The minimum inhibitory concentration and exposure time of the combination of quaternary ammonium compound with ethylendiamintetraacetic acid (EDTA) against methicillin-resistant Staphylococcus aureus

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ABSTRACT

The aim of this study was to determine The Minimum Inhibitory Concentration (MIC) and the exposure time of the combination of quaternary ammonium compound with ethylendiamintetraacetic acid (EDTA) towards Methicillin-resistant Staphylococcus aureus so that the combination can be applied as a disinfectant against MRSA.The tested bacteria MRSA was isolated from the RSGM of the Faculty of Dentistry Padjadjaran University. This laboratory experimental study was conducted based on a serial dilution method. The combination of quaternary ammonium compound with EDTA was diluted in 1/1000, 1/2000, 1/4000, 1/8000, 1/16000, 1/32000, 1/64000, and 1/128000 concentration.The result showed that the combination of quaternary ammonium compound with EDTA inhibited the MRSA on 1/1000, 1/2000 and 1/4000 concentrations. The effective exposure time to reduce the number of MRSA colonies are 2.5 and 3 minutes with 1/1000 concentration from the combination. In conclusion that the MIC of the combination of quaternary ammonium compound with EDTA is in 1/4000 concentration and the effective exposure time is more than 3 minutes.

Key words: The combination of quaternary ammonium compound with EDTA, MRSA, MIC, exposure time

INTRODUCTION

MRSA was initially found in United Kingdom in 1961 after meticillin is used as the medicine for *Staphylococcus aureus* infection. MRSA now becomes endemic in hospitals. In 2004 in United Kingdom, 100,000 people came to the hospital with MRSA infection and 5,000 of them died. MRSA appears due to prolonged broad spectrum antibiotic use.[1-2] MRSA is a bacterium that is resistant to poor environment, can live for months in dry condition, in 7.5-10% NaCl and resistant to heating with a temperature of 50^0 C for 30 minutes.

Quaternary Ammonium has stronger antibacterial property for Gram positive bacteria compared to Gram negative bacteria. This chemical also has sporostatic property.[3] In basic environment, the bactericide property increases and will disappear totally in acid environment of pH < 3. The quaternary ammonium activity can be stopped by anion elements such as soap and organic elements, especially protein and pus, certain ionic elements, calcium and metals such as ferrum, alluminum and magnesium.[4]

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EDTA is an antibacteria material against Gram negative bacteria but not Gram positive bacteria.[5-8] EDTA is often added to detergent and has a synergic effect that the combination of quartenary ammonium and EDTA can give maximum effect as disinfectant.[9]

The aim of this study is to determine antibacteria power of the combination of quartenary ammonium and EDTA towards MRSA by determining the Minimum Inhibitory Concentration (MIC) and the contact time.

This study is also aimed at using combination of quartenary ammonium and EDTA to be used as disinfectant in managing MRSA spread.

The study was performed from February to July 2007 in Microbiology Laboratory, Faculty of Dentistry, University of Padjadjaran, Jatinangor.

METHODS

This study is a laboratory experiment study to analyze antibacterial activity of the combination of quartenary ammonium and EDTA towards MRSA by determining the Minimum Inhibitory Concentration (MIC) and contact time.

The instruments and materials used include experimental tubes, experimental tube holder, petri dish, innoculator, pipette, incubator, sterile cotton bud, microscope, object glass, markers, glass pencil, ruler and filter paper, combination of quartenary ammonium and EDTA (Corium 95 consists of 30% quartenary ammonium, 60% EDTA, 5% sodium metasilicate, and 5% materials added by PT Magna), glucose bullion slanted blood agar, LAB, LAD, and Gram staining materials. The sample consists of isolated swab from the instruments of the Oral and Dental Hospital, Faculty of Dentistry, UNPAD Bandung.

The study procedure consists of sampling and culturing, isolation and MRSA identification, making specimen suspension, determining Minimum Inhibitory Concentration (MIC) and contact time.

The Minimum Inhibitory Concentration (MIC) is used to determine the lowest concentration of combination of quaternary ammonium and EDTA needed to inhibit test bacterial growth based on serial dilution method.

The combination of quaternary ammonium and EDTA was diluted 1000 times to get standard solution. Three test tubes were marked A, B, and C and were filled with 4.5 ml sterile glucose bullion. Combination of quaternary ammonium and EDTA, 0.5 ml, was added to tube A and then the tube was shaken until homogenous solution was gained and the concentration would be 10^{-1}. From tube A, 0.5 ml solution was transferred to tube B and shaken to homogeneity. From tube B, 0.5 ml was transferred to tube C to get a standard solution with a concentration of 10^{-3}.

Ten test tubes were then marked 1-10. Tube 1 was filled by 4 ml dilution of combination of quaternary ammonium and EDTA from tube C. Tube 2-8 were filled with 2 ml of bullion, and then from tube 1, 2 ml was taken and transferred to tube 2 which was then shaken to homogeneity to get a concentration of 1/2000. Two ml was taken tube 2 and transferred to tube 3 and was shaken to homogeneity to get a concentration of 1/4000. The same method was applied to tube 4, 5, 6, 7, 8. Tube 9 as the negative control was filled by 2 ml bullion from tube 8. Tube 10 as a positive control was filled with bullion plus 0.1 ml test bacteria suspension. In tube 1-8, 0.1 ml test bacteria was given in a same way as tube 10.

The smallest concentration of combination quaternary ammonium and EDTA that shows inhibition towards test bacteria growth is the minimum inhibitory concentration (MIC) of the solution towards the tested bacteria.

The turbidity of the culture that shows the test bacteria growth is sometimes hardly visible by naked eyes. Therefore, to assure that the growth happens, 1 innoculator of the tube material was taken from each tube and then cultured sectorally on LAB which is then incubated in 37°C for 18-24 hours. When there is a bacteria growth on the LAB, it is stated that by that concentration the bacteria growth can be inhibited. Repetition was done three times using the same method.

After the MIC was determined, the contact time test was performed by diluting the combination of quaternary ammonium and EDTA solution according to MIC, 2 x MIC and 4 x MIC. And then, into the tube, 0.1 ml MRSA bacterial suspension with Mc Farhland turbidity of 0.5 was filled and cultured immediately on the LAB that had been divided into several sectors for different contact time. This manipulation was performed for 0.5 minute, 1 minute, 1.5 minutes, 2 minutes,

2.5 minutes and 3 minutes contact time.

On the next day, the bacterial growth was observed on the LAB that showed the least colony growth in the shortest contact time according to the length of someone's habit of washing hand was chosen by the most effective contact time. The Minimum Inhibitory Concentration (MIC) and contact time was analyzed using *Kruskal-Wallis* statistical test.

RESULTS AND DISCUSSION

Turbidity in determining MIC of the combination of quaternary ammonium and EDTA is seen starting from tube 5 or dilution of 1/16000. The MRSA growth is proven through examination using sectoral culturing on LAB. The result of incubation shows that sector one and two was not colonized by MRSA and that the colonies were found in sector three to eight. In sector nine, i.e. the negative control, there was no MRSA while in sector ten as the positive control, MRSA growth was found.

Based on sectoral MIC examination result for the combination of quaternary ammonium and EDTA, it is apparent that in a concentration of 1/2000 (tube 2) and 1/1000 (tube 1) there is no MRSA growth against the three test bacteria. The MRSA starts to grow in a concentration of 1/4000 (tube 3), so that it can be stated that the MIC is in the concentration of ≤ 1/4000.

Based on this study, it can be said that the combination of quaternary ammonium and EDTA has good antibacterial power to kill MRSA. The combination of quaternary ammonium and EDTA has very low MIC towards MRSA, i.e. 1/4000. The low concentration shows that the effectiveness of the combination quaternary ammonium and EDTA is high enough and that this combination can be used as a more economic disinfectant compared to other disinfectants.

In its utilization as disinfectant, to reduce the possibility of unexpected dilution, the best concentration of quaternary ammonium and EDTA is 4 x MIC (1/1000) with more than 3 minutes time. The unexpected dilution may be caused by the residual rinsing water that stay on the instrument to be cleaned. A concentration of 1/1000 is in line with the statement of Lawrence and Block[11], that stated the recommended quaternary ammonium concentration that will not trigger skin irritation is between 1/1000 and 1/5000.

CONCLUSION

Based on the study results it can be concluded that: The effective Minimum Inhibitory Concentration (MIC) of the combination of quaternary ammonium and EDTA towards MRSA is 1/4000. The effective contact time of the combination of quaternary ammonium and EDTA towards MRSA is more than 3 minutes.

SUGGESTION

In the application of the combination of quaternary ammonium and EDTA, it is better to use a concentration of 1/1000 (4 x MIC). A further study is needed to see the effect of the combination of quaternary ammonium and EDTA as instrument disinfectant, especially in dentistry. A further study is needed to study other bacteria often found in hospitals. It is necessary to do further research with longer contact time.

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