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Evaluation of The Antiseptic Efficacy of 4% Chlorhexidine Gluconate and 10% Povidone Iodine On Methicillin-Resistant Staphylococcus aureus-Infected Wounds In White Rat (Rattus norvegicus)

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA), an opportunistic bacterium that harms immunocompromised hosts, was 8.1% at our hospital in 2014, with 8.2% of cases in the surgical wards. Chlorhexidine gluconate (CHG) and povidone iodine (PVP-I) are excellent antiseptics for MRSA-infected wounds.

Groups of male Wistar rats (n=15) were wounded and inoculated with MRSA then divided into three groups of intervention; 4% CHG, 10% PVP-I, and saline (control). After a six-hour incubation, each wound was intervened. Swab samples were taken before (first result), after intervention (second result), and 24 hours after intervention (third result), followed by a tissue sample taken in the end (fourth result). Using colony-forming units (CFU)/ml, the bacterial colonies were divided into three logarithmic groups: $\log_{10}$ 1, ≤ $\log_{10}$ 5 (≠ 0), and > $\log_{10}$ 5. All data were analysed using a statistic with a significance level of $P < 0.05\%$.

Only the second result of all groups showed a reduction in colony number, while the other results showed > $\log_{10}$ 5 CFU/ml. Sixty percent of the second result of PVP-I group showed no bacteria, the rest, 40%, showed ≤ $\log_{10}$ 5 (≠0) CFU/ml. All of the second result of CHG group showed no bacteria. We found a significant difference in the second results of all groups ($P = 0.009$) and in the comparison between CHG versus control group and PVP-I versus control group ($P = 0.003$ and 0.049, respectively).

The effectiveness of 4% CHG and 10% PVP-I is equivalent in MRSA-infected wound care in this study.

Keywords: Wound care, Wistar rats, *Rattus novergicus*, chlorhexidine gluconate, povidone iodine, methicillin-resistant *Staphylococcus aureus*, MRSA-infected wound

Introduction

Staphylococcus aureus (*S. aureus*), a Gram-positive bacterium, is one of the most common opportunistic pathogens that can cause nosocomial infections in both immunocompetent and immunocompromised patients in healthcare facilities. As humans serve as natural reservoirs for *S. aureus,* it is a potential threat to public health. (1,2) Upon superficial penetration, *S. aureus* is known to cause minor skin infections such as folliculitis. (3) However, upon deeper penetration, it has the potential to cause bacteraemia and severe disease manifestations such as sepsis and infective endocarditis, with significant morbidity and mortality. This can result in a prolonged length of stay and an increased burden on the patient, the patient's family, and the healthcare providers, both physically and mentally, as well as an economic burden to the nation. (4,5)

Shortly after the introduction of methicillin as an antibiotic, *S. aureus* developed the ability to resist new antimicrobials, leading to the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the early 1960s. Since that time, MRSA has continued to be the world's leading nosocomial pathogen, attracting the attention of the health community. (5-7) Over the decades, its prevalence

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has continued to increase, as has its resistance to a variety of antimicrobial agents. (4,8,9) As of present, MRSA constitutes over 50% of the hospital-acquired *S. aureus* strains in the majority of nations. It is estimated that approximately 50 million individuals worldwide are colonised with MRSA. (7) In 2019, the global incidence of antimicrobial resistance has resulted in over 100,000 mortalities, with a significant proportion of these mortalities being attributed to MRSA. (9) In 2011, more than 50% of MRSA specimens were detected in ICU in the United States. (10) In 2019, CDC still declared MRSA as one of serious threats as estimated cause 323,700 infection cases with 2,700 deaths in the United States. (11)

Asia is recognized as one of the regions with the highest incidence of MRSA worldwide. (12,13) Reports from Southeast Asian countries indicate a highly variable prevalence rate ranging from 2% to 80%. (13,14) Currently, there is a scarcity of data regarding the prevalence of MRSA in Indonesia. In 2011, a study was conducted in three teaching hospitals located in Denpasar, Bali; Semarang, Central Java; and Malang, East Java, that revealed a prevalence of 4% MRSA from 1,502 patients screening for nares, throat, and skin lesion discharge. (15) During a 4-month period in 2001, a study identified the presence of 0.3% MRSA in 329 nasal carriage isolates obtained from patients in area of Java, Indonesia. (16) In 2014, a study carried out at our hospital, Dr. Soetomo General Academic hospital, Surabaya, East Java which reported a prevalence of MRSA at 8.1% among a sample of 643 patients over a duration of four months. That study revealed the incidence rate was 8.2% among patients in surgical wards and 8.0% among patients in non-surgical wards. (17) As samples were obtained from the same region, those data showed a notable rise of MRSA prevalence in 13 years from 0.3% in 2001 to 8.1% in 2014. The extent of MRSA prevalence in this nation may yet be ascertained.

The primary mode of transmission of MRSA is via patient-to-patient contact, which encompasses airborne MRSA pneumonia and the utilization of ventilators, or potential contamination through the unclean hands of healthcare providers. (10,11,18) The capacity of MRSA to persist on inanimate surfaces for a duration ranging from seven days to seven months. (18,19) Given this information, it is postulated that MRSA may have exhibited a wide distribution that particular timeframe. The healthcare system has established a globally accepted protocol to mitigate the transmission of MRSA, which consists of standard precautions, contact precautions, active surveillance cultures (ASC), environmental cleaning, an even rational distribution ratio between healthcare providers and patients, judicious use of antimicrobial agents, and a MRSA decolonization regimen. (5,20) The wound infected with MRSA may serve as a potential origin for the dissemination of MRSA, while concurrently increasing the likelihood of bacteraemia in patients who have that wounds. To date, a universally accepted protocol for managing MRSA-related wound care has not yet to be established, and this aspect must also taken into account.

In Indonesia, including rural areas, povidone iodine (PVP-I) and chlorhexidine gluconate (CHG) remain the primary antiseptics utilized despite the emergence of novel alternatives globally. Both PVP-I and CHG have demonstrated efficacy in eliminating MRSA colonies present in wound sites. (21) The bactericidal properties of CHG increase proportionally with its concentration. (22) At a concentration of 9.6%, PVP-I exhibits bactericidal properties against *S. aureus*. (23) Our search yielded no prior studies that employed the same methodology and investigated the same topic as ours. However, there existed certain studies that bore resemblance. In 2013, Kulkarni et al. examined the comparative effectiveness of 2% CHG and 10% PVP-I for skin disinfection. (24) In 2015, Vestby et al. examined the efficacy of chlorhexidine (CHX), pyrisept, and iodine in relation to *S. aureus* biofilm. (25) In 2016, Kanno et al. investigated the effectiveness of wound irrigation using 1% iodine solution. (26) In 2018, Lakhi et al. examined the differences in vaginal cleansing between 4% CHG and 10% PVP-I. (27) Based on several research studies, it can be inferred that CHG exhibits greater efficacy as compared to PVP-I. (27-29) Our hypothesis posits that the aqueous form of 4% CHG is superior to the aqueous form of 10% PVP-I in reducing MRSA colonies in the wound of Wistar rats. The objective of this investigation was to assess the efficacy of 4% CHG and 10% PVP-I in eradicating MRSA in animal model that monitored twice, once immediately following intervention and again 24 hours post intervention. Our result study hopefully will provide a support data for future research on humans with MRSA-infected wounds that will be used to establish MRSA-infected wound care protocols in our hospital located in East Java, as well as throughout our nation, Indonesia.

Material and Methods

Randomized controlled trials were conducted on Wistar strain white rats (*Rattus novergicus*) without blinding. This study was approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia, with ethical clearance (Number 2.KEH.098.08.2022). This study was conducted on August 20th, 2022, in the laboratory at the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia. This study's protocol followed the HARRP guideline designed by International Council for Laboratory Animal Science (ICLAS) for randomized trial protocol in animal study, and National Institutes of Health Guidelines. (30,31) The subject of this research was a 2x2 cm MRSA-infected wound on the dorsal region of a rat. The study employed three independent variables, namely treatment with normal saline as a control, 4% CHG, and 10% PVP-I; the dependent variable was colony-forming unit (CFU)/ml. This study's inclusion criteria were male Wistar strain white rats of the *Rattus novergicus* species, aged three months, weighing 250–300 grams, and in good health as certified by a certificate. The rats that gained weight loss > 10%, experienced hair loss, decreased activity, or had exudative eyes before therapy were excluded. Our study included three samples: 1) MRSA-infected wounds intervened with saline; 2) MRSA-infected wounds intervened with 10% PVP-I; and 3) MRSA-infected wounds intervened with 4% CHG. According to the ANOVA resource equation variant [E = number of animals − number of groups], our sample sizes ranged between 13 and 23, with constant E values of 10 and 20. A total of 15 rats were utilized, with each group consisting of five rats. (32)

Bacterial Strains and Culture Conditions: The suspension of MRSA was provided by the Institute of Tropical Diseases at Universitas Airlangga. The MRSA was isolated from a human clinical specimen and cultivated until it attained the required suspension concentration. The suspension was cultivated on Müller-Hinton (MH) agar (Oxoid®, Thermo Fisher Scientific, United Kingdom) at 37°C and pH 7.2-7.4 for 24 hours. Then, discs containing antibiotics (methicillin, penicillin, tetracycline, doxycycline, erythromycin, and gentamicin) (Oxoid®, Thermo Fisher Scientific, United Kingdom) was placed on MH agar and stored for 24 hours to determine the MIC (Minimally Inhibitory Concentrated). The presence of MRSA was verified on discs lacking the MIC data for each antibiotic. The bacteria suspension preserved in a medium consisting of 85% glycerine (Emsure®, Merck, Germany) and stored at a temperature of -80°C.

Animal Model: Fifteen rats were subjected to the experiment, with every two to three rats being placed in an individual cage that had undergone a two-week acclimation period in the laboratory setting prior to the intervention. A balanced and standardised pellet diet, water, and the same controlled conditions were ensured. Anaesthesia was induced using 10% ketamine 40 mg/kg (KTM-100®, PT. Guardian Pharmatama, Indonesia) and 2% xylazine 5mg/kg (Xyla®, Interchemie, Holland), intramuscularly. The operating table, instruments, disposable surgical clothing, and surgical site were prepared following aseptic and antiseptic protocols. The schematic of the experimental procedure see Figure 1.

After the rat was shaved and cleaned, a disposable surgical cloth with a 2.5x2.5cm hole was positioned on its dorsum. We incised according to a 2x2 cm design and then excise skin until the wound bed fascia was exposed. The MRSA suspension using McFarland 0.5 as equal to $1.5 \times \log_{10}$ 8 was applied to each wound using a sterile cotton swab until it had complete coverage, leaving the periwound. Then covered with a transparent dressing to prevent external contamination. Six hours later, the dressing was removed, and the first swab specimen (first result) (Figure 2) was collected by gently pressing a sterile cotton swab into the wound center until it contacted the wound bed. The sterile cotton bud tip is deposited in a tube containing tryptic soy broth (TSB) and processed for bacteria growth on MH agar with 24-hour storage (no CO_2). Then, CFU/ml was calculated. The first specimen determined the initial number of CFU per ml.

The wounds were intervened with irrigation using 2 ml of 10% PVP-I (Povidine®, OneMed, Indonesia) (Figure 3) for the second group and 4% CHG (OneSCRUB®, OneMed, Indonesia) (Figure 4) for the third group. A syringe and abbocath were used to administer the irrigation for a duration of 30 seconds, commencing from the center and extending outward and centripetally to cover the entire wound bed. Then, 20 ml of normal saline (Otsu-NS®, Otsuka, Indonesia) was irrigated for 30 seconds to eliminate PVP-I and CHG. We placed a second specimen swab (second result) in the wound center, which was not wet, and then covered it with transparent dressing. After 24 hours of intervention, we opened the

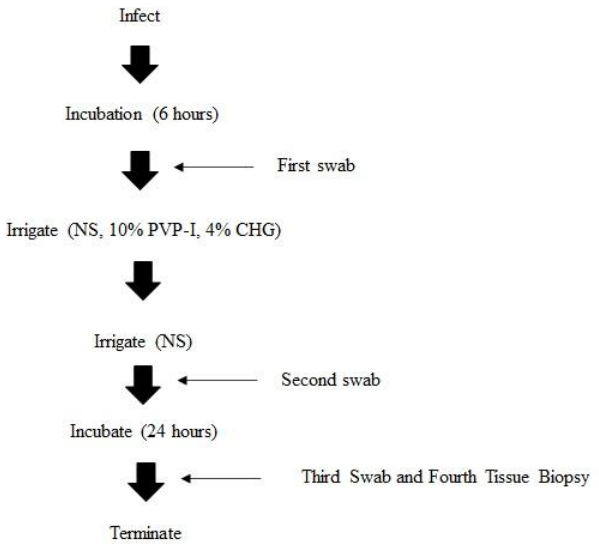

Fig. 1. Schematic of The Experimental Procedure

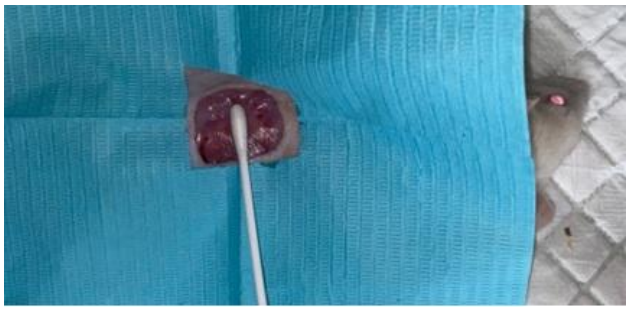

Fig. 2. Collecting A Swab Specimen

wound and collected the third specimen swab (third result) and excised the entire wound bed fascia for a tissue biopsy specimens (fourth result). As with the other two groups, the control group received 22 ml of normal saline and was intervened the same. The specimens were sent to the Institute of Tropical Diseases at Universitas Airlangga for processing. The entire subject was euthanized per the protocol for disposing of infectious medical waste.

Statistical Analysis: The data were analysed using the Kruskal-Wallis, Mann-Whitney, and Wilcoxon-T tests when it was known that the data had an abnormal distribution (the Shapiro-Wilk test was used to test the data distribution). Statistical significance was determined when *P*-values were < 0.05. We used Statistical Package for The Social Sciences (SPSS) version 26.0 IBM, New York 10504-1722, United States, 914-499-1900, for statistical analysis.

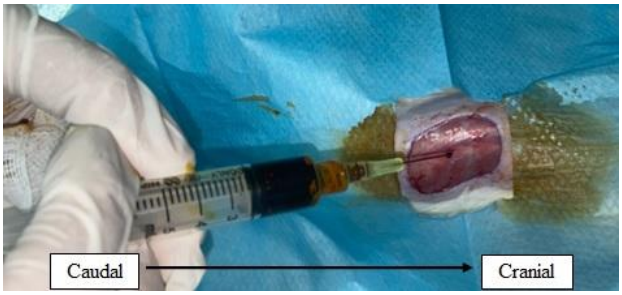

Fig. 3. Irrigating with 10% PVP-I

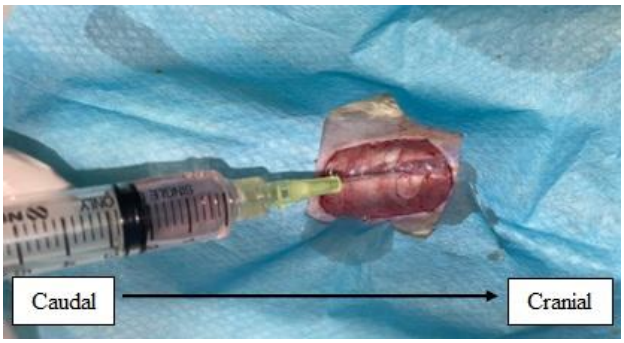

Fig. 4. Irrigating with 4% CHG

Results

Final Sample Size: In this study's final analysis, there were five rats in each of the three groups (total number of rats n = 15).

Microbiological Findings: Table 1 presents alterations in MRSA colony numbers in this study. Five out of five (100%) of the first result of PVP-I, CHG and control group showed > $\log_{10}$ 5 CFU/ml. Three out of five (60%) of the second result of PVP-I group showed no bacteria, two out of five (40%) showed ≤ $\log_{10}$ 5 (≠0) CFU/ml. Five out of five (100%) of the second result of CHG group showed no bacteria. The third and fourth results of all groups showed 100% (5 out of 5) > $\log_{10}$ 5 CFU/ml.

Based on Figure 5, it appears that 4% CHG is more effective at eliminating MRSA bacteria than 10% PVP-I and normal saline. The Kruskal-Wallis test revealed a statistically significant difference in the number of MRSA colonies between all three groups (P = 0.009). Regarding the first, third, and fourth results, the number of MRSA colonies in each analysis was not statistically significant (P = 1.0) (Table 1). In the comparison between two groups based on the second result's MRSA colony number (Table 2), both 4% CHG and 10% PVP-I to normal saline revealed statistically significant results, with P values of 0.003 and 0.049, respectively, using the Mann-Whitney test. Meanwhile, there was no significant difference in

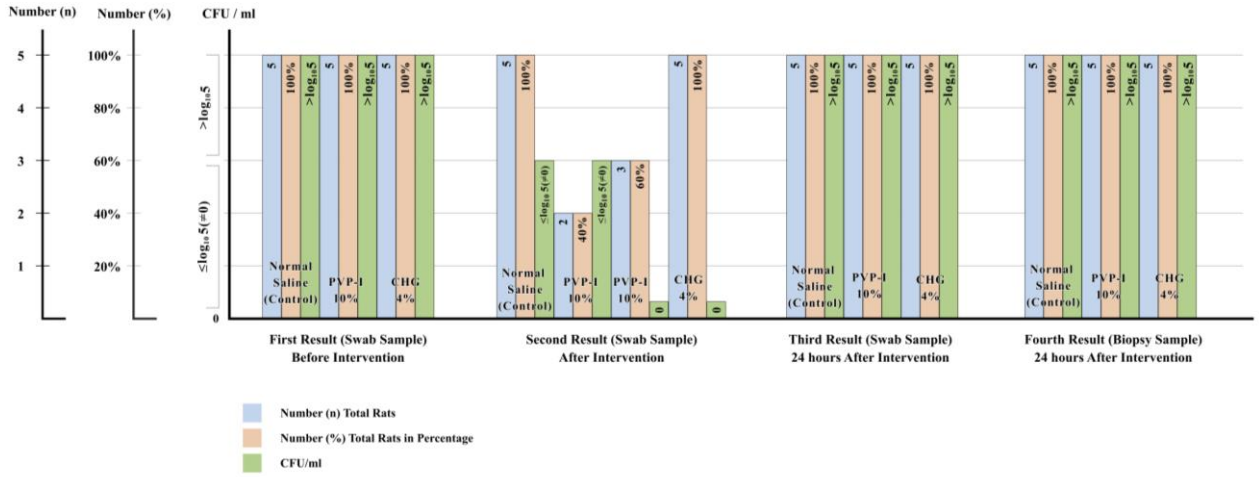

Fig. 5. Alterations in MRSA Colony Numbers Across Diverse Intervention Cohorts At Varying Time Intervals

the comparison of colony number between 4% CHG group and 10% PVP-I group with *P* statistical test results between the first and third result; and between the first and fourth result in each= 0.134. Further Wilcoxon testing revealed significant differences in the number of colonies in each group: 10% PVP-I, 4% CHG and normal saline, respectively, with *P* values of 0.038, 0.025, 0.025. The same group did not differ substantially (*P* = 1.0) (Table 2).

Discussion

S. aureus is the most common opportunistic pathogen in acute and chronic wounds. (21) As *S. aureus* can become MRSA, the diverse virulence of MRSA and its quasi-dormant characteristics contribute to the recurrence of infections. The distinct and perilous characteristics of MRSA have instigated endeavors to eliminate it through the implementation of a decolonization regimen. (20) However, there is no specific procedure for the treatment of MRSA-infected wound. Due to their omnipresence and rapid proliferation, a colonized wound has the potential to exhibit signs of infection within a matter of hours. (21) Thus, the reduction of microbial contamination in the wound is crucial in the management of infection. Wound care may be painful for patients, yet strong analgesics are restricted to wards for wound care in our hospital. Wound maceration can occur with prolonged wound care. All of these factors require a realistic and effective wound treatment strategy that minimizes pain and bacterial load quickly, especially in our hospital. Yasuda et al. reported that PVP-I destroyed 20 bacterial strains in 20 seconds. (33) Meanwhile, CHX affects microorganisms in 20 seconds. (22,34,35) Given these challenges, the rapid action of CHG and PVP-I, and knowing that our hospital has consent concerning MRSA, we investigated the antiseptic effects of 10% PVP-I and 4% CHG in rat models with MRSA-infected wounds. This pilot study will provide data for forthcoming research to our institution in order to set up a MRSA-infected wound care protocol.

In theory, CHX exhibits bactericidal properties at elevated concentrations through the mechanism of destabilizing the cell wall, resulting in structural integrity loss within the cells. (21,28,36) High concentrations of CHX induce cell mortality by forming an intracellular precipitate with adenosine triphosphate (ATP) and nucleic acid that is irreversible. (37) The CHX has demonstrated effectiveness against Gram-positive and Gram-negative bacteria, as well as certain fungi. Novel antimicrobial nanoparticles based on a hexametaphosphate salt on CHX, that rapidly adhere to specimens of glass and titanium also exhibited antimicrobial efficacy against MRSA in both planktonic and biofilm growth conditions. (21) At the other hand, PVP-I is an iodophor that is soluble in water and comprises a complex of iodine and polymer polyvinylpyrrolidone (povidone/ PVP). Upon dissolution in water, the iodine is released in the form of free iodine I_2, which can effectively infiltrate microorganisms and induce oxidation of proteins, nucleotides (with a particular emphasis on cysteine), and fatty acids. This process results in a reduction of protein synthesis and damage to the cell membrane or wall, ultimately leading to cell death. (21,37) The PVP-I has a broad spectrum of activity against Gram-positive (including MRSA)

Table 1. Alterations in MRSA Colony Numbers Across Diverse Intervention Cohorts at Varying Time Intervals

The Time of Intervention	Antiseptic	Frequency (n=5)	Percentage	CFU/ ml	P Value*
First result from swab specimen					
After 6 hours inoculation	Normal Saline (control)	5	100%	> $\log_{10}$ 5	1.000
	10% PVP-I	5	100%	> $\log_{10}$ 5	
	4% CHG	5	100%	> $\log_{10}$ 5	
Second result from swab specimen					
After intervention	Normal Saline (control)	5	100%	≤ $\log_{10}$ 5 (≠0)	0.009
	10% PVP-I	2	40%	≤ $\log_{10}$ 5 (≠0)	
		3	60%	0	
	4% CHG	5	100%	0	
Third result from swab specimen					
24 Hours after intervention	Normal Saline (control)	5	100%	> $\log_{10}$ 5	1.000
	10% PVP-I	5	100%	> $\log_{10}$ 5	
	4% CHG	5	100%	> $\log_{10}$ 5	
Fourth result from tissue biopsy					
24 Hours after intervention	Normal Saline (control)	5	100%	> $\log_{10}$ 5	1.000
	10% PVP-I	5	100%	> $\log_{10}$ 5	
	4% CHG	5	100%	> $\log_{10}$ 5	

*Kruskal-Wallis test was used

and Gram-negative bacteria, fungi, viruses, protozoa, and bacterial spores. (21,38) Different mechanisms of action between CHG and PVP-I may have contributed to this study's disparate results. We suspected the dosage forms of PVP-I and CHG differ also contributed to our study result. The water-soluble form of PVP-I facilitated the rapid passage of liquid through the convex-shaped dorsal wound of the rat. Conversely, CHG in the form of soap-based products adhered more to the wound bed, so it worked longer.

In this study, changes in the number of MRSA bacterial colonization only appeared significant in the second swab results, namely immediately after the intervention compared to the results of the other specimens. This finding is supported by another study. Watts found in 2016 that irrigating the wound and implant surface with CHG 0.05% for one minute and flushing with normal saline was a safe and effective alternative to antibiotic irrigation. (34) In an *in vitro* experiment, a 0.05% CHG solution effectively reduced MRSA and Methicillin-Sensitive *Staphylococcus aureus* (MSSA) microbial recovery by five to six log at one and five minutes, respectively. (39) Previous studies have shown that PVP-I kills all types of MRSA strains (33/33 strains, compared to CHX which only kills 3/33 strains) within 15-30 seconds. (38)

A third study in an orthopaedic patients population using a bundling strategy of active surveillance for MRSA, decolonization in all positive patients (nasal mupirocin, twice daily and total body wash with 4% CHG aqueous-based for 5 days), found a significant decrease in the rate of selective MRSA infections (P = 0.032) and total surgical site infections (SSI) (P = 0.0093) during

Table 2. Mann-Whitney and Wilcoxon Statistical test Results

The Time of Treatment	Antiseptic	P Value
Mann-Whitney test results		
Results on second swab specimen results	Normal Saline (control)	0.049
	10% PVP-I	
	Normal Saline (control)	0.003
	4% CHG	
	10% PVP-I	0.134
	4% CHG	
Wilcoxon test results		
Between first and second specimen results	Normal Saline (control)	0.025
	10% PVP-I	0.038
	4% CHG	0.025
Between first and third specimen results	Normal Saline (control)	1.000
	10% PVP-I	1.000
	4% CHG	1.000
Between first and fourth specimen results	Normal Saline (control)	1.000
	10% PVP-I	1.000
	4% CHG	1.000

the intervention period (N = 7,019). (40) A randomized clinical trial by Darouiche on 849 subjects demonstrated a lower surgical site infection (SSI) rate in the CHX group than PVP-I (RR 0.59; 95% CI 0.41-0.85). (41) Xiao's meta-analysis revealed that rinsing with CHX antiseptic reduced the incidence rate ratio of MRSA infections by 0.65. (28) According to the research conducted by Wade, CHX is twice as efficient as PVP-I in avoiding SSI. (29)

At the other hand, in 2010, Hill's study on the *in vitro* biofilm model demonstrated that iodine-based dressings completely eradicated a seven-day-old mixed Pseudomonas and Staphylococcus biofilm. (42) In an *in vitro* study it was also found that the logarithmic reduction factor (LRF) was greater in the treatment with PVP-I compared to CHX. Compared with PVP-I, the microbicidal effect of CHX on MRSA was also found to be lower (3.07 vs 3.47). (38)

Previous studies supported our finding that PVP-I and CHG are better than normal saline. Non-significant difference between PVP-I and CHG in our study is similar to Srinivas (43) and Kulkarni (24) findings and Belo study on dogs. (44) The study found no significant difference including in the logarithmic decrease in the number of bacteria PVP-I (6.51 ± 1.94 $\log_{10}$) vs CHX (6.46 ± 2.62 $\log_{10}$). (44) A randomized trial comparing 10% PVP-I to 4% CHG for vaginal hysterectomy revealed that CHG is as secure for vaginal tissues as PVP-I. (45)

Some data suggest PVP-I in concentrations used in clinical situations, i.e. > 10% (0.9% iodine), would be cytotoxic to all cells involved in wound healing. (46) In contrast, research in 2001 by Bennett on porcine models of wound healing has shown that application of 10% PVP-I solution is associated with increase in the number of proliferating fibroblasts at day four and enhanced angiogenesis at day seven as compared to the controls. (47) Fibroblasts and keratinocytes exposed to 0.05% CHX for 15 minutes lose viability, according to in vitro studies, within 24 hours.(5c Level). (45) Another *in vitro* investigation revealed a substantial decrease in fibroblast proliferation (P = 0.05) after 96 hours of exposure to CHX at a concentration of 0.0032%. In contrast, fibroblast proliferation was significantly increased at a concentration of 0.0004% CHX (16% versus 7%, P = 0.05). (5c Level). (48) This aligns with our research initiative to expedite the implementation of CHX and PVP-I antiseptic irrigation as a treatment for wounds infected with MRSA.

MRSA recolonization during observation 24 hours after antiseptic irrigation may occur due to migration of bacteria from the tissue to the surface. Intracellular invasion of MRSA allows the bacteria to become protected from various extracellular host defences and wound cleanings. This is supported by the findings of MRSA colonization on tissue biopsy examination in this

study. (49) A cross-sectional study by Ferrara on the duration of the antimicrobial effect of CHX found persistent bactericidal activity for up to four hours post application. (50) Another study on the skin of a pig model showed antibacterial activity of PVP-I for up to 12 hours. (51) This finding also is in line with research conducted by Ghaddara, who described that suppression of MRSA was only effective in the first one to six hours after application using PVP-I. (52)

Cookson's 1991 study cited evidence of MRSA resistance to CHX. (53) While the likelihood of PVP-I resistance is said to be extremely low. (21) In general, resistance (or diminished sensitivity) can be caused by intrinsic (biofilm, endospore) or extrinsic (acquired mutation, genetic transfer) alterations. Resistance to CHG is predominantly mediated by mutase and the presence of the efflux protein qacA, whereas resistance to PVP-I is associated with iodine's multimodal effect. Meta-analysis performed by Aftab et al. found the lowest minimal change in bactericidal concentration (Minimum Bacterial Concentration/ MBC) in CHG (2 mg/L) against MRSA ($P<0.001$). The reduction of CHX susceptibility still lacks efficacy data. (36) In the meantime, there are insufficient data for synthesizing pooled PVP-I's MBC. (54)

This study was subject to certain limitations, including its narrow focus on a singular concentration of each antiseptic, limited interval time parameters, different types of antiseptic solvents, and the lack of histological analysis of the wound observation.

In our study, as for the MRSA-infected wounds of rats, the use of 4% CHG antiseptic was as effective as 10% PVP-I in eradicating bacteria. Further studies are needed to investigate whether CHG alone or in combination with PVP-I, which has a wider range of antiseptic concentrations and solvent variation, is more frequent in measuring colony bacteria, and considers the specific type of bacteria present, whether in animal models or clinical trials on humans, as the basis for setting up a MRSA-infected wound care protocol in our Hospital, which hopefully could be adapted in healthcare facilities in Indonesia.

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Conflicts of Interest: The authors report no potential conflicts of interest.

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