

Comparison of the antiseptic effectiveness of octenidine dihydrochloride with povidone-iodine for *Acinetobacter baumannii* contaminated wounds in Wistar rat

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ABSTRACT

Introduction: Effective antiseptic use is essential in healthcare settings to prevent the spread of diseases, especially in areas with high patient traffic and exposure to various pathogens. One essential pathogenic germ is *Acinetobacter baumannii*. Octenidine and povidone-iodine have been demonstrated to be effective against *A. baumannii* in vitro. This study will compare octenidine dihydrochloride and povidone-iodine as wound-cleansing solutions for wounds contaminated with *A. baumannii* in vivo.

Materials and Methods: Twenty-four rats were divided into three groups: normal saline, octenidine dihydrochloride and povidone-iodine. Wounds were made on the rats' backs, and *A. baumannii* germs were inoculated into the wounds. After 3 hours, the wound was irrigated with wound cleansing solution according to the group for 30 seconds. Each wound was taken swab culture before and after wound irrigation and tissue culture 5 hours after wound irrigation.

Results: All specimens showed bacterial colony growth with a median value of 1.22×10^5 CFU before irrigation. Wound irrigation with normal saline did not reduce colony counts, while there was a 3-log reduction to 5-log reduction in the octenidine and povidone-iodine groups. Statistically, there was no significant difference in the mean number of colonies between the octenidine and povidone-iodine groups after irrigation ($p = 0.535$). However, 3 hours after irrigation, all specimens that experienced 3-log reduction showed regrowth to more than 1×10^5 CFU. In contrast, specimens subjected to 5-log reduction did not exhibit any regrowth.

Conclusion: The antiseptic effectiveness of octenidine dihydrochloride is equivalent to povidone-iodine in eradicating *A. baumannii* colonies in wounds in vivo.

KEYWORDS:

Antiseptic, octenidine dihydrochloride, povidone-iodine, *A. baumannii*

INTRODUCTION

Infection wound care is a global problem that requires innovative strategies to fight microorganisms and biofilms.

Antiseptic irrigation is expected to reduce germ colonies and help eradicate infection. One essential pathogenic germ is *Acinetobacter baumannii*. This opportunistic pathogen can cause nosocomial infections, especially in operating rooms and intensive care units.^{1,2} Based on data from the Clinical Microbiology Unit of Dr. Soetomo General Hospital Surabaya in January–December 2019, it was reported that *A. baumannii* bacteria were the most commonly found bacteria in the burn unit, surgical ward, high care unit and intensive observation room, besides that these bacteria were resistant to 7 of 11 antibiotics including gentamycin, amoxicillin-clavulanate, ceftazidime, piperacillin-tazobactam, levofloxacin and chloramphenicol.

Infection by these bacteria leads to impaired wound healing. It can also spread to the circulatory system resulting in sepsis, with a high mortality rate if patients are not adequately treated.³ *A. baumannii* has a faster rate of biofilm development than other species.⁴ The biofilm matrix surrounding the bacteria allows the germs to survive in extreme circumstances and become antibiotic-resistant. As a result, drugs now available to treat *A. baumannii* biofilm-related infections have become ineffective.⁵ Given that any injury carries a high risk of infection, effective management of bacterial biobload is a crucial component of wound care.

A crucial step in preventing further infectious incidences is using antiseptics to fight colonisation and infection directly at the portal of entry. Many experiments have been undertaken in the previous few decades to produce novel antiseptic agents like chlorhexidine, iodine or iodophores to attain the best circumstances in killing or inhibiting bacteria.⁶ Denysko et al.⁷ found that among the tested antiseptics, decamethoxin and octenidine showed the greatest activity against clinical strains of *A. baumannii*, followed by povidone-iodine, polyhexanide, chlorhexidine and miramistine.⁷ Commercially available products containing decamethoxin and miramistine are not available in Indonesia. Chlorhexidine-cetrimide and povidone-iodine are the recommended antiseptics for wound care at Dr. Soetomo General Hospital Surabaya. In addition, Pradnyana's⁸ comparative analysis revealed that povidone-iodine exhibited superior efficacy in reducing bacterial colony counts in *A. baumannii*-infected wounds compared to chlorhexidine-cetrimide. Povidone-iodine is an antiseptic solution commonly used in healthcare settings to prevent

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and treat infections. It is effective against a broad range of bacteria, viruses, fungi and protozoa, making it a versatile antiseptic agent used in various healthcare settings. However, povidone-iodine carries the risk of affecting thyroid function.⁹ Considering these factors, exploring alternative antiseptics to replace previously used ones is crucial. Currently, octenidine is offered as an alternative antiseptic in Indonesia. Octenidine dihydrochloride is a novel cationic antiseptic that belongs to the bispyridine class.¹⁰ It disrupts the microbial cell envelopes and eukaryotic cell membranes through strong adherence to lipid components and binding to negatively charge microbial surfaces. Preliminary results imply a particularly strong adherence to lipid bacterial cell membrane components explaining the high antimicrobial efficacy without adversely affecting human epithelial or wound tissue.¹¹⁻¹³ Comparison of its biocompatibility with other disinfectants, such as povidone-iodine or chlorhexidine, showed that octenidine is an antiseptic with low cytotoxicity and high microbicidal effect.^{14,15} Octenidine was found to have the most potent efficacy against biofilms of multidrug-resistant clinical microorganisms, including *A. baumannii* in vitro.⁷

However, no data have been reported for the effectiveness of octenidine and povidone-iodine as an antiseptic solution against *A. baumannii* in vivo. This study aims to determine the antiseptic effectiveness of octenidine dihydrochloride 0.1% and povidone-iodine 10% as antiseptic by comparing the bacterial colony reduction on wounds contaminated with *A. baumannii*.

MATERIALS AND METHODS

Animal Preparation

Animals Male Wistar Rat (200–300g, 7–10 weeks of age) were obtained from Animal Lab of Pharmacy Department Universitas Airlangga and maintained under a 12-hour light/dark cycle. Food and water were available ad libitum. The rats were divided into three groups (Group 1 was the control group; Group 2 was octenidine dihydrochloride group; and Group 3 was the povidone iodine group). Sample size was eight rats per group using the resource equation method.¹⁶

$E = \text{Total number of animals} - \text{Total number of groups}$

According to this method, a value “E” is measured, which is nothing but the degree of freedom of analysis of variance (ANOVA). The value of E should lie between 10 and 20. If E is less than 10 then adding more animals will increase the chance of getting more significant result, but if it is more than 20 then adding more animals will not increase the chance of getting significant results. Any sample size that keeps E between 10 and 20 should be considered as an adequate. The sample size of eight rats per group in this study is considered adequate for the purposes of statistical analysis.

Wound Creation

Animal handling was performed under anaesthesia induced by ketamine (20 mg/kg; Ketamine HCl, Bernofarm Pharmaceutical Company, Indonesia). The rat's back was shaved then a 2 × 2 cm deep full-thickness wound was made on the skin using a scalpel. At the end of the experiments, the animals were killed with an overdose of pentobarbital.

Wound Contamination and Bacterial Counts

A. baumannii strains tested were obtained from the Institute of Tropical Disease Airlangga University, a positive culture isolate of patients at Dr. Soetomo General Hospital Surabaya. *A. baumannii* were inoculated into the wounds at the dose of 1.5×10^8 CFU/mL (0.5 mL McFarland), and the wound was covered with a transparent dressing. After 3 hours, the wound was irrigated with wound cleansing solution according to the group using a 20 cc syringe with constant pressure for 30 seconds. The first group was designated as the control group. This group was only applied normal saline 0.9% (Ecosol NaCl®, B-Braun Medical Ltd., Indonesia); the second group was applied octenidine dihydrochloride 0.1% (Octadin®, Infion Ltd., Indonesia) and the third group was applied povidone iodine 10% (Betadine®, Mahakam Beta Farma Ltd., Indonesia).

The wounds were swabbed with sterile cotton-tipped applicators before and after wound irrigation. In addition, tissue cultures were taken at 3 hours after irrigation. The samples were plated onto Mueller-Hinton agar (Oxoid CM0337, UK) to quantify the number of viable bacteria and incubated at 37°C overnight. Specimens were ground and plated immediately, and colony-forming units (CFUs) were read 24 hours after plating using biological microscopes (Olympus CX23, Japan).

Statistical Analysis

Data analysis was performed using a statistical software package (Statistical Package for Social Sciences 15 for Windows). The significance level was considered to be $P < 0.05$.

Ethical Clearance

All experimental protocols described in the present study were approved by the Health Research Ethics Committee Faculty of Dentistry Universitas Airlangga (151/HRECC.FODM/II/2023).

RESULTS

All specimens showed bacterial colony growth with a median value of 1.22×10^5 CFU (range 1.02×10^5 – 1.43×10^5). As shown in Table I, wound irrigation with normal saline did not reduce colony counts. At the same time, there was a 3-log reduction to 5-log reduction in colony counts in the octenidine and povidone-iodine groups. However, 3 hours after irrigation, all specimens that experienced 3-log reduction showed regrowth to more than 1×10^5 CFU, whereas specimens subjected to 5-log reduction did not exhibit any regrowth (Table II). Statistically, there was no significant difference in the mean reduction of colonies between the octenidine and Povidone-iodine groups after irrigation ($p=0.535$), as shown in Table III.

DISCUSSION

A. baumannii multidrug-resistant strains tend to evolve quickly, which is concerning. In intensive care units, it makes up between 2 and 10% of all gram-negative hospital-acquired infections.¹⁷ The use of antiseptics as the primary active ingredient and potentiator of antibiotics is crucial for treating patients with infected burns, post-traumatic wounds

Table I: Frequency of bacterial reduction post irrigation on *A. baumannii*-contaminated wounds

Group	No Reduction	Log-3 Reduction	Log-5 Reduction
Normal Saline	8 (100%)	-	-
Octenidine	-	6 (75%)	2 (25%)
Povidone-Iodine	-	7 (87.5%)	1 (12.5%)

Table II: Frequency of bacterial growth 3-hour post irrigation on *A. baumannii*-contaminated wounds

Group	No growth	>10 ⁵ CFU
Octenidine	2 (25%)	6 (75%)
Povidone-iodine	1 (12.5%)	7 (87.5%)

Table III: Mean reduction and mean growth of CFU on antiseptic applications

	Octenidine (%)	Povidone-iodine (%)	p-value*
Mean reduction post irrigation	99.25	99.13	0.535
Mean growth 3 hours post irrigation	99.00	99.00	0.535

* Mann-Whitney U test.

and surgical wounds in the context of the global rise in antibiotic resistance.^{18,19}

This study compared the effectiveness of octenidine antiseptic with povidone-iodine for wounds contaminated with *A. baumannii* in Wistar rat. This was the first in vivo study in this field to compare the efficacies of two antiseptics for treating *A. baumannii* contamination wounds in an experimental model.

The use of octenidine and povidone-iodine resulted in a significant bacterial count reduction. However, our findings indicate no significant difference between the effectiveness of povidone-iodine and octenidine antiseptics in reducing *A. baumannii* colonies in vivo. We found the mean CFU reduction after the application of the antiseptics were as follows: octenidine 99.25% and povidone-iodine 99.13% ($p=0.535$). Denysko et al. (2022) found that both octenidine and povidone-iodine had bactericidal action against *A. baumannii* in an in vitro study. Among the tested antiseptics, decamethoxin and octenidine showed the greatest activity against clinical strains of *A. baumannii*, followed by povidone-iodine, polyhexanide, chlorhexidine and miramistine.⁷ In an in vitro study, Koburger et al. described the most effective antiseptics. Prioritising the agent of choice should be octenidine = povidone-iodine >> polyhexanide > chlorhexidine > triclosan when an immediate effect is needed. If a longer contact time is necessary (as in wound antiseptics and mucosal infection therapy), polyhexanide = octenidine > chlorhexidine > triclosan > povidone-iodine should be prioritised.²⁰

The difference in antiseptic effectiveness against *A. baumannii* observed in the previous study and the absence of significant differences in the in vivo study may be due to several factors. In vitro studies are conducted under controlled laboratory conditions and may not fully reflect the human body's complexities and the in vivo environment. In vivo studies, on the other hand, are conducted in living organisms and may involve factors such as immune response, wound healing and tissue damage that can affect the effectiveness of antiseptics.

This study also examined the number of colonies three hours after irrigation using tissue culture to determine whether the antiseptic effect was still in effect. Specimens that underwent a 3-log reduction demonstrated regrowth to levels exceeding 1×10^5 CFU, whereas specimens that underwent a 5-log reduction did not exhibit any regrowth. While complete eradication of bacteria is ideal, it is often not achievable in clinical settings, particularly in the presence of biofilm or other factors that may promote bacterial growth. Therefore, a reduction to log 3 CFU can still be considered a good outcome, especially since it was achieved after a single 30 seconds irrigation treatment in this study. However, further research is needed to determine if repeated irrigation or prolonged treatment could completely eradicate the bacteria in this wound model.

CONCLUSIONS

In this study, it can be concluded that octenidine dihydrochloride and povidone-iodine irrigation effectively reduce the number of colonies of *A. baumannii* colonies. The antiseptic effectiveness of octenidine dihydrochloride is equivalent to povidone-iodine in eradicating colonies of *A. baumannii* bacteria in vivo.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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