

BENZALKONIUM CHLORIDE AND PINE OIL-CONTAINING CLEANING FLUID IS NOT EFFECTIVE AGAINST *Pseudomonas Aeruginosa*

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ABSTRAK

Infeksi nosokomial merupakan salah satu penyebab utama meningkatnya morbiditas dan mortalitas di rumah sakit. Pseudomonas aeruginosa termasuk dalam salah satu yang paling tiga bakteri yang menyebabkan infeksi oportunistik pada manusia termasuk infeksi nosokomial. WHO pada tahun 2002 telah menerbitkan pedoman pencegahan infeksi nosokomial, termasuk infeksi oleh Pseudomonas aeruginosa. Salah satu tindakan pencegahan adalah menjaga lingkungan rumah sakit yang bersih, baik makroskopis dan mikroskopis. Salah satu langkah untuk mencegah infeksi nosokomial adalah lingkungan rumah sakit yang bersih dengan menggunakan bahan rutin membersihkan lantai, dinding, kamar mandi, dll Di antara banyak merek bahan pembersih lantai zat aktif antimikroba yang paling banyak digunakan adalah benzalkonium klorida dan minyak pinus. Oleh karena itu peneliti sedang meneliti efektivitas pembersih lantai cairan yang mengandung benzalkonium klorida dan minyak pinus. Tujuan dari penelitian ini adalah untuk mengetahui perbandingan efektivitas antara pembersih lantai cairan yang mengandung antimikroba benzalkonium zat klorida dan minyak pinus terhadap penelitian Pseudomonas aeruginosa. This adalah penelitian eksperimental yang benar yang dilakukan metode modifikasi difusi di laboratorium mikrobiologi. Pembersih lantai cairan yang digunakan adalah membersihkan lantai agen yang mengandung 450 ml dari 1,5% benzalkonium klorida yang kemudian diencerkan sesuai dengan aturan penggunaan yang tertera pada kemasan dan membersihkan lantai agen yang mengandung 450 ml minyak Pine 2,5% kemudian diencerkan sesuai dengan aturan penggunaan yang tertera pada kemasan. Kemudian bahan-bahan pembersih lantai ditempatkan pada sumur yang telah dibuat pada cawan agar yang telah ditanami Mueller Hinton bakteri Pseudomonas aeruginosa dengan metode goresan. Agar piring dan kemudian dimasukkan ke dalam inkubator, dan mengamati pembentukan bakteri zona hambatan pertumbuhan sekitar sumur. Hasil penelitian menunjukkan bahwa tidak ada zona hambatan pertumbuhan bakteri terbentuk di sekitar sumur yang telah memberikan cairan pembersih lantai di dalamnya. Dalam kesimpulan, lantai cairan pembersih yang mengandung zat antimikroba apakah benzalkonium klorida atau minyak pinus tidak terhadap resiko yang efektif Pseudomonas aeruginosa. (FMI 2012;48:121-125)

Kata kunci: cairan pembersih lantai, *Pseudomonas aeruginosa*, benzalkonium chloride, minyak pinus, difusi

ABSTRACT

Nosocomial infection is one of the leading cause of the increasing of morbidity and mortality in the hospital. Pseudomonas aeruginosa is included in one of the most three bacteria that cause opportunistic infections in human including nosocomial infections. WHO in 2002 has issued guidelines prevention of nosocomial infections, including infections by Pseudomonas aeruginosa. One preventive measure is to maintain a clean hospital environment, both macroscopically and microscopically. One of the measures to prevent nosocomial infection is a clean hospital environment by using materials routinely cleaning floors, walls, bathrooms, etc. Among the many brands of floor cleaner ingredients antimicrobial active substances most widely used is benzalkonium chloride and pine oil. Therefore the researcher are examining the effectiveness of floor cleaning fluid which contain benzalkonium chloride and pine oil. The objective of this study was to know the effectiveness comparison between the floor cleaner fluid which is containing antimicrobial substance benzalkonium chloride and pine oil against Pseudomonas aeruginosa. This research is true experimental research which is done diffusion modification method in the microbiology laboratory. Floor cleaner fluid being used is floor cleaning agents containing 450 ml of 1.5% benzalkonium chloride which was then diluted in accordance with the rules of usage indicated on the packaging and floor cleaning agents containing 450 ml of 2.5% Pine oil is then diluted in accordance with the rules the use indicated on the packaging. Then the floor cleaning materials are placed on wells that have been made on agar plates that had been planted with Mueller Hinton bacterium Pseudomonas aeruginosa with streaking method. In order for the plate and then put into an incubator, and observed the formation of bacterial growth inhibition zones around the wells. Results revealed that no bacterial growth inhibition zone was formed around the wells which has given floor cleaning fluid therein. In conclusion, the floor cleaning fluid which is containing antimicrobial substance whether benzalkonium chloride or pine oil are not effective againts Pseudomonas aeruginosa. (FMI 2012;48:121-125)

Keywords: floor cleaning fluid, *Pseudomonas aeruginosa*, benzalkonium chloride, pine oil, diffusion.

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INTRODUCTION

Nosocomial infections are a major cause of morbidity and in-hospital mortality (World Health Organization 2002). The increase in the number of events is a reflection of the health care services that are less good. Nosocomial infections can be caused by naturally occurring microorganisms are pathogens that exist around the patient and also by the normal flora that exist in the patient's body. This can occur due to a decrease in the patient's immune system (immunocompromised condition). Increased incidence of nosocomial infections caused by two factors: the condition of the patient's body resistance decreases and diagnostic measures and treatments that are invasive, that should be acceptable to the patient (World Health Organization 2002). As the installation of endoscopy equipment, catheters, and ventilators which can lead to the entry of microorganisms into the patient's body.

Based on the results of a survey conducted by the American Medical Association in the intensive care (Intensive Care Unit) in seventeen Western European countries stated that from 4501 patients treated infections in intensive care, 45.9% of them suffer from nosocomial infections, the most frequent were pneumonia (46.9%), and lower respiratory tract infections (17.8%), urinary tract infection (17.6%), and bloodstream infection (12%). Pneumonia due to the incidence of nosocomial infections has 3% per day of the total number of patients in hospitals (World Health Organization 2002). 28.7% of nosocomial infections occurring in intensive care in Western Europe in 2002 caused by the bacterium *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa is a gram-negative bacterium that produces toxins which damage the tissue. These microorganisms are ubiquitous, especially in damp areas (Brooks et al 2005) and includes one of the three bacteria most likely to cause opportunistic infections in man including nosocomial infection (Stover et al 2000). In these bacteria infect hospital patients who experienced a decrease in endurance. Some examples of these are bacterial infections: urinary tract infections, such as pneumonia, respiratory system, motion system of the body such as the bones and joints, gastrointestinal digestive system, bacteremia, dermatitis, and other systemic infections. Bacteria spread from one patient to another patient by hospital staff, direct contact with a patient - a contaminated (reservoir) or through food and beverages ingested by the patient.

WHO (2002) has issued a guide to the prevention of nosocomial infections, including infection by *Pseudomonas aeruginosa*. One precaution is to keep the

hospitals clean environment both macroscopically and microscopically. It is important to do because it is different with a very invasive surgery but do indoors right - its sterility guarded with UV light while invasive procedure that many causes of nosocomial infections such as catheters and ventilators much done in the intensive care are not sterilizing process perfect as the operating room.

Almost all floor cleaning materials on the market today have the words "to be antimicrobial" on the packaging, but its effectiveness in killing *Pseudomonas aeruginosa* located where - where and cause nosocomial infections needs to be proved further. Benzalkonium chloride and Pine oil is an antimicrobial active agent most widely used in floor cleaning agents but which demonstrate the effectiveness of the test and to compare the ability of floor cleaning materials with active substance Benzalkonium chloride or Pine oil in killing *Pseudomonas aeruginosa* has never been done, therefore it is necessary to effectiveness comparison test floor cleaning agents containing Benzalkonium chloride and Pine oil to determine and compare the ability of both to kill *Pseudomonas aeruginosa*. Expected later this research can be input to the hospitals and other health facilities in choosing the right floor cleaning agents that are effective for preventing nosocomial infections by *Pseudomonas aeruginosa*. The purpose of this study was to determine the effectiveness of floor cleaning materials containing Benzalkonium chloride and Pine oil in killing the bacteria *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

This study was an experimental study with laboratory observational study design to examine the effectiveness of an antimicrobial agent Benzalkonium chloride and Pine oil on floor cleaning materials in killing *Pseudomonas aeruginosa*, as well as comparison of the effectiveness of both. Samples were *Pseudomonas aeruginosa* obtained from the Laboratory of Microbiology, Faculty of Medicine, University of Airlangga. *Pseudomonas* bacterial suspension with a turbidity *aeruginosa* adjusted to the turbidity of 0.05 mL of a mixture of 1.175% solution of barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), and 9.95 mL of 1% sulfuric acid (H_2SO_4). While the antimicrobial compounds used Benzalkonium chloride Benzalkonium chloride is a compound contained in floor cleaning material. Benzalkonium chloride concentration used was 1.5% in 450 ml of floor cleaner diluted with sterile distilled water in accordance with the rules for the use indicated on the packaging material of the floor cleaner.

Antimicrobial compounds used Pine oil Pine oil is a substance contained in the floor cleaning material. The concentration of pine oil used was 2.5% in 450 ml of floor cleaner diluted with sterile distilled water in accordance with the rules for the use indicated on the packaging material of the floor cleaner. Effect power to kill bacteria or bacteriostatic properties and bacteriocyte of Benzalkonium chloride and Pine oil looks of inhibition zone formed around the wells that contain one of the two solutions. Observation of the effect of the power to kill bacteria is done after the agar plate that has been planted bacteria were incubated for about 24 hours at 37 ° C. This research was conducted with sixteen times replication. Data from the control group and the treatment group were processed and tested statistically with SPSS method. The study was conducted at the Laboratory of Microbiology, Faculty of Medicine, University of Airlangga. The study was conducted from September 2011 to December, 2011.

RESULTS

From the comparison of the effectiveness of laboratory observation floor cleaning agents containing an antimicrobial agent and Pine Oil Benzalkonium chloride against *Pseudomonas aeruginosa* obtained the following results:

Table 1. Results of comparative diffusion test the effectiveness of floor cleaning materials containing an antimicrobial agent and Pine Oil Benzalkonium chloride against *Pseudomonas aeruginosa*

<i>Benzalkonium chloride</i>		<i>Pine oil</i>	
Replication	Bacterial Growth Inhibition Zone (mm)	Replication	Bacterial Growth Inhibition Zone (mm)
1	0	1	0
2	0	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	0	7	0
8	0	8	0
9	0	9	0
10	0	10	0
11	0	11	0
12	0	12	0
13	0	13	0
14	0	14	0
15	0	15	0
16	0	16	0
Control (+)	0	Control (+)	0
Control (-)	0	Control (-)	0

Of the 16 replication conducted to compare the effectiveness of floor cleaning materials containing antimicrobial Benzalkonium chloride and pine oil against *Pseudomonas aeruginosa* invisible bacteria growth inhibition zone formation. Pitting around the

place where the antimicrobial look more bluish green indicates that the growth of the bacteria *Pseudomonas aeruginosa* more rapidly in the area.

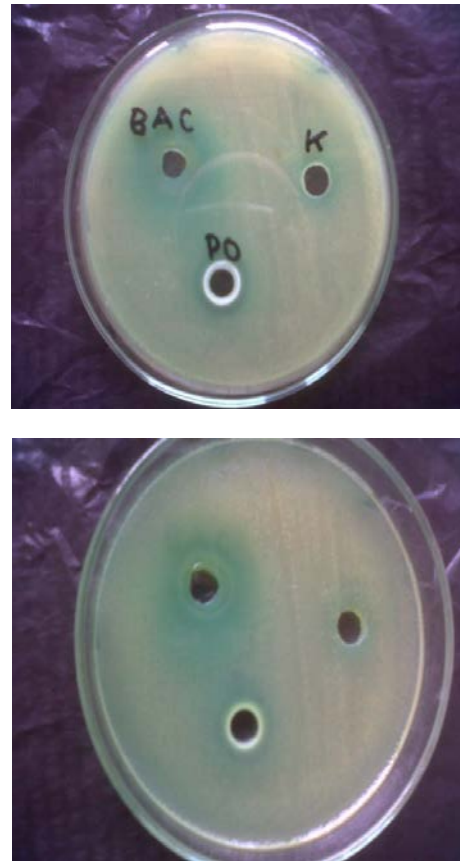


Figure 1. The results of comparative effectiveness diffusion test floor cleaning agents containing Benzalkonium chloride and pine oil seen from two sides Muller Hinton To Plate.

DISCUSSION

Biocide benzalkonium chloride is the main working mechanism through activity-dependent intrinsic detergent concentrations (ranging from 0.004 to 0.02% on products on the market). Its bactericidal/microbicidal mechanism is to damage (disruption) intermolecular interactions of microorganisms causing the dissociation of the bilayer membrane of cells that cause disruption of the permeability and cause leakage of cell contents, biomolecular other cells can also undergo dissociation, most enzymes that play a role in the mechanism of cell respiration and metabolism are also vulnerable undergo deactivation.

Some research has been done previously by experts have proved the effectiveness of Benzalkonium chloride in killing bacteria, among which the study reported by Tebbs and Elliott (1993), research conducted by placing the catheter hose Benzalkonium chloride has been given over the nutrient agar inoculated with various first previous types of bacteria, the results seem formation after incubation of bacterial growth inhibition zone around the catheter against five strains of *Staphylococcus epidermidis* and two strains of *Staphylococcus aureus*, but it also formed a smaller zone of inhibition against gram-negative rod bacteria and *Candida albicans*.

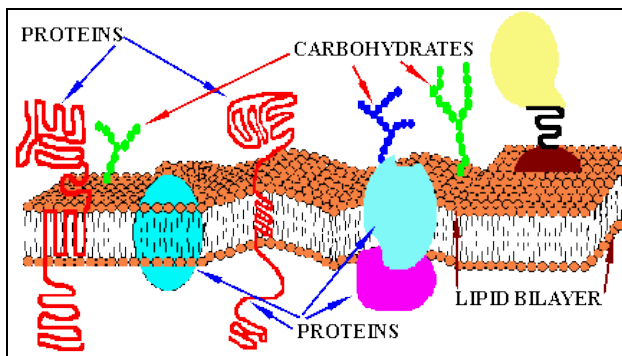


Figure 2. Bacterial Bilayer Membrane

Gainor et al (1997) revealed the results of their study, the comparison of the results of bacterial cultures were obtained from swabs of beef that had been flushed with Benzalkonium chloride by culturing bacteria from swabs of beef that has been irrigated with normal saline. Negative bacterial culture results obtained from beef that is irrigated with Benzalkonium chloride, while the use of normal saline showed the opposite.

Conroy et al (1999) also reported the results of a similar study with two previous studies. Conroy et al (1999) found a negative bacterial culture results derived from contaminated orthopedic wounds that have been irrigated with 0.03% Benzalkonium chloride. The two other cultures derived from contaminated orthopedic wounds were irrigated with bacitracin and use another one with Castile soap showed the opposite result.

The different results obtained from this study, sixteen after planting replication *Pseudomonas aeruginosa* on Mueller Hinton media made benzalkonium chloride pitting and added, in each preparation does not seem formation of bacterial growth inhibition zone. Shows that the floor cleaning agents containing Benzalkonium chloride that is currently circulating in the community did not effectively kill *Pseudomonas aeruginosa*.

Pine oil is a biocide that has the properties of disinfectants, sanitizers, microbicide/microbiostat, virucidal, and insecticides. According to the United States Environmental Protection Agency (2006) states that the pine oil effectively kill *Pseudomonas aeruginosa* by an unknown mechanism. Research reported by Herbert et al (1989) for US Patent Broad Spectrum Antimicrobial titled Hard Surface Cleaner System for in 1989 stated that Pine oil does not have a broad-spectrum anti-microbial properties but effective way to kill gram-negative bacteria and has the effect of synergism when combined with oil soluble organic acid that is able to kill gram-negative bacteria and is also positive. The different results obtained from this study, after study with sixteen replication planting *Pseudomonas aeruginosa* on Mueller Hinton media were given pitting and added pine oil, in each preparation does not seem formation of bacterial growth inhibition zone. It was shown that the floor cleaning agents containing Pine oil that is currently circulating in the community did not effectively kill *Pseudomonas aeruginosa*.

Based on the research that has been conducted to prove the effectiveness of the two active substances most widely used in floor cleaning agents against *Pseudomonas aeruginosa* in the prevention of nosocomial infection in the hospital, it is evident that they are not effective in killing *Pseudomonas aeruginosa*. The ineffectiveness of the active substance can be based on a variety of factors, including, *Pseudomonas aeruginosa* characteristics that proved resistant to weak antiseptic and some kinds of antibiotics. These bacteria are included in the three bacteria most likely to cause opportunistic infections in humans including nosocomial infection (Stover et al 2000). Possess unique properties so as to cause nosocomial infection incidence is quite high, this bacterium has a combination of natural resistance properties that exist in itself, the ability to acquire new resistance properties through mutations induced by a wide - range of treatment directed against him, and high enough role in causing a variety of serious infections. In fact, some isolates of *Pseudomonas aeruginosa* obtained at the hospital proved to be resistant to a variety of commonly used antibiotics (Livermore 2012).

In addition to the characteristics possessed by *Pseudomonas aeruginosa* as described above, the ineffectiveness of anti microbial active substances can also be caused by the concentration of an antimicrobial agent that is used (in accordance with the instructions listed on the packaging floor cleaning agents) have not reached the minimum concentration that gave inhibition or MIC (minimum Inhibitory Concentration), as expressed by McCay et al (2010) that the use of

biocides in subinhibitori levels can increase the resistance of *Pseudomonas aeruginosa*. The continuous use of the environment at a level mikrobiosida subinhibitori can also improve immunity against *Pseudomonas aeruginosa* antibiotics, this event is commonly called the cross-resistance.

CONCLUSION

Floor cleaning materials with active substance Benzalkonium chloride and Pine oil is not effectively kill the bacteria *Pseudomonas aeruginosa*.

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