Research Article

BIOCHEMICAL CHARACTERIZATION AND ANTIBIOTIC PATTERN OF STREPTOCOCCUS MUTANS ISOLATED FROM DENTAL UNIT, SICK-BAY, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

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Abstract

Fifty carious tooth swab samples were collected from patients presented with tooth decay at the Dental Unit, Sick-Bay, Ahmadu Bello University, Zaria, and characterized biochemically. The isolates were subjected to antibiotic susceptibility test. Total of 41 (82%) isolates were isolated. Streptococcus mutans was found to be more prevalent amongst age group 21-30 years, with more females (70%) than males (60%) based on the gender distribution. The isolates are sensitive to Ampicillin, Penicillin and Tetracycline but resistant to Cefuroxime and Gentamicin. Determination of antibiotic susceptibility profile of the bacterium is very important in the determination of first line drugs of choice.

Key words: Dental caries, Isolation, Streptococcus mutans, Antibiotic susceptibility, and Tooth decay.

INTRODUCTION

Dental caries is a chronic infectious disease, generally believed to be caused by acid-producing mutants- Streptococci and Lactobacilli bacteria (Beighton et al., 2004). Streptococcus mutans is the major pathogen in human dental caries. One of its important virulence properties is the ability to form biofilms (dental plaque) on the tooth surface. The eradication of such biofilms is extremely difficult. Tooth decay (dental caries) is a disease of multifactorial etiology that includes: appropriate number and species of bacteria; the types and frequency of consumption of fermentable carbohydrates; and susceptible tooth surfaces. Theoretically, tooth decay can be prevented by eliminating any one of these interacting factors (Balakrishnan et al., 2000). Dental caries is a result of progressive destruction of enamel by bacteria and their product within the oral environment and thus due to the number of factors necessary for caries to occur, these factors includes: host interaction with oral flora and with substrate (sucrose) (Ikroes, 2009).

Streptococcus mutans is a facultative anaerobic, Gram-positive coccus shaped bacterium commonly found in the oral cavity and is a significant contributor of tooth decay (Carlsson et al., 2007). The microbe was first described by J. Kilian Clarke in 1924. Streptococcus is a genus of spherical Gram-positive bacteria belonging to the phylum firmicutes and the lactic acid bacteria group. Streptococcus mutans, a member of the human or alflora, is widely recognized as the main etiological agent of dental caries. Conditions in the oral cavity are diverse and complex, frequent changes from one extreme to another. Thus to survive the oral cavity, S. mutans must tolerate rapidly harsh environmental fluctuation that expose it to various antimicrobial agents in order to survive. However, the mechanisms under which this cariogenic pathogen can survive and proliferate under such extreme environmental conditions are largely unknown, as little research has been done on this matter. There are twenty five (25) bacterial species of oral cavity, each species have developed specific specialized properties for colonizing different oral cavity and constantly changing conditions to fight competing bacteria and to withstand external challenges. Mutans streptococci are the most important bacteria associated with tooth decay.

Streptococcus mutans, the microbial species most strongly associated with carious lesion is naturally present in the human oral microbiota. The taxonomy of the complex bacteria remains tentative. A 1970 study found that Streptococcus mutans was more prevalent on the pits and fissures, constituting 39 percent of the total streptococci in the oral cavity. Fewer Streptococcus mutans were found on the oral surface (Holt et al., 2004). The surface of the teeth are colonized by a complex microbial community dental plaque, which if provided with certain substrates derived the hosts diet, will initiate carious lesion (Orland et al., 2004). In an elegantly simple experiment, (Fitzgerald, 2002), demonstrated that penicillin inhibitory for most Gram-positive bacteria decrease the level of caries activity probably by increasing the number of Gram-negative bacteria present at the caries-prone sites. This narrowed the field of potential etiological agent of tooth decay to the Gram-positive component of dental plaque. This includes primarily the streptococci, Actinomycyes spp and Lactobacillus, all of which are commonly present at caries-prone site of human teeth (Loeshe and Syed, 2003; Bowden et al., 2001).

Early colonization of the tooth surface is mainly caused by Neisseria spp and streptococci including Streptococcus mutans. The growth and metabolism of these pioneer species change local environmental condition (e.g. pH, coaggregation and substrate availability) thereby enabling more fastidious organisms to further colonize after them, forming dental plaque. S.mutans plays a major role in decay metabolizing sucrose to lactic acid using enzyme glucansucrase. The acidic environment created in the mouth by this process is what causes the highly mineralized tooth enamel to be vulnerable to decay. Dental caries are described first by the Miller chemo-parasitic theory in 1890 (Miller, 2003) is a result of the dissolution of teeth by acid produced by metabolic of dietary carbohydrates by oral bacteria through a pathological process of destruction of tooth surface by such microorganisms. It is also known as tooth decay and is commonly called cavities (Nester et al., 2008). The mouth which is the entrance to the digestive system, provides an environment that supports a large and varied microbial population (Gerald et al., 2008) the mucous membrane of the mouth and pharynx are often sterile at birth but may be contaminated by passage through the birth canal. Within 4-12 hours after birth, Streptococcus viridans become established as the most prominent member of the resident flora and remains so for life (Jawetz et al., 2012).

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There is a great diversity of microorganism in the oral flora found in the dental plaque, there are Gram-positive and Gram-negative rods (bacilli), spherical (coci) and spirals (spirillae) (Slade, 1997; and Holt et al., 2004). Prime amongst these bacteria is their ability to colonize the caries-prone site on the teeth and to withstand and flourish despite the environmental pressures present at such sites. The major problem that bacteria have to cope with is the rapid production of acid and consequent rapid fall in pH (acidogenicity). The ability of mutants streptococci to survive producing acid at low pH (aciduricity) is reflected in their ability to continue producing acid at low pH levels at which non-cariogenic bacteria such as Streptococcus sangius cease to be able to metabolize carbohydrates and produce acid (Kleinberg, 2002). A second feature is the production of cell bound glycosyl transferase enzymes. An accompanying and perhaps major effect this enzyme activity is to increase the water activity around the bacterial cell. The water activity is lowered as the concentration of sucrose increases; this occurs when sucrose is eaten and reduced water activity is deleterious to bacterial growth and metabolism (Carlsson, 2007; and Nyvad et al., 2009). This study will provide baseline information necessary for health planning, since the information on this subject is scanty in the study area.

**MATERIALS AND METHODS**

The study populations were students of Ahmadu Bello University attending Dental Unit of Sick-Bay Hospital, Zaria. Fifty (50) samples were collected from the Dental Unit of Sick-Bay, Ahmadu Bello University Zaria, Nigeria. The decayed teeth were extracted by a qualified dentist, and the carious teeth were swabbed using a sterile disposable swab stick. The samples were transported immediately to the microbiology laboratory for analysis. Using aseptic techniques, the swabs were inoculated on the prepared blood agar plates and incubated at 37°C for 24 – 48 hours under reduced Carbon (iv) oxide (CO₂) atmosphere to observe hemolysis and white grey colonies. The isolates were Gram stained and observed microscopically (Cheesbrough, 2000). According to the method of Marimon et al. (1996) and Cheesbrough (2000), the following biochemical tests were carried out after reactivation of the isolates in peptone water for two hours at 37°C of incubation.

**Table 1. Prevalence of Streptococcus mutans in relations to age and gender**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Number of cases with Streptococcus mutans</th>
<th>Male</th>
<th>Female</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>0-10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11-20</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>21-30</td>
<td>33</td>
<td>12</td>
<td>60</td>
<td>21</td>
<td>70</td>
</tr>
<tr>
<td>31-40</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>&gt;40</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>20</td>
<td>60</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>

**Catalase Test**

A drop of 3% hydrogen peroxide was placed on a clean glass slides and a bit of growth colony from the slant was taken with sterile wireloop and immersed in the hydrogen peroxide solution. Active bubbling and frothing which was absent in negative test indicated a positive test. This was carried out for all the isolates.

**Indole Test**

The isolates were grown in 5ml peptone water for 24 hours at 37°C after which three drops of Kovac’s reagent was added and mixed gently. The Indole reagent retained its yellow colour indicating a negative test. While a positive reaction was determined by the development of a red colour on the reagent layer floating above the broth within one minute. This was carried out for all the isolates (Cheesbrough, 2000).

**Motility Test**

The motility medium in a test tube was inoculated by stabbing to a depth of 2mm. The tubes were incubated at 37°C for 24 hours. A positive result was indicated by a cloudy medium and distinct line of inoculation. A negative result was identified with sharp indistinct inoculation line.

**Methyl Red- Voges Proskauer (MR- VP)**

About 5ml of MR- VP broth were inoculated and incubated for 48 hours at 37°C, after the period of incubation, 1ml of the broth was transferred to a small serological test tube and 2 drops of methyl red was added. Following addition of the indicator, a red colour signifies positive methyl red test. While a yellow colour signify a negative test.

A heavy inoculum of the test organism will be inoculated into VP medium contained in different test tubes. The tubes were incubated at 37°C for 48 hours. After which 0.5ml of alpha naphthol was added followed by 0.5ml of 40% KOH. It was then agitated and allowed to stand for 30 minutes; a red to pink colour signifies a positive testThis was carried out for all the isolates (Cheesbrough, 2000).

**Antibiotics Sensitivity Studies (Kirby – Bauer Method)**

The inocula of the isolates were prepared by streaking the organisms on nutrient agar plates to obtain discrete colonies.

**Table 2. Incidence of caries in relation to site and types of teeth extracted, frequency and percentage of carious patients**

<table>
<thead>
<tr>
<th>Teeth Types</th>
<th>Number and site of extraction</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.  %</td>
<td>No.  %</td>
</tr>
<tr>
<td>Molar</td>
<td>15</td>
<td>71.4</td>
</tr>
<tr>
<td>Premolar</td>
<td>2</td>
<td>9.5</td>
</tr>
<tr>
<td>Roots</td>
<td>4</td>
<td>19.0</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>100</td>
</tr>
</tbody>
</table>

**KEY:**

UR=Upper right tooth, UL=Upper left tooth, LL=Lower left tooth, LR=Lower right tooth.
RESULTS

Out of fifty (50) decayed tooth samples collected from the patients, isolates that appeared as white, gray colonies on blood agar as well as catalase negative were identified to belong to the genus Streptococcus. A total of 41 (82%) isolates were Streptococcus mutans giving a prevalence rate of 82%. While 9 (18%) isolates were negative organisms (Table 1).

Table 1 showed the prevalence of Streptococcus mutans in relation to age and sex. Females had higher prevalence (70%) than the male (60%). Age group 21-30 years was observed to have the highest prevalence both among males and females, which recorded 70% and 60% respectively. Table 2: Shows the incidence of caries by the site and type of teeth extracted and the number and percentage of carious patients. The molar, especially the lower right teeth were most affected next to the premolar then the roots. Table 3: Represents the antibiotic sensitivity profile of the isolates to different antibiotics used on the isolated Streptococcus mutans (n=41).

DISCUSSION

The total prevalence of dental caries obtained in this study (82%) is high compared to the report of a previous study (Udoye et al., 2008). The difference in both studies may be due to the differences in socioeconomic backgrounds, patterns of dental visit, lifestyle, oral hygiene practices, parents education and job. In Italian children 6-11 year olds, a lower prevalence rate of 17.4% was reported using similar methods (Raemy et al., 2013). However, in a Brazilian study using polymerase chain reaction (PCR) amplification method, a higher prevalence of 85.7% was observed (Franco e Franco et al., 2007). The upsurge in the prevalence of dental caries especially in our urban cities has been reported by several studies. Osurhor, (2007) also reported that, sugar consumption (lifestyle) in underdeveloped countries was said to have exceeded that of the industrialized countries. In Western countries, the decline has been associated with the changing patterns of sugar consumption in which artificial sweeteners are now being used, widespread availability of fluoride toothpaste, possibly frequent use of antibiotic and also a good supply of fluorinated water. This research work showed that (70%) of females had caries compared to (60%) in male patient between the age group 21-30 years, having the highest number of caries cases caused by the organism. It is, however noteworthy that there appears to be a slight sex difference in the age related isolation of dental caries.

From the nutrient agar plate, bacterial colonies were transferred into McCartney bottles containing sterile normal saline to obtain bacterial density of 3×10^8 organism per milliter as determined by McFarland standard scale number 1. The culture was streaked uniformly onto freshly prepared Muller Hinton agar plates using disposable sterile swabs. The plates were allowed to dry briefly, and then discs of multiple antimicrobials were mounted on the surface of the streaked inoculum. The plates were incubated at 37°C for 24 hours. The culture plates were examined for evidence of inhibition. A meter rule was used to measure the zones of growth inhibition (Cheesbrough, 2000; and Bauer et al., 2006). The isolates were recorded as sensitive or resistant to the antibiotics by comparing with value recommended standard charts given by NCCLS (1990 and 2008). Antibiotic susceptibility patterns of the isolated organisms were therefore tested against some antibiotics which include Tetracycline (30mg), Chloramphenicol (15mg), Ampicillin (10mg), Penicillin (10mg), Gentamicin (10mg), Erythromycin (10mg) and Streptomycin (10mg) using Kirby and Bauer disc diffusion methods of determining susceptibility as described by Cheesbrough (2000).

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This concurred with the findings of the World Health Organization (1997), that reported that, despite initial sex differences in the prevalence of caries that might occur in early age, studies have shown that caries prevalence in both sexes tend to be similar in later life. But, some researchers, however, did not obtain any significant difference between the sexes (Brekus, 1931; Daneshkazemi et al., 2005). However, closely related results were found in Lagos, Nigeria by Sofola et al., (2014) who reported that 75% of females had dental caries compared to 62.1% in the male patients between the age group 5-9 having the highest number of cases with dental caries. This may be due to poor oral hygiene as reported by Alamondi and Mosoud (1995), that inefficient oral hygiene control, and an increase in the plaque index, can predispose one to dental caries. Henshaw, (2004) also reported 58% and 49% carious individuals between the ages of 10 – 20 years in Kwara and Lagos state respectively.

However, patients indicated by several studies were noted to present to the dental clinic only when they developed a toothache. Dental clinic is largely ignorance about the disease in asymptomatic individuals, which may therefore play a role in the late presentation to the dental clinic. The antimicrobial susceptibility pattern of the isolated Streptococcus mutans showed that 36% were sensitive to Penicillin, 32% Ampicillin, and 29% Tetracycline. While, the predominant bacterial isolates were found to be resistant to Cefuroxime (30%) and Gentamicin (33%).

This agrees with the findings of Jarvinen et al. (1995), who reported that, majority of Streptococcus mutans isolates are susceptible to Amoxillin, Penicillin and Tetracycline. In conclusion, from the analysis, the occurrence of dental caries is high amongst the age range of 21-30 years with females accounting for more prevalence than males, and these can be attributed to high exposure to sugary substances such as sweets, jams, chocolates and cakes by females, which are the main factors necessary for caries to occur. In this study, it was found that the prevalence of dental caries is high, and the age group 21-30 years constituted the ages at greater risk. Based on the antibiotic susceptibility profile of the bacterium, antibiotics such as Ampicillin (32%), Penicillin (36%) and Tetracycline (29%) were found to be the first line drugs of choice in the treatment of dental infections caused by Streptococcus mutans. The bacterium was found to be resistant to Cefuroxime (30%) and Gentamicin (33%).
Recommendations

It is therefore recommended that since dental caries is a complex multifactorial disease, then periodic determination of antibiotic sensitivity profile of the bacterium is essential for early detection of any emergence of drug resistance. Taking fibrous food such as carrot, coconut, groundnut, orange and tomatoes that help to reduce caries risk should be taken into consideration and such foods should be included in the dietary formulation of people at risk; eating fresh fruits and vegetables that require chewing by stimulating the gum and help in keeping them healthy should be encouraged; regular brushing and use of fluorinated toothpastes to minimize oral decay should be of paramount importance; and systemic health campaign and public enlightenment should be intensified.

Acknowledgment

We wish to express our gratitude to the Laboratory Technologists of Dental Unit, Sick-Bay, Ahmadu University, Zaria (ABU) – Kaduna state, Nigeria for their contributions during sample collections. We also wish to acknowledge the supports and contributions of the entire staff of Nigerian Institute of Leather and Science Technology, Zaria, Kaduna State, Nigeria for their guidance and technical support.

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