International Journal of Latest Research in Science and Technology **ISSN (Online):2278-5299**
Volume 5, Issue 2: Page No. 98-101, March-April 2016
https://www.mnkpublication.com/journal/ijlrst/index.php

IN- VITRO ANTIMICROBIAL ACTIVITY OF FRAMYCETIN AND PARAFFIN WOUND DRESSING TO *METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS* (MRSA) AND *PSEUDOMONAS AERUGINOSA*

Yeva Rosana[1], Vanya Utami Tedhy[2], Indah Lestari[2]
[1]Microbiology Department, Faculty of Medicine University of Indonesia, Jakarta 10320
[2] Faculty of Medicine University of Indonesia, Jakarta 10430

Corresponding author:
Jl. Pegangsaan Timur No. 16, Jakarta 10320, Indonesia
Emial: yeva.rosana@ui.ac.id, Phone: (62-21) 3160492

Abstract - ***A good dressing provides good barrier against bacterial infection to promote natural wound healing. Framycetin and paraffin wound dressings have long been used to cover wounds to prevent infection. This study aims to determine in-vitro antimicrobial activity of framycetin and paraffin wound dressing to Methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa, multidrug resistant bacteria which are frequently found on superficial wound infection.***
In vitro study was conducted by exposing suspension of MRSA and Pseudomonas aeruginosa to framycetin and paraffin wound dressing. The suspension was diluted in ten time serial dilution. Plating on agar plates was done at exposure time of 0, ½, 2, 4, 6, and 24 hours. Antimicrobial activity of dressing is defined as its ability to inhibit bacterial growth.
The result showed that framycetin wound dressing had antimicrobial activity against MRSA at ½ to 24 hours exposure time with bactericidal effect at 4, 6, and 24 hours. Its antimicrobial activity against Pseudomonas aeruginosa was shown at 4, 6, and 24 hours. Paraffin wound dressing showed antimicrobial activity to both bacteria at 4 and 24 hours with addition of 6 hours for MRSA. Antimicrobial activity of framycetin wound dressing against MRSA and Pseudomonas aeruginosa were superior to paraffin wound dressing.

Keywords: Antimicrobial activity, Framycetin wound dressing, Methicillin-resistant Staphylococcus aureus (MRSA), Paraffin wound dressing, Pseudomonas aeruginosa

I. INTRODUCTION

Skin is the body's largest organ that plays an important role in body defense. Skin wound, such as burn wound, traumatic wound, and surgical wound can enable the entry of pathogenic bacteria [1,2]. *Methicillin resistant Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* are most common bacteria found on wound infection. Infection of these bacteria is hard to treat, contributed to their resistance against many antimicrobial agents [3,4].

Two important components of wound management are infection prevention and moisture retention which can be achieved by applying wound dressing impregnated with antimicrobial agents. These measures are important since wound infection can cause prolonged healing and moist environment is necessary for natural healing [5].

Wound dressing impregnated with framycetin have long been used in wound management to prevent infection. Framycetin is an aminoglycoside with broad spectrum activity. This antibiotic is effective against Gram positive cocci and Gram negative rods [6,7]. Antimicrobial activity of framycetin wound dressing has not been further evaluated while bacteria rapidly mutate and develop resistance. Therefore, this study is conducted to determine the antimicrobial activity against MRSA and *Pseudomonas aeruginosa*, which are the most common bacteria found on wound infection that have developed multi-drug resistant property.

II. MATERIAL AND METHODS

This research was experimental in-vitro. This study used dressing antimicrobial activity method which was adopted from Aramwit P et al. [8] with some modification. Bacteria used were *Methicillin resistant Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* those have been identified with the culture, Gram stain, and Vitek2 GN card®.

Dressing tested (paraffin dressing and dressing containing framycetin) cut into a size of 1 cm^2 were prepared in aseptic manner. Each of square was put into a different sterile tube. As many as 800 µL of distilled water was added to the tube containing dressing for pretreatment and allowed to stand for 10 minutes. Tryptone soy broth 2.2

Publication History
Manuscript Received : 23 April 2016
Manuscript Accepted : 28 April 2016
Revision Received : 29 April 2016
Manuscript Published : 30 April 2016

ml was then added to each tube containing dressing in order to obtain a total volume of 3 mL.

A suspension of each bacteria was prepared in broth from fresh colonies after overnight incubation. The turbidity of each bacterial suspension was adjusted to 0.5 McFarland standard (equivalent to 1.5 x 10^8 c.f.u (*Colony Forming Unit*)/mL). The concentration of bacterial suspension was measured using a nephelometer. Of the 10 µL aliquot bacterial suspension was put into the tube containing a solution of broth and dressing. This mixture was then incubated at 35° C in a shaking incubator. Control tube containing a broth with and without the bacteria were also prepared.

Of the 10 µL aliquots of the bacterial broth were taken from each tube at intervals of 0, ½ , 2, 4, 6 and 24 hours and serial 10-fold dilutions for each aliquot were prepared in broth. Of the 25 µL duplicate aliquots from each samples were spread on nutrient agar plate. The plates were then incubated overnight at 35 ° C and colonies counted (CFU/mL). Colonies that allowed to quantify were 10-150 colonies and the mean counts calculated. To obtain a mean value of CFU counts, plate counts were measured in duplicate and each experiment was repeated three times.

Antimicrobial activity was indicated by a reduction in average bacterial counts presented as $\log_{10}$ c.f.u/mL were compared to positive control [9]. The normal growth rate of each bacteria was represented by the growth control which contained no antimicrobial dressing. Data normality of the colonies number was assessed using the Shapiro Wilk test. Hypothesis testing was done using an unpaired T-test comparing the mean of the two groups if the data were normally distributed. Mann Whitney test was used if the data distribution was not normal. Statistical analysis was considered to be significant if p value was ≤0.05.

III. RESULT AND DISCUSSION

The results showed exposure with framycetin wound dressing gives the average number of MRSA colonies were decreased when compared to the positive control. The inhibitory effects of framycetin wound dressing against MRSA was obtained from an exposure time of ½ to 24 hours. The inhibitory effects of framycetin wound dressing is statistically significant at the time of exposure of 2, 4, 6 and 24 hours (p <0.05). The framycetin wound dressing has bactericidal effect to MRSA on the exposure time of 4, 6, and 24 hours as indicated by the average number of colonies of MRSA as 0 colony. The result is illustrated in Fig. 1 and the data for average number of colonies with standard deviation is presented in Table 1

The average number of MRSA colonies was exposure with paraffin wound dressing continues to increase at any time exposure. Paraffin wound dressing inhibited MRSA growth at an exposure time of 4, 6, and 24 hours. However, these results did not statistically significant (p> 0.05). It seems mechanism action of paraffin wound dressing through hydrophobicity properties could inhibit the growth of bacteria by binding to the bacterial cell surface hydrophobic structures [10,11]. MRSA has lower hydrophobic structures so that the bond between paraffin wound dressing with bacteria was not sufficiently effective to inhibit the growth of MRSA [12] .

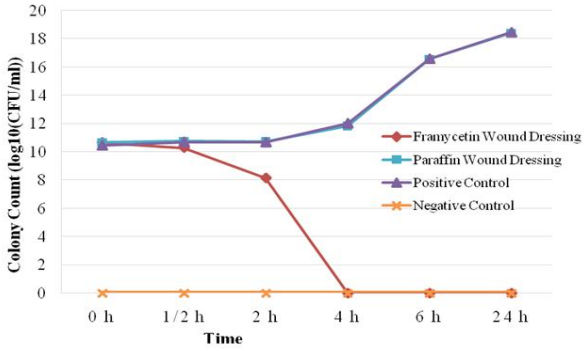

Fig. 1 Logarithmic Graph of MRSA Colony Count (CFU/mL) after Exposure with Framycetin and Paraffin Wound Dressing

The inhibition effect of framycetin wound dressing against MRSA better than paraffin wound dressing on *in-vitro* testing. The result showed the significant differences between the framycetin wound dressing compared to paraffin wound dressing at the exposure time of 2, 4, 6, and 24 hours (p <0.05). The result indicated that framycetin in wound dressing effectively inhibit the growth of bacteria while paraffin wound dressing was not contain antimicrobial could not inhibit the bacterial growth.

Table 1 Average number of MRSA Colonies (CFU/mL) after Exposure with Framycetin and Paraffin Wound Dressing

Time	Framycetin Wound Dressing	Paraffin Wound Dressing	Positive Control	Negative Control
0 h	$5{,}00\times10^{10}$ (SD $2{,}42\times10^{10}$)	4.17×10^{10} (SD $1{,}45\times10^{10}$)	3.00×10^{10} (SD $3{,}46\times10^{9}$)	0
1/2 h	$2{,}53\times10^{10}$ (SD $2{,}05\times10^{10}$)	5.87×10^{10} (SD $2{,}20\times10^{10}$)	5.33×10^{10} (SD $9{,}24\times10^{9}$)	0
2 h	$6{,}93\times10^{8}$ (SD $2{,}57\times10^{8}$)	5.27×10^{10} (SD $9{,}02\times10^{9}$)	5.07×10^{10} (SD $5{,}77\times10^{9}$)	0
4 h	0	7.47×10^{11} (SD $3{,}64\times10^{11}$)	1.18×10^{12} (SD $4{,}85\times10^{11}$)	0
6 h	0	3.60×10^{16} (SD $9{,}17\times10^{15}$)	4.07×10^{16} (SD $2{,}31\times10^{15}$)	0
24 h	0	2.56×10^{18} (SD $3{,}86\times10^{17}$)	3.00×10^{18} (SD 0)	0

Antimicrobial activity framycetin and paraffin wound dressing against *Pseudomonas aeruginosa* were tested at exposure time of 0, ½, 2, 4, 6, and 24 hours. Table (2) showed the number of colonies (CFU / ml) *Pseudomonas aeruginosa* after exposure with framycetin wound dressing, paraffin wound dressing, and control.

Table 2 Average Number of *Pseudomonas aeruginosa* (CFU/ml) after Exposure with Framycetin and Paraffin wound Dressing

Time	Framycetin Wound Dressing	Paraffin Wound Dressing	Positive Control	Negative Control
0 h	$1{,}26\times10^{10}$ (SD 5 x 10^{9})	$8{,}8\times10^{9}$ (SD 6,21 x 10^{9})	$9{,}07\times10^{9}$ (SD 2,19 x 10^{9})	0
1/2 h	$1{,}47\times10^{10}$ (SD 5,21 x 10^{9})	$1{,}11\times10^{10}$ (SD 6,12 x 10^{9})	$1{,}05\times10^{10}$ (SD 6,7 x 10^{9})	0
2 h	$2{,}78\times10^{10}$ (SD 3,81 x 10^{9})	$1{,}53\times10^{10}$ (SD 9,26 x 10^{9})	$1{,}41\times10^{10}$ (SD 2,89 x 10^{8})	0
4 h	$1{,}82\times10^{10}$ (SD 1,28 x 10^{10})	$1{,}71\times10^{11}$ (SD 1,21 x 10^{10})	$7{,}47\times10^{11}$ (SD 1,62 x 10^{11})	0
6 h	$8{,}67\times10^{10}$ (SD 7,26 x 10^{10})	$3{,}6\times10^{13}$ (SD 1,06 x 10^{13})	$2{,}57\times10^{13}$ (SD 7,51 x 10^{12})	0
24 h	$2{,}24\times10^{18}$ (SD 7,23x 10^{17})	$2{,}57\times10^{17}$ (SD 7,39 x 10^{16})	3×10^{18} (SD 0)	0

These results showed that the number of *Pseudomonas aeruginosa* colonies (CFU/ mL) after exposure with framycetin woun dressing were lower than the positive

control at 4, 6, and 24 hours. While the number of *Pseudomonas aeruginosa* colonies (CFU/ mL) after exposure with paraffin wound dressing were lower than the positive control at 4 and 24 hours. Normality test by test Shapiro Wilk showed normal distribution number of colonies (CFU / mL) in exposure with framycetin wound dressing for 1/2 , 2, 4, 6 and 24 hours, while the exposure with paraffin wound dressing for 1/2, 2, 4, and 6 hours. The unpaired T test was used for analysis of normal distribution data and Mann Whitney test was used for analysis of abnormal distribution data. The average number of *Pseudomonas aeruginosa* colonies (CFU/ mL) after exposure with framycetin wound dressing was significantly lower than the positive control and paraffin wound dressing at 4 and 6 hours.

Normalization of data is done by finding a delta value of the average number of colonies. Value delta (Δ) sought to reduce the number of colonies at a given time by the number of colonies at 0 minutes. Δ value of the average number of colonies used in the subsequent data analysis. Δ value of the average number of colonies at any time of the exposure is shown in Figure 2.

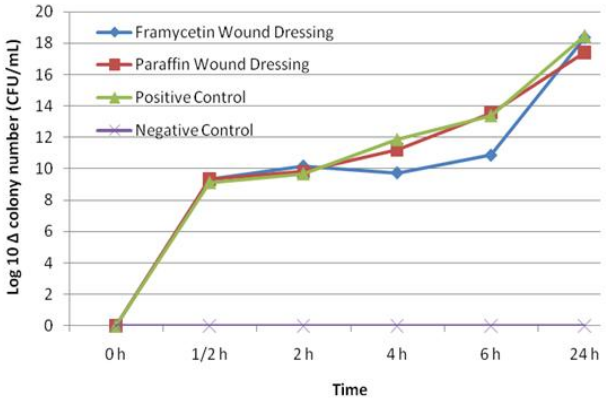

Fig. 2 Logarithmic Graph of *Pseudomonas aeruginosa* Colony Count (CFU/mL) after Exposure with Framycetin and Paraffin Wound Dressing

Statistical analysis showed a significant difference between framycetin wound dressing and others after 4 and 6 hours exposure. These results show that framycetin have antimicrobial activity against *Pseudomonas aeruginosa*. Maximal inhibition of framycetin wound dressing against *Pseudomonas aeruginosa* is 4 hours of exposure. The inhibition was be found at 4 hours exposure in accordance of the study by Aramwit P et al which states that the antimicrobial activity of the new dressing is shown in the incubation time of more than an hour [8]. Although Δ average number of *Pseudomonas aeruginosa* colonies increased at 6 hours exposure but it was significantly lower than the positive control. Thorn et al reported that this phenomenon can be caused by *Pseudomonas aeruginosa* can survive in unfavorable environmental conditions [13]. *Pseudomonas aeruginosa* is among the largest in the bacterial world allowing for great genetic capacity and high adaptability to environmental changes [14]. This is important challenge in the management of wound infection because of *Pseudomonas aeruginosa*.

On exposure to paraffin wound dressing, the number of *Pseudomonas aeruginosa* colonies increased from time to time. Δ the average number of colonies after 4 and 24 hours exposure to paraffin wound dressing was lower than the positive control. These results were statistically significant and showed inhibition of paraffin wound dressing to *Pseudomonas aeruginosa*. However, because of inhibition was demonstrated at exposure times that are not consecutive, it can not be explained whether this was due to the hydrophobic nature of paraffin wound dressing.

IV. CONCLUSION

From this study, it can be concluded that framycetin wound dressing have *in-vitro* antimicrobial activity against MRSA and *Pseudomonas aeruginosa*. *In-vitro* antimicrobial activity framycetin wound dressing against MRSA obtained from an exposure time of ½ hour (30 minutes). Statistical significance of inhibition against MRSA was obtained at an exposure time of 2, 4, 6, and 24 hours. Framycetin wound dressing have started killing power against MRSA at the time of exposure 4, 6, and 24 hours. Paraffin wound dressing have *in-vitro* antimicrobial activity against MRSA on exposure time of 4, 6, and 24 hours, but it was not statistically significance. Framycetin have inhibitory effect on the growth of MRSA in vitro better than paraffin wound dressing.

Framycetin wound dressing showed *in-vitro* antimicrobial activity against *Pseudomonas aeruginosa* at an exposure time of 4, 6, and 24 hours with optimal inhibition occurred after 4 hours of exposure. Framycetin wound dressing have in-vitro antimicrobial activity against *Pseudomonas aeruginosa* better than paraffin wound dressing at 4 to 6 hours of exposure.

ACKNOWLEDGMENT

We like to express sincere appreciation and deep gratitude to all participants in this study.

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