Prevelence And Characterization Of Antibiotic Resistance Enterococci From Human With Dental Plague

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Abstract: Two hundred and fifty patients with dental plague aged 8 to 80 years were screened for *Enterococcus* species. Susceptibility of the isolates to various antibiotics and antimicrobial agents including enzymatic and haemolytic reactions were determined. 44 patients were positive for Lactic acid bacteria (LAB), out of which 23 (9.2%) of them were positive for *Enterococcus* species, mostly from men between 21 to 40 years of age. The *Enterococcus* species isolated includes; *E. munditti, E. faecium, E. facealis, E. gallinarum, E. dispar, E. avium, E. hirae, E. porcinus, E. cecorum* and *E. ratti*. *E. faecalis* had the highest occurrence of 21.74% while *E. ratti* had percentage occurrence of 4.34%. All the isolates exhibited multiple antibiotics resistance and were sensitive to 30mg calcium hydroxide, 22%v/v ethanol, 35%w/v sodium chloride, 85%w/v sucrose and 6%v/v hydrogen peroxide. None of the isolates have lipolytic activity but some have amylolytic and proteolytic activity, produced gelatinase and haemolysed red blood cell. There is need to investigate the pathogenicity and adhesion mechanisms of these isolates in human with dental caries.

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1. Introduction

Dental plague (caries) is a cavity or decay of a tooth, a localized disease that begins at the surface of the tooth and may progress through the dentine into the pulp cavity (Santos et al., 2002). Dental caries result due to an infection by cariogenic bacteria. The bacteria include the mutants, *Lactobacilli, Streptococci* and the *Enterococci* all of which are resident in the human mouth (Jason et al., 2001). These strains are considered as commensal organisms (normal microbiota) of the large and small intestine (Prescott et al., 2005), well suited to survival in the intestinal, vaginal tracts and the oral cavity, but can induce human caries, following its resistant to most antibiotics and all conventional antimicrobial drugs (Nester and Roberts 2004). They are also found in the soil, water and in the foods we eat (Richard et al., 2000).

It is believed that the action of microorganisms in the mouth on ingested sugars and carbohydrates produce acids that eat away the enamel, the protein structure of the dentine is then destroyed by enzymatic action (Santos et al., 2002). Of all these species of microorganisms in the mouth, *Enterococci* were under study with little information. No human data support a significant role of *enterococci* in the development of human carious lesions or in their prevalence in the human mouth (Jason et al., 2001). This leads to more research and attention on the mutant *enterococci* either as a normal commensal, probiotics and bacteriocin (enterocin) used in fermentation purposes in relation to other Lactic acid bacteria (LAB) or as a virulence factor in relation to human caries. The clinical *enterococci* isolates from human dental plaques may be useful in the studies of the virulence mechanism of this microorganism.

This study was made to determine how frequently *enterococci* could be recovered from human with dental plaques in Nigeria and determine their antibiotic resistance.

2. Materials and Methods

Sample collection

Two hundred and fifty (250) dental plague samples were collected from the anterior and molar teeth of both jaws of human aged 8 to 80 years, from Oyo State Hospital Management Board and Dental Centre Dugbe, Ibadan Oyo State, Nigeria. Sterile swab sticks were used for picking the plague samples

while sterile specimen bottles were used for saliva and excavated teeth collection. The uprooted teeth were diluted by 100 fold in phosphate buffered saline prior to analysis (Yoo et al., 2007).

Culture Media

For Isolation of *Enterococus* strains, MRS medium, Brain Heart Infusion (BHI) agar supplemented with 10µg/mil of streptomycin sulphate antibiotic and Oxoid Bile Esculin agar (BEA) were used for isolation. The medium were weighed using a Microwa Swiss electronic weighing machine (7730 model). Preparation procedure was in accordance with the manufacturer's directive. The medium were sterilization with autoclave at 121^{o}C for 15 minutes, allowed to cool to 45^{o}C before used.

Isolation of Microoganisms

Swabs with dental plaque samples were inoculated on dried Brain heart infusion agar plates, incubated in a candle jar which supplied approximately 5 – 10 $^{o}/_{o}$ carbon dioxide, at 37^{o}C for 24-48 hours (Osoba, 1979) and later sub-cultured on Bile Esculin agar plates using the same procedure. The pure cultures were obtained by streaking out in Bile Esculin agar slants and stored in the refrigerator at 4^{o}C for subsequent use and periodically transferred into sterile Bile Esculin agar slant and in Hogness Freezing Medium at -20^{o}C from which representative colonies were then prepared for identification. Statistical parameters and Sturgis rule was used for determining percentage occurrence and age distribution of dental plague patients with LAB and *Enterococcus* species.

Characterization of Isolates

The Isolates were characterized based on the morphological, physiological and biochemical tests such as Gram's stain, catalase, oxidase, indole, methyl red, Voges – Proskauer, hydrogen sulphide, pigmentation, motility, starch hydrolysis, gelatin hydrolysis, proteolysis, lipolysis, haemolysis, production of ammonia from arginine, growth at temperatures of 10^{o}C, 35^{o}C, 37^{o}C, 45^{o}C and 60^{o}C, growth at pH of 9.6, 6.5% and 7.5% sodium chloride, gas production and Carbohydrate fermentation tests.

Antibiotic susceptibility testing

Instead of Mueller-Hinton media for antibiotic susceptibility by National committee on clinical laboratory standards (NCCLS), Brain heart infusion (BHI) agar was used for effective growth of the bacteria strain. Twenty milliliter (20ml) of sterile BHI agar was poured into disposable Petri-dishes (100mm in diameter, selected for uniform flat bottoms). The agar plates were dried for two hours after setting and the entire surface were seeded with each bacterium isolate using sterile swab sticks. The commercial Gram positive paper discs and few broad spectrum Gram negative discs containing 10 µg Amoxicillin, 30 µg Augumentin, 15 µg Azithromycin, 30 µg Cefazolin, 30 µg Cefuroxime, 30 µg Cephalexin, 30 µg Chloramphenical, 15 µg Ciprofloxacin, 25 µg Co-trimoxazole, 15µg Erythromycin, 10 µg Gentamicin, 10 µg Nitrofrantion, 5 µg Oxfloxacin, 10 µg Penicillin, 100 µg Piperacillin, 30 µg Nalidixic, 30 µg Vancomycin, 30 µg Streptomycin and 30 µg Tetracycline were placed flat side down on the seeded agar surfaces using sterile forceps. The discs were pressed firmly but carefully into the agar with the sterile forceps to ensure complete contact with the agar. The plates were incubated in a candle jar at 37^{o}C for 24hours. The relative susceptibility of each isolate to each antibiotic was shown by a clear zone around the growth, inhibition was measured by means of calibrated ruler in millimeter from the under side of the plates. The data obtained were used to group the isolates as sensitive ''S", and resistance "R".

Determination of calcium hydroxide ($Ca(OH)_2$) susceptibility to enterococci species

Disc diffusion method was used against membrane filter model by Wen Chai et al. (2007). 30mg sterile solution of calcium hydroxide was prepared. Sterile filter paper discs (Whatman #1 filter paper) which measured 6mm in diameter were incorporated into the solutions of calcium hydroxide. The discs asceptically transferred into freshly inoculated BHI agar plates. The relative susceptibility of each isolate to calcium hydroxide was shown by a clear zone around the growth; inhibition was measured as described above for antibiotics.

Screening for enzymes production of Enterococcus species

Gelatinase production was detected by using method of Vergis et al. (2002); amylase by using method of Seeley and Van Demark (1972); protease by using method of Dovat et al. (1970) while lipase production was detected using method of Kenneally et al. (1998).

Lethal treatments on Enterococcus species

The selected representative ten (10) strains of *enterococci* specie were subjected to lethal stress conditions and viability assay. Cultures were grown at 37^{o}C in MRS broth to the mid-exponential phase and harvested by centrifugation. 6.5% (w/v) sodium chloride were prepared in fresh MRS broth and added to the harvested shock adapted cells which were incubated for two hours. The Control (non-adapted)

cells were re-suspended in fresh MRS broth without 6.5% NaCl. Protein synthesis was blocked by the additions of 10µg/ml of chloramphenicol at the zero time of adaptation cells (before 2 h incubation). Adapted cells were further challenged after 2h incubation by 28.5 % (w/v) NaCl, 85 % (w/v) sucrose, 22 % (v/v) ethanol, 6:4 ml (v/v) hydrogen peroxide in MRS broth as diluents. Viability of adapted and non-adapted cells was determined by spreading 1ml of dilutions on small and bigger agar plates respectively containing 0.5% glucose, followed by incubation at 62 ^{o}C for adapted and 37 ^{o}C for control cells for 48hours. Survival was determined as the ratio of the number of CFU after challenge treatment to the number of CFU at the zero time point (Amezaga et al., 1995).

3. Results

Two hundred and fifty (250) patients with dental plague aged eight (8) to eighty (80) years were screened for Lactic acid bacteria (LAB) in which one hundred and twenty six (126) patients were female while one hundred and twenty four (124) were males. A total of forty four (44) patients were positive for lactic acid bacteria (17.6%) out of which seventeen (17) and twenty seven (27) of them were female and male patients respectively. Out of the 44 patients positive for LAB, only 23 (9.2%) of them were positive for *Enterococcus* in which fourteen (14) of them were male while nine (9) were female (Table 1).

The age distribution of patients with dental plague positive for both LAB and *Enterococcus* are shown in Figure 1a-c. The age group between 28 and 36years with midpoint 32 had the highest frequency (55) occurrence for dental plague, those between 23 and 33years of age with midpoint 28 had the highest frequency occurrence of 16 for LAB and the age group between 17 and 24years with midpoint 20.5 had the highest frequency occurrence of 8 for *Enterococcus*.

Enterococci genus were differentiated from other LAB based on their morphological appearance in Brain heart infusion (BHI) agar and in Bile Esculin Agar (BEA) which turned BEA to black while other LAB remain brownish. All catalase negative, non spore former and gram positive bacteria isolated using Brain Heart infusion (BHI) agar were considered presumptive LAB but microorganisms that hydrolysis bile in the presence of Esculin (BEA) were consider as *Enterococci* and further characterized by negative gas production from sugars in MRS broth, growth at 10^{o}C and 45^{o}C, growth at pH of 9.6, growth in MRS broth of 6.5% NaCl and deamination of arginine in which some produced ammonia from arginine (Table 2). All the species were small, whitish to creamy in colour in BHI but dark and slightly yellow in BEA. The cellular shape revealed Gram positive cocci in short chains. Biochemical test showed that all species were negative for catalase, oxidase, indole, hydrogen sulphide production and Voges Proskauer test and were homofermenters. All grew at temperature 10^{o}C, 35^{o}C, 37^{o}C and 45^{o}C but only one strain grew at 60^{o}C and at 7.5%w/v sodium chloride in MRS broth. All grew in medium containing 6.5% sodium chloride and at pH of 9.6 in MRS broth. None was motile, some were positive to methyl red test and few were negative. Some haemolysed red blood cells completely, some partially while few do not.

Sugar fermentation tests were used to differentiate the Enterococci into species (Table 3). Almost all species fermented cellobiose, lactose, fructose and sucrose with no gas production while none fermented sorbose and rhamnose. Variations were found in the fermentation of sorbitol, glycerol, xylose, maltose, galactose, glucose and mannitol. The isolates were identified as *E. munditti, E. faecium, E. faecalis, E. gallinarum, E. dispar, E. avium, E. hirae, E. porcinus, E. cecorum* and *E. ratti*.

Percentage occurrence of *Enterococcus* species is shown in Figure 2. *E. faecalis* had the highest occurrence of 21.74%, followed by *E. faecium* (17.44%). and *E. ratti* had percentage occurrence of 4.34%.

The isolates were screened for enzyme production. It was observed that some of the strains produced amylase, protease and gelatinase but none produce lipase (Table 4).

Antibiotic susceptibility pattern including calcium hydroxide susceptibility of *Enterococcus* species presented in Table 5 shows that there were variations in the inhibition pattern even within species. Some antibiotics were more susceptible in sensitivity and resistance towards the bacterial strains than others. Oxfloxacin have the highest zone of inhibition (13mm) against *E. munditii* while caphelexin and penicillin have the lowest zone of inhibition (3mm each) against *E. munditii* and *E. cecorum*. All the isolates were resistance to all the antibiotics used in this work.

Lethal treatment was performed on the *Enterococci* isolates with 35% w/v sodium chloride, 85% w/v sucrose, 22% v/v ethanol and 6% Hydrogen peroxide (H_2O_2) using MRS broth as diluents. All the challenged isolates show no growth on the MRS broth supplemented with sodium chloride, sucrose, ethanol and Hydrogen peroxide (H_2O_2) while the unchallenged control cells had viable bacterial count that ranges between 1.0 x 10^6 cfu/ml and 3.4 x 10^6 cfu/ml (Table 6).

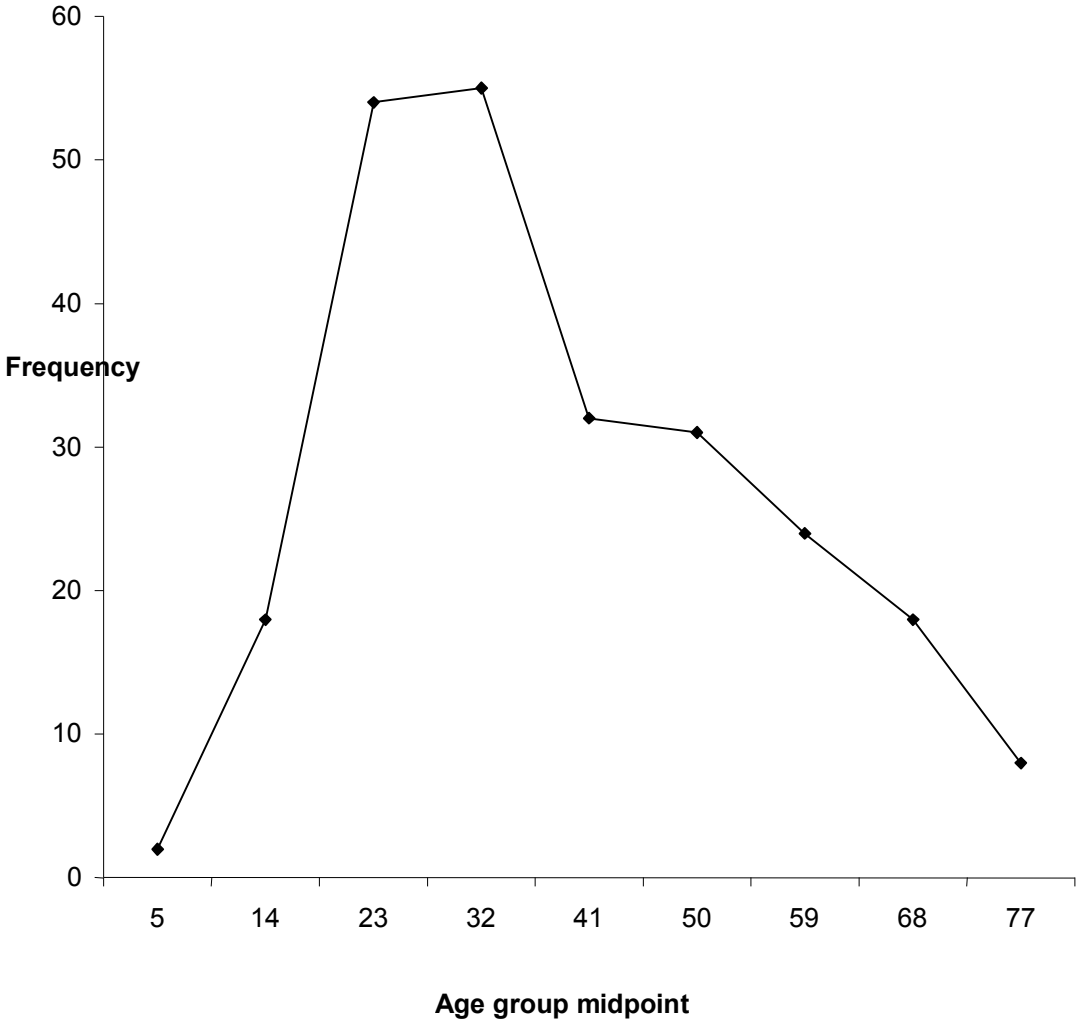

Fig 1a: Age distribution of dental plague patients

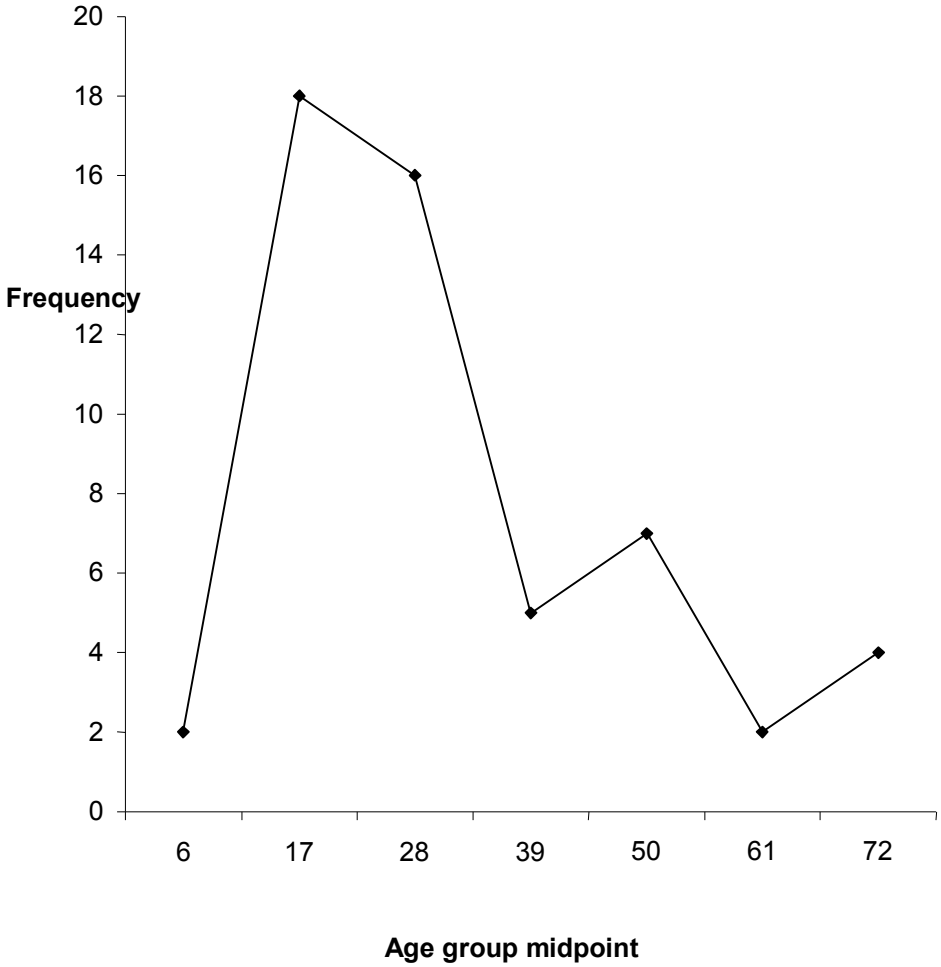

Fig 1b: Age distribution of patients with Dental plague positive for LAB

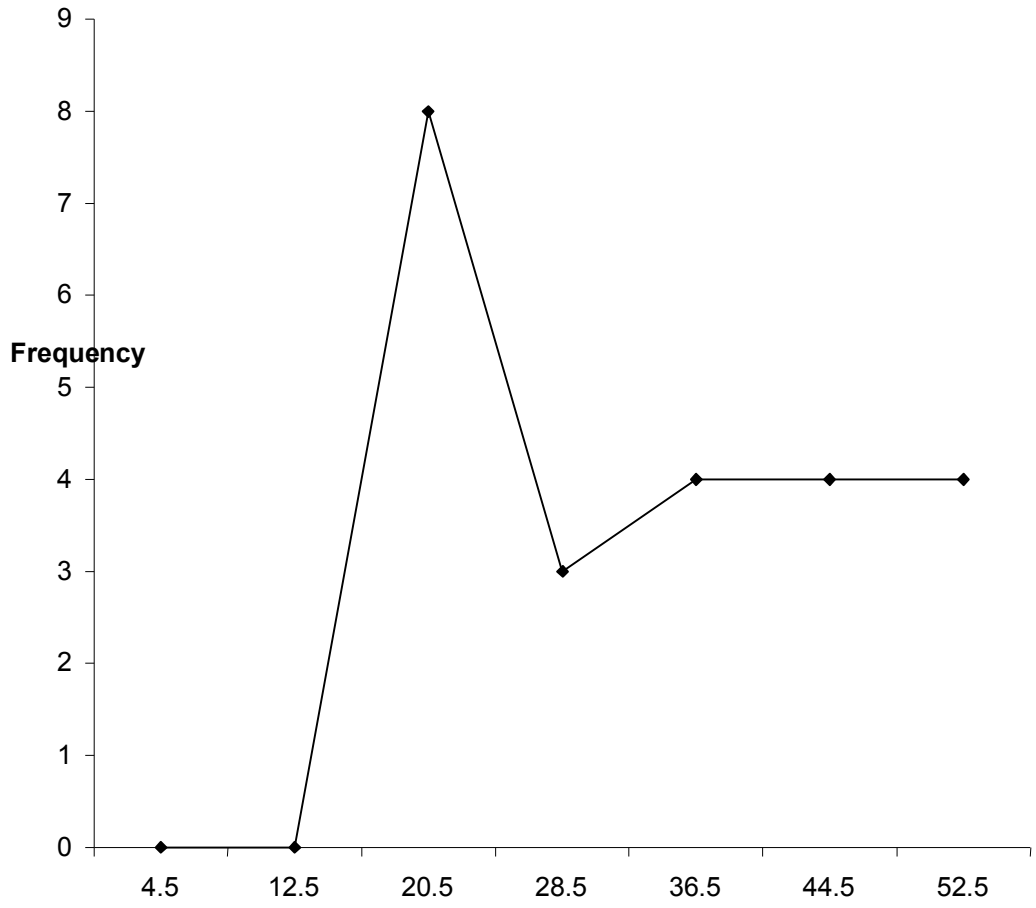

Fig 1c: Age distribution of patients with Dental plague positive for *Enterococci*

TABLE 1: The relationship in age range, sex, number of patient positive for LAB and *Enterococci* Isolated from Human with dental plague

Age Range	No of samples collected		No of samples positive for LAB:		No of samples positive for *Enterococci*	
	Male	Female	Male	Female	Male	Female
1-20	15	18	7	2	4	2
21-40	62	54	10	10	5	5
41-60	31	36	7	4	5	2
61-80	16	18	3	1	-	-
TOTAL	124	126	27	17	14	9
GRAND TOTAL	250		44		23	

Table 2: Biochemical Characteristics of *Enterococcus* Species isolated from Human with Dental plaque

Characteristics	Percentage Positive	Percentage Negative
Catalase	0	100
Gram's	100	0
Oxidase	0	100
Indole Test	0	100
Arginine Hydrolysis	72.7	27.3
Hemolysis	90	10
Growth at pH 9.6	100	0
Growth at 6.5% NaCl	100	0
Growth at 7.5% NaCl	5	95
Growth at 10^{o}C	100	0
Growth at 30^{o}C	100	0
Growth at 35^{o}C	100	0
Growth at 37^{o}C	100	0
Growth at 45^{o}C	100	0
Growth at 60^{o}C	10	90
Methyl red test	80	20
Motility	0	100
Hydrogen sulphide Test	0	100
Voges-Proskauer Test	0	100
Gas Production in MRS Broth	0	100
Homo fermentation	100	0
Heterofermentation	0	100
Bile Esculin Hydrolysis	100	0

Table 3: Sugar fermentation pattern of *Enterococcus* species isolated from human with dental plague

Isolate code	Inositol	Inulin	Sorbitol	Glycerol	Xylose	Cellobiose	Sorbose	Rhamnose	Maltose	Lactose	Fructose	Sucrose	Galactose	Glucose	Arabinose	Melibiose	Mannitol	Probable Identity
P.185	-	+	-	-	+	+	-	-	+	+	+	+	+	+	-	-	+	*E. munditii*
P.190	-	-	-	-	-	+	-	-	-	+	+	+	-	+	+	+	-	*E. faecium*
P.246	-	-	-	(+)	+	+	-	-	-	+	+	+	+	+	-	-	+	*E. faecalis*
P.188	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	(+)	+	*E. gallinarum*
P.192	-	-	(+)	+	-	+	-	-	-	+	+	+	+	-	-	-	-	*E. dispar*
P.177	-	-	+	-	(+)	+	-	-	+	+	+	+	+	+	-	-	+	*E. avium*
P.164	-	-	-	-	-	+	-	-	(+)	+	+	+	+	+	-	-	-	*E. hirae*
P.138	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	-	*E. porcinus*
P.193	-	+	-	+	+	+	-	-	+	+	+	(+)	+	+	-	-	+	*E. cecorum*
P.198	++	-	++	-	++	+	-	-	-	+	++	+	+	+	-	-	+	*E. ratti*

Key:

P	=	Patient number
++	=	Strong Positive reaction
+	=	Positive reaction
(+)	=	Weakly positive reaction
-	=	Negative reaction

TABLE 4: Enzymatic profile of *Enterococcus* species isolated from human with dental plague

Isolate	Enzymes			
	Amylase (Hydrolysis of starch)	Protease (Hydrolysis of casein)	Lipase (Hydrolysis of Olive oil)	Gelatinase (Hydrolysis of Gelatin)
E. porcinus P.138	+	-	-	-
E. gallinarum P.188	-	+	-	-
E. hireae P.164	+	-	-	-
E. avium P.177	-	-	-	+
E. faecium P.190	-	-	-	+
E. faecalis P.246	-	-	-	+
E. munditi P.185	-	-	-	-
E. cecorum P.193	+	+	-	-
E. ratti P.198	+	+	-	+
E. dispar P.192	-	-	-	-

+ = Hydrolysis; - = No Hydrolysis

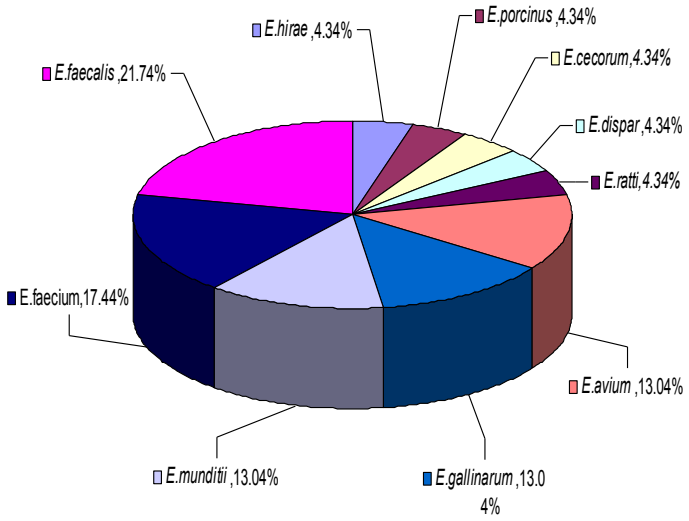

Fig 2: **Percentage occurrence of *Enterococcus* species isolated from human with dental plague.**

Table 5: **Antibiotics and Calcium Hydroxide ($CaCOH)_2$ susceptibility of *Enterococcus* species isolated from Human with Dental Plaque**

Name/ code of Isolates	PG (10 µg)	AMX(10 µg)	COT (25 µg)	CP (30) µg	CF (30 µg)	CR (30 µg)	Ery (15 µg)	CK (30 µg)	CI (15 µg)	OF (5 µg)	PC (100 µg)	AZ (15 µg)	TE (30 µg)	Van (30 µg)	Strep (30 µg)	AUG(30 µg)	GEN (10 µg)	NAL (30 µg)	NIT (10 µg)	$Ca(OH)_2$(30 mg)
E. porcinus(p138)	R	R	R	R(10)	R	R	R	R(9)	R	R(8)	R	R	R	R(9)	R(3)	nd	nd	nd	nd	S(4)
E. facium(p190)	R(8)	R	R	R	R	R	R	R	R(11)	R(10)	R(10)	R	R(12)	R(10)	R	nd	nd	nd	nd	S(3)
E. avium(p177)	R	R	R	R	R	R	R	R	R(9)	R(10)	R	R	R(4)	R	R	nd	R(13)	R	nd	R
E. hirae(p164)	R(8)	R(4)	R	R	R(4)	R(3)	R(9)	R	R(8)	R(8)	R(3)	R(4)	R(8)	R(9)	R(10)	nd	nd	nd	nd	S(8)
E. dispar(p192)	R(9)	R(10)	R(10)	R(13)	R(10)	R(11)	R(12)	R(8)	R(9)	R(9)	R(8)	R(8)	R(9)	R(8)	R(9)	R(8)	nd	nd	nd	S(9)
E.ceacorum(p193)	R(3)	R(12)	R(4)	R(8)	R(10)	R(11)	R(9)	R(9)	R(8)	R(10)	R(8)	R(9)	R(8)	R(4)	R	nd	R(9)	R(10)	nd	S(6)
E. munditi(p185)	R(8)	R(10)	R	R(3)	R	R(8)	R(10)	R(9)	R(11)	R(13)	R(10)	R(10)	R(9)	R(13)	R(3)	nd	nd	nd	R(11)	R
E. feacalis(p246)	R(8)	R(9)	R(7)	R(8)	R(8)	R(9)	R(8)	R(8)	R(11)	R(9)	R(4)	R(8)	R(11)	R(12)	R(9)	nd	nd	nd	nd	R
E. galinarum(p188)	R	R	R	R	R	R(4)	R	R	R	R	R	R	R	R	R	nd	nd	R	nd	R
E. ratti(p198)	R(3)	R(3)	R	R	R	R	R(4)	R(8)	R(8)	R(10)	R	R(3)	R(9)	R(10)	R	R	nd	nd	nd	S(3)

Key: PG-Penicillin, AMX-Amoxicillin, COT-Cotrimoxazole, CP-Cephalexin, CF-cefazolin, CR-Cefuroxime, ER-Erythromycin, CK-Chloramphenicol, CI-Ciprofloxacin, OF-Oxfloxacin, PC-Piperacillin, AZ-Azithromycin, TE-Tetracyclin, GEN-Gentamicine, NIT-Nitrofurantion, AUG-Augumentin, Van-Vancomycin, Strep-Stroptomycin, NAL-Nalidixic, $Ca(OH)_2$- Calcium Hydroxide, nd-not determined, R-resistance, S-Sensitive.

TABLE 6: Lethal treatment of the *Enterococcus* species isolated from Human with dental plague

Isolate	Lethal Treatment				
	35%w/v Nacl	85%w/v sucrose	22%v/v ethanol	6%v/v Hydrogen peroxide(6:4mls)	Unchallanged (control) cfu/ml
E. faecium 221	-	-	-	-	$1x10^6$
E. ratti 198	-	-	-	-	$2.9X10^6$
E. munditi 247	-	-	-	-	$2.3X10^6$
E. hirae 164	-	-	-	-	$3.4X10^6$
E. dispar 192	-	-	-	-	$3.2X10^6$
E. cecorum 193	-	-	-	-	$2.3X10^6$
E. avium 177	-	-	-	-	$2.8X10^6$
E. faecalis 178	-	-	-	-	$2.9X10^6$
E. Porcinus 193	-	-	-	-	$3.3X10^6$
E. gallinarum 188	-	-	-	-	$2.9X10^6$

- = **No growth**

4. Discussion

A total of 44 strains of lactic acid bacteria (LAB) were isolated from 250 human with dental plague out of which 23 of them were identified to be *Enterococccus species*. Patients between 21 and 40 years of age (students and early working class) had the highest occurrence of *Enterococccus species*, probably due to more active life and little time to take care of themselves, eating mostly junk foods which can easily accumulate on the dentine for the bacteria to invade. This study portrayed higher prevalence of LAB which is 17.6% against 9.2% of *Enterococci species* isolated. *Enterococci* are capable of surviving for long durations under adverse circumstances (Figdor et al., 2003). They are also present in vegetables, olives and plant materials (Giraffa 2002), mostly fermented food products (Andrighetto et al., 2001) suggesting their sources in the mouth.

The twenty three (23*) Enterococci* species isolated and identified in this work were differentiated from other LAB based on their cultural, morphological, physiological and biochemical characteristics. Their carbohydrate fermentation pattern differentiates the Enterococci to species level as described by Facklam and Collins (1989); Manaro and Blanch (1999) and Bergey's manual of systemic Bacteriology (Sneath et al., 1986).

Baldassari *et al.* (2004) reported that the virulence mechanism of *Enterococci* is not well understood but surface proteins and polysaccharide (that is the enzymatic activity) is possibly involved in virulence.

In this study *E. gallinarum*, *E. munditii*, *E. faecium* and *E. dispar* with exception of few strains did not hydrolyze starch. According to Wie-Shing-Lee (1976) all strains of *S. bovis* showed positive amylase reaction, whereas all strains of enterococcus showed negative amylase on starch agar.

None of the *Enterococcus* species isolated in this work produced lipolytic activity.

LAB are weakly lipolytic compared to other organism and thus believed to play an important role in the determination of the special aroma of many different cheeses (Foulquie-Moreno et al., 2006).

E. ratti had strong proteolysis reaction followed by *E. cecorum* but *E. gallinarum* showed weak reaction whereas *E. faecalis* showed no Proteolytic activity. The association between an *Enterococcal* protease and virulence was first suggested by Gold et al. (1975) who found that a gelatin liquefying human oral *E. faecalis* isolate induced caries formation in germ free rats while non-proteolysis strain did not. Proteolytic and peptidolytic activities are generally low for *Enterococcus* strain with the exception of *E. faecalis* (Carresco, 1989).

E. faecali had gelatinase activity in this study. Gelatinase (Gel) is an extra-cellular metallo-endopeptidase involved in the hydrolysis of gelatin, collagen, hemoglobin and other bioactive peptides (Su et al., 1991). According to Tavaria and Malcata (1998), *E. faecium* did not produce gel but hydrolyze milk fat more extensively than *Lactococcus lactis*, but in this study, *E. avium*, *E. ratti* and *E. faecium* had gelatinase activity while *E. faecalis* had weak reaction.

Most of the *Enterococcus* species isolated in this work have complete heamolysis while few have partial haemolysis on Blood Agar, however, *E. porcinus* and *E. hirae* were non- hemolytic. Other hemolytic stains were even regarded as non-hemolytic or the third type of hemolysis called gamma (Ryan and Ray 2004) due to their brownish appearance as a result of normal reaction of the blood to the growth conditions used (37° C in the presence of carbon dioxide).

Antibiotic susceptibility pattern including calcium hydroxide susceptibility of *Enterococcus* species showed that there were variations in the inhibition pattern even within species. Some antibiotics were more resistance towards the bacterial strains than others. Despite the fact that some of the

antibiotics used in this work inhibited some of the isolates, yet they were being regarded as resistant according to CLSI (2011) and may not be effective for treatment. The rise in prevalence of allergic disease in Westernized country mostly is due in part to a disease in exposure to infection and an increase in the use of antibiotics early in life (Farooqi and Hopkin 1998; Hopkin, 1999). Many living organisms can acquire some degree of resistance through gradual increased exposure to a drug (Ishiwa and Iwata 1980).

Sixty percent (60%) of *Enterococci* species isolated in this work were sensitive to Calcium hydroxide. Calcium hydroxide has been shown to eliminate most micro organism when used as a 7–day dressing (Sundqvist et al., 1988) of the root canal space in dental plague. Comparism between the antimicrobial effects of calcium hydroxide and iodine –potassium iodide (IKI) in a clinical study revealed that fewer culture reversals occurred when calcium hydroxide was used compared with IKI (Safavi et al., 1985). Other salts and antimicrobial agents used to eliminate the growth of isolates in this study include high concentration of sodium chloride (NaCl), sucrose, hydrogen peroxide (H_2O_2) and ethanol at 60^0C and with 10µg/ml addition of chloramfenicol. All the isolates in this work grew at 6.5 %w/v NaCl but not at 35%w/v NaCl. Higher concentration of sucrose (85%w/v), hydrogen peroxide (45mM) and ethanol (22%v/v) were also lethal to all *Entorococcus* species isolated in this work. The ability to eliminate *Entorococcus* species at higher concentration of salt and antimicrobial agents is one of the criteria that commonly characterize the *Enterococci* (Mundt, 1986).

Conclusion

This research work establishes the presence of *Enterococci* in the mouth of human with dental plague. All the isolates were multidrug resistant but sensitive to higher concentration of sucrose (85%w/v), hydrogen peroxide (45mM) and ethanol (22%v/v). However, there is need to investigate the pathogenicity of these isolates in dental caries.

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