Original Research Article

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

ABSTRACT

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 human oral cavity. Then, changes in susceptibility of planktonic bacteria to chlorhexidine and cetylpyridinium chloride were investigated by mixing each of the bacterium in different combinations. 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 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. 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 varied degrees, with different MIC and MBC values.

Keywords: viridans streptococci, chlorhexidine, cetylpyridinium chloride, minimum inhibitory concentration, minimum bactericidal concentration

1. INTRODUCTION

Although bacteria in plaques are present as a mixed population comprising various species, the mechanism underlying the differences in susceptibility between the mixed population of bacteria and each individual bacterium to antimicrobial agents is yet unknown. 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 [1-3]. Chlorhexidine introduces negative

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Backgroud and Objectives
Materials and Methods
Results
Conclusion

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charges on the bacterial surface and is reported to damage the cytoplasm and cell membrane [4]. Cetylpyridinium chloride is an effective amphipathic compound and also exerts antimicrobial activity by introducing negative charges on bacterial surfaces [5]. Cetylpyridinium chloride is also reported to destruct lipid bilayers in cell membranes, resulting in the leakage of bacterial contents [6,7].

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 [8].

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 of the bacterium in different combinations.

2. MATERIAL 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. The selected strains are shown in Table 1. The selected bacteria were mixed, with two strains in each combination.

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

	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

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.) were 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

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the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) [9]. 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 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 (Hangang, 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 was repeated twice again.

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3. RESULTS AND DISCUSSION

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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 Figures 1 and 2. MIC and MBC values of combinations showed frequently in group 1, which showed a higher susceptibility value for mixed population than that for the individual bacteria. The MIC and MBC values of cetylpyridinium chloride in two groups—group 1 and group 5—showed higher susceptibility value for the mixed population for the individual bacteria.

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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 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.

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The reason of change of MIC and MBC in mixture of bacteria from individual bacteria in planktonic state is not clear. It is assumed that each bacteria in mixed state might affect the other bacteria in 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 mixture of bacteria.

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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.

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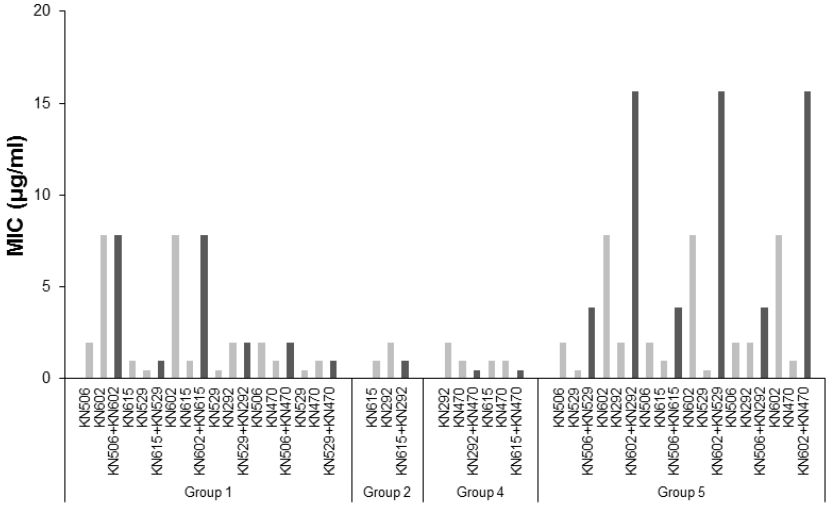
(A) Chlorhexidine



(B) Cetylpyridinium chloride

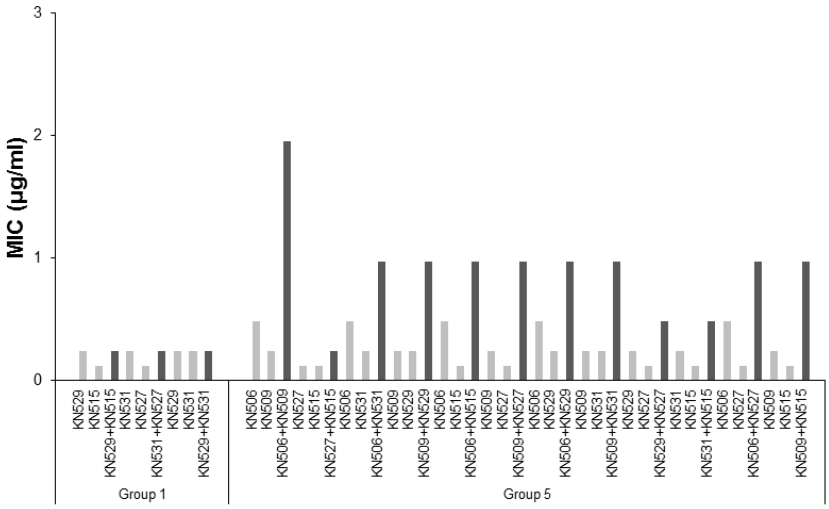
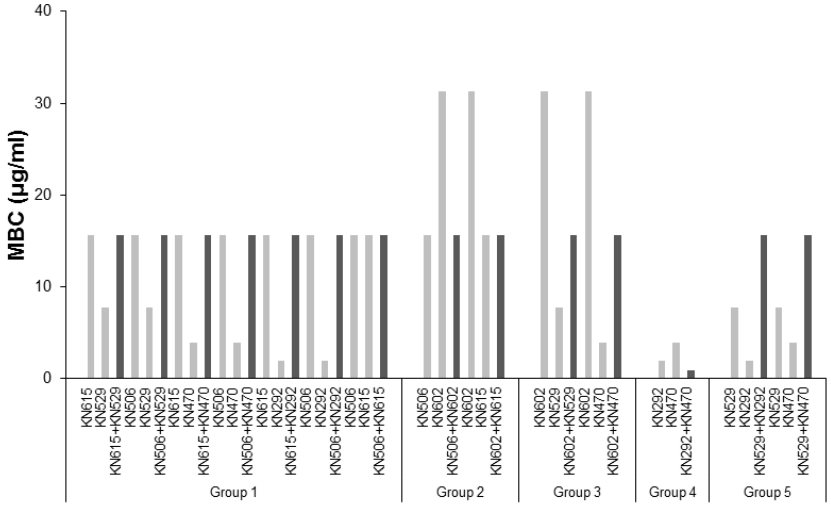


Fig 1. MIC values of chlorhexidine and cetylpyridinium chloride before and after mixing bacteria.

(A) Chlorhexidine



(B) Cetylpyridinium chloride

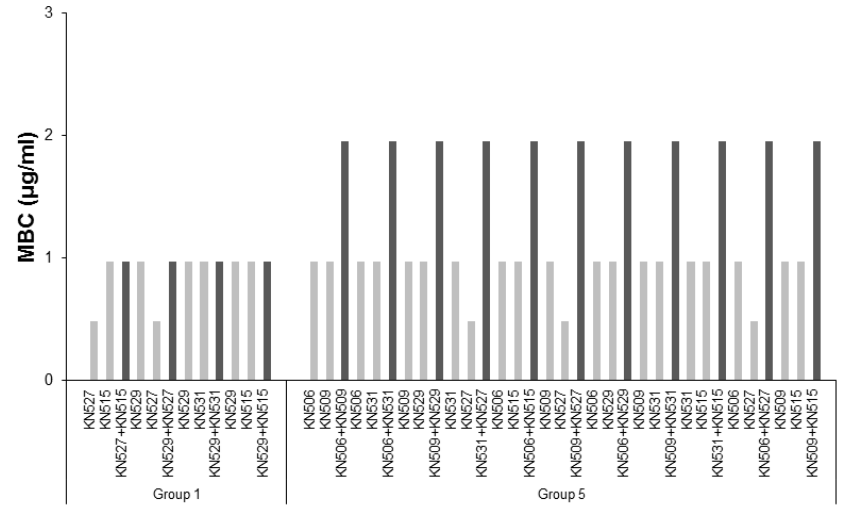


Fig. 2. MBC values of chlorhexidine and cetylpyridinium chloride before and after mixing bacteria.

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4. CONCLUSION

In this study, we found that the susceptibility of mixed bacterial population to antimicrobial agents can change in various ways.

5. COMPETING INTERESTS

The authors declare that there are no competing interests.

6. REFERENCES

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