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*Short Communication*

# Inhibition of Cetylpyridinium Chloride against the Growth of *Porphyromonas gingivalis*

Ernie M Setyawatie[1]*, Irma J Savitri[1], Nova A Hepitaria[2], Ferlina DAYP Asano[2], Gusti IA Agung Putra[2], Esi Y Fitrina[2], Rizka Valentina[3]

[1]*Departement of Periodontology, Faculty of Dental Medicine, Universitas Airlangga, 60132, Surabaya, Indonesia*
[2]*Periodontology Specialist Study Program, Faculty of Dental Medicine, Universitas Airlangga, 60132, Surabaya, Indonesia*
[3]*Dental Medicine Education Study Program, Faculty of Dental Medicine, Universitas Airlangga, 60132, Surabaya, Indonesia*

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ABSTRACT

Periodontal disease is a prevalent dental and oral health issue within the community, typically commencing with symptoms of gingivitis and potentially advancing to periodontitis. *Porphyromonas gingivalis* is an obligate anaerobic Gram-negative bacterium that is the primary aetiological agent in developing periodontal disease. One strategy to avert periodontal disease is the utilization of antibacterial medicines such as cetylpyridinium chloride. This study seeks to evaluate the inhibitory efficacy of cetylpyridinium chloride against *Porphyromonas gingivalis*. The *in vitro* inhibitory efficacy of *cetylpyridinium* chloride against *Porphyromonas gingivalis* was assessed using the colony count method and compared to the positive control, Chlorhexidine. The percentage of inhibition for each concentration was determined to ascertain the minimal inhibitory and bactericidal concentrations. The minimum inhibitory concentration of cetylpyridinium chloride was 92.66% at a concentration of 0.05% and >99.9% at 0.06%. Cetylpyridinium chloride effectively inhibits the development of *Porphyromonas gingivalis* bacteria at a concentration of 0.05% with a MIC value of 92.66%. These results indicate that cetylpyridinium chloride shows significant antibacterial activity against *Porphyromonas gingivalis*, suggesting its potential as an effective agent in preventing and managing periodontal disease.

***Keywords***: Periodontal disease, *Cetylpyridinium chloride*, *Porphyromonas gingivalis*, Minimum inhibitory concentration, Bacterial colonization.

## Introduction

Periodontal disease is an inflammatory pathological illness affecting the gingiva and its supporting structures, including the cementum, alveolar bone, periodontal ligament, and gingiva.[1,2] *Porphyromonas gingivalis* is a principal causal agent in the formation and progression of inflammatory episodes of periodontal disease. *Porphyromonas gingivalis*, an obligate anaerobic Gram-negative bacterium, exhibits various virulence factors, including collagenase, fimbriae/pili, lipopolysaccharide (LPS), gingipain, capsule, protease, superoxide dismutase. These components are essential in the initial phases of periodontitis, releasing metabolites and toxic chemicals that might damage periodontal tissues.[13] Alongside dental treatments, there are other accessible alternatives for avoiding periodontal disease, including mechanical measures such as brushing and flossing and chemical ones, including toothpaste and mouthwash containing antimicrobial agents [4,5]. Most mouthwash formulations comprise key active chemicals that serve as antimicrobials, including cetylpyridinium chloride, povidone-iodine, and chlorhexidine. [6,7]

*Corresponding author. Email: ernieperiodonsia@gmail.com
Tel.: + (031) 5030255

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Chlorhexidine is regarded as the gold standard antibacterial agent for periodontal disease treatment; however, its long-term use is not recommended due to adverse effects such as tooth and tongue discolouration, parageusi*a*, xerostomia, irritation, altered taste, burning sensations from its alcohol content, and hypersensitivity of the oral mucosa. [8,9,10] Cetylpyridinium chloride (CPC), a broad-spectrum antibacterial derived from quaternary ammonium cationic biocide compounds, is one of the principal active ingredients commonly present in commercial mouthwashes. [11] CPC is considered a safe and effective antibacterial agent with little adverse effects, making it a viable alternative for reducing plaque-forming bacteria and preventing periodontal disease. [8,12,9] This study aimed to assess the inhibitory efficacy of cetylpyridinium chloride against *Porphyromonas gingivalis.*

## Materials and Methods

This research is an experimental laboratory study employing a post-test-only control group design. The *Porphyromonas gingivalis* bacteria samples included in this investigation were divided into 13 categories: one negative control group, one positive control group, and ten treatment groups. Each group was administered three repeats of CPC concentrations: 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, and 0.1%.

*Preparation and intervention of Porphyromonas gingivalis*

Brain Hearth Infusion-Broth (BHI-B) media was utilized for culturing *Porphyroons gingivalis* bacteria. The media was dissolved in 1 L of distilled water in an Erlenmeyer flask until homogeneous and then autoclaved for fifteen minutes at 121ºC. Bacterial colonies cultivated on agar media were extracted with a sterile oese needle, transferred to BHI-B media in a test tube, and incubated for 24 hours at 37ºC to produce a suspension of *Porphyromonas gingivalis* bacteria.[13]



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*Minimum Bactericidal and Minimum Inhibitory Concentration Test*
The BHI-B media in test tubes 1–10, corresponding to treatment groups, contain CPC concentrations of 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, and 0.1%. The negative control group utilized BHI-B and bacteria, the positive control group employed BHI-B and CHX, while the media control consisted only of BHI-B 0.1 mL of bacterial suspension was introduced into each test tube, excluding the medium control tube, and the tubes were thereafter incubated for two 24-hours intervals at 37ºC. 0.1 mL of suspension from each tube was collected and disseminated onto the MHA medium. The Mueller Hinton Agar (MHA) medium was subsequently incubated for two consecutive 24-hour intervals at 37ºC. A tally of the colonies on MHA media was subsequently performed.[13]

*Statistical Analysis*
The Shapiro-Wilk normality test, Levene's homogenity test, and the One-Way Anova parametric test were utulized for data analysis using SPSS IBM 25 Software, 2017.

## Results and Discussion

Chlorhexidine (CHX) is widely employed as a positive control and regarded as the gold standard for oral antiseptics due to its superior efficacy in inhibiting pathogens responsible for periodontal disease and reducing the plaque index. The antibacterial mechanism of chlorhexidine, a bis-biguanide derivative, closely resembles that of cetylpyridinium chloride: the positively charged molecule of chlorhexidine interacts with the negatively charged bacterial membrane, penetrating the cell membrane and ultimately resulting in bacterial death. Chlorhexidine exhibits a broad-spectrum antimicrobial action against Gram-positive and Gram-negative bacteria, functioning as both bactericidal and bacteriostatic.[14, 15] Two concentrations of chlorhexidine are available: 0.12% and 0.2%. Research indicates that gargling with 0.12% chlorhexidine (CHX) exhibits significantly lower antibacterial efficacy than 0.2% CHX; nevertheless, the 0.2% concentration is reported to have a higher incidence of side effects than the 0.12% concentration. [16]
This study indicates that *Porphyromonas gingivalis* bacteria did not proliferate in MHA media containing Cetylpyridinium Chloride at concentrations of 0.06% to 0.1%. Conversely, *Porphyromonas gingivalis* bacteria proliferated at concentrations ranging from 0.05% to 0.01%. Figure 1 illustrates the fraction of inhibition of *Porphyromonas gingivalis* bacterial growth (Table 1). *Porphyromonas gingivalis* locally infiltrates periodontal tissues by producing several virulence factors. This enables the bacteria to evade the host's defence mechanisms and disrupt the innate immunological and inflammatory responses, fostering an environment favourable to the pathogen's survival and ultimately leading to the onset of periodontal disease. [1718]

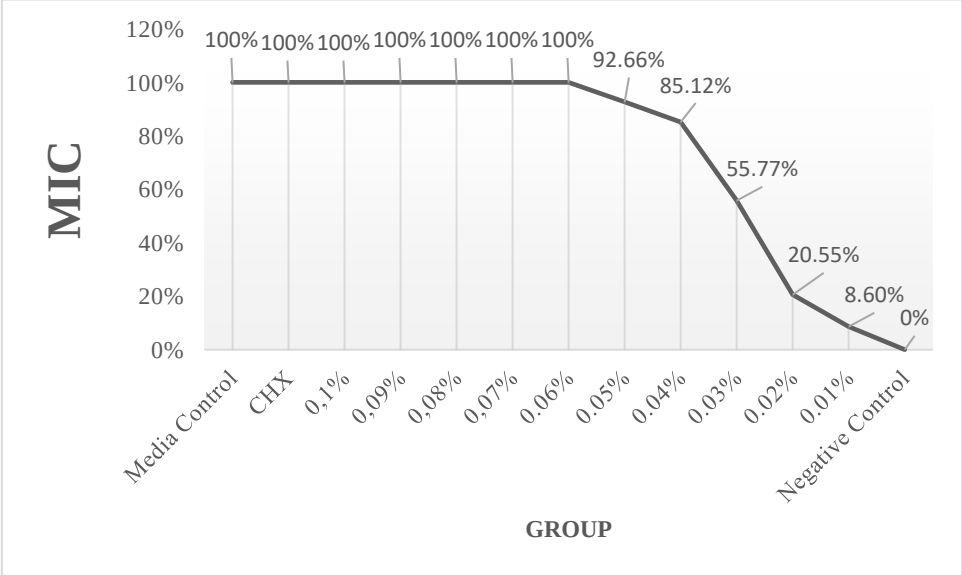


**Figure 1:** Growth inhibition of *Porphyromonas gingivalis* showing the MIC

Cetylpyridinium chloride (CPC), a quaternary ammonium compound, is a broad-spectrum antibiotic effective against Gram-positive and Gram-negative bacteria.[19] The cationic group in CPC facilitates attachment to the negatively charged bacterial cell membrane, resulting in altered permeability and membrane damage, which causes the leakage of cellular components and ultimately leads to cell death. [20, 21]
In the negative control group, the Shapiro-Wilk test, employed in this work to assess normality, yielded significance values over 0.05 ($p$>0.05) for concentrations of 0.05%, 0.04%, 0.03%, 0.02%, and 0.01%. Levene's test produced a p-value of <0.05, signifying that the data about the number of colonies in each sample group is inconsistent. The One-Way ANOVA test was utilized as the parametric analysis, accompanied by a Games-Howell post-hoc test because the data was normally distributed yet not homogeneous. The ANOVA test findings indicated a substantial disparity in the formation of *Porphyromonas gingivalis* bacterial colonies across the sample groups, with a significance value of 0.000 ($p$ < 0.05) (Table 2). The One-way ANOVA test produced a significant $p$<0.05 (0.000), demonstrating that Cetylpyridinium Chloride influenced *Porphyromonas gingivalis* inhibition by 0.06%. The Games-Howell follow-up test results for CPC, at concentrations of 0.05%, 0.04%, 0.03%, 0.02%, and 0.01%, indicate significant differences ($p$<0.05) across all sample groups, including CHX as a positive control. Compared to CHX, CPC concentrations of 0.06%, 0.07%, 0.08%, 0.09%, and 0.1% exhibited no significant differences. Cetylpyridinium chloride (CPC) demonstrates bactericidal efficacy at concentrations ranging from 0.05% to 0.1%. Concentrations beyond that level may harm humans, and its antibacterial efficacy against pathogenic oral bacteria diminishes below that threshold.[12] Researchers use these sources to determine the Minimum Inhibitory Concentration (MIC), which is 0.05% of the concentration of CPC and exhibits an inhibition percentage exceeding > 90% (MIC 90). Determining the optimal KHM value is essential as it will impact and modify the therapeutic efficacy of the antimicrobial's application. [22] Previous studies established that CPC can suppress the proliferation of many oral pathogenic bacteria, including *Porphyromonas gingivalis*, corroborating this study's findings. [23]


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**Table 1:** Result of growth inhibition of *Porphyromonas gingivalis*

| Sample Group | Colony Count (CFU/mL) | | | Inhibition |
|---|---|---|---|---|
| | Replication I | Replication II | Replication III | |
| Negative Control group (K-) | 156 | 163 | 158 | 0% |
| Positive control group (CHX) | 0 | 0 | 0 | 100% |
| Media control | 0 | 0 | 0 | 100% |
| 0.01% concentration | 141 | 151 | 144 | 8.60% |
| 0.02% concentration | 112 | 131 | 136 | 20.55% |
| 0.03% concentration | 64 | 69 | 78 | 44.77% |
| 0.04% concentration | 26 | 20 | 25 | 85.12% |
| 0.05% concentration | 12 | 13 | 10 | 92.66% |
| 0.06% concentration | 0 | 0 | 0 | 100% |
| 0.07% concentration | 0 | 0 | 0 | 100% |
| 0.08% concentration | 0 | 0 | 0 | 100% |
| 0.09% concentration | 0 | 0 | 0 | 100% |
| 0.1% concentration | 0 | 0 | 0 | 100% |

**Table 2.** A table comparing the differences between groups that follow a normal distribution

| **ANOVA** | | | |
|---|---|---|---|
| **Variable** | **df** | **F** | **Sig.** |
| Colonization | 12 | 654.973 | 0.000 |

Alcohol-free CPC at a dose of 0.075% may prevent oral pathogenic microorganisms by up to 99.9% [24]. Alcohol-free 0.05% CPC can inhibit 24 supragingival plaque bacteria by up to 90%. This study also indicated chlorhexidine effectively inhibited bacterial growth by > 98%. [9] This indicates that the study's results align with previous research, particularly that a reduced bacterial presence occurs due to CPC's ability to effectively bind and concentrate the negative charge of bacteria within the cell membrane, thereby influencing permeability and inhibiting bacterial proliferation through cell death.[25, 26] CPC can be formulated without alcohol and has fewer detrimental dental and mucosal discolouration effects. This study suggests that cetylpyridinium chloride could be an alternative antibacterial ingredient in mouthwash formulations. [15]

## Conclusion

Cetylpyridinium chloride exhibits a Minimum Inhibitory Concentration (MIC) of 92.66% at 0.05% and a Minimum Bactericidal Concentration (MBC) of 100% at 0.06%, effectively inhibiting the growth of *Porphyromonas gingivalis.*

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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