Bacteria associated with tooth infection among school children in Ijadu, Ado Ekiti Vol. 7(3) pp 11-13, December, 2018
DOI: http:/dx.doi.org/10.14303/irjm.2018.028
Available online http://www.interesjournals.org/IRJM

Full Length Research Paper

Bacteria associated with tooth infection among school children in Ijadu, Ado Ekiti

Adekunle OC*, Adeniji KM

Department of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria

***Corresponding e-mail:** toyintoro@yahoo.com

ABSTRACT

Good oral health helps to minimize any etiologic agents of disease in the mouth. WHO pointed that the global problem of oral disease still persists despite great improvements in the oral health of population in several countries. WHO claimed that poor oral health may have a profound effect on general health as well as quality of life, and several oral diseases are related to chronic diseases (Petersen et al. 2005). There is great need to encourage good oral health in children hence this study was carried out. The aim was to study the occurrence of tooth infection among primary school children in Ado-Ekiti.

Tooth swab samples were collected from 60 primary school children between aged 5-10 after obtaining informed consent from their parents. Samples were cultured and colonies formed were identified morphologically and biochemically characterized. Drug resistance profile was determined using different antibiotics. Forty one children were found with different bacteria on their teeth. The bacteria isolated were *Streptococcus mutans, Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumonia* and *Streptococcus mitis.*

Majority of the organisms showed that they were mostly multi-drug resistant strains. Cefuroxime is the best drug of choice to treat Gram negative organisms that cause tooth infection; it has 100% success on the gram negative organisms isolated.

Keywords: WHO, oral diseases, antibiotics, facultatively and obligately anaerobic bacteria

INTRODUCTION

The children suffering from poor oral health are 12 times more likely to have restricted activity days as compared to those who did not (Cohen et al., 2008). Dental (tooth) infection, decay, injury, or loss of a tooth are the most common causes of dental pain (Gherunpong et al., 2004). Although some bacteria are found in the mouth which are normal flora such as Streptococcus spp, Neisseria species, lactobacillus, Veillonella and Actinomycetes. However, certain bacteria growing inside the mouth can contribute to gum disease and dental decay, both of which can cause pain (Luo et al., 2007). Untreated dental decay has been reported as the most important reason for toothache which can impact routine daily activities such as eating, studying, concentrating on delicate tasks (Peterson et al., 2005). The predominant flora differ with age in children (Onyejeka, 2016) during beginning of development of tooth, oral flora changes predominantly to gram positive anaerobic bacteria such as Streptococcus, Peptostreptococcus in youth Streptococcus, Peptostreptococcus, Veillonella spp, Bacteroides and Treponema spp. in old due to teeth lost, predominant flora are Gram negative bacillus e.g. Veillonella (Rahim et al., 2010).

During the last few decades, the incidence of microbial diseases has amplified drastically. Microorganisms are the super bug agent responsible for causing dental infections which may even lead to caries. Many facultatively and obligately anaerobic bacteria

dominate the microbial community of dental caries. The prevalence pattern and severity of dental infection varies with age, sex, race, socio-demographic characteristics, economic status, geographical location, food practice and oral hygiene habits within the same country or region in various parts of the world (Yadav et al., 2015).

A study among Nigerian children identified the child's age, gender and frequency of sugar consumption as possible risk factors for developing tooth decay Yadav et al., 2017. Information on tooth decay prevalence among Nigerian preschool children is limited. Earlier reports indicated lower dental infection prevalence among pre-school children in Nigeria when compared to older Nigerian children (Sowole et al., 2007) and pre-school children in developed countries.

Little or inadequate research has been done on this field especially in Nigeria, many perceive that oral health has little to do with the general wellbeing of the body. This study intends to enlighten the general public about microorganisms that cause tooth infection in children and how to ensure good dental health among them. The aim is to study the occurrence of tooth infection among primary school children in Ado-Ekiti.

MATERIALS AND METHODS STUDY AREA

The study was carried out at Ijadu Primary school off Olorunsogo Area, Ado-Ekiti in Ado local government of Ekiti State, Nigeria. Ado-Ekiti is a city in Southwest Nigeria.

Subject and sample size

Primary school children between aged 5-10 were the subject. Ten children were taken from each class from primary 1-6 making a total of 60 children altogether. Of these 30 were male and 30 were female. The Study was carried out in January 2018.

Parental consent

Parental consent was sought before commencing sample taking, parental consent forms were distributed which explain what the study was all about and assuring that taking of sample will cause no physical inconveniency to the children.

Materials

Blood Agar, MacConkey Agar, Nutrient Agar, Mueller Hinton Agar, Microscope, Cover slips, Antibiotic disk (positive and negative disks), Petri dishes, Incubator, Gram's stain reagent, Slides, Standard wire loop, Autoclave .

Sterilization of media and materials

All glass wares were washed with detergent and rinsed with water, then allowed to dry. The glass wares were sterilized in a hot air oven at 160◦C for 3 hours. The media used were sterilized by autoclaving at 121°C for 15 minutes.

Specimen collection

A sterile cotton swab was taken and dipped in 1% glucose solution. The swab was then squeezed on the wall of clean, dry, sterile test tube and pressed gently on the portion of teeth cavity. The swab was rotated 2-3 times on the cavity and again dipped into the same tube containing glucose solution.

Specimen processing

All culture media were prepared as instructed by the manufacturer company. A loopful of inoculum from glucose broth was streaked on Blood Agar (BA) and MacConkey Agar (MA) plates. Plates were then incubated at 37°C for 24 hours. The significant growth of isolates were subcultured on Nutrient Agar (NA) and BA plates and incubated at 37°C for 24 hours. The biochemical tests carried out include; Gram Staining, Motility, Oxidase, Urease, Catalase, Coagulase, Indole, Citrate, Lactose.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test (AST) was performed using the disk diffusion (DD) break point assay, according to clinical and laboratory standards institute guidelines (CLSI, 2010). All of the required antibiotic disks were purchased from Mast Co. Ltd, UK and used as per manufacturer's instructions. The following antibiotic disks were used for antimicrobial susceptibility testing: Positive antibiotics disk containing the following antibiotics was used; Ceftazidime 30 µg, Cefuroxime 30 µg, Gentamicin 10 µg, Ceftriaxone30 µg, Erythromycin 5 µg , Cloxicillin 5 µg, Ofloxacin 5 µg, Amoxycillin/Clavulanate 5 µg . Negative antibiotics disk containing the following antibiotics was used; Ceftazidime 30 µg, Cefuroxime 30 µg, Gentamicin 10 µg, Cefixime 5 µg, Ofloxacin 5 µg, Augmentin 30 µg, Nitrofurantion 300 µg,Ciprofloxacin 5 µg.

RESULTS

A total of 60 samples were obtained from 60 primary school children between the ages of 5-10 years. Number of male was 30 and that of female was also 30 (Table 1).

According to the non-parametric Mann-Whitney test analysis, it was concluded that there was a significant distinction between tooth infection and the child's

gender ($p<0.001$). In this age group, caries was observed more frequently in girls than in boys (Table 2).

The bacteria isolated are *Streptococcus mutans, Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumonia* and *Streptococcus mitis.* The bacteria isolated were not part of children normal microbiota.

Children grouped according to organisms isolated, a total of 41 children have different microorganisms in their dental cavity which is 68.33%.

Table 1. Grouping of the school children according to organisms isolated.

Group A	1,5,7,10,21,42,45,49,54,60
Group B	10,15,16,32,36,37,40,46,55
Group C	9,11,13,30,33,36,37,40,42,43,48,54
Group D	1,2,3,12,17,18,19,28,29,52,58,60
Group E	7,4,5,6,16,23,32,34,39,47,50
Group F	1,10,11,15,16,21,28,40,46,52,56,57

Children free of microorganisms in the teeth. A total of 19 children had no visible growth of microorganisms from their dental swab after 24 hours of incubation, this gives a percentage of 31.67% (Tables 3-8).

Table 2. Group of Children free of microorganisms.

Group G	8,12,14,20,22,24,25,26,27,31,35,38,41,44,45,51,53,57,59

Table 3. Age Distribution of Organisms.

Age	No. of Organisms
5	6
6	9
7	11
8	11
9	13
10	4

Table 4. Culture on Blood Agar and MacConkey Agar.

GROUP A-F	Mixed colony seen both on blood Agar and MacConkey Agar after 24 hours of incubation at 37°C
GROUP G	No visible growth after 24 hours of incubation at 37°C

Table 5. Shows the growth seen on blood agar and Nutrient agar after subculturing to obtain pure culture.

SWAB NO	ON BLOOD AGAR	ON NUTRIENT AGAR
A	Dry colonies after 24 hours(Alpha heamolysis)	Requires enriched media only
B	Pink colonies on MacConkey agar	Big creamy colonies
C	Beta heamolysis	Golden yellow colonies
D	Alpha heamolysis (creamy colonies on chocolate agar)	Creamy, dry colonies
E	Partial heamolysis, Yellow/pale colonies on MacConkey agar	Greenish-blue pigment, large colonies
F	Mucoid colonies	Requires enriched media only

Table 6. Shows the gram reaction of colonies obtained.

SWAB NO.	GRAM REACTION
A	Gram positive cocci in chains (short chains)
B	Gram negative rod
C	Gram positive cocci in clusters
D	Gram positive cocci in pairs
E	Gram negative rod
F	Gram negative rod

Table 7. shows the various biochemical tests done to be able to name the organisms viewed.

Group	Motility	Catalase	Coagulase	Oxidase	Indole	Citrate	Urease	Lactose	Organism Identified
A	Non motile	_	_	_	_	_	_	+	*Streptococcus mitis*
B	Motile	+	_	_	+	_	_	_	*Escherichia coli*

C	Non Motile	+	+	_	_	+	+	+	*Staphylococcus aureus*
D	Non motile	_	_	_	+	_	_	+	*Streptococcus mutans*
E	Motile	_	_	+	_	+	+	_	*Pseudomonas aeroginosas*
F	Non Motile	+	_	+	_	+	+	+	*Klebsiella pneumoniae*

Table 8. Shows the sensitivity reaction of organisms isolated.

SWAB NO.	Organism Identified	Drug profile/Zone of Inhibition
A	*Streptococcus mitis*	Resistance to the antibiotics used
B	*Escherichia coli*	Erythromycin 5µg-0.05mm
		Ofloxacin 5µg-0.03mm
C	*Staphylococcus aureus*	Ceftazidime 30µg-0.06mm
		Augumentine-30µg-0.06mm
D	*Streptococcus mutans*	Resistance to the antibiotics used
E	*Pseudomonas aeruginosa*	Ceftrazone 30µg-0.02mm
		Cefuroxine 30µg-0.05mm
F	*Klebsiella pneumonia*	Amoxicillin/Clavulanate 30µg-0.04mm
		Cefuroxime 30µg-0.03mm
		Ceftriaxone 30µg-0.1mm

DISCUSSION

Dental infection is a complex chronic oral disease. It is the most prevalent chronic disease of childhood, yet oral health is often neglected within the health care system (Folayan et al., 2015). A common perception is that tooth infection rates are decreasing in developed countries but the trend in developing countries is not clear.

The results of most surveys of the prevalence of dental infection among school children showed that children in rural communities had lower prevalence than those in the urban.

All the children that participated in this research indicated that they brush once per day which was the main factor to cause tooth infection because after eating and drinking if teeth is not cleaned then there may be the chances of plague formation quickly by the fermentable bacteria called *Streptococcus mutans* (Sukumaran et al., 2017).

Factors considered to have effect on decay development in children include; age, sex, parents social status and sugar consumption (Folayan et al., 2012).

Gender influence caries distribution from the result obtained in which females have a slightly higher percentage in dental infection formation than males, this can be favourably compared to the study conducted by Olajokun et al. (2008) in which the initiator *Streptococcus mutans* have 56.8% in female and 44.2% in males and also the work of Yadav and Prakash (2015) who also have higher occurrence in female than in male. It may be due to female's dental eruption six month earlier than males, so they are exposed to cariogenic factors or may be due to the increasing sugar consumption, low exposure to fluoride containing toothpaste and poor access to oral health care (Huong et al., 2017).

Olajokun et al.,(2008) also went on to indicate that organisms appear to be more prevalent in 6-10 years in which the initiator *Streptococcus mutans* is 73.1% while ages 1-5 years were least affected with 5.8% (which suggest the reason while we have high occurrence of organisms in the age groups (5-10 years) used for this study.

The high percentage of *Streptococcus mutans* supports previous reports of some researchers (Folayan, 2015) that the initiation and progression of dental caries is closely associated with *Streptococcus mutans*.

The presence of Klebsiella support the work of Huong et al. 2017 that Gram negative rods were isolated from the mouth and that diet has a marked influence on the relative composition of mouth flora.

The high number of *Staphylococcus aureus* also collaborate with the work of Yadav and Prakash (2015) in which they are the second most common Gram positive bacteria associated with caries. Unfortunately *Steptococcus mutans* and *S. mitis* were resistant to all antibiotics used. They can be referred to as Multi-Drug resistant (MDR) species (Yadav et al., 2015)

Though the incidence of tooth infection is low in the study population, the bacteria which cause dental carries are present in 68% of the total population. The main causative agent, *Streptococcus mutans* and other Gram positive organisms as well as Gram negative organisms such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeroginosa, Klebsiella pneumoniae* and *Steptococcus mitis* were isolated in large numbers from the children's dental cavity.

Unfortunately *Steptococcus mutans* and *S. mitis* were resistant to all antibiotics used. A study conducted on dental caries by Yadav et al. 2015 at Department of

Microbiology, Janaki Medical College, Ramdaiya, Janakpurd-ham, Nepal reported 66.15% isolates of *S. mutans* were resistant to Penicillin; 60.76% were to tetracycline and 20% were resistant to cotri-moxazole. *S. aureus* was highly resistant towards penicillin (91.48%), tetracycline (86.17%) and ampicillin (61.70%). *S. mitis* was resistant to tetracycline (78.12%), ciprofloxacin (65.62%). Pseudomonas spp were highly resistant to tetracycline followed by cotrimoxazole (90.90%) (Hoffmeister et al., 2016)

The study conducted at Dental Unit of General Hospital Minna, Nigeria reported *Staphylococcus aureus* had the highest degree of occurrence with 31 isolates, followed by *Streptococcus mutans* with 23 isolates, while the least was *Lactobacillus spp* with 4 isolates. All the strains of *Streptococcus mutans*, and *Staphylococcus aureus* were sensitive to ofloxacin and nitrofurantoin and totally resistant to co-trimozazole and erythromycin. Only a small percentage of these strains were sensitive to chloramphenicol, ceftriaxone, gentamycin, tetracycline, cefuroxime and cefotaxime.

Poor dental health can affect a child self-esteem causing mouth odor and some diseases as well, therefore children are advised to brush their teeth twice daily with flouride containing toothpaste, reduce intake of food and snacks containing refined sugar and lastly notify their parents if they feel any pain in their teeth.

Parents are advised to supervise their underage wards while brushing their teeth, buy good quality brand of toothpaste for them and lastly refrain from using self-medication to treat their ward's toothache, instead they should visit a dentist (Onyejaka et al., 2016).

In conclusion, the study was able to establish different bacteria associated with tooth infection in Ado-ekiti children and these isolates are multi-drug resistance, but Cefuroxime is effective on the gram negative isolates.

REFERENCES

Cohen LA, Bonito AJ, Akin DR, Manski RJ, Macek MD, Edwards RR, Cornelius LJ (2008). Toothache pain: a comparison of visits to physicians, emergency departments and dentists. J Am Dent Assoc. 139(9): 1205-16.

Gherunpong S, Tsakos G, Sheiham A (2004). The prevalence and severity of oral impacts on daily performances in Thai primary school children. Health Qual Life Outcomes 2:57.

Luo Y, McMillan AS, Wong MC, Zheng J, Lam CL (2007). Orofacial pain conditions and impact on quality of life in community-dwelling elderly people in Hong Kong. J Orofac Pain. 21(1):63-71.

Petersen PE, Bourgeois DS, Ogawa H, Estupinan-Day S, Ndiaye C (2005). The global burden of oral diseases and risks to oral health. Bull World Health Organ. 83(9): 661-669.

Rahim-Williams B, Tomar S, Blanchard S, Riley JL (2010). Influences of adult-onset diabetes on orofacial pain and related health behaviors. J Public Health Dent. 70(2):85-92.

Yadav K, Prakash S, Yadav NP, Sah RS (2015). Multi-Drug resistance of Bacterial isolates among Dental Caries Patients. Janaki Medical College J Med. Sci. 3(1):37-44.

Yadav K, Prakash S (2017). Dental Caries: A Review. Asian J of Biomed and Pharmaceutic Sci. 6:1-7.

Folayan M, Sowole A, Kola-Jebutu A (2008). Risk Factors for Caries in Children from South-Western Nigeria. J clin pediatr dent. 32(2):171-175.

Folayan MO, Kolawole K, Oziegbe E (2015). Prevalence, and early childhood caries risk indicators in preschool children in suburban Nigeria.BMC Oral Health 15(1):72.

Sukumaran A, Pradeep SA (2017). Early Childhood Caries: Prevalence, Risk Factors, and Prevention. Front in Pediatr.5:157.

Folayan MO, Sofola OO, Oginni AB (2012).Caries incidence in a cohort of primary school students in Lagos State, Nigeria followed up over a 3 years period. Eur Arch Paediatr Dent. 13(6):312-318.

Huong DO, Hang LTT, Ngoc VTN, Anh LQ, Son LH, Chu DT, Le DH (2017). Prevalence of early childhood caries and its related risk factors in preschoolers: Result from a cross sectional study in Vietnam. Pediatr Dent J. 27(2).

Hoffmeister L, Moya P, Vidal C, Benadof D (2016). Factors associated with early childhood caries in Chile Gac Sanit. 30(1):59-62.

Onyejaka NK, Amobi EO (2016). Risk Factors of Early Childhood Caries among Children in Enugu, Nigeria. Braz Res Pediatr. Dent. Integr Clin. 16(1): 381-391.