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Isolation of Mouth Infected Bacteria

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ABSTRACT

The human oral cavity harbors a diverse microbial population, including both beneficial and pathogenic bacteria. Oral infections caused by pathogenic microorganisms can lead to dental caries, gingivitis, periodontitis, and other oral health complications. This study focuses on the isolation and identification of bacteria associated with mouth infections. Oral swab samples were collected from individuals exhibiting symptoms of oral infections under sterile conditions. The samples were cultured on selective and differential media to promote bacterial growth and facilitate identification. Morphological examination, Gram staining, and biochemical tests were performed to characterize the isolated bacterial strains. The results revealed the presence of several pathogenic bacteria commonly associated with oral diseases, including *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus* spp., and *Pseudomonas aeruginosa*. The study highlights the prevalence of diverse bacterial species in infected oral cavities and emphasizes the importance of early diagnosis and microbial identification for effective treatment and prevention of oral diseases. The findings contribute to a better understanding of oral microbial infections and may aid in the development of improved therapeutic and preventive strategies for maintaining oral health.

Keywords: Oral Microbiology, Mouth Infection, Bacterial Isolation, Oral Pathogens, *Streptococcus mutans*, Gram Staining, Microbial Identification, Dental Health

1. INTRODUCTION

The microbial residents of the oral cavity constitute one of the most varied and numerous floras in the human body. More than 500 bacterial taxa are found in the oral cavity, of which approximately 22 predominant ones have been identified. The microbial oral flora is a complex mixture of microbial species, which include bacteria, fungi, protozoa and occasionally viruses. A multitude of organisms is normally present in oral cavity because of wide range of sites of different environmental characteristics. Some of these distinct ecological niches are:

- The tongue
- The saliva
- Various surfaces of teeth or dentures
- The tonsils
- The mucous membrane lining lips, cheek and palate.

Each niche mentioned above has a unique set of conditions that permits the organisms to establish residency and thrive, including receptors for selective adherence, appropriate nutrients and oxygen tension, or simply physical protection from unfavorable conditions. Although the subtypes and proportions of organisms differ among individuals, the general patterns of the indigenous micro floras are similar in healthy individuals. However, changes in systemic disease patterns and concurrent use of medications result in the presence of unusual organisms as part of the "normal" flora and an increase in disease caused by normal organisms that usually are considered to have low pathogenicity. Generally, in the orofacial region, most bacterially mediated conditions involve disturbances of the normal flora or displacement of normal organisms to abnormal sites. The oral cavity is home to a diverse ecosystem of microorganisms, including dental plaque biofilms, the cariogenic S. mutans and oral bacteria that are associated with periodontal disease (e.g., Porphyromonas gingivalis). S. mutans is especially resilient in attaching to tooth surfaces and other S. mutans bacteria. However, numerous biological modifying factors, such as low salivary flow, diet, oral hygiene, acid reflux disease and other gastrointestinal conditions, can affect an individual patient's risk of dental caries. Saliva plays a protective role by reducing exposure to the plaque acids that can demineralize tooth surfaces.

Gram Negative Bacteria in Relation to Oral Cavity

A large number of gram-negative bacteria are present in the oral cavity. Gram-negative, facultative cocci designated as Neisseria have been found in the oral cavity and nasopharynx of humans. N. sicca is the major species, although it may be a type of N. subflava. This organism can produce inflammation of the oral mucosa and may produce lesions resembling oral gonorrhea. Gram-negative bacilli constitute another important group. Four species of Haemophilus are usual inhabitants of oral cavity. These are: H. aphrophilus, H. parainfluenzae, H. paraprophilus, H.haemolyticus and H. segnis. H. aphrophilus is occasionally found in dental plaques. Though H. parainfluenzae is the commonest isolated species of haemophilus from oral cavity, recent evidence has incriminated H. segnis as an important cause of dental plaque.

Other common organisms include species of Campylobacter, Actinobacillus and Helicobacter. In the healthy mouth these may constitute nearly 25% of the flora, but the proportions double with the development of gingivitis or periodontitis. Most infections are polymicrobial, involving various combinations of gram-negative and gram positive cocci and gram-negative rods. Pathogen(s) isolated from such infections or the proportions of such pathogens may vary depending on the stage of the infection. As conditions within the lesion change because of environment, microbial metabolism (e.g., oxygen scavenging by aerobes), or treatment, the organisms present or dominant at one stage may decrease or even disappear at a later stage while others become dominant.

2. REVIEW OF LITERATURE

Enright et al. from Mellon Institute of Dentistry, University of Pittsburgh studied the cause and nature of dental caries. They found Lactobacilli of dental and intestinal origins, the evidence indicates that there are two main types that may be differentiated quite easily by a combination of morphological, fermentation, agglutination, and growth (at 15°C.) characteristics [7]. Besic D.D.S. from College of Dentistry, University of Illinois, Chicago studied in 1943 the Fate of bacteria sealed in Dental Cavities [4]. In the 10 cases studied by him in detail, streptococci (short chain) were present in 8 (gamma streptococci in 6 and Streptococcus viridans in 2).

Lactobacilli were present in 5 and Staphylococci were present in 2. It was found that Streptococci were the most prevalent and resistant and in onethird of the cases still remained positive after being sealed for more than 1 year. The cultivable microbic flora of dental plaques has been studied by Hemmens et al. (Department of Bacteriology and Parasitology, University of Chicago, Chicago) in 1946, beginning with the newly erupted tooth and continuing through the period of early enamel decay until after carious lesions had become well established. Changes in the frequency of isolation in culture of several types of bacteria have been observed. Leptotrichia and actinomyces, alpha hemolytic streptococci, fusiform bacteria and several species of Neisseria were among those forms which decreased in incidence with the progress of the lesion. Lactobacilli were the only bacteria for which there was definite evidence of increased incidence in association with the development of carious lesions [12].

Kraus et al. from Tufts College Dental School, Boston, Mass, in 1953 worked on the classification of nonhemolytic streptococci recovered from Bacteremia of dental origin. One hundred sixty strains of aerobic nonhemolytic streptococci, isolated from the blood of patients after dental extractions, were classified by serologic and physiologic tests. Although Streptococcus saltivarius is one of the more common members of the oral flora, they found only a small number of it in their collection. Streptococcus sanguis has been isolated from a high percentage of cases of subacute bacterial endocarditis, yet they have identified few in the bacteremia studies. On the strength of the data available they concluded that a few of the streptococci recovered from bacteremia of dental origin are potential producers of subacute bacterial endocarditis [15]. Yardeni et al. in 1959 studied Streptococci in Dental Caries. The subject of this paper was the description of methods in caries investigation devised to verify the fact of bacterial invasion. The methods employed were: (a) tapping bacteria directly from clinical cases in routine preparation of cavities, and (b) identification of the organisms by cultural, morphological, biochemical, and serological methods [26].

The results have shown that the organisms most frequently found in the floor of the cavities of initial caries are microaerophilic streptococci not fully identified as yet. Krasse (Royal Dental School, Malmo, Sweden, 1963) studied the Oral Aggregations of Microbes. He observed that Oral aggregations of microbes are found mainly in three locations—on those areas of the teeth not cleaned by mastication, in the gingival crevice, and on the tongue. The proportional distribution of various organisms is quite different, and saliva does not give a representative picture of the bacterial flora of the plaques. The number of bacteria in dental plaques and in scrapings from the gingival pocket seems to be of about the same order of

magnitude, but the proportional distribution of various organisms seems to be quite different. It is obvious that the oral aggregations of microbes are of determinative importance for dental diseases, but their specific role in the dynamic hostmicrobial relationship is not properly understood [14]. Balenseifen And Madonia (Department of Oral Biology and Microbiology, Loyola University, Illinois) established the study of Dental Plaque in Orthodontic Patients in 1970. They collected and studied the plaque samples from orthodontic patients before and after placement of orthodontic bands and arch wires.

The samples were assayed for changes in pH, carbohydrate content, and microbial populations of streptococci and Lactobacilli. Statistically significant increases of plaque pH, carbohydrate content, and microbial populations were noted. [3] Loesche and associates (Department of oral Biology and Pedodontics, University of Michigan, Michigan studied in 1975 the Association of Streptococcus mutans with Human Dental Decay. The association of Streptococcus mutans with human dental decay was investigated by them using several types of samples. Sixty-five percent of the pooled plaque samples from the children with rampant caries had S. mutans accounting for more than 10% of the viable flora, whereas 40% of the pooled samples from children that were caries free had no detectable S. mutans. [20]

Hamada et al. in 1980 worked on the isolation and immunobiological classification of Streptococcus sanguis from human tooth surfaces. A total of 113 pure cultures of S. sanguis were obtained from dental plaque samples of 64 subjects. All isolates synthesized glucan from sucrose, elaborated peroxide, and were alpha-hemolytic. [11]

The microbiological study of various dental diseases has not been a very common practice. In 1988 Genco et al. studied the origin of periodontal infections. Periodontal diseases are recognized as bacterial infections, and some forms are associated with specific organisms, such as Actinobacillus actinomycetemcomitans in juvenile periodontitis, and Bacteroides gingivalis and others in adult periodontitis. The source of the periodontal organisms, whether they are part of the indigenous or resident flora and overgrow to become opportunistic oral pathogens, or whether they are exogenous oral pathogens, is important to determine. The chain of periodontal infection, microbial agent(s) and their transmission, and host response are reviewed with respect to the role of A. actinomycetemcomitans in localized juvenile periodontitis and B. gingivalis in adult periodontitis. They hypothesized that some periodontal organisms may be exogenous pathogens. [9]

3. MATERIALS & METHODS

Specimen Processing, Isolation and Identification

The isolation, identification and susceptibility testing of microorganisms associated with infection are important components in determination of the diagnosis and appropriate therapy for many infectious diseases. This testing usually is accomplished by microscopic examination and culture of specimens representative of the site of infection. The infectious diseases associated with oral region have unique microbiological features because of the abundance and variety of microorganisms in this region. Culture detection of a specific organism or the determination of an organism's significance from culture is often

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complicated. The normal flora of the oral cavity consists up to 10 bacteria per gram of tissue, with anaerobic bacteria composing the majority of the flora present. In some individuals this represents more than 300 species of bacteria. Because of the large numbers of flora, the risk of specimen contamination with bacteria unrelated to infection is increased. Thus the careful collection of appropriate specimens is extremely important in the accurate diagnosis of infections from this region.

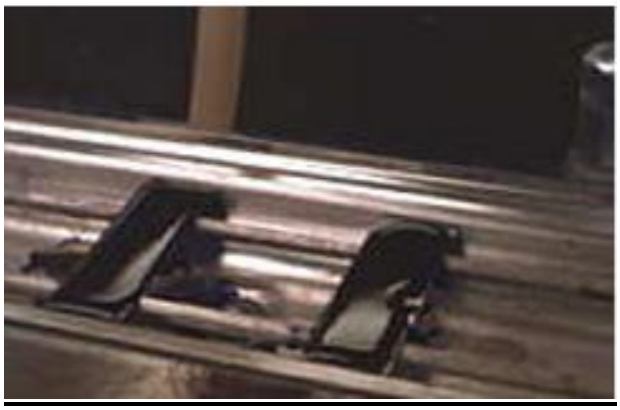
Specimen acquisition and transport

The results obtained from microbiological examination and culture depends on care observed in specimen collection. Because the optimal site and time of collection have an important influence on the usefulness of microbiology results, specimens should always be collected from a site representative of infection. Improper collection techniques or collection from an inappropriate site results in a specimen of little clinical value and may produce misleading results. Although the collection of an appropriate specimen is the first step in the achievement of quality, clinically relevant microbiological results, proper collection should be followed by appropriate transportation to the laboratory.

All specimens for microbiological examination and culture should be transported to the laboratory promptly because they are subject to deterioration and overgrowth during transit. Extremes of temperature, delay, dehydration and the presence of nutrients in body fluids results in the death of more fastidious organisms, thus producing misleading reports. The optimum time for specimen transport to the laboratory is less than 2 hours.

Gram-Staining Procedure:

STEP 1: Place your slide on a slide holder or a rack. Flood (cover completely) the entire slide with crystal violet. Let the crystal violet stand for about 60 seconds. When the time has elapsed, wash your slide for 5 seconds with water. The specimen should appear blue-violet when observed with the naked eye.



STEP 2: Now, flood your slide with the iodine solution. Let it stand about a minute as well. When time has expired, rinse the slide with water for 5 seconds and immediately proceed to step three. At this point, the specimen should still be blue-violet.

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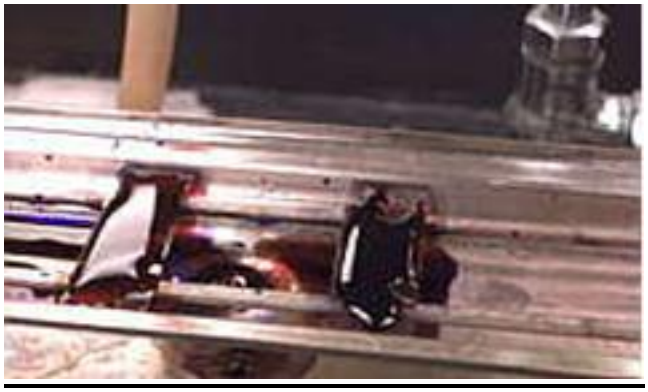
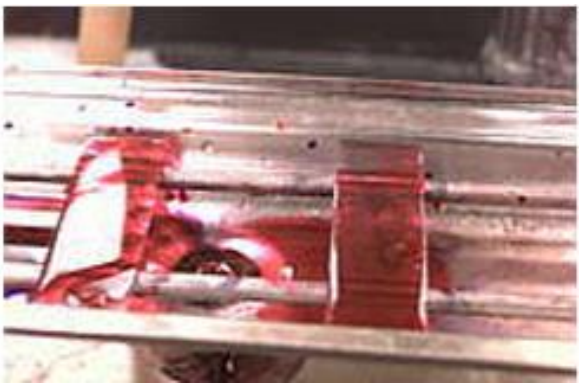


STEP 3: This step involves addition of the decolorizer, ethanol. Step 3 is somewhat subjective because using too much decolorizer could result in a false Gram (-) result. Likewise, not using enough decolorizer may yield a false Gram (+) results. To be safe, add the ethanol dropwise until the blue-violet color is no longer emitted from your specimen. As in the previous steps, rinse with the water for 5 seconds.

STEP 4: The final step involves applying the counterstain, saffranin. Flood the slide with the dye as you did in steps 1 and 2. Let this stand for about a minute to allow the bacteria to incorporate the saffranin. Grampositive cells will incorporate little or no counterstain and will remain blue-violet in appearance. Gram-negative bacteria, however, take on a pink color and are easily distinguishable from the Gram positives. Again, rinse with water for 5 seconds to remove any excess of dye.



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After you have completed steps 1 through 4, you should blot the slide gently with bibulous paper or allow it to air dry before viewing it under the microscope.

Procedure:

DAY 1 – The samples were taken from different OPD's like pedodontics, endodontics, maxillofacial, etc through a sterilized swab of infected and healthy individuals collected from gums, tongue, soft palate, cheeks, throat, etc. Use a sterile cotton swab to sweep the site of infection. After obtaining the specimen, the swabs were rolled down back and forth several times on the suitable agar media plate. Now with the help of a sterilized inoculating loop streaking was done. As the inoculum passes over the agar, bacteria are deposited, and after incubation, each develops into a colony visible to the naked eye. This technique will hopefully assure that the culture will show isolated colonies, instead of just a big smear of many colonies. Incubate the plate overnight at 37oC.

DAY 2 – The plates were observed, colony characters were observed. If mixed colony seen then pure cultures are made. A single colony is then passed in nutrient broth with the help of a sterilized loop or a needle and kept at 37oC for 24 hours. Smears were prepared on a clean slide and Gram staining was done. Slides were observed under the microscope in 100 X oil immersion and morphological characters were observed.

DAY 3 – The colonies passed in the nutrient broth on day 2 were then again inoculated in Nutrient agar and MacConkey plates. Plated were then incubated at 37oC for 24 hrs.

DAY 4 - Colony characters were observed. Separated colonies (pure cultures) were then picked up with the help of a sterilized loop and passed in nutrient broth. The tubes were then incubated at 37oC or kept at room temperature for 24 hrs. Smears were prepared and Gram staining was done. The slide was then observed under microscope and the morphological characters were observed as done in day 2.

DAY 5 – The biochemical tests were performed on the separated colonies passed in the nutrient broth on day 4.

DAY 6 – The results which were obtained on the basis of microscopic observation and biochemical tests helped in identifying the particular species of the bacteria.

4. OBSERVATION & RESULT

This research work is undertaken to study the bacterial flora in and around the teeth and gums of healthy and infected individuals.

Total 10 healthy and 20 infected individuals were studied. All the samples were taken from the various OPD's of Peoples College of Dental Sciences. All the samples belonging to the healthy individuals as well as the infected individuals yielded microbes.

Step 1 describes the bacterial flora in teeth and gums of healthy individuals. Total subjects studied were 10. The bacterial flora isolated were as follows – S.mutans, S.salivarius, S.mitis, S.sanguis and Staph epidermidis. It was found that the frequency of S.mutan was maximum

(whose frequency was 8 times out of the 10 subjects), followed by Staph epidermidis (whose frequency was 5 times out of the 10 subjects). S.salivarius (4 out of 10), S.sanguis (3out of 10) and S.mitis (2 out of 10).

Step 2 describes the bacterial flora in teeth and gums of infected individuals. Total subjects studied were 20. The bacterial flora isolated were S.mutans, S.sanguis, S.mitis, Staph epidermidis, Haemophilus haemolyticus and Neisseria catarrhalis. It was found that the frequency of S.mutans was maximum (10 times out of 20 subjects), followed by S.sanguis (4 out of 20), S.mitis (3out of 20), Staph epidermidis, Haemophilus haemolyticus and Neisseria catarrhalis (1 out of 20). It is very clear from the above data that S.mutans is the major disease causing pathogen in the oral cavity. Colony characters were studied alongwith the types of haemolysis on Blood agar. Coagulase, Catalase, Phosphatase tests were done to differentiate Streptococci from Staphylococci. S.aureus was positive for these tests excepting for Staph epidermidis which was coagulase negative, indicative of being a commensal in the oral cavity. One case each of Neisseria catarrhalis and Haemophilus haemolyticus was observed. Both these organisms are commensal of oral cavity. In case of Haemophilus haemolyticus, the smear showed gram negative thin bacilli with lot of pleomorphism in the size of bacilli. The smear was very typical.

Step 3 describes the no. of subjects studied, age, sex and the bacterial flora isolated from the healthy individuals.

Step 4 shows the no. of cases studied, age, sex and the bacterial flora isolated from the teeth and gums of the infected individuals along with the diagnosis of the disease caused.

Step 5 describes the percentage of bacteria isolated from 10 healthy individuals. 22 bacteria were isolated from 10 subjects of which the percentage was calculated.

Step 6 is showing the percentage of bacterial flora isolated from 20 infected cases.

5. DISCUSSION

In the present study, effort has been made to collect and correlate the work carried out since 1924 to the present year, 2006.

It has been reported by Asikainen and Alaluusua [2] that most common dental caries, dental diseases, periodontal diseases are chronic infections caused by normal oral flora. When these bacteria increase in number, and, irritation exceeds the host defense threshold, disease arises. Alterations in diet, medication, smoking, denture wearing, general health, etc. can also lead to overgrowth of minor components of bacterial flora which can predispose a site to disease. Disease is a result of a shift in the balance of the resident flora due to a change in local environmental conditions which are the predisposing factors.

In most of the infectious samples collected during our study, the common diagnosis was of dental caries, dental plaque and dentoalveolar abscess. Dental caries is the destruction of the enamel, dentin or cementum of teeth due to bacterial activities. Caries are initiated by direct demineralization of the enamel of teeth due to lactic acid and other organic acids which

accumulate in dental plaque. Dental plaque is defined as the material that accumulates on the tooth surface. Plaque is essentially a deposit of dense gelatinous material consisting of protein, polysaccharide and an enormous mass of bacteria that can be S.sanguis, S.mutans, S.mitis, etc. Abscess is an infection of either the tooth, gums or bone. Commonly appears clinically as a gum boil on the tissue. Marsh and Martin proposed ecological plaque hypothesis that the organisms associated with disease may also be present at sound sites, but at levels too low to be clinically relevant [21].

For establishing a cause and effect relationship between a single microorganism in oral flora and diseases has been very difficult. Most of the investigators believe that development of diseases of enamel is preceded by the formation of microbial plaque in the tooth. Infectious nature of dental infections has been brought out in our study. All the samples belonging to healthy as well as infectious group yielded bacteria. Although the frequency of various species of bacteria varied. As described earlier by various researchers and thereby taking inspiration from their work, we tried to find out the bacterial flora in and around teeth and gums of healthy and infectious individuals. The microbiota isolated from healthy individuals in our study were – S.mutans (36.36%), S.salivarius (18.18%), S.sanguis (13.63%), S.mitis (9.09%) and Staph epidermidis (22.72%). All these microbes were reported as normal flora of oral cavity in various publications by Elmer W. Koneman et al. [13], J.G.Collee and associates [6], D.R.Arora [1], Rajesh Bhatia and R L Ichhpujani [5].

It is to be noted that we haven't found any bacteria which has not been reported/ established as normal flora. During the study of the bacterial flora in infectious teeth and gums, we isolated most of the commensal bacteria that played a significant role in dental infections. In our study S.mutans was found in the 50% of the total number of bacteria which caused dental caries. It is to be noted that S.mutans was also reported as opportunistic bacteria by Clarke, Loesche et al. [19], JDB Featherstone [8], and Saini et al [21]. On the surface of a tooth, S.mutans is the first important colonizer, particularly in people with a high sucrose diet. This bacterium metabolizes sucrose to produce extracellular polysaccharide (glucans) that enable the bacterial cells to stick onto the surface of the tooth.

6. CONCLUSION

The present study successfully isolated and identified bacterial species associated with oral infections from mouth swab samples. Microbiological analysis revealed the presence of several pathogenic bacteria that are known to contribute to dental caries, gingivitis, periodontitis, and other oral diseases. The use of culture techniques, Gram staining, and biochemical characterization proved effective in identifying the bacterial isolates. The findings demonstrate that the oral cavity serves as a reservoir for a diverse range of microorganisms, some of which can become pathogenic under favorable conditions. Early detection and identification of these bacteria are essential for accurate diagnosis, effective treatment, and prevention of oral infections. This study enhances the understanding of oral microbial diversity and provides valuable information for improving oral healthcare practices and developing targeted antimicrobial therapies. Maintaining proper oral hygiene and regular

dental examinations remain crucial for reducing the incidence and severity of bacterial oral infections.

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