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Antibiotics Susceptibility and Multiple Antibiotics Resistant Pattern of Enterococcus Spp. Isolated from a Tertiary Hospital in Abuja, Nigeria

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Abstract:
The research was carried out at National Hospital, Abuja where 120 samples comprising stool, urine, and wound swabs were collected and cultured onto Bile esculin azide agar. The stool yielded 25 enterococcal strains, urine 11 strains while wound swabs yielded 3 strains making a total of 39 isolated strains. Enterococcus mundtii and E. gallinarum exhibited resistance to 8 antibiotics disk, E. faecium and E. dispar exhibited resistance to 7 antibiotics disk while E. faecalis was resistant to 4 antibiotics disk tested. Ten multidrug resistance pattern were exhibited by the isolates with 87% of them having MAR index of 0.2 and above.

Keywords: *Isolates, resistance, Enterococcus species, susceptibility, antibiotics.*

1. Introduction

Enterococcus spp. have become important organisms in the health care sector because of their ability to cause infectious diseases such as urinary tract infection, endocarditis, bacteremia and wound infections with increasing resistance to different antibiotics (Chakraborty et al., 2015). They are normal flora in the gastro-intestinal tract of humans (Miller *et al*., 2014). The adaptation of these organisms to environmental selective pressure has made them to survive in hospital environment (Miller *et al*. 2014). Antibiotics use in the Nigeria has increased among the populace both in the hospitals and within the community. Enterococci easily acquire resistance from neighbouring organism or when exposed to antibiotics. Emergence of multidrug resistant enterococci has become a significant public health threat (Magiorakos *et al*., 2012). There is a rapid spread of resistance to antimicrobials especially to vancomycin which used to be the drug of last resort for the treatment of infections cause by enterococci (Boneca and Chiosis, 2003). Resistance to penicillin, high level aminoglycosides, linezolid, daptomycin have been reported. (Agudelo *et al*., 2014)

Enterococcus faecium has been implicated to be intrisincally more resistant than *E. faecalis* and has emerged as the leading cause of multidrug-resistant enterococcal infection (Hidron, *et al*., 2008). Other enterococcal species such as *E. durans, E. avium, E. casseliflavus, E. hirae, E. gallinarum, E. raffinosus*, and *E. muntdii* are rarer causes of human infection (Gordon, *et al*., 1992) as they are not commonly isolated like *E. faecalis* that account for 85-90% of infections and 5-15% of infections cause by *E. faecium* (Gordon *et al*., 1992; Olawale *et al*., 2011). The need to carry out susceptibility and determine the multiple antibiotics resistant (MAR) index of the *Enterococcus* spp in the study population will provide the profile of multiple resistant enterococci in the study population and thus serves as guide for healthcare policy makers or formulators.

2. Result

A total of 39 strains were isolated from 120 clinical samples comprising of urine, stool and wound swabs at National Hospital, Abuja. They were inoculated on Bile Esculin Azide agar and incubated for 24 hours after which the colonies were tested for catalase, growth

at 45c°, growth on 40% bile and growth in 6.5% NaCl broth. The strains were further confirmed to be *Enterococcus* spp. with microgen test kits. Seventy urine samples collected yielded 11 isolates with a percentage of 15.7; 30 stool samples yielded 25 isolates with a percentage of 83.3; 20 wound yielded 3 isolates with a percentage 15.0. There was a significant association between isolates and sample source (>0.001).

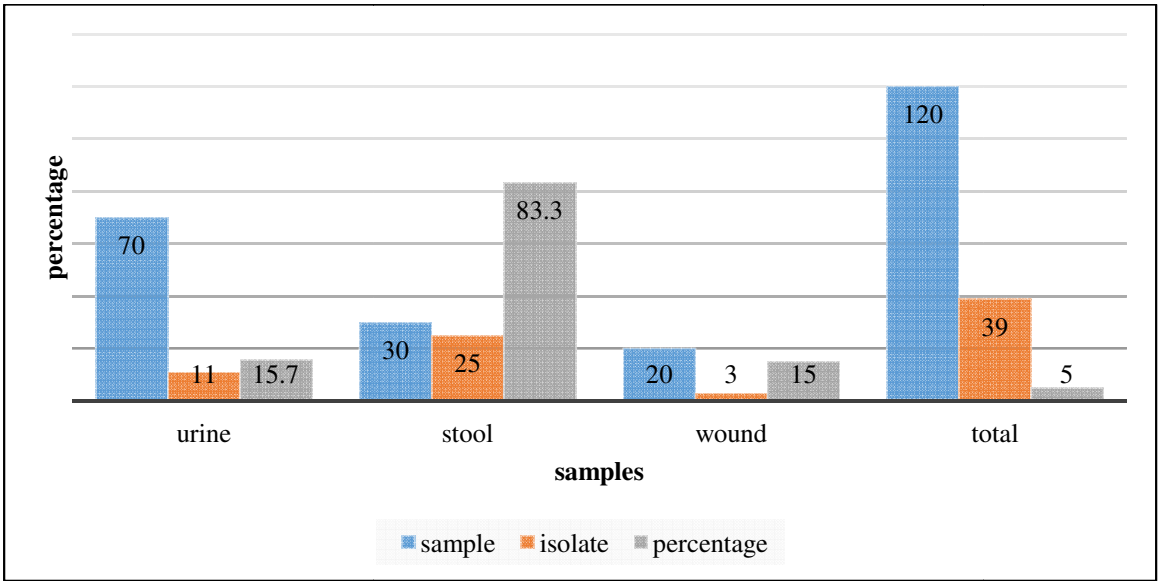

Figure 1

The isolates were subjected to Kirby-Bauer antibiotics susceptibility testing with 10µg of ampicillin, ciprofloxacin (µg5), rifampin (µg5), Linezolid (µg30), vancomycin (µg30), doxycycline (µg30), teicoplanin(µg30), erythromycin (µg5), chloramphenicol(µg30), nitrofurantoin(µg300), gentamicin(µg120) and streptomycin () as shown in figure 2. More than 7 antibiotics showed greater than 50% inactivity against the isolates with 87.2% of the strains resistant to nitrofurantoin, 76.9% strains resistant to erythromycin, 71.8% of the strains resistant to doxycycline, 61.5% of the strains resistant to both vancomycin and linezolid, 59.0% of the strains resistant to teicoplanin, 53.8% of the strains resistant to chloramphenicol. Nitrofurantoin, streptomycin, gentamicin, ciprofloxacin and ampicillin gave good activity of >50% activity.

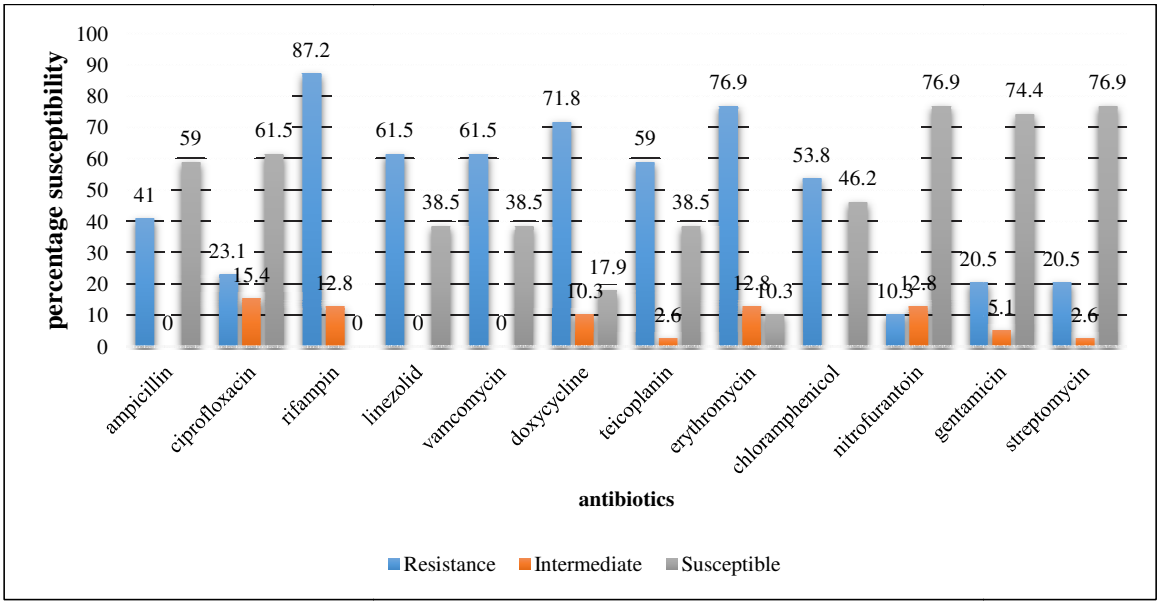

Figure 2

The table below shows the resistance profile of the *Enterococcus* species isolated from the hospital. *Enterococcus mundtii* exhibited the most resistance with more than 50% of the strains resistant to 8 antibiotics followed by *E. faecium* with >50% resistant strains to 7 antibiotics and *E. faecalis* with >50% of the strains resistant to 4 antibiotics among the commonly isolated species. Only one strain each of *E. gallinarum* and *E. dispar* were isolated with exhibition of resistance to 8 antibiotics by *E. dispar* and 7 by *E. gallinarum*.

antibiotics	*E. faecalis* (19)	*E. faecium* (11)	*E. gallinarum* (1)	*E. mundtii* (7)	*E. dispar* (1)
Ampicillin	3(15.8)	6(54.5)	-	6(85.7)	1(100)
Ciprofloxacin	4(21.1)	3(27.3)	-	2(28.6)	-
Rifampin	14(73.7)	11(100)	1(100)	7(100)	1(100)
Linezolid	7(36.8)	8(72.7)	1(100)	7(100)	1(100)
Vancomycin	7(36.8)	8(72.7)	1(100)	7(100)	1(100)
Doxycycline	13(68.4)	6(54.5)	1(100)	7(100)	1(100)
Teicoplanin	6(31.6)	8(72.7)	1(100)	7(100)	1(100)
Erythromycin	12(63.2)	9(81.8)	1(100)	7(100)	1(100)
Chloramphenicol	13(68.4)	3(27.3)	1(100)	4(57.1)	-
Nitrofurantoin	1(5.3)	1(9.1)	-	2(28.6)	-
Gentamicin	4(21.1)	2(18.2)	-	1(14.3)	1(100)
Streptomycin	4(21.1)	2(18.2)	-	2(28.6)	-

Table 1: Resistance profile of the isolated Enterococcus spp

The table below shows the multiple antibiotics resistance (MAR) index and pattern of the isolates. Ten resistant pattern were observed. Eighty seven percent (87%) of the isolates had MAR index of 0.2 and above. The class of resistance shows that 21(53.8%) of the isolates were multidrug-resistant (MDR) while 3(7.7%) were extremely drug resistant (XDR).

NA	AWP	Resistance pattern/phenotype	RI	%	CR
1	4	RIP	0.1	13	Nil
	1	STR			Nil
2	1	RIF, LIN	0.2	5	nil
	1	RIF, DOX			nil
3	1	CIP, GEN, STR	0.3	8	mdr
	1	RIF, DOX, ERY			mdr
	1	DOX, ERY, CHL			mdr
4	2	CIP, DOX, ERY, CHL	0.3	8	mdr
	1	RIF, DOX, ERY, CHL			mdr
5	2	RIF, LIN, VAN, TEIC, ERY	0.4	8	mdr
	1	AMP, RIF, VAN DOX, ERY			mdr
6	2	RIF, LIN, VAN, DOX, TEIC, ERY	0.5	10	mdr
	1	CIP, RIF, ERY, CHL, GEN, STR			mdr
	1	AMP, RIF, LIN, VAN, TEIC, CHL			mdr
7	2	RIF, LIN, VAN, DOX, TEIC, ERY, CHL	0.6	18	mdr
	1	CIP, RIF, DOX, ERY, CHL, GEN, STR			mdr
	4	AMP, RIF, LIN, VAN, DOX, TEIC, ERY			mdr
8	2	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, CHL	0.7	18	mdr
	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, NIT			mdr
	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, CHL			mdr
	1	CIP, RIF, LIN, VAN, DOX, TEIC, CHL, NIT			mdr
	1	RIF, LIN, VAN, DOX, TEIC, ERY, GEN, STR			mdr
	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, GEN			mdr
9	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, ERY, NIT	0.8	5	mdr
	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, GEN, STR			mdr
10	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, ERY, GEN, STR	0.8	8	xdr
	1	AMP, CIP, RIF, LIN, VAN, DOX, TEIC, ERY, CHL, NIT			xdr
	1	AMP, RIF, LIN, VAN, DOX, TEIC, ERY, CHL, NIT, STR			xdr

Table 2: Antibiotics Resistance Pattern and resistance index of NHA Isolates
MDR: multidrug-resistance, XDR: extremely drug resistance, NA: number of antibiotics. AWP: Antibiotics with pattern. RI: Resistance Index. CR: classification of resistance

3. Discussion

Multiple-antimicrobial resistance in *Enterococcus* species has been reported worldwide (Arias *et al*. 2010; Wei *et al*., 2014). According to Courvalin *et al*. (2006), isolation of enterococci resistant to multiple antibiotics has become increasingly common in the hospital setting. Resistance in *Enterococcus* spp. are mostly due to intrinsic and acquired resistance genes they pose (Cetinkaya *et al*., 2000). A total of 39 strains were isolated from the samples collected in this research and were subjected to Kirby-Bauer disk diffusion test using 12 different antibiotics. Seven out of the 12 antibiotics showed less activity against the isolates as > 50% were resistant. The

strains exhibited the most resistance to rifampin with 87.2% which is similar to the result obtained by Wei *et al*. (2014), followed by 76.9% to erythromycin, 71.8% to doxycycline, 61.5% to vancomycin and linezolid, 59.0% to teicoplanin and 53.8 to chloramphenicol. Some of these drugs such as rifampin, chloramphenicol, and doxycycline are not commonly used for the treatment of enterococcal infections but acquire resistance due to exposure of commensal enterococci to different antibiotics and exchange of resistance genes between bacteria. Some of these drugs are used as combination therapy such as the use of doxycycline, rifampin and Quinupristin-dalfopristin for successful treatment of patients with endocarditis (Arias *et al*., 2010).

Among the isolated strains in this research, *E. mundtii* and *E. dispar* exhibited resistance to 8 antibiotics, *E. faecium* and *E. gallinarum* exhibited resistance to 7 antibiotics while *E. faecalis* exited resistance to 4 antibiotics. The high resistance seen in *E. faecium* has been reported worldwide as it has been implicated to be responsible for most vancomycin-resistant enterococci (VRE) infections (Fraser *et al*., 2016). The resistance observed in *E. mundtii* and *E. dispar* could be because of the phylogenetic relatedness of these strains to *E. faecium* (Moellering, 1992). The resistance observed in *E. gallinarum* could be associated with the presence of intrinsic resistant gene which makes *E. gallinarum* and *E. casseliflavus* naturally resistant to some antibiotics use for treatment of enterococcal infection (Cetinkaya *et al*., 2000). *Enterococcus faecalis* are isolated most frequently from clinical samples accounting for 80-90% while *E. faecium* account for 5-15% and other *Enterococcus* spp. account for 5% (lewis and Zervos, 1990; Moellering, 1992; Cetinkaya *et al*., 2000).

Ten resistant patterns were exhibited by the isolates. According to Magiorakos *et al*. (2012), multidrug-resistance (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial categories while extensive drug-resistance (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories. The isolates exhibited multi drug-resistant patterns ranging from 2, 3, 4,5,6,7,8,9 and 10. Three (7.7%) isolates were resistant to 10 out of 12 antibiotics disk tested in this research indicating they were extensively drug resistant while 21(53.8%) had resistance to 2-9 antibiotics disk indicating they were multi drug-resistant. Four strains were resistant to single antibiotic disk indicating they were not multiresistant. Eighty seven percent (87%) of the isolates had MAR index of 0.2 and above indicating that the isolates had been pre-exposed to the antibiotics tested which agrees with the works of Krumperman (1983), Olayinka *et al*. (2004) and Olonitola *et al*. (2009) where they observed that MAR index greater than 0.2 indicates that an organism must have originated from environment where antibiotics are habitually used without prescription. The implication of this multidrug-resistant strains is that they disseminate resistant genes to the environment which are acquired by neighbouring bacteria living as normal flora which in turn cause infections in humans.

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