RESEARCH PAPER

Effectiveness of Normal Saline Solution, Chlorhexidine Gluconate, and Vinegar (Apple Cider Vinegar) as Wound Cleansers for Wounds Contaminated with Pseudomonas Aeruginosa

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ABSTRACT

Wound contamination with Pseudomonas aeruginosa can impair healing and complicate wound management. Although normal saline is widely used for wound cleansing, antiseptic and acidic solutions may provide greater antimicrobial activity. This study compared the effectiveness of normal saline, chlorhexidine-cetrimide, and apple cider vinegar in reducing P. aeruginosa contamination in experimentally contaminated wounds. A randomized experimental study was conducted using 30 healthy male Wistar rats (Rattus norvegicus), allocated equally to three groups: normal saline, chlorhexidine-cetrimide, and apple cider vinegar. Following experimental wound contamination with P. aeruginosa, the assigned cleansing solution was administered. Bacterial contamination was assessed by quantifying colony-forming units per gram of tissue. Differences among groups were analyzed using one-way analysis of variance, followed by Mann–Whitney U post hoc comparisons. The mean bacterial burden was substantially higher in the normal saline group (100,681 ± 1,438 CFU/g; range, 100,000–103,571) than in the chlorhexidine-cetrimide group (110.90 ± 68.59 CFU/g; range, 31–262) and apple cider vinegar group (115.50 ± 72.34 CFU/g; range, 33–260). Overall, significant differences were observed among the three groups ($P < 0.001$). Post hoc analysis demonstrated significant differences between normal saline and chlorhexidine-cetrimide ($P < 0.001$) and between normal saline and apple cider vinegar ($P < 0.001$), whereas no significant difference was observed between chlorhexidine-cetrimide and apple cider vinegar ($P = 1.000$). In this experimental wound model, chlorhexidine-cetrimide and apple cider vinegar were significantly more effective than normal saline in reducing P. aeruginosa contamination. Apple cider vinegar demonstrated antibacterial efficacy comparable to chlorhexidine-cetrimide.

Keywords: Pseudomonas aeruginosa; Chlorhexidine; Sodium Chloride; Wounds and Injuries; Rats

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INTRODUCTION

Skin loss wounds (full thickness) are skin damage conditions that involve the entire epidermis and dermis layers, often requiring intensive medical treatment to accelerate healing and prevent complications such as infection. The optimal wound healing process involves complex interactions between inflammatory, proliferation, and tissue remodeling factors, thus requiring effective therapeutic agents [1].

A wound is the loss of body tissue due to various factors that disrupt the body's protective system. These factors include trauma, chemicals, temperature changes, explosions, electric shocks, or animal bites. Several complex stages are required for the wound healing process. The hemostasis phase occurs at the onset of the wound, followed by the inflammatory phase and the proliferation phase, and finally the remodeling phase. Each phase is influenced by various cellular interactions and the release of local chemical signals such as cytokines, chemokines, growth factors, and inhibitory factors [2].

The wound healing process is closely related to wound management or treatment techniques, which include cleansing, removal of dead tissue, and dressing. These three components of wound care support each other in the healing process. However, wound cleansing is a key indicator of successful wound care and is a standard practice that is always applied by healthcare professionals, both in Indonesia and in other countries [3].

In the United States, Pseudomonas aeruginosa is the most common pathogen in hospitals and the second most

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common pathogenic bacterium found in patients with *ventilator acquired pneumonia* [4]. Research conducted by Asadullah (2015) on patients with nosocomial pneumonia at Dr. Soetomo General Hospital in Surabaya from January 1, 2011 to March 30, 2012 showed that the most common pathogenic bacteria causing nosocomial pneumonia was *Pseudomonas aeruginosa* [5].

The ability of *Pseudomonas aeruginosa* to form biofilms has made *Pseudomonas aeruginosa* a feared pathogen due to the combined effects of its various intrinsic virulence factors and its resistance to antimicrobial drugs. Contamination with Pseudomonas can lead to death [6].

Antiseptic solutions commonly used in wound care include chlorhexidine-cetrimide, more commonly known as Savlon®. In addition to this ingredient, other ingredients such as polyhexanide and chlorhexidine alone are also available. Although these ingredients are highly effective, they carry the risk of causing resistance and undesirable effects on tissue, especially fibroblast tissue [7].

Common wound cleaning solutions used in medical practice include normal saline, chlorhexidine gluconate, and apple cider vinegar [8]. Normal saline is an isotonic solution often used to clean wounds, but its effectiveness in treating bacterial contamination still needs to be explored further. Chlorhexidine gluconate, on the other hand, is an antiseptic known to have a broad spectrum of activity against various microorganisms, including *Pseudomonas aeruginosa* [9] Apple cider vinegar, which contains acetic acid, has also been reported to have antimicrobial properties and may be an attractive alternative in wound care [10]. Several studies on apple cider vinegar have shown its potential as an antimicrobial against some of the pathogens mentioned above. This literature review aims to summarize and draw conclusions from the available studies on apple cider vinegar [11].

This study aims to compare the effectiveness of normal saline, chlorhexidine gluconate, and apple cider vinegar as wound cleansing solutions for wounds contaminated with Pseudomonas aeruginosa in Wistar rats (*Rattus norvegicus*).

METHODS

This study is an experimental study with a randomized controlled trial design, which is a design used to measure the effect of treatment on the experimental group after intervention. The sample was divided into three groups, namely the first group, which was the positive control group given normal saline; the second group, which was the positive control group given chlorhexidine-cetrimide; and the third group, which was given vinegar (apple cider vinegar).

The test animals used were healthy male rats (*Rattus norvegicus*) of the Wistar strain, approximately 3 months old and weighing 250-300g. Each rat was kept in the same cage, in the same room, and fed the same amount and type of food. The rats were kept in polypropylene cages measuring 30 cm x 40 cm x 15 cm with ventilation and a room temperature of around 32°C. Each cage contained 4 test animals and was partitioned so that the rats could not interact with or disturb other rats. The cages were covered with wire mesh and lined with husks and adapted for 1 week. The test animals were fed PAR-G pellet feed at a rate of 20 grams/day/test animal [12, 13]. The test animals were given Aqua brand bottled water ad libitum using a special bottle that dispensed water when sucked by the test animals. The sacrificed test animals were disposed of by cremation. To reduce bias, the researchers determined the inclusion and dropout criteria for selecting the research subjects. The inclusion criteria included: male white rats (Rattus norvegicus), Weight 250-350 grams, aged 3-4 months, and healthy, while dropout criteria include: weight loss >10% during the adaptation period in the laboratory, symptoms of illness (hair loss, eye discharge, reduced activity) during the adaptation period in the laboratory, and removal of dressings or wound coverings during the study period.

The data on colony forming units per gram of tissue after wound cleansing in each treatment group is presented in the table. The results of the study were then statistically analyzed using one-way ANOVA followed by post-hoc Mann-Whitney tests.

RESULTS

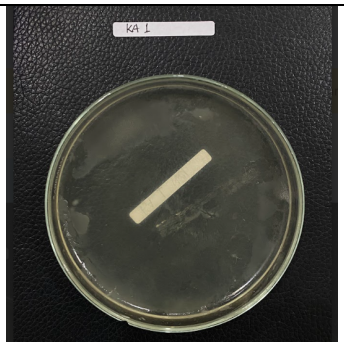
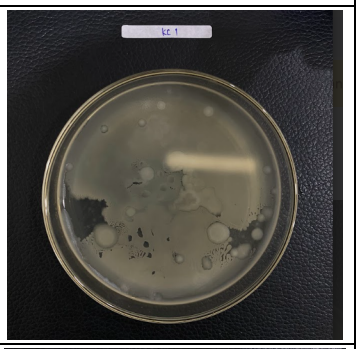
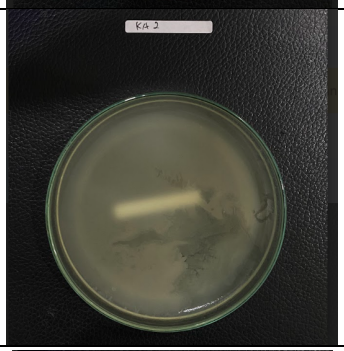
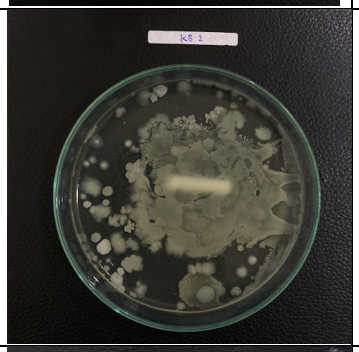
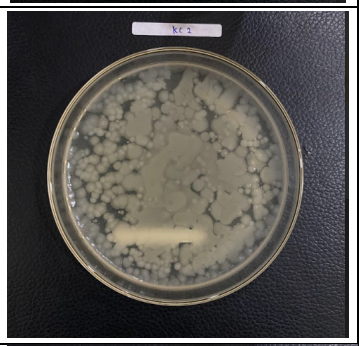
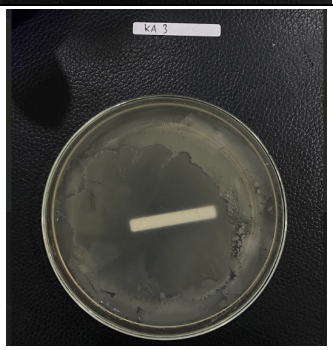
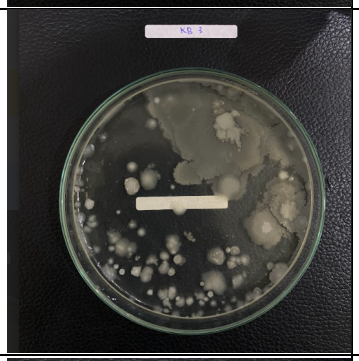
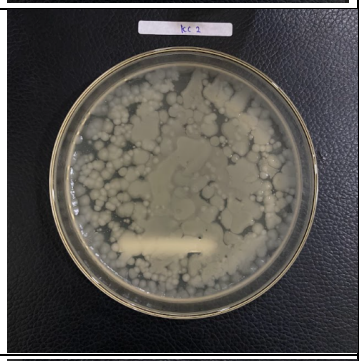
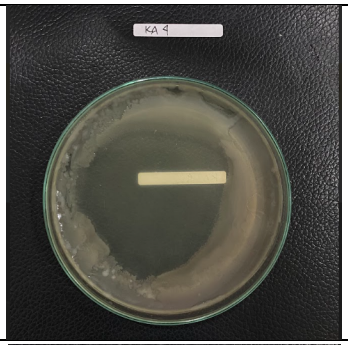
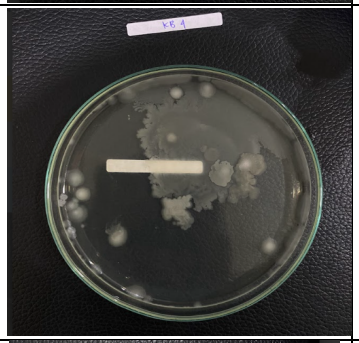
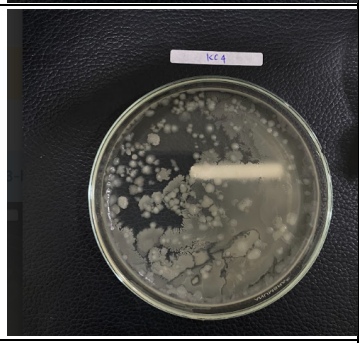
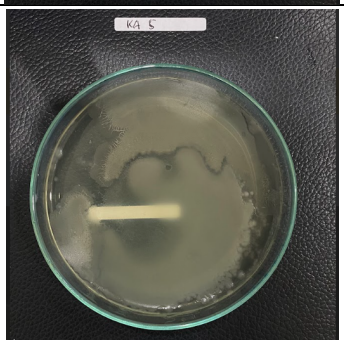
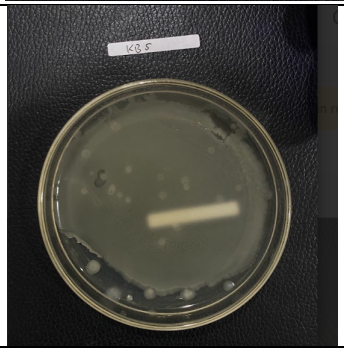
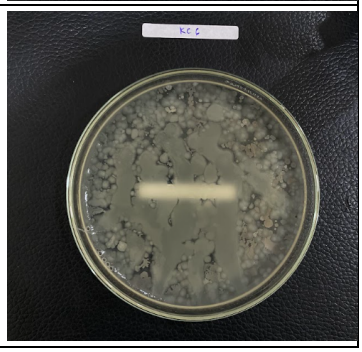
The study was conducted at the Animal Laboratory of the Faculty of Medicine, Hasanuddin University, and the HUM-RC Laboratory of the Faculty of Medicine, Hasanuddin University, over a period of three weeks in December 2025. The research samples were divided into three groups: the first group was the positive control group given normal saline, the second group was the positive control group given chlorhexidine-cetrimide, and the third group was given vinegar (apple cider vinegar).

Univariate analysis in this study includes sample characteristics, descriptive results of macroscopic and microscopic examinations, which will be presented in the following table:

Table 1. Sample Characteristics

Group	n	%
Nacl	10	33.3%
Clorheksidin-setrimid	10	33.3%
Vinegar	10	33.3%
Total	30	100%

Table 1 shows that this study involved 30 male Wistar rats as subjects, consisting of 10 rats in the NaCl group (33.3%), 10 rats in the chlorhexidine-trimethoprim group (33.3%), and 10 rats in the control group with very high bacteria levels.

Day	Nacl	Clorheksidin-Setrimid	Vinegar
1			
2			
3			
4			
5			

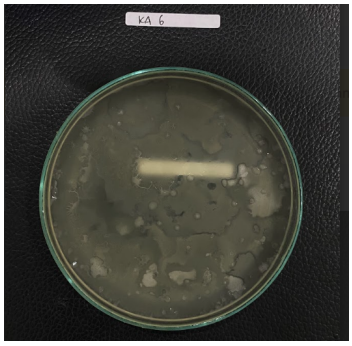
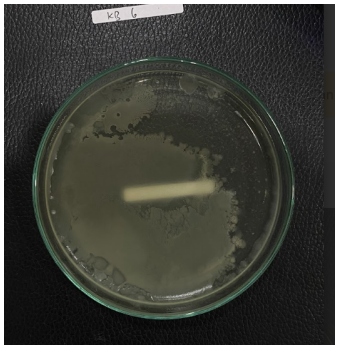
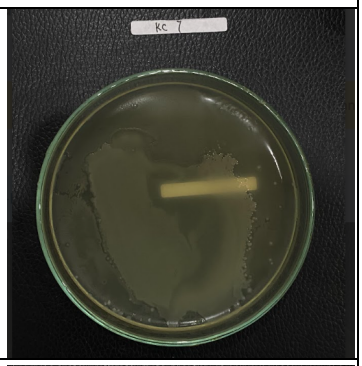
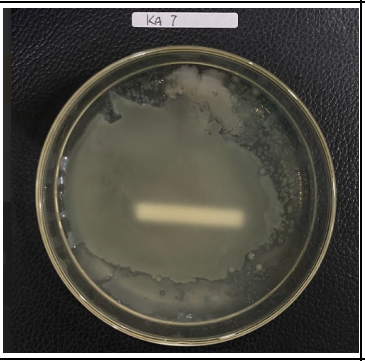
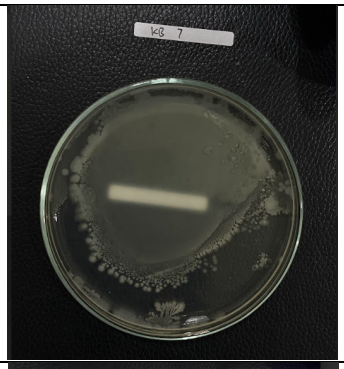
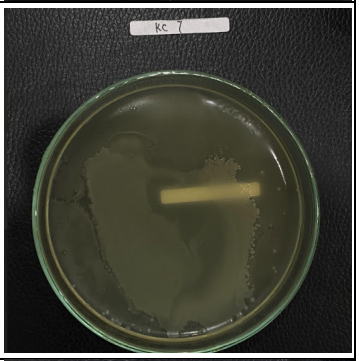
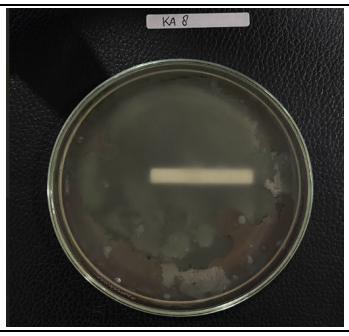
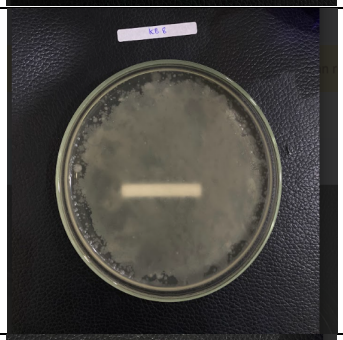
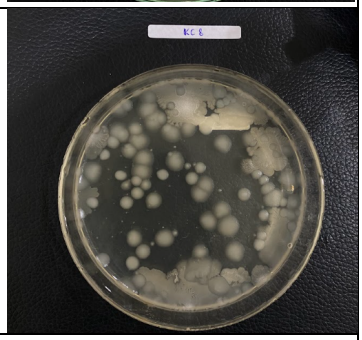
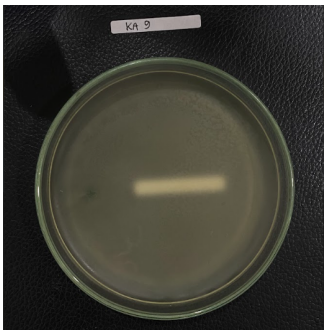
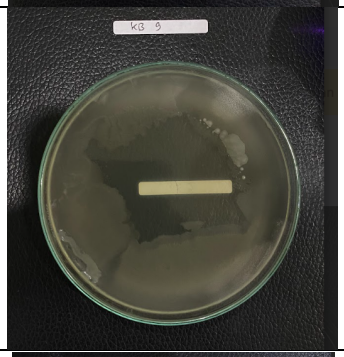
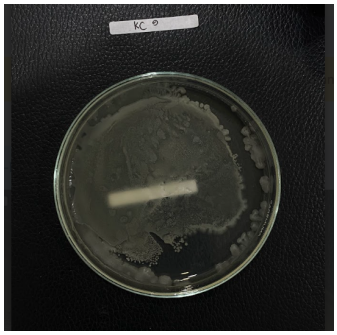
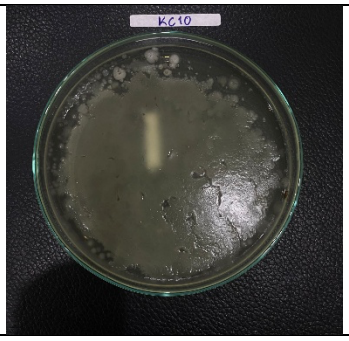
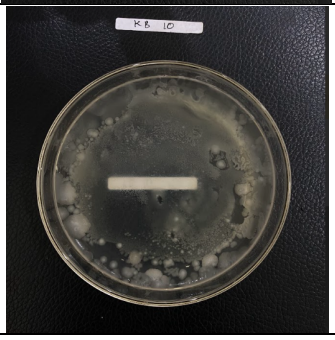
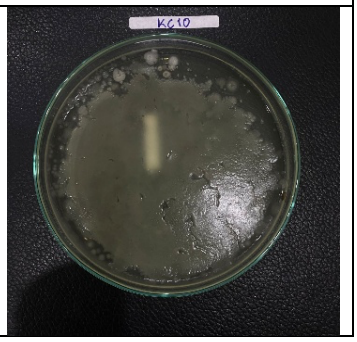
Day	Nacl	Clorheksidin-Setrimid	Vinegar
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7			
8			
9			
10			

Figure 1. Final Culture Results by Group

Figure 1 shows the final culture results based on the three types of liquids tested, namely Chlorhexidine, Vinegar (Apple Cider Vinegar), and NaCl. Although NaCl has a much higher value, reaching hundreds of thousands, so it is not shown in this graph, Chlorhexidine and Vinegar show more comparable results. Chlorhexidine (yellow) has a wider range with several extreme values, while Vinegar (green) tends to be more centered with less variation, but both show similar final culture values, with Chlorhexidine being slightly more effective in controlling microbial growth than Vinegar (**Figure 2**).

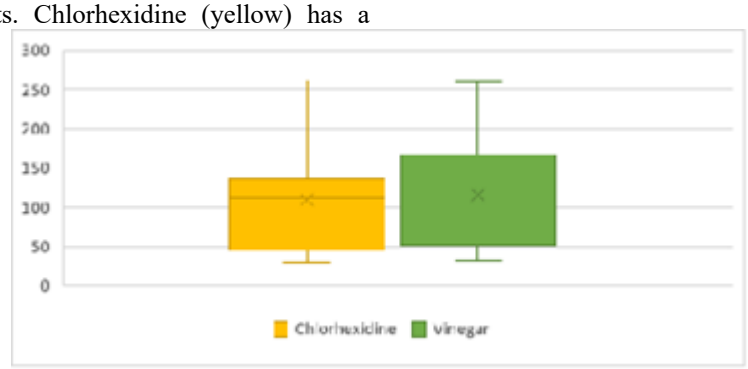


Figure 2. Comparison Diagram between Intervention Group

Table 2. Mean (SD) and Min-Max Values of Pseudomonas Aeruginosa Contamination

Macroscopic Results	Mean (SD)	Min-Max	P value
Nacl	100681 (1438)	100000-103571	0.000
Clorheksidin-setrimid	110.90 (68.59)	31.00-262.00	
Vinegar	115.500 (72.34)	33.00-260.00	

** Anova Test Analysis*

Based on **Table 2**, the highest Pseudomonas aeruginosa contamination value was found in the NaCl group, with an average value of 100,681 ± 1,438 and a range of 100,000–103,571. These results indicate a very high level of bacterial growth in the control group.

In the chlorhexidine-trimethoprim group, there was a significant decrease in the number of colonies, with an average value of 110.90 ± 68.59 and a range of 31.00–262.00, reflecting the effectiveness of this antiseptic compound in suppressing the growth of *Pseudomonas aeruginosa*. Meanwhile, the vinegar (apple cider vinegar) group showed an average contamination value of 115.50 ± 72.34 with a range of 33.00–260.00. This value is relatively comparable to the chlorhexidine-trimethoprim group, indicating that apple cider vinegar has almost equivalent antibacterial potential in inhibiting the growth of *Pseudomonas aeruginosa*. The results of the anova test showed a p-value, indicating that there were significant differences between groups.

Table 3. Mann-Whitney U Post-Hoc Test Results

Comparison	p-value	Conclusion
Nacl vs Clorheksidin-setrimid	0.000	Difference in meaning (p<0.05)
Nacl vs Vinegar	0.000	Difference in meaning (p<0.05)
Clorheksidin-setrimid Vs Vinegar	1.000	No difference in meaning

** Post-hoc Mann Whitney Analysis*

The results of the post-hoc test using the Mann-Whitney U method in this study show a comparison between NaCl, Chlorhexidine-cetrimide, and Apple Cider Vinegar (**Table 3**). Based on the analysis results, the comparison between NaCl and Chlorhexidine-cetrimide produced a p-value of 0.000, which means there is a significant difference ($p < 0.05$) between the two groups. A similar finding was observed in the comparison between NaCl and Apple Cider Vinegar, which also showed a p-value of 0.000, indicating a significant difference ($p < 0.05$). Conversely, the comparison between chlorhexidine-cetrimide and apple cider vinegar showed a p-value of 1.000, which is greater than 0.05, meaning that there was no significant difference between these two groups. Thus, it can be concluded that NaCl has a significant difference compared to Chlorhexidine-Cetrimide and Apple Cider Vinegar, while Chlorhexidine-Cetrimide and Apple Cider Vinegar do not show a significant difference.

DISCUSSION

The Effectiveness of Normal Saline Solution on Wounds Contaminated with *Pseudomonas aeruginosa*

Normal saline has traditionally been used as a wound irrigation fluid due to its isotonic properties, which do not damage tissue, and its ability to mechanically clean debris and exudate, [14, 15]. As an isotonic fluid, Normal Saline is compatible with human tissue, does not cause new tissue damage, and does not affect the function of fibroblasts and keratinocytes in wound healing [8, 16].

These results consistently demonstrate that Normal Saline, although essential for cleaning wounds, does not possess inherent antibacterial activity against Pseudomonas

aeruginosa. Its primary function is as a negative control in this study, allowing us to compare the effectiveness of other antimicrobial agents [17].

Other studies also support this view, showing that Normal Saline is used as a control in comparing the effectiveness of other antimicrobial agents, such as acetic acid, in treating wounds infected with P. aeruginosa. [17].

The effectiveness of Normal Saline in preventing infection has not been confirmed. Other studies also support this view, showing that Normal Saline is used as a control in comparing the effectiveness of other antimicrobial agents, such as acetic acid, in treating wounds infected with P. aeruginosa. This is in line with its main function as a negative control in this study, which allows us to compare the effectiveness of other antimicrobial agents [17].

Several recent studies have shown the effects of normal saline on bacteria, especially in hypertonic concentrations. For example, a study by Michon et al. found that a 6% NaCl solution can inhibit the growth of P. aeruginosa, and a 10% concentration has bactericidal activity on most P. aeruginosa isolates from cystic fibrosis patients [18]. Similarly, Lin et al. reported that hypertonic saline can increase the efficacy of antibiotics against P. aeruginosa. [19].

However, the concentration used in that study was much higher than Normal Saline (0.9%), which explains why in our study, Normal Saline did not show significant antimicrobial effects. The role of Normal Saline in modern clinical practice has shifted more towards mechanical cleaning and providing an optimal moist environment for wound healing, rather than as a direct bactericidal agent.

Other comparative studies also often use Normal Saline as a standard comparator. Kim et al. compared the results of using Normal Saline with antiseptic solutions in negative pressure wound therapy with instillation for infected wounds [20]. Although the context is different, the results of these various studies consistently emphasize that Normal Saline alone is not sufficient to treat active bacterial infections, especially those caused by P. aeruginosa, which is known for its high resistance.

Therefore, our research findings strongly support the objective of this study that Normal Saline is not directly effective in proving antimicrobial efficacy against P. aeruginosa in contaminated Wistar rat wounds. Instead, it serves as a benchmark for assessing the antimicrobial potential of other interventions.

In conclusion, while Normal Saline plays a crucial role in general wound management, its role is limited as a non-antimicrobial agent that provides a basis for comparison for evaluating the effectiveness of other materials in controlling P. aeruginosa infections. Our research reaffirms the role of Normal Saline as a standard irrigation fluid without direct bactericidal effects on P. aeruginosa.

The Effectiveness of Chlorhexidine-Cetrimide on Wounds Contaminated with Pseudomonas aeruginosa

Chlorhexidine-Cetrimide is an antiseptic combination that has long been recognized for its broad spectrum of antimicrobial activity, including against Pseudomonas aeruginosa[21]. In this study, the Chlorhexidine-Cetrimide group showed a significant reduction in P. aeruginosa contamination.

The effectiveness of Chlorhexidine-Cetrimide stems from its mechanism of action. Chlorhexidine works by disrupting the integrity of the bacterial cell membrane, causing leakage of intracellular components and ultimately cell death. Cetrimide, as a cationic agent, also has disinfectant properties and helps clean wound surfaces, which is synergistic with Chlorhexidine[22].

At high concentrations, chlorhexidine also causes cytoplasmic coagulation. Cetrimide, as a cationic surfactant, helps clean wound surfaces and has a non-specific damaging effect on bacterial membranes. The damage caused by surfactants to cell membranes causes irreversible changes in permeability and ultimately leads to fatal massive leakage [21]. This combination is effective in killing Pseudomonas aeruginosa cells. [23].

Recent studies continue to support the role of chlorhexidine as a powerful antiseptic. They developed a nanofilm system containing chlorhexidine acetate and demonstrated its antibacterial effectiveness against P. aeruginosa both in vitro and in vivo [24]. This demonstrates innovation in chlorhexidine formulations for wound applications.

Additionally, Rippon et al. evaluated the effectiveness of wound cleansing solutions containing antimicrobials, including those based on chlorhexidine, against P. aeruginosa biofilms. They found that some of these solutions were effective in eradicating P. aeruginosa biofilms, although P. aeruginosa showed higher tolerance compared to Staphylococcus aureus [25]. This highlights the challenge of overcoming P. aeruginosa biofilms, which are known to be resistant.

A systematic review showing that bacterial sensitivity to chlorhexidine can change over time, although the clinical implications remain unclear [22]. This highlights the need for ongoing monitoring of the effectiveness of this antiseptic amid rising antimicrobial resistance.

Our study, which showed a significant decrease in the Chlorhexidine-Setrimid group, is consistent with most of the existing literature confirming its effectiveness against P. aeruginosa. Penn-Barwell et al. also compared the antimicrobial effects of irrigation with chlorhexidine gluconate versus saline in a contaminated open fracture model, demonstrating the superiority of chlorhexidine [15].

Overall, the results of this study reinforce the position of Chlorhexidine-Cetrimide as a highly effective antiseptic agent in reducing the burden of P. aeruginosa on contaminated wounds, supporting its use in clinical wound management.

The Effectiveness of Vinegar on Wounds Contaminated with Pseudomonas aeruginosa

Wounds that are difficult to heal are often caused by the presence of debris and tissue that allow for heavy bacterial growth and biofilm development [26]. The group given vinegar showed average contamination values that were relatively comparable to the chlorhexidine-trimethoprim group, and significantly lower than the normal saline group. These results demonstrate the strong antibacterial potential of apple cider vinegar against Pseudomonas aeruginosa.

The effectiveness of apple cider vinegar is largely attributed to its acetic acid content 10. Acetic acid works by lowering the pH of the environment, which effectively inhibits bacterial growth and damages their cell membranes [27–29]. In addition, apple cider vinegar contains other compounds that may have synergistic or additive effects in antimicrobial activity [30, 31]. It also has anti-inflammatory properties that can help reduce inflammation in wounds [10]. In addition, apple cider vinegar contains other compounds that may have synergistic or additive effects in antimicrobial activity [30, 32].

Several recent studies have highlighted the potential of acetic acid and apple cider vinegar in wound management. Agrawal et al. showed that acetic acid is effective not only against Pseudomonas aeruginosa but also against various other microorganisms that infect wounds, even at low concentrations such as 1% [29]. Bjarnsholt et al found that acetic acid not only kills planktonic bacteria but also eradicates bacteria growing in biofilms, including P. aeruginosa [27].

A more recent study by Tawre et al. demonstrated the antibiofilm and anti-persister activity of acetic acid against drug-resistant P. aeruginosa [33]. This is particularly relevant because P. aeruginosa often forms biofilms that make it very difficult to treat with conventional antibiotics. Feng et al. also evaluated the effects of acetic acid on colistin-resistant P. aeruginosa, demonstrating promising antibacterial, antibiofilm, and anti-virulence activity [34].

Specifically regarding apple cider vinegar, Al-Khalisy and Al-Imam found that apple cider vinegar can accelerate wound healing, reduce contamination, and effectively combat P. aeruginosa, including those resistant to many drugs, without causing the emergence of resistant strains [35]. Syeda Mahanoor et al. also found that apple cider vinegar at concentrations of 2.5% and 5% showed significant inhibition of bacterial growth and could increase the sensitivity of P. aeruginosa to certain antibiotics [36].

These findings suggest that given that apple cider vinegar is relatively inexpensive, easily accessible, and demonstrates efficacy equivalent to standard antiseptics such as chlorhexidine-cetrimide, it has the potential to be an attractive alternative, especially in resource-limited areas or as part of an integrative approach to wound care [35, 37]. Our study results support that apple cider vinegar is a promising antimicrobial agent against P. aeruginosa. However, further research is needed to optimize the concentration, application duration, and formulation of apple cider vinegar for clinical use on wounds. Yagnik et al. have also investigated the antimicrobial activity of apple cider vinegar against various microorganisms, including E. coli and S. aureus, confirming its broad spectrum [31, 32].

Overall, our research results strongly prove the effectiveness of Apple Cider Vinegar in reducing Pseudomonas aeruginosa contamination in Wistar rat wounds, positioning it as a natural antimicrobial agent that deserves further consideration in the treatment of wound infections.

CONCLUSION

This study shows that Normal Saline solution is ineffective in reducing Pseudomonas aeruginosa contamination. In contrast, Chlorhexidine-Cetrimide and Vinegar (apple cider vinegar) solutions were found to be significantly effective in reducing bacterial colony counts, with apple cider vinegar demonstrating efficacy nearly equivalent to Chlorhexidine-Cetrimide.

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Author's Contributions

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Conflict of Interest

The authors declare no conflict of interest

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