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PREVALENCE AND ANTIBIOTICS SUSCEPTIBILITY PROFILE OF *ENTEROCOCCUS* SPP. ISOLATED FROM SOME HOSPITALS IN ABUJA, NIGERIA.

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ABSTRACT
This study investigated the prevalence and antibiotics susceptibility of *Enterococcus* spp. isolated from patients and some selected hospital environment in Abuja, Nigeria. The samples included clinical and environmental. The clinical samples included stool, urine and wound swabs while the environmental samples included swabs samples taken from the health care givers hands, floor, beds, door handle, BP cuff, stethoscope, sink, toilet seats. The samples were cultured on bile aesculinazide agar and the isolates were identified with microgen test kit. The enterococcal strains isolated include *Enterococcus faecalis*, *Enterococcus faecium, Enterococcus mundtii, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus dispar, Enterococcoushirae* and *Enterococcus avium*. The susceptibility testing was done with vancomycin, teicoplanin, gentamicin, streptomycin, linezolid, ampicillin, ciprofloxacin, chloramphenicol, doxycycline, nitrofurantoin, erythromycin and rifampin. More than 50% of the isolates were resistant to erythromycin, rifampin and doxycycline. E-test M.I.C confirmed 12 out of 34 strains to be intermediately resistant to vancomycin. *Enterococcus faecium*and *Enterococcus mundtii* exhibited more resistance than *Enterococcus faecalis*.

Key Word: *Enterococcus* spp., samples, Isolates, Hospitals, susceptibility, resistance, vancomycin.

Prévalence ET PROFIL DE SENSIBILITÉ AUX ANTIBIOTIQUES DES ENTEROCOCCUS SPP. Isolées DE CERTAINS HÔPITAUX À ABUJA, NIGERIA.

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ABSTRACTThis étude examine la prévalence et la sensibilité aux antibiotiques des Enterococcus spp. isolées de patients et certains hôpitaux à Abuja, Nigeria. Les exemples inclus et de l'environnement clinique. Les échantillons cliniques inclus les selles, l'urine et d'écouvillons plaie tandis que les échantillons environnementaux inclus écouvillons prélevés sur des fournisseurs de soins de santé les mains, étage, lits, poignée de porte, un brassard, stéthoscope, lavabo, toilettes sièges. Les échantillons ont été mis en culture sur gélose bile aesculinazide et les isolats ont été identifiés avec microgen trousse d'essai. Les souches isolées d'entérocoques : Enterococcus faecalis, Enterococcus faecium, Enterococcus mundtii, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus dispar, Enterococcoushirae et Enterococcus avium. La sensibilité a été fait avec la vancomycine, teicoplanine, la gentamicine, la streptomycine, le linézolide, à l'ampicilline, le chloramphénicol, la ciprofloxacine, la doxycycline, l'érythromycine, la nitrofurantoïne et la rifampicine. Plus de 50 % des isolats étaient résistants à l'érythromycine, la rifampicine et la doxycycline. E-test M.I.C confirmé 12 des 34 souches à intermédiaires résistantes à la vancomycine. Enterococcus Enterococcus mundtii feciumand ont présenté plus de résistance qu'Enterococcus faecalis.

Mots clés: Enterococcus spp., les échantillons, les isolats, les hôpitaux, la sensibilité, la résistance, la vancomycine.

INTRODUCTION

Enterococci are facultative anaerobic Gram-positive cocci that share their morphology and Lancefield antigenicity with group D streptococci. The genus *Enterococcus* includes at least 17 species, distinguished on the basis of pigment production, motility, and ability to produce acids from various carbohydrates (1). These coccoid-shaped bacteria are common in environments affected by animal and human faecal material. *Enterococcus* spp. could be spread via hand contact with open wounds containing the bacteria, or by touching contaminated environmental surfaces, where the organisms can survive for weeks. Recent years have witnessed increased interest in enterococci because of their ability to cause serious infections such as endocarditis, bacteraemia, intra-abdominal and urinary tract infection (UTI) and also because of

their increasing resistance to many antimicrobial agents (2).

Acquisition of microorganisms resistant to multiple antibiotics represents a threat to patients' safety. Enterococci easily acquire resistance when exposed to antibiotics or when they acquire genetic resistance factors from neighboring organisms (3). Therefore, VRE can spread through the population via human, environmental or animal reservoirs.The treatment problem such as prolong hospital stay by patients translates to increase healthcare bills and eventual death of the patients due to multi-resistant nature of VRE to antibiotics.

METHODOLOGY

Five hundred samples were collected fromKuje and Kubwa general hospitals which are secondary care hospitals; University of Abuja Teaching Hospital and National Hospital which are tertiary care hospitals. Ethical approval was obtained from the management of the hospitals. The 500 samples included 400 clinical and 100 environmental samples. The clinical samples collected included 100 stool, 240 urine, 60 wound swabs. From the 400 clinical samples, 97 strains were isolated while 5 strains were isolated from the environment. The procedure included inoculation of the stool, urine and swabs onto bile esculinazide agar, incubation for 24 hours at 37c^{o}, observation of the characteristic dark brown colonies is assumed presumptive of isolation of *Enterococcus* spp. The isolates were further subjected to growth at 45c^{o}, growth in 6.5% salt (NaCl) broth, growth on 40% bile agar, catalase test before being subjected to further confirmatory test with microgen test kit. The enterococcal strains isolated include Enterococcus faecalis, Enterococcus faecium, Enterococcus mundtii, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus dispar, Enterococcoushirae and Enterococcus avium. Antibiotics susceptibility of the isolates were conducted using Kirby-Bauer disk diffusion method using vancomycin (30µg), teicoplanin, erythromycin (15ug), doxycycline (30ug), ampicillin (10ug), chloramphenicol (30ug), linezolid (30ug), rifampicin (5ug), (30ug), ciprofloxacin (5ug), nitrofurantoin (300ug), gentamicin (120ug) and Streptomycin (300ug).

RESULT

Table 1 shows the prevalence of the species isolated from the various hospitals. A total of 102 isolates made up of 8 *Enterococcus* spp. were isolated from the various hospitals. The various samples yielded 59(57.8%) *Enterococcusfaecalis,* 24(23.5%) *Enterococcus faecium,* 11(10.8) *Enterococcus mundtii*, 3(2.9%) *Enterococcus gallinarum*, 2(2.0%) *Enterococcus dispar*, 1(1.0%) each of *Enterococcus casseliflavus*, *Enterococcus avium* and *Enterococcus hirae*. Most of the isolates were from stool with 68, followed by urine with 24, wound and environmental swabs with 5 each. Table 2 shows the antibiotics susceptibility profile of the isolates from the various hospitals. Susceptibility of all the species to ampicillin (10µg) was 72.5%, 57.8% to ciprofloxacin(5µg), 20.6% to rifampin(5µg), 57.8% to linezolid (30µg), 66.7% to vancomycin(30µg), 25.5% to doxycycline(30µg), 65.7% to teicoplanin(30µg), 16.7% to erythromycin(15µg), 51.0% to chloramphenicol(30µg), 84.3% to nitrofurantoin(300µg), 70.6% to gentamicin(120µg), 57.8% to streptomycin(300µg).

TABLE 1: PREVALENCE OF *ENTEROCOCCUS* SPECIES ISOLATED FROM SOME HOSPITALS IN ABUJA

Source	No. +ve for *Enterococcus*	E.f (%)	E.fc (%)	E.c (%)	E.g (%)	E.m (%)	E.a (%)	E.d (%)	E.h (%)
Urine	**24**	**22(91.7)**	**1(4.2)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	**1(4.2)**	**0(0.0)**	**0(0.0)**
Stool	**68**	**29(42.6)**	**23(33.8)**	**1(1.5)**	**2(2.9)**	**11(16.2)**	**0(0.0)**	**1(1.5)**	**1(1.5)**
Wound	**5**	**5(100.0)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	**0(0.0)**	**0(0.0)**
Environmental	**5**	**3(60.0)**	**0(0.0)**	**0(0.0)**	**1(20.0)**	**0(0.0)**	**0(0.0)**	**1(20.0)**	**0(0.0)**
Total	**102**	**59(57.8)**	**24(23.5)**	**1(1.0)**	**3(2.9)**	**11(10.8)**	**1(1.0)**	**2(2.0)**	**1(1.0)**

Key: +ve: positive, *E.f:E.faecalis, E.fc:E.faecium, E.c:E.casselliflavus, E.g:E.gallinarum, Em: E. mundtii, E.a:E.avium, E.d: E.dispar, E.h: E.hirae.*

Table 2 also shows that *E.faecalis* was the most susceptible of all the species while more resistance was exhibited by *E.feacium* and *E.mundtii* in this study. Table 3 confirmed 12 out of 34 strains that had resistance to vancomycin by disk diffusion method to be intermediately resistant by E-test minimum inhibitory concentration (M.I.C).

TABLE 2: SUSCEPTIBILITY PROFILE OF *ENTEROCOCCUS* SPP. ISOLATED FROM THE HOSPITALS

Antibiotics	Sus	*E.faecalis* 59(%)	*E.faecium* 24(%)	*E.cas* 1(%)	*E.ga* 3(%)	*E.mundtii* 11(%)	*E.avium* 1(%)	*E.dispar* 2(%)	*E.hirae* (1(%)	Total 102(%)
AMP 10µg	R	9(15.3%)	10(41.7)	-	1(33.3)	6(54.5)	-	1(50)	1(100)	28(27.5)
	I	-	-	-	-	-	-	-	-	-
	S	50(84.7)	14(58.3)	1(100)	2(66.7)	5(45.5)	1(100)	1(50)	-	74(72.5)
CIP 5µg	R	10(16.9)	9(37.5)	-	1(33.3)	4(36.4)	-	-	1(100)	25(24.5)
	I	14(23.7)	3(12.5)	-	-	-	-	1(50)	-	18(17.6)
	S	35(59.3)	12(50)	1(100)	2(66.7)	7(63.6)	1(100)	1(50)	-	59(57.8)
RIF 5µg	R	37(62.7)	18(75.0)	-	-	11(100)	-	1(50)	1(100)	68(66.7)
	I	10(16.9)	2(8.3)	-	-	-	1(100)	-	-	13(12.7)
	S	12(20.3)	4(16.7)	1(100)	3(100)	-	-	1(50)	-	21(20.6)
LIN 30µg	R	13(22.0)	13(54.1)	-	1(33.3)	9(81.8)	-	1(50)	1(100)	38(37.3)
	I	3(5.1)	1(4.2)	-	-	-	1(100)	-	-	5(4.9)
	S	43(72.9)	10(41.7)	1(100)	2(66.7)	2(18.2)	-	1(50)	-	59(57.8)
VAN 30µg	R	10(16.9)	12(50.0)	-	1(33.3)	9(81.8)	-	1(50)	1(100)	34(33.3)
	I	-	-	-	-	-	-	-	-	-
	S	49(83.1)	12(50.0)	1(100)	2(66.7)	2(18.2)	1(100)	1(50)	-	68(66.7)
DOX 30µg	R	34(57.6)	16(66.7)	-	3(100)	9(81.8)	1(100)	1(50)	1(100)	65(63.7)
	I	8(13.6)	3(12.5)	-	-	-	-	-	-	11(10.8)
	S	17(28.8)	5(20.8)	1(100)	-	2(18.2)	-	1(50)	-	26(25.5)
TEIC 30µg	R	9(15.3)	12(50.0)	-	1(33.3)	9(81.8)	-	1(50)	1(100)	33(32.4)
	I	2(3.4)	-	-	-	-	-	-	-	2(1.96)
	S	48(81.4)	12(50.0)	1(100)	2(66.7)	2(18.2)	1(100)	1(50)	-	67(65.7)
ERY 15µg	R	28(47.5)	17(70.8)	1(100)	3(100)	9(81.8)	1(100)	1(50)	1(100)	61(60.0)
	I	21(35.6)	1(4.2)	-	-	2(18.2)	-	-	-	24(23.5)
	S	10(16.9)	6(25.0)	-	-	-	-	1(50)	-	17(16.7)
CHL 30µg	R	28(47.5)	10(41.7)	-	1(33.3)	6(54.5)	1(100)	-	-	46(45.1)
	I	2(3.4)	1(4.2)	-	1(33.3)	-	-	-	-	4(3.9)
	S	29(49.2)	13(54.1)	1(100)	1(33.3)	5(45.5)	-	2(100)	1(100)	52(51.0)
NIT 300µg	R	4(6.8)	3(12.5)	-	-	2(18.2)	-	-	-	9(8.8)
	I	3(5.1)	3(12.5)	-	-	1(9.1)	-	-	-	7(6.9)
	S	52(88.1)	18(75)	1(100)	3(100)	8(72.7)	1(100)	2(100)	1(100)	86(84.3)
GEN 120µg	R	14(23.7)	8(33.3)	-	-	3(27.3)	1(100)	1(50)	-	27(26.5)
	I	1(1.7)	2(8.3)	-	-	-	-	-	-	3(2.9)
	S	44(74.6)	14(58.3)	1(100)	3(100)	8(72.7)	-	1(50)	1(100)	72(70.6)
STR 300µg	R	24(40.7)	12(50.0)	-	-	4(36.4)	1(100)	-	-	41(40.2)
	I	1(1.7)	-	-	-	-	-	-	1(100)	2(1.96)
	S	34(57.6)	12(50.0)	1(100)	3(100)	7(63.6)	-	2(100)	-	59(57.8)

Key: E.ca: *E.casseliflavus*, E.ga: *E.gallinarum*, Sus: Susceptibility,R: Resistance, I: intermediate, S: susceptible, Cassel: *casselliflavus*, AMP: Ampicillin, CIP:Ciprofloxacin, RIF: Rifampicin,LIN: Linzolid, VAN:Vancomycin, DOX: Doxicyclin, TEC:Teicoplanin,ERY:Erythromycin,CHL:Chloramphenicol,NIT:Nitrofurantoin,GEN:Gentimicin,STR:Streptomycin.

DISCUSSION

Enterococci are part of human and animal intestinal flora which have emerged as community acquired pathogens and a leading cause of hospital acquired infections. In this study, we investigated the prevalence of *Enterococcus* spp. isolated from 500 samples collected from some selected tertiary and secondary care hospitals in Abuja, Nigeria. Eight different species were isolated with *E faecalis* as the majority with a percentage of 57.8 followed by *E.faecium* with percentage of 23.5, *E. mundtii* (10.8%), *E.gallinarum*(2.9%), *E.dispar*(2.0%), *E. casseliflavus*(1.0), *E.avium*(1.0%) and *E.hirae*(1.0%). This result is comparable to other work on *Enterococcus* spp. in other parts of the world where *E.faecalis* predominated followed by E.faecium while others account for less than 5% (4), (5) however Baragundi*et al*. (6), Anjana*et al*., (7) and Azza*et al*.,(8), reported more isolation of *E.faecium* in their studies. The more isolation of *E.faecium* could be responsible for the multidrug resistance reported in their studies as it has been implicated to be the

most causative agent of nosocomial infection and vancomycin resistance. This findings also confirmed the report of Cetinkaya*et al.*(9) where *E. gallinarum*, *E. casseliflavus*, *E. disparand E. avium* were isolated less frequently and account for less than 5% of clinical isolates. More isolation of *E.faecalis* (68) from stool in this study could be due to the normal floral nature of *Enterococcus* spp. in the gastrointestinal track of most organisms especially humans unlike the other samples in this study such as urine, wound that are sterile unless there is infection.

TABLE 3: ZONE DIAMETER INTERPRETIVE STANDARDS AND EQUIVALENT MINIMUM INHIBITORY CONCENTRATION (MIC) BREAKPOINTS FOR *ENTEROCOCCUS* SPECIES

S/N	Isolate code	Sample	Strain	R(<14mm)	Etest Van MIC(ug/ml) <4	8-16	>32
1	Kw2	Stool	*E.faecium*	0	4	-	-
2	Kw3	Stool	*E.mundtii*	0	-	8	-
3	Kw4	Stool	*E.mundtii*	0	-	8	-
4	Kw5	Stool	*E.faecalis*	0	2	-	-
5	Kw6	Stool	*E.faecium*	0	2	-	-
6	Kw10	Stool	*E.hirae*	0	4	-	-
7	UA14	Stool	*E.faecalis*	0	1	-	-
8	UA15	Stool	*E.faecium*	0	1	-	-
9	UA17	Stool	*E.faecium*	0	1	-	-
10	UA18	Stool	*E.faecalis*	0	4	-	-
11	NH2	Stool	*E.gallinarum*	0	-	8	-
12	NH4	Stool	*E.mundtii*	0	2	-	-
13	NH5	Urine	*E.faecalis*	0	2	-	-
14	NH7	Stool	*E.mundtii*	0	1	-	
15	NH8	Stool	*E.faecium*	0	-	8	-
16	NH9	Stool	*E.mundtii*	0	-	8	-
17	NH10	Stool	*E.faecium*	0	2	-	-
18	NH11	Stool	*E.faecium*	0	2	-	-
19	NH12	Stool	*E.faecium*	0	4	-	-
20	NH17	Stool	*E.faecalis*	0	-	8	-
21	NH18	Stool	*E.faecium*	0	4	8	-
22	NH19	Urine	*E.faecalis*	0	2	-	
23	NH20	Urine	*E.faecalis*	0	-	8	-
24	NH21	Urine	*E.faecalis*	0	4	-	-
25	NH24	Stool	*E.faecium*	0	1	-	-
26	NH25	stool	*E.mundtii*	0	-	8	-
27	NH26	stool	*E.faecium*	0	4	-	-
28	NH27	stool	*E.faecalis*	0	-	8	-
29	NH31	stool	*E.mundtii*	0	1	-	-
30	NH32	stool	*E.mundtii*	0	4	-	-
31	NH33	stool	*E.faecium*	0	-	8	-
32	NH34	stool	*E.mundtii*	0	4	-	-
33	NH35	stool	*E.dispar*	0	-	8	-
34	NH36	urine	*E.faecalis*	0	2	-	-

The susceptibility profile of the isolates shows above average susceptibility of the strains to commonly used recommended antibiotics by CLSI, 2014 (10). Out of the 12 antibiotics tested, 9 showed good activity against the strains except for rifampin, doxycycline and erythromycin that had more than 50% of the isolates resistant to them. The resistance to this 3 antibiotics could be associated to their abuse since they are over the counter medication and accessible to patients without doctor's prescription due to proliferation of patent medicine stores and pharmacies. Also, consumption of poultry or animal product reared with this antibiotics as growth supplement could have contributed to the resistance as the susceptibility profile is comparable to the work of Schwaiger*et al.*,(11) where *Enterococcus* spp. isolated from hens showed high resistance to rifampicin, erythromycin, fosfomycin and doxycycline. Good susceptibility to glycopeptides, ampicillin and high level aminoglycosides in this research gives reassurance for synergistic treatment of vancomycin resistant enterococcal infections such as endocarditis, urinary tract infections and bacteriamia. The above average activity of high level aminoglycoside (120ug gentimicin and 300ug of streptomycin) in this study is encouraging as ampicillin, penicillin, or vancomycin (for susceptible strains) can be combined, plus an aminoglycoside to work synergistically for the treatment of serious enterococcal infections, such as endocarditis, unless high-level resistance to both gentamicin and streptomycin is documented (10). The susceptibility profile of the isolates in this study showed *E.mundtii* and *E.faecium* to be more resistant than *E.faecalis*.

In this research, 33.3% of the enterococcal isolates were resistant to vancomycin by Kirby Bauer disk diffusion method. Most of the VRE isolates were isolated from National Hospital Abuja. Previous studies accounted for 100% susceptibility of *Enterococcus faecalis* to vancomycin(12) however most of our resistant strains were *E.mundtii* with 81.8% resistance and *E.faecium* with 50.0% resistance unlike *E.faecalis* that showed a lower percentage (16.9%) of resistance. It has been reported that

E.faecium is responsible for most vancomycin resistant enterococci (VRE) infections (1). The higher resistance of *E.mundtii* in this study could be because of its close relatedness to *E.faecium* by phylogeny (13). Minimum inhibitory concentration of the 34 resistant enterococcal strains confirmed 12(11.8%) strains to have intermediate susceptibility of≤ 8 µg/ml by E-test strips (oxoid) method using CLSI, 2014 antibiotics susceptibility interpretive guideline. E-test MIC confirmed 4(36.4%) *E.mundtii*, 3(12.5%)*E.faecium* and 3(5.1%)*E.faecalis* which are the most frequently isolated to have intermediate susceptibility of 8µg/ml each.Non was extremely resistant with MIC of ≥32 µg/ml. The possibility of acquisition of resistant genes and exposure to different antibiotics could have caused the emergence of low or intermediate enterococcal resistance to vancomycin in this study. Enterococci acquire drug resistance through plasmids, conjugative transposition or by mutations which leads to the rapid spread of multidrug resistant enterococcal infections (7). In Nigeria, VRE may soon become a great threat since 33.3% of the 102 isolates exhibited resistance to vancomycinby by disk diffusion method even though only 4 were phenotypically confirmed by minimum inhibitory concentration. Adequate measures aimed at curtailing its spread needs to be implemented.

CONCLUSION

The result showed *E.faecalis* as the major isolates among the *Enterococcus* spp. isolated with stool urine, wound and environmental swabs as the major sources. Most of the isolates showed greater than 50% susceptibility to the antibiotics tested except for erythromycin, doxycycline and rifampicin with < 50% susceptibility.

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