ANTIBIOGRAM OF BIOFILM FORMING ORAL *STREPTOCOCCI* SPECIES ISOLATED FROM DENTAL CARIES PATIENTS VISITING FEDERAL COLLEGE OF DENTAL TECHNOLOGY AND THERAPY, ENUGU NIGERIA

ABSTRACT

Background and objectives: The potential roles of diverse bacterial communities in oral cavity are influence by biofilm formation which promote colonization and lead to increase severity of dental caries and poor respond to antimicrobial therapy. Information on bacterial biofilm features are limited in dental disease were it challenges patient management in Nigeria. This current study was designed to determine the Antibiogram of biofilm forming Oral *Streptococci* species isolated from Dental Caries Patients Visiting FCDT &T Enugu.

Methodology: A total of four hundred and fifty (450) swab sample which include one hundred and fifty (150) each for dental caries, dental plaque and saliva of infected dental caries patient were collected and analyzed using Standard Microbiological protocol for isolation and identification. Detection of biofilm formation in oral *Streptococci* species was performed using Qualitative Biofilm Assay Technique. Antibiogram studies of biofilm forming oral *Streptococci* species was performed using the Kirby–Bauer disk diffusion method and the results were interpreted using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints. Multiple antibiotic resistance indices (MARI) was determined for MDR oral *Streptococci* species

Results: Of the 317 (70.4%) Oral *Streptococci* species isolated, greater proportion were *S. mutans* 167 (37.1%), followed by *S. salivarius* 61 (13.5%), *S. mitis* 56 (12.5%) and *S. sanguis* 33(7.3%) as the least isolate. Oral *Streptococci* species were highly predominant in dental caries sample 167(37.1) followed by dental plaque 104(23.1%) and saliva 46(10.2). oral *Streptococci* species were common amongst age 21-35years 162 (36.0%). High proportion of oral *Streptococci* species 181(40.2%) was observed in male subject (significant at $p \leq 0.05$) over female 136(30.2%) with no association between presences of oral *Streptococci* species and dental caries at $p \leq 0.05$ in female patient. Biofilm formers comprises of higher detection rate in dental caries sample 78 (46.7 %) accompanied by dental plaque 76 (44.2%) and saliva samples 13(28.2%) with no identified association between biofilm production in oral *Streptococci* species and sample source ($p \leq 0.05$). The isolates exhibited high percentage of resistance within the range of 50-100% against tetracycline Chloramphenicol, Trimethoprim-Sulfamethoxazole, Vancomycin, Ceftriaxone, Oxacillin and penicillin G and also exhibit MDR with MARI value of ≥0.4 but were susceptible to Ciprofloxacin 87.5%, Amoxicillin 97.1% and Gentamicin 100%.

Conclusion: This study report high prevalence of Oral *Streptococci* species among dental caries patient and emphasize the need for good oral hygiene to minimize occurrence of plaque mediating biofilm formation and assemblage of biofilm bacteria. Amoxicillin, Gentamicin and ciprofloxacin were highly effective and require judicious application in dental care while further antibiotic treatment for dental patient should rely on appropriate surveillance and screening for selection of antimicrobial therapy.

Keyword: Biofilm-forming, Oral *Streptococci* species, Dental caries

Introduction

Oral cavity is a moist environment which is kept at a relatively constant temperature (34-36°C) and a pH close to neutrality in most areas [1], which may promote the colonization, proliferation and growth of diverse community of Gram positive and Gram negative bacterial species. Within the oral cavity, there are few obligate anaerobic, aerobic species and mostly with higher prevalence of facultative anaerobes [2, 3]. The oral microflora shows highly distinct diverse of microbial species and constitute more than 500 species of bacteria present in the oral cavity [4]. Majority of the oral microbial species are commensal. However, shift in oral *Streptococci* species community dynamics may cause pathological changes within oral cavity and distant sites [5]. Complex interactions among oral *Streptococci*, other bacteria species and host tissues may result in the formation of microbial biofilms on dental surface. On dental surfaces, if biofilms left unwashed for days, plaque is formed [6]. Oral *Streptococci* species in dental plaque have

been reported in dental diseases including gingivitis, periodontitis and dental caries; the most reported prevalent oral bacterial infections in humans [7]. Dental caries is a transmissible infectious disease which hampers the attainment and protection of oral health in different age groups. It is a multifactorial disease link to *mutans Streptococci* group as the major etiologic agent. *S. mutans* is the best-known species that includes other seven species in the *mutans Streptococci* group [8]. Considerable epidemiologic evidence links oral *Streptococcus* species to caries [9, 10]. Oral bacteria such *Streptococcus species*, *Lactobacillus acidophilus* etc., which are the earlier colonizer of oral cavity, accumulate to form a whitish thick layer on the dental surface known as dental plaque or biofilm through homogenous combination with the saliva and fermentable food remains [10]. This embedded matrix of organism produces acid from the fermented foods, which causes demineralization and destruction of the tooth surface resulting in the formation of holes and cavities in the teeth region [10, 11] Dental plaque is considered as a biofilm and most of the biofilm accumulated on dental surface are made up of oral *Streptococci* species and other oral flora which may serve as the primary or initial etiologic agent of dental caries. This oral microorganism interaction within the biofilm community by various organized and recognized mechanism including assemblage and co-aggregation, quorum sensing i.e., cell-cell communication, exchange of metabolic and genetic material. This inherent features may benefit interspecies bacterial survival while presenting infected dental caries patient with associated biofilm forming oral *Streptococci* species expressing difficult therapeutic targets in dental disease, were this strains could become non-susceptible to antibiotic agent which it was initially sensitive or vulnerable. As antibiotic is cardinal in treatment of dental caries, the degree of resistance to antibiotics is directly proportional to the degree of their use; the greater the use of an antibiotic, the greater the chance of emerging of resistant bacterial populations to that particular antibiotic [12]. Majority of biofilm forming oral *Streptococci* species from dental caries may exhibit multidrug resistant (MDR) genetic determinant. According to earlier study, bacterial cells in biofilms matrix are 10-1000 times more non-susceptible to antimicrobial agents, when compared to their planktonic equivalents [13]. However, better knowledge and understanding of Antibiotic profile of biofilm forming oral *Streptococci* species from dental caries will be significant in guiding the selection of antibiotic for treatment

MATERIALS AND METHODS

Patient recruitment and Assessment of oral conditions

The study was carried out at Federal College of Dental Technology and Therapy, Trans-Ekulu, located at latitude 6°29'07.1"N and longitude 7°29'42.5"E in Enugu, Nigeria. Subject presenting any of the following criteria was excluded from the study which includes; subjects on antibiotics prophylaxis for dental treatment and recurrent dental caries while inclusion criteria involve subject who are not in any form of antibiotics, subject with dental caries infection. A designed robust questionnaire was administered to patient to collect socio-demographic relevant data (Educational status, marital status, gender, age) and other relevant demographic information about dental caries. World Health Organization 1997 criteria for indexes DMF (Decayed, missing and Filled) was used as reference for assessment of oral health condition with signs of dental caries seen amongst the patient [14]**.** Assessment of Debris and calculus index were used as criteria for Simplified Oral hygiene Index (SOHI), oral hygiene was evaluated in ambient conditions, under natural light, with the use clinical mirror and probe for the removal of debris[15].

Sample collection

A total of four hundred and fifty (450) swab sample which include one hundred and fifty (150) each for dental caries, dental plaque and saliva of patient was collected for this study. Aseptically, short sterile hypodermic needle and endodontic file was used to collect dental plaque sample by scraping, 5 times from mesial central fissures to distal and pits from the upper and lower teeth quadrant. The hypodermic needle was aseptically suspended in 5 mL

double strength Brain-heart infusion broth (BHI) (Thermo Fisher Scientific, Inc., USA), supplemented with 20 µL and 20 % of bacitracin and sucrose. Thereafter, it was anaerobically incubated at 37°C for 24 hours to use undiluted for further bacteriological analysis.

Saliva Samples of dental caries patient were collected into a tubes containing 5 mL double strength Brain-heart infusion broth (BHI) (Thermo Fisher Scientific, Inc., USA), supplemented with 20 µL of bacitracin and 20% sucrose and was incubated anaerobically at 37°C for 24 hours to use for the subsequent analysis**.**

Following standard procedure for collection of dental caries sample described by Loyola-Rodriguez *et al.* [9] with slide modification. Disinfection of the patient tooth canal was performed with 30% volume of hydrogen peroxide before using 5 % volume tincture of iodine and 5 % of sodium hypochlorite.

A sterile bur was used to remove the caries lesion while access to the caries lesion was performed without using water coolant to avoid contamination. Considering patient with purulent infections, a sterile physiological buffer saline solution was introduced aseptically into the canal, then a sterile swab was dipped and pressed gently on the portion of the affected tooth cavity. The adherent bacterial sample were aseptically transferred to sterilized 5 mL double strength Brain-heart infusion broth (BHI) (Thermo Fisher Scientific, Inc., USA), supplemented with 20 µL of bacitracin and 20 % sucrose and was incubated anaerobically at 37°C for 24 hours

Culture and Identification Procedure

After overnight incubation, the turbid broth culture was inoculated on blood agar and *Mitis*/*salivarius* agar (Thermo Fisher Scientific, Inc., USA) plates. The culture plate were kept in 5 % $C0_2$ at 37°C for 37-72 hours in an Isotemp incubator (Thermo Fisher Scientific, Waltham, MA, USA). Colonies of oral *Streptococcal* species were identified based on their morphological characteristic (such as color, consistency) and observed hemolysis pattern. *Streptococcus salivarius* grew and appeared pale-blue, large, mucoid (i.e., gum-drop) glistering colonies, *Streptococcus mitis* appeared as domed center colonies, with blue coloration, flat, small, hard colonies, *Streptococcus mutans;* displayed characteristic appearance of pale-blue coloration, with colonies that are appear as frosted glass or granulated, opaque, convex, raised and undulate, colonies also exhibit a glistening bubble on the surface. *S. sanguis* – appeared as dark hard colonies embedded in agar that are raised and smooth. In order to confirm the growth specific biochemical test such as catalase test which showed negative tests were carried out for identification of oral *Streptococcus* species using standard microbiological techniques described in Microbiology Practical Handbook [16]. VITEK 2 System (bioMerieux, France) was use for further confirmation test according to manufacturer's guideline.

Biofilm Production Assay

Qualitative Assay (Congo red Method)

Qualitative assay of biofilm producing *Streptococci* species was performed by the growing the *Streptococci* species on Congo red agar (CRA). Briefly, the Brain Heart Infusion (Thermo Fisher Scientific, Inc., USA). The broth (37g/l) was supplemented with sucrose (50 g/l) (Sigma-Aldrich, Germany), agar (10 g/l) and Congo red dye (0.8 g/l) was used for Congo Red agar method [17, 18].

Aqueous Concentrated solution of Congo red was prepared and autoclaved separately from other constituents. After cooling to $55^{O}C$ it was added to the other mixture. The oral *Streptococci* isolate were plated on the sterilized solidified CR agar. The strains were kept in 5% $C0_2$ at 37°C for 37-72 hours in Isotemp incubator (Thermo Fisher Scientific, Waltham, MA, USA). After 72 hours incubation, the results were interpreted as follows: red and Bordeaux red with smooth colonies was considered to be non-biofilm producers while strains producing intensive black, black, and reddish black colonies with a rough, dry, and crystalline consistency was classified as biofilm producers [18].

Quality Control

Control strains *Enterococcus faecalis* ATCC 29212 (positive control or biofilm former) and *Staphylococcus epidermidis* ATCC 12228 (negative control or biofilm nonformer) were used for *in vitro* biofilm evaluation procedures [19, 20].

***In vitro* Antibiotic susceptibility testing**

The response of all biofilm producing *Streptococci* species were screened against the following conventional antibiotics; Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Amoxicillin (30 µg), Chloramphenicol (30 µg), Ampicillin (10 µg), Erythromycin (15 µg), Gentamicin (30 µg), Penicillin G (10 µg), Streptomycin (10 µg), Tetracycline (30 µg), Trimethoprim-Sulfamethoxazole (30 µg) , Vancomycin (30 µg) (Oxoid, UK) performed using Kirby-Bauer disc diffusion Method according to Clinical and Laboratory Standards Institute [21]. Bacteria inoculum equivalent to 0.5 McFarland standard of the isolate was streaked on entire Mueller-Hinton agar plate before antibiotic discs were carefully placed. The culture plate were kept in 5% $C0_2$ at 37°C for 37-72 hours in Isotemp incubator (Thermo Fisher Scientific, Waltham, MA, USA). After overnight incubation, zone of inhibition was measured and was used to interpret the level of resistance and susceptibility to each of the tested antibiotic [21].

Multiple antibiotic resistance (MAR) index

Multiple antibiotic resistance (MAR) index was described using the formula **MAR=a/b**, where

a= the total number of antibiotics which test isolate displayed resistance

b=the total number of antibiotics which the test organism has been evaluated for sensitivity [22].

Statistical Analysis

The Fisher's exact and Chi-squared tests were employed to evaluate gender association between dental caries experience, oral hygiene index and presence of Oral *Streptococci* species. One-way ANOVA was use to estimate association between biofilm production in Oral *Streptococci* species and sample source (dental caries, dental plaque and saliva of patients). The level of statistical significance $p \leq 0.05$ was adopted for all analyses data**.**

RESULT AND DISCUSSION

Association between dental caries experience and oral hygiene index in Patient

Of the 450 samples collected, Caries Index evaluation showed 56.2% and 43.8% of DMF in male over female counterpart as statistical significant ($p \leq 0.05$) was identified between caries experience and patient (Table 1). Upper Quadrant was mostly affected in female 22.9 % while lower Quadrant was highly affect in male 34.9%. Relationship between Quadrant affected and caries experience in patient was statistical significant **(**$p \leq 0.05$) in both patient. Dental caries severity, showed high risk of dental caries 31.7% in male, in contrast to low risk 7.3% observed in female as dental caries severity was statistically significant ($p \leq 0.05$) in both patient. Through Fisher's exact test there was association between Simplified Oral Hygiene Index and patient caries experience ($p \leq 0.05$). Poor oral Hygiene was common in male 37(8.2%) while Regular Oral Hygiene Index 30.7% was observed in female. *Streptococcus mutans* was more predominant in both male and female subject 89(19.8%) and 78(17.3%) respectively compare to other oral *Streptococci* species. Higher proportion of oral *Streptococci* species 181(40.2%) was observed in male subject ($p \leq 0.05$) over female 136(30.2%) in female. However, it can be seen in Table 1 that there was no association between presences of oral *Streptococci* species and dental caries ($p \leq 0.05$) in female patient.

Overall Presence of oral *Streptococci* species and Comparative overview of demographics characteristic of sample population

Regarding age, oral *Streptococci* species were common amongst age 21-35years 162 (36.0%), followed by 36-50year 73 (16.2%) while ≥51 year and above harbored 37 (8.2%) as the least occurrence rate of the bacteria as displayed in Table 2. Oral *Streptococci* species was more prevalent in male 181(40.2%) than female 136(30.2%). Marital Status information among patient, showed increase frequency of oral *Streptococci* species 186 (41.3%) in Single patient (unmarried) compared to other subject with isolation rate of 117 (26.0%), 11 (2.4%) and 3 (0.7%) recorded against Married, Widowed and Divorced patient. Patient with Formal level of education harbor high prevalence of oral *Streptococci* species 293 (65.1%) over Informal educated patient recording 24 (5.3%). Within the Occupational framework, presence of oral *Streptococci* species were frequent in Student patient 117 (26.0%)

accompanied by Unemployed patient 86 (19.1%) while the lowest proportion of the isolate was seen among Public servant 10 (2.2%). Of the 317 (70.4%) Oral *Streptococci* species isolated, greater proportion were *S. mutans* 167 (37.1%), followed by *S. salivarius* 61 (13.5%) and *S. mitis* 56 (12.5%) while the least was *S. sanguis* 33(7.3). Oral *Streptococci* species were highly predominant in dental caries sample 167(37.1) followed by dental plaque 104(23.1%) and saliva 46(10.2) as the least prevalence rate shown in Table 3.

Detection rate of Biofilm and non-biofilm forming Oral *Streptococci* species

Biofilm formers comprising of higher detection rate in dental caries sample 78 (46.7 %) which (include *Streptococcus mutans* 31.7%, *Streptococcus salivarius* (9.6%, *Streptococcus mitis* 5.4% and *Streptococcus sanguis* 0.0%), followed by dental plaque 76(44.2 %) (comprising of *Streptococcus mutans* 20.2%, *Streptococcus salivarius* 14.4%, *Streptococcus sanguis* 4.8% and *Streptococcus mitis* 3.8%) and 28.2% in saliva samples (consisting of *Streptococcus mutans* 15.2%, *Streptococcus salivarius* 8.7% and *Streptococcus sanguis* 4.3%) shown in Table 4. There was no identified association ($p \leq 0.05$) between biofilm production in oral *Streptococci* species and sample source (Table 4). The proportion of non-biofilm forming Oral *Streptococci* species in dental caries sample accounted 53.3%, 55.7% and 71.7% in dental caries sample, dental plaque and saliva respectively.

Antibiogram pattern of biofilm forming Oral *Streptococci* species

Biofilm forming *S. mutans, S. mitis, S. salivarius,* and *S. sanguis* were more sensitive to ciprofloxacin 100%, 90.0%, 85.7% and 87.5% respectively. Amoxicillin was also effective against biofilm forming *S. mutans* 96.3%, *S. salivarius* 97.1% and 100% for both *S. mitis* and *S. sanguis*. The result also showed that biofilm forming Oral *Streptococci* species were 100% susceptible in high portion to Gentamicin (Table 5). This study strongly opined that all biofilm forming Oral *Streptococci* species were susceptible to Amoxicillin, ciprofloxacin and Gentamicin at high proportion. The isolates demonstrated high level of resistance to Chloramphenicol, Trimethoprim-Sulfamethoxazole, Vancomycin, Ceftriaxone, Oxacillin and penicillin G recording 100% respectively across the biofilm forming Oral *Streptococci* species isolates. Tetracycline resistance ranges between 50.0%−100%. Resistance to Ampicillin and erythromycin was 91.4% and 97.1% for *S. salivarius, S. mitis* 100% and 75.0%, *S. mutans* 100% and 91.4%, *S. sanguis* 87.5% and 62.5%. Biofilm forming *S. mutans* were 100% resistant Streptomycin while other biofilm forming strain*; S. mitis, S. salivarius* and *S. sanguis* demonstrated low level of susceptibility to Streptomycin recording 5.0%, 20. 0%, 25.0% respectively. The result showed that all the biofilm forming Oral *Streptococci* species were resistant to two or more antibiotic inferring multidrug resistant with MARI ranging from 0.4-0.8 (Table 6).

Table 1: Clinical relevant data and Association between dental caries experience and oral hygiene index in Patient (**n**=450).

Dental Caries Experience	**Male (%)**	**Female (%)**	**p-*value****
Caries Index			
Decayed	168(37.3)	197 (43.8)	**0.00001**
Missing	85 (18.9)	0 (0.0)	
Filled	0 (0.0)	0 (0.0)	
Total	**253(56.2)**	**197 (43.8)**	
Quadrant Affected			
Upper	96(21.3)	103 (22.9)	**0.003**

Lower	157(34.9)	94 (20.9)	
Dental caries Severity			
Low risk	61(13.6)	33(7.3)	**0.00001**
Moderate risk	49(10.9)	69(15.3)	
High risk	143(31.7)	95(21.1)	
Simplified Oral Hygiene Index			
Good	151(33.6)	47(10.4)	**0.00001**
Regular	65(14.4)	138(30.7)	
Poor	37(8.2)	12(2.7)	
Oral *Streptococci* species			
Streptococcus salivarius	18(4.0)	43(9.6)	**X^2 =29.6, *p*=.00001 *Male***
Streptococcus mitis	41(9.1)	15(3.3)	
Streptococcus mutans	89(19.8)	78(17.3)	**X^2 =3.0, *p*=. 58 *Female***
Streptococcus sanguis	33(7.3)	0(0.0)	
Total OSP	**181(40.2)**	**136(30.2%)**	

n=number of sample**, Fisher Exact test**; $p < 0.05$; **X^2 –**Chi Square $p < 0.05$; **OSP-** Oral *Streptococci* species

Table 2: Overall *Streptoccocci* species growth and Comparative overview of demographics data of sample population

Demographics data	**No. Sampled**	***Streptoccocci* species growth (%)**
Age (years)		
6-20	77	45 (10.0)
21-35	204	162 (36.0)
36-50	110	73 (16.2)
≥51 and above	59	37 (8.2)
Gender		
Male	253	181 (40.2)
Female	197	136 (30.2)
Marital Status		
Married	183	117 (26.0)
Singles	231	186 (41.3)
Divorced	5	3 (0.7)
Widowed	31	11 (2.4)
Educational Qualification		
Formal	407	293 (65.1)
Informal	43	24 (5.3)
Occupation		
Business/Artisan	65	45 (10.0)
Civil servant	64	26 (5.8)
Public servant	23	10 (2.2)
Retired	48	33 (7.3)
Student	142	117 (26.0)
Unemployed	108	86 (19.1)

Table 3: **Distribution of Oral *Streptococci* species isolates from dental caries, dental plaque and saliva of patient**

	Oral *Streptococci* species				
Sample Matrix	***S. salivarius* (%)**	***S. mitis* (%)**	***S. mutans* (%)**	***S. sanguis* (%)**	**Prevalence (%)**
Dental caries	24(5.3)	35(7.8)	97(21.6)	11(2.4)	**167(37.1)**
Dental plaque	20(4.4)	21(4.7)	46(10.2)	17(3.8)	**104(23.1)**
Saliva	17 (3.8)	0(0.0)	24 (5.3)	5(1.1)	**46(10.2)**
	61 (13.5)	**56 (12.5)**	**167 (37.1)**	**33(7.3)**	**317 (70.4)**

Total sample= 450

Table 4: Biofilm and non-biofilm forming Oral *Streptococci* species isolated from dental caries, dental plaque and saliva of patients

Sample matrix	**Number of isolate**	**Biofilm forming (%)**	**Non-biofilm forming (%)**	**p-*value****
Dental caries				
Streptococcus salivarius	24	16(9.6)	8(4.8)	
Streptococcus mitis	35	9(5.4)	26(15.6)	
Streptococcus mutans	97	53(31.7)	44(26.3)	
Streptococcus sanguis	11	0(0.0)	11(6.6)	
Total	**167**	**78(46.7)**	**89(53.3)**	
Dental plaque				.410195
Streptococcus salivarius	20	15(14.4)	5(4.8)	
Streptococcus mitis	21	4(3.8)	17(16.3)	
Streptococcus mutans	46	21(20.2)	25(24.0)	
Streptococcus sanguis	17	6(4.8)	11(10.6)	
Total	**104**	**46(44.2)**	**58 (55.7)**	
Saliva				
Streptococcus salivarius	17	4(8.7)	13(28.3)	
Streptococcus mutans	24	7(15.2)	17(36.9)	
Streptococcus sanguis	5	2(4.3)	3(6.5)	
Total	**46**	**13 (28.2)**	**33(71.7)**	

One-way ANOVA $p < 0.05$

Table 5: Antibiogram of biofilm forming oral *Streptococcus* species isolated from dental caries patients

Classes	**Antibiotic (µg)**	***S. salivarius* (n=35)**		***S. mitis* (n=20)**		***S. mutans* (n=81)**		***S. sanguis* (n=8)**	
β-lactam		**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
	Ampicillin (30 µg)	32(91.4)	3(8.6)	20(100)	0(0.0)	81(100)	0(0.0)	7(87.5)	1(12.5)
	Amoxicillin (30 µg)	1(2.9)	34(97.1)	0(0.0)	20(100)	3(3.7)	78(96.3)	8(100)	0(0.0)
	Penicillin G (10 µg)	35(100)	0(0.0)	20(100)	0(0.0)	81(100)	0(0.0)	8(100)	0(0.0)
	Oxacillin (30 µg)	35(100)	0(0.0)	20(100)	0(0.0)	81(100)	0(0.0)	8(100)	0(0.0)
Aminoglycoside	Gentamicin (30 µg)	0(0.0)	35(100)	0(0.0)	20(100)	0(0.0)	81(100)	0(0.0)	8(100)
	Streptomycin (10 µg)	28(80.0)	7(20.0)	19(95.0)	1(5.0)	81(100)	0(0.0)	6(75.0)	2(25.0)
Cephalosporin	Ceftriaxone (30 µg)	35(100)	0(0.0)	20(100)	0(0.0)	81(100)	0(0.0)	8(100)	0(0.0)
Macrolide	Erythromycin (5 µg)	34(97.1)	1(2.9)	15(75.0)	5(25.0)	74(91.4)	7(8.6)	5(62.5)	3(37.5)
Glycopeptide	Vancomycin (30 µg)	35(100)	0(0.0)	20(100)	0(0.0)	81(100)	0(0.0)	81(100)	0(0.0)
Fluoroquinolone	Ciprofloxacin (5 µg)	5(14.3)	30(85.7)	2(10.0)	18(90.0)	0(0.0)	81(100)	1(12.5)	7(87.5)
Sulfonamide	Trimethoprim-Sulfamethoxazole (30 µg)	35(100)	0(0.0)	20(100)	0(0.0)	81(100)	0(0.0)	8(100)	0(0.0)
Other	Tetracycline (10 µg)	35(100)	0(0.0)	10(50.0)	10(50.0)	79(97.5)	2(2.6)	6(75.0)	2(25.0)
	Chloramphenicol (30 µg)	35(100)	0(0.0)	20(100)	0(0.0)	81(100)	0(0.0)	81(100)	0(0.0)

Key: **R**=Resistance, **S**=Susceptibility, **n**=number of isolate, **%-Percentage**

Table 6: Multiple Antibiotic Resistant of Biofilm forming Oral *Streptococcus* species isolated from dental caries patients

Oral *Streptococcus* species	Resistant Antimicrobial Agent	Mean Average MARI
Streptococcus salivarius	**AMP, AMX, P, OX, S, CRO, E, CIP, SXT, TE, C**	0.7
Streptococcus mitis	**AMP, P, OX, S, CRO, E, VA, CIP, SXT, TE, C**	0.6
Streptococcus mutans	**AMP, AMX, P, OX, S, CRO, E, VA, SXT, TE, C**	0.4
Streptococcus sanguis	**AMP, AMX, P, OX, S, CRO, E, VA, CIP, SXT, TE, C**	0.8

Key: **MARI**- Multiple Antibiotic Resistant Index**, AMP**=Ampicillin, **AMX**= Amoxicillin, **CRO**=Ceftriaxone**, C**= Chloramphenicol**, CIP**=Ciprofloxacin, **E=** Erythromycin, **OX**=Oxacillin, **P**= Penicillin G, **S**= Streptomycin, **TE**=Tetracycline, **SXT**= Trimethoprim-Sulfamethoxazole, **VA**=Vancomycin

DISCUSSION

When comparing Oral *Streptococci* species growth according to gender, male patient accounted 40.2% a higher frequency over female patient 30.2%. There are reports that active oral infection is more frequent in males, as such, this result is in line with the findings of other study of which higher occurrence in males 64.0% over than females 34% [23] and 65.22 % male and 38.89 % of females [24] has been reported. Similar findings was seen in the study done by Loyola-Rodriguez *et al.* [9] and Majdy *et al.* [25] in Mexican and Saudi population. This study has observed male gender association between dental caries experience and occurrence of Oral *Streptococci* species ($p \leq 0.05$) was statistically significant. This association further substantiate the data obtained from Simplified Oral Hygiene Index (SOHI) which revealed poor oral hygiene in male in this current study. This current study strongly opined that males are likely more prone to develop dental caries diseases, because they usually display poorer oral hygiene than females. Earlier study in Nigeria as reported has linked chronic Periodontitis to male gender with Kola-nut eating and smoking habits [26]. Additionally, the observed gender disparity may result from exposure to cariogenic factors or may be due to the increasing sugar consumption, low exposure to fluorides containing toothpaste and poor access to oral health care as supported by Khushbu and Satyam. [27]

Isolation rate of oral *Streptococci* species was observed among age group 21–35 years (36.0%), followed by 36-50year 16.2%. Regarding age, it's worth noting that dental caries has been documented in all ages, ranging from children to adults [8, 9, 28, 29]. But is important to reveal that those who consumed a high volume of carbonated soft drink may have a significantly higher rate of dental disease than those had high juice, high milk, and high water in their diet supported by earlier study [30]. The observed age differences noted in our study between the aforementioned ages could be linked to by negligible difference in regular tooth cleaning frequency among patient.

Patient with Formal level of education harbor high prevalence 293 (65.1%) oral *Streptococci* species over Informal educated patient recording 24 (5.3%). It could be envisage that formal education attendee are aware of the risk, severity and threat of dental caries, thus their visitation to dental clinic may have influence the sample size of the study population. In contrast with our findings, perusal of studies has reported similar higher occurrence of dental pathogens in population with lower educational background or attainment [26, 27, 31, 32, 33, 34] and may reflect or depict lack of awareness on dental hygiene and associated disease. Within the Occupational framework, presence of oral *Streptococci* species were frequent in Student 26.0 and Unemployed 19.1 %. It should be noted with credence to

this two group (Student and Unemployed patient) as most of the are known to resorting to ready-to-eat food marketed by vendors and prefer sapid foods such as beverages, chocolate, premium ice cream, refined carbohydrate, and more curried, smooth and oily fried food items which may increase proliferation of oral *Streptococci* species and shelter the caries to progression amongst the studied population. Clearly, the observation in this result may be influence by the sample size as revealed in the result section where high proportion of the studied population were drawn from this two group (Student and Unemployed patient). Our findings is in concordant with Khushbu and Satyam. [27] who reported that the epidemiology trend of oral disease differs with sex, race, age, socioeconomic status, oral hygiene practices, food habits and geographical location.

In our study, 70.4% of the patient harbored oral *Streptococci* species (*S. mutans* 37.1%, *S. salivarius* 13.5% *S. mitis* 12.5% and *S. sanguis* 7.3%) from active dental disease. The high proportion of *S. mutans* was recovered from the studied samples but however, the presence of other oral *Streptococci* species found in this current study as earlier been reported in active dental infections [35, 36, 37]**.** Additionally**,** the predominant of *S. mutans* over other Oral *Streptococci* from our study tandem with reported by other researchers [8, 9, 10, 38, 39]. Existing literature as shown that *S. mutans* are frequently isolated from carious lesions and is a considerable indicator of caries. In general, it has been postulated that the greater the amount of *Streptococcus mutans* in the oral cavity, the greater the chance of developing this dental disease [8, 9, 10, 15]. However, the high prevalence of *S. mutans* only make it lucid that the oral cavity is deem suitable for the proliferation and progression of dental caries to more complicated dental disease such periodontitis, gingivitis etc.

Biofilm formers comprising of higher detection rate in dental caries sample 46.7 % followed by dental plaque 44.2 % and saliva sample 28.2 % recording the least detection rate. In addition, *S. mutans* followed by *S. salivarius* significant biofilm producing strain. However, there was no identified association between biofilm production in oral *Streptococci* species and sample source ($p \leq 0.05$) as presented in result section. Similar result from So-Young *et al.* [40] has shown that majority of *Streptococcus mutans* 100 % isolated from dental caries were capable to forming biofilm. Although limited studies exist for comparative assessment of other Oral *Streptococci* species but its relevant to know that formation of biofilm could possibly be as a result of ecological imbalance in the equilibrium between tooth minerals and oral biofilms and secondly biofilm is developed due to microbial succession [41, 42]. It challenging that biofilm forming oral *Streptococci* species formation is a threat in dental care.

Since antibiotic are cardinal treatment of dental disease, most studies have considered amoxicillin as the drug of choice for oral infections [9], nevertheless it has been evidence from our study that Amoxicillin as one of the β-lactam antibiotic was effective against biofilm forming *S. mutans* 96.3%, *S. salivarius* 97.1% and 100% for both *S. mitis* and *S. sanguis*. Our findings substantiate reported from other study [9, 43, 44, 45]. In most developed countries notably Germany, recommends the use of β-lactam especially amoxicillin for empiric antibiotic therapy in their guidelines for treatment of odontogenic infections [46].

The result also showed that biofilm forming Oral *Streptococci* species were 85.7-100% susceptible in high portion to Ciprofloxacin and Gentamicin. This observation support the earlier reported that bacteria from oral cavity were sensitive to gentamicin by Gaetti-Jardim *et al*. [47]. Also, Sonia *et al.* [48] reported that biofilm forming strains isolated from chronic periodontitis patients were sensitive to gentamicin (80.5 %). In Ogun state, Nigeria, *Streptococcus viridians* was most sensitive to Ciprofloxacin 85.7% [49] and in Minna, Nigeria, *Streptococcus mutans* was sensitive to gentamicin [50] while study by Jesse and Ramteke. [51] proved that *Streptococcus* species were susceptible to gentamicin and norfloxacin of fluoroquinolone class while Devi *et al.* [45] in their study, reported that out of 320 isolates of *Streptococcus* species, 80 % of the recovered isolates were sensitive activity to ciprofloxacin and gentamycin. Aminoglycosides, are heterocyclic antibiotics and also known as amino sugars, which inhibit bacterial protein synthesis [52]. For the treatment of oral infections, they are not recommended singly or individually but they are used in combination or jointly with other antimicrobial agent particularly β-lactams in oral surgery [52]. These studies is supportive for considering the use of gentamicin against oral *Streptococcus* species. Our study strongly opined that judicious use while prescribing this antibiotics will maintain the bacteria with less resistance. The isolates exhibited high level of resistance to Chloramphenicol, Trimethoprim-Sulfamethoxazole, Vancomycin, Ceftriaxone, Oxacillin and penicillin G 100% respectively across the biofilm forming Oral *Streptococci* species isolates and is consistent with study in Germany [46], Spain [53] and elsewhere [54, 55]. This explanation for the observed higher resistance biofilm forming oral *Streptococcus* species to those drugs may be due indiscriminate and widespread use, as a result of its ease of accessibility in most pharmacies as Over the Counter (OTC) which can lead to selective pressure and increase resistant biofilm forming oral *Streptococcus* species. Addition it worth noting that, biofilm has been reported to increase resistance to various antimicrobial agents especially antibiotics [56].

Thus, the biofilm forming Oral *S. mitis, S. mutans,* and *S. sanguis* and *S. salivarius* predominated the community of Antibiotic Resistant (AR) oral *Streptococci*, including Multidrug Resistant in this studied setting. Most our isolates displayed multidrug resistance patterns with MAR index value of ≥0.4 while similar MDR pattern has been reported [8, 9, 35, 57], higher MDR among biofilm former may emerge from increased properties of higher plasmid transfer, efflux mechanism, modification of target genes and metabolic pathway that allow for occurrence of antimicrobials resistance may also be the mainstay of the reported multidrug resistant in our study. So, being in one of the most densely populated biotopes of a microbial species, biofilm forming Oral *Streptococcus* species can mediate the spread of resistance determinants to more clinically susceptible pathogenic, which requires careful monitoring of their pattern of susceptibility to antimicrobial agents [35].

CONCLUSION

The findings of this study report the prevalence of biofilm forming Oral *Streptococcus* species in dental caries patient. The biofilm forming Oral *Streptococcus* expressed multidrug resistant phenotype to most of the antibiotic; ceftriaxone, tetracycline, Penicillin, Trimethoprim-Sulfamethoxazole, chloramphenicol, and ampicillin. The high Multiple Antibiotic Resistant index found in our present investigation accentuate the need for antibiotic susceptibility testing to be conducted before treatment. Majority of the biofilm former were 87.5% -100% susceptible to amoxicillin, ciprofloxacin and gentamicin. Therefore this agent can be used for the empirical management of biofilm forming Oral *Streptococcus* causing dental caries. This would not only help in judicious use of the aforementioned antibiotics but would also restrain and curtail the transfer of antibiotics resistant determinant in Nigerian dental clinics and community settings as well. To develop new treatments for biofilm destruction such as nano-drug delivery is necessary in dentistry to contain extreme increase of MDR biofilm forming bacteria facilitating recalcitrant dental infection.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

All authors declare that written informed consent was obtained from the patient or care-giver of the patient before collection of sample

ETHICAL CONSIDERATION AND APPROVAL

The approval and consideration for this study was gotten from the research and ethical committee of Federal College of Dental Technology and Therapy, Enugu with ethical clearance number FCDTT/VOL056/2021/R099.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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