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Chlorhexidine gluconate is an excellent antimicrobial solution against *Streptococcus mutans* to maintain oral hygiene

Dr. Arijit Chatterjee

Technical Officer in Dr. B.C.Roy Post Graduate Institute of Paediatric, Sciences, Kolkata, West Bengal, India

Abstract
One of the major oral health problems in most of the countries is dental plaque or dental caries. *Streptococcus viridians group, especially Streptococcus mutans* is recognized as the solely responsible pathogen of dental caries in human. In this study, we assessed the antimicrobial activity of Chlorhexidine gluconate solution against oral microorganisms, especially *Streptococcus mutans*, the major one responsible for the same. 50 samples were collected at different dental clinics in kolkata. All the isolates of *S.mutans* were identified biochemically and were selected for antimicrobial activity in vitro i.e. on artificial culture media. Chlorhexidine gluconate (0.2% w/v) was purchased from nearby pharmacy shop and diluted with distilled water eg. 1:5, 1:10, 1:15 and 1:20 respectively to observe the antimicrobial activities in each case against *S.mutans* isolates. All the concentration of chlorhexidine gluconate solution showed excellent antimicrobial activity in vitro. This study proves that chlorhexidine gluconate solution is essential to maintain oral hygiene.

Keywords: Chlorhexidine gluconate solution, Antimicrobial activity, *Streptococcus mutans*, Dental caries

1. Introduction
This study was carried out at SRL Reference Laboratory in salt lake, Kolkata. In this study, 50 samples were collected at different dental clinics in kolkata. All the isolates of *S.mutans* were identified biochemically and were selected for antimicrobial activity in vitro i.e. on artificial culture media.
Streptococcus mutans is believed to be the most common bacteria associated with dental caries (Rani, 2006) [3]. Bacteria in the oral cavity colonize in the form of communities known as dental biofilms or plaques. (Hojo, 2009) [2] defined dental plaque as "a biofilm community that accumulates through sequential and ordered colonization of multiple oral bacteria". Streptococci are the first isolated genus to play a role in the formation of dental plaque and development of caries of the human oral cavity (Semyari, 2011) [1].
Therefore, maintenance of oral hygiene is necessary by controlling the bacterial biofilm on teeth with the use of chlorhexidine gluconate solution. The main objective of our work was to assess the antibacterial activity in vitro against *Streptococcus mutans*. Chlorhwxidine considered the gold standard (Van Leeumen, 2011) [5].

2. Materials and methods
The chemicals used in this study were purchased from pharmacy shop. Samples were collected at dental clinics and *Streptococcus mutans* were isolated from laboratory.

Isolation and identification of the organism
5% Sheep Blood Agar (SBA) was the enriched media used for the isolation of *S.mutans*. Swabs collected from dental plaques were inoculated immediately i.e. within 2 hours of collection by 'Koch's Plating Technique' with the help of plastic disposable wire loop and incubated in a CO_2 incubator for 24 hours at 37^0C. After 24 hours of incubation, small, circular, tiny, glossy colonies were observed on SBA plate.
Gram stain of the isolated organism shows gram positive cocci in chains. Then, catalase test was performed to differentiate staphylococci which is catalase positive from streptococci which is catalase negative. Colonies on SBA show α-haemolysis. Growth was tested with 5 µg optochin disc which shows resistance to the antibiotic indicated α-haemolytic streptococci other than *S. pneumonia.* Other tests included were Voges proskauer positive whereas Oxidase, Indole, Methyl red and Citrate utilization were negative. Sugar fermentation was positive. All these tests indicated the organism as *Streptococcus mutans.*

Preparation of the Chlorhexidine gluconate solution
Chlorhexidine gluconate was diluted with distilled water eg.1:5, 1:10, 1:15 and 1:20 respectively

Antimicrobial Assay Procedure
The SBA was punched with sterile nichrome wire to made wells. The inoculums were spread on to the agar plates using sterile cotton swabs and then the wells were filled with different dilutions of chlorhexidine gluconate, each with 100µl of solution. The plates were incubated at 37 ºC for 24 hours in a CO_2 incubator. After incubation, zone of growth inhibition for each dilution was measured in millimeters.

3. Results
In this study, 42 (84%), out of 50 isolates of *S.mutans* were identified by grams stain and biochemical reactions and antibiotic assay tests were performed. Different dilutions of chlorhexidine gluconate solution eg. 1:5, 1:10, 1:15 and 1:20 were tested against the pathogenic microorganism i.e. *Streptococcus mutans* and the antimicrobial assay report was listed in the Table 1.
Chlorhexidine gluconate solution was effective showing different zone of inhibition against each dilution.

Table 1: Antimicrobial activity of chlorhexidine gluconate solution against *Streptococcus mutans*

S. No.	Isolates	Zone of inhibition (mm)	S. No.	Isolates	Zone of inhibition (mm)
1.	Isolate 1	24	22.	Isolate 22	24
2.	Isolate 2	24	23.	Isolate 23	23
3.	Isolate 3	22	24.	Isolate 24	26
4.	Isolate 4	24	25.	Isolate 25	25
5.	Isolate 5	20	26.	Isolate 26	24
6.	Isolate 6	23	27.	Isolate 27	22
7.	Isolate 7	21	28.	Isolate 28	21
8.	Isolate 8	22	29.	Isolate 29	23
9.	Isolate 9	23	30.	Isolate 30	24
10.	Isolate 10	24	31.	Isolate 31	20
11.	Isolate 11	24	32.	Isolate 32	21
12.	Isolate 12	20	33.	Isolate 33	24
13.	Isolate 13	20	34.	Isolate 34	23
14.	Isolate 14	23	35.	Isolate 35	22
15.	Isolate 15	24	36.	Isolate 36	20
16.	Isolate 16	22	37.	Isolate 37	21
17.	Isolate 17	25	38.	Isolate 38	23
18.	Isolate 18	26	39.	Isolate 39	25
19.	Isolate 19	21	40.	Isolate 40	24
20.	Isolate 20	20	41.	Isolate 41	20
21.	Isolate 21	22	42.	Isolate 42	24

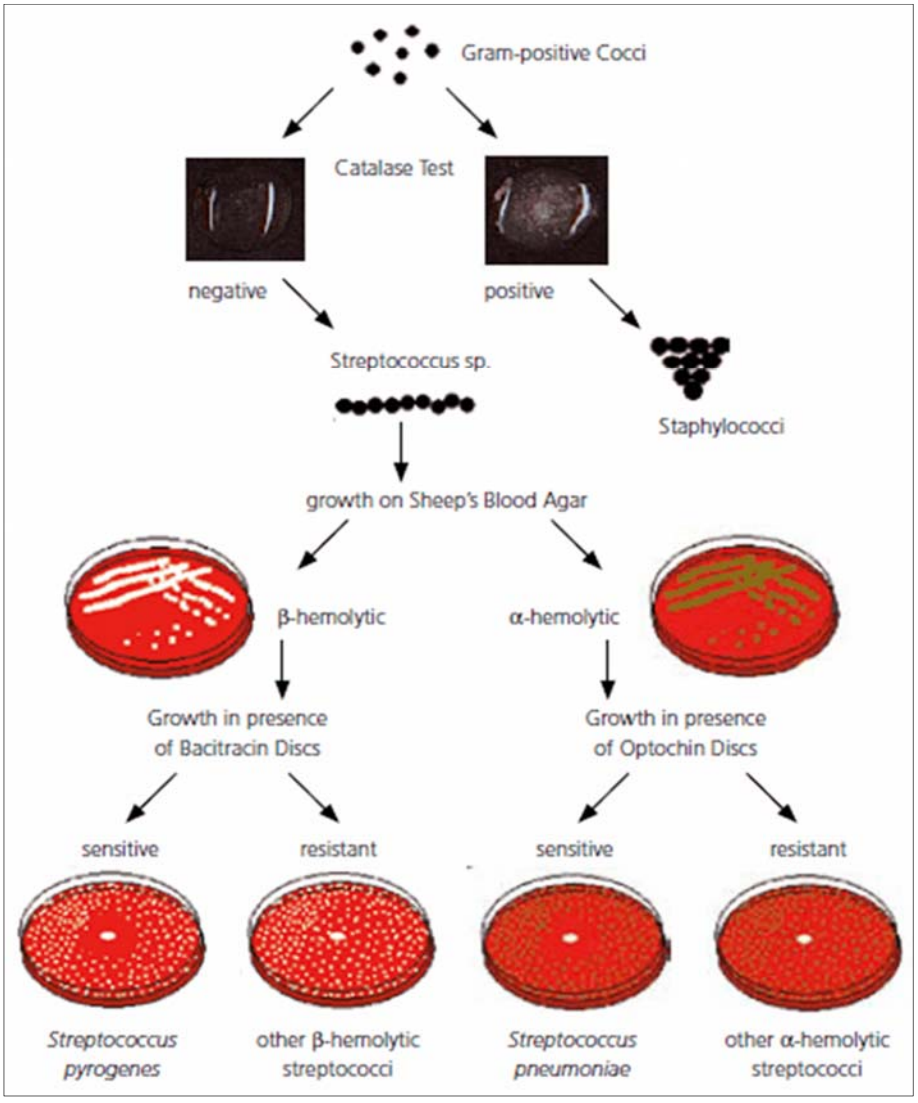

Fig 1

4. Discussion

Anderson (1997) reported that the use of chlorhexidine gluconate oral rinse contributes to improving oral hygiene. The use of chlorhexidine gluconate reduces the levels of *S.mutans*, gingival index and gingival bleeding (Eldridge, 1998).

The anionic nature of chlorhexidine gluconate molecules damages the cytoplasmic membrane causing loss of cytoplasmic constituents with enzyme inhibition, coagulation of cytoplasm, precipitation of proteins and nucleic acids (Jones, 2000) [6].

5. Conclusion

This study clearly indicates that chlorhexidine gluconate soulution is an excellent antibiotic solution for mouth rinsing and also for maintaining oral hygiene. Further study is necessary to establish its activity against all the cocci in viridians group.

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7. References

1. Semyari HP, Owlia S, Farhadi, Moghadami Tabrizi S. Evaluation of antimicrobial effect of Ammi Visnaga against oral streptococci, Journal of Microbiology and Antimicrobials. 2011; 3:126-129.
2. Hojo K, Nagaoka S, Ohshima T, Maeda N. Bacterial interactions in dental biofilm development. J Dent Res. 2009; 88(11):982-90.
3. Rani A, Chopra A. Isolation and identification of root canal bacteria from symptomatic non-vital Tooth with periapical pathosis. Endodontology 2006; 18:12-7.
4. Bouwsma OJ. The status, future, and problems of oral antiseptics. Curr Opin Periodontol 1996; 3:78-84.
5. Van Leeumen MPC, Slot D.E and Van Der Weijden. Essential oils compared to chlorhexidine with respect to plaque and parameters of gingival inflammation: A systematic Review. Journal of Periodontology 2011; 82(2):174-194.
6. Jones CG. Chlorhexidine: Is it still the gold standard? Periodontol. 2000, 1997; 15:55-62.