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Article

Isolation, identification and antibiogram profile of bacteria isolated from dental caries patients of Mymensingh district of Bangladesh

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Abstract: This study was carried out for the evaluation of causative agents of dental plaques which leads to dental caries. A total of 76 samples collected from the dental caries patients of various age and sex were processed on different bacterial isolation media like Tryptose Soya Agar, Nutrient Agar, and MacConkey Agar. Total viable count of bacteria was ranged from 0.8×10^5 to 2.9×10^5. Out of total 184 isolates bacterial species were *Streptococcus mutans* (28.80%), *Streptococcus mitis* (23.91%), *Staphylococcus aureus* (28.26%) and *Streptococcus salivarius* (19.02%). The biochemical properties of the isolates were tested according to Bergey's Manual of Systematic Bacteriology and UK Standards for Microbiology Investigation. Antibiotic Susceptibility testing of different species of bacteria were performed according to Kirby Bauer disc diffusion method on Muller Hinton Agar by using commercial antibiotic discs. Effect of the bacterial isolates on different age groups was studied where the age group of 26 to 35 years was found to have highest prevalence (32.60%) of infection while the lowest prevalence (10.86%) of infection was seen in the group of below 15 years of age. A relative study was also performed on caries patients according to their economic status and gender where Higher Middle Economic Class was in highest percentage (35.32%) of infection. Different species of bacteria showed various sensitivity patterns to several kinds of antibiotics. Regular use of tooth paste containing triclosan and fluoride may be beneficial for prevention of dental caries instead of using antibiotics presented in this study.

Keywords: dental caries; total viable count; bacteria; antibiotic sensitivity

1. Introduction

Human oral cavity is usually having certain temperature and moisture as well as containing different nutritional compound such as carbohydrates, lipids and proteins that shelter the growth of normal flora and sometimes playing as an incubator for some pathogenic bacteria (Gibbons *et al.* 1990; Mohapatra *et al.* 2012). Tooth has a unique structure and composition so that it is the only organ of the body not subject to metabolic inversion. In early stage bacteria usually brace to the substratum of the teeth. Then the growth and multiplication of bacteria colonize the surrounding area of teeth and initiate the formation of biofilm by agglomerating into long chain. That agglomeration of latent and actively growing bacterial colonies form complex heterogeneous structure with the aid of their enzymes and excretory products (Mohapatra *et al.* 2012). Bacterial colonies heap on the

surface of teeth is the primary etiological agent of dental caries. Bacteria interact between them on the dental surface by co-aggregation, metabolic exchange, cell-cell communication and reciprocation of genetic material. These are the mechanisms that make the biofilm difficult to destroy when the therapeutic drugs are used in dental diseases. Dental caries usually demolish the enamel and dentin by bacterial activity (Chandrabhan *et al.* 2012; Kolenbrander *et al.* 2006; Li *et al.* 2002; Roberts *et al.* 2001). It is now avowed that the formation of bacterial biofilm is responsible for a variety of human diseases such as osteomyelitis, middle ear infections, dental caries, medical instrument and device related infections, native valve endocarditis, ocular implant infections and chronic lung infections (Donlan *et al.* 2002). Biofilm can resist to different antibiotics, alcohol, and different bacteriocidal and bacteriostatic agents at concentration of 10 to 100 times than that needed to destroy and is also exceptionally escape phagocytocis that helps to exist inside host's oral cavity and makes it extremely difficult to kill (Lewis *et al.* 2001).

Dental caries is one of the most significant and common infectious disease in human oral cavity. Dental caries also known as cavity or decay is a disease where bacteria cause damage in hard tooth structure. Lactic acid is produced as a by-product of carbohydrate metabolism by bacterial species which can demineralize enamel, dentin and cementum (Yoo *et al.* 2007). The mineral composition of teeth is sensorial to increased acidic condition produced due to lactic acid. The dynamic breakdown of tissues enhanced by *Streptococcus mutans*, *Staphylococcus aureus* and *Lactobacillus* spp. produce dental caries (Kohler *et al.* 1984; Dwivedi *et al.* 2011). The untreated condition of dental caries can lead to pain, tooth loss, infection, inflammation and finally dead in severe cases. The exhibition of dental caries may be different where stages of development and risks factors are similar. In the beginning it may appear in a tiny area that may progressively develop to a large cavity. Dental caries can be seen directly however some diagnostic method such as Radiographic Imaging is used to detect the less visible decay and to measure the extent of decomposition (Dwivedi *et al.* 2011).Almost all the types of bacteria found in the oral cavity have efficient pathogenic potential to enhance inflammatory response on teeth and soft tissues. Existence of bacterial flora may be different in various areas of teeth such as dentin enamel junction beneath white spot lesion, gaps between cavity walls and restoration, areas of penetrated caries, fissures, root channels and remaining carious dentin beneath restoration (Simonovic *et al.* 2002). The purpose of this study was to isolate and identify the bacterial species associated in dental caries and evaluate their antibiogram profile.

2. Materials and Methods

Seventy six patients of different hospitals, clinics and dentist's OPD of different areas of Mymensingh district were selected for the evaluation of associated bacterial species that are responsible for dental caries as well as different types of oral infections. Food habit of the people of different location of the district is almost similar but differences were in variation of awareness about the notion of teeth plaque and dental caries which includes alteration of brush timing and variety of age.

2.1. Sample collection

Samples were taken from the patients who exhibited the sign and symptoms of plaque and dental caries. Every sample was collected from infected teeth of patients by sterile cotton bud sticks. The swab sticks were implanted into the area of caries lesion and placed for 2 minutes. Then the sticks were taken out and put separately into previously prepared Tripticase Soya Broth (TSB) containing McCartney bottles for transporting to the Bacteriology Laboratory at Department of Microbiology and Hygiene, Bangladesh Agricultural University to ensure further processing. Then every sample was subjected to microbial investigations. A total of seventy six samples were taken up from different location of Mymensingh district in a random collection, fifty samples from the dental unit of Mymensingh Medical College Hospital, sixteen samples from Khandakar Dental Clinic and ten samples from Kabir's Dental Clinic and Orthodontics. After describing the intention of the study, all patients or their relatives were provided information consent in written documents. All patients have provided their samples voluntarily. After all, proper medical ethics were maintained for collecting samples by following the issues published by Nurunnabi *et al.* 2010 and Cheah *et al.* 2014.

2.2. Total viable count of bacteria

Collected samples in TSB were incubated at 37^{o}C temperature for 24 hours. Then every sample was serially diluted with sterile peptone water (PW) up to 10^{-6} dilution and 100µl of each dilution was inoculated on to Plate

Count Agar (PCA) and incubated overnight at 37°C temperature. After 24 hours of incubation of PCA, the viable colonies were counted by using colony counter.
Calculation: CFU/ml of sample = Number of colonies counted / Dilution factor × Volume Plated.

2.3. Isolation of bacteria

For the isolation of etiological agents involved in causing teeth plaques and dental caries all samples were inoculated into Triptic Soy Agar, Nutrient agar, McConkey Agar and Sheep Blood Agar media. A loopful of suspension of every sample was streaked into each culture medium. Then the inoculated plates were incubated at 37°C for 24 hours. After appearing mixed growth of different bacteria, separate colonies were picked up and streaked into Nutrient Agar media for producing pure culture.

2.4. Cultural and morphological characteristics

The isolated microbes were characterized on the basis of size and shape of the colony as well as staining characteristics and cultural properties according to Prescott and Harley, 2002.

2.5. Biochemical characterization

The discrete colonies from pure culture plates were taken up and series of biochemical tests were performed for identifying bacterial species. The biochemical properties of the isolates were tested according to Bergey's Manual of Systematic Bacteriology (Krieg, 1984) and UK Standards for Microbiology Investigation.

2.6. Antibiogram profile

Antibiotic Susceptibility testing of different species of bacteria were performed according to Kirby Bauer disc diffusion method (Bauer *et al.* 1966) on Muller Hinton Agar (MHA). Different commercial antibiotic discs were used to evaluate the sensitivity pattern of different isolated species. The zone of inhibition of each antibiotic disc against different bacterial species was interpreted on the basis of CLSI guidelines (Sharp, 2013).

3. Results

There are a large number of bacterial species which are responsible for causing dental plaques and dental caries. From seventy six samples of dental plaques and dental caries 184 bacteria were isolated and from those isolates four different species of bacteria were identified.

3.1. Enumeration of viable count of bacteria

The results acquired from the microorganisms amalgamated with dental plaques and dental caries asserted that the total aerobic counts for the sample site 1 ranged from 1.6×10^5 to 2.9×10^5 cfu/ml of samples where sample site 2 ranged from 0.8×10^5 to 1.9×10^5 cfu/ml of samples and sample site 3 ranged from 1.1×10^5 to 2.6×10^5 cfu/ml of samples.

Table 1. Total viable count of bacteria gained from different sample collection sites of Mymensingh town.

Sample collection site	Number of samples	Viable count
Dental Unit, Mymensing Medical College and Hospital	50	1.6×10^5 to 2.9×10^5 cfu/ml
Khondokar Dental Clinic	16	0.8×10^5 to 1.9×10^5 cfu/ml
Kabir's Dental Clinic and Orthodontics	10	1.1×10^5 to 2.6×10^5 cfu/ml

3.2. Cultural and morphological characteristics of bacteria

Four different bacterial species were ascertained from the total seventy six samples of dental plaque and dental caries. Bacterial species were *Streptococcus mutans* (28.80%), *Streptococcus mitis* (23.91%), *Staphylococcus aureus* (28.26%) and *Streptococcus salivarius* (19.02%).*Streptococcus mutans* are gram positive cocci, usually 0.70µm to 0.75µm in diameter, found to form medium long chain under the microscope and showed nonmotile characteristics by hanging drop preparation. Colonies of *Streptococcus mutans* are small grey white and γ hemolytic on Sheep Blood Agar (SBA), diameter of the colony ranged from 0.8mm to 1.2mm, become rough and heaped after two days of growth. *Streptococcus mitis* are gram positive cocci, usually 0.7µm to 0.9µm in diameter, found to arrange in long chain under the microscope and exhibited nonmotile characteristics. They showed appearance of S and R type mucoid colonies on blood agar and expressed α hemolytic characteristic.

Staphylococcus aureus are gram positive cocci, 0.8 to 1µm in diameter, found to appear grape like cluster under the microscope and showed nonmotile characteristics. Colonies of *Staphylococcus aureus* are circular opaque, yellowish, 0.5 to 2mm in diameter, S type and β hemolytic on Sheep Blood Agar. But on Nutrient Agar the colonies are rough and dry texture, smooth and golden yellow in color. *Streptococcus salivarius* are gram positive cocci, 0.8-1.0µm in diameter, found to appear from short to long chains under microscope. Colonies on Sheep Blood Agar are small, nonpigmented, smooth and often showed weak α hemolytic reaction.

Limning the diverse consolidation of bacterial isolates plainly pointed out that there was maximum number of *Streptococcus mutans* (53) followed by*Staphylococcus aureus* (52), *Streptococcus mitis* (44), *Streptococcus salivarius* (35) in Table 2.

Table 2. Distribution of different bacterial species.

Species	Number of bacteria isolated	Percentage
Streptococcus mutans	53	28.80%
Streptococcus mitis	44	23.91%
Staphylococcus aureus	52	28.26%
Streptococcus salivarius	35	19.02%
Total number of bacterial isolates	184	100%

3.3. Biochemical characterization

Four different bacterial species were characterized on the basis of biochemical properties. *Streptococcus mutans* expressed positive reaction to voges-proskauer (VP), esculin hydrolysis, glucose, fructose, sorbitol, trehalose, melibiose, mannitol, inulin, salicin, arginin, lactose and raffinose with the production of acid while acid was not produced from ribose but negative reaction to pyrrolidonearylamidase, β galactosidase, β glucuronidase and alkaline phosphatase. *Streptococcus mitis* showed glucose, raffinose, sorbitol, lactose, salicin, sucrose, inuline, trehalose and maltose positive with acid production while acid was not produced from glycerol, arabinose, mannitol and xylose but catalase, VP, hydrolysis of esculin, pyrrolidonearylamidase, α and β galactosidase, hydrolysis of hippurate, β glucuronidase and alkaline phosphatase negative. *Staphylococcus aureus* exhibited positive recompose with catalase, citrate, urease, lactose, sucrose, glucose, mannitol and lipid hydrolysis but oxidase, indole, methyl red, voges proskauer, H_2S production, starch hydrolysis, gelatin liquefaction negative. *Streptococcus salivarius* expressed negative reaction to α galactosidase, arginin, β galactosidase, pyrrolidonearylamidase, alkaline phosphate and β glucoronidase but positive reaction to VP, sucrose, inuline, lactose, raffinose, glucose, trehalose and maltose with the production of acid while acid was not produced from arabinose, glycerol, xylose, mannitol and sorbitol. Identification of bacterial isolates was carried out according to Bergey's Manual of Systematic Bacteriology.

Effect of the bacterial isolates on different age groups was measured in this study. All the patients were distributed into five different age groups where the age group of 26 to 35 years was found to have highest prevalence (32.60%) of infection while the lowest prevalence (10.86%) of infection was seen in the group of bellow 15 years of age. The second highest prevalence (28.26%) was observed in the group of 36 to 45 years. Another two age groups 16 to 25 years and 46 to 55 years were showing the prevalence rate 13.58% and 14.67% respectively. Table 3 is recounting the distribution of bacteria in various age groups.

Table 3. Ordination of bacterial isolates in various age groups of patients.

Age groups	Number of collected samples	Number of isolated bacteria	Percentage of infection
Bellow than 15 years	10	20	10.86%
16-25 years	8	25	13.58%
26-35 years	28	60	32.60%
36-45 years	19	52	28.26%
46-55 years	11	27	14.67%

A relative study was also performed on caries patients according to their economic status and gender where all patients were divided into five different economic groups. Result of this variant stated that, 6 male and 2 female patients belonging to Lower Economic Class (LEC) yield a total of 22 bacteria (11.95%) where female were

infected less than the male. From 16 Lower Middle Economic Class (LMEC) patients also the number of affected female was less than the male. Highest cases (23) belong to Higher Middle Economic Class (HMEC) which yielded a total of 65 bacteria (35.32%) where males were infected less than females. A total of 13 cases existing in Upper High Economic Class (UHEC) which yielded 29 bacteria (15.76%) indicate females were more infected than the males. Table 4 is recounting the sex wise distribution of patients in various economic classes.

Table 4. Ordination of bacterial isolates on the basis of gender in several economic classes.

Economic Class	Male	Female	Number of Isolated Bacteria	Percentage
Lower Economic Class (LEC)	6	2	22	11.95%
Lower Middle Economic Class (LMEC)	11	5	28	15.21%
Higher Middle Economic Class (HMEC)	10	13	65	35.32%
Middle High Economic Class (MHEC)	7	9	40	21.73%
Upper High Economic Class (UHEC)	5	8	29	15.76%

3.4. Antibiogram

Different species of bacteria showed various sensitivity patterns to several kinds of antibiotics. Here every individual bacterial species demonstrated zone of inhibition to several antibiotics was measured as sensitive (30mm - 45mm), moderate sensitive (10mm – 29mm)and resistant (0 mm).

Isolates of *Streptococcus mutans* found sensitive to Livofloxacin, Ciprofloxacin, Amikacin and Netilmicin while moderate sensitive to Doxycycline, Stereptomycin, Azythromycin and Cotrimoxazole. In this study multiple antibiotic resistant *Streptococcus mutans* species were observed. The total isolates of this species of bacteria were seen resistant to Novobiocin, Erythromycin, Amoxicillin, Cephotaxime, Ceftriaxone and Tetracycline. Table 5 is showing sensitivity, moderate sensitivity and resistance of *Streptococcus mutans* to several antibiotics.

Table 5. Antibiogram of *Streptococcus mutans.*

Sensitive	Moderate Sensitive	Resistant
Livofloxacin	Doxycycline	Novobiocin
Ciprofloxacin	Streptomycin	Erythromycin
Amikacin	Azythromycin	Amoxicillin
Netilmicin	Cotrimoxazole	Cephotaxime
-	-	Ceftriaxone
-	-	Tetracycline

Isolates of *Streptococcus mitis* also found resistant to several broad spectrum antibiotics. *Streptococcus mitis* showed resistant to Streptomycin, Amikacin, Amoxicillin, Tetracycline and Ceftriaxone while sensitive to Ciprofloxacin, Gentamicin, Norfloxacin, Netilmicin and Doxycycline but moderate sensitive to Azythromycin, Cotrimoxazole, Erythromycin and Roxythromycin.Sensitivity, moderate sensitivity and resistance of *Streptococcus mitis* to various antibiotics is expressed in Table 6.

Table 6. Antibiogram of *Streptococcus mitis.*

Sensitive	Moderate Sensitive	Resistant
Ciprofloxacin	Azythromycin	Streptomycin
Gentamicin	Cotrimoxazole	Amikacin
Norfloxacin	Erythromycin	Amoxicillin
Netilmicin	Roxythromycin	Tetracycline
Doxycycline	-	Ceftriaxone
-	-	-

Staphylococcus aureus species isolated from the patients having dental caries exhibited resistance to multiple broad spectrum antibiotics such as Erythromycin, Novobiocin, Vancomycin, Cefixime, Cefuroxime, Ceftriaxone and Tetracycline. Isolates of *Staphylococcus aureus* species were found sensitive to a few kinds of antibiotics such as Levofloxacin, Linezolid and Doxycycline while moderate sensitive to Amikacin, Ciprofloxacin, Netilmicin and Norfloxacin. Table 7 is showing sensitivity, moderate sensitivity and resistance behavior of *Staphylococcus aureus* to several antibiotics.

Table 7. Antibiogram of *Staphylococcus aureus.*

Sensitive	Moderate Sensitive	Resistant
Levofloxacin	Amikacin	Erythromycin
Linezolid	Ciprofloxacin	Novobiocin
Doxycycline	Netilmicin	Vancomycin
-	Norfloxacin	Cefixime
-	-	Cefuroxime
-	-	Ceftriaxone
-	-	Tetracycline

In this study isolates of *Streptococcus salivarius* were found sensitive to Vancomycin, Netilmycin, Gentamycin, Doxycycline and Amikacin while moderate sensitive to Amoxicillin and Azythromycin but resistant to a variety of antibiotics such as Cefotaxime, Ceftriaxone, Tetracycline, Erythromycin, Cotrimoxazole and Ceftriaxone. Table 8 is showing sensitivity, moderate sensitivity and resistance behavior of *Streptococcus salivarius* to multiple antibiotics.

Table 8. Antibiogram of *Streptococcus salivarius.*

Sensitive	Moderate Sensitive	Resistant
Vancomycin	Amoxicillin	Cefotaxime
Netilmycin	Azythromycin	Ceftriaxone
Gentamycin	-	Tetracycline
Doxycycline	-	Erythromycin
Amikacin	-	Cotrimoxazole
-	-	Ceftriaxone

4. Discussion

Proper medical ethics were maintained for collecting samples by following the issues published by Nurunnabi *et al.* (2010)and Cheah *et al.* (2014). Viable count of bacteria obtained in this study is almost similar with the findings of Handelman *et al.* (1976) but much lower than the findings of Jensen *et al.* (1980) while significantly higher than the results of Going *et al.* (1978) and Weerheijm *et al.* (1993). Placement of sealant progressively reduced the total bacterial counts of caries lesions in the studies of Going *et al.* (1978) and Weerheijm *et al.* (1993), that's why the results are variable with the current study. The viable counts of bacteria counted from the samples of Dental Unit, Mymensingh Medical college Hospital are almost similar to those that reported by Stecksen *et al.* (2008); Stecksen *et al.* (2007). On the other hand the total viable counts of bacteria obtained from the samples of Khondokar Dental Clinic and Kabir's Dental Clinic and Orthodentics are little bit different from those that exhibited by Ella *et al.* (2008); Truman *et al.* (2002) and Ahovou *et al.* (2004).The cultural and morphological characteristics of *Streptococcus mutans* were found similar with the previous studies of Holt *et al.*, 1994 and Facklam *et al.* (2002). Andrewes, 1906 and Elliot *et al.* (1990) were reported the morphological characteristics of *Streptococcus mitis* are almost similar with the current study. *Streptococcus salivarius* showed the cultural and morphological characteristics were found closely similar with those that reported by Kathry *et al.* (1984), Facklam *et al.* (1977) and Amstein *et al.* (1973). The cultural and morphological characteristics of *Staphylococcus aureus* recorded in this study is agreed with those that reported by Comolai *et al.* (2008) and Hanselman *et al.* (2009). According to the present investigation the number of different species of Streptococci is much higher than the findings that expressed by Cawson *et al.* (1987) and Mohapatra *et al.* (2012). No lactobacilli were found in this study is disagreed with the findings of Cawson *et al.* (1987) but completely agreed with the findings that reported by Mohapatra *et al.* (2012). The biochemical properties of

Stereptococcus mutans found in this experiment is highly agreed with Holt *et al.* (1994) but a little bit variables with Richard *et al.* (2002).

Dental caries is a disease caused by bacteria where it progressively damaging the teeth and causing tooth lose to the younger people. The ultimate upshot of caries is to decay enamel, dentin and cementum to shell a pathway for bacteria to enter into the underlying tissue. In this study we have collected samples of different types of caries such as fissure caries, enamel caries, dentinal caries, pulpal exposure, pus forming and root apex. Pit and fissure caries was highest in number which is agreed with a previous study done by Mohapatra *et al.* (2012). Isolation of microorganisms from fissure caries suggested that this phase is providing mechanical shelter to the microorganisms for expanding destruction. The least number of microorganisms were isolated from enamel caries which is almost similar with the findings that reported by Cawson *et al.* (1987). Enamel is the main hindrance to onslaught of bacteria, once invade, bacteria accelerate the infection to dentine with relatively lower impediment. The rapid adjacent prolongation of the lesion befalls through cross branching between tubules but keep on barge into the pulp in a comparatively ample front. If the pulp is invaded, it becomes incited and ultimately undergoes infraction. Though the pulp is impregnated with arteries, veins, lymphatic vessels and nerves via apical foramen, infections of this tissue provide a portal of entry to bacteria into various part of the body. The most pyogenic microorganisms Streptococci and Staphylococci were found in pus forming dental caries where the galling and necrosis leads to create periapical abscess, this findings are highly agreed with those that reported by Schuster *et al.* (1980) and Mohapatra *et al.* (2012).

The biochemical properties of *Streptococcus mutans* are agreed with the study that exhibited by Holt *et al.* (1994) but slightly variable with the experiment that reported by Facklam *et al.* (2002). *Streptococcus mitis* isolated in this study showed biochemical characteristics are found almost similar with those that debriefed by Andrewes*et al.* (1906) and Elliot *et al.* (1990). The basic differentiation between *Streptococcus mutans* and *Streptococcus mitis* in biochemical properties is that *S. mutans* are VP and esculin hydrolysis positive but *S. mitis* are VP and esculin hydrolysis negative, this Data is agreed with that enlisted in UK Standards for Microbiology Investigations and also similar with those that reported by Hardie *et al.* (1986); Thompson *et al.* (2013); Schleifer *et al.* (1984) and Naser *et al.* (2005). The biochemical behavior showed by *staphylococcus aureus* in this current study is highly agreed with the findings that expressed by Hanselman *et al.* (2009) and Comolai *et al.* (2008) but found slightly variable with the findings that reported by Cole *et al.* (2001) and Kluytmans *et al.* (1997), the variability showed by *S. aureus* in this study may be caused by point mutation. The basic differences in biochemical behavior between *S. mutans* and *S. salivarious* is arginin positive to *S. mutans* and arginin negative to *S. salivarius* while arginin is also positive to *S. mitis* but VP is positive to *S. salivarius*, this data of the current study is acceded with the preceding studies that disclosed by Holt *et al.* (1994); Elliott *et al.* (1990); Amstein *et al.* (1973) and Thompson *et al.* (2013).

The maximum incidence of caries was between the age group 26-35 years, moderate in age group 46-55 years and minimum in age group bellow than 15 years. Dental caries is a progressive disease; when it takes place in the pit of teeth it gradually destroy the structure and building block of the teeth. The data obtained from the age wise ordination of dental caries in this study is in consonance with the quondam findings of Mohapatra *et al.* (2012). An assimilation of the patients suffering from dental caries associating to various economic groups to that of lingam transpired that females belonging to higher middle economic class, middle high economic class and upper high economic class asserted a higher aptitude of caries than males. The dentition commences to decay earlier in females and the male patients often have been at risk for a brief period of time in comparison with females. The people of high economic classes who have solvency prefer sapid foods like refined carbohydrate, chocolate, premium ice cream, beverages and more oily fried items and curries which may shelter the caries to progress. This data of caries in various economic groups is unanimous with the previous data of Gluck *et al.* (1999) and Mohapatra *et al.* (2012).

Antibiotic administration must be adjunctive to proper medical remedy. Selection of antibiotic should be based on the specification of efficacy for the particular pathogenic bacterial agents, because dental caries infections are ecosystem of bacteria where products excreted by one species of bacteria may be consumed as nutrients by another species of bacteria (Reday *et al.,* 2003; Dwivedi *et al.,* 2011). Antibiotics showed sensitivity, moderate sensitivity and resistance to *S. mutans, S. mitis* and *S. salivarius, S. aureus* are in consonance with the previous studies that has been reported by Murat *et al.* (1997), Mohapatra *et al.* (2012) and Mahalle *et al.* (2014) but slightly variable with the studies that reported by Chandrabhan *et al.* (2012) andAntipa *et al.* (2014).

5. Conclusions

In this current study we observed that acid producing *Streptococcus* spp. and *Staphylococcus aureus* mostly colonized in dental plaque of patients which are mainly responsible to cause caries regardless of sex and age in this geographical area. All the bacteria isolated in this study are showing resistant to almost all broad spectrum antibiotics. It is becoming a threat to control and treat this bacterial species. Practicing the use of antibiotics without proper recommendation of registered physician is imposing the threat. Selling of all kinds of antibiotics as over the counter drug should be stopped by implementing medical rules and regulations. Regular use of tooth paste containing triclosan and fluoride is better for prevention instead of using antibiotics presented in this study.

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Conflict of interest

None to declare.

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