Isolation and Identification of *Candida albicans* and *Staphylococcus aureus* from Oral Swabs among Primary School Pupils in Uzuakoli, Abia State, Nigeria

Mbakwem – Aniebo C, Odoemelam HA and Okonko IO

Department of Microbiology, University of Port Harcourt, East-West Road, P.M.B. 5323, Choba, Port Harcourt, Rivers State, Nigeria;
mac2finney@yahoo.com; iheanyi.okonko@uniport.edu.ng

ABSTRACT: *Candida albicans* and *Staphylococcus aureus* were isolated from oral swabs of one hundred pupils, (aged 8 – 11 years) of Ngwu/ Amankwo Community Primary School, Uzuakoli, Abia – State. Sterile swab sticks were used for the collection of the specimens. Out of the 100 specimens collected, *Staphylococcus aureus* was isolated from (61.0%) samples, (65.0%) samples contained yeast – like organisms and (30.0%) samples had both *Staphylococcus aureus* and *Candida albicans,* forty of the yeast – like isolates were identified as *Candida albicans*. The highest carriage of *Staphylococcus aureus* and *Candida albicans* was found in the mouth of pupils using Charcoal and Chewing stick as dental agent. These pupils, who used chewing stick only, as their method of oral hygiene maintenance, had a carriage of 67.0%. Pupils that used the toothbrush infrequent had a carriage of 75.0%, those that alternated the use of tooth brush with chewing stick had a carriage of 42.0%, while those that used toothbrush regularly, as their method of oral hygiene, had the least carriage of 4.0%. This study showed that the use of tooth brush regularly, is the best method of oral hygiene.
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1. INTRODUCTION

Bacteria are the most obvious inhabitants of the oral cavity but other microorganisms are often seen. These include several species of fungi, viruses and protozoa (George et al., 1988). *Candida albicans* and *Staphylococcus aureus* are normal flora of the oral cavity and they are opportunistic pathogens which means that they are generally harmless in its normal environment but become pathogenic in a compromised host. A compromised host is seriously debilitated and has a lowered resistance to infection (Prescott *et al*., 2002).

The oral cavity is inhabited by more than 700 microbial species and many intrinsic and extrinsic factors affect the composition, metabolic activity and pathogenicity of the highly diversified oral microflora (Samaranayake et al., 2002; Aas et al., 2005; Nejad et al., 2011). This fact has been correlated mainly to the use of broad-spectrum antibacterials, corticosteroids, anti-tumoral agents, oral contraceptives and increase in the number of immunocompromised patients (Eggimann et al., 2003; Nejad et al., 2011).

Yeasts, especially Candida spp. are the normal oral flora and their isolation from the mouth can be investigated in excessive consumption of fermentable carbohydrates (Samaranayake al., 1986; Nejad et al., 2011), dental caries risk and denture-wearing status (Beighton et al., 1991; Nejad et al., 2011). *Candida albicans* is the most common cause of oral fungal infection. *Candida albicans* is a diploid fungus (a form of yeast) and a causal agent of opportunistic oral and genital infections in human (Ryan and Ray 2004). Candida is not harmful in healthy hosts, but may cause opportunistic infections in immunocompromised hosts, such as patients suffering from AIDS, leukemia and diabetes (Nejad et al., 2011). Oral candidiasis, which is frequently caused by *Candida albicans*, is one of the most common fungal opportunistic infections in immunocompromised patients (Klein et al., 1984; Nejad et al., 2011).

Systemic fungal infections (fungaemias) have emerged as important causes of morbidity and mortality in immune compromised patients (e.g., AIDS, Cancer chemotherapy, organ or bone marrow transplantation). *Candida albicans* biofilms readily form on the surface of implantable medical devices. In addition, hospital-acquired infection in patients not previously considered at risk (e.g., patients in an intensive care unit) has become a cause of major health concern (Ryan and Ray, 2004). Importantly, many non-albicans Candida have decreased susceptibility to antifungal agents. Specifically, *Candida krusei* and *Candida glabrata* demonstrate decreased susceptibility to fluconazole

(Lynnet al., 2003; Nejad et al., 2011). Clinicians now depend on identification of Candida species for accurate selection of antifungal agent and to provide the best treatment possible to the patient (Nejad et al., 2011).

Staphylococcus aureus is also an inhabitant of the oral cavity. *Staphylococcus aureus* is a facultative anaerobic, gram positive cocci, which appears as grape like clusters, when viewed under the microscope, it has large, round golden yellow colonies, often with haemolysis, when grown on blood agar plate (Tolan, 2007). The term Staphylococcus is derived from Greek term stapyle, meaning a bunch of grapes. Staphylococci are non motile, non spore –forming and catalase - positive bacteria. The cell wall contains peptidoglycan and teichoic acid. The organisms are resistant to temperatures as high as 50^{o} c, to high salt concentrations, and to drying (Tolan, 2007). Colonies are usually large (6 – 8 mm in diameter), smooth and translucent. The colonies of most strains are pigmented, ranging from cream – yellow to orange (Tolan, 2007).

The ability to clot plasma continues to be the most widely used and generally accepted criterion for the identification of *Staphylococcus aureus*. One such factor, bound coagulase, also known as clumping factors, reacts with fibrinogen to cause organisms to aggregate (Tolan, 2007). Another factor extracellular staphylothrombin, which convert fibrinogen to fibrin. Approximately 97% of human *Staphylococcus aureus* isolates possess both of these forms of coagulase (Tolan, 2007).

A recent review has highlighted the paucity of both clinical and laboratory data on the role of *Staphylococcus aureus*, in the oral cavity in both healthy and disease people (Smith *et al*., 2001, 2003). Some oral infections are caused at least in part by *Staphylococcus aureus*, for example, angular cheilitis, parotitis and staphylococcal mucositis (Smith *et al*., 2003).

The aim of this study was to evaluate the prevalence of *Candida albicans* and *Staphylococcus aureus* among oral isolates from primary school pupils using different methods of oral hygiene.

2. MATERIALS AND METHODS

2.1. Study Area

The study was carried out among the pupils of Ngwu/Amankwo Community Primary School, Uzuakoli, Abia state. Nigeria

2.2. Study Population

Samples of oral swab were collected from pupils (8 to 11 years) of Ngwu/Amankwo community primary school, Uzuakoli, Abia state. Before the collection of the specimens, age, sex, educational background of parents, and what they use for oral hygiene. Specimens were collected by rubbing the sterile swab sticks on the surface of the tongue, below the tongue and on the surface of the gum behind the molars. A total of 100 specimens were collected from 100 pupils and information on the sources of specimens is given in Table 1.

2.3. Isolation and identification of isolates

Isolated colonies on Nutrient agar were subcultured on to Mannitol Salt Agar. The confirmation of the colonies as *Staphylococcus aureus* was made when growth occurred on the Mannitol salt Agar. The isolated colonies on Sabouraud Agar plates were also Subculture on Corn Meal Agar. Pure cultures were obtained by streaking and restreaking colony on to nutrient agar plates and Sabouraud Agar plate until single colonies were obtained after incubation. All tests were performed on pure culture. The smears of colonies were prepared and gram stained (Cheesbrough, 2006). Slide coagulase test was performed using the method of Cheesbrough (2006). A loopful of a pure colony was emulsified in saline of a clean microscopic slide to produce a thick milky suspension. A loopful of citrated plasma was then added to the suspension. Both were mixed using a sterile wire loop and examined microscopically for the presence of clumps which indicate a positive result. The *Staphylococcus aureus* was identified based on their biochemical reactions. *Candida albicans* was identified based on the Chlamydospore formation test and the biochemical reactions.

3. RESULTS ANALYSIS

3.1 Frequency of isolation of *Candida albicans* from different methods of oral hygiene

Table 1 shows the frequency of isolation of *Candida* isolates from pupils using different methods of oral hygiene. It showed that isolation of *Candida albicans* was most predominant 40(61.5%). This was followed by *C. krusei* 6(9.2%), *C. guilliermondii* 5(7.7%), *C. parapsilosis* 4(6.2%), *C. tropicalis* 4(6.2%), *C. stellatoidea* 3(4.6%) and *C. pseudotropicalis* 3(4.6%) as shown in Table 1.

Table 1: Frequency of isolation of *Candida* isolates from pupils using different methods of oral hygiene

Candida Isolates	No. (%)
Candida albicans	40(61.5)
C. krusei	6(9.2)
C. stellatoidea	3(4.6)
C. guilliermondii	5(7.7)
C. pseudotropicalis	3(4.6)
C. parapsilosis	4(6.2)
C. tropicalis	4(6.2)
Total	**65(100.0)**

3.2. Frequency of isolation of bacterial isolates (*Staphylococcus aureus* and *Streptococcus* sp) from different pupils using different methods of oral hygiene

Table 2 shows the frequency isolation of bacterial isolates (*Staphylococcus aureus* and *Streptococcus* sp) from different pupils using different methods of oral hygiene. It showed that the isolation of *Staphylococcus aureus* was most predominant 61(71.8%), followed by *Streptococcus* spp. 23(27.0%). Isolation of *S. aureus & Streptococcus spp.* coinfection was least predominant 1(1.2%) as shown in Table 2.

Table 2: Frequency isolation of bacterial isolates (*Staphylococcus aureus* and *Streptococcus* sp) from different pupils using different methods of oral hygiene

Bacterial isolates	No. (%)
Staphylococcus aureus	61(71.8)
Streptococcus spp.	23(27.0)
S. aureus & Streptococcus spp. Coinfection	1(1.2)
Total	**85(100.0)**

3.3. Coinfections of *S. aureus*, *Streptococcus* spp. and *Candida* species from different pupils using different methods of oral hygiene

Table 3 shows the coinfections of *Staphylococcus aureus*, *Streptococcus* sp. and *Candida albicans* from different pupils using different methods of oral hygiene. It showed that coinfections of *Staphylococcus aureus* and *Candida albicans* were most predominant 43(78.2%). This was followed by *Candida* species *& Streptococcus* spp. 10(18.2%). Coinfections of *S. aureus & Streptococcus* spp. and that of *S. aureus, Streptococcus* spp. and *Candida* species were least predominant 1(1.8%) as shown in Table 3.

Table 3: Coinfections of *S. aureus*, *Streptococcus* spp. and *Candida* species from different pupils using different methods of oral hygiene

Isolates	No. (%)
S. aureus & Streptococcus spp.	1(1.8)
S. aureus & Candida spp.	43(78.2)
Candida species *& Streptococcus* spp.	10(18.2)
S. aureus, Streptococcus spp. and *Candida* species	1(1.8)
Total	**55(100.0)**

3.4. Frequency of isolation of both *Candida albicans and Staphylococcus aureus* from different pupils using different methods of oral hygiene

Table 4 shows the frequency of isolation of *Staphylococcus aureus* and *Candida albicans* from different pupils using different methods of oral hygiene. The highest frequency of isolation of *Candida albicans* was obtained from pupils using charcoal and chewing stick 2(100.0%). Those that do infrequent tooth brushing had a frequency of 75.0%. Those that use chewing stick alone had 73.0%. Those that use both charcoal and tooth brush had 0.0%. Those that use tooth brush only had a frequency of 19.0%, while the pupils that use tooth brush and chewing stick had 48.0% isolation of *Candida albicans.* The highest frequency of isolation of *Staphylococcus aureus* was obtained from the mouth of pupils that use chewing stick and charcoal (100.0%), tooth brush and charcoal (100.0%), infrequent tooth brushing (100.0%). Those that use tooth brush had a frequency of 34.0% isolation of *S. aureus*. The pupils that use chewing stick had an isolation frequency of 80.0% for *S. aureus*, while those that use both toothbrush and chewing stick had 83.0% isolation for *S. aureus* (Table 2). The highest frequency of isolation of *S. aureus* and *C. albicans* was obtained from the mouth of pupils using charcoal and chewing stick (100.0%). Those that use chewing stick only had the frequency isolation of 67.0%. Those that do infrequent tooth brushing had isolation frequency of 75.0%. The pupils that use both tooth brush and chewing stick had 42.0%, while those that use tooth brush had isolation frequency of 4.0% (Table 4).

Table 4: Frequency of isolation of *Candida albicans and Staphylococcus aureus* from different pupils using different methods of oral hygiene

Method of oral hygiene	No. Examined (%)	No. Positive for *Candida* isolates (%)	No. Positive for bacterial isolates (%)	No. Positive for both *Candida* and bacterial isolates (%)
Toothbrush	47(47.0)	9(19.0)	16(34.0)	2(4.0)
Toothbrush and Chewing stick	31(31.0)	15(48.0)	26(83.0)	13(42.0)
Chewing stick	15(15.0)	11(73.0)	12(80.0)	10 (67.0)
Charcoal and Chewing stick	2(2.0)	2(100.0)	2(100.0)	2(100.0)
Charcoal and Toothbrush	1(1.0)	0(0.0)	1(100.0)	0(0.0)
Infrequent tooth brushing	4(4.0)	3(75.0)	4(100.0)	3(75.0)
Total	**100(100.0)**	**40(40.0)**	**61(61.0)**	**30(30.0)**

Table 5 shows frequency of isolation of *Candida* and bacterial isolates from different pupils using different methods of oral hygiene in relation to their demographic characteristics. It showed that males (96.4%) had higher carriage rate of the isolates compared to their female counterparts (91.1%). Pupils within ages 8 years old had higher carriage rate compared to those in ages 9 years old with lower carriage rate of 83.3%. Isolation of pathogens were more prevalent among pupils whose parents were illiterate (98.1%) than those who had educated parents (89.4%). Other details are shown in Table 5.

Table 5: Frequency of isolation of *Candida* and bacterial isolates from different pupils using different methods of oral hygiene in relation to their demographic characteristics

Demographic Characteristics	No. Tested (%)	No. positive (%)	*Candida* spp. (%)	*S. aureus* (%)	*Streptococcus* spp. (%)	Coinfections of *Candida* and bacterial isolates (%)
Sex						
Male	55	53(96.4)	4(7.5)	9(16.9)	10(18.9)	31(58.5)
Female	45	41(91.1)	8(19.5)	5(12.2)	3(7.3)	24(58.5)
Age (year)						
8	23	22(95.6)	4(18.2)	4(18.2)	4(18.2)	11(50.0)
9	12	10(83.3)	2(20.0)	1(10.0)	3(30.0)	5(50.0)
10	21	20(95.2)	1(5.0)	3(15.0)	2(10.0)	14(70.0)
11	44	42(95.4)	5(11.9)	6(14.3)	4(9.5)	25(59.5)
Educational background of parents						
Illiterate	53	52(98.1)	6(11.5)	9(17.3)	3(5.8)	32(61.5)
Educated	47	42(89.4)	6(14.3)	5(11.9)	10(23.8)	23(54.8)
Total	**100**	**94(94.0)**	**12(12.8)**	**14(14.9)**	**13(13.8)**	**55(58.5)**

4. DISCUSSION

This study demonstrates clearly that *Staphylococcus aureus* and *Candida albicans* are part of the normal oral flora in most humans. From the results, it is clear that out of the 100 samples examined, 65 samples contained yeast –like organisms. The various tests carried out confirmed and to differentiate the species of *Candida*. It was realised that majority of these yeast – like organisms were *Candida albican*s. This result agrees with that reported by Rosenthal and Blecham (1962) and Budtz- Jorgensen et al. (1975).

The role of *S. aureus* in some types of oral disease may be more important than previously recognized (Smith et al., 2003). Some oral infections are caused at least in part by *S. aureus*, for example, angular cheilitis (MacFarlane and Helnarska, 1976; Smith et al., 2003), parotitis (Goldberg, 1981) and staphylococcal mucositis (Bagg et al., 1995). Furthermore there is now a growing body of evidence to suggest that staphylococci can be frequently isolated from the oral cavity of particular patient groups such as children (Miyake et al., 1991), the elderly (Bagg et

al., 1995) and some groups with systemic disease, such as the terminally ill (Jobbins et al., 1992), rheumatoid arthritis (Jacobson et al., 1997) and patients with haematological malignancies (Jackson et al., 2000).

This study highlights the potential role of *S. aureus* in a number of oral diseases. However, it is difficult from this study to ascribe a pathogenic role to the *S. aureus* isolates, which may have been colonizing rather than infecting the oral cavity. *S. aureus* infection is commonly associated with oral diseases and the findings of this study have confirmed those of earlier workers, suggesting a *S. aureus* isolation rate of 71.8% from oral cavity of primary school pupils.

There was no particular trend to increased recovery of *S. aureus* isolates from younger or older children in agreement with previous work (Percival et al., 1991) which found no age related trend for the recovery of *S. aureus* from a healthy population. This finding is in contrast to that of Smith et al. (2003). It is unclear whether this reflects changes in the oral flora associated with increasing age, medication, increased incidence of prosthetic oral devices or referral patterns (Smith et al., 2003). The percentage of isolation of *Staphylococcus aureus* revealed that the pupils that use charcoal and chewing stick, charcoal and toothbrush, and infrequent tooth brushing had the frequency percentage of 100.0%. Those that use Toothbrush and Chewing stick had frequency percentage of 83.0%, while pupils that use Chewing stick had frequency percentage of 80.0% and pupils that use Toothbrush had the least percentage (34.0%).

This study showed that the isolation of *Staphylococcus aureus* was most predominant (71.8%), followed by *Streptococcus* spp. (27.0%) and isolation of *S. aureus & Streptococcus spp.* coinfection was least predominant (1.2%) from oral swab of pupils using different methods of oral hygiene. In a study of 110 patients attending a dental hospital with a range of oral diseases there was an observed prevalence of *S. aureus* in saliva of 21.0% and from gingival swabs of 11.0% (Kondell et al., 1984; Smith et al., 2003). Salivary carriage of *S. aureus* in a cohort of patients with reduced salivary flow rates attending an oral medicine clinic was found in 41.0% of patients (Samaranayake et al., 1986; Smith et al., 2003).

Isolates of *S. aureus* are capable of producing a wide range of exotoxins which has been noted in oral isolates. A study of staphylococcal carriage in children attending a paedodontic department found that 19.0% of the *S. aureus* isolates produced exfoilotive toxin and 40.0% produced enterotoxin (Miyake et al., 1991; Smith et al., 2003). In line with more recent surveys (Smith et al., 2001, 2003), this study suggests that *S. aureus* may be a more frequent isolate from the oral cavity than hitherto suspected.

During the past two decades, there has been a significant increase in the prevalence of fungal infections caused by *Candida* species (Nejad et al., 2011). Oral candidiasis is a common opportunistic infection of the oral cavity caused by yeast fungi of the genus Candida on the mucous membranes of the mouth (Nejad et al., 2011). The study showed that isolation of *Candida albicans* was most predominant 40(61.5%) among pupils using different methods of oral hygiene. In agreement with findings of others (Back-Brito et al., 2009; Nejad et al., 2011), the majority of yeast isolates from oral cavity swabs were *C. albicans* (61.5%), but it was often recovered in association with other yeasts. This was followed by *C. krusei* 6(9.2%), *C. guilliermondii* 5(7.7%), *C. parapsilosis* 4(6.2%), *C. tropicalis* 4(6.2%), *C. stellatoidea* 3(4.6%) and *C. pseudotropicalis* 3(4.6%). These values for *Candida* species is comparable to what was reported by Donbraye-Emmanuel et al. (2010), Alli et al. (2011), and Nejad et al. (2011). *Candida* species that cause vaginitis most often are C. *albicans,* C. *g/abrata* and C. *tropicalis*. *Candida* species that rarely causes infection includes C. *parapsilosis,* C. *pseudotropicalis,* C. *krusei,* C. *guilliermondi* and C. *stellatoidea* (Alli et al., 2011).

In agreement with findings of others (Nejad et al., 2011), the most common mixtures observed in the present study were *C. albicans* plus *C. krusei* or *C. albicans* plus *C. tropicalis*. Although, *Candida* species been less common than bacterial infections, serious fungal infections occur in the immunocompromised patient both as new infection and as reactivation of latent disease (Donbraye-Emmanuel et al., 2010; Alli et al., 2011). The percentage reported for *Candida albicans* (61.5%) in this study is lower than the 75.0% reported by Nejad et al. (2011).

The result also showed that out of the 100 samples examined, *Staphylococcus aureus* was isolated from 61 samples. Both *Staphylococcus aureus and Candida albicans* were isolated from 30 samples. The comparison of samples made revealed that the percentage isolate of *Staphylococcus aureus and Candida albicans* is higher in the pupils that use charcoal and chewing stick for their oral hygiene. In the pupils that use charcoal and chewing stick for their oral hygiene had a

frequency percentage of 100.0%. The pupils that practice infrequent tooth brushing had frequency percentage of 75.0%. The pupils that use chewing stick had frequency percentage of 67.0%, the pupils that use tooth brush and chewing stick had frequency percentage of 42.0% while the pupils that use tooth brush only as their source of oral hygiene had frequency percentage of 4.0% and the pupils that use charcoal and Tooth brush had frequency percentage of 0.0%.

The percentage of isolation of *Candida albicans* revealed pupils that use charcoal and chewing stick had the highest frequency percentage (100.0%). The pupils that practice infrequent tooth brushing had a frequency percentage of 75.0%. Those that use Chewing stick had frequency percentage of 73.0%. Those that use Tooth brush and chewing stick had frequency percentage of 48%. While those that use Tooth brush had 19.0% and pupils that use Toothbrush and Charcoal had 0.0%.

From the percentage isolations of *Candida albicans*, the pupils that use charcoal and chewing stick as their method of oral hygiene had the highest frequency percentage (100.0%) in all the isolations, followed by pupils that practice infrequent tooth brushing. The pupils that use toothbrush had the least frequency percentage of organisms in all isolations. This might have been because toothbrushes are used with toothpastes. Toothpastes are a pastes or gel dentifrices used with a tooth brushes as accessories to clean and maintain the aesthetics and health of the teeth. They are also used to promote oral hygiene. There were no growths seen in a few Nutrient and Sabouraud's agar plates. This may be as a result of the absence these organisms as part of the normal oral flora of some of the pupils.

The ages of the subjects used in this study ranged from 8 to 11 years. This conforms to the findings of previous studies. Konje et al. (1991) showed that the infections were almost uniformly distributed in all age groups studied. In this study, 55.0% of the subjects were males while 45.0% were females. Isolation of *Candida* species were higher among females (19.5%) than the males (7.5%), however, there was no association with any of the demographic characteristics studied. Klufio et al. (1995) also reported that infections by *C. albicans* had no association with any of the sociodemographic characteristics studied. According to Adad et al. (2001), infection by *Candida sp* were most frequent among younger patients, especially those ages under 20 years, in all decades. Alli et al. (2011) also reported that infections by *C. albicans* had no association with any of the sociodemographic characteristics studied.

Toothpastes are derived from a variety of components, including three main ones: abrasives, fluoride and detergents or surfactants. Abrasives constitute at least 50.0% of typical toothpaste. These insoluble particles help remove plaque from the teeth. The removal of plaque and calculus prevents cavities and periodontal disease. Representative abrasives include Aluminium hydroxide ($Al(OH)_3$,Calcium carbonate ($CaCO_3$) etc. Fluoride in various forms is the most popular active ingredient in toothpaste to prevent cavities. It has beneficial effects on the formation of dental enamel and bones. Sodium fluoride (NaF) is the most common source of fluoride but Stannous fluoride (SnF_2), Olaflur (an organic salt of Fluoride), and Sodium monofluorophosphate (Na_3PO_3F) are also used. Many, although not all, toothpastes contain Sodium Lauryl sulphate (SLS) or related surfactants (detergents).SLS is mainly a foaming agent, which enables uniform distribution of toothpaste, improving its cleansing power other components include antibacterial agents which prevent gingivitis (Wolfgang, 2005). Other components of toothpaste include antibacterial agents which prevent gingivitis.

Candida albicans and *Staphylococcus aureus* had been isolated from several clinical specimens from different part of Nigeria (Donbraye-Emmanuel et al., 2010) and different parts of the world (Smith et al., 2003; Nejad et al., 2011). The differences in the frequency of isolation of these organisms in our study and that reported by other workers could be due to geographic, ethnic, and socioeconomic factors, as well as differences in sampling and culturing techniques. Variations may also reflect differences in sexual practice and environmental factors such as hygiene and nutrition (Donbraye-Emmanuel et al., 2010; Alli et al., 2011).

5. CONCLUSION

The result of this study shows that the method of oral hygiene affects the percentage isolation of *Staphylococcus aureus* and *Candida albicans*. The highest percentage of isolation was recorded from pupils that use charcoal and chewing stick as their method of oral hygiene, which is a poor method of oral hygiene. The least percentage of isolation was recorded from pupils that use tooth brush only as their method of oral hygiene which is a good method of oral hygiene. This study suggests that oral carriage of *S. aureus* may be more common

than previously recognized and the data collected suggests a reappraisal of the role of *S. aureus* in the health and disease of the oral cavity. The use of Toothbrush is the best method of oral hygiene, as confirmed by this study. Parents both illiterate and educated should therefore provide their children, with toothbrush and toothpaste and also teach them how to brush their teeth. This is because most of the cleaning is achieved by the mechanical action of the toothbrush, and not by the paste. Quality tooth paste should be used and also brushing regularly is highly recommended.

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CORRESPONDENCE TO:
Iheanyi O. Okonko
Department of Microbiology,
University of Port Harcourt,
East/West Road, P.M.B 5323, Choba,
Port Harcourt, River State, Nigeria;
E-Mail: mac2finney@yahoo.com;
iheanyi.okonko@uniport.edu.ng;
Tel.: +234-080-3538-0891

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