

The effectiveness of dalethyne dressings for reducing bacteria in diabetic foot ulcers



Original article

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Received: 25 October 2020; Accepted: 16 November 2020; Published: 20 June 2021

Abstract: **Objective:** This study evaluates the effectiveness of a dalethyne dressing for decreasing bacteria in diabetic patients with infected foot ulcers.

Methods: This study was conducted from March to September 2018 with a sample of 30 participants from the outpatient Kitamura Wound Clinic in Pontianak City, Indonesia. A quasi-experimental non-equivalent pretest–posttest control group design was used for the study. Participants were divided into two groups: an intervention group (treated with a dalethyne dressing) and a control group (treated with a standard dressing). Two trained research assistants collected the data using the Wagner wound classification system and a bacteria counter. The assistants swabbed each wound surface with sterile cotton, and the swabs were used to conduct a bacteria culture and count.

Results: The study population was 50% female and 50% male with no significant differences between each other in age, HbA1c, blood pressure, or ankle-brachial index (ABI; $P > 0.05$). Both groups had a significant reduction in the number of bacteria from the pretest to posttest ($P < 0.05$). Mann–Whitney analysis of posttest data indicated a significant difference in bacteria reduction between the control group (median = 2.25) and the intervention group (median = 7.6; $P = 0.018$). It was noted that *Staphylococcus aureus* was found in the control group at posttest, but not in the intervention group.

Conclusions: This study provides evidence that a dalethyne dressing is effective for killing *S. aureus* in the infected foot ulcers of diabetic patients.

Keywords: diabetic ulcer • dalethyne • infection • *Staphylococcus aureus*

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1. Introduction

In 1983, the prevalence of diabetes mellitus (DM) in Indonesia was 1.63%. This percentage increased to 5.7% in 2007 and is predicted to reach 6.0% by 2030. Furthermore, DM affected 8.5 million patients in

Indonesia in 2013, a number that is expected to reach 14.1 million by 2035.¹ Diabetic foot ulcers (DFUs) are a common and serious DM complication that significantly increase the DM treatment costs.² The most common

How to cite this article: Jais S, Priyono D. The effectiveness of dalethyne dressings for reducing bacteria in diabetic foot ulcers. *Front Nurs*. 2021;2:153–158.

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cause of morbidity and mortality in DFU is an infection, which occurs in 40–80% of cases.² The primary causative pathogen, *Staphylococcus aureus*, may be resistant to multiple drugs.^{3–5} Therefore, managing DFU infections with antimicrobial agents without increasing antibiotic resistance is an important goal requiring the exploration of alternative treatments. Dalethyne, a herbal product that promotes wound healing, has been shown to be effective against antibiotic-resistant of *S. aureus* and *Pseudomonas aeruginosa*.^{6,7} This new active compound, extracted by ozonization, consists of a combination of compounds: essential oil (aldehyde), fatty acids (stearic, oleic, linoleic, and palmitic acids), iodine, and peroxide.⁷ When applied to a dressing, dalethyne serves as an antimicrobial agent that can kill bacteria and help to promote new tissue formation on wounded skin.⁷ While dalethyne has been used to treat acute and chronic wounds in Indonesia in all clinical settings, no previous study has examined its use to treat DFU infections. Therefore, the present study evaluated the hypothesis that using a dalethyne in wound dressing would reduce the bacterial counts in infected DFU.

2. Methods

This study was conducted in the outpatient Kitamura Wound Clinic in Pontianak, Indonesia, using a quasi-experimental, non-equivalent, and pretest–posttest control group design. Using non-probability purposive sampling, 30 patients with DFU were selected, enrolled, and distributed into two groups (15 each in the control and experimental groups). The inclusion criteria were patients aged 30–70 years with a DFU who had one or more local infection signs or symptoms, a Wagner wound classification of 2–4 without spreading tissue damage, no complications, a regular wound size, without digit amputation, and complied with follow-up care.

Two registered nurses certified in diabetic wound care at the Kitamura Wound Clinic were trained to collect samples using the study protocols. The intervention group was treated with a dalethyne dressing, whereas the control group was treated with a standard dressing according to Kitamura Wound Clinic's wound care management guidelines. The following data were collected for each patient: demographic characteristics (age and sex), HbA1c level, ankle-brachial index (ABI), Wagner ulcer classification, wound duration, DM duration, and blood pressure reading.

The two trained nurses performed bacterial count assessments for patients in both groups. To collect the samples, the research assistants swabbed the longest axis of the ulcer once, using a cotton-tipped sterilized swab. The swab was then placed in a normal saline bottle that was inserted into the bacteria counter. The

per swab bacterial count was measured at the bedside within 60 s.

The bacterial culture samples were investigated twice at intervals of 4 weeks (on the first week at the first treatment and again on the fourth week after treatments). Bacterial counts were measured after wound cleansing to evaluate the bacterial bioburden on the wound surface. The swabs were transported immediately to the Department of Microbiology at the Faculty of Medicine, Tanjungpura University, where the bacterial culture was analyzed by a microbiologist. The rapid bacteria quantification system (Bacteria Counter, DU-AA01NP-H; Panasonic Healthcare Co. Ltd., Tokyo, Japan) was used to measure the bacterial counts.⁸ This device can quantify data ranging from 1.0×10^5 colony-forming units (CFU)/mL to 1.0×10^8 CFU/mL. Bacterial counts $>1.0 \times 10^7$ CFU/mL were considered high.

Institutional ethical clearance was obtained from the Institute of Nursing Muhammadiyah (Board no. 76/II.I.AU/Ket.ETIK/S-1/III/2019), and informed consent was obtained from all the subjects. The participants were followed and observed for 4 weeks. After discharge, all participants were continued to receive care from the staff nurses at the Kitamura Wound Clinic.

Descriptive statistics were calculated for each group (mean and standard deviation values) before the mean bacteria count scores were analyzed. Statistical analyses were performed using a Mann–Whitney–Wilcoxon test and *t*-test, with a value of <0.05 considered statistically significant. SPSS version 20.0 (SPSS Inc., Chicago, IL) was used to analyze all data.

3. Results

Data were collected from 30 participants recruited from among the Kitamura Wound Clinic outpatients treated for local DFU infections from March to September 2018 (Figure 1). Half of the study participants were male and half were female. At the first assessment, 40% of the participants had wounds that had lasted for 2 weeks and 36.7% had wounds that had lasted for 1 week. More than half the participants (53.3%) had suffered from DM from 4 years to 7 years (Table 1). The control and intervention groups did not differ significantly in the HbA1c level, age, blood pressure level, or ABI ($P > 0.05$; Table 2). The Wilcoxon sign rank test ($P < 0.05$) indicated that both groups had statistically significant bacteria count reductions from pretest to posttest (Table 3). The Mann–Whitney test showed that the intervention group's bacteria count reduction was significantly greater than that of the control group ($P = 0.018$; Table 4).

The pretest and posttest levels of the four types of bacteria are shown in Table 5. In both groups, *Proteus mirabilis* and *Citrobacter freundii* bacteria were found in

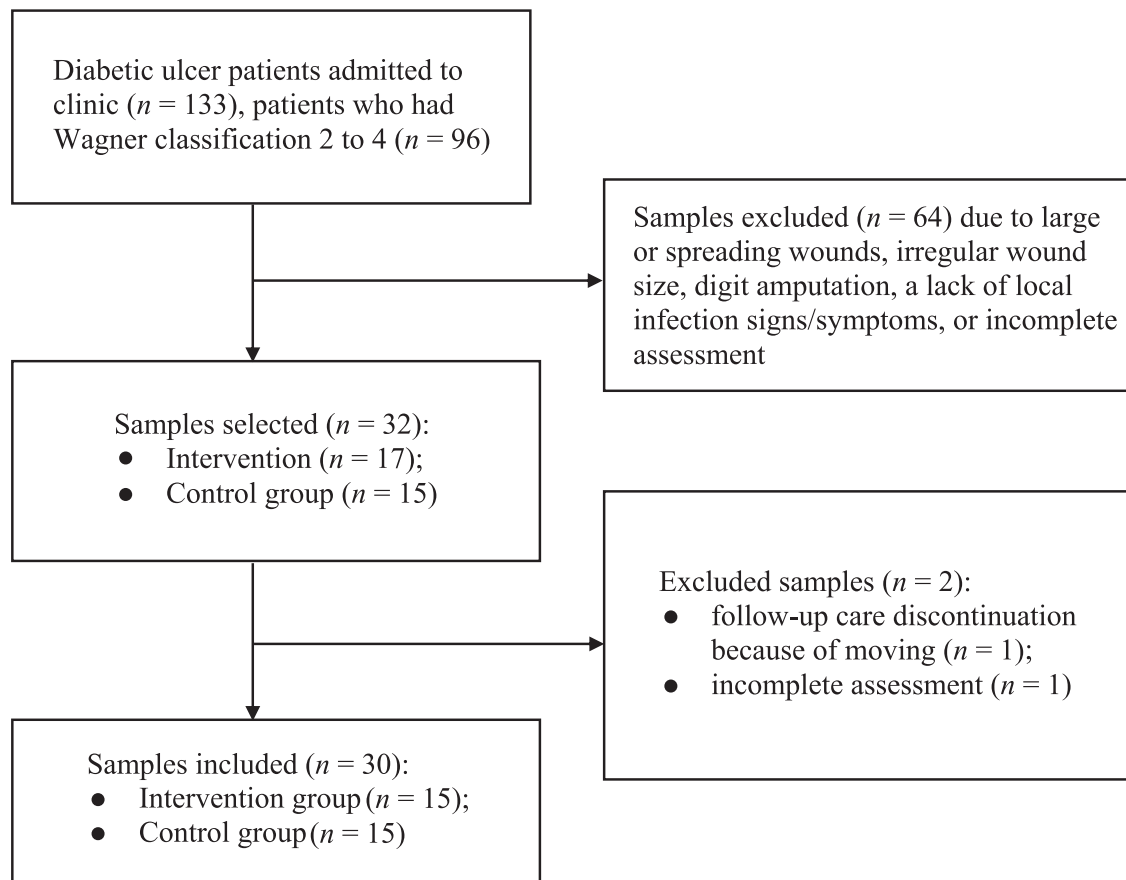


Figure 1. Flowchart of study participants.

Characteristics	<i>n</i>	%
Sex		
Female	15	50
Male	15	50
Wound duration at week		
1	11	36.7
2	12	40.0
3	6	20.0
4	1	3.3
DM duration in years		
1–3	10	33.3
4–7	16	53.3
>7	4	13.3

Table 1. Participant characteristics.

the pretest and posttest samples (Table 5). *S. aureus* bacteria were found in both group's pretest samples and the control group's posttest sample, but not in the intervention group's posttest sample. *Bacillus subtilis* was found in the intervention group's pretest and posttest samples, but not in either of the control group's samples. Finally, *Bacillus megaterium* was found only in the

Items	Control group	Intervention group
Age	49.7 ± 8.4*	52.5 ± 8.1*
HbA1c	9.0 ± 1.0*	9.6 ± 1.9*
Blood pressure		
Systole	122.9 ± 10.2*	121.5 ± 10.1*
Diastole	81.2 ± 9.0*	78.8 ± 7.1*
Ankle Brachial Index (ABI)	0.9 ± 0.07*	0.9 ± 0.6*

Note: * $p > 0.05$; SD, Standard Deviation.

Table 2. Differences between control group and intervention group ($M \pm SD$).

control group's pretest sample, not in the intervention group's pretest sample, or either groups' posttest samples.

4. Discussion

The findings of this study support the hypothesis that using a dalethyne dressing reduces the bacterial counts in infected DFUs. In accordance with previous

Variables	Pretest (bacteria count $\times 10^5$ CFU/mL)			Posttest (bacteria count $\times 10^5$ CFU/mL)			P-value
	Mean	Median	SD	Mean	Median	SD	
Control	30.19	63.7	683	23.80	2.25	37.62	0.004*
Intervention	1946.50	227.0	2790	0.97	0.76	0.83	0.003*

Note: *Wilcoxon sign rank test, significance at $P < 0.05$.
CFU, colony-forming units.

Table 3. Pretest and posttest independent bacteria counts.

Variables	Control group posttest		Intervention group posttest		P-value
	Median	95% CI	Median	95% CI	
Bacteria count, $\times 10^5$ CFU/mL	2.25	2.20–16.35	7.6	7.82–62.57	0.018*

Note: *Mann–Whitney, Significance at $P < 0.05$.
CFU, colony-forming units.

Table 4. Posttest bacteria count differences.

Bacteria type	Control <i>n</i> (%)		Intervention <i>n</i> (%)	
	Pre (<i>n</i> = 15)	Post (<i>n</i> = 15)	Pre (<i>n</i> = 15)	Post (<i>n</i> = 15)
<i>Staphylococcus aureus</i>	8 (50)	10 (62.5)	10 (58.8)	–
<i>Proteus mirabilis</i>	3 (18.75)	3 (18.75)	1 (5.9)	1 (14.3)
<i>Citrobacter freundii</i>	3 (18.75)	3 (18.75)	2 (11.8)	3 (28.6)
<i>Bacillus megaterium</i>	2 (12.5)	–	–	–
<i>Bacillus subtilis</i>	–	–	4 (23.5)	4 (57.1)

Table 5. Type of bacteria present in pretest and posttest samples.

findings, *S. aureus* was the most frequently isolated pathogen in this study.^{3,9,10} In contrast to previous reports, the present study does not support the contention that *Escherichia coli* and *P. aeruginosa* are common pathogens in DFU infections.¹¹ In this study, the number of bacteria decreased in the intervention group after treatment with a dalethylene dressing. As in previous studies, dalethylene eliminated *S. aureus*, a common infection in DFU ulcers in the present study's intervention group.^{6,7} *S. aureus* is dangerous because it secretes toxins that can lead to tissue necrosis. These toxins play an important role in deepening and spreading DFU infections.¹² However, *B. subtilis* was found in the intervention group at pretest and posttest, indicating that dalethylene is not effective in killing these bacteria. Fortunately, most types of *B. subtilis* are non-pathogenic.¹³ The findings of the present study also showed that dalethylene was not effective for killing *P. mirabilis* or *C. freundii*. While previously recognized as an environmental contaminant or colonizer with low virulence, *C. freundii* is now known to cause a wide spectrum of infections involving the urinary tract, peritoneum, respiratory tract, wounds, soft tissues,

urinary tract, liver, biliary tract, peritoneum, intestines, bone, respiratory tract, endocardium, meninges, and the bloodstream.¹⁴ Both *P. mirabilis* and *C. freundii* are frequently isolated in infected wounds.¹⁵ Confirming this fact, the present study also found that both bacteria are virulent and frequently found in DFU infections.^{14–16}

The findings from this study show a greater decrease in the number of bacteria in the intervention group compared with the control group and confirm that dalethylene has some benefits for wound treatment. Bacterial bioburden has two aspects: bacterial count and pathogenicity. The culture method, which is widely used in clinical settings, quantifies the bacterial count.⁸ Having a decreased number of bacteria in a wound is associated with reduced inflammation.⁸ However, pathogenicity also plays an important role in clinical outcomes. In this study, both groups had bioburdens; all cultures were pathogenic and had more than one microbe. Furthermore, the number of bacteria decreased in both groups. The key fact here is that all chronic wounds are colonized by bacteria. Therefore, the most important goal in wound care management is to reduce the

number of bacteria to accelerate the wound healing process.¹⁷ Both groups experienced a significant reduction in the number of bacteria, which can facilitate the wound healing process. The desired result in wound care management is to attain the greatest possible bacteria-level reduction without introducing other complications. In the present study, the key finding was that, compared with the control group, the intervention group experienced a greater reduction in the number of bacteria as well as a complete elimination of *S. aureus*, the virulent bacteria that causes most DFU infections. These results suggest that treating infected wounds with dalethyne can effectively kill *S. aureus*.

The results of this study suggest the benefits of using dalethyne dressings in the management of chronic infectious wounds in diabetic patients. In addition to reducing the virulent bacteria of *S. aureus* and promoting wound healing, dalethyne offers the additional advantage of not having negative effects on wounds.

5. Conclusions

The results of this study indicate that dalethyne is effective in killing *S. aureus* and can reduce the bioburden in DFU infections.

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Limitations

The limitations of this study include the small sample size, which limits the statistical power and generalizability of these findings. Furthermore, because this study did not continue documenting follow-up care until all the wounds had healed completely, any between-group differences in long-term healing acceleration remain unknown. Future studies should include larger samples at multiple centers to identify other factors that affect wound progression and healing and detect biofilm in wounds.

Acknowledgment

The authors thank Dermozone and the Soho Company, which supported the study, and the staff nurses at Kitamura Wound Clinic, who participated in this study.

Ethical approval

Ethical issues are not involved in this paper.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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