

**ANTIBACTERIAL ACTIVITY OF BIOLOGICAL FLUID –  
THE SALIVA**

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**Summary**

The morning saliva samples collected and inoculated in the optimized Nutrient medium, incubated at  $37 \pm 2^\circ\text{C}$  for 24 hours showed the organisms with specific gram character using Gram's staining method. Using Dimethylsulfoxide (DMSO) as control, saliva as the test material and Chloramphenicol as the standard; the potential antibacterial property of saliva was evaluated on *B.subtilis*, *S.aureus* and *E.coli* bacterial species by measuring the zone of inhibitions. The pH conditioned medium at 7.4 gave highly populated colonial growth of the cocci species from the saliva sample after 24 hrs of incubation and were observed to be Gram negative species and displayed significant inhibitory zones when checked with Chloramphenicol conferring to its antibacterial nature. Thus the biological fluid, saliva confirmed the presence of cocci which were proven to be possessing potent antibacterial properties for immune and non-immune defence systems.

**Keywords:** Saliva, antibacterial, Zone of Inhibition

## **Introduction**

Saliva in humans is a mouth fluid possessing several functions involved in oral health and homeostasis with an active protective role in maintaining oral healthiness. It aids in bolus formation, protection of oral mucosa, preliminary digestion of food, taste perception, teeth enamel mineralization and defence function against pathogen microorganisms. Saliva is one of the most complexes, versatile and important body fluids, supplying a large range of physiological needs. In the digestive tract, saliva plays an important role in oesophageal physiology, the digestive process, and gastric cell protection(1, 2). In the oral cavity, saliva takes part in mastication, speech, deglutition, gustatory sensitivity, tissue lubrication, mucosal protection against invasion, antibacterial (3), antifungal, and antiviral activity, post-eruptive maturation, ionic balance regulation at enamel remineralisation, deposition of acquired enamel pellicle, and acid diffusion limitation (4). There are three pairs of major salivary glands namely, parotid, submandibular, sublingual and minor salivary glands namely, mucosa of tongue (Von Ebner glands), cheeks, lips, palate (5).

Secretion of it is controlled by ANS, and involves two processes; viz., secretions of aqueous plasma like fluid by acinar cells and modification by water impermeable ductal cell system. Saliva is mainly composed of water 99.5%, proteins 0.3% - glycoproteins, enzymes ( $\alpha$ -amylase, carbonic anhydrase, immunoglobulins), inorganic/traces – electrolytes (Na, K, Cl,  $\text{HNO}_3$ ), peptides (cystatins, statherin, histatin, proline rich protein). The rate of secretion of saliva confers to about 0.3-7ml/min i.e. 500-1500ml/day. The value of saliva as a diagnostic tool for oral and systemic diseases has been an area of study for many researchers with the aim of increasing its use as a possible complementary exam. The ability to use saliva to monitor an individual's health and disease state is a highly desirable objective for healthcare research and promotion (6, 7). The present paper examines studies done to evaluate the anti-bacterial potential of this biological fluid.

## **Materials and methods**

### **Collection of Saliva**

The morning saliva samples (1-2ml) before brushing teeth or mouth rinse were spilled directly in pre-sterilised collector vials.

### **Storage of Saliva**

The collected salivary vials were then stored within half-an-hour in the refrigerator at 5°C and used thereafter.

### **Preparation of Media**

#### **Nutrient agar Medium**

Nutrient agar medium was weighed and dissolved in distilled water. This was then adjusted to pH  $7.4 \pm 2$  and autoclaved for 20min at 15lbs.

#### **Optimization of Nutrient Medium**

The nutrient medium was prepared and made in to two flasks before adjusting the pH. One of which was pH adjusted and then both were incubated. Thus the media with the adjusted pH showed optimized growth of the microbial colonies than the unadjusted medium. The medium with pH  $7.4 \pm 2$  was selected for further experimental work.

#### **Sample Spreading Technique**

The sterilised cotton swab was placed in the saliva sample and was spread on the Nutrient agar petri plates. These agar plates were further incubated for 24hrs at  $37 \pm 2^\circ\text{C}$ .

#### **Evaluation of Organisms**

The plates removed after incubation were Gram stained and the colonies were observed microscopically for gram positive and gram negative type of bacteria. It was observed as gram negative cocci species.

### **Study of Growth Characteristics in Post-prandial Samples**

Some saliva samples were collected one hour after having meals and refrigerated as described before. These also were then spread over the pH adjusted nutrient agar medium and incubated for 24hrs. The colonial growth observed after 24 hrs when compared with the morning samples was found to be very scant. This showed that the post prandial samples had more of acid secretion which proved non-conducive for the coccilary growth.

### **Determination of Antibacterial Effect**

Three different petri plates with the nutrient agar medium; each being spread with the bacterial species *E.coli*, *B.subtilis* and *S.aureus* were taken. Test sample, i.e. saliva of about 15µl/ml was wellled in each plate in 5mm bore. Similarly another plate of nutrient agar was kept for the standard taken as Chloramphenicol 30mg/ml. A control plate of dimethyl sulfoxide was kept to observe the distinctness of the zones. All these plates were then incubated for 24hrs at  $37 \pm 2^{\circ}\text{C}$ .

### **Results and Discussion**

The saliva was found to be containing Gram negative cocci species. Varied inhibitory zones were observed when saliva sample was spread over the bacterial species when compared with the zone of inhibition of 27mm from the standard plate of Chloramphenicol. The readings of the zones of inhibition were obtained as given in the following table:

**Table 1: ZONES OF INHIBITION OF ORGANISMS**

Organisms	Zone of Inhibition (mm)
<i>E. coli</i>	17
<i>B. subtilis</i>	16
<i>S. aureus</i>	15

The salivary gram negative cocci gave significant inhibitory zones with the three organisms, which prove it antibacterial property. The salivary peroxidase system contained in the saliva gets enhanced by

generation of hydrogen peroxide in to the oral cavity. The advantages of using saliva for diagnosis compared to other biological specimens are the easy availability, simple and non-invasive collection that can be done by the individual themselves, and easy, low-cost storage.

In addition to these advantages; saliva collection offers a painless alternative, eliminating the stress that the patient can feel which can be useful for geriatric, pediatric and obese patients or patients with mental deficiency, prisoners, etc. Thus saliva can prove to be a great tool for defending the immune and non-immune system. The salivary peroxidase system contained in it is enhanced in vivo by generation of small concentrations of hydrogen peroxide into the oral cavity, contributing to its effective antibacterial property.

### **Conclusion**

The biochemical and physicochemical properties of saliva contribute to the numerous functions of saliva in maintaining oral and general health and for its potential to diagnose bacterial diseases. Thus with the study done it can be said that the saliva has fountain of opportunities for its antibacterial property to be put in use for research purposes. Thus there is necessity to elaborate the antibacterial activity by other sophisticated techniques.

### **References**

1. Lima DP, Diniz DG, Moimaz SA, et al. Review Saliva: reflection of the body. *International Journal of Infectious Diseases* 2003; 3 0:xxx.e1–xxx.5.
2. Chiappin S, Antonelli G, Gatti R, et al. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. *Clinica Chimica Acta* 2007; 383: 30–40.
3. Tenovuo J. Antimicrobial Agents in Saliva-Protection for the Whole Body. *Journal of Dental Research* 2002; 81: 807.
4. Schipper RG, Silletti E, Vingerhoeds MH. Saliva as research material: Biochemical, physicochemical and practical aspects. *Archives of oral biology* 2007; 52: 1114 – 1135.

5. Michael WJ, Johnson DA, Chih-ko yeh. Health benefits of saliva: a review. *Journal of Dentistry* 2005; 33: 223–233.
6. Ihalin R, Loimaranta V, Tenonuo J. Origin, structure, and biological activities of peroxidases in human saliva. *Archives of Biochemistry and Biophysics* 2006; 445: 261–268.
7. Johan KM, Martens LC. Review: The physiology of saliva and transfer of drugs into saliva. *Forensic Science International* 2005; 150: 119–131.