*ejpmr, 2021, 8(3), 267-272*

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# EFFECT OF OCTANIDINE DIHYDROCHLORIDE AND CHLORHEXIDINE DIGLUCONATE ON *PORPHYROMONAS GINGIVALIS* AND *AGGREGATIBACTER ACTINOMYCETEMCOMITANS*

**Dr. Komal Gaikwad*, Dr. Yogesh Doshi, Dr. Mona Shah, Dr. Vasundhara Rikame**

PDU Dental College Solapur, Maharashtra, India.

**Corresponding Author: Dr. Komal Gaikwad**

PDU Dental College Solapur, Maharashtra, India.

**Article Received on 05/01/2021** **Article Revised on 26/01/2021** **Article Accepted on 16/02/2021**

## INTRODUCTION

In clinical practice, antiseptic mouthwashes are either used as an adjunct to improve the efficacy of mechanical oral hygiene or as the only measure for plaque control, e.g., in oral trauma patients, after oral surgical interventions but also in ventilated, long-term hospitalized, terminally ill patients. Antiseptics are primarily intended to reduce the overall bacterial load in the oral cavity and the precipitation and proliferation of bacteria on nonshedding tooth surfaces. Antiplaque agents affect antibacterial action and inhibit plaque growth and plaque-associated inflammation.[1]

During a step-wise evaluation process of new formulations and products, the mode of action should be investigated as precisely as possible.

Besides in vitro experiments, clinically controlled trials play a major role before regulatory approval for drug is granted.

A new formulation containing the same active ingredient as an already marketed product does not mean that efficacy is equivalent.[2] Therefore, each new formulation has to undergo the testing process.

Currently, Chlorhexidine (CHX) is regarded as the gold standard of antiplaque agents due to its efficacy and safety verified by a multitude of clinical studies over the past 45 years.

Nevertheless, adverse effects of CHX use like tooth discoloration, taste disturbance, and in rare occasions allergic reactions led to the search for an equivalent alternative.[3]

Octenidine dihydrochloride (OCT), a bispyridinamine, developed in the 1980s may qualify as a suitable candidate. It has been licensed as an antiseptic agent in 20 European countries since 1995.

Like CHX, it unspecifically binds to negatively charged sites of bacterial cell walls as well as to all soft and hard tissue surfaces of the oral cavity due to its cationic nature. Binding to bacteria subsequently results in autolysis and cell death.[4]

Thus, OCT is used as a preventive or therapeutic antiseptic for disinfecting skin, mucosa, and wound surfaces.[5] In the oral cavity, it proved to reduce the overall bacterial load[6,7] by its broad-spectrum efficacy affecting Gram-positive and Gram-negative organisms as well as yeasts.[8]

Octenidine dihydrochloride (OCT; N, N′-[1,10 decanediyldi-1[4H]-pyridinyl-4ylidene]bis[1-octanamine]dihydrochloride) is a bispyridine antimicrobial compound that carries 2 cationic active centers per molecule and demonstrates broad-spectrum antimicrobial effects, covering both gram-positive and gram-negative bacteria, fungi, and several viral species.[9]

It exerts bactericidal/fungicidal effects by interfering with cell walls and membranes. OCT is currently widely used in the medical field for skin burns and decontaminating mucous membranes and open wounds.[10]

It is also used in mouthwash formulations and other dental applications. Nonetheless, OCT is not currently popular as endodontic irrigants because insufficient information is available about its properties in vivo. OCT is unique due to its relative non-cytotoxicity at the site of action[11] and good antimicrobial activity. Exerting a sustained antimicrobial effect made OCT also suitable for being used as an antiplaque agent.[12] Clinical trials verified its pronounced plaque and gingivitis-reducing properties.[13–16]

In therapeutic concentrations, OCT is well tolerated without relevant local or systemic toxicity and does not induce bacterial resistance. The oral cavity possesses several features that make it a distinct habitat for a menagerie of microorganisms.

The surfaces in the oral cavity are continuously bathed in saliva most of the time at a narrow temperature range (34 to 36◦C) and a pH close to neutrality (Marcotte and Lavoie, 1998). With such an ideal environment, various classes of microflora are found to be distributed in various ecological niches **(Parahitiyawa et al., 2010)**.[17]

***Actinobacillus actinomycetemcomitans*** and ***Porphyromonus gingivalis*** are major putative periodontopathic bacteria. ***Aggregatibacter Actinomycetemcomitans*** has been closely associated with periodontitis in young individuals and with cases of refractory adult periodontitis ***Porphyromonas gingivalis (Pg)*** occurs in severe adult periodontitis, failing guided tissue regeneration and acute periodontal abscesses.[18]

So, the present study aims to check and compare the antimicrobial efficacy of Chlorhexidine Digluconate and Octanidine Dihydrochloride on ***Porphyromonas gingivalis (Pg)*** and ***Aggregatibacter Actinomycetemcomitans (Aa).***

## MATERIALS AND METHODS

### 1. Agar diffusion method

Both microorganisms were previously subcultured in appropriate culture media and under gaseous conditions to confirm their purity.

The anaerobes and the facultative anaerobic strains were individually inoculated into tubes containing 5mL of sterile 0.9% saline solution. The suspension was adjusted spectrophotometrically at 800 nm (Optical Density) to match. The turbidity of 1.5 x$10^{8}$CFUmL$^{-1}$ (equivalent to 0.5 McFarland standards). Five hundred µL of each test microorganism suspension was used to inoculate glass bottles containing 50 mL of BHI Agar at 46°C, mixed and poured onto 130 mm plates containing a previously set layer of Mueller Hinton agar. The isolated anaerobic microorganisms were suspended spectrophotometrically at 800 nm to match the turbidity of 3.0 x $10^{8}$CFU mL$^{-1}$ (equivalents McFarland standard). Sterile swabs were dipped into the bacterial suspension and were used to inoculate pre-reduced 70 mm plates containing 5% sheep-blood- Fastidious Anaerobe Agar.

Sterilized stainless steel tubes of 8.0 x 1.0 x 10 mm inner diameter, (6 mm) will be added to the surfaces of the media and filled with 40 µL of each test substance and controls. The plates were maintained for 2 hours at room temperature in the appropriate gaseous conditions to allow the diffusion of the agents through the agar and then incubated at 37°C again under the appropriate gaseous conditions for an appropriate period: aerobes, 24 hours; facultative, 24 - 48 hours in a CO2 incubator, in an atmosphere of 10% CO2 and anaerobes in the anaerobic workstation in an atmosphere of 10% H2, 10% CO2, 80% N2 for 7 days.

Zones of inhibition of microbial growth around the cylinder containing the tested substances were measured and recorded after the incubation period. The inhibitory zone will be considered to be the shortest distance (mm) between the outer margin of the cylinder and the initial point of the microbial growth.

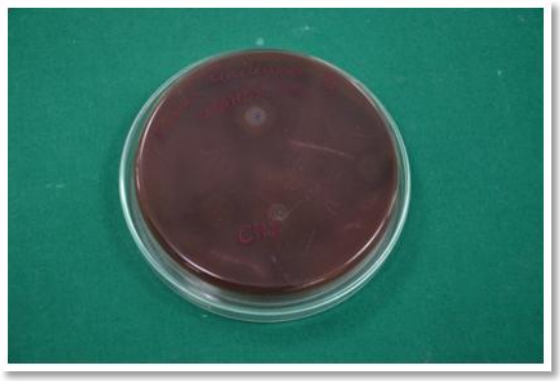


**Figure 1: Agar diffusion: *Porphyromonas gingivalis.***

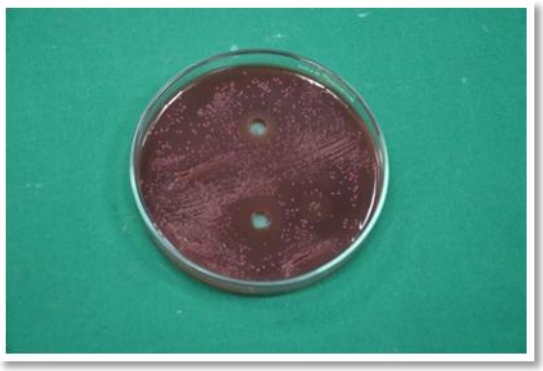


**Figure 2: Agar Diffusion *Aggregatibacter Actinomycetemcomitans.***

## RESULT

Mean values of microbial growth inhibition (in mm) produced by Octanidine Dihydrochloride and Chlorhexidine Digluconate sterile water are shown in graph no 1 & 2.

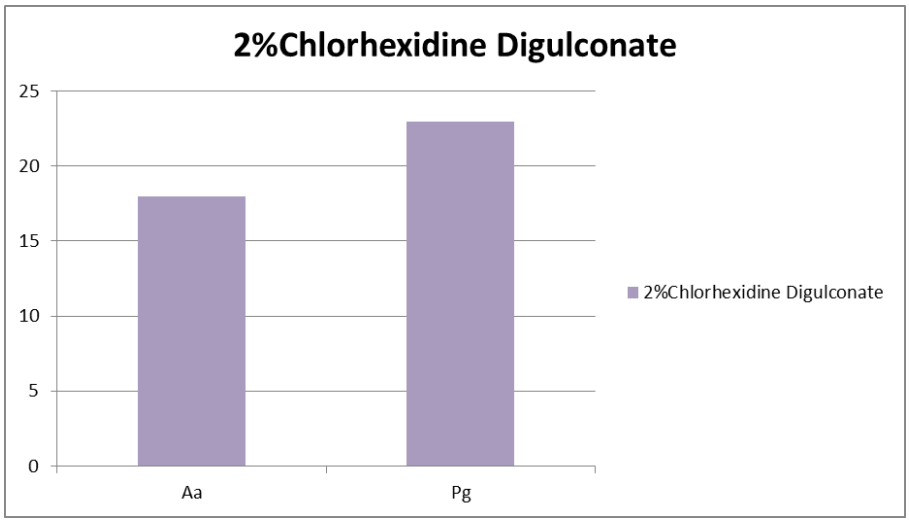
However, 2% CHX alone demonstrated the strongest antimicrobial action, showing the largest inhibitory growth zones, for ***Aggregatibacter Actinomycetemcomitans*** is 18 mm and for ***Porphyromonas gingivalis*** is 23mm, whereas 0.1 % Octanidine Dihydrochloride demonstrated the strongest antimicrobial action, showing the largest inhibitory growth zones, for ***Aggregatibacter Actinomycetemcomitans*** is 12 mm and for ***Porphyromonas gingivalis*** is 18 mm.

The 2% CHX alone required up to 1 minute to eliminate the tested microorganisms and the smallest zone of

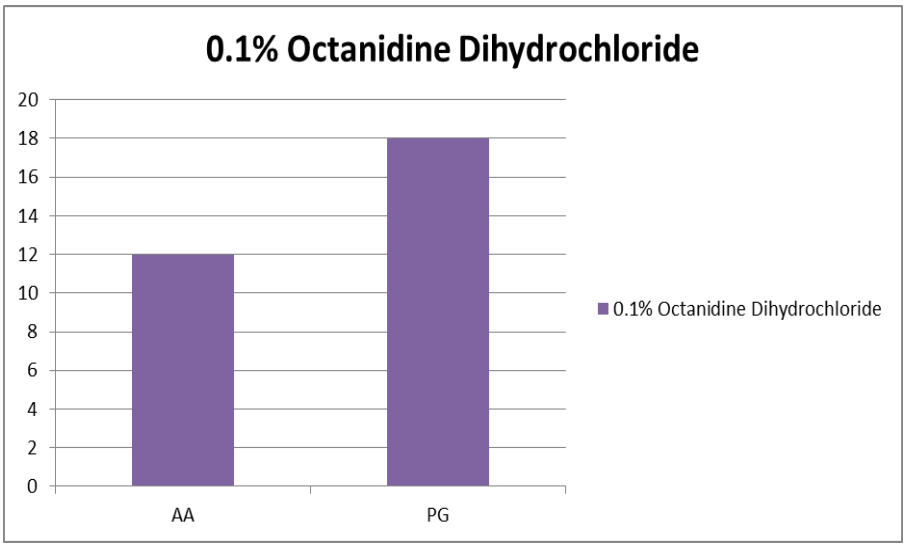
inhibition measured 4.33 mm. OCT 0.1% CHX gel required up to 6 hours to eliminate the tested microorganisms and the smallest zone of inhibition growth measured 3.83 mm.

Strict anaerobes (***Porphyromonas gingivalis***) were the most susceptible microorganisms, showing the largest inhibition zones, which ranged from 0 to 21.67 mm.

The facultative anaerobes (***Aggregatibacter Actinomycetemcomitans***) were more resistant to all medicaments used, producing inhibition zones ranging from 0 to 9.67mm.




**Graph no. 1: Mean values of microbial growth inhibition (in mm) produced by Chlorhexidine Digluconate on *Porphyromonas gingivalis* and *Aggregatibacter Actinomycetemcomitans.***




**Graph no.2: Mean values of microbial growth inhibition (in mm) produced by Octanidine Dihydrochloride on *Porphyromonas gingivalis* and *Aggregatibacter Actinomycetemcomitans.***

## DISCUSSION

In this study, the antibacterial effect of OCT on **Porphyromonas gingivalis** and **Aggregatibacter Actinomycetemcomitans** was evaluated and compared with that of CHX. In the present study, all the tested solutions significantly reduced the microorganisms in a period of 3 minutes whereas 0.1% OCT less effective than CHX. CHX is a broad-spectrum antimicrobial agent, that can be used effectively as a mouthwash, and irrigant, disinfect the dentinal tubules and be absorbed into the dentin. Several researchers have pointed out the potential advantages of CHX as an antimicrobial medicament in endodontic therapy. Chlorhexidine is a positively-charged molecule that binds to the negatively-charged sites on the cell wall; it destabilizes the cell wall and interferes with osmosis. The bacterial uptake of the chlorhexidine is very rapid, typically working within 20 seconds. In low concentrations, it affects the integrity of the cell wall. Once the cell wall is damaged, chlorhexidine then crosses into the cell itself and attacks the cytoplasmic membrane (inner membrane). Damage to the cytoplasm's delicate semipermeable membrane

allows for leakage of components leading to cell death. In high concentrations, chlorhexidine causes the cytoplasm to congeal or solidify. The accumulation of supragingival dental plaque on tooth surfaces is the major etiological component in the development of caries and periodontal disease. Numerous reports have documented that the flora change from primarily Gram-positive to Gram-negative bacteria in conditions leading to gingivitis.[20,21] Even though several other studies have tested the effects of CHX[22-24] and polyvinylpyrrolidone-iodine complex PVP-I,[25] there are only a few studies that tested OCT[26–30] to evaluate its contribution to oral hygiene by determining its effects on the number of total and cariogenic bacteria during its usage time.

In the study ***Dorgan et al 2009*** [35], ***Streptococccus mutans*** was reduced significantly with CHX ($P \leq .05$) on T1 and T2 periods and began to increase on-time period T3. Significant reduction in ***Streptococccus mutans*** levels was similar to the results of studies in which CHX varnish[31–34] and mouth rinse[22-25] were used, but the increase in ***Streptococccus mutans*** count on the third day opens the lasting antibacterial effect of CHX to debate.

***Beiswanger et al 1990*** [36] also have reported that the group rinsing with 0.1% OCT, had significantly lower levels of plaque, less gingivitis, and fewer bleeding sites when compared with the control group.
***Slee and O'Connor 1983*** [37] found comparably favorable effects of OCT compared with CHX on ***Streptococccus mutans, Streptococcus sanguis, Actinomyces viscosus,*** and ***Actinomyces naeslundii*** concerning overall antiplaque potency in vitro, whereas ***Samet et al 2006*** [38] found that the kinetics of OCT in killing ***Staphylococcus aureus*** depended on its concentration but was independent of bacterial genotype.

The characterization of different antiplaque agents by both ***Addy 1986*** [39] and ***Kornman 1981*** [40] in terms of persistence or substantivity can now be modified; persistence per se is not enough for antiplaque activity. Chlorhexidine's superior antiplaque effect can be explained in terms of its superior degree of persistence at the tooth surface or, more correctly, its superior persistence of antibacterial effect (both bactericidal and bacteriostatic) at the tooth surface.

Chlorhexidine has a wide spectrum of activity encompassing gram-positive and gram-negative bacteria, yeasts, dermatophytes, and some lipophilic viruses.[41] Its antimicrobial activity is of the membrane-active type, used to describe an antimicrobial agent that damages the inner (cytoplasmic) membrane.

Chlorhexidine may reduce the salivary bacterial counts - a single rinse with chlorhexidine can reduce the oral flora by over 90% for several hours[42] many millions of bacteria present in the saliva and on the oral surfaces are still not affected. As the oral cavity cannot be sterilized, there must be a continual challenge to the tooth surface by bacteria that can begin the process of plaque formation. As the salivary bound chlorhexidine patently has not eradicated putative plaque-forming bacteria, it would seem logical therefore to assume that the process of plaque prevention occurs at the tooth surface itself - by tooth-bound chlorhexidine.

In this present study, antimicrobial properties of Chlorhexidine Digluconate and Octanidine Dihydrochloride were compared with the help of the Agar Diffusion method.

With the regard to the Agar Diffusion method, Chlorhexidine Digluconate was shown more microbial inhibition effect compared to Octanidine Dihydrochloride.

Followed by Octanidine Dihydrochloride which failed to reach their efficacy microbial inhibition after 24 hr. The maximum values for CHX were ~ 3 times higher.
In summary, concerning its high antimicrobial effect as well as its biocompatibility, 2% CHX represents a promising antimicrobial activity against ***Porphyromonas gingivalis*** and ***Aggregatibacter Actinomycetemcomitans.***

## CONCLUSION

In conclusion, our study support, this investigation of antimicrobial efficacy under standardized
and harmonized conditions allows the user to choose the most efficacious agent.

For indications such as plaque control and treatment of periodontal infections, where a prolonged contact time for antimicrobial treatment is feasible, the following ranking for the investigated antiseptic agents regarding their effective microbicidal concentration was set:
OCT >CHX. Thus, by understanding the properties and limitations of the chlorhexidine molecule, the dental profession can ensure that the efficacy of the agent is maximized, and the side effects associated with the agent are minimized, allowing chlorhexidine to rightly remains the gold standard against which other antiplaque agents are measured.

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