Oral Microbial Isolates from Patients Attending the Dental Clinic, University College Hospital, Ibadan, Nigeria.

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Key words: Oral microbial isolates, Antibiotic therapy, *Enterobacteria.*

ABSTRACT

Aim

To evaluate microbial isolates from the buccal mucosa, carious teeth cavities and root tips of extracted carious teeth from patients attending the dental clinics of the University College Hospital, Ibadan, Nigeria. The isolates may guide the choice of preoperative antibiotics which hitherto has been based on "best guess".

Materials and Method

Consecutive consenting patients seen over a period of six months were included in the study.

Pre-tested questionnaires were administered for socio- demographic and oral hygiene information. This was followed by an intra-oral examination. Oral swab was performed using a sterile microscopic culturing swab stick which was run along the buccal and labial sulci of the jaws. The swab tips were cut into brain-heart infusion broth and the cultured micro-organisms plated out on blood agar. Microscopy, culture and sensitivity of the isolates were carried out using standard microbiological techniques.

Data was analyzed using SPSS version 19. Frequency tables were generated and measures of central tendency were calculated.

Results

One hundred and eighty-three patients were seen. The non- commensal micro-organisms isolated from the oral sulcus were predominantly coliforms (*Klebsiella species* in 15.8%, *Escherichia coli* in 13.70%) with normal oral flora found in 12 (6.6%) isolates. The carious cavities were also dominated by coliforms; *Escherichia coli* (20.6%), *Klebsiella species* (12%) and *Proteus species (*8.62%) with 10.34% being the normal flora isolates. The isolates from the root tip of extracted carious teeth yielded mainly *Escherichia coli* (28.6%) with no growth in over half of the samples.

High sensitivity of the isolates to quinolones and marked resistance to ampicillin and tetracycline were observed.

Conclusion

The isolation of invasive microorganisms from the oral cavity of our patients suggests the need for precautions that would reduce the oral microflora burden preoperatively. The antimicrobial antibiotic sensitivity pattern may guide the choice of prophylactic antibiotics in maxillofacial surgery.

Introduction

The oral cavity is constantly bathed by saliva, which could be considered an environmental agent influencing the state of the oral health. Saliva has a buffering effect as a result of its high bicarbonate content and it augments the clearance of food particles in the mouth thereby reducing the population of the oral microbes.[1] The saliva also contains antibodies and immunoglobullins; IgE, IgA and IgM with their inherent antibacterial activities. In addition it also contains cyanates, a lysozyme-like substance and other theoretically antibacterial substance. [1, 2]

The composition of the oral microflora is highly complex and variable and to date at least 300 different species are known to be associated with the oral cavity and it is suggested that only half of them can be cultured.[3] The resident or normal bacterial flora in the oral cavity in the developed and the developing world may differ considering the variety in environmental conditions, dietary habits, contaminants and immune status. [3]

The mucous membranes of the mouth are often sterile at birth but may be contaminated by passage through the birth canal within 4 – 12 hours. After birth, Streptococcus viridians become established as the most prominent member of the resident flora in the oral cavity and remain so for life. [2] It is thought that these organisms (*Streptococcus viridians*) probably originated in the respiratory tracts of the mother and birth attendants.

Early in life (before teeth erupts) aerobic and anaerobic Staphylococcus, gram negative diplococcus (*Neisseriae, Braxell catarrahalis*), *Diptheroids* and occasional *Lactobacilli* are added to the oral microbial flora. [4, 5] However, when teeth begin to erupt, anaerobic spirochete such as *Prevotella species*, especially *Prevotella melaninogenica*, *Fusobacterium species, Rothia species and Capnocytophage species* establish themselves along with some anaerobic *Vibros* and *Lactobacilli* in the oral cavity. [5] Along with this bacteria load, *Actinomyces species* are normally present in tonsillae tissue and gingivae in adults. Various protozoan and yeasts may also be present. [5] In effect the mouth supports a wide variety of microorganisms including bacteria, yeasts, viruses and occasional protozoan, with bacteria being the predominant resident microflora. [5]

The mouth is therefore colonized by a characteristic commensal microflora, also referred to as normal flora that remains relatively stable as a result of a dynamic balance between inter bacterial and host bacterial interactions. These commensal oral flora together with other oral defense mechanisms play an important role in protecting the oral cavity from infection by exogenous organisms. [6]

The composition of the microflora of the mouth is highly complex and varies considerably from site to site and at different times because of the potential habitats for attachment in the oral cavity which include the non-shedding hard tooth surfaces and the soft, constantly replaced epithelia surfaces.[7] At these sites in the oral cavity, there are variations in anaerobiosis, availability of nutrients, exposure to salivary secretions or gingival crevicular fluids and masticatory forces, all of which contribute to the variation in the types of microflora. [8]

Pattern of hand hygiene are developed and established in early life. For children with inadequate and poor toilet hygiene, enteric bacteria can pose a potential threat and danger

by penetrating the body via the mouth as a result of nail-biting; so they can lead to various infections in the oral cavity. The need to provide proper hygiene instructions as a preventive measure was advocated. [9, 10]

Therefore, an alteration in the delicate balance between the resident commensal or normal oral flora and the host, predispose to endogenous oral infection. The factors that predispose to oral infection include physiological factors such as pregnancy, trauma (local or general), malnutrition, immunosuppression, antimicrobial therapy, chemotherapy, oral malignancies, salivary flow dysfunction, dehydration and loss of masticatory muscle function. [11]

Aim

The aim of this study was to evaluate the microbial isolates in the buccal mucosa of the oral cavity, carious teeth cavities and root tips of extracted carious teeth from patients attending the dental clinic at the University College Hospital (UCH), Ibadan, Nigeria, with the intent of mapping out the characteristic commensal microbial isolates and assessing the appropriate antibiotic therapy in anticipation of dental (oral) infections.

Materials and Methods

Consecutive patients presenting at the oral diagnosis and oral surgery clinics of the University College Hospital (UCH), Ibadan over a period of six months were entered into the study. A self-administered questionnaire was completed by the patients after informed consent was obtained and swabs of the buccal mucosa, carious tooth cavities (where present) and the tips of roots of the extracted carious tooth were taken. Oral swab was performed using a sterile microscopic culturing swab stick (FL Medical Swab, Plastic stick. w/, Rayon tip; 12 x 150 in polypropylene test tubes) which was run along the entire buccal and labial sulci of both the upper and lower jaws. Swabs of carious cavities (where present) and swabs from root tips of extracted carious teeth were obtained using sterile swab sticks. The swab tips were cut into brain – heart infusion broth and then cultured on chocolate, blood and McConkey agars in duplicates. One set was incubated aerobically at 37^{o}c while the other set was incubated anaerobically in an anaerobic gas pack system (Oxoid HP0011A).

Isolates were identified by the API 20A and microbacteria identification kits for anaerobes and aerobes respectively. Antimicrobial susceptibility was performed on confirmed pathogens by the Kirby- Bauer disk diffusion method.

Data were entered into a computer spread sheet and analysed using SPSS version 19. Frequency tables were generated and measures of central tendency calculated.

Results

One hundred and eighty-three consecutive patients were seen during the study period. There were 104 (56.8%) females and 70 (38.3%) and in 9 (4.9%) cases, the sex was not indicated. Among the respondents, 50 (27.3%) were students, 18 (9.8%) were traders, 6 (3.3%) were housewives respectively and 4 (2.2%) lecturers. The others were priests, youth corper, business men and civil servants, caterer, farmers and researcher. Forty-five (45) of the respondents did not indicate their profession.

Fifty-three (29.0%) of the respondents claim to brush their teeth twice daily (morning and night), 29 (15.8) brushed their teeth only in the morning, while 2 (1.5%) used chewing stick to clean their teeth.

Twenty-nine patients (15.8%) had previous tooth extraction, 46 (25.1%) had other forms of

dental procedures including scaling and polishing and surgical reconstructive procedures while 11(6.0%) were visiting the dentist for the first time.

Buccal mucosa swabs revealed normal flora, (S*treptococcus albus*) isolates in 12 (6.6%) cases. Non-commensal micro-organisms were also isolated from the buccal mucosa and these included *Klebsella species* in 31 (15.8%) cases, *Escherichia coli* in 23 (12.6%) and *Staphlococcus aureus* in 13 (7.0%) cases. *Proteus species* was isolated in 10 (4.37%) and *Brahamma catarrhalis* in 7 (3.82%) cases (Table 1)

Table 1: Oral microbial isolates from all patients

Microbial isolates from all patients.	**Buccal Sulcus**		**Carious Cavities**		**Root Tips**	
Klebsiella Species	31	16.9%	7	12.1%	-	-
E. coli	23	12.6%	12	20.8%	8	28.6%
Normal flora	12	6.5%	6	10.4%	-	-
Staph. aureus	13	7.1%	6	10.4%	-	-
Beta Haemolytic Strep.	10	5.5%	-	-	-	-
Aerobic spore bearers (ASB)	9	4.9%	2	3.5%	5	17.9%
Proteus	10	5.5%	5	8.6%	-	-
Brahamalis catarrhalis	7	3.8%	6	10.4%	-	-
Pseudomonas	8	4.2%	1	1.7%	-	-
Strep Pneumoniae	4	2.1%	4	6.9%	-	-
Micrococcus serreti	1	0.5%	-	-	-	-
Anaerobic coliforms	1	0.5%	-	-	-	-
Non hemolytic Strep	2	1.1%	-	-	-	-
H. Influenzae	1	0.5%	-	-	-	-
Klebsiela species	1	0.5%	-	-	-	-
No growth	28	15.3%	9	15.5%	15	53.6%
Nil	22	12.0%	-	-	-	-
TOTAL	**183**		**58**		**28**	

In all the 58 carious teeth cavities that were swabbed, *Escherichia coli* was isolated in 12 (20.6%) cases, *Proteus species* in 5 (8.62%), *Klebsiella species* in 7 (12.0%), *Staphylococcus aureus* in 6 (10.3%), *Pseudomonas* in 1 (1.72%), *Streptococcus pneumoniae* in 4 (6.89%), Normal flora in 6 (10.34%) while 9 (15.5%) of the swabs yielded no growth (Table 1).

Twenty-eight root tips were swabbed immediately the teeth were extracted and the culture yielded *Escherichia coli* in 8 (28.6%), *Brahamma catarrhalis* in 5 (17.9%) and the remaining 15 (53.6%) swabs yielded no growth.

Fifty of the patients (27.3%) were on antibiotics within 2 weeks prior to the study. The combination of Amoxyl and Flagyl was the prescribed antibiotics in 11 (4.45%), Amoxyl alone in 10 (5.5%) cases, while 29 (15.85%) of

the patients used various combinations of other types of antibiotics.

The microbial isolates in those patients on antibiotics are shown in (Table 2) reflecting the predominance of *Escherichia coli* and *Kleibsella species* in the 8(17.4%) buccal swabs respectively and *Escherichia coli* in 4 (19%) carious cavity swabs.

Table 2: Patients on antibiotics

Organisms isolated	**Buccal sulcus**		**Carious cavities**		**Root tips**		**Total**
Brahamalis catarrhalis	**7**	**15.2%**	**2**	**9.5%**	**6**	**66.7%**	**15(19.7%)**
E. coli	**8**	**17.4%**	**4**	**19.0%**	**-**	**-**	**12(15.8%)**
Kleb. Species	**8**	**17.4%**	**1**	**4.8%**	**-**	**-**	**9(11.8%)**
Strep aureus	**3**	**6.5%**	**1**	**4.8%**	**2**	**22.2%**	**6(7.9%)**
Proteus species	**2**	**4.4%**	**3**	**14.3%**	**-**	**-**	**5(6.6%)**
Non haemolytics Strep.	**3**	**6.5%**	**1**	**4.8%**	**-**	**-**	**4(5.3%)**
Beta haemolytic Strep.	**1**	**2.2%**	**-**	**-**	**-**	**-**	**1(1.3%)**
Pseudomons species	**3**	**6.5%**	**1**	**4.8%**	**-**	**-**	**4(5.3%)**
Strep peumoniae	**2**	**4.4%**	**2**	**9.4%**	**1**	**11.1%**	**5(6.6%)**
H. influenzae	**1**	**2.2%**	**-**	**-**	**-**	**-**	**1(1.3%)**
Normal flora	**4**	**8.7%**	**3**	**14.3%**	**-**	**-**	**7 (9.2%)**
No growth	**4**	**8.7%**	**3**	**14.3%**	**-**	**-**	**7(9.2%)**
TOTAL	**46**		**21**		**9**		**76**

Table 3 shows the antibiotic sensitivity pattern for the commensal isolates from the buccal cavity, carious cavities and root tips. Higher sensitivity to Quinolones (Ciprotab and Sparflotab) and greater resistance to Ampicillin and tetracycline were observed.

Table 3: Oral microbial isolates/ antibiotic sensitivity pattern

Microbial isolates	**Ampicillin/ Tetracycline**	**Augmentin/ Cephalexin**	**Cefuroxime/ Ceftazidime**	**Quinolones Ciprotab/Sparflotab**	
BS Sensitivity	10(43.5%)	15(65.5%)	20(87.0%)	20(87.0%)	23(100%)
BS Resistance	13(56.5%)	8(34.8%)	3(13.0%)	3(13.0%)	0
CC Sensitivity	2(16.7%)	6(50.0%)	10(83.3%)	11(91.7%)	12(100%)
CC Resistance	10(83.3%)	6(50.0%)	2(16.7%)	1(8.3%)	0
RT Sensitivity	1(12.5%)	2(25.0%)	6(75.0%)	8(100%)	8(100%)
RT Resistance	7(87.5%)	6(75.0%)	2(25.0%)	0	0

Key: BS - Buccal Sulcus
CC - Carious Cavities
RT - Root Tips

Discussion

The mucous membrane of the mouth is sterile at birth but may be contaminated by passage of the baby through the birth canal. However, over time, the mouth becomes colonized by various organisms and to date, almost 300 micro-organisms have been documented as non-commensal microorganisms in the mouth. The predominant normal microflora includes *Streptococcus viridians* and *Lactobacillus species.* [12]

The oral cavity of healthy individual contains hundreds of different bacterial, viral and fungal species. Many of these can associate to form biofilms. They can become pathogenic as a result of changes in the oral environment or other triggers including an individual's personal hygiene. Their presence, therefore contribute to both health and diseases.[13] Since disease states caused by diverse number of various microbial communities have been identified, microbial genomes have been paid a lot of attention in the era of personalized medicine. [14]

The isolation of coliforms in the buccal mucosa, carious teeth cavities and the root tips (following extraction of non-salvageable carious teeth) would suggest that the microbes in the buccal mucosa may be responsible for the root tip infection following propagation from the carious cavities.

In a healthy individual, the normal oral microbial ecosystem is remarkably stable despite its complexity but many endogenous factors may affect the composition and metabolic activity of the oral microflora .[15] The isolation of *Pseudomonas aeruginosa* from the oral cavity may be a pointer to the fact that some of these patients have had previous dental treatment and they could have acquired them as nosocomial infections.

Pseudomonas aerugenosa has been recognized as a pathogen of hospital patients in modern era of intensive treatment and antibiotic administration. [16, 17] Its ability to grow in most condition with simple nutrients and its comparative resistance to antibiotic and disinfectants has allowed it to become established and colonize the mucous membrane and skin of patients. [18]

The periodontal pockets in the gingival are particularly rich sources of organisms including anaerobes that are rarely encountered elsewhere. When implanted in another site, attention is drawn to them, for example *Rothia dentocariosa* and *Capnocytophaga Species* have been known to cause infective endocarditis and bacteremia in granulopenic host respectively. [19]

Proteus Species which are enterobacteria, frequently cause endogenous infections. It is present in rotten meat and sewage faeces, garden soil and vegetables. [20] *Proteus* infection is very difficult to treat with a mortality of 18 – 88% depending on the severity of the underlying disease; in addition, individual species differ in resistance to antibiotics. They may cause osteomyelitis of the jaws and are also linked to rheumatoid arthritis. [21]

A ready transmission of these emerging organisms has demonstrated the need to contain certain infections.[22] *Staphylococcus aureus* is said to be carried by many normal people while streptococci is readily shed from the upper respiratory tract by coughing, sneezing and singing while *Staphylococcus* is shed in the skin squames during physical activity. [23, 24]

The most important microorganisms spread by hand contact are *Staphlococcus aureus* and gram- negative bacilli such as *Klebsiella* and

Serratia species [25]. *Escherichia coli* and *Klebsiella* are the most frequently encountered bacteria in hospital acquired infections [26]. *Klebsiella* is said to be carried by faecal route and transmitted by the hands, therefore poor general hygiene and specifically poor oral habits and deficiency in hand washing of carriers is suspected [27].

Poeta *et al*., (2009) did not recover *Enterococci* or *Escherichia coli* from healthy volunteers, whereas 10 isolates were obtained from 19.5% of patients with fixed appliances. [28] However, Hable *et al*. studied 490 Minnesota children and found 4.7% prevalence of *Enterobacteriaceae.* [29]

Chang and Foltz (1960) in their study on 254 adult college students isolated coliform bacteria from oral cavity of 22 (8.6%) adults, of these; three were identified as *Escherichia coli* [30]. Leitch *et al*., in their study on Caucasians isolated coliforms from 5% of the dental plaque samples [31], while Mobbs *et al*., showed a 6.6% prevalence of *Enterobacter* spices, *Pseudomonadaceae*, and *Acinetobacter* spices in 120 healthy individuals. [32]

Studies have been done on oral carriage of *Enterobacteriaceae* in patients with systemic diseases. Back-Brito *et al*., (2011) in their study on 45 human immunodeficiency virus (HIV) positive and 45 HIV negative patients detected a significantly higher number of *Enterobacteriaceae* and *Pseudomonas* in the oral cavities of the HIV positive patients compared to HIV negative patients. [33] *Escherichia cloacae* were the most frequently isolated species in both groups. Similar studies have been performed on individuals suffering from conditions like HIV-1 [34] and burning mouth syndrome. [35]

Enterobacteriaceae usually live in the intestinal tract. Up to 15% of the population may harbor these organisms in the oral cavity, mostly as transient commensals. Their oral carriage rate may increase in old age, and in conditions leading to reduced salivary flow. [36] *Escherichia* coli, *Enterobacteriaceae* and *kliebsiella*, *Proteus* and *Serratia* are mainly in soil, water, plants and animals and are attribute to feco-oral route of infection. They cause diseases such as meningitis, bacillary dysentery, typhoid fever and food poisoning. [10]

The oral cavity is a potential portal of entry for organisms into the body, but the commensal oral flora, together with other oral defense mechanisms, plays an important role in protecting the oral cavity from infection by exogenous organisms. These organisms are implicated in chronic inflammatory diseases in the oral cavity. They colonize the mouth by the suppression or modulation of oxygen radical production.

These oxygen radicals induce the production of Reactive Oxygen Species (ROS) in host immune cells which trigger off immune response against the invading microbes. Once suppressed, immune defenses are subverted thereby supporting self-survival and long term carriage of these pathogenic microorganisms in their target host since the effective clearance of colonizing microorganisms is highly dependent on ROS. [37]

The normal commensal or oral flora along with the other defense mechanisms, play an important role in protecting the oral cavity from infection by exogenous organisms. In this study, normal oral commensals were found in 10% of the buccal mucosa isolates, however, the isolation of *Escherichia coli* and *Klebsiella*

species which are predominantly colonic commensals may suggest poor oral hygiene.

The antimicrobial sensitivity pattern is similar to that reported in previous studies. [38, 39] The isolates may guide the choice of preoperative antibiotics which hitherto has been based on "best guess".

Greater resistance to Ampicillin and Tetracycline which are Over- The- Counter medications and are frequently used in inadequate doses in our environment was observed. Higher sensitivity to Quinolones (Ciprotab and Sparflotab) presents them as a cheap and available and efficient alternative for use in the management of orofacial infections.

Conclusion

The isolation of invasive microorganisms in our patients would suggest the need for precautions that would reduce the microflora burden in the perioperative period. The antimicrobial antibiotic sensitivity pattern may also guide the choice of prophylactic antibiotic in our dental clinics. This will obviate the use of best guess antibiotics and therefore reduce the risks of infection in the dental patients.

Acknowledgement

The authors wish to acknowledge the members of the microbiology laboratory of the University College Hospital, Ibadan for facilitating the Laboratory aspect of this study.

I declare no conflict of interest.

Research was personally financed by the authors.

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