

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

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Aims: The implication of *Enterococcus faecalis* in dental infections is rising and resistance to common antibiotics is increasing in the recent time. In this study, the association of *E. faecalis* with different forms of dental infections and their antibiotic resistance profile were investigated.

Place and Duration of Study: The samples were collected from a tertiary health institution in Ekiti State, Nigeria and transferred to the Laboratory of the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria for immediate processing. The study was carried out between June, 2009 and March 2010.

Methodology: Oral interview was conducted among the patients to collect useful data while sterile culturette was used to collect the samples after proper diagnoses. Antibiotic susceptibility of the isolates was determined by disc diffusion method.

Results: The proportion of the subjects with dental infections who were males was 46.60% while 53.40% were females. Students accounted for a total of 58.25% of the total subjects followed by traders (16.50%). *Enterococcus faecalis* was isolated from 52.08% of the students, 22.92% traders and 4.17% of the farmers. The highest occurrence of dental infection (37.86%) was noticed among patients within the age bracket 21 and 30 years while dento-aveola abscess (DAA) followed by dental caries were the predominant dental infections in that order. A total of 46.60 % of the dental infections were colonized by *E. faecalis* which were mostly resistant to amoxicillin (93.90 %) and cloxacillin (92.68 %). Resistance to the fluoroquinolones tested was low, ranging from 8.54 to 25.61 % in sparfloxacin and perfloracin, respectively. We observed 1.22 % resistance to vancomycin among the isolates.

Conclusion: The isolates were resistant to common antibiotics however, vancomycin proved to be better in the inhibition of the isolates.

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

INTRODUCTION

Dental disease is the second most common health problem after the common cold [1], it develops as a result of bacterial infections or damage in the supporting tissues [2, 3] or loosing or chipping of the fillings [4]. Dental disease may be acute or chronic, affecting the gums, teeth and/or bone supporting the teeth which may lead to loss of teeth [5]. 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 [6].

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 [7]. *E. faecalis* accounts for 90% of human enterococcal infections which have increased substantially during the last 10 years [8].

Though an opportunistic pathogen, *E. faecalis* colonises oral infections including marginal periodontitis, infected root canal, and periradicular abscesses [9]. It is association with failure in root-filled tooth and 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 [10] and has been reported to be most resistant pathogen to antimicrobials commonly used in treatment of oral infections [11]. It is the most prevalent species isolated from root canals of previously root-filled teeth with chronic apical periodontitis [12].

The purpose of this study was to determine the association of *E. faecalis* with different dental diseases and to determine the pattern of resistance to antibiotics commonly used in the clinical treatment of the infections.

2. MATERIALS AND METHODS

2.1 Collection of Samples and Isolation of *Enterococcus* spp

All the patients were informed about the aim of the study and gave their consents. Oral interview was conducted among the patients to obtain their demographic data including occupation and age. The diagnostic procedure to confirm tooth infections was carried out by the dentists. Teeth with acute apical abscess were clinically identified by swelling and pains 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 24hrs. Colonies with black hallow were subcultured on MacConkey Agar No 2 and incubated as stated above.

Identification of isolates was carried out using standard methods of Olutiola *et al.* [13] and Fawole and Oso [14]. The results were interpreted according to Schleifer and Kilpper-Balz [15].

2.2 Antibiotic sensitivity test

The antibiotic susceptibility of the isolates was determined by the disk diffusion method of CLSI [16] on Mueller-Hilton agar. The inoculum used was standardized by adjusting its density to equal the turbidity of barium sulphate (BaSO₄) solution (0.5 McFarland turbidity standard), and incubated at 35°C for 18hrs. The following 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 Guidelines [16].

3. RESULTS AND DISCUSSION

In this study, association of *E. faecalis* with different dental infections was determined in 103 patients diagnosed of dental infections. The incidence of dental infections in patients with various occupations is shown in Table 1. Students accounted for a total of 60 (58.25 %), followed by traders with a total number of 17 (16.50 %). This finding is similar to the report of Younai *et al.* [17] which equally showed a high incidence of dental infections among students. From the samples *E. faecalis* was isolated from 52.08%, 22.92% and 4.17% of 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. Out total of 103 subjects with dental infections in this study 48 (46.60 %) were males while 55 (53.40 %) were females. Cohen and Goldman (18) reported females to be more susceptible to dental infections; an observation similar to what was obtained in this study.

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	58.25	25	52.08
Civil servants	3	2.91	1	2.08
Trader	17	16.50	11	22.92
Farmer	5	4.85	2	4.17
Health workers	6	5.82	1	2.08
Others	12	11.65	8	16.67
Total	103	100.00	48	100.00

As shown in Table 2, *E. faecalis* was isolated from two out of the three patients that were less than 10 years old. The highest occurrence (37.86 %) of dental infection was among patients within the ages 21 and 30 bracket. This group also had the highest number of enterococci-associated dental infections. Young adults between the age of 23 and 25 years are prone to dental infections [19, 20]. Among young adults, females experience high dental infections due to hormonal changes during

pregnancy which compromises health status including dental health [21, 22]. The percentage occurrence of dental infections in age groups 10-20 years and 31-40 years was 16.50% and 18.44%, respectively. *Enterococcus faecalis* was isolated from 52.00% of the patients with dental infections in age group 10-20 years and from 47.36% in age group 21-30 years.

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	2.91	2	66.66
10-20	17	16.50	9	52.94
21-30	39	37.86	18	46.15
31-40	19	18.44	9	47.36
41-50	12	11.65	3	25.00
>50	13	12.62	7	53.85
Total	103	100.00	48	46.60

Most dental infections may defy treatment with both chemicals and antibiotics and *E. faecalis* has been implicated in the majority of these cases [23, 24]. Dento aveola abscess [DAA] followed by dental carries [DC] were the most encountered dental infections in that order among the samples examined in this study. The association of *E. faecalis* in the samples ranged between 25.00 % and 62.50 % and acute apical periodontitis [AAP] had the highest association with the organism followed by DAA (Table 3). 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 [25, 26]. As part of survival mechanisms *E. faecalis* forms an irreversible three dimensional, sophisticated architecture of cells also known as biofilm [27, 28]. 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.

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

Dental infections	Occurrence		Number positive for <i>E. faecalis</i>	
	Number	percentage	Number	Percentage
Dental carries (DC)	15	14.56	8	53.33
Dento aveola abscess (DAA)	19	18.45	11	57.89
Chronic apical periodontitis (CAP)	14	13.59	6	42.86
Retained root (RR)	11	10.68	6	54.54
Acute apical periodontitis (AAP)	8	7.77	5	62.50
Curious lesson (CL)	14	13.59	6	42.86
Periodontitis (P)	9	8.74	3	33.33
Others (O)	13	12.62	3	25.00
Total	103		48	46.60

Our results showed that 46.60 % of the dental infections were colonized by *E. faecalis*, however, this finding was higher than the figure (22.00 %) reported by Fouad et al. [29] and lower than 77.00% reported by Siqueira and Rocas [30]. The intrinsic ruggedness of *Enterococcus* spp. confers on them a strong tolerance to many antibiotics [31, 32]. The misuse and abuse of antibiotics used for oral infection treatment can also lead to widespread resistance among the isolates. The high propensity of the organisms to acquire resistance genes may contribute significantly to their high level of resistance to these usually potent drugs [33]. The *E. faecalis* isolates from the dental infections had varying degrees of resistance to the 14 different antibiotics tested belonging to various classes. The organisms were most resistant to amoxicillin (93.90 %), and cloxacillin (92.68 %) and least resistant to vancomycin (1.22%). This may probably be accounted for by the volume of usage of these drugs in the study area. Resistance of the isolates to the fluoroquinolones tested in this study was relatively low, ranging from 8.54% to 25.61 % in sparfloxacin and perfloxacin, respectively.

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

Antibiotics	<i>E. faecalis</i> resistance	
	Number (n=82)	Percentage
Amoxicillin	77	93.90
Cotrimoxazole	70	85.37
Gentamicin	36	43.90
Cloxacillin	76	92.68
Ofloxacin	15	18.29
Levofloxacin	9	10.96
Perfloxacin	21	25.61
Spafloxacin	7	8.54
Ciprofloxacin	18	21.95
Augmentin	51	62.20
Tetracycline	53	64.63
Vancomycin	1	1.22
Chloramphenicol	21	25.61
Erythromycin	51	62.20

Conclusively, this study shows that *E. faecalis* recovered from dental infections were resistant to common antibiotics while fluoroquinolones and vancomycin can be preferred as alternatives 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 molecular study of the *E. faecalis* associated still opened to investigation.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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