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ORIGINAL ARTICLE

Saliva as a medium for antibiotic sensitivity: A new tool in diagnosis

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ABSTRACT:

Salivary diagnostics is a dynamic and emerging field utilizing nanotechnology and molecular diagnostics to aid in the diagnosis of oral and systemic diseases. An antibiotic is any substance that delays with the ability of bacteria to function normally. Data from the previous literature shows that there has been continuous research for utilization of human saliva as medium for testing antibiotic sensitivity. Hence; we have tried to summarize some of the important aspects of human saliva and their role in acting as a medium for antibiotic testing.

Key words: Antibiotic, Saliva, Sensitivity

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INTRODUCTION

Oral fluid is a diagnostic medium that can be easily collected and with minimal invasion but it has been neglected in the past. Today, saliva is being used more often to diagnose: HIV virus, oro-facial and systemic tumors, cardiovascular disease and in detecting addictive substances. Neutrophil levels in saliva may also indicate successful bone marrow transplant. Oral fluid is now systematically being researched and oral fluid analysis is being compared with the analysis of other diagnostic media such as blood and urine. A number of recent studies have focused on oncogenic marker detection and its monitoring in saliva. Data from previous literature shows that there has been continuous research for utilization of human saliva as medium for testing antibiotic sensitivity.^{1,2}

PROPERTIES OF SALIVA

Human saliva is a clear, slightly acidic (pH 6.0 to 7.0) heterogeneous biofluid composed of water (99%), proteins (0.3%), and inorganic substances (0.2%). On average, individual salivation can range from 0.3 to 0.7 ml of saliva

per minute, producing a range of 1 to 1.5 liters daily. Saliva is multifunctional, serving not only to facilitate digestion, swallowing, tasting, and tissue lubrication but also as a protective barrier against pathogens.³

USING SALIVA AS A DIAGNOSTIC FLUID

There is a large amount of literature which describes the use of saliva, as well as gingival crevicular fluid and oral mucosal transudates to both monitor medication and also detect various oral and systematic diseases. Salivary analysis, just like blood analysis, has two main objectives: to identify specific pathologies in patients and monitor change in those patients undergoing treatment.⁴

SALIVA VERSUS BLOOD

Like saliva, blood is a complex bodily fluid known to contain a wide range of molecular components, including enzymes, hormones, antibodies, and growth factors. While cells, tissues, stool, and other alternatives are routinely pursued, blood serum or plasma is traditionally and most frequently the source of measurable biomarkers. Although

life-saving in many instances, the procedures required to collect and eventually analyze blood samples can often be expensive, problematic, and physically intrusive. Employing salivary fluids as a medium for biomarker development and evaluation alleviates subject/patient discomfort through the provision of a noninvasive method of disease detection.⁵

EFFECTS OF SALIVA AND ALPHA-AMYLASE ON ANTIBIOTIC SENSITIVITY OF BACTERIA

Rotimi VO et al in their study, analysed the effect of saliva and alpha-amylase on antibiotic sensitivity of bacteria. Two hundred and ninety-six bacterial isolates were investigated for the effects of saliva and alpha-amylase on their susceptibility to ampicillin, tetracycline, chloramphenicol and gentamicin. When the test organisms were primed with normal and 'diseased' saliva there were no observable differences in the MICs of ampicillin and chloramphenicol for group-A streptococci, but alpha-amylase significantly reduced the MIC of tetracycline from 2 to 0.25 mg/1. With *Staphylococcus aureus*, priming with saliva and alpha-amylase had no effect on the MICs of gentamicin and ampicillin, whereas the MICs of tetracycline and chloramphenicol were increased. The effect of saliva on the susceptibility of *E. coli* to tetracycline was also significant; MIC₅₀ and MIC₉₀ were reduced from 128 to 8 and 32 mg/1 respectively. Chloramphenicol was however increased from less than 0.125 to 1 and 2 mg/1 when *E. coli* was primed with amylase and saliva respectively.⁶

Efforts on the discovery of analytes in the saliva of normal and diseased subjects suggest an additional function of saliva, a local and systematic diagnostic tool. Analytes used for disease detection range from proteins, to antibodies, and nucleic acids that are of either human microorganism origins. Highly sensitive and high-throughput assays such as mass spectrometry, RT-PCR, microarray, and nano-scale sensors that can measure proteins and nucleic acids with a minimal amount of sample requirement in a short period of time allowed scientists to broaden the utility of saliva as a diagnostic tool.⁷⁻⁹

ORAL BACTERIA AND CHARACTERIZATION FOR BACTERIOCIN PRODUCTION AND ANTIMICROBIAL SENSITIVITY

Various species of the genus *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Staphylococcus*, *Corynebacterium*, *Veillonella* and *Bacteroids* are the prominent bacteria commonly found in the oral cavity. Among the oral bacteria, *Streptococcus* and *Enterococcus* are two important members because they can shift their lifestyle from beneficial microflora on the surface of oral cavity and oropharynx to destructive pathogens when they gain access into the oral tissue and blood stream. Among the diseases caused by oral bacteria include dental caries, periodontitis, endocarditis, pharyngitis, pneumonia, meningitis etc. Most of the oral *Streptococcus* are gram

positive facultative anaerobes demonstrating highly efficient survival strategies such as the ability to adhere hard and soft tissues, cell-cell communication, biofilm formation and to cope up with the rapidly changing oral environment.¹⁰

A bacterium has to compete with other bacteria to colonize in the oral cavity. Therefore, they undergo extensive intra-species and inter-species communication which confer survival advantage in the harsh environment of oral cavity.¹¹

Production of bacteriocin is an important mean of outcompeting other bacteria in this heterogeneous environment. Many gram positive bacteria produce bacteriocins which act like toxin against other bacteria, however, the producer strain is immune to its own bacteriocin due to immunity factor. Since the oral environment is very competitive, it is speculated that bacterial species isolated from such environment will produce inhibitory substances against other bacteria.¹²

Rahman M et al isolated and identified oral bacteria and characterize their ability to produce bacteriocin against other oral bacteria as well as their sensitivity to common antibiotics. We have employed deferred antagonism bacteriocin assay for bacteriocin production and disk diffusion assay for antibiotic susceptibility testing. They identified eight bacterial strains belonging to the genera *Streptococcus* and *Enterococcus* based on colony morphology, biochemical assays, 16S rDNA sequence analysis, and species-specific PCR. Antibiotic susceptibility assay indicated that some of the strains are resistant to one or more antibiotics. Their study revealed that the isolated strains are capable of producing one or more bacteriocins against other oral bacteria. Further molecular and biochemical studies are required to understand the nature of observed bacteriocin.¹³

Viridans streptococci represent a group of 24 currently described *Streptococcus* species that are nutritionally fastidious and mainly alpha-hemolytic on blood agar. These gram-positive cocci are commensals of the oral cavity, upper airway, and gastrointestinal and genitourinary tracts. Despite the overall low virulence, Viridans group streptococci continue to be the most common cause of both native valve endocarditis and late prosthetic valve endocarditis. They have also been implicated in serious pyogenic infections moreover as emerging pathogens in neonates and neutropenic patients, and they appear to be a serious problem in patients with hematologic malignancies receiving cytotoxic chemotherapy.¹⁴

Antibiotic resistance is most prevalent in countries with a high level of antibiotic consumption, supporting the link between antibiotic use and the emergence of bacterial resistance. Resistance to beta-lactams, macrolides and other antibiotics among blood cultures of VGS is a major concern and could compromise currently available prophylactic and therapeutic regimens. The antibiotic resistance of oral and gut flora may be due to the selection

of clones with reduced susceptibility during antibiotic treatment, but also to the transfer of resistance genes from exogenous bacteria.¹⁵

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