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## **Association of *Enterococcus faecalis* with Different Forms of Dental Diseases among Patients Visiting a Tertiary Hospital in Ekiti State, Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors OMD and OF designed the study, author OMD performed the analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors OMD, IBF and OF managed the analyses of the study. Author IBF managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** The implication of *Enterococcus faecalis* in dental infections is rising and resistance to common antibiotics continues to rise globally as well. In this study, the association of *E. faecalis* with different forms of dental infections was investigated and the antibiotic resistance profile of the isolates was determined.

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

**Methodology:** Oral interview was conducted among the patients in order to collect relevant data while sterile culturette was used to collect the samples after proper diagnoses, plated and incubated appropriately using standard microbiological techniques. The susceptibility of the isolates to commonly and frequently used antibiotics was determined by the disc diffusion method on Müller-Hinton agar.

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**Results:** Overall, 46.0% of the subjects had dental infections. The subjects with dental infections were 46.60% males and 53.40% females. Students accounted for 58.25% of the total subjects followed by traders with 16.50%. *Enterococcus faecalis* was isolated from 52.08% of the students, 22.92% traders and 4.17% of the farmers. The highest occurrence (37.86%) of dental infection was recorded among patients within the age bracket 21 and 30 years while dento-aveola abscess (DAA) followed by dental caries (DC) were the predominant dental infections recorded in that order. A total of 46.60% of the dental infections were colonized by *E. faecalis* that were mostly resistant to amoxicillin (93.90%) and cloxacillin (92.68%). Resistance to the fluoroquinolones tested was relatively low, ranging from 8.54 to 25.61% in sparfloxacin and perfloxacin, respectively whilst it was 1.22% to vancomycin among the isolates.

**Conclusion:** The isolates were resistant to common antibiotics tested, however, vancomycin proved to be the most effective in the inhibition of the isolates.

*Keywords: Enterococcus faecalis; dental infections; antibiotic resistance; fluoroquinolones; vancomycin.*

## 1. INTRODUCTION

Dental disease is the second most common health problem after the common cold [1] which develops as a result of bacterial infections, damage in the supporting tissues [2,3], loss 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 [5,6]. Detailed knowledge of the microflora and opportunistic pathogens can be useful in the choice of the appropriate treatment strategies or the materials for retrograde root-canal fillings and prevent the recurrence of tooth lesion [7].

*Enterococcus faecalis* is a Gram-positive coccus and facultative anaerobic bacterium that inhabits the oral cavity and other human parts such as the gastrointestinal tract as well as the vagina [8]. *Enterococcus faecalis* accounted for 90% of human enterococcal infections which increased substantially since the 1980s [9,10].

Though an opportunistic pathogen, *E. faecalis* colonises the oral cavity, causing infections which may include marginal periodontitis, infected root canal, and periradicular abscesses [6,11]. It is associated with failure in root-filled tooth and has been reported to be involved in mixed infections of the necrotic root canal among other dental infections. *Enterococcus faecalis* often infects root canals during endodontic dental treatments of patients with persistent apical periodontitis [12] and has been reported to be the most resistant pathogen to antimicrobials commonly used in the treatment of oral infections [13]. The organism is the most prevalent species recovered from the root canals of previously root-filled teeth with chronic apical periodontitis [14].

The purpose of this study therefore, was to determine the association of *E. faecalis* with different dental diseases and to determine the pattern of resistance to antibiotics commonly or frequently prescribed and used in the clinical treatment of the dental 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 upon which they gave their consents. Oral interview was conducted among the patients to obtain their demographic data while 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 with the aid of cotton swab [culturette]. The samples were immediately plated on Bile aesculin agar [Oxoid, Basingstoke, Hampshire, UK] and incubated at 37°C for 24hrs. Colonies with black hollow typical of *E. faecalis* were subcultured on MacConkey Agar No 2 and incubated as stated above. The isolates were identified by standard methods described by Olutiola et al. [15] and Fawole and Oso [16] while the results were interpreted according to Schleifer and Kilpper-Balz [17].

### 2.2 Antibiotic Sensitivity Test

The antibiotic susceptibility of the isolates was determined by the disk diffusion method on Müller-Hinton agar according to CLSI [18]. The inoculum used was standardized by adjusting its density to equal the turbidity of barium sulphate (BaSO<sub>4</sub>) solution (0.5 McFarland turbidity standard), and incubated at 35°C for 18hrs. The isolates were tested against the following antibiotic disks (Abtek Biologicals, UK) with their concentrations (in µg): 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 [18].

## 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 presented in Table 1. Of the 103 subjects included in this study, students accounted for a total of 60 (58.25%), and followed by traders with 17 (16.50%). Overall, 48 (46.60%) patients were infected with *E. faecalis*. This finding is similar to the observation of Younai et al. [19] that equally reported a high incidence of dental infections among students.

*Enterococcus faecalis* was isolated from 52.08%, 22.92% and 4.17% of students, traders and farmers respectively. This study cuts across diverse ages among the patients, and the ratio of the infected male to female was very close to unity. Out of a total of 103 subjects with dental infections in this study, 48 (46.60%) were males while 55 (53.40%) were females. Cohen and Goldman [20] reported that females are more susceptible to dental infections; an observation that is corroborated by 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

Table 2 shows that *E. faecalis* was isolated from two out of the three patients aged <10 years while the highest occurrence (37.86%) of dental infection was recorded among patients within the age bracket 21 and 30 years. This group also had the highest number of enterococci-associated dental infections. The percentage occurrence of dental infections in the 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 the age group 10-20 years and from 47.36% among the age group 21-30 years. Young adults between the ages of 23 and 25 years are prone to dental infections [21,22] and females among the young adults have been reported to experience high dental infections attributed to hormonal changes during pregnancy, which compromise the health status including dental health [23,24].

**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 the cases of infection [25,26]. Dento-aveola abscess [DAA] followed by dental carries [DC] were the most encountered dental infections among the samples examined in this study. The association of *E. faecalis* with infection among the samples ranged between 25.00% and 62.50% whereas the organism had the highest association with acute apical periodontitis [AAP] followed by DAA (Table 3). Association of *E. faecalis* with dental infections may be attributed to ability to survive in the dental intracanal and form an irreversible three dimensional, sophisticated architecture of cells known as biofilm and hence, cause prolonged root canal treatments [6,27,28,29,30]. This structure is sustained by the exo-polymers that allow circulation of nutrients but 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 considerably higher than that (22.00%) reported by Fouad et al. [31] and much lower than 77.00% reported by Siqueira and Rocas [32]. The intrinsic ruggedness of *Enterococcus* spp. confers on them a strong tolerance to many antibiotics [33,34]. The misuse and abuse of antibiotics employed for the treatment of oral infection may also be a source of widespread resistance among the isolates. The high propensity of the organisms to acquire resistance genes may as well contribute significantly to their high level of resistance to these usually potent antimicrobials [35]. The *E. faecalis* isolates recovered from the dental infections showed varying degrees of resistance to the 14 different antibiotics tested which belong to various classes.

Table 4 shows that the test organisms were mostly 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 agents 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 spafloxacin and perfloxacin, respectively which may likewise be explained by the level of usage. Perfloxacin is one of the fluoroquinolones commonly prescribed in the study environment. Resistance to antibiotics in the same class is often not unexpected with time, even where that member of the class is less commonly or frequently used as it is the case with perfloxacin in the study area. This is often due to the possession of common chemical structures.

**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

#### 4. CONCLUSION

Conclusively, this study shows that *E. faecalis* recovered from dental infections were resistant to commonly used antibiotics in the study environment while fluoroquinolones and vancomycin can be preferred as alternatives in the treatment of the infections. To our knowledge, this is the first report on the association of *E. faecalis* with dental infection in Western part of Nigeria. However, molecular study of the *E. faecalis* isolates associated with dental infection remains open to investigation and this is ongoing in our laboratories.

#### CONSENT

Not applicable.

#### ETHICAL APPROVAL

Not applicable.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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