Survey of Enterococci in Public Hospitals in Uyo, Akwa Ibom State, Nigeria

***Onwuezobe IA,[1] Abasiubong VN,[1] Udoh IA,[2] Isong NF[1]**

ABSTRACT

Enterococci are gut flora of man and animals. They are implicated in various human infections especially nosocomial infection which is often resistant to antibiotic treatment. The study objectives were to determine the prevalence, antibiotic sensitivities and virulence factors of Enterococcus species from clinical samples. A descriptive cross-sectional study of three clinical samples from patients admitted in two public hospitals in Uyo. Sample collection and initial processing were done within two hours with standard techniques being followed for further isolation, identification, antibiotic susceptibility testing and detection of Enterococci virulence factors. A total of 200 samples from inpatients, aged between 16- 46 years, comprising 99(49.5%) females and 101(50.5%) males, were included in this study. Urine samples were 113(56.5%), while wound and stool samples were 54(27.0%) and 33(16.5%) respectively. The Enterococci isolates from urine were 9(47.4%), mostly Enterococcus faecalis 7(77.8); stool 6(31.6%), mostly E. faecium 3(50.0%) and wound 4(21.1%) which had both E. faecalis and E. durans. Only the stool sample had an isolate of E. gallinarium 1(16.7%). All the isolates were resistant to Ciprofloxacin, Gentamycin and Erythromycin, but showed some sensitivity to Ampicillin. Only E. durans and E. faecalis were sensitive to Vancomycin. The virulence factors expressed by the various strains were biofilm and haemolysin. Enterococus faecalis was the most prevalent Enterococcus in Public hospitals in Uyo. Although some of the isolates were sensitive to Ampicillin and Vancomycin, most were resistant to common antibiotics amidst the production of Biofilms and Gamma haemolysins as virulence factors.

Keywords: Enterococci, Public hospitals, antibiotic sensitivity, virulence factors

INTRODUCTION

Enterococci are gram-positive cocci bacteria that are normally present in the human intestine and the female genital tract and are often found in the environment and even harboured by animals. Although they were previously believed to be harmless commensals however, they are known now to cause diseases, most commonly, nosocomial infection.[1] The prevalence of Enterococci from various specimen has been reported to be up to 22.19% in a survey.[2]

The genus *Enterococcus* includes more than 17 species,[3] although only a few are known to cause clinical infection in humans.[1] Before they were assigned their genus they were classified as group D Streptococcus. The species known to cause human infection include *Enterococcus faecalis* and *Enterococcus faecium* which are the most prevalent species and account for more than 90% of clinical isolates.[4] Others are *Enterococcus avium, Enterococcus durans, Enterococcus raffinose, Enterococcus gallinarium and Enterococcus mundtii*. *Enterococci* have both an intrinsic and acquired resistance to antibiotics, making them important nosocomial pathogens. Intrinsically, Enterococci tolerate or resist beta-lactam antibiotics because of their low affinity to penicillin-binding protein (PBPs).[5] They are intrinsically resistant to penicillinase susceptible penicillin, penicillinase-resistant penicillins, cephalosporins, nalidixic acid, aztreonam, macrolides and low concentrations of clindamycin and aminoglycosides. They use already formed folic acid, which allows them to bypass the inhibition of folate synthesis, resulting in resistance to sulfamethoxazole. Vancomycin-resistant Enterococci (VRE), particularly *E.faecium* strain are frequently resistant to all antibiotics that are effective against it, *t*hereby leaving little or no options of treatment with antibiotics. However, newer antibiotics such as quinupristin-dalfopristin, linezolid, daptomycin, tigecycline with activity against many VRE

Department of Medical Microbiology,[1] Faculty of Clinical Sciences, University of Uyo.

Department of Surgery,[2] Faculty of Clinical Sciences, University of Uyo.

*Corresponding author: ifeanyionwuezobe@uniuyo.edu.ng

strains have improved this situation. This notwithstanding, a mutation in the domain V of the 23s rRNA of enterococci is now known to trigger linezolid resistance, whereas resistance to quinupristin-dalfopristin may be as a result of several mechanisms which includes modification of enzymes, active efflux, and target modification.[6] The resistance of *E*nterococc*i faecalis* and *Enterococci faecium* to daptomycin, newer cyclic lipopeptide antibiotics that act on the bacterial cell membrane, has also been reported.[7] Virulence factors used by Enterococci in the causation of infection exist as pathogenicity islands (PAIs), where virulence genes are often clustered in the genome in distinct regions.[8] Most prominent among these virulence determinants are the surface adhesions including *Enterococcal* surface proteins and aggregation substance, a microbial surface components recognizing adhesive matrix molecules, secreted toxin cytolysin, gelatinase and serine protease, *Enterococcal* capsule, cell wall polysaccharides and extracellular superoxide.[9] Due to paucity of data, this study was carried out to determine the prevalence, antibiotic susceptibility and virulence factors of *Enterococci* isolates from urine, wound and stool samples of patients attending Secondary and Tertiary Hospitals in Uyo, South-South Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out for six months in two hospitals with high patient patronage and admission capacity, located in Uyo, Akwa Ibom State. The two selected hospitals were the University of Uyo Teaching Hospital (UUTH) and Anua General Hospital (AGH), a tertiary and secondary hospital respectively.

Study Design

It was a descriptive cross-sectional, hospital-based study consisting of clinical samples (pus/ wound swab, urine and stool samples) from inpatients admitted into various wards of University of Uyo Teaching Hospital and Anua General Hospital, Uyo, Nigeria.

Study Population

A total of 200 consented patients on admission for various infections were included in the study. Relevant samples where indicated and collected accordingly from these patients. The sample size of 200 was calculated based on Enterococci seroprevalence of 23% found among patients in Oshogbo, Osun State, Nigeria. to give a 95% confidence level and margin of error of ±5%. A total of 123 and 77 patients (totalling 200), from the University of Uyo Teaching Hospital and Anua General Hospital respectively were included based on patients' admission capacity of the hospitals. All consented patients on admission in the hospitals were included while nonconsenting patients on admission and outpatients were excluded from this study.

Ethical Consideration

Ethical approval was sought and obtained from the Research Ethic committees of both hospitals before commencing the study.

Sample Collection and Processing

Sterile swab sticks were used for the collection of pus and wound samples while patients themselves, collected stool samples and clean catch mid-stream urine into sterile universal bottles. All samples were collected with strict adherence to asepsis and patients were clearly instructed on these procedures. The collected samples were transported using a ice-box to the Medical Microbiology laboratory where initial processing (microscopy and inoculation on CLED, and Bile-Esculin agar) was done within two hours. Biochemical identification of *Enterococci* and antibiotic sensitivity test followed 18-24 hours after, using recommended techniques.[10] These included catalase negativity, growth on and blackening of Bile esculin, growth in the presence of 6.5% sodium chloride, pigment production, and the generation of acid from Mannitol, Arabinose and Sorbitol. The carbohydrate fermentation reaction was performed using Brain heart infusion broth containing 1% carbohydrate with bromocresol purple as an indicator.[10] The colonies were

identified and confirmed as *Enterococcus* using the following test: Gram stain positivity test, negative catalase test, positive bile-esculin test, mannitol, and sorbitol and arabinose fermentation reaction.[10]

Isolates were further tested for the detection of two virulence factors; Haemolysin and Biofilm production. For Hemolysin detection, the various isolates were inoculated on Brain heart infusion agar supplemented with 5% sheep blood and incubated at 37^{0}C for 24-48 hours.[11] Biofilm detection was done using the tube method by Christensen *et al.*[12] The isolates were aseptically inoculated into sterile tubes containing 10mls of Trypticase soy broth and incubated at 37^{0}C for 24 hours. After 24 hours, the supernatant in each tube was decanted and washed with phosphate buffer saline and allowed to dry in an inverted position. They were stained with crystal violet (0.1%) for 30minutes, washed and left to dry in an inverted position for the observation of biofilm formation. Antibiotic sensitivity testing was performed on Mueller Hinton Agar using the Kirby Bauer disk diffusion method.[10]

RESULTS

A total of 200 patients on admission, 123 from the University of Uyo Teaching Hospital and 77 from Anua General Hospital (table 1) were included in the study. Among these were 101(50.5%) males and 99(49.5%) females, with age range from 16-46 years (Table 1). The clinical samples were mainly urine 113(56.5%), pus/wound 54(27.0%) and stool samples 33(16.5%) as in Table 2. Only 19(9.5%) varying species of *Enterococci* were isolated, 5(26.3%) from Anua General Hospital and 14(73.7%) from University of Uyo Teaching Hospital, Table 3. These isolates include *E.faecalis* 11(57.9%), *E.faecium* 5(26.5%), *E.durans* 2 (10.5%) and *E.gallinarium* 1(5.3%), Table 4. Their antibiotics sensitivity patterns revealed complete resistance by all the isolated species to Ciprofloxacin, Gentamycin and Erythromycin. However, they were all mostly sensitive to Ampicillin. Only an isolate (50%) of *E.durans* and 3 (27.3%) of *E.faecalis* were sensitive to Vancomycin as in Table 5. Most of the *E.faecalis* strains were shown to strongly produce Biofilms 7(63.6%) and Gamma Haemolysin 8(72.7%). However, *E.faecium* produced Biofilms 3(60.0%) and Alpha Haemolysin 3(60.0%) strongly as in Table 6.

Table 1: Age and Sex Distribution of Subjects

Age	Females	Males	Total
16-25	25(12.5%)	15(7.5%)	40(20.0%)
26-35	35(17.5%)	25(12.5%)	60(30.0%)
36-45	13(6.5%)	37(18.5%)	50(25.0%)
≥46	26(13.0%)	24(12.0%)	50(25.0%)
Total	99(49.5%)	101(50.5%)	200(100.0%)

Table 2: Distribution of Clinical Samples Based on Gender

Clinical samples	Males (n =101)	Females (n=99)	Total (%)
Urine	56(56.5%)	57(56.4%)	113(56.5%)
Pus/Wound	29(28.7%)	25(25.3%)	54(27.0%)
Stool	15(14.9%)	18(18.2%)	33(16.5%)
Total	101(100.0%)	99(100.0%)	200(100%)

Table 3: Distribution of isolates from the different hospitals

Clinical samples	No. of isolates	No. of isolates from Anua Gen. Hosp. (AGH)	No. of isolates from Univ. of Uyo Teach. Hosp.(UUTH)
Urine	9(47.4%)	3(60.0%)	6(42.8%)
Pus/Wound	6(31.6%)	2(40.0%)	4(28.6%)
Stool	4(21.1%)	0(0%)	4(28.6%)
Total	19 (100%)	5(26.3%)	14(73.7%)

Table 4: Distribution of Enterococci isolates according to Clinical samples

Clinical samples	No. of samples (%)	No. of isolates (%)	Species of isolates *E.faecalis*	*E.durans*	*E.faecium*	*E.gallinarium*
Urine	113(56.5)	9(47.4)	7(77.8)	0(0)	2(22.2)	0(0)
Pus/Wound	54(27.0)	6(31.6)	2(33.3)	0(0)	3(50.0)	1(16.7)
Stool	33(16.5)	4(21.1)	2(50.0)	2(50.0)	0(0)	0(0)
Total	200(100)	19(9.5)	11(57.9)	2(10.5)	5(26.5)	1(5.3)

Table 5: Antibiotic sensitivity pattern of isolates

Isolates	Ciprofloxacin			Gentamycin			Vancomycin			Aztreonam			Erythromycin			Ampicillin		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
E.faecalis	0	3	8	0	0	11	3	0	8	4	0	7	0	1	10	5	0	6
E. faecium	0	1	4	0	0	5	0	2	3	0	0	5	0	1	4	4	0	1
E. durans	0	1	1	0	2	0	1	0	1	1	0	1	0	0	2	1	0	1
E.gallinarium	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0

Key: S = Sensitive, I = Intermediate, R = Resistance

Table 6: Virulence factors produced by isolates

Isolates	Total No. of isolates (%)	No. with Biofilm production (%)			No. with Haemolysin production (%)		
		Strong	Moderate	Weak	Gamma	Alpha	Beta
E.faecalis	11(57.9)	7(63.6)	4(36.4)	0(0)	8(72.7)	3(27.3)	0(0)
E. faecium	5(26.5)	3(60.0)	2(40.0)	0(0)	0(0)	3(60.0)	2(40.0)
E. durans	2(10.5)	0(0)	0(0)	2(10.5)	0(0)	0(0)	2(100.0)
E. gallinarium	1(5.3)	0(0)	0(0)	1(5.2)	0(0)	0(0)	1(100.0)

DISCUSSION

The overall prevalence of *Enterococci* species from this study is 9.5%. This is higher than the prevalence rate of 5.9% obtained from a similar study from Oshogbo.[13] The higher probability of having patients who are on admission for a longer time in the referral hospitals used for the study and the higher risk of nosocomial infection,[14] may have contributed to the higher prevalence. Expectedly, there is was a higher prevalence of *Enterococcus* in the University of Uyo Teaching hospital (73.7%), a tertiary health facility with more referrals when compared to the prevalence of 26.3% from Anua General Hospital, a secondary health facility with lower admissions. The prevalence of

Enterococcus in accordance with the various samples, urine samples have the highest rate of 47.4%. This prevalence rate although high is less than what was obtained in a survey by the Center for Disease Control(CDC) on nosocomial infections in which *Enterococcus,* accounted for 63.9% of urinary tract infections being next to *Escherichia coli* as a causative agent of hospital-acquired urinary tract infection.[2,15] This finding differed from that by Vandamme *et al*., who recorded more isolates from pus samples.[16] There are reports of an increase in the isolation of *E. faecium* and other Enterococcal species,[17] however, this study revealed *E.faecalis* (57.8%) as the major isolate, followed by *E.faecium* (26.5%). This conforms with reports by Facklam *et al*.[18] While majority of the *E.faecalis* were gotten from urine samples, most of the *E.faecium* were from pus/wound samples. This goes to suggest that these *Enterococcus species* are probably common Gram-positive nosocomial pathogens of urinary and post-surgical wound infection in the two hospitals respectively. As regards antibiotic susceptibility, the increasing resistance of *Enterococcus* to various antibiotics is worrisome globally.

Of note, is the complete resistance of the various species of this pathogen from this study, to Ciprofloxacin, Gentamycin and Erythromycin. This is a deviation from reports of previous similar studies which showed more than 50% sensitivity of the various *Enterococcus species* to Ciprofloxacin.[16,19] Revealed also, was the high resistance of the various species to Vancomycin. Only *E. durans (*50%) and *E.faecalis (27.3%)* showed sensitivity to Vancomycin while all the other species showed complete resistance to the drug. The high resistance of these pathogens to Vancomycin has also been reported in India[20]. A total of 11 (57.9%) isolates of *Enterococcus species* were sensitive to Ampicillin making it the antibiotic with the highest sensitivity, though with a resistance rate of 42.1%. other studies have reported higher sensitivity and lower resistance to Ampicillin.[16,19] The reason for this may be attributable to the abuse of this antibiotic by the populace. The most prevalent isolates in this study, *E.faecalis* and *E.faecium s*trongly expressed Biofilm production as a virulence factor. However, while *E.faecalis* expressed more of Gamma Haemolysin as another virulence factor, *E.faecium e*xpressed mostly Alpha Haemolysin. This may be one of the reasons why these species are responsible for a greater number of infections[21] as revealed by this study despite the absence of a statistical correlation. Nevertheless, *E durans* and *E.gallinarium* were observed to only weakly express Biofilm and Beta Haemolysin and therefore may have lesser abilities to cause infection.

CONCLUSION

The prevalence of Enterococci species among in-patients in Uyo, Nigeria, is 9.5% with isolates comprising mostly of *E.faecalis, E.faecium, E.durans* and *E.gallinarium.* The isolates were completely resistant to Ciprofloxacin, Gentamycin and Erythromycin while more than 50% of them were sensitive to Ampicillin. Only *E. durans* (50%) and *E.faecalis* (27.3%) showed sensitivity to Vancomycin while all the other species showed complete resistance to the drug. Their expressed virulence factors were biofilm production and haemolysins.

CONFLICT OF INTEREST

I declare no conflict of interest. The article has been read and approved by all the authors.

REFERENCES

1. Lopez-salas P, Llaca-Diaz J, Morfin-Otero R. Virulence and antibiotic resistance of *Enterococcus faecalis* clinical isolates recovered from three States of Mexico. Detection of linezolid resistance. *Arch Med Res*. 2013;44:422-8.
2. Desai PJ, Pandit D, Mathur M, Gogate A. Prevalence, identification and distribution of various species of enterococci isolated from clinical specimens with special reference to urinary tract infection in catheterized patients. *Indian J Med Microbiol*. 2001;19:132-7
3. Patel SN, Memari N, Shahinas O, Toye B, Jamieson FB, Farewell DJ. Linezolid resistance in *Enterococcus faecalis* isolated in Ontario, Canada. *Diagn microbial infect Dis*. 2000; 67: 4321-6.
4. Murray IK, Baron EJ, Lanry ML, Forgensen JH, Faller MA. Manual of microbi. 9th ed. Washington DC: *ASM press*; 2007;p. 67-9
5. Willems RJ, Bonten MN, Baquero F, Grudmann H. Glycopeptide-resistance Enterococci: deciphery virulence, resistance and epidemicity. *Curr Opin Infect Dis*. 2009; 20:384-390.

6. Hershberger *E. Quinupristin-dalfopristin resistance in Gram positive resistance and Epidemiology. Clin Infec Dis.* 2004;38:92-98.
7. Montero CI, Stock F, Murray PR. Daptomycin, a bacterial lipopeptide synthesized by non-ribosomal machinery. *Antimicrob Agents Chemother*. 2008;52:1167-70.
8. Shankar N, Baghdayan AS, Gilmore MS. Modulation of virulence within pathogenicity and vancomycin-resistant Enterococcus faecalis nature. *J Med Microbiol*. 2002;417:746-50.
9. Upadyyaya PM, Ravikumar KL, Umpathy BL. Review of virulence factors of *Enterococci*, an emerging nosocomial pathogen. *Int j Med Microbiol*. 2009;27:234-9.
10. Teixeira LC, Carvalho MS, Shewmaker PL, Facklam RR. Enterococcus in Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML Warnock DW. Manual of clinical microbiology 10th ed. Washington DC: *ASM presss*; 2011; 354-5.
11. Vergis EN, Shankar N, Chav JW, Hayden MK, Snydman DR, Zervos MJ *et al*. Association between the Presence of Enterococcal virulence factors, Haemolysin and Enterococcal surface protein and mortality among Patients with Bacteraemia due to *Enterococcus faecalis*. *Clin Infec Dis.* 2001; 35:570-5.
12. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime producing strains of Staphylococcus epidermidis to smooth surfaces. *Infect Immun.* 1982;37:318-6.
13. Kafayat OO, Solomon OF, Samuel ST. Prevalence of hospital acquired Enterococci in two Primary care hospital in Oshogbo, South Western Nigeria. *Afr J Infec Dis.* 2011;5:40-6.
14. Newman MJ. Nosocomial and community-acquired infection in Korle Bu Teaching Hospital, Ghana. *West Afr J Med*. 2009;28:300-3.
15. Center for disease control and prevention (CDC), vital signs: Carbapenms-resistants Enterobactericeae. *MMWR mortal wkly Rep*.2013,62:165-170.
16. Vandamme P, Vercauteren E, Lammens C, Pensart N, Ieven M, Pot B. *Et al*. Survey of Enterococcal Susceptibility Patterns in Belgium. *J Clin. Microbiol*. 1996;34:2572-6
17. Jain S, Kumar A, Kashyap B, Kaur IR. Clinico-epidemiological profile and high-level aminoglycoside resistance in enterococcal septicemia from a tertiary care hospital in east Delhi. *Int J Appl Basic Med Res.* 2011; 1:80-3.
18. Facklam RR, Collins MD. Identification of *Enterococcus* species Isolated from Human Infections by a Conventional Test Scheme. *J Clin. Microbiol*. 1989;27:731-4.
19. Milkiyas T, Getnet B, Tesfaye K, Zeleke G, Rawleigh H, Biruk Y. Prevalence and phenotypic characterization of *Enterococcus species* isolated from clinical samples of pediatric patients in Jimma University Specialized Hospital, south west Ethiopia. Published online 2018 May 8. doi: 10.1186/s13104-018-3382-x. Accessed 20/02/19.
20. Karmarkar MG, Gershom ES, Mehta PR. *Enterococcal* infections with special reference to phenotypic characterization and drug resistance. *Indian J Med Res*. 2004;119 (suppl): 22-5.
21. Giridhara Upadhyaya PM, Umapathy BL, Ravikumar KL. Comparative study for the presence of enterococcal virulence factors gelatinase, hemolysin and biofilm among clinical and commensal isolates of *Enterococcus faecalis*. *J lab. Phy.* 2010;2:100-4.