ANTIBIOTIC PROFILING OF DENTOGINGIVAL BACTERIAL FLORA OF PRIMARY SCHOOL PUPILS IN AGO IWOYE, OGUN STATE, NIGERIA

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ABSTRACT

The dentogingival bacterial flora was determined using cultural method while the antibiotic susceptibility testing was carried out using agar disc diffusion techniques and interpreted as described by clinical laboratory standard institutes. Also, the levels of bacterial counts were determined on appropriate selective media prior to confirmation using biochemical test. Results obtained, showed on alarming trend of important dental caries pathogens in the following proportion *Streptococcus sobrinus* 22 (21.8%), *Streptococcus oralis* 22 (21.8%, *Streptococcus mutans* 26 (25.7%, *Streptococcus mitis* 17 (16.8%), *Actinomyces, naeslundii* 4 (3.96%) and *Neisseria mucosa* 10(9.9%). The antibiogram assay potrayed ciprofloxacin and ofloxacin as the most resisted by the isolated organisms while ampicillin was found to be the most efficacious. The levels of bacterial counts ranged from 1 X 10^3 to 3 X 10^4 cfulg of the food sediments examined. It can therefore, be concluded, that the studied population harbour arrays of dental caries agent in their mouth.

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INTRODUCTION

The presence of food residue on the dentogingival surface may represent a significant favourable microenvironmental conditions for the growth and survival of cariogenic and periodontopathic bacteria. These microorganisms have been implicated in the pathogenesis and perpetuation of important oral diseases (Asikainen *et al*., 1991; Asikainen *et al*., 1996; Papaiannou *et al*., 1997) and transmission between individuals can also occur through kisses, personal contact and even from parents to children (Troit – Linden *et al*., 1995; Papaionnou *et al*., 1997) .These microflora are part of a complex ecosystem referred to clinically as dental plaque (Bowden, 1991; Van Haute, 1994). Dental plaque formation is a function of the interaction between the adhesiveness of plaque to the tooth surfaces and the physical shear forces which serve to dislodge and remove the plaque (Roberts, 2005). According to Benson *et al*.(2004), dental plaque is a complex biofilm found on tooth surface and capable of causing dental caries. Although, it has been reported, that bacteria form an important group of microorganisms found in both healthy and diseased mouths (Simmonovic *et al*., 2002; Roberts, 2005), the accumulations of these organisms on oral surfaces are a major factor in the development of most of the common dental diseases including dental caries and periodontal disease (Williams and Cummins, 2003). In view of this and due to the dearth of information regarding the subject matter, this study was designed to assess the level of bacterial flora on the dentogingival surfaces and their antibiotics spectrum.

MATERIALS AND METHODS

Subjects and Sample Population

Pupils attending public primary schools in Ago-Iwoye (Igan Wesley, Idode Wesley, Ago Wesley, St. Paul Anglican, Imere and Oke Padie) were asked to participate in the study, after their parent or guardian consent have been sought and approved by the ethics committee on Human Research and a signed consent was completed. Inclusion parameters included voluntary participation, medically well fitted, pupils between 6 – 12years old. Subjects with history of

antibiotics use 3 months before the examination were excluded. In all, a total of hundred pupils (50 male and 50 female participated).

MICROBIOLOGICAL SAMPLING AND PROCESSING

The dentogingival sample was taken using sterile paper points inserted into the dentogingival surfaces for 15seconds. Each paper point was placed back in its container and processed within 12h of sampling. These samples were collected in triplicates and the microbiological analyses were carried out as follows;

Total viable bacterial counts was done on plate count agar (Oxoid, UK), *Actinomyces naeslundii* was isolated on gelatin based medium (containing Metronidazole 10µglml, Calcium Sulfate 20µglml and gelatin Agar (Oxoid, UK), *Neisseria mucosa* was cultured on Thayer-martin selective agar while *Streptococcus* species was selected on Blood agar base medium. The identification of each of the isolates was done following standard bacteriological procedure (Cheesbourough, 2005).

RESULTS

The bacterial count of food debris removed from the teeth of primary school pupils in Ago Iwoye, Ogun State, Nigeria, are depicted in table 1. The total viable bacterial counts ranged from 1.7×10^4 to 7×10^4 cfu/g of the food debris. Results from this study, revealed a relatively very high total viable bacterial counts in pupils from Igan Wesley, this was followed by pupils from Oke padie and then Ago Wesley. The differential counts of the analyzed samples also ranged from 1×10^3 to 3×10^4 cfu/g. The differentials of the analyzed teeth samples revealed six bacterial species viz; *Actinomyces naeslundii* 4 (3.96%), *Streptococcus sobrinus* 22 (21.8%), *Streptococcus oralis* 22 (21.8%, *Streptococcus mutans* 26 (25.7%), *Neisseria mucosa* 17 (16.8%), and *Streptococcus mitis* 17 (16.8%). The antibiogram spectrum potrayed ciprofloxacin and ofloxacin as the most resisted by the isolated organisms. On the other hands, ampicillin was found to be the most efficacious of the tested antibiotics 85(84.2%), followed by augmentin 79 (78.2%) and then clindamycin 74 (73.3%).

Table 1. Bacterial counts of food debris on teeth of primary schools pupils

Primary School	Differential Counts (Cfulg)						Total Viable Bacterial Count Cfulg
	ATC	SSC	SOC	SMIC	SMC	NMC	
Igan Wesley	2×10^4	5×10^3	3×10^3	2×10^3	6×10^3	3×10^4	7×10^4
Idode Wesley	--------	1×10^3	1×10^4	1.0×10^3	4×10^3	1×10^4	2.8×10^4
Ago Wesley	1×10^4	3×10^3	1.5×10^3	1.5×10^3	4×10^3	2×10^3	5.2×10^4
St. Paul Anglican	---------	4×10^3	2×10^4	3×10^3	4×10^3	6×10^3	4.1×10^4
Imere	---------	5×10^3	3×10^3	2×10^3	2×10^3	2×10^3	1.7×10^4
Okepadie	1×10^4	4×10^3	5×10^3	3.3×10^3	3×10^{34}	6.7×10^3	5.4×10^4
Total	4×10^4	2.2×10^4	4.25×10^4	1.28×10^4	5.0×10^4	5.67×10^4	2.72×10^4

ATC= *Actinomyces naeslundii* counts, SSC= *Streptococcus sobrinus* counts,
SOC = *Streptococcus oralis* counts, SMC *Streptococcus mutans* counts
NMC= *Neisseria mucosa* counts, SMIC = *Streptococcus mitis* counts

Table 2: Sensitivity pattern of bacterial flora Of food debris from teeth on some selected primary school pupils in Ago – Iwoye, Ogun State, Nigeria

ISOLATES	N	%	NUMBER OF SENSITIVE STRAINS							
			CLN	AMP	AUG	OFL	ERY	CIP	GEN	COT
STREPTOCOCCUS SOBRINUS	22	21.8	18 (81.8)	19 (86.4)	15 (68.2)	4 (18.2)	14 (63.6)	4(18.2	16(72.7	11(50
STREPTOCOCCUS ORALIS	22	21.8	18 (81.8)	19 (86.4)	13 (86.4)	7(31.8)	10(45.5)	4(18.2)	13(59.1)	8(36.4)
STREPTOCOCCUS MUTANS	26	25.7	21(80.8	22(84.6	20(76.9	4(15.4	18(69.2	4(15.4)	17(65.4	10(38.5)
ACTINOMYCE, NAESLUNDIC	4	3.96	1 (25)	2 (50)	4(100)	2 (50)	3 (75)	2(50)	3(75)	2(50)
NEISSERIA MUCOSA	10	9.9	2(20)	8(80)	10(100)	2(20)	10(100)	6(60)	2(20)	8(80)
STREPTOCOCCUS MITIS	17	16.8	14(82.4)	15(88.2)	17(100)	6(35.3)	6(35.3)	2(11.8)	14(82.4)	6(35.3)
TOTAL	101	(100)	74(73.3	85(84.2	79(78.2	25(24.8*	61(60.4	22(21.8)	65(64.4)	45(44.6)

CLN = Clindamycin, AMP = Ampicillin, AUG= Augmentin, OFL= Ofloxacin,
ERY = Erythromycin CIP = Ciprofloxacin, GEN = Gentamycin COT = Cotrimoxazole

TABLE 3: Resistant pattern of bacterial flora Of food debris from teeth on some selected primary school pupils in Ago – Iwoye, Ogun State, Nigeria

ISOLATES	N	%	NUMBER OF RESISTANT STRAINS							
			CLN	AMP	AUG	OFL	ERY	CIP	GEN	COT
STREPTOCOCCUS SOBRINUS	22	21.8	4 (18.2)	3 (13.6)	7 (31.8)	18(81.8)	8(36.4)	18(81.8)	6(27.3)	11(50)
STREPTOCOCCUS ORALIS	22	21.8	4 (18.2)	3 (13.6)	9(40.9)	15(68.2)	12(54.5)	18(81.8)	9(40.9)	14(63.6)
STREPTOCOCCUS MUTANS	26	25.7	5(20)	5(19.2)	6(23.1)	22(84.6)	8(30.8)	22(84.6)	9(34.6)	16(61.5)
ACTINOMYCE, NAESLUNDIC	4	3.96	3(75)	2(50)	0(0)	2(50)	1(25)	2(50)	1(25)	2(50)
NEISSERIA MUCOSA	10	9.9	8(80)	2(20)	0(0)	8(80)	0(0)	4(40)	8(80)	2(20)
STREPTOCOCCUS MITIS	17	16.8	3(176)	2(11.8)	0(0)	11(64.7)	11(64.7)	15(88.2)	3(17.6)	11(64.7)
TOTAL	101	(100)	27(26.7)	16(15.8)	22(21.8)	76(75.2)	40(39.6)	79(78.2)	36(35.6)	56(55.4)

DISCUSSION

Bacteriological determinations of dentogingival flora are often used to assess the number and the types of microorganisms present in the mouth or on the dentogingival surfaces. Among the four *Streptococcus species* isolated, *Streptococcus mutans* and *Streptococcus sobrinus* are regarded to as primary etiologic agents of coronal and root caries (Ellen *et al*., 1985; Loesche,1986; Bowden, 1990;Van Houte,1994;Van Houte *et al*.,1994) while the non-mutans *Streptococcus* particularly the acid tolerant strains such as *Streptococcus oralis* has been implicated in coronal caries (Van Houte *et al*., 1991;Sansone *et al*., 1993). *Actinomyces naeslundii* which was the least isolated organisms in this study, is part of the commensal oral microbiota (Bowden *et al*., 1990; Li *et al*., 2004) and may play a role in pathogenesis of caries (Bowden *et al*., 1990). Also, several culture based studies, have indicted *Actinomyces* species as gaining increased prominence at the expense of *Streptococci* during maturation of the biofilm (Syed and Loesche,1987;Van-Palestein, 1981). This biofilm develops readily on tooth surfaces because of the optimal temperature, the rich nutrient supply in the oral cavity and the hard non shedding surface (Digi *et al*., 2009). The presence of *Neisseria mucosa* on the dentogingival surfaces in this study, is somehow surprising, as this organism is not known to be a frequent human pathogens. This is because, this organisms is part of the normal human nasopharyngeal flora though has been implicated in causing meningitis (Stotka *et al*., 1991). According to simonovic *et al*.(2002), *Neisseria mucosa* may represent about 16.6% of the isolated organisms in the infected root canal. This observation is parallel to our findings, as *Neisseria mucosa* was only present in 9.9% of the total organisms isolated. The variation that occurs, in the two studies, may be due to difference in the study location, our sample size and the study population. The fact that, the differentials counts of the isolated bacteria ranged from 1×10^3 to 3×10^4 cfu/g corroborates that of Simmonovic *et al*., (2002), who observed that a healthy oral cavity represents a complex microecosystem, changeable in number and type of bacteria, fungi, viruses and protozoa, which can be found there as part of the oral normal flora or commensal. However, the type of bacteria found in this study are all oral pathogens, otherwise the counts were still within the acceptable limit of 4 or 5×10^7 bacteria according to Simmonovic *et al*., (2002). The antibiogram spectrum revealed ciprofloxacin and ofloxacin as the most resisted by the bacterial isolate. This may be due to the abuse of this antibiotic, both in human and animal health (Chikwendu *et al*., 2008). The fact that, the two antibiotics mentioned above are fluoroquinolones, further stressed that, the isolates may

possess fluoroquinolones resistant genes on their plasmids (Rooney *et al*., 2009). The high sensitivity of the isolated organisms to ampicillin, clindamycin and augmentin, suggest that these antibiotics may be useful in the treatment of caries caused by these isolates. It can also be said, that the isolates are not betalactamase producing organisms due to the effectiveness of ampicillin and augmentin in controlling their growth (Efuntoye and Amuzat, 2007). It can therefore be concluded, that antimicrobial susceptibility profile of dentogingival bacterial flora be requested for, to guide treatment.

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