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Use of Mitis *Salivarius* Agar Base for Presumptive Identification of *Streptococcus salivarius* Collected from Clinical Samples

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Abstract: ***S.salivarius are the normal commensals which inhabitats in oral mucosa Considering it's deleterious virulence towards various destructive dental - periodontal diseases and belonging to same background we were fascinated to find the most efficient way to isolate and study this species. Oral swabs with a sample size of 20 were collected cultured on blood agar and MSA simultaneously. Blood agar showed mixed growth including various streptococcal species whereas MSA showed blue gum dropped colonies of S.salivarius. Hence, we terminated this study to a conclusion that MSA over blood agar is more efficient and less time consuming for isolation of salivarius species.***

Keywords: S.salivarius, dental and periodontal diseases, blood agar, mitis salivarius agar (MSA), blue gum dropped colonies

1. Introduction

The human oral streptococci are commensals which often inhabit the gastrointestinal and genitourinary tracts, as well as the oral mucosa and tooth surfaces. In healthy individuals, streptococci can constitute more than 50% of the oral microbiota and these bacteria generally possess low pathogenic potential. However, oral streptococci can invade the bloodstream, and have the potential to cause infective endocarditis (IE) or post-antineoplastic septicaemia in neutropenic patients with haematological disease.[2]

Humans are introduced to S. salivarius within a few hours after birth, inhabiting the mouth and upper respiratory tract, making it one of the first commensal bacteria humans are exposed to. Early exposure allows humans to acquire immunity, so S. salivarius is usually considered harmless. However, while immunity is established during infancy, the bacteria are opportunistic pathogens, proving to be detrimental under certain circumstances, such as entrance to the bloodstream. Due to its opportunistic nature, S. salivarius has been linked to cases of sepsis in immunocompromised patients with neutropenia, a disease associated with a depleted level of white blood cells in the body. [2]

As belonging from a dental background my interest towards isolation of these species specifically is due to it's virulence towards dental and periodontal diseases. Use of MSA over blood agar is more effective as it shows growth on culture in 24 hours while on blood agar it requires 48 hours for growth. Also other species show various haemolytic reactions making it difficult to differentiate while high selectivity of MSA does it all at ease Mitis Salivarius Agar is formulated as per Chapman [6]. This medium (with 1% potassium tellurite) is a highly selective medium, which enables to isolate streptococci from highly contaminated specimens like exudates from body cavities and faeces etc., as it inhibits a wide variety of bacteria. Some authors have also used sodium azide in this medium to inhibit the growth of gram- negative bacteria like Proteus, Casein enzymic hydrolysate and peptic digest of animal tissue in the medium provide the essential growth nutrients. Dextrose and sucrose are the fermentable carbohydrates. Dipotassium phosphate buffers the medium. Trypan blue is an acidic, blue diazo dye while crystal violet is a basic dye and also, a bacteriostatic agent, which inhibits many gram-positive organisms. Potassium tellurite also helps to make the medium selective for streptococci.[6]

Patients consent:
Patients were explained about our study, informed about the procedure to be performed for the collection of samples and oral consent was taken.

Inclusion criteria:
Patients with usually high caries index and poor periodontal health, moderate to severe periodontitis were included in this study. Also, samples from patients with poor oral hygiene were a part of the study.

Exclusion criteria:
Patients with low caries index, good oral hygiene were excluded. Those on prophylactic antibiotics were not considered.

2. Methods and Materials

Sample collection
Sterile oral swabs were used to collect specimens from the clinical sample size of 20 patients that routinely showed high caries index and poor periodontal health. The plaque and debris were excavated from the tooth surface with a probe followed by immediate securing of the swab in a sterile test tube. They were transferred to the laboratory from the respective department within as short span as

possible.

Culture: .The samples were then inoculated on two separate media, blood agar and MSA with 1% potassium tellurite prepared as per manufacturer's instructions, respectively. They were incubated in an incubator at 37 degree celcius for 24 hours.

Sample results

BLOOD AGAR: Some S-type and some R- type colonies were seen and alpha haemolytic colonies were observed. S.salivarius strains shows weak alpha reaction with small non pigmented rough colonies. *Image-1*

MSA: S.Salivarius shows a specific blue green gum drop appearing colonies on MSA. *Image-2*

Microbial assessment*:* Gram staining was performed; Gram positive cocci were seen varying in length from small to very long chains. *Image-3*

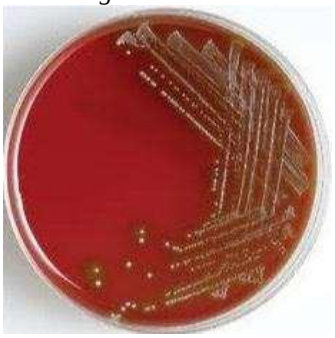


Image 1: Alpha haemolysis on blood agar

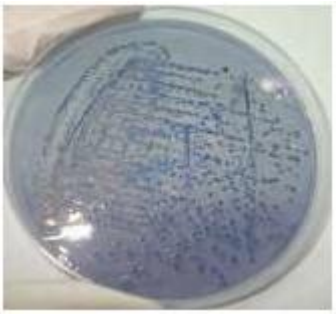


Image 2: Blue gum dropped colonies on MSA.

Image 3: Microscopic appearance showing long chain cocci

3. Results

Following results were obtained as demonstrated in *table-1*.

A mixed kind of growth was seen on BA, as shown in *table -1,* which showed candidal growth in 12 specimens out of 20 that is 60%. Entercoccal species sure 75% of growth that is 15 out of 20 specimens. S. Salivarius indicated 18 specimens showing positive growth that is 90% from the complete growth while staphylococcus aureus Sure 70% of growth.

When studied on MSA, E.faecalis showed 35% of growth in the form of blue black colonies while blue gum dropped colonies of S.salivarius was seen in 90% of specimens.

As we can conclude that various organisms show more than 50% of growth in blood agar which makes it difficult isolate S.salivarius species specifically Whereas on MSA a concentrated growth of the respective organism was seen.

Table 1

S.salivarius	18	90%	18*	90%
S. aureus	14	70%	0	0%

BA: blood agar; MSA: mitis salivarius agar
*On MSA, E.faecalis shows blue black colonies while Characteristic blue gum dropped colonies seen with s.salivarius.

When studied on pie diagram, mixed growths seen on blood agar 25% showed growth of C.albicans, 30% of E.faecalis, 20% S.salivarius, while S.aureus showed 24% gowth. (*Diagram-1)*

On the other hand, on MSA S.salivarius showed 72% gowth and E.faecalis shows 28% growth.

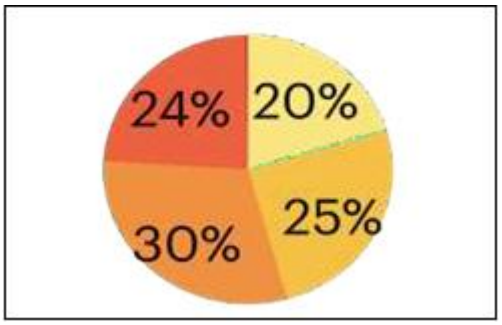
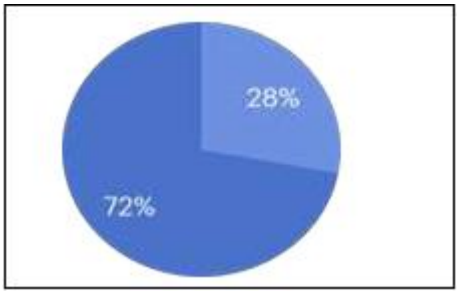


Diagram 1: Growth on blood agar

% Growth	*Organism*
25%	C.albicans
30%	E.faecalis
20%	S.salivarius
24%	S.aureus



Pie Diagram 2: Growth on Mitis Salivarius agar

Organism	Growth on BA	% Growth on BA	Growth on MSA	% Growth on MSA
C. albicans	12	60%	0	0%
E.faecalis	15	75%	7*	35%

Organism	% Growth
S.salivarius	90%
Others	10%

Biochemical tests for s.salivarius:
S.salivarius shows negative test to enzymes catalase and oxygenase. On fermentation with sugar,acid production production is seen with glucose fructose maltose while lactose shows variable reaction for acid production. [7]

4. Discussion

Streptococcus salivarius, a viridans group of streptococcus that is prone to cause nasolacrimal or iatrogenic central nervous system infections. As studied by centers of disease control and prevention,S. Salivarus are most frequently associated with causes of bacterial meningitis following spinal procedure eg. anaesthesia. [4]

Streptococcus salivarius the species usually found as part of the normal human oral flora. Humans are introduced to S. salivarius within a few hours after birth, inhabiting the mouth and upper respiratory tract, making it one of the first commensal bacteria humans are exposed to it. It is found occasionally in neonates, and more than 75% in infants[9].Early exposure allows humans to acquire immunity hence considered harmless, while immunity is established during infancy, the bacteria are opportunistic pathogens, proving to be detrimental under certain circumstances, such as entrance to the bloodstream. Due to its opportunistic nature, S. salivarius has been linked to cases of sepsis in immunocompromised patients with neutropenia. [5]

When cultured on blood agar, these strains form non-hemolytic or alpha-hemolytic green colonies S. salivarius has been noted to be isolated. They are gram positive cocci in chains, that is catalase and oxygenase negative.[1]

According to study of C. E. Scafford, J.M. Sherman and H.M. Hodge [3] , when oral specimens were collected and inoculated on blood agar the reaction varied from gamma, to "*weak alpha*" being characteristic. Very few were showing gamma type of reaction while other few remaining gave variable reaction that terminated in a typical alpha reaction . There is no growth occurring at 10 degrees celsius neither above 47 degrees celsius. Maximum favourable temperature being 45 degrees they incubated the specimen at 37° celsius for 24 hours. [3]

Also studies by Holt. J. R., et al [7] that is mentioned in Bergey's manual of determinative bacteriology, demonstrated that most strains shows *weak alpha reaction* on blood agar or gamma reactions. Small non-pigmented smooth to rough colonies were seen on blood sgar when grown at 37 degrees. [7]

As studied by J. M. Sherman et al [8]. To identify the characteristic of non-haemolytic streptococci of human throat, they found that when cultured collect Specimen work culture on blood agar they showed indifferent reaction in more than 95% of the cultures there was *no greening* seen [8]. Alpha haemolysis partially breaks down the red blood cells and leave a greenish colour behind. This is referred to as alpha haemolysis addressing the greening.

Similar results were obtained by us on blood agar as mentioned above. Whereas on MSA the growth was obtained after 24 hours of incubation had a characteristic *blue gumdrop* appearing colonies were seen. This characteristic appearance is typically associated with S.salivarius. Also some blue black colonies were observed indicating growth of Enterococcus faecalis. But as our study was focused to S. salivarius, we included the growth from the gumdrop colonies for the further biochemical tests.

5. Conclusion

As discussed S. Salivarius is a lethal opportunistic organism due to its deleterious effects on humans directly attacking the central nervous system and its adverse effects on dental health though it is found in the normal habitat of every individual it's invasion in bloodstream can be highly virulent.

When cultured on blood agar A mixed kind of growth was obtained including all other species like candidal, Enterococcal, staphylococcus, streptococcal.On the other hand when culture on MSA blue gumdrop colonies were observed that indicated the growth of s. Salivarius

For isolation from a mixed culture a selective differential agent is necessary to inhibit the growth of other organisms and promote the growth of streptococcus salivarius. Considering the cascade of the events to be followed this becomes time consuming, expensive and a tedious job. While MSA contains 1%potassium tellurite as a selective agent resulting in an isolated growth of s. Salivarius.

Hence we conclude that mitis salivarius agar is more cost efficacious over conventional blood agar as it is more efficient less time consuming and easy to practice routinely.

6. Future Scope

The scope in this topic is deep due to occurrence of the organism as normal commensal in various habitats of human body. As discussed earlier, inspite of oral cavity, S.salivarius is a resident of genitourinary tract, gastrointestinal tract, hence can potentiate pathologies via invasion through blood stream. My research was limited to samples from oral cavity, one can study by collecting various other samples.

As isolation under MSA over blood agar is concluded to be more effective by us, we can even focus on other selective agents to be used on the same agar base to isolate other species of streptococcus.

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