

Research paper

Association of *Enterococcus faecalis* with different forms of dental diseases among patients visiting a tertiary hospital in Ekiti State, Nigeria

ABSTRACT

The implication of *Enterococcus faecalis* in dental infections and high antimicrobial resistance to common antibiotics is increasing in recent time. In this study, the association of *E. faecalis* with different forms of dental infections and their antibiotic resistant profile were investigated. Standard aseptic method was used to recover the organisms from infected teeth while antibiotic susceptibility of the isolates was determined by disc diffusion method. Forty eight (46.6 %) of the subjects with dental infections were male while 55 (53.4 %) were female. Students accounted for a total of 60 (58.3 %) of the total subjects followed by traders, 17 (16.5 %). *E. faecalis* was isolated from 25 (52.1%), 11 (22.9 %) and 2 (4.2 %) from students, traders and farmers respectively. The highest occurrence (37.86 %) of dental infection was found among patients within the age of 21 and 30 years while dento-aveola abscess followed by dental carries were the most encountered dental infections. A total of 46.6 % of the dental infections were colonized by *E. faecalis* which were mostly resistance to amoxicillin (93.9 %) and cloxacillin (92.7 %). Resistance to the fluoroquinolones tested was low, ranging from 8.54 to 25.6 % in spafloxacin and perfloxacin respectively. We observed 1.2 % resistance to vancomycin among the isolates. The isolates were resistant to common antibiotics however, vancomycin proved to be better in the growth inhibition of the isolates.

Keywords: *Enterococcus faecalis*, dental infections, fluoroquinolones, vancomycin.

INTRODUCTION

Teeth are a set of tissues which are used for biting and chewing, they are mechanically modified for creating a larger surface area for big chunks of food (Wehmeier, 2001; Touger, 2003). Dental disease is the second most common health problem after the common cold (Harris *et al.*, 2004). It develops as a result of bacterial infections or damage in the supporting tissues (Marinho *et al.*, 2003; Touger, 2003) or loosening or chipping of the fillings (Amir *et al.*, 1997).

Dental disease may be acute or chronic affecting the gums, teeth and/or bone supporting the teeth which may lead to loss of teeth (Jeffcoat *et al.*, 2001). Detailed knowledge of the microflora and opportunistic pathogens can be useful for choosing the appropriate treatment strategies or the materials for retrograde root-canal fillings and prevent recurrence of tooth lesion (Siqueira *et al.*, 2000).

Enterococcus faecalis is Gram-positive coccus facultative anaerobic bacterium which inhabits the oral cavity and other human parts like gastrointestinal tract as well as the vagina (Jett et al., 1994). *Enterococcus faecalis* accounts for 90% of human enterococcal infections which have increased substantially during the last 10 years (Fisher and Phillips, 2009).

Though an opportunistic pathogen, *E. faecalis* colonises oral infections including marginal periodontitis, infected root canal, and periradicular abscesses (Cogulu et al., 2007). Its association with failure in root filled tooth reported to be involved in mixed infection of necrotic root canal and other dental infections. *Enterococcus faecalis* often infects root canals during endodontic dental treatments of patients with persistent apical periodontitis (Pei et al., 2012) and has been reported to be most resistant pathogen to antimicrobials commonly used in the treatment of oral infections (Fisher and Phillips, 2009). It is the most prevalent species isolated from root canals of previously root-filled teeth with chronic apical periodontitis (Peciuliene et al., 2001).

We investigated the association of *E. faecalis* with different dental diseases and susceptibility pattern to antibiotics commonly used in the clinical treatment of the dental infections.

MATERIALS AND METHODS

Collection of samples and Isolation of *Enterococcus* spp

Samples were collected from patients attending Dental Unit of a tertiary hospital in Ekiti State, Nigeria. The diagnostic procedure to confirm tooth infections was carried out by the Dentists. Tooth with acute apical abscess were clinically identified by swelling and pain on biting, percussion and palpation. Care was taken to avoid cross-contamination during sampling. The samples were collected with the aid of sterile cotton swab (culturette). Strict aseptic procedures were employed throughout the sampling periods. The samples were immediately plated on sterile plates of Bile aesculin agar (Oxoid, Basingstoke, Hampshire, UK) and incubated at 37 °C for 24 h. Colonies with black halos were subculture on MacConkey Agar No 2 and incubated as stated above.

The pure isolates were characterized and identified with 24 h old culture using standard methods of Olutiola et al. (2001) and, Fawole and Oso (2001) and the results were interpreted according to Schleifer and Kilpper-Balz (1984).

Antibiotic sensitivity test

The antibiotic susceptibility of the isolates was determined using the disk diffusion method of CLSI (2005) on Mueller-Hilton agar. The inoculum used was standardized by adjusting its density to equal the turbidity of barium sulphate (BaSO₄) solution which is the 0.5 McFarland turbidity standard, and incubated at 35°C for 18h. The following commercially prepared antibiotic disks (Abtek Biologicals, UK) with the following concentrations (in µg) were tested against the isolates: amoxicillin (25), augmentin (30), chloramphenicol (30), cloxacillin (5), cotrimoxazole (25), erythromycin (5), gentamicin (10), vancomycin (5), tetracycline (10) levofloxacin (5), ofloxacin (30), perfloxacin (5), sparfloxacin (10) and ciprofloxacin (10). The diameter of the zone of inhibition was measured and interpreted using the CLSI guideline (CLSI, 2005).

RESULTS AND DISCUSSION

In this study, association of *E. faecalis* with different dental infections was determined in 103 patients diagnosed for dental infections. Out total of the total subjects with dental infections in this study 48 (46.60 %) were male while 55 (53.40 %) were female. Cohen and Goldman (1996) reported females to be more susceptible to dental infections; an observation similar to our result.

Incidence of dental infections in patients with various occupations show that students account for a total of 60 (58.3 %) followed by the traders with a total number of 17 (16.5 %) (Table 1). This finding is similar to the finding of Younai *et al.* (2001) who reported high incidence of dental infections among students. From the samples *E. faecalis* was isolated from 25 (52.08%), 11 (22.92 %) and 2 (4.17 %) from students, traders and farmers respectively. This study cuts across various ages of patients, and the ratio of the infected male to the female was very close to unity.

As shown in Table 2, the highest occurrence (37.86 %) of dental infection was noticed among patients within the age of 21 and 30. This group also had the highest number of enterococci-associated dental infections. Your adults between the age of 23 and 25 years are prone to dental infections in this stage of their life (Yamaoka *et al.*, 2008; Forner *et al.*, 2006). Among young females experience of dental infections are high due to hormonal changes during pregnancy which compromises dental health (Dunning, 1962; DePaola *et al.*, 1982). The percentage occurrence of dental infections in age groups 10-20 years and 31-40 years were 16.5 and 18.4 % respectively. *Enterococcus faecalis* was isolated from 52% of the patients with dental infections in ages group of 10-20 were and 47.4 % in age group 21-30.

Most dental infections may defile treatment with both chemical and antibiotic treatments. *E. faecalis* has been implicated in the majority of this cases (Molander *et al.*, 1998; Hancock *et al.*, 2000). Dento aveola abscess (DAA) followed by dental carries (DC) were the most encountered dental infections among the patient sampled in this study. The association of *E. faecalis* in the samples ranged between 25.00 % and 62.50 %. Acute apical periodontitis (AAP) had the highest association with the organism followed by DAA. The association of *E. faecalis* with these infections may be attributed to their ability to survive in the dental intracanal and form biofilm and cause prolonged root canal treatments (Molander and Dahlen, 2003; Zoletti *et al.*, 2011). As part of survival mechanisms *E. faecalis* forms an irreversible three dimensional, sophisticated architecture of cells also known as biofilm (Distel *et al.*, 2002; Flemming and Wingender, 2010). The structure is sustained by the exo-polymers that allow circulation of nutrients and prevent antimicrobial agents from reaching the cells in the core of the biofilm.

Our results show that 46.6 % of the dental infection were colonized by *E. faecalis*, however, this report was higher than the figure (22.0 %) reported by Fouad *et al.* (2005) and lower than 77.0 % reported by Siqueira and Rocas (2004). The intrinsic ruggedness of *Enterococcus* spp. confers on them a strong tolerance to many antibiotics (Megran, 2001; Ott and Wirick, 2008). The misuse and abuse of antibiotics used for oral infection treatment may have led to widespread resistance among the isolates. The propensity of the organisms to acquire resistance genes contribute significantly to their high level of resistance to these potent drugs (Schurek *et al.*, 2006).

Fourteen different antibiotics belonging to various classes were tested against the *E. faecalis* isolated from the dental infections. The isolates had varying degrees of resistance to the antibiotics. The organisms were most resistant to amoxicillin (93.9 %)

next to it was cloxacillin (92.7 %). Resistance of the isolates to the fluoroquinolones tested in this study was low, ranging from 8.5 to 25.6 % in sparfloxacin and pefloxacin respectively. Resistance to vancomycin was the least (1.22 %). Only one out of all the isolates (n=82) was resistant to vancomycin (Table 4). This study showed an increased in the rate of vancomycin resistance as Pinheiro et al. (2004) observed no resistance to vancomycin in the isolates they recovered.

This report showed that *E. faecalis* recovered from dental infections were resistant to common antibiotics. Fluoroquinolones and vancomycin can be used in the treatment of the infections. This is the first report on the association of *E. faecalis* to dental infection in Western part of Nigeria however, further studies are needed in other parts of the country to assist in knowing the prevalence and resistant patterns of the organism to antibiotics.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Table 1: Occupational distribution and incidence of *E. faecalis* among screened patients

Occupation	Occurrence		Samples positive for <i>E. faecalis</i>	
	Number	percentage	Number	percentage
Students	60	58.3	25	52.1
Civil servants	3	2.9	1	2.1
Trader	17	16.5	11	22.9
Farmer	5	4.9	2	4.2
Health workers	6	5.8	1	2.1
Others	12	11.7	8	16.7
Total	103	100.0	48	100.0

Table 2: Age distribution and incidence of *E. faecalis* among screened patients

Age (Years)	Occurrence		Number positive for <i>E. faecalis</i>	
	Number	percentage	Number	Percentage
<10	3	2.9	2	66.7
10-20	17	16.5	9	52.9
21-30	39	37.9	18	46.2
31-40	19	18.4	9	47.4
41-50	12	11.7	3	25.0
>50	13	12.6	7	53.9
Total	103	100.0	48	46.6

259

260 Table 3: Incidence of dental infection and association of *E. faecalis* among screened
261 patients

Dental infections	Occurrence		Number positive for <i>E. faecalis</i>	
	Number	percentage	Number	Percentage
Dental carries (DC)	15	14.6	8	53.3
Dento aveola abscess (DAA)	19	18.5	11	57.9
Chronic apical periodontitis (CAP)	14	13.6	6	42.9
Retained root (RR)	11	10.7	6	54.5
Acute apical periodontitis (AAP)	8	7.8	5	62.5
Curious lesson (CL)	14	13.6	6	42.9
Periodontitis (P)	9	8.7	3	33.3
Others (O)	13	12.6	3	25.0
Total	103		48	46.6

262

263 Table 4: Antibiotic resistance of representative *E. faecalis* isolated from patient with
264 dental infections

Antibiotics	<i>E. faecalis</i> resistance	
	Number (n=82)	Percentage
Amoxicillin	77	93.9
Cotrimoxazole	70	85.4
Gentamicin	36	43.9
Cloxacillin	76	92.7
Ofloxacin	15	18.3
Levofloxacin	9	11.0
Perfloxacin	21	25.6
Spafloxacin	7	8.5
Ciprofloxacin	18	22.0
Augmentin	51	62.2
Tetracycline	53	64.6
Vancomycin	1	1.2

265	Chloramphenicol	21	25.6
266	Erythromycin	51	62.2