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Minimum Inhibitory Concentration of Chlorhexidine and Cetylpyridinium Chloride against a Mixture of Two Species of Oral Streptococci

So Yeon Lee[1] and Si Young Lee[1*]

[1]Department of Oral Microbiology, Research Institute of Oral Science, College of Dentistry, Gangneung-Wonju National University, Gangneung, 210-702, Korea.

Authors' contributions

This work was carried out in collaboration between both authors. Author So Yeon Lee performed experiments and wrote the first draft of the manuscript. Author Si Young Lee designed the study, managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Background and Objectives: Although bacteria in plaques are present as a mixed population comprising various species, mechanisms underlying differences in susceptibility between the mixed population of bacteria and each individual bacterium to antimicrobial agents is yet unknown. In this study, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of chlorhexidine and cetylpyridinium chloride were determined against various streptococci isolated from the human oral cavity. Then, changes in susceptibility of planktonic bacteria to chlorhexidine and cetylpyridinium chloride were investigated by mixing each of the bacteria in different combinations.

Materials and Methods: MIC and MBC were measured by the micro-dilution method according to the standards recommended by Clinical and Laboratory Standards Institute (CLSI).

Results: The MIC and MBC values of cetylpyridinium chloride against each bacterium tended to be high or more than the high susceptibility values for the two mixed bacteria in all combinations. Most of the MIC and MBC values of chlorhexidine against individual bacterium were higher than those

**Corresponding author: E-mail: siyoung@gwnu.ac.kr;*

against the mixtures of two bacteria. However, in some combinations, susceptibility values for two mixed bacteria were low or lesser than the low values for the individual bacterium.
Conclusion: When two antimicrobials were applied to mixed bacteria, cetylpyridinium chloride was observed to inhibit the growth of all combinations, with higher MIC and MBC values, whereas chlorhexidine was observed to inhibit the growth to varying degrees, with a different MIC and MBC values.

Keywords: Cetylpyridinium chloride; chlorhexidine; minimum inhibitory concentration; minimum bactericidal concentration; viridans streptococci.

1. INTRODUCTION

Bacteria in plaque are present in mixed populations that contain a variety of species, but the mechanism to account for differences in susceptibility to antimicrobials between mixed populations and individual bacteria is not yet known. When bacteria are mixed, characteristics, such as their metabolism, are inevitably changed, depending on their interactions with each other. Studies on the susceptibility of a single bacterium to antimicrobial agents have been well documented, but those on the effect of antimicrobial agents on a mixed bacterial population are lacking.

Chlorhexidine and cetylpyridinium chloride are generally used in dentistry and have been reported to be effective antimicrobial agents [1-5]. Chlorhexidine introduces negative charges on the bacterial surface and is reported to damage the cytoplasm and cell membrane [6]. Cetylpyridinium chloride is an effective amphipathic compound and also exerts antimicrobial activity by introducing negative charges on bacterial surfaces [7-9]. Cetylpyridinium chloride is also reported to destruct lipid bilayers in cell membranes, resulting in the leakage of bacterial contents [10,11].

Generally, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) evaluations are performed to determine the sensitivity of bacteria toward an antimicrobial agent. MIC of an antimicrobial agent is defined as the minimum concentration of the antimicrobial agent required to inhibit the growth of bacteria, and MBC is defined as the minimum concentration at which 99.9% of the bacteria are killed [12,13].

MIC and MBC are measured against individual bacteria. However, in the oral cavity, the bacteria exist in a mixed state, and there is no study on how the MIC and MBC of the bacteria change with the MIC and MBC of the individual bacteria in the mixed state. In this study, MIC and MBC of chlorhexidine and cetylpyridinium chloride were determined against various streptococci isolated from the oral cavity. Further, the differences in susceptibility of planktonic bacteria to chlorhexidine and cetylpyridinium chloride were investigated by mixing each bacterium in different combinations.

2. MATERIALS AND METHODS

2.1 Selection of Bacteria

From the bacterial stock list of isolated oral streptococcal strains available in the Department of Oral Microbiology, Gangneung-Wonju National University, strains were selected for preparing a mixed bacterial population in this experiment. Bacterial strains were selected as bacteria with various MIC results with relatively high or low MIC values. The selected strains are shown in Table 1. The selected bacteria were mixed, with two strains in each combination.

2.2 Determination of MIC and MBC of Antimicrobial Agents against Mixed Bacterial Population

Chlorhexidine (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and cetylpyridinium chloride (Sigma-Aldrich Chemical Co.) was used and diluted in Brain Heart Infusion (BHI) broth (Becton, Dickinson and Company, Sparks, MD, USA) to prepare a concentration of 1000 μg/ml. To investigate the sensitivity of mixed bacteria to chlorhexidine and cetylpyridinium chloride, MIC was determined using the micro-dilution method according to the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) [14]. For preparing the mixed bacterial population, the concentration of the bacterial suspension was adjusted to 0.5 McFarland (1×10^8 CFU/ml), and the mixture was used such that the combined concentration of the two bacteria was

Table 1. Selection of bacteria for determining the susceptibility of a mixed bacterial population to chlorhexidine and cetylpyridinium chloride

Antimicrobial agent	Species	Strain	MIC (μg/ml)	MBC (μg/ml)
Chlorhexidine	*S. mitis*	KN602	7.8125	31.2500
		KN506	1.9531	15.6250
	S. mutans	KN529	0.4883	7.8125
		KN615	0.9766	15.6250
	S. salivarius	KN470	0.9766	3.9063
		KN292	1.9531	1.9531
Cetylpyridinium chloride	*S. mitis*	KN509	0.2441	0.9766
		KN506	0.4883	0.9766
	S. mutans	KN531	0.2441	0.9766
		KN529	0.2441	0.9766
	S. oralis	KN515	0.1221	0.9766
		KN527(2)	0.1221	0.4883

5×10^5 cells/ml. The antimicrobial agent was diluted serially in a 96-well plate (SPL Life Sciences, Pocheon-si, Gyeonggi-do, Korea) and the mixed bacterial population was inoculated. After incubation in 5% CO_2 incubator at 37℃ for 18 hours, the turbidity was visually observed and the minimum concentration at which the growth of the bacteria was inhibited was determined as the MIC. After determining the MIC, the bacterial culture solution along with the antimicrobial agent at concentrations same or more than the MIC was applied to a blood agar plate (Hanging, Gunpo-si, Gyeonggi-do, Korea), and the concentration at which 99.9% of the bacteria were killed was determined as the MBC. MIC and MBC values were determined at least twice. If different results were observed, determination of MIC and MBC has repeated twice again.

3. RESULTS

The results for the mixed bacterial population were divided into the following 5 groups:

a. Group 1: The susceptibility values of the mixed bacterial population were higher than those of the two individual bacteria.
b. Group 2: The susceptibility values of the two bacteria were low.
c. Group 3: Following the average susceptibility values of the two bacteria.
d. Group 4: The susceptibility values were lower than the low values for the two bacteria.
e. Group 5: The susceptibility values were higher than the high values for the two bacteria.

The MIC and MBC values for the mixed strains are shown in Figs. 1 and 2. MIC and MBC values of combinations showed frequently in group 1 (group 1 - the susceptibility values of the mixed bacterial population were higher than those of the two individual bacteria), which showed a higher susceptibility value for the mixed population than that for the individual bacteria. The MIC and MBC values of cetylpyridinium chloride in two groups—group 1 and group 5 (group 5 - the susceptibility values were higher than the high values for the two bacteria) — showed higher susceptibility value for the mixed population for the individual bacteria.

4. DISCUSSION

Chlorhexidine susceptibility studies have been carried out on oral streptococci, but there are not many studies on susceptibility to cetylpyridinium chloride. In some studies, MICs for clinical isolates of *S. mutans* and *S. sobrinus* in chlorhexidine were reported to range from 0.25 ~ 1 μg/ml and 1 ~ 2 μg/ml, respectively [15]. The MIC for chlorhexidine was about 0.5 ~ 1 μg/ml in the case of *S. mutants* used in this study and showed a range of MIC values similar to the previous experiment. However, in other studies, MICs of chlorhexidine and cetylpyridinium chloride for *S. mutans* were observed to be 3 μg/ml and 2 μg/ml, respectively [16]. In this study, the MIC for cetylpyridinium chloride was measured at 0.2 μg/ml, and our results showed lower MIC values than these studies.

When two bacteria with different susceptibility values are mixed, it is generally considered that the susceptibility value of their mixture will follow the high value of MICs and MBCs of two individual bacteria. In the present study, the MIC

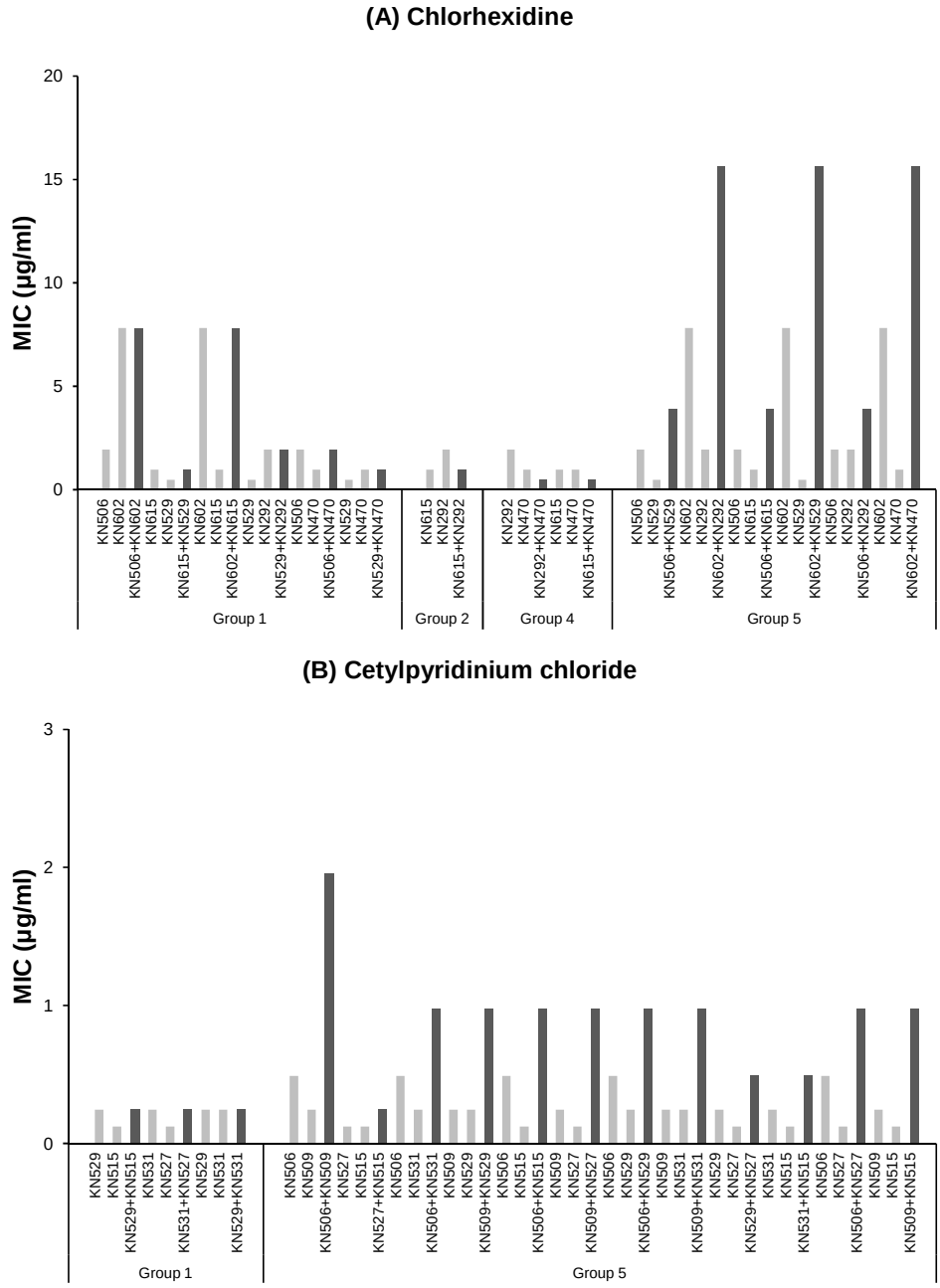

Fig. 1. MIC values of chlorhexidine and cetylpyridinium chloride before and after mixing bacteria

and MBC values of cetylpyridinium chloride followed high or more than the high susceptibility values in all combinations. The MIC and MBC results for chlorhexidine followed higher susceptibility values between the two bacteria. In addition, other results, such as those following the low or lesser than the low susceptibility value were also observed.

The reason for change of MIC and MBC in a mixture of bacteria from individual bacteria in the planktonic state is not clear. It is assumed that each bacteria in the mixed state might affect the other bacteria in the mixture. Also, the bacterial coaggregation of two bacteria could affect the MIC and MBC of mixed bacterial state. The further studies will be needed for the reason of change of MIC and MBC in a mixture of bacteria.

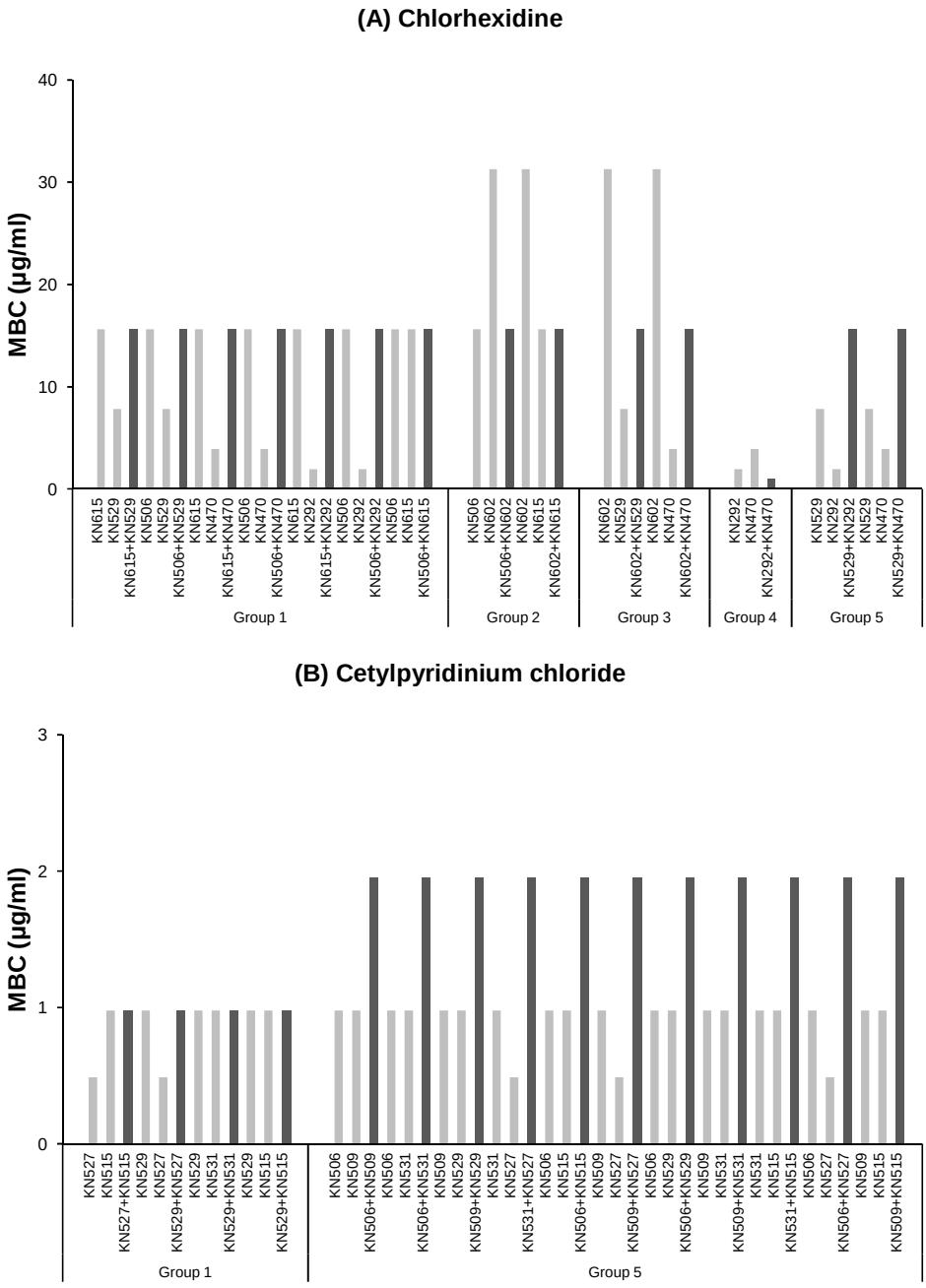

Fig. 2. MBC values of chlorhexidine and cetylpyridinium chloride before and after mixing bacteria

Bacteria in the oral cavity are present in mixed dental plaques. There has been no study yet on what biological changes in the biofilm affect the bacterial MIC and MBC. In this study, the effects of mixing of planktonic bacteria on MIC and MBC were investigated. However, in the oral cavity, bacteria are present as biofilms, which are a mixture of various bacteria. Based on the results of this study showing the effect of planktonic bacterial mixing on MIC and MBC, the effect of bacterial mixing on biofilm condition on MIC and MBC should be studied.

We observed the changes in susceptibility of streptococci isolated from the human oral cavity to chlorhexidine and cetylpyridinium chloride

when they were present in a mixed bacterial population compared with that when they were present as individual bacteria. Further, the interaction between the bacteria in the mixture should be clarified.

5. CONCLUSION

This study concludes that the susceptibility of the mixed bacterial population to antimicrobial agents can change in various ways.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Addy M, Moran J. The effect of a cetylpyridinium chloride (CPC) detergent foam compared to a conventional toothpaste on plaque and gingivitis. A single-blind crossover study. J Clin Periodontol. 1989;16:87-91.
2. Jenkins S, Addy M, Newcombe RG. A comparison of cetylpyridinium chloride, triclosan and chlorhexidine mouthrinse formulations for effects on plaque regrowth. J Clin Periodontol. 1994;21:441-4.
3. Jenkins S, Addy M, Wade W, Newcombe RG. The magnitude and duration of the effects of some mouthrinse products on salivary bacterial counts. J Clin Periodontol. 1994;21:397-401.
4. Steinberg D, Moldovan M, Molukandov D. Testing a degradable topical varnish of cetylpyridinium chloride in an experimental dental biofilm model. J Antimicrob Chemother. 2001;48:241-3.
5. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. J Clin Periodontol. 1988;15:488-98.
6. Hope CK, Wilson M. Analysis of the effects of chlorhexidine on oral biofilm vitality and structure based on viability profiling and an indicator of membrane integrity. Antimicrob Agents Chemother. 2004;48:1461-8.
7. Sreenivasan PK, Haraszthy VI, Zambon JJ. Antimicrobial efficacy of 0.05% cetylpyridinium chloride mouthrinses. Lett Appl Microbiol. 2013;56:14-20.
8. Myc A, Vanhecke T, Landers JJ, Hamouda T, Baker JR,Jr. The fungicidal activity of novel nanoemulsion (X8W60PC) against clinically important yeast and filamentous fungi. Mycopathologia. 2002;155:195-201.
9. Ramalingam K, Amaechi BT, Ralph RH, Lee VA. Antimicrobial activity of nanoemulsion on cariogenic planktonic and biofilm organisms. Arch Oral Biol. 2012;57:15-22.
10. Sutherland IW. Biosynthesis and composition of gram-negative bacterial extracellular and wall polysaccharides. Annu Rev Microbiol. 1985;39:243-70.
11. Ioannou CJ, Hanlon GW, Denyer SP. Action of disinfectant quaternary ammonium compounds against *Staphylococcus aureus*. Antimicrob Agents Chemother. 2007;51:296-306.
12. Lorian V. Medical relevance of low concentrations of antibiotics. J Antimicrob Chemother. 1993;31:137-48.
13. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The calgary biofilm device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol. 1999;37:1771-6.
14. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 8th ed. CLSI. Wayne, PA; 2009.
15. Jarvinen H, Pienihakkinen K, Huovinen P, Tenovuo J. Susceptibility of *Streptococcus mutans* and *Streptococcus sobrinus* to antimicrobial agents after short-term oral chlorhexidine treatments. Eur J Oral Sci. 1995;103:32-5.
16. Kitagawa H, Izutani N, Kitagawa R, Maezono H, Yamaguchi M, Imazato S. Evolution of resistance to cationic biocides in *Streptococcus mutans* and *Enterococcus faecalis*. J Dent. 2016;47:18-22.

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