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CARRIAGE OF MULTIDRUG RESISTANT ENTEROCOCCUS FAECIUM AND ENTEROCOCCUS FAECALIS AMONG APPARENTLY HEALTHY HUMANS

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Abstract

Background: Enterococci are indigenous flora of the gastro-intestinal tracts of animals and humans. Recently, interest in two major species, *E. faecium* and *E. faecalis*, has heightened because of their ability to cause serious infections and their intrinsic resistance to antimicrobials. This study was aimed at determining the prevalence of *E. faecium* and *E. faecalis* in human faecal samples and evaluating the susceptibility of the isolates to antibiotics.
Materials and Methods: One hundred faecal samples were collected from apparently healthy individuals and analysed using conventional bacteriological methods. The susceptibility profile of the isolates to nine antibiotics were determined using disk diffusion method.
Results: Seventy-three (73) *Enterococcus* were phenotypically identified and 65 of the isolates were differentiated into 36 (55.4%) *E. faecium* and 29 (44.6%) *E. faecalis*. Eight (8) isolates could not be identified by the conventional biochemical methods employed. No dual colonization by the *E. faecalis* and *E. faecium* was observed and isolation rate was not dependent on sex of the participants. All the isolates were resistant to ceftriaxone, cefuroxime and ceftizoxime. *Enterococcus faecium* exhibited resistance to erythromycin (88.9%), gentamicin (77.8%), amoxicillin-clavulanate (63.9%), ofloxacin (44.4%), teicoplanin (19.4%) and vancomycin (16.7%). *Enterococcus faecalis* showed the least resistance to vancomycin (13.8%) and teicoplanin (27.7%). Remarkable multiple antibiotic resistances to the classes of antibiotic tested were observed among the two species.
Conclusion: The high carriage rate of antibiotic resistant *E. faecium* and *E. faecalis* in this study provides information on the local antibiotic patterns of our enterococci isolates thereby suggesting that they could present as important reservoir and vehicle for dissemination of resistant genes in our community.

Keywords: *Enterococcus faecium*, Human faecal samples, *Enterococcus faecalis*, Biochemical identification, Antibiotic resistance.

Introduction

Members of the genus *Enterococcus*, are characterized by individual, paired, or short-chain Gram-positive catalase-negative cocci (Ciftci *et al*., 2009). Although, ubiquitous, they are primarily localized to the human gut and are found in human faeces, where they represent a minority population (up to 1%) within the gut microflora (Song *et al*., 2005; Bibalan *et al*., 2015). Once considered as bacteria of minimal clinical impact, enterococci, particularly *Enterococcus faecium* and *Enterococcus faecalis*, have now emerged as one of the major causes of human clinical infections (Silva *et al*., 2011). They have assumed a vital role as etiologic agent of urinary tract infections, hospital-acquired bloodstream infections, endocarditis, abdominal and pelvic abscesses and chronic periodontitis (Sydnor and Perl, 2011; Qian-Qian *et al*., 2012). Their importance in such infections is reinforced by their intrinsic and acquired resistance to various antimicrobial agents which renders them difficult to treat.

Enterococcal colonization is a known risk factor for developing associated infections and is most commonly caused by the patients' own commensal flora (Maschieto *et al*., 2004). Studies have also confirmed that colonising strains of enterococci serve as reservoir for antibiotic resistance genes which can be transferred among enterococci or acquired by other bacteria (Schjørring and Krogfelt, 2011; Boehm and Sassoubre, 2014). High-density colonization by antibiotic-resistant enterococci increases the risk of infections like bacteremia (Lebreton *et al*., 2014; Tedim *et al*., 2015). These infections are difficult to treat; chronic, recurrent and sometimes fatal. Thus, there is a need to assess colonisation of multiple antibiotic-resistant enterococci especially in developing countries where the control of antibiotic use is derisory. In Nigeria, most studies on enterococci have been conducted on food, animals and environmental samples (Oladipo *et al*., 2013; Olawale *et al*., 2014; 2015; Ayeni *et al*., 2016) and a few have examined samples from clinical sources (Iregbu *et al*., 2002, Olawale *et al*., 2011; Ekuma *et al*., 2016). We report here the prevalence of multidrug resistant faecal *E. faecium* and *E. faecalis* isolates among apparently healthy humans.

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Materials and Methods

Ethical issues and Collection of samples

Verbal consent in collection of samples was sought and obtained from each person after explaining the procedure. The voiding was done by each participant without any form of coercion. They were provided with wide-mouthed, leak-proof sterile plastic containers. One hundred (100) faecal samples were collected from the participants and their sexes were documented. Other demographic data were not available because most of the participants declined to give the required information. The samples were carefully labelled and processed immediately at the Microbiology and Parasitology Laboratory, University of Lagos, Idi-Araba.

Isolation and Biochemical Identification

Sterile inoculating loop was used to pick a small portion of the sample which was then streaked on Bile-esculin agar (Oxoid, UK) plates and incubated at 37ºC for 24 hours. Discrete colonies of suspected *Enterococcus* species were sub-cultured for purity on MacConkey and blood agar plates which were also incubated at 37ºC for 24 hours. Species identification was carried out based on Gram stain, cultural characteristics and various biochemical tests including catalase reaction, growth in 6.5% NaCl broth, pyruvate utilization test, growth at 45°C and 60°C for 24 hours in a nutrient broth, haemolytic reaction on blood agar and carbohydrate (mannitol, lactose, glucose, sorbitol, raffinose, fructose, dextrose, xylose, trehalose, galactose, dulcitol, and arabinose) fermentation (Facklam *et al*., 1999). The carbohydrate fermentation tests were performed in agar containing 1% of each sugar.

Antibiotics susceptibility testing

Testing of susceptibility to ceftizoxime (30µg), cefuroxime (30µg), vancomycin (30µg), erythromycin (25µg), gentamicin (10µg), teicoplanin (25µg), ceftriaxone (30µg), ofloxacin (5µg) and amoxicillin-clavulanate (30µg) was performed by disk diffusion method. The results were interpreted in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2009). Isolates were adjudged as multidrug resistant (MDR) if resistance to three or more antibiotics of different antimicrobial classes was demonstrated (Magiorakos *et al.*, 2012). Reference strain *Enterococcus faecalis* ATCC 29212 was used as control.

Results

Of the 100 faecal samples analysed, 73 isolates were identified as *Enterococcus*. The biochemical scheme differentiated 65 of the 73 isolates into *E. faecalis* (29; 44.6%) and *E. faecium* (36; 55.4%). The phenotypic characteristics of eight isolates could not be determined through the conventional methods used and were excluded in the overall analyses. All the *Enterococcus faecalis* isolates fermented sorbitol, mannitol, glucose and lactose but not arabinose while *E. faecium* was able to ferment arabinose, mannitol, glucose and lactose but not sorbitol (Table 1). The isolation rate was independent of sex (Table 2) and no individuals exhibited colonisation with dual isolates. The isolated strains of *E. faecalis* showed resistance to ceftriaxone, cefuroxime and ceftizoxime. The overall antimicrobial susceptibility pattern of the isolates is shown in Table 3. A large proportion (96.6%) of the *E. faecalis* isolates was resistant to gentamicin and erythromycin. Three percent (10.4%) of the isolates were resistant to vancomycin. Similarly, *E. faecium* significantly exhibited high degree of resistance to gentamicin (88.9%) and erythromycin (91.7%). *Enterococcus faecalis* showed the least resistance to vancomycin (13.8%) (Figure 1). Marked multiple antibiotic resistances to the classes of antibiotic tested were observed among the two species (Table 4).

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Table 1: Sugar Utilisation Reaction of *Enterococcus faecalis* and *Enterococcus faecium*

	No of Positive Isolates (%)	
	E. faecalis	*E. faecium*
Arabinose	0 (0%)	36 (100%)
Sorbitol	29 (100%)	0 (0%)
Mannitol	29 (100%)	36 (100%)
Glucose	29 (100%)	36 (100%)
Lactose	29 (100%)	36 (100%)
Trehalose	29 (100%)	36 (100%)
Raffinose	29 (100%)	36 (100%)
Fructose	29 (100%)	36 (100%)
Dextrose	29 (100%)	36 (100%)
Xylose	0 (0%)	0 (0%)
Galactose	29 (100%)	36 (100%)
Dulcitol	0 (0%)	0 (0%)

Table 2: Gender-wise Distribution of *Enterococcus faecium* and *Enterococcus faecalis*

Sex	No of Samples Collected	No of Enterococci Isolated	No of :	
			E. faecium	*E. faecalis*
Male	50	38	16	15
Female	50	35	20	14
Total	100	73	36	29

Table 3: Antimicrobial susceptibility of *Enterococcus* Isolates to antimicrobial agents

Antibiotics	***E. faecalis***			***E. faecium***			**Total**		
	S (%)	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**
Vancomycin	25(86.2)	1(3.5)	3(10.4)	30(83.3)	-	6(16.7)	55(84.6)	1(1.5)	9(13.8)
Teicoplanin	18(62.1)	-	11(37.9)	29(80.6)	-	7(19.4)	47(72.3)	-	18(27.7)
Amoxicillin-clavulanate	10(34.5)	1(3.5)	18(62.1)	9(25.0)	4(11.1)	23(63.9)	19(29.2)	5(7.7)	41(63.1)
Ofloxacin	13(44.8)	3(10.4)	13(44.8)	17(47.2)	3(8.3)	16(44.4)	30(46.2)	6(9.2)	29(44.6)
Erythromycin	1(3.5)	-	28(96.6)	1(2.8)	3(8.3)	32(88.9)	2(3.1)	3(4.6)	60(92.3)
Gentamicin	1(3.5)	-	28(96.6)	8(22.2)	-	28(77.8)	9(13.8)	-	56(86.2)
Ceftriaxone	-	-	29(100)	-	-	36(100)	-	-	65(100)
Cefuroxine	-	-	29(100)	-	-	36(100)	-	-	65(100)
Ceftizoxime	-	-	29(100)	-	-	36(100)	-	-	65(100)

Abbreviation: S= Susceptible, I= Intermediate, R= Resistant

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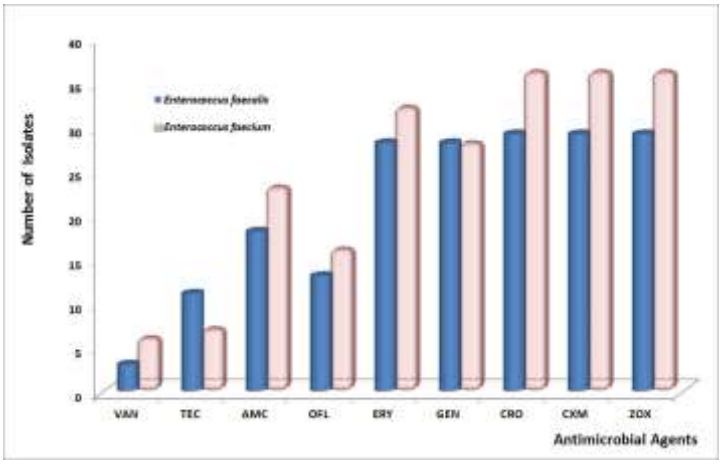

Figure 1: Antimicrobial resistance rates of *Enterococcus faecalis* and *Enterococcus faecium* isolates from Apparently Healthy Humans. **Abbreviations:** VAN-Vancomycin; TEC-Teicoplanin; AMC - Amoxicillin-clavulanate; OFL-Ofloxacin; ERY-Erythromycin; GEN-Gentamicin; CRO - Ceftriaxone; CXM - Cefuroxime; ZOX- Ceftizoxime.

Table 4: Resistance Rate of the Isolates to Classes of Antimicrobials Tested

Classes of Antimicrobials	Antimicrobial agent	Species	No. of resistant isolates (%)
Glycopeptide	Vancomycin	*E. faecium*	6 (16.67)
		E. faecalis	3 (10.34)
	Teicoplanin	*E. faecium*	7 (19.44)
		E. faecalis	11 (37.9)
Beta-lactam inhibitor	Amoxicillin-clavulanate	*E. faecium*	23 (63.9)
		E. faecalis	18 (62.1)
Fluoroquinolone	Ofloxacin	*E. faecium*	16 (44.4)
		E. faecalis	13 (44.8)
Macrolide	Erythromycin	*E. faecium*	32 (88.9)
		E. faecalis	28 (96.6)
Aminoglycoside	Gentamicin	*E. faecium*	28 (77.8)
		E. faecalis	28 (96.6)
Cephalosporins	Ceftriaxone	*E. faecium*	36 (100)
		E. faecalis	29 (100)
	Cefuroxime	*E. faecium*	36 (100)
		E. faecalis	29 (100)
	Ceftizoxime	*E. faecium*	36 (100)
		E. faecalis	29 (100)

Discussion

Enterococcal species are found in high concentrations in alimentary tract of humans and animals and may be transferred to other humans through contaminated food animals or the environment (Fisher and Phillips, 2009). *Enterococcus faecium* and *E. faecalis* have long been known to be significantly important human pathogens that are specially responsible for nosocomial infections. In this study, *E. faecium* was the most dominant species with prevalence of 55.4%. This finding is consistent with studies that have established the preponderance of *E. faecium* in healthy human samples with 44.9% *E. faecium* and 34.7% *E. faecalis* (Barreto *et al.* 2009). A similar distribution of enterococcal species was observed in poultry (Krocko *et al*., 2007). *Enterococcus faecium* has also been reported as the most common species in other studies involving chicken, turkey, pork, and beef (Hayes *et al*., 2003). However, the results presented by authors elsewhere showed a relatively low occurrence of *E. faecium* compared with *E. faecalis*

among human faecal isolates (Salem-Bekhit *et al*., 2012). These differences could be attributed to variable host dynamism imposed by gender or sex, diet, age or other environmental conditions. We however found no differences in the frequency of isolation of either *E. faecium* or *E. faecalis* between the samples from male and female, suggesting that colonization rate was not sex dependent.

Most biochemical identification schemes proposed for enterococci are based on the phenotypic patterns of clinical isolates. Results from previous studies have suggested wide variability in the biochemical profile of *E. faecium* and *E. faecalis* isolates from various sources (Facklam and Sahm, 1995, Day *et al*., 2001). In our case, all the isolates exhibited typical metabolic reaction to key phenotypic characteristics investigated. For instance, the *E. faecalis* isolates were able to utilize sorbitol unlike the *E. faecium* strains, contrary to some reports (Teixeira *et al*., 1995; Castillo-Rojas *et al*., 2013). Typically, strains of *E. faecium* would not produce acid from sorbitol but Day *et al*. (2001) showed that 16 out of 18 *E. faecium* isolates utilized sorbitol. On the other hand, Facklam and Sahm (1995) speculated that over 97% isolates of *E. faecium* could not utilize sorbitol. Therefore, combined with other biochemical identification criteria, sorbitol fermentation might be a useful marker for the differentiation of *E. faecium* from *E. faecalis* isolates in our setting. We, nonetheless, need to confirm this with a larger number of isolates in further study.

In this present communication, very high percentage of resistance to almost all antimicrobial tested were demonstrated by the *Enterococcus* isolates. The multiple antibiotic resistances of our isolates agree with those found in other studies (Rams *et al*., 2013; Komiyama *et al*., 2016). The recovery of MDR colonising strains of *E. faecium* and *E. faecalis* highlights the ability to these organisms to develop resistance to an array of antimicrobial drugs. *E. faecium* were more resistant to most of the antibiotics than *E. faecalis*, particularly, gentamicin and erythromycin. This is similar to those reported by Mengeloğlu and colleagues (Mengeloğlu *et al*., 2011). However, resistance to erythromycin and gentamycin varies substantially in other studies depending on the setting. Frequencies of 100% and 93.6% erythromycin resistance had been documented for *E. faecalis* and *E. faecium* respectively in a trial conducted in Spain (Aarestrup *et al*., 2002). In another instance, Peters and others observed that 74% of their *E. faecalis* strains and all their *E. faecium* strains were moderately or completely resistant to erythromycin (Peters *et al*., 2003). They also noted that the isolates showed low gentamicin resistance with 1% among *E. faecalis* isolates and 5% among *E. faecium* (Peters *et al*., 2003). The high resistance of our isolates to gentamicin is consistent with findings of some researchers who examined the carriage rate of vancomycin resistant enterococci among patients on prolonged hospitalization in Lagos University Teaching Hospital in Lagos, Nigeria (Ekuma *et al*., 2016). It has however been suggested that gentamicin resistance in enterococci is caused by the difficulty of penetration of this agent through the cell membrane and the secreting of the enzymes modifying gentamicin as a result of genes acquired by plasmids and transposons (Shepard and Gilmore, 2002).

In this study, the sensitivity pattern observed for ofloxacin (a quinolone) was close to that reported by Mengeloglu *et al*. (2011). The workers observed that all *E. faecium* isolates investigated were resistant to ciprofloxacin, but the resistance rate in *E. faecalis* group was 65%. Both ofloxacin and ciprofloxacin belong to fluoroquinolone class of antimicrobial agents. Fluoroquinolones have been the preferred antibiotics for treatment of *Enterococcus* and resistance to the agent could have arisen principally from its misuse since antibiotics are readily available over–the–counter in Nigeria. Resistance to several antibiotics has also been reported in other Nigerian studies (Olawale *et al*., 2011, Ayeni *et al*., 2016).

Glycopeptides (teicoplanin and vancomycin) are drugs that are rarely sold across the counter. Surprisingly, resistance to vancomycin was exhibited by some of our isolates. The resistance was however, higher in *E. faecium* (16.7%) than *E. faecalis* (10.4%). This was in contrast to the reported rate by Aarestrup *et al*. (2002) where 100% susceptibility to vancomycin in all *E. faecalis* isolates was recorded. Nevertheless, there was an agreement between our findings and that of Metiner *et al*. (2013) who observed that 26.6% of the *E. faecalis* strains isolated from pig faecal samples in Istanbul, Turkey were resistant to vancomycin while 6.4% of *E. faecium* were resistant to the antibiotic. Similar report from Northwest Ethiopia (Abebe *et al*., 2014) showed that prior antibiotic treatment was associated with vancomycin resistant enterococci colonization among clients with and without HIV. Although, we are not able to provide information on the use of antibiotics of the participants but expression of resistance is usually favoured by antibiotic misuse which conceivably can cause the emergence of vancomycin resistant isolates. As proposed by Teymournejad and others, expression of resistance genes and selection of strains already expressing these genes may alter the competing microbial flora in the GI tract, thereby increasing vancomycin resistant enterococcal concentration in the stools (Teymournejad et al., 2015). Based on this point, it is apparent that over-use or inappropriate use of this antibiotic may seriously compromise the treatment options for these organisms.

Conclusion

Our data indicate that while gender was not a determinant in *Enterococcus* carriage, high prevalence of resistant *E. faecium* and *E. faecalis* was identified among the individuals investigated. Resistance to gentamicin, erythromycin, ceftriaxone, ceftizoxime and cefuroxime were the most commonly detected among the two species. This infers that these antibiotics may not be *suitable* candidates for the *treatment* of enterococcal infections in our environment. We therefore stress the need for development of strategies to stop the sales of antibiotics across the

counter and create awareness among the populace by Government and Health Agencies on the consequences of unregulated antibiotic use. More detailed microbiological studies using genotypic methods are warranted.

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