

# Comparison of the effectiveness of various topical treatment on the growth of bacteria in burns infection in vitro

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## Abstract

**Introduction:** Burns lead to significant morbidity due to infection risks. This study compares the effectiveness of petroleum jelly silver sulfadiazine and a combination of hyaluronic acid with silver sulfadiazine on bacterial growth common in burn infections in vitro.

**Methods:** An in vitro experimental design was utilized, involving cultures of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Staphylococcus aureus*. The study assessed the inhibitory effects of the treatments on bacterial growth using the Kirby-Bauer disk diffusion method.

**Results:** Results indicated varying levels of bacterial growth inhibition by the treatments, with significant differences observed between the treatments' effectiveness on gram-positive and gram-negative bacteria. The combination of hyaluronic acid and silver sulfadiazine showed superior inhibitory effects on gram-positive bacteria, while silver sulfadiazine alone was more effective against gram-negative bacteria.

**Discussion:** The study suggests the importance of treatment selection based on bacterial type in burn infections. The combination of hyaluronic acid and silver sulfadiazine may offer a better therapeutic option for gram-positive bacterial infections, highlighting the need for targeted antimicrobial strategies.

**Keywords:** burn infections, bacterial growth, petroleum jelly silver sulfadiazine, hyaluronic acid, in vitro study.

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## 1. Introduction

Burns comprise a spectrum of traumatic injuries to the body and occur when the skin comes into direct contact with a heat source at or above the energy threshold to cause permanent damage. Thermal burns denature proteins, destroy cell membranes, and release oxygen free radicals. Three different zones can be distinguished in a burn wound, such as dead tissue (coagulation zone), living tissue, which is at risk of ongoing damage (stasis zone) and normal skin with minimal injury, which still shows blood flow (coagulation zone). hyperemia). Furthermore, local and systemic inflammatory responses are also initiated after burn injury, which has detrimental effects on both the burn wound and several distant organ systems. The cardiovascular, respiratory, gastrointestinal and renal systems are all affected. The process that occurs immediately after a burn is the loss of natural microbial flora on the skin surface. This makes the wound susceptible to infection. Previous studies reported that in larger and deeper burn wounds, with partial destruction of the dermis, the risk of subsequent burn wound colonization and infection is increased (Elloso and Cruz, 2017; Wardhana *et al.*, 2017).

Previous research at H. Adam Malik Hospital, Medan, examined the profile and sensitivity patterns of bacteria in burn wounds at H. Adam Malik Hospital for the period January – December 2019, where the data collected was secondary data from the microbiology laboratory unit at H. Adam Malik Hospital. The bacteria commonly found in burn wound specimens were *Pseudomonas aeruginosa* (47.6%), *Acinetobacter baumannii* (12.9%), *Staphylococcus aureus* (8.1%), *Klebsiella pneumoniae* (6.5%) and *Enterobacter cloacae complex* (3.2%) (Wahyuni *et al* , 2020).

The use of topical antimicrobial agents has played an important role in the control of burn wound infections. Topical antimicrobial therapy may be helpful to overcome the adverse effects of bacteria in certain circumstances. Management of microbial contamination of burn wounds to prevent sepsis is a routine need of acute care leading to the development of various therapeutic agents for topical use. Before the advent of topical antimicrobial agents, the overall mortality rate in the burn population was reported to be 38–45%. However, after the use of topical antimicrobial therapy, the overall mortality rate is reduced to 14-24%. Furthermore, the risk of developing superinfections and antibiotic-resistant bacteria, as well as organ toxicity, is minimal compared with the use of systemic antibiotics in clean surgery (Choi *et al.*, 2019; D'Abbondanza and Shahrokhi, 2021).

In certain clinical circumstances, topical agents may be used to treat incipient or early burn wound infections. Currently, available preparations fulfill most of the characteristics of ideal topical agents but their bacteriostatic or bacteriocidal activity is still little discussed. Based on the above background, researchers were interested in comparing the effectiveness of *Petroleum Jelly* , *Silver Sulfadiazine* and a combination of *Hyaluronic Acid* with *Silver Sulfadiazine* against the bacteria that most often cause infections in burn wounds in vitro.

## 2. Methods

This is in vitro laboratory experimental research using *Posttest Only Control Group Design* . In this study, 16 petri dishes were used, divided according to the bacteria *Pseudomonas aeruginosa* , *Acinetobacter baumannii* and *Staphylococcus aureus*. as well as interventions to assess comparison of the effectiveness of *Petroleum Jelly* , *Silver Sulfadiazine* and a combination of *Hyaluronic Acid* with *Silver Sulfadiazine* . This research was conducted at the Department of Microbiology, Faculty of Medicine, University of North Sumatra - Prof. Hospital. Dr. Chairuddin Panusunan Lubis University of North Sumatra with approval from the USU Health Research Ethics Commission. Research and data collection will be carried out starting from September 2023 until November 2023. This research was carried out by culturing the ATCC bacteria *Pseudomonas aeruginosa* , *Acinetobacter baumannii* and methicillin resistant and methicillin sensitive *Staphylococcus aureus* on *Muller Hinton Agar* (MHA) petri dishes .

*Muller Hinton Agar* (MHA) plates are used where the size is 5/6 mm ( *blank disk* ). Incubate the germs for 1x24 hours, repeating approximately twice. Antibacterial activity testing was carried out using the Kirby-Bauer method, known as the paper disc method. The total sample in the study was 48 bacterial cultures in petri dishes. In this study, researchers looked at the Minimum Inhibitory Concentration (MIC) in bacteria that had been cultured from burn patients and were given *Petroleum Jelly*, *Silver Sulfadiazine* and a combination of *Hyaluronic Acid* and *Silver Sulfadiazine* and gentamicin.

Analysis was used for bacterial profile data that had been cultured. The inferential analysis used was the ANOVA test or the alternative was *the Kruskal-Wallis test* used to test the comparative effectiveness of *Petroleum Jelly* , *Silver Sulfadiazine* and a combination of *Hyaluronic Acid* with *Silver Sulfadiazine* and gentamicin on the growth of bacteria that most often cause infections in burn wounds in vitro. The research

data were analyzed statistically with the help of statistical software where differences were considered statistically significant if  $p < 0.05$ .

### 3. Results

In this examination, cultures were carried out on the three most common bacteria found in burn wounds in accordance with previous research by Wahyuni in 2020 which examined the profile and sensitivity patterns of bacteria in burn wounds at H. Adam Malik General Hospital for the period January – December 2019, where the data was collected is secondary data from the microbiology laboratory unit of H. Adam Malik Hospital. There are four types of ATCC bacteria, namely *Methicillin-sensitive Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

**Table 1.** Diameter of the inhibition zone in *Methicillin-sensitive bacteria Staphylococcus aureus* (MSSA)

Treatment	Inhibition Zone Diameter Repetition (mm)			Averag e	p value
	I	II	III		
MEBO	0	0	0	0	0.017 <sup>a</sup>
<i>Silver Sulfadiazine</i>	15	15.5	14.75	15,083	
Combination of <i>Hyaluronic Acid</i> and <i>Silver Sulfadiazine</i>	16.25	16.25	15.25	15,916	
Gentamicin	26.5	26.25	26	26.25	

<sup>a</sup>Kruskal-Wallis

Table 1. shows the diameter of the inhibition zone in *Methicillin-sensitive bacteria Staphylococcus aureus* where the average diameter of the inhibition zone in the *Petroleum jelly treatment* was 0 mm, *Silver Sulfadiazine* was 15,083 mm, the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* was 15,916 mm and gentamicin was 26.25 mm. Based on CLSI, it can be concluded that the interpretation of the inhibition zone diameter category of gentamicin against *Methicillin-sensitive bacteria Staphylococcus aureus* is *susceptible* (S), which means the organism is successfully inhibited by the drug.

From these results, it appears that the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* has the highest inhibitory effect close to the control, namely the Gentamicin disc with a difference of 10,334 mm, followed by *Silver Sulfadiazine* with a difference to the control of 11,167 mm. Inhibition zones were not formed in the *petroleum jelly treatment*. The p value was found to be 0.017 ( $p < 0.05$ ), so there was a significant difference between the *Staphylococcus aureus* bacteria given the Petroleum Jelly disk, *Silver Sulfadiazine* and the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* and gentamicin ointment.

**Table 2.** Diameter of the inhibition zone in *Methicillin-resistant Staphylococcus aureus* (MRSA) bacteria

Treatment	Inhibition Zone Diameter Repetition (mm)			Averag <sup>e</sup>	p value
	I	II	III		
<i>Petroleum jelly</i>	0	0	0	0	0.021 <sup>a</sup>
<i>Silver Sulfadiazine</i>	11.5	11	11.25	11.25	
Combination of <i>Hyaluronic Acid</i> and <i>Silver Sulfadiazine</i>	12.25	11	12	11.75	
Gentamicin	21.75	21	22	21,583	

<sup>a</sup> Kruskal-Wallis

*Methicillin-resistant Staphylococcus aureus* bacteria where the average diameter of the inhibition zone in the *Petroleum jelly* treatment was 0 mm, *Silver Sulfadiazine* was 11.25 mm, the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* was 11.75 mm and gentamicin was 21.583 mm. Based on CLSI, it can be concluded that the interpretation of the inhibition zone diameter category of gentamicin against *methicillin-resistant Staphylococcus aureus* bacteria is susceptible (S), which means the organism was successfully inhibited by the drug.

From these results, it appears that the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* has the highest inhibitory effect close to the control, namely the Gentamicin disc with a difference of 9,833 mm, followed by *Silver Sulfadiazine* with a difference to the control of 10,333 mm. Inhibition zones were not formed in the *petroleum jelly* treatment. The p value was found to be 0.021 ( $p < 0.05$ ), so there was a significant difference between *Methicillin-resistant Staphylococcus aureus* bacteria that were given *Petroleum Jelly* discs, *Silver Sulfadiazine* and a combination of *Hyaluronic Acid* and *Silver Sulfadiazine* and gentamicin ointment.

**Table 3.** Diameter of the inhibition zone in *Acinetobacter baumannii* bacteria

Treatment	Inhibition Zone Diameter Repetition (mm)			Averag <sup>e</sup>	p value
	I	II	III		
<i>Petroleum jelly</i>	0	0	0	0	0.014 <sup>a</sup>
<i>Silver Sulfadiazine</i>	12.25	12.75	12	12.34	
Combination of <i>Hyaluronic Acid</i> and <i>Silver Sulfadiazine</i>	10.25	11	10.5	10.58	
Gentamicin	23	21.25	22	22,083	

<sup>a</sup> Kruskal-Wallis

*Acinetobacter baumannii* bacteria where the average diameter of the inhibition zone in the *Petroleum jelly* treatment was 0 mm, *Silver Sulfadiazine* was 12.34 mm, the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* was 10.58 mm and gentamicin was 22.083 mm. Based on CLSI, it can be concluded that the

interpretation of the inhibitory zone diameter category of gentamicin against *Acinetobacter baumannii* bacteria is *susceptible* (S), which means that the organism was successfully inhibited by the drug.

From these results, it appears that *Silver Sulfadiazine* has the highest inhibitory effect close to the control, namely the Gentamicin disc with a difference of 9,743 mm, followed by the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* with a difference to the control of 11,503 mm. Inhibition zones were not formed in the *petroleum jelly treatment*. The p value was found to be 0.014 ( $p < 0.05$ ), so there was a significant difference between the *Acinetobacter baumannii* bacteria that were given *Petroleum Jelly* disks, *Silver Sulfadiazine* and a combination of *Hyaluronic Acid* and *Silver Sulfadiazine* and gentamicin ointment.

**Table 4.** Diameter of the inhibition zone in *Pseudomonas aeruginosa* bacteria

Treatment	Inhibition Zone Diameter Repetition (mm)			Average	p value
	I	II	III		
<i>Petroleum jelly</i>	0	0	0	0	0.021 <sup>a</sup>
<i>Silver Sulfadiazine</i>	12.75	12.75	12.75	12.75	
Combination of <i>Hyaluronic Acid</i> and <i>Silver Sulfadiazine</i>	13	11.5	12.5	12.34	
Gentamicin	27.5	28.5	29.5	28.5	

<sup>a</sup> Kruskal-Wallis

*Pseudomonas aeruginosa* bacteria where the average diameter of the inhibition zone in the *Petroleum jelly treatment* was 0 mm, *Silver Sulfadiazine* was 12.75 mm, the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* was 12.34 mm and gentamicin was 28.5 mm. Based on CLSI, it can be concluded that the interpretation of the inhibition zone diameter category of gentamicin against *Pseudomonas aeruginosa* bacteria is *susceptible* (S), which means that the organism was successfully inhibited by the drug.

From these results, it appears that *Silver Sulfadiazine* has the highest inhibitory effect close to the control, namely the Gentamicin disc with a difference of 15.75 mm, followed by the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* with a difference to the control of 16.16 mm. Inhibition zones were not formed in the *petroleum jelly treatment*. The p value was found to be 0.021 ( $p < 0.05$ ), so there was a significant difference between the *Pseudomonas aeruginosa* bacteria given the *Petroleum Jelly* disk, *Silver Sulfadiazine* and the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* and gentamicin ointment.

#### 4. Discussion

In this study, it was seen that the combination of *silver sulfadiazine* with *hyaluronic acid* had a better inhibitory diameter than the combination of *silver sulfadiazine* alone on *Staphylococcus aureus* bacteria, both *methicillin-sensitive* and *methicillin-resistant*. Meanwhile, for the bacteria *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, *silver sulfadiazine* alone has a better inhibitory diameter compared to the combination of *silver sulfadiazine* and *hyaluronic acid*. It should be taken into consideration that *Staphylococcus aureus* bacteria are gram positive and *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are gram negative bacteria. It can be interpreted that the combination of *silver sulfadiazine* and *hyaluronic acid* is able to work better on gram-positive bacteria, when compared to *silver sulfadiazine* alone.

This can be explained by the differences in the cell wall structure of gram-positive and gram-negative bacteria. Gram-positive bacteria have cell walls composed of a thicker peptidoglycan layer, while gram-negative bacteria have a thinner peptidoglycan layer and have a thick lipopolysaccharide structure (Ueda *et al.*, 2020; Zamboni, Wong and Collins, 2023).

*Silver sulfadiazine* is reported to have a broad spectrum of activity against both bacteria (gram-positive and gram-negative organisms) by damaging the cell membrane or cell wall of the organism. *Silver sulfadiazine* has a bacteriocidal effect against gram-positive bacteria. However, when combined with *hyaluronic acid*, it seems that the effect of this bacteriocid is better compared to single administration, proven in this study where a smaller inhibitory diameter was found compared to *silver sulfadiazine* alone on gram-positive bacteria (Aliakbar Ahovan *et al.*, 2022; Liang *et al.*, 2022). This can be explained by the fact that *hyaluronic acid* has a bacteriostatic effect and contains glycosaminoglycans which are natural components of the extracellular matrix. This component has high water retention capacity, biocompatibility and hygroscopic qualities. It has been proven that *hyaluronic acid* is able to reduce inflammation by suppressing the production of several pro-inflammatory mediators, including metalloproteinase and interleukin-1 $\beta$  (Dalmedico *et al.*, 2016; Fatima *et al.*, 2022b). The *anti-biofouling* properties of *hyaluronic acid* are based on hydrophilicity and negative charges on the cell walls which interact with the balance of ions in the positively and negatively charged cell walls of bacteria, thus providing the best antimicrobial effect on gram-positive bacteria which have a thick peptidoglycan structure. The combination of *silver sulfadiazine* and *hyaluronic acid* is reported to have polycationic properties that facilitate its interaction with gram-positive bacterial membranes with thick polypeptide walls, where the modified cell permeability causes leakage of intracellular components (Della Sala *et al.*, 2022; Zamboni, Wong and Collins, 2023).

On the other hand, in gram-negative bacteria it appears that *silver sulfadiazine* alone works better to kill bacteria with a better inhibition zone diameter when compared to the combination of *silver sulfadiazine* with *hyaluronic acid*. This related explanation is related to differences in cell wall structure between gram-positive and gram-negative bacteria, where gram-negative bacteria have thicker cell walls, lipopolysaccharide. The bacteriocidal mechanism of silver sulfadiazine can be explained by three different mechanisms: (1) production of reactive oxygen species derived from dissolved oxygen through its catalytic activity; (2) cross-linking with *silver* at hydrogen bond sites between DNA double strands; and (3) inhibition of enzyme activity by intracellular *silver ions*. *Sulfadiazine*, a sulfonamide, inhibits intracellular folate metabolism in bacteria, thereby stopping proliferation. Research reports that *silver sulfadiazine* has been selected among different compounds because of its high and broad bacteriocidal effect. Moreover, when combined with *hyaluronic acid*, it is necessary to consider that *hyaluronic acid* is hydrophilic which poses pharmacological challenges, especially in the treatment of intracellular bacterial infections. Due to its high hydrophilicity, the combination of *silver sulfadiazine* and *hyaluronic acid* has poor penetration into cell membranes, especially with the thickness of lipopolysaccharide making it difficult to penetrate the cell walls (Di Domenico *et al.*, 2020; Ueda *et al.*, 2020; Zamboni, Wong and Collins, 2023).

With this research, it needs to be considered that administering *silver sulfadiazine* 1% either alone or in combination with *hyaluronic acid* has a much cheaper cost than the cost of treating sepsis in burn patients. Preventing infection in burn wounds is of the utmost importance; However, carelessly used antibiotics can lead to the emergence of infections, increased drug resistance, and the failure of most topical and systemic treatments to eradicate these bacteria, thereby increasing morbidity and mortality (Czarnowicki *et al.*, 2016; Fatima *et al.*, 2022b).

## 5. Conclusion

There is a significant difference between the inhibitory diameter of *Petroleum Jelly*, *Silver Sulfadiazine*, the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* and gentamicin on bacteria, where the combination of *Hyaluronic Acid* and *Silver Sulfadiazine* has the highest inhibitory effect on gram-positive bacteria (*Methicillin-sensitive Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus*) and *Silver Sulfadiazine* alone had the highest inhibitory effect compared to other treatments on gram-negative bacteria (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*).

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