staphylococcus aureus         | 10     | 17.2       |
| Staphylococcus epidermidis     | 2      | 3.4        |
| Streptococcus pneumoniae      | 1      | 1.7        |

Table 5: Resistance pattern of hospital-acquired enterococci isolates in Osogbo, Nigeria

| No of antibiotic | Antibiotic Resistance Pattern | No of isolate (%) |
|------------------|-------------------------------|-------------------|
| 4                | Amp, Oxa, Ceft, Gen           | 2 (28.6)          |
| 5                | Amp, Oxa, Ceft, Gen, Cot      | 2 (28.6)          |
| 8                | Amp, Oxa, Ceft, Gen, Cot, Cip, Ery, Van | *3 (42.8) |
| Total            |                               | 7 (100)           |

*Amp=ampicillin, Oxa=oxacillin, Gen=Gentamicin, Ceft=Ceftazidime, Cot=Cotrimoxazole, Cip=Ciprofloxacin, Ery=Erythromycin, Van=Vancomycin* include the only E. faecium isolated

Table 6: % Resistance of other Gram positive bacteria isolates in two primary-care hospitals in Osogbo

| Isolate                  | Antibiotics (%) |
|--------------------------|-----------------|
| S. aureus (n=10)         | Amp 10 (100) Oxa 5 (50) Gen 10 (100) Van 2 (20) Ery 6 (60) Cot 10 (100) Ceft 10 (100) Cip 5 (50) |
| S. epidermidis (n=2)     | Amp 2 (100) Oxa 2 (100) Gen 2 (100) Van 0 (0) Ery 2 (100) Cot 2 (100) Ceft 2 (100) Cip 2 (100) |
| S. pneumoniae (n=1)      | Amp 1 (100) Oxa 1 (100) Gen 1 (100) Van 0 (0) Ery 1 (100) Cot 1 (100) Ceft 1 (100) Cip 1 (100) |

*Amp=Ampicillin, Oxa=Oxacillin, Gen=Gentamicin, Van=Vancomycin, Ery=Erythromycin, Cot=Cotrimoxazole, Ceft=Ceftazidime, Cip=Ciprofloxacin*

Table 7: % Resistance of Gram negative bacteria isolates in two primary-care hospitals in Osogbo, Nigeria

| Isolates                  | Antibiotics (%) |
|---------------------------|-----------------|
| Klebsiella spp (n=18)     | Amp 18 (100) Gen 12 (66.7) Tet 18 (100) Cot 18 (100) Ceft 18 (100) Cip 13 (72.2) |
| Pseudomonas sp (n=12)     | Amp 12 (100) Oxa 12 (100) Gen 12 (100) Van 12 (100) Ery 12 (100) Cot 12 (100) Ceft 12 (100) Cip 12 (100) |
| Escherichia coli (n=7)    | Amp 7 (100) Oxa 7 (100) Gen 7 (100) Van 7 (100) Ery 7 (100) Cot 7 (100) Ceft 7 (100) Cip 7 (100) |
| Serratia spp (n=1)        | Amp 1 (100) Oxa 1 (100) Gen 1 (100) Van 1 (100) Ery 1 (100) Cot 1 (100) Ceft 1 (100) Cip 1 (100) |

*Amp=Ampicillin, Oxa=Oxacillin, Gen=Gentamicin, Van=Vancomycin, Ery=Erythromycin, Cot=Cotrimoxazole, Ceft=Ceftazidime, Cip=Ciprofloxacin*
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Discussion

Before now in our environment, enterococci have been largely regarded as commensal flora and generally disregarded when isolated from clinical specimens such as wound and urine. However, there are increasing reports that this opportunistic bacterium can become pathogenic when it colonizes ecological niche where it is not normally found with potential to become invasive. Since the beginning of the 21st century in the United States of America, enterococci have become major reservoir of antibiotic-resistant genes and VRE a major cause of nosocomial infections especially of the bloodstream, urinary tract and surgical sites (Cetinkaya et al, 2000). Also recently in our teaching and specialist hospitals, enterococci especially E. faecalis are increasingly isolated in pure cultures from patients with clinical evidence of infections (Taiwo et al, 2008; Fadiola et al, 2009). The aim of this study was to determine the prevalence of hospital-acquired enterococci infections in primary health care institutions in our environment.

This study recorded hospital-acquired infection rate of 22.5% for the two hospitals. This rate is higher than what is reported in developed countries with rates of 5-10% (Meers et al, 1980; Moro et al, 1986; Mayon-White et al, 1988; Scheel & Stormark, 1999) and also higher than rates reported from hospitals in developing countries such as Ghana (Newman, 2009) with 6.7% and Ethiopia (Gedebo, 1988) with 17%. There is need to strengthen infection control activities in Nigerian hospitals in order reduce the prevalence, mortality, morbidity, and cost of care associated with HAIs.

The prevalence rate of 5.9% for hospital-acquired enterococci infection recorded in this study is considered high in view of the fact that all the isolates were from clinically infected patients over a period of just 6 months. There is need for clinicians in our environment to be aware of the role enterococci plays in clinical infections especially of urinary tract and surgical site infections. Most of the hospitalized patients (79.7%) in the two hospitals were in the age group 20-50 years and all the enterococci infections occurred in this age group. Because this age group constitutes the work force of any society, it becomes imperative to be critically aware of danger of enterococci infections and the need for prompt diagnosis, treatment and prevention.

Identification of Enterococcus species in this study was done using the conventional physiologic test scheme described by Facklam & Collins (1989). This scheme has been shown to correlate well with miniaturized-dehydrated tests and DNA hybridization techniques of identifying enterococci (Facklam et al, 1989). The scheme allowed identification of two Enterococcus species in this study; 85.7% E. faecalis and 14.3% E. faecium which agrees with the trend reported worldwide where E. faecalis is said to be responsible for about 80 to 90% of all enterococcal infections and E. faecium accounts for most of the others (Gordon et al, 1992; Facklam et al, 2002). This scheme is appropriate for identification of enterococci in resource limited countries such as ours as it allowed the identification of E. faecium, the specie that has not before been reported in Nigeria from previous studies (Iregbu et al, 2002; Taiwo et al, 2002; Taiwo et al 2008; Fadiola et al, 2009).

The enterococci isolates were resistant to multiple antibiotics with 100% of the isolates resistant to ampicillin, oxacillin, ceftazidime and gentamicin; 71.4% resistant to cotrimoxazole and 42.9% resistant to erythromycin, ciprofloxacin and vancomycin. Three isolates including the only E. faecium isolate were resistant to all tested antibiotics including vancomycin. Some enterococci are known to be intrinsically resistant to β-lactam antibiotics as well as many aminoglycosides while some are known to have acquired multidrug resistance to tetracycline, erythromycin, chloramphenicol and fluoroquinolones. A previous Nigerian study (Iregbu et al, 2002) reported 100% susceptibility of E. faecalis to ampicillin and vancomycin but exhibited 11% and 34% high level resistance (HLR) to gentamicin and streptomycin respectively. This present study revealed resistance rate of 100% to ampicillin and gentamicin and 43% to vancomycin. VRE may have gradually emerged in Nigeria at the turn of the century.

The emergence of VRE strains at the turn of the 20th century has generated major concern among clinicians (Cetinkaya et al, 2000) particularly in the last two decades; virtually these strains have emerged in nosocomial infections of hospitalized patients in the USA. In this study, VRE form about 43% of all the enterococci isolates, a figure that is high when one considers the fact that vancomycin is not available for clinical use in Nigeria. The VRE isolates include two E. faecalis and the only E. faecium isolate and were resistant to all the eight antibiotics tested. This observation agrees with a recent study on wound infections in the teaching hospital in Osogbo, Nigeria which reported two E. faecalis isolates to be resistant to all antibiotics tested in that study (Fadiola et al, 2009). The clinical implication is that VRE in Nigeria may soon become a great threat unless proper control measures are initiated.

A major problem is the fact that enterococci harbor transferable genetic elements, which have an unusually broad host range and can be transferred to both Gram-negative and Gram-positive bacteria species by conjugation systems involving plasmids and transposons (Clewell and Dunny, 2002). The danger of such transmission to bacteria such as S. aureus and the enterobacteriaceae in our environment is apparent as some Nigerian studies have reported vancomycin resistance among clinical S. aureus isolates (Olayinka et al, 2005, Onolitola et al, 2007) without previous exposure to vancomycin. A cursory look at our study revealed that 50% and 20% of the S. aureus isolates are oxacillin and vancomycin resistant respectively. Transfer of vancomycin resistance (von) genes has been specifically reported in patients co-colonized with VRE and MRSA (Furuno et al, 2005). One limiting factor in our study is that we could not perform molecular analysis to detect the genes responsible for vancomycin resistance because of lack of facilities. Nevertheless, we believe that hospital acquired infections with VRE will
become a significant health problem in this era of sophisticated medical and surgical procedures unless strategy of systematic surveillance and infection control put in place.

Other Gram positive bacteria (S. aureus, S. epidermidis and S. pneumoniae) isolated from the patients demonstrated multiple resistances to all antibiotics except vancomycin. The Gram negative isolates (Klebsiella spp, Pseudomonas spp, E. coli and Serratia spp) were also multiple resistant and only ciprofloxacin appeared effective. These findings are in agreement with reports of recent studies in our environment on bacterial isolates of hospital environment (Taiwo et al, 2006), catheter associated urinary tract infection (Taiwo & Adedunmu, 2006), ear infection (Tobih et al, 2006), blood stream infections (Taiwo et al, 2008) and superficial wound infections (Fadiora et al, 2009). This high antibiotic resistance situation has arisen from poor antibiotic prescription policy and guidelines, with over-the-counter availability of most antibiotics including the fluoroquinolones in Nigeria. The need to regulate antibiotic consumption, prescription and usage is highly imperative in Nigeria. Multicentre studies are necessary to determine the national prevalence of enterococci infections in Nigeria and to study the evolution of vancomycin resistance strains, their distribution and spread in the country using molecular method.

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