



Epidemiology of Enterococcal Infections in Enugu State, Nigeria

G. A. C. Ezeah^{1*}, M. C. Ugwu², C. O. C. Ibe², O. C. Ike³ and A. O. Ekundayo⁴

¹Department of Microbiology, Enugu State University Teaching Hospital Parklane, Enugu State, Nigeria.

²Department of Medical Laboratory Science, College of Health Sciences, Nnamdi Azikiwe University, P.M.B. 5001, Nnewi, Anambra State, Nigeria.

³Department of Industrial Chemistry, Enugu State University of Science and Technology, P.M.B. 01660, Agbani, Enugu State, Nigeria.

⁴Department of Microbiology, Ambrose Alli University, P.M.B. 14, Ekpoma, Edo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Enterococci despite being a normal commensal is of great health concern since it can become virulent. Hence the study investigated the prevalence of Enterococci infection and two tertiary health institutions (Enugu State University of Technology (ESUT) Teaching Hospital, Parklane and University of Nigeria Teaching Hospital (UNTH), Ituku/Ozalla in Enugu State) were used. Isolation and identification were based on standard procedures and biochemical tests. The results showed that age ranges of 21-25years 10(14.7%) and 26-30 years 8(11.8%) had the highest prevalence. Also, more females were infected by the organism than males though the difference was not statistically significant ($p= 0.371$).The possible predisposing factor showed that 16(23.5%) were unknown while 10(14.7%), 6(8.8%), 14(20.6%), 9(13.2%), 8(11.8%) and 5(7.6%) were catheterization, surgery, pregnancy, Diabetes, HIV/AIDS and previous history of enterococcal

*Corresponding author: E-mail: ezeahg@yahoo.com, princeconsult@yahoo.com;

infection, respectively. The frequency of enterococcal isolates from different specimens showed that 24(35.3%) of the isolates were from urine samples, 12(17.6%) were from high vaginal swab, 7(10.2%) were from ear swab and wound swab respectively, 4(5.9%) were from urethral swab and aspirates respectively and 2(2.9%) were from sputum samples. Furthermore, departmental sources of *enterococcus* sp. isolated showed that 21(30.9%) were from Surgery department followed by general out-patients department 14(20.6%), Urology 8(10.3%), Gynaecology 7(10.3), Medicine 6(8.8%), Antenatal, Children out-patients, Neurology and Children Emergency Department had 3(4.4%) each. Also, 41(60.3%) out of the 68 isolates were nosocomially acquired and 27(39.7%) isolates were community acquired. There was no significant difference ($p= 0.486$) when nosocomially acquired enterococcal isolates were compared with community acquired enterococcal isolates. Monthly frequencies of the isolates showed that July had the highest frequency 10(14.7%) followed by the month of May 7(10.3%). April, August and October had 6(8.8%) respectively. February, March November and December had 5(7.4) respectively; January and September had 4(5.9%) respectively while June had 3(4.4%). Seasonal comparison (rainy and dry season) of the distribution of the isolates within the years of the study showed that rainy season had 38 number of isolates while dry season had 30 number of isolates and there was no significant difference between the frequencies of occurrence in the two seasons ($p= 0.271$). Therefore, there is an increasing prevalence of Enterococci and can be hospital acquired, screening for this organism is important in hospital setting.

Keywords: *Enterococci; infection; prevalence; nosocomial.*

1. INTRODUCTION

Enterococci can cause a wide range of infection [1]. The infections caused by enterococci are urinary tract infections, bacteremia, endocarditis, meningitis, hematogenous osteomyelitis, septic arthritis, pneumonia, intra-abdominal, pelvic, soft tissue infections [1,2].

Pathogenesis of *Enterococcus* sp: The healthy intestine habits more than 400 distinct bacteria species that compete with each other and help maintain the colon balance. Taking antibiotics, however, can destroy some bacteria and interrupt this balance, and species such as the vancomycin-resistant *Enterococcus* (VRE) can proliferate and cause local diseases. Previous use of antibiotics is a risk factor for VRE infection [3]. Other risk factors include a compromised immune system, cancer, chronic diseases such as diabetes or kidney failure [4]. Also, infection is most probable if the mucosal membrane (lining) of the intestine breaks or a patient undergoes a gastrointestinal surgery or operation [5]. Indwelling catheters or intravenous lines increase the risk of infection because they interfere with normal mucosal or skin barriers and provide a type of artificial reef where organisms can grow [5].

Adult females are the largest group of patients with urinary tract infection. With age and sexual activity, the incidences rise. Due to bladder or uterine prolapse causing incomplete bladder or

uterine emptying, infection rates are high in postmenopausal females. Loss of estrogen with associated modifications in the vaginal flora, lactobacilli loss, allowing peri urethral colonization with gram negative aerobes such as *E coli* and increased probability of concomitant health problem such as diabetes [6]. Factors involved in urinary tract infection in women include [5]:

A relatively short urethra: The urethra's position makes it subject to faecal contamination and colonization with potential pathogenic intestinal bacteria that can spread to the bladder only a few centimeters away.

Sexual Intercourse: Approximately one third of female urinary tract diseases are linked to sexual intercourse. Bladder infection is caused by the massaging impact of sexual intercourse on the urethra that introduces bacteria from the urethra into the bladder. After their first sexual intercourse, many females create their first urinary tract infection, a medical condition referred to as honey moon cystitis.

Use of Diaphragm for Contraception: The diaphragm ring compresses the urethra and prevents urine flow; this increases the risk of urinary tract infection. A history of prior cystitis episode and frequent or recent sexual activity are the most significant risk factors for acute cystitis

in young females. According to Scholes *et al.*, [7], about four out of five women with urinary tract infection get another in 18 months and many women get even more. Using spermicidal agents increases the probability of urinary tract infection by a factor of two or three regardless of whether exposure happens with the use of a diaphragm or a spermicidal coated condom [7]. Women with cystitis are likely to have a maternal cystitis history and to have had early age cystitis [7].

Urinary tract infection risk increases with age and debility as well as those with problem of impaired voiding and poor hygiene of the perineum [8]. Deficiency of estrogen may also contribute to urinary tract infection in women. Generally, among healthy postmenopausal women, sexual activity is a less significant predictor of cystitis than in younger women and women with diabetes who require pharmacological therapy. Women with diabetes have approximately twice high risk of cystitis compared to non-diabetes women [4]. Also, women who have had genitor-urinary surgery and urinary incontinence or cystoceles usually experience recurrent cystitis [4].

Infections of the urinary tract are uncommon in men until about 50 when the prostate gland compresses the urethra and makes it difficult to empty the bladder entirely. Also, catheter insertion increases the risk of bacteria entry into the bladder. Bacteria can form biofilms on the catheter, thereby posing therapeutic challenge with antibacterial drugs [9,10]. At each day the catheter remains in place, the risk of infection increases at 5% [5].

Among neonates, boys are slightly more probable to have urinary tract infection than girls. Pre-school children have approximately two percent of infection and in girls; it's ten times more common. Five percent of school age girls experience urinary tract infection. It is uncommon in school-aged boys [6]. According to Nester *et al.*, [5] three types of urinary tract infections include:

Cystitis: This is an inflammation of the bladder commonly caused by *E. coli*, *Proteus* sp., *Klebsiella* sp., *Enterococcus* sp. and *Staph. Saprophyticus* infection.

Pyelonephritis: This is an inflammation of the Kidney (renal parenchyma, calyces and renal pelvis) caused by *E. coli*, and *Enterococcus* sp infection

Urethritis: This is an inflammation of the urethra (the tube that conveys urine from the bladder to outside the body). It is caused by infection with *Neisseria gonorrhoea*, *Enterococcus* sp. and *Chlamydia trachomatis*. Andrew *et al.*, [11] in a study; showed that *Enterococcus faecalis* has tropism for the kidneys in the urinary tract of mice and can be used to elucidate the pathogenesis of urinary tract infection.

Furthermore, among the bacteria recently used as probiotics, Enterococci represent the largest risk medically to human. The significance of the presence of enterococci in food, particularly in dairy products, their possible use as faecal contamination indicators and contribution in flavour development has been a long-standing source of debate. Also, *Enterococcus* species used as probiotics can become virulent by acquiring virulent factors through the process of gene transfer [12].

2. MATERIALS AND METHODS

2.1 Area of the Study

This study was carried out in Enugu State. The two tertiary health institutions used were Enugu State University of Technology (ESUT) Teaching Hospital, Parklane and University of Nigeria Teaching Hospital (UNTH), Ituku/Ozalla in Enugu State, Nigeria.

Study Design: The study comprised of three categories of patients; 504 in-patients, 504 out-patients and controls (20 male and 20 female volunteers who did not have symptoms of any infection. They were selected from outside the hospital environment).

Specimen Collection: Sterile universal containers containing boric acid preservative were used for urine sample collection while sputum, stool, aspirates and CSF were collected with sterile plain universal bottles. Sterile swabs were used to collect high vaginal, urethral, wound, nasal, ear, anal sample. For blood culture, five milliliters of blood was collected with syringe and put aseptically into fifty milliliters of sterile brain heart infusion (BHI) broth contained in a bijoux bottle.

2.2 Analytical Techniques

Culture/Isolation Considerations: The urine samples were inoculated into 5% sheep blood agar and cysteine lactose electrolyte deficient

(CLED) agar plates with quantitative sterile wire loops (0.001 ml). Identification of organisms was based on the method described by Baker *et al.*, and Cheesbrough [13,14]. API 20 Strep by Biomerieux was used for species identification [15,16] using the identification software. Reference type *E. faecalis* strain (ATCC 29212) was used as control.

Out of one thousand and eight (1008) samples processed in this study, six hundred and thirty two (632) yielded different species of bacteria and fungi. Then, from six hundred and thirty two (632) microorganisms, sixty eight were Enterococci [16].

2.3 Statistical Analysis of Results

The results obtained from this work were analyzed statistically using percentage and Student t-test of computer program SPSS version 18. The student t-test was used to show significant different.

3. RESULTS

3.1 Age/Sex Distribution of Patients with Enterococcal Infection

The distribution of patients with enterococcal infection according to age and sex are shown on Table 1. The age range of 21-25 years (adolescents) had the highest frequency of 14.7% followed by 26-30 (also adolescents) which had 11.8% and infants between 0-5 who had 11.8%. The lowest frequency was found among the age range of 96-100 because there was no subject in the data collected. Furthermore, more females (57.4%) had the infection than males (46.6%).

Table 2 showed that there was no significant difference ($p= 0.371$) when the frequency of enterococcal isolates of male subjects were compared with the female subjects in the study.

Table 1. Age/sex distribution of patients with enterococcal infection

| Age(years) | Male | Female | Total | Percentage |
|------------|------|--------|-------|------------|
| 0 - 5 | 4 | 4 | 8 | 11.8 |
| 6 - 10 | 2 | 2 | 4 | 5.9 |
| 11 - 15 | 3 | 2 | 5 | 7.4 |
| 16 - 20 | 1 | 2 | 3 | 4.4 |
| 21 - 25 | 2 | 8 | 10 | 14.7 |
| 26 - 30 | 5 | 3 | 8 | 11.8 |
| 31- 35 | 1 | 5 | 6 | 8.8 |
| 36 - 40 | 1 | 3 | 4 | 5.9 |
| 41- 45 | - | 2 | 2 | 2.9 |
| 46 - 50 | - | 4 | 4 | 5.9 |
| 51 - 55 | 1 | 1 | 2 | 2.9 |
| 56 - 60 | 2 | - | 2 | 2.9 |
| 61 - 65 | - | 1 | 1 | 1.5 |
| 66 - 70 | 1 | 1 | 2 | 2.9 |
| 71 - 75 | 1 | - | 1 | 1.5 |
| 76 - 80 | 1 | - | 1 | 1.5 |
| 81 - 85 | 1 | - | 1 | 1.5 |
| 86 -90 | 1 | - | 1 | 1.5 |
| 91 - 95 | 2 | 1 | 3 | 4.4 |
| 96 -100 | - | - | - | 0 |
| Total | 29 | 39 | 68 | 100 |
| Percentage | 46.6 | 57.4 | 100 | |

Table 2. Comparison of the frequency of the enterococcal isolates among males and females

| Gender | N | Mean | SD | t-value | df | p-value |
|--------|----|------|------|---------|----|---------|
| Male | 29 | 1.45 | 1.32 | -.905 | 66 | 0.371 |
| Female | 39 | 1.95 | 2.09 | | | |

0.371 ($p > 0.05$)
Key: Not Significant

3.2 Possible Predisposing Factors to the Enterococcal Infections

The possible predisposing factors to the enterococcal infection were deduced from the folders of patients that had the enterococcal infection as shown on Fig. 1. 10(14.7%) of the positive patients were catheterized. 6(8.8%) underwent surgery prior to infection. 14(20.6%) patients were pregnant. 9(13.2%) were Diabetes, 8(11.8%) were HIV/AIDS patients. 5(7.6%) had previous history of enterococcal infection. 16(23.5%) of the patients did not have any leading information to the possible predisposing factors.

3.3 Frequency of Enterococcal Isolates from Different Specimens

Different specimens with the number and the percentages of isolates were displayed in Fig. 2. 24(35.3%) of the isolates were from urine samples. 12(17.6%) were from high vaginal swab. 7(10.2%) were from ear swab and wound swab respectively. 4(5.9%) were from urethral swab and aspirates respectively. 2(2.9%) were from sputum samples.

3.4 Departmental Sources and Number of *Enterococcus* sp. Isolated

Departmental distribution of the 68 *Enterococcus* isolates was determined as shown in Table 3.

Surgery department had a total (percentage) of 21(30.9%) followed by general out-patients department. 14(20.6%). Urology had 8(10.3%). Gynaecology 7(10.3) Medicine 6(8.8%); Antenatal, Children out-patients, Neurology and Children Emergency Department had 3(4.4%) each.

From the folders of positive patients, the sources of *Enterococcus* infection were determined. It was ascertained that 41(60.3%) out of the 68 isolates were nosocomially acquired and 27(39.7%) isolates were community acquired.

Table 4 showed that there was no significant difference ($p= 0.486$) when nosocomially acquired enterococcal isolates were compared with community acquired enterococcal isolates in the study.

3.5 Summary of Monthly Frequencies of Enterococcal Isolates from 2012 to 2014

This study was carried out for 24 months and the number (frequency) of isolates per month determined as shown in Table 5. The month of July had the highest frequency 10(14.7%) followed by the month of May 7(10.3%). April, August and October had 6(8.8%) respectively. February, March November and December had 5(7.4) respectively; January and September had 4(5.9%) respectively while June had 3(4.4%).

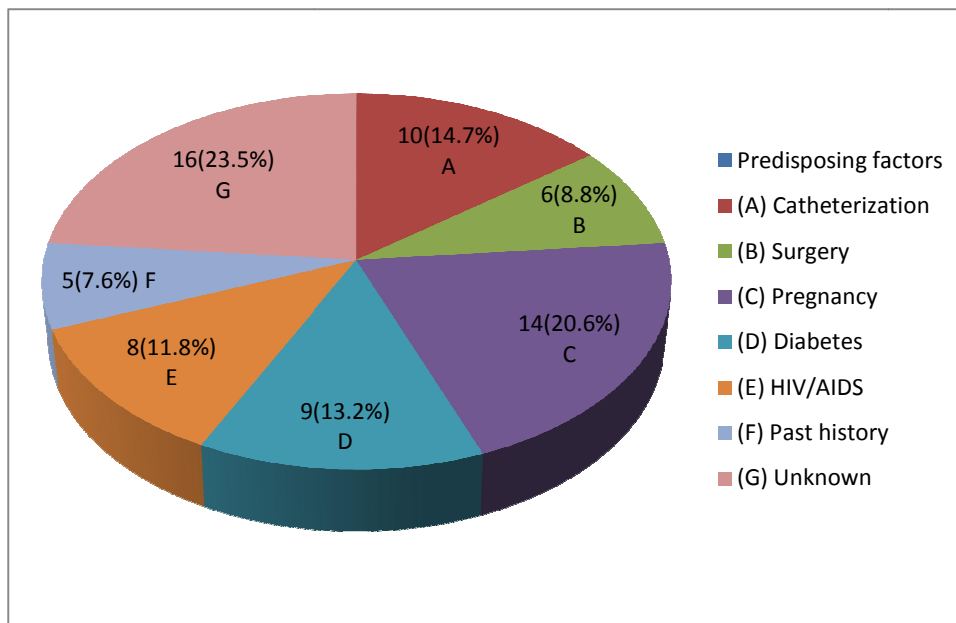


Fig. 1. Possible predisposing factors to the enterococcal infections

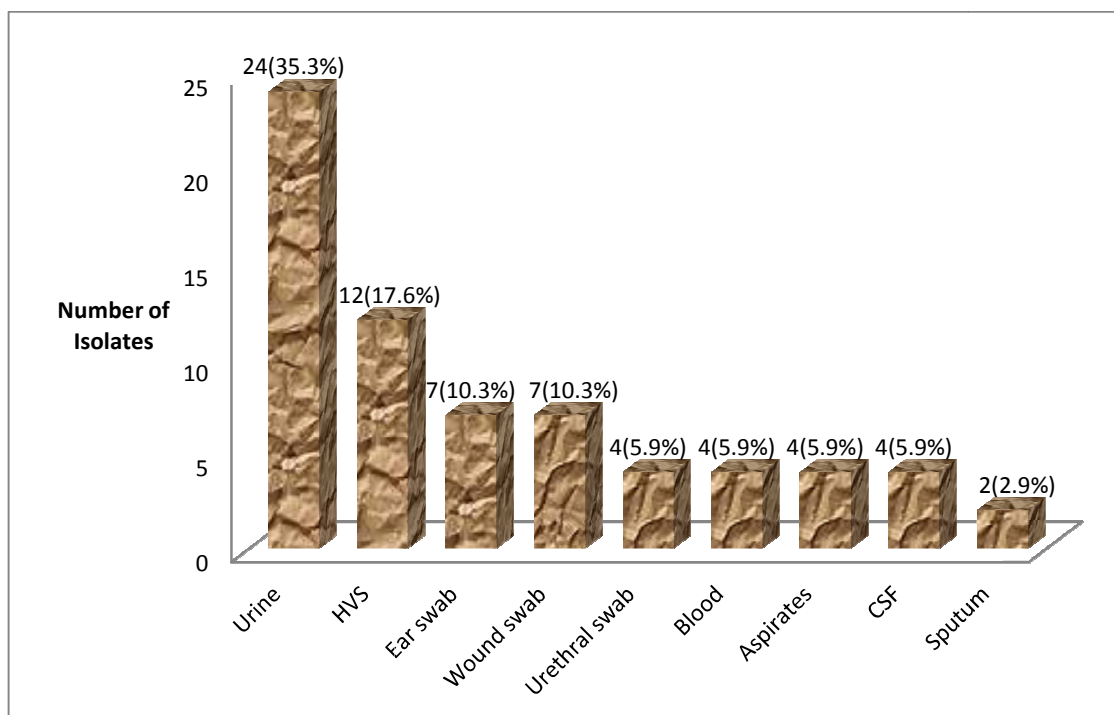


Fig. 2. Frequency of enterococcal isolates from different specimens

HVS: High vaginal swab
CSF: Cerebrospinal fluid

Table 3. Sources and number of enterococcus sp. Isolated

| Dept/unit | Nosocomially acquired | Community acquired | Total | Percentage |
|-------------|-----------------------|--------------------|-------|------------|
| Surgery | 19 | 2 | 21 | 30.9 |
| GOPD | 4 | 10 | 14 | 20.6 |
| Urology | 5 | 3 | 8 | 10.3 |
| Gynaecology | 6 | 1 | 7 | 10.3 |
| Medicine | 5 | 1 | 6 | 8.8 |
| Antenatal | 0 | 3 | 3 | 4.4 |
| CHOP dept | 0 | 3 | 3 | 4.4 |
| Neurology | 2 | 1 | 3 | 4.4 |
| CHE | 0 | 3 | 3 | 4.4 |
| Total | 41 | 27 | 68 | 100 |

Key: GOPD → General Out-patient Department, CHOP → Children Out-patient, CHE → Children Emergency, Dept → Department

Table 4. Comparison of the nosocomially acquired and community acquired enterococcal isolates

| Source | N | Mean | SD | t-value | df | p-value |
|-----------------------|----|------|------|---------|----|---------|
| Nosocomially Acquired | 41 | 4.56 | 5.92 | .714 | 66 | 0.486 |
| Community Acquired | 27 | 3.00 | 2.78 | | | |

0.486 ($p > 0.05$)
Key: Not Significant

Table 5. Summary of monthly frequency of isolation of *Enterococcus* sp

| | 2012 | 2013 | 2014 | Total (%) n=68 |
|-----------|------|------|------|----------------|
| January | -- | 2 | 2 | 4(5.9) |
| February | -- | 3 | 2 | 5(7.4) |
| March | -- | 3 | 2 | 5(7.4) |
| April | -- | 3 | 3 | 6(8.8) |
| May | -- | 3 | 4 | 7(10.3) |
| June | -- | 5 | 0 | 3(4.4) |
| July | 6 | 4 | -- | 10(14.7) |
| August | 3 | 3 | -- | 6(8.8) |
| September | 2 | 2 | -- | 4(5.9) |
| October | 3 | 3 | -- | 6(8.8) |
| November | 2 | 3 | -- | 5(7.4) |
| December | 3 | 2 | -- | 5(7.4) |
| Total | 19 | 36 | 13 | 68(100) |

Table 6. Seasonal comparison of the distribution of the enterococcal isolates

| Seasons | N | Mean | SD | t-value | df | p-value |
|---------|----|------|------|---------|----|---------|
| Rainy | 38 | 6.33 | 2.58 | 1.164 | 66 | 0.271 |
| Dry | 30 | 5.00 | 1.10 | | | |

0.271 ($p > 0.05$)

Key: Not Significant

These months in Table 5 were grouped into dry seasons (from November to April) and rainy seasons (from May to October) and total number of isolates per season was determined as shown in Table 6. Dry season had 30 isolates and rainy season had 38 isolates.

The table further showed that there was no significant difference between the frequencies of occurrence in the two seasons ($p > 0.05$).

4. DISCUSSION

Enterococci can be a source of disease for patients with compromised immunity or as a result of catheterization in the hospital. These pathogens possess the potential to become virulent thereby constituting a serious global health concern. The age ranges of 21-25 years 10(14.7%) and 26-30 years 8(11.8%) had the highest prevalence and this could be due to the fact that they are sexually active group in line with the report of Ellis [6], that the rate of infection increases with increase in sexual activities.

The result showed that more females were infected by the organism than males though the difference was not statistically significant. This could be related to the anatomical position of the vagina which is very close to the anus, creating possible easy transfer of the organism as reported by Scholes *et al.*, [7].

The frequency of isolation was more in rainy season than in dry season. This could be due to the fact that rain water washes faecal materials into streams and rivers where drinking/domestic water could be obtained especially in rural areas where treated water is not supplied as reported by Odeyemi *et al.*, [17] and where bush method of toilet disposal is practiced.

Pregnancy 14(20.6%) was the most common possible predisposing factor observed in this study and this was in accordance with the report of Boyko *et al.*, [4] and Nester *et al.*, [5] who established that reduced immune system can predispose patients to the infection. Catheterization 10(14.7%) is also a predisposing factor that was high in the study. Catheters increase the risk of infection because they can impede the normal barrier function of the mucosa [5].

This organism was more nosocomially acquired 41(60.3%) with a prevalence of 6.5% than community acquired 27(39.7%) with a prevalence of 4.3% [16]. In Nigeria, Kafayat *et al.*, [18] reported a prevalence of hospital-acquired enterococcal infection of 5.9% in two primary hospitals in Osogbo Southwestern Nigeria. This shows an increase in the prevalence rate. The reason for this increase is unclear, but an important contributory factor could be the selection pressure of increasing

consumption of β -lactam-based antibiotics and cephalosporins. This promotes enterococci, which are inherently resistant to this group of antibiotics [19].

5. CONCLUSION

Therefore, there is an increasing prevalence of Enterococci and can be hospital acquired, screening for this organism is important. The practice of good hygiene should be promoted at home and in clinical setting.

CONSENT AND ETHICAL APPROVAL

Ethical clearance from the ethical committees of the two institutions and informed consent from the patients were obtained.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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