IJBPAS, June, 2024, 13(6): 2614-2629

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# BIOCHEMICAL CHARACTERIZATION AND MULTI-DRUG RESISTANCE IN *Streptococcus species* ISOLATES FROM ORAL CAVITY

**PRASAD J, VERMA J AND KUSHWAHA SK***

Bhavdiya Institute of Pharmaceutical Sciences and Research, Sewar, Sohawal, Ayodhya, UP, India- 224126

***Corresponding Author: Dr. Sanjay Kumar Kushwaha: E Mail: sanjaykushwaha78927@rediffmail.com**

**Received 15th April 2023; Revised 8th June 2023; Accepted 1st Sept. 2023; Available online 1st June 2024**

https://doi.org/10.31032/IJBPAS/2024/13.6.8048

## ABSTRACT

The human body is home to many indigenous microorganisms, with distinct communities at different anatomical sites. Recent studies have shown the importance of the gut microflora but its role in the oral cavity is one of the least appreciated topics in microbiology. With the application of modern approaches, further research can be applied in this valuable area.

A total of 180 samples were collected from dental plaque/ carries of the oral cavity of students aged between 8-21 years.

A total of 31 Gram-positive *Streptococcus* bacterial species were isolated and studied for various biochemical tests. Glucose was fermented by 45.16% isolates with the production of acid but no gas was produced. Other sugars like sucrose, fructose, maltose, mannitol, lactose, and sorbitol were also fermented by 16-45% of the isolates.

The analysis leading to the production of enzymes by these isolates shows that they were capable of producing amylase, protease, catalase, and urease by 61.29%, 77.41%, 25.80%, and 58.06% isolates respectively.

In the Antibiotic sensitive assay, none of the isolates were found to be sensitive to all the 16 antibiotics used for the present study. Penicillin-G, Cefoxitin, and Clavulanic acid were found to be resistant by all the isolates showing their Multi Drug Resistance nature.

Based on the results and findings, we can conclude that species from dental plaques constitute approximately 19.22% of the bacterial consortium in the oral cavity. These organisms possess typical characteristics required for their survival in the oral cavity. Multi-drug resistance nature of these micro-organisms in the cavity is a major concerned area.

***Keywords: Oral cavity, Antibiotics, Streptococcus, Resistance***

## INTRODUCTION

Various studies have shown the importance of the gut microflora, but the oral cavity is also home to microbial communities, with important implications for human health and disease (Marsh, 2000**)** **[1]**. The microbial diversity of the oral cavity promotes the establishment of distinct microbial communities, such as supragingival plaque, subgingival plaque, and tongue coating.

The properties of the environment determine which microorganisms can occupy a site. Non-mutans *Streptococci* and *Actinomyces* are predominant in the supragingival ecosystem and their metabolic activities cause acidification, resulting in both demineralization of tooth surface and introduction of more cariogenic microorganisms mutans *Streptococci,* to the ecosystem (Carlsson *et al*., 1987) **[2]**; (Rotimi *et al*., 1981) **[3]**; (Pearce *et al*., 1995) **[4]**.

At birth, the oral cavity is sterile but rapidly becomes colonized from the environment, particularly from the mother in the first feeding. The complexity of the oral flora continues to increase with time and it includes *streptococci, lactobacilli, staphylococci,* and *corynebacteria,* etc. with a great number of anaerobes in oral cavity like *Streptococcus, Actinomyces*, *Arachnia*, *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *Lactobacillus*, *Leptotrichia*, *Peptococcus*, *Peptostreptococcus*, *Propionibacterium*, *Selenomonas*, *Treponema*, *Veillonella Capnocytophaga, Prevotella, Tannerella, Eikenella,* and *Fusobacterium (*Sutter*, 1984)* **[5]***;* Sweeney *et al*., 2004*)* **[6]**.

The entire mouth is continuously in touch with saliva and this influences the ecology of the mouth (Scannapieco, 1994) **[7]**. The pH of saliva varies and ranges from 6.75 to 7.25 and is favorable for microbial populations. The ionic components of saliva promote its buffering properties.

The supragingival area consists of the stable environment of the tooth surface coated with salivary components such as proteins and glycoproteins. This continuous supply of saliva acts as a nutrient supply for the microorganisms while carbohydrates derived from foods also serve as nutrients to the microflora (Takahashi, 2005) **[8]**. *Streptococcus* and *Actinomyces* are saccharolytic and degrade carbohydrates derived from foods through the Embden–Meyerhof–Parnas pathway to form lactic, formic, acetic, succinic, and other organic acids. Other sugars like sucrose, maltose, mannitol, lactose, and sorbitol were also fermented by these species.

Subgingival sites provide a stable tooth surface and an unstable epithelial surface, the later, it desquamates continuously. Both surfaces are bathed with a continuous efflux of GCF, derived from blood plasma and thus nutritionally rich in nitrogenous compounds such as amino acids, peptides, and proteins. Proteolytic bacteria can degrade nitrogenous compounds into small peptides and amino acids by cell membrane-bound and/or extracellularly secreted proteases for subsequent use as metabolic substrates (Takahashi, and Yamada, 2000 **[9]**. Other enzymes that are produced by these microbes are amylase, catalase, and urease which also cause the degradation of proteins and other nitrogenous compounds (Ruby and Gerencser, 1974) **[10]**.

These metabolic activities of bacterial species create an acidic and anaerobic condition that leads to the demineralization of enamel. (Takahashi, and Yamada, 1999) **[11]**. The formation of dental plaques and dental caries sequentially takes place, leading to the introduction of more pathogenic micro-organisms into the microbial cavity.

Most oral infections are poly-microbial because of the involvement of Gram-positive and Gram-negative bacteria in anaerobic and aerobic habitats. The antibiotics used frequently for the treatment of dental infections are Amikacin, Penicillin-G, Ciprofloxacin, Ofloxacin, Gatifloxacin, Norfloxacin, Azithromycin, Gentamycin, etc.

Resistance to antibiotics can be conferred by chromosomal or mobile genetic elements (e.g., plasmids) (Kumar *et al*., 2013) **[12]** and can be achieved using four main strategies: (i) decrease in cell wall and membrane permeability to antibiotics, (ii) drug inactivation, (iii) rapid efflux of the antibiotic, and (iv) alteration of cellular target(s) by mutations (Krulwich *et al*. 2005) **[13]**. Microbial resistance to antibiotics and metal ions is a potential health hazard because the genes for these traits are generally associated with the same transferable plasmids (Novick and Roth 1968 **[14]**; Calomiris *et al*. 1984) **[15]**. Bacteria acquire antibiotic resistance genes through mobile elements, *viz*., plasmids, transposons, and integrons (Rubens *et al*. 1979) **[16]**. The resistance in bacteria through mobile genetic elements is related to the mutations in genes responsible for the uptake of antimicrobial agents or binding sites or activation of portions of bacterial chromosomes (Sayah *et al*. 2005) **[17]**.

With the above views, we proposed to study *Streptococcus* species in the oral cavity to ascertain their presence and role in oral health. It was proposed to characterize the *Streptococcus* species isolates using microbiological and biochemical tests. The isolates were subjected to antibiotic sensitivity assay for the profiling of drug sensitivity among the isolates from dental plaque.

## MATERIALS AND METHODS

**Sampling of dental sites for bacterial isolation:** A total of 180 samples from students aged between 8-21 years from different schools and colleges were collected. The students were selected randomly to obtain samples from dental plaque/dental carries of the oral cavity of individuals. Sterile (autoclaved) toothpicks were used for scraping the plaque/carries **(Figure 1)**. The scrapings were then inoculated into 5 ml sterile nutrient broth in a screw-capped culture tube. The inoculated culture tubes were incubated for 24h at 37° on a shaking incubator.

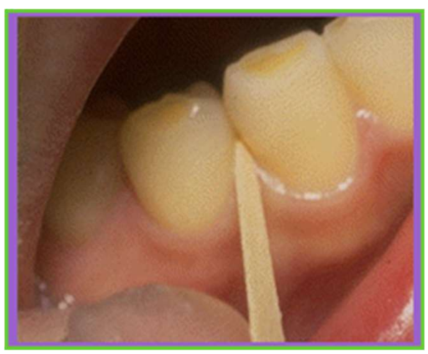

**Figure 1: Sample collection from the dental cervical area in the oral cavity using sterile tooth-pick**

**Isolation of bacterial cultures:** After incubation at 37° for 24h, the bacterial cultures from tubes presenting turbidity due to bacterial growth were streaked on nutrient agar medium in Petri-dishes using a sterile inoculation loop. The inoculated Petri dishes were incubated for 24h at 37°. On the subsequent day appearance of well-isolated colonies at the last streaks on the medium were considered colonies of pure culture. The pure culture was restreaked one medium to a certain purity of the culture. The single pure colonies obtained from each sample were streaked on agar slants and incubated for 24h at 37°. Following the growth the slants were preserved in the refrigerator at 4°. The cultures were sub-cultured on a regular interval of 30 days.

## Culture Identification

**Gram staining reaction:** The bacterial cultures were transferred on a clean glass slide in a drop of water using a sterile loop. The bacterial culture was mixed homogenously in the water to form an emulsion. The emulsion was spread evenly in the form of a thin film to develop a smear. The smear was allowed to air dry at room temperature with intermittently quickly passing over the flame for heat fixing of the bacteria. The bacterial smears were stained by following Gram's staining method. The smear on the slide was flooded with crystal violet solution for a minute and washed in running tap water followed by treatment of Gram’s iodine solution for a minute as a mordant. The iodine from the slide was washed using running tap water. The color of the crystal violet iodine (CVI) complex was decolorized using 95% alcohol for 10s, avoiding over-decolorization. The slide was then washed and shaken off to remove excess water and counterstained using safranin solution for the 20s and examined under the 100X oil objective. Pink background and violet bacterial cells confirm the presence of Gram-positive bacteria.

**Negative staining**: A loopful bacterial culture was placed in a drop of nigrosine dye on a clean slide. The bacterial culture and dye were mixed properly and spread by placing another clean slide at an angle of 30º on a slide containing culture in dye and allowing the droplet to spread across the edge of the top slide **(Figure 2)**. The dye-mixed bacterial culture was spread across the bottom slide with the help of top slide and allowed the smear to air dry. The size of bacterial cells was measured using calibrated ocular micrometer and putting the specimen under a 100X objective lens in a compound microscope.


**Figure 2: Process of negative staining. A. Place and mix drops of nigrosine and culture. B. the mix was spread using a backstroke of the second slide**

## Biochemical tests

**Carbohydrate fermentation tests:** Five ml medium for fermentation medium was prepared and inoculated with a loopful bacterial culture isolate. An uninoculated medium in the tube acted as a negative control. The media in culture tubes was incubated at 37° for 24-48h for the appearance of yellow color due to acid production by the bacteria by utilizing carbohydrates and collection of gas in inverted Durham tubes inserted in the medium during medium preparation. The results were recorded as observed.

**Amylase production test:** Starch agar medium in Petri-dishes was used for the amylase test. The bacterial culture was inoculated as a single line streak on the medium surface using a sterile inoculation loop **(Figure 3)**. The inoculated Petri dishes were incubated at 37ºC for 48h. Upon the appearance of bacterial culture, the medium surface was flooded with iodine solution using a dropper for the 30s. The excess iodine solution was decanted. The starch hydrolysis was observable as a hollow zone on the Petri dishes around the bacterial culture streak.

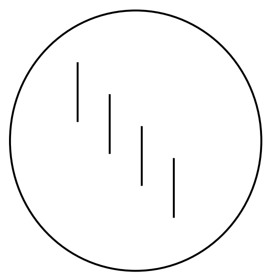

**Figure 3: The inoculation of culture for amylase production. A single short line for culture was streaked on agar medium to allow growth of culture followed by the addition of indicator and detection of amylase activity**

**Proteinase production test (Casein hydrolysis)**: Casein hydrolysis was studied by inoculating the bacterial cultures on Skimmed Milk agar medium. A single line streak of bacterial cultures was made using a sterile inoculation loop. The inoculated Petri dishes were incubated at 37º for 24-48h.

**Catalase test:** A loopful of freshly grown bacterial culture in Yeast Extract broth was placed on clean slides and mixed with a drop of hydrogen peroxide. The appearance of bubbles from bacterial cultures indicated the presence of catalase enzyme.

**Urease test:** The slants containing urea agar were inoculated with the bacterial culture. The inoculated slants were incubated at 37º for 24-48h. The appearance of pink color indicated the presence of urease while the yellow color was representative of the absence of the enzyme.

**Hydrogen sulfide ($H_2S$) production test:** The agar medium for $H_2S$ production in the culture tube was inoculated by stabbing with bacterial culture on a sterile inoculation wire for $H_2S$ production. The stabbed tubes were incubated at 37º for 48h. The development of black coloration along the line of stab indicated the production of $H_2S$.

**Antibiotic sensitivity assay**

The isolates were subjected to antibiotic sensitivity assay as per the method of Bauer *et al*. 1966 **[18]** to evaluate the pattern of clinical efficacy and multiple antibiotic resistances (MAR) pattern. The details of antibiotics are presented in **Table 1**. For the antibiotic assay, bacterial isolates were grown overnight in LB medium for the preparation of inoculum containing $10^8$cfu $ml^{-1}$. The inoculum (100 µl) was aseptically spread evenly on the surface of Muller Hinton agar, followed by placing antibiotic discs (6 mm dia.) using a disc dispenser (Hi-Media, India). The Petri dishes were incubated at 37° for 24h.

The inhibition zone was measured using a ruled template (Hi-Media, India). The isolates were classified as sensitive, resistant, or intermediate according to the performance standards for antimicrobial disk susceptibility tests, Clinical and Laboratory Standards Institute (CLSI). The experiments were performed in triplicate, and the average values were considered for antibiotic resistance/sensitivity.

**Table: 1 Antibiotic used for sensitivity assay**

| S. No. | Antibiotics | Concentration (per disc) | Abbreviation |
|---|---|---|---|
| 1 | Amikacin | 30µg | AK |
| 2 | Penicillin-G | 2 unit | P |
| 3 | Ciprofloxacin | 5µg | CIP |
| 4 | Ofloxacin | 5µg | OF |
| 5 | Gatifloxacin | 5µg | GAT |
| 6 | Norfloxacin | 10µg | NX |
| 7 | Piperacillin | 100µg | PI |
| 8 | Cefotaxime clavulanic acid | 100µg | CEC |
| 9 | Ceftazidima | 30/10µg | CAZ |
| 10 | Cefoxitin | 30µg | CX |
| 11 | Amoxyclavamoxycillin/clavulanic acid | 30µg | AMC |
| 12 | Azithromycin | 15µg | AZM |
| 13 | Gentamicin | 10µg | GEN |
| 14 | Erythromycin | 15µg | E |
| 15 | Vancomycin | 30µg | VA |
| 16 | Doxycycline hydrochloride | 10µg | DO |

## RESULTS

**Isolation of bacterial cultures from oral cavity:** Out of a total of 180 bacterial cultures isolated from the oral cavity of 180 individuals in various locations, 31 were studied in the present project. Detailed microbiological and biochemical studies were carried out for the characterization of *Streptococcus* species isolates.

**Microbiological characteristics**

**Colony morphology of bacterial isolates:** Different type of colonies appeared on the surface of nutrient agar containing Pteri-dishes. The colony morphology of the 29.0%, 19.0%, 24.0%, 12.90%, and 6.0% isolates was white smooth, white rough, purple pigment, transparent smooth, and transparent colonies, respectively **(Figure 4)**.

**Microbiological features of bacterial isolates:** The isolates were stained for Gram's reaction and identified as Gram-positive *Streptococcus* based on their microscopic appearance and Gram's reaction. Negative staining was performed for size measurement. The spherical cells were measured around 0.5-0.8 µm in diameter.

**Biochemical characterization of bacterial isolates**

**Carbohydrate fermentation Test:** Seven different carbohydrates (Glucose, Sucrose, Fructose, Maltose, Lactose, Mannitol, and Sorbitol) were fermented by *Streptococcus* species isolates. A representative panel of the fermentation test is shown in **Figure 5**.

Out of 31 *Streptococcus* species isolates the carbohydrates glucose, fructose, lactose, maltose, sucrose, mannitol, and sorbitol were fermented respectively, by 45.16%, 32.25%, 22.58%, 9.67%, 29.0%, 16.12%, and 3.22% bacterial cultures. These cultures produced acid but no gas was produced. The results are presented in **(Figure 6)**.

**Production of enzymes by *Streptococcus* species isolates**

**Amylase production test:** Nineteen isolates out of 31 *Streptococcus* species produced amylase enzyme to hydrolyze starch and produced a clear zone around the colony on starch agar upon treatment with the iodine solution **(Figure 7 and 8)**.

**Protease production test:** Casein hydrolysis was observed in Petri-dishes containing medium for the estimation of protease production by the bacterial isolates indicated by the development of a hollow zone around bacterial colonies. Protease production reported in 24 *Streptococcus* bacterial cultures could hydrolyze casein **(Figure 8)**.

**Catalase production test:** Eight isolates belonging respectively to the *Streptococcus* group were catalase positive **(Figure 8 and 9)**.

**Urease production test:** Out of 31 *Streptococcus* isolates 18 bacteria produced urease enzyme **(Figure 8)**.

**Hydrogen Sulfide ($H_2S$) Gas production test:** Thirteen out of 31 *Streptococcus* bacteria produce $H_2S$ gas. The results of hydrogen sulfide gas production are presented in **(Figure 8 and 10)**.

**Antibiotic sensitivity assay**

Antibiotic sensitivity assay of the bacterial isolates was carried out using 16 antibiotics Amikacin, Penicillin-G, Ciprofloxacin, Ofloxacin, Gatifloxacin, Norfloxacin, Piperacillin, Cefotaxime, Clavulanic acid, Ceftazidime, Cefoxitin, Amoxy-clavamoxycillin/ Clavulanic acid, Azithromycin, Gentamicin, Erythromycin, Vancomycin, and Doxycycline hydrochloride.

The antibiotic sensitivity assay results were obtained as shown in **Figure 11**. The clear zone around the antibiotic disc was measured to estimate the sensitivity/resistance of isolates. The percentage of bacterial isolates showing sensitive/resistant/intermediate response against antibiotics used is presented in **Table 2**.

There was no antibiotic found effective against 100 percent bacterial isolates. The most effective antibiotic against the bacterial isolates was Amikacin and Norfloxacin**,** which inhibited the growth of about 83.87% of isolates, while Penicillin-G, Cefotaxime, Clavulanic acid, Cefoxitin, and Erythromycin were fully ineffective as all the isolates showed resistance against these antibiotics. The results are shown in **Table 2** and **Figure 11 and 12**.

**Multi-drug resistance**

The assay for multi-drug resistant (MDR) isolates was carried out. There was no culture found sensitive to the entire range of employed antibiotics. These all cultures were resistant to more than 3 antibiotics. The results are shown in **Figure 13**.

**Figure 4: Petri-dish showing colony morphology on an agar plate**

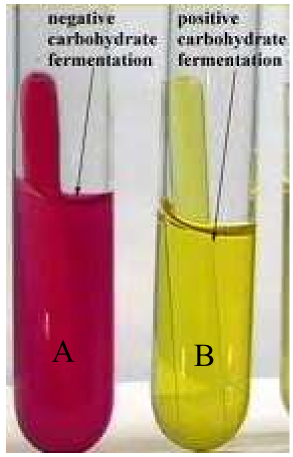


**Figure 5: Carbohydrate fermentation test observations. Tube (A) is an uninoculated control to showing the negative fermentation. Tube (B) shows the production of acid**

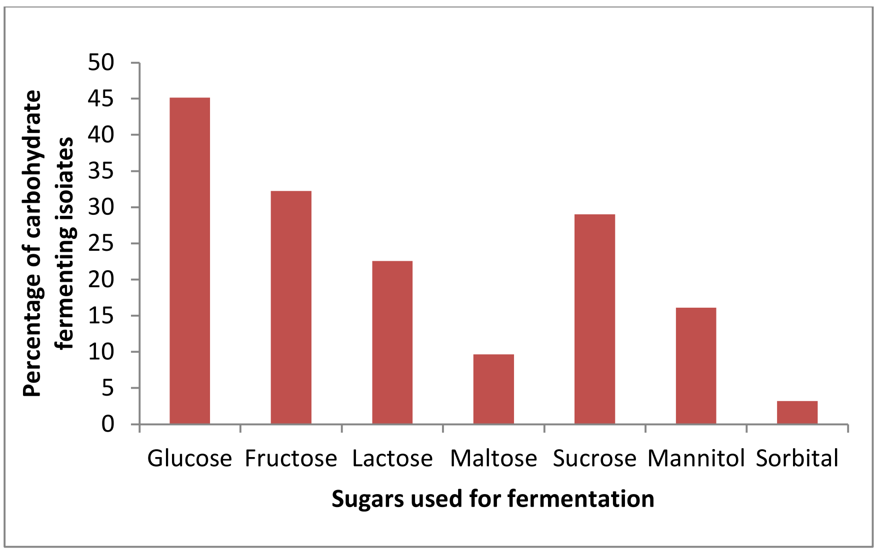


**Figure 6: Carbohydrate fermentation test of *Streptococcus* species isolates**

**Figure 7: Amylase-producing bacterial colonies identified due to the development of clear zone around them following treatment with iodine solution**

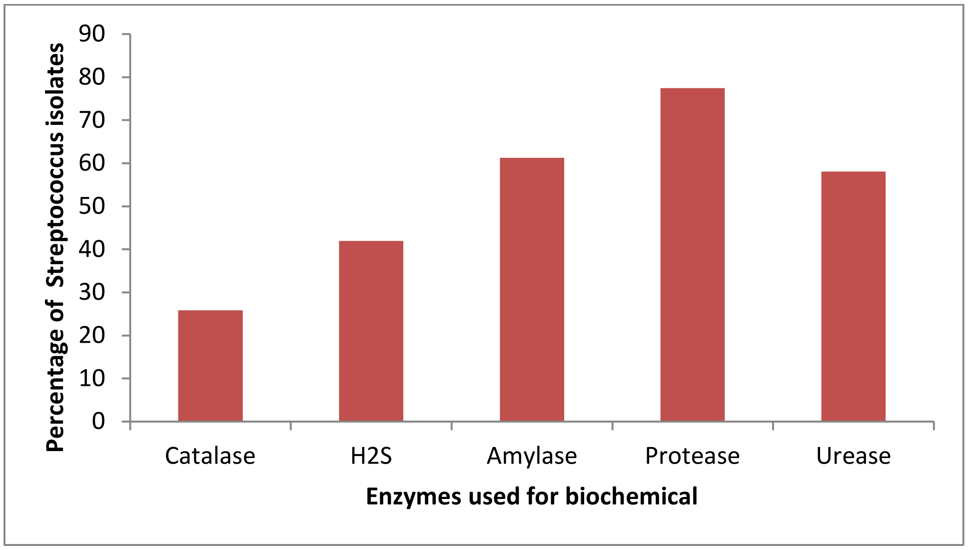


**Figure 8: Biochemical test of *Streptococcus* species isolates**

**Figure 9: Catalase tests, First (left) drop of culture shows catalase negative test while, the second (right) drop shows the appearance of bubbles due to catalase activity**

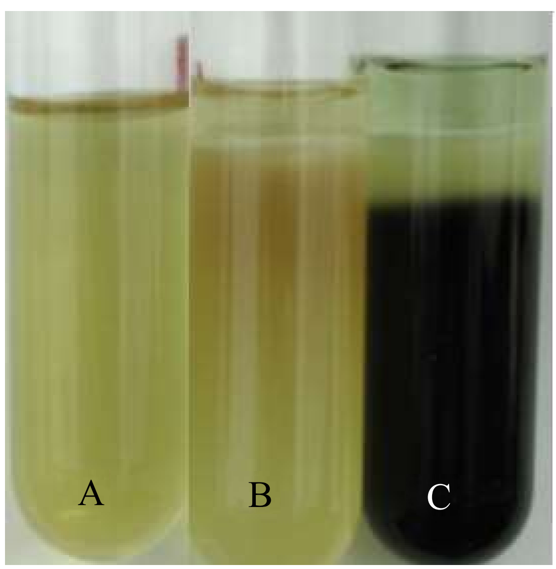


**Figure 10: $H_2S$ production by bacterial isolates. Tube (A) is controlled representing no precipitate and hence negative result, tube (B) shows low-level production of $H_2S$ gas while tube (C) shows high production of gas indicated by a black precipitate of FeS**


**Figure 11: Antibiotic sensitivity assay test in Petri-dish containing Müller Hinton agar medium**

**Table 2: Responses of bacterial isolates against antibiotics used in the study**

| Antibiotics | Response of bacterial isolates (percent) against antibiotics | | |
|---|---|---|---|
| | Sensitivity | Resistance | Intermediate |
| AK 30 | 83.87 | 6.45 | 9.67 |
| P 2 | 0.00 | 100.00 | 0.00 |
| CIP 5 | 19.35 | 29.03 | 51.61 |
| OF 5 | 77.41 | 12.90 | 9.67 |
| GAT 5 | 54.83 | 29.03 | 16.12 |
| NX 10 | 83.87 | 12.90 | 3.22 |
| P 100 | 9.67 | 48.38 | 41.93 |
| CEC 30/10 | 0.00 | 100 | 0.00 |
| CAZ 30 | 25.80 | 45.16 | 29.03 |
| CX 30 | 0.00 | 83.87 | 16.12 |
| AMC 30 | 3.22 | 90.32 | 6.45 |
| AZM 15 | 45.16 | 35.48 | 19.35 |
| GEN 10 | 83.87 | 16.12 | 0.00 |
| E 15 | 0.00 | 77.41 | 22.58 |
| VA 30 | 3.22 | 93.54 | 3.22 |
| DO 10 | 9.67 | 77.41 | 12.90 |

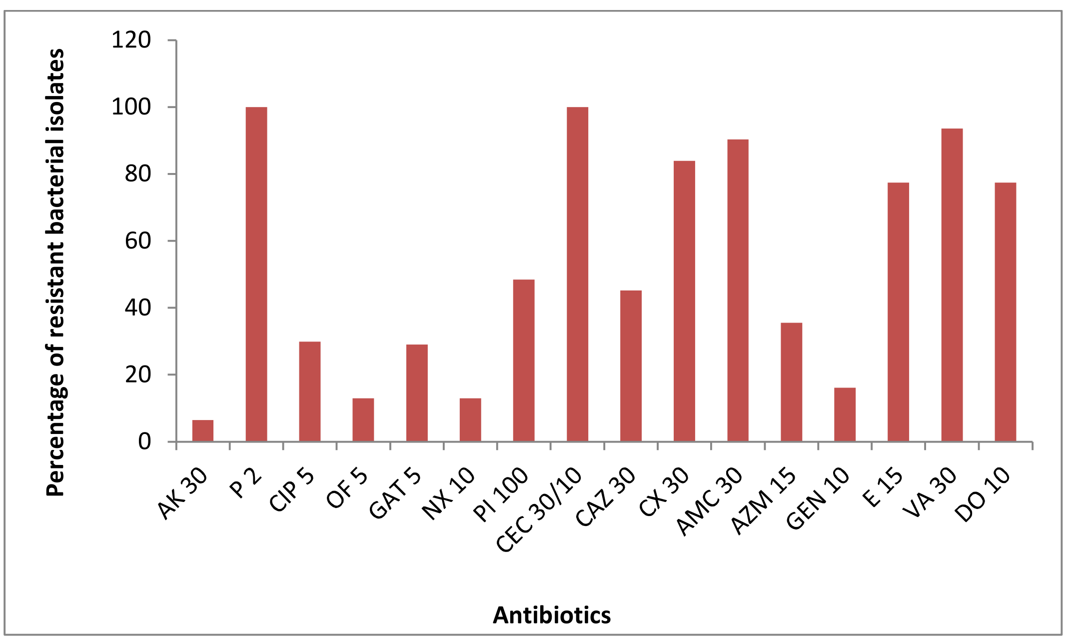


**Figure 12: Antibiotic resistance in bacterial isolates from the oral cavity**

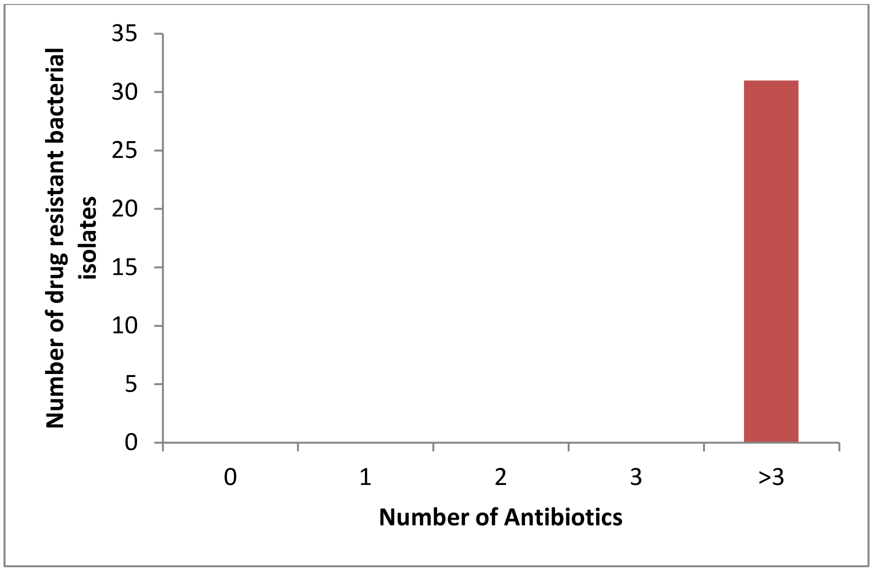


**Figure 13: Multi-drug resistant bacterial isolates from the oral cavity**

## DISCUSSION

There are several reports for the *Streptococcus* spp. isolates in the oral cavity. There is a long list of *Streptococcus* isolates including *S. mutants* and *S. sanguis* and *Streptococcus oralis, S. mitis*biovar and *S. salivarius*were the numerically dominant species. Most of the oral bacterial isolates possess sugar fermentation activity. In the present study, 45.16% of *Streptococcus* species efficiently fermented glucose, while, sucrose was also fermented by 29% isolates. Sugar fermentation favors acid production and helps in shifting the ecological niche in the oral cavity for the development of aciduric bacteria and the establishment of pathogenic conditions required for dental carries, etc.

The bacteria residing in the oral cavity are expected to have several enzymatic properties, mainly carbohydrate-degrading enzymes such as amylase. Our study results have also presented a number (61.29%) of isolates possessing amylase activity, which helps in the digestion of remains of carbohydrates trapped in the oral cavity following food ingestion. Carbohydrate digestion helps increase acidic conditions in the oral cavity, which are not in favor of the host but support demineralization and help in shifting ecological conditions and establishment of bacterial infections by successive organisms.

The oral microorganisms are also known for their proteolytic activity which helps organisms utilize peptides and proteins in the oral cavity available through, GCF, ingested food, etc. Our results are in agreement of Takahashi's finding as a large number of

77.41% isolates produced proteolytic enzymes. The presence of bacteria producing different enzymes in the oral cavity is dependent on the feeding habit of the host. Therefore, varying numbers of isolates have represented the production of these enzymes.

The isolates have shown multi-drug resistance against the antibiotics employed for the study. The characteristic development of multiple drug resistance among the normal microflora of healthy hosts is an alarming situation. Normal microorganisms may lead to an opportunistic infection. The diseases caused by drug-resistant bacteria may be difficult to treat. Such development may be a result of the indiscriminate use of antibiotics by the people. Most of the time inadequate usage of antibiotics is responsible for the pressure created for the development of drug resistance.

Given the above results we can conclude that the oral cavity is a habitat of microorganisms whose sustainability is dependent upon nutrients available in GCF, the remaining particles of food ingested. The resident bacteria help in the development of acid and further establishment development of aciduric bacteria leading to dental carries and other infectious diseases. The development of drug resistance in bacteria from the oral cavity is a dangerous situation and can turn into a very ugly face of opportunistic infections including *Streptococcal* endocarditis.

**CONCLUSION**

Based on studies and results obtained through this work, we can shows that the Gram-positive *Streptococcus* species from dental plaques constitute approximately 19.22% of the bacterial consortium in the oral cavity. These organisms possess typical characteristics required for their survival in the cavity. This study compels us to examine various aspects of antibiotics and their resistance to the possible opportunistic pathogens residing in healthy oral cavities in human beings. Multi-drug resistance nature of these micro-organisms in the cavity is a major concerned area.

**ACKNOWLEDGEMENT**

First and foremost, I would like to express my heartfelt gratitude to my teacher and esteemed academician Dr. Shailendra Kumar, Professor/Head, Department of Microbiology, Dr. Ram Manohar Lohia Avadh University, Ayodhya. His patience, enthusiasm, dedication to work, positive attitude, and critical comments are largely responsible for the successful completion of this work.

**REFERENCES**

**[1]** Marsh, P.D. (2000). Role of the oral microorganisms in health. Microbial ecology in health and disease 12: 130-137.

**[2]** Carlsson, J., and M. B. (1987), Edlund. Pyruvate oxidase in *Streptococcus sanguis* under various growth conditions. Oral Microbiol. Immunol; 2:10-14

**[3]** Rotimi VO, Duerden BI (1981). The development of the bacterial flora in normal neonates. J Med Microbiol; 14:51-62.

**[4]** Pearce, C., Bowden, G.H., Evans, M., Fitzsimmons, S.P., Johnson, J. and Sheridan, M.J. *et al* (1995). Identification of pioneer viridian's Streptococci in the oral cavity of human sneonates. J Med Microbiol 42: 67-72.

**[5]** Sutter, Vera L. (1984). Anaerobes as Normal Oral Flora. Clin Infect Dis. 6(1): S62-S66.

**[6]** Sweeney LC, Dave J, Chambers PA, Heritage J. (2004). Antibiotic resistance in general dental practice: a cause for concern? J Antimicrob Chemother, /53:/567_76.

**[7]** Scannapieco, (1994). Scannapieco FA. (1984). Saliva-bacterium interactions in oral microbial ecology. Crit Rev Oral Bio Med, 5: 203-48.

**[8]** Takahashi, N. (2005). Microbial ecosystem in the oral cavity. Metabolic diversity in an ecological niche and its relationship with oral diseases. International congress series 1284; 103-112.

**[9]** Takahashi, N. and Yamada, T. (2000). Pathways for amino acid metabolism by Prevotellaintermedia and Prevotellanigrescens, Oral Microbiol. Immunol. 15 (2): 96–102.

**[10]** Ruby, J.D. and Gerencser, V.F. (1974). Amylase activity of bacterial origin from human dental plaque. J. Dent. Res. 53(2): 498

**[11]** Takahashi, N. and Yamada, T. (1999). Acid-induced acidogenicity and acid tolerance of non-mutants streptococci, Oral Microbiol. Immunol. 14 (1) (1999) 43–48.

**[12]** Kumar, S., Tripathi, V.R. and Garg, S.K. (2013). Antibiotic resistance and genetic diversity in waterborne Enterobacteriaceae isolate from recreational and drinking water sources. International Journal of Environmental Science and Technology DOI: 10:789-798.

**[13]** Krulwich, T.A.; Lewinson, O.; Padan, E. and Bibi, http://www.nature.com/nrmicro/jour nal/v3/n7/abs/nrmicro1181.html -a2#a2 E. (2005). Do physiological roles foster persistence of drug/multidrug- efflux transporters? A case study.*NatureRev.Microbiol*., 3: 566-572.

**[14]** Novick, R. P. and Roth, C. (1968). Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*.*J. Bacteriol*. 95: 1335-1342.

**[15]** Calomiris, J. J. Armstrong, J.L. and Seidler, R.J. (1984). Association of metal tolerance with multiple antibiotic resistance of bacteria isolated from drinking water. *Appl. Environ. Microbiol*. 47(6): 1238-1242.

**[16]** Rubens, C.E.; McNeill, W.F. and Farrar, Jr. W.E. (1979). Evolution of multiple antibiotic resistance plasmids mediated by transposable plasmid deoxyribonucleic acid sequences. *J. Bacteriol*., 140: 713-719.

**[17]** Sayah, R.S.; Kaneene, J.B.; Johnson, Y. and Miller, R.A. (2005). Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. *Appl. Envviron. Microbiol*., 71(3): 1394-1404.

**[18]** Bauer, A.W.; Kirby, W.M.; Sherris, J.C. and Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*. 45: 493-496.