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Chlorhexidine – A Gold Standard proved against Oral Streptococci

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Abstract
Dental caries is a well known major oral health problem in most countries. *Streptococcus mutans* is recognized as the main pathogen of dental caries in human. This study was to assess the antimicrobial effectiveness of Chlorhexidine diacetate against oral microorganism. Ten samples were collected at dental clinics in and around Tirupur. All the isolates of *S.mutans* were identified by biochemically and five isolates were selected for antimicrobial activity. Chlorhexidine diacetate was prepared at different concentrations (0.05%, 0.10%, 0.20% and 0.25%) against *S.mutans* isolates. All the concentrations of CHX were showed the maximum inhibition in antimicrobial activity. This study one again proved that CHX is a gold standard against clinical oral pathogens.

Keywords: CHX, Dental caries, *S.mutans*, Antimicrobial activity.

1. Introduction

Bacteria in the oral cavity colonize in the form of communities known as dental biofilms or plaques. Dental plaque is defined as "a biofilm community that accumulates through sequential and ordered colonization of multiple oral bacteria" (Hojo, 2009) [1].
Oral streptococci are the first isolated species to play a role in the formation of dental plaque and development of caries (Semyari, 2011) [2]. *S. mutans* is believed to be the most common bacteria associated with dental caries (Rani, 2006) [4].
Therefore, control of the bacterial biofilm on teeth is essential for the maintenance of oral health. A few antiseptics, like chlorhexidine has been used in cariology (Bouwsma, 1996) [10].
Chlorhexidine is used as a positive control in many clinical trials of new mouth rinse formulations and is considered the gold standard. (Van Leeumen, 2011) [3]. The main objective of this work was to assess antibacterial activity against *S.mutans*.

2. Materials and methods

All the chemicals were procured from Hi media, Mumbai. Samples of *S.mutans* isolates were collected at dental clinics and were isolated from laboratory.

Isolation and identification

Mitis-salivarius [MS] Agar was the selective media used for the isolation of *S.mutans*. Grams reaction and Voges proskauer were positive whereas Catalase, Oxidase, Indole, Methyl red and Citrate utilization were negative. Sugar fermentation was positive [Table 1].

Table 1: Identification of Streptococcus mutans

S. No	Investigation	Results
1.	Gram stain	Positive
2.	Indole	Negative
3.	Methyl red	Negative
4.	VP test	Positive
5.	Citrate utilization	Negative
6.	Oxidase	Negative
7.	Catalase	Negative
8.	Sucrose	Positive
9.	Glucose	Positive
10.	Mannitol	Positive

Preparation of Chlorhexidine diacetate stock solution

CHX powder was dissolved directly into the distilled water and prepared at respective concentrations. Stock solution (0.1mg/ml) and its different concentrations were prepared.

Antibacterial assay

The solid agar was punched with steel cork borer to made wells. The inoculums were spread on to the agar plates using sterile swabs and then the wells were filled and the concentrations of the CHX were 50µl, 100µl and 150µl. The plates were incubated at 37 ºC for 24 hours. After incubation, zone of growth inhibition for each concentration was measured in millimeters.

3. Result

Totally ten isolates were isolated in this study. The isolates of *S.mutans* were identified by grams reaction and biochemical characterization and only five *S.mutans* isolates were selected for further process of antibacterial assay. All the concentrations [0.05%, 0.10%, 0.20% and 0.25%] of Chlorhexidine diacetate exerted for antibacterial

activity against dental caries pathogen *S.mutans*. The antibacterial activity of the test agent was in direct proportion with the concentration used. The effect of different concentrations of Chlorhexidine diacetate 0.05%, 0.10%, 0.20% and 0.25% were on selected isolates tabulated in Table 2 and Figure 1. The antibacterial assay values are presented in Figure 2.

Chlorhexidine diacetate was most effective agent showing highest zone of inhibition at the highest concentration against *S.mutans*. Also it was most consistent of all the medicaments tested, showing inhibitory effect against the pathogens at all selected concentrations.

Table 2: Antibacterial activity of Chlorhexidine Diacetate

S.No	Strain No	Zone of Inhibition (mm)
1.	KK1	23
2.	KK2	20
3.	KK3	24
4.	KK4	22
5.	KK5	24

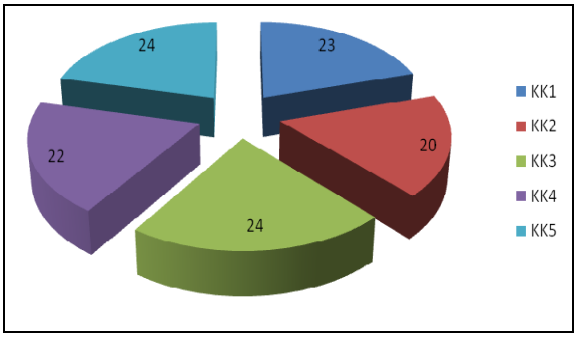

Fig 1: Effect of Chlorhexidine diacetate on *S.mutans*

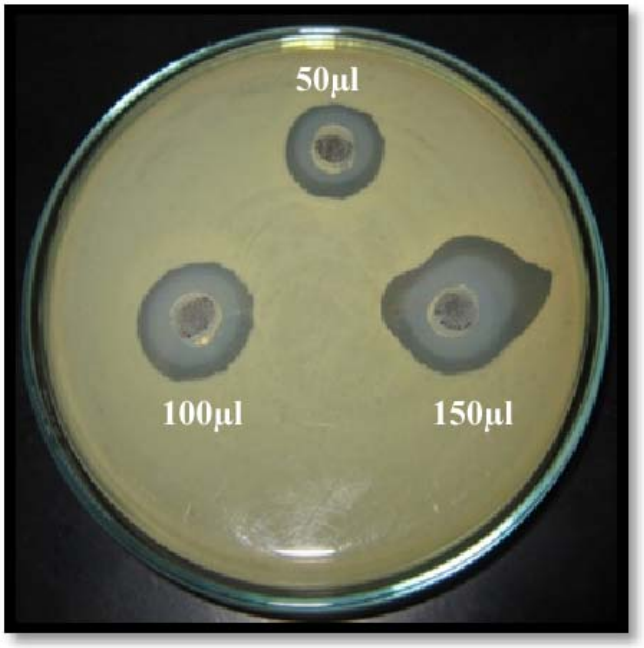

Fig 2: Effect of Chlorhexidine diacetate at different Concentrations on *S.mutans*

4. Discussion

This study argued that based on the inhibition zone of the antibacterial assay. 0.25 % concentration of Chlorhexidine diacetate was showed the maximum antibacterial activity against the isolates with the zone of inhibition 24mm and least activity was observed at 20mm. The least concentrations of CHX were similarly reported and the concentration often used in endodontic therapy is upto 2% [Mistry, 2014] and one more report was proved that found to be more effective in the least concentration and compared with other concentrations of CHX ranging from 0.002% to 2% [Lessa, 2010].

The antibacterial effect is because of reaction of CHX molecule with negatively charged groups on the cell surface, causing an irreversible loss of cytoplasmic constituents, membrane damage and enzyme inhibition. At higher concentrations CHX results in extensive cell damage, coagulation of cytoplasm and precipitation of proteins and nucleic acids [Jones, 2000]

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

5. Conclusion

This study reveals that, chlorhexidine diacetate provided better results in its antibacterial assay against *Streptococcus mutans.* Further research could study the antibacterial efficacy of chlorhexidine diacetate in greater depth and in vivo clinical testing is essential to confirm the in vitro results.

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