The Effect of Irrigation to the Amount of Bacterial Colony Formation in Open Fracture

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Authors' contributions

This work was carried out in collaboration between both authors. Author UHH collected the data, performed irrigation and debridement, took swabs, calculated the number of colonies, analyzed data. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: To determine the effectiveness of 3, 6, and 9 liters of physiological saline for wound irrigation in grade II open fracture at lower extremity in reducing the number of bacterial colonies

Study Design: This observational study determined the quantity of physiological saline for wound irrigation in grade II open fracture at lower extremity which is effective in reducing the number of bacterial colonies.

Place of Study: Moewardi Hospital Emergency Room and the Microbiology Laboratory of the Faculty of Medicine, Universitas Sebelas Maret.

Methodology: 16 patients with grade II open fracture at lower extremity who came and received treatment was included. Patients with grade II long bone open fracture less than six hours, patients with multiple open fractures of the long bones taken only in one place, patients who had not received medical treatment since the incident were included. Patients suffering from previous bone and/or soft tissue infections at the fracture site and suffering from multiple trauma who should receive immediate life-saving measures were included.

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Results: There were 12 patients who met criteria inclusion. After 3 L irrigation, there was a decrease in the amount of bacterial colony, same as 6 L irrigation, while there was no increase in the number of bacterial colonies in 9 L irrigation. There were significant differences in 3L, 6L, 9L irrigations (p = 0.001), but the most effective irrigation fluid was in 9 liters compared with 6 liters (p <0.05).
Conclusion: Wound irrigation with 3, 6, and 9 liters of normal saline will significantly reduce the number of bacteria. However, irrigation with 9 liters of normal saline dilution in grade II open fracture at the lower extremity is the most effective.

1. INTRODUCTION

There are several principles to be considered in the management of open fracture for a good result. Open fracture should be considered an emergency case so that it should be treated as soon as possible [1]. Thus, it is necessary to conduct an initial evaluation and determine the presence of abnormalities that can cause death. Infection is still one of the main complications in the treatment of open fracture. A prospective study conducted at County Medical Center, Minnesota, showed that 70.3% of open fractures were contaminated with positive culture results; most (50–70%) of open fracture wounds had been contaminated with bacteria that were potentially pathogenic at the time of incident or sometime after that including contamination in the hospital emergency room [2]. Gustilo et al. reported a correlation between fracture type and the risk of infection. Grade I open fracture has an incidence rate of infection between 0–2%, for grade II 2–5% and 4–10% in grade IIIA, 10–50% IIIB, and 25–50% IIIC [3]. Several factors can reduce the incidence of infection in open fractures. They are adequate wound debridement, use of prophylactic antibiotics, fracture stabilization, and early wound closure. Adequate wound debridement at the start of open fracture management is the single most important factor that can be controlled by the surgeon [1].

To date, most of the literature states that the volume of irrigation fluid to be given is copious, ample, adequate, or arbitrary without mentioning the specific volume recommended [4]. The common wound irrigation fluid is physiological saline [5]. Therefore, research is needed to determine the effectiveness of 3, 6, and 9 liters of physiological saline for wound irrigation in grade II open fracture at lower extremity in reducing the number of bacterial colonies.

2. METHODOLOGY

2.1 Study Design

This observational study determines the quantity of physiological saline for wound irrigation in grade II open fracture at lower extremity which is effective in reducing the number of bacterial colonies. It was conducted at the Moewardi Hospital Emergency Room and the Microbiology Laboratory of the Faculty of Medicine, Universitas Sebelas Maret.

2.2 Population and Sample

The study population covers all patients with grade II open fracture at lower extremity who came and received treatment at the Emergency Room of Moewardi Hospital. The sample included 16 patients with grade II open fracture at lower extremity who came and received treatment at the Emergency Room of Moewardi Hospital who met the inclusion criteria. Inclusion criteria included patients with open fractures of grade II long bones that occurred less than six hours before admission to the hospital, patients with multiple open fractures of long bones taken from only one place where the fracture occurred in one long bone, patients with open fractures of grade II long bones who had not yet been received medical treatment since the incident, and patients who agreed and signed the informed consent. The exclusion criteria included patients suffering from previous bone and/or soft tissue infections at the fracture site and patients suffering from multiple trauma who should receive immediate life-saving measures. Sampling was conducted purposively. All subjects who met the inclusion criteria who came were included in the study until the required number of subjects was met.

2.3 Research Variable

The independent variable in this study is the amount of irrigation fluid (3, 6, and 9

liters of physiological saline) and the dependent variable is the number of bacterial colonies.

2.4 Research Instrument

The independent variable in this study is the amount of irrigation fluid, which is the amount of physiological saline that is used to rinse blood clots and other impurities, allowing it to inspect the wound to remove foreign objects and avital tissue. This variable was measured by observation and expressed in liters on a nominal scale. Meanwhile, the dependent variable is the number of bacterial colonies, which is the number of bacteria calculated and obtained from the results of swabs carried out on open fracture bones before and after debridement which can be used as a predictor factor for infection. The number of bacterial colonies was measured using nutrient agar plate media and expressed in Colony Forming Unit (CFU)/ml on a ratio scale.

2.5 Data Analysis

The frequency and distribution are displayed in tables and graphs. Wilcoxon signed-ranks statistical test for paired data was used for data analysis to determine the p-value. SPSS for desktop was used for all analyses.

3. RESULTS

3.1 Sample Characteristics

During the study period, 12 people with grade II open fracture at lower extremity who met the inclusion criteria were collected and participated in this study. Patients who came and received treatment at the Emergency Room of RSDM consisted of 8 men and 4 women. There were 8 patients (66.7%) with grade II open fracture at lower extremity while the rest were female, namely 4 patients (33.3%) with a mean age of 31.7 years, with the youngest being 12 years and the oldest 53 years.

The largest percentage of open fracture was grade II with most fractures occurring in the tibia-fibula fractures in six patients (50%); then, there were only three patients who had tibial fractures (25%), and three patients had femur fractures (25%). In accordance with the study conducted by Clifford, the Tibia Bone (21.6%) was the long bone that most often experienced open fractures, followed by femur (12.1%), radius-ulna (9.3%), and humerus (9.3%). The most common bacterium found in grade II open fracture wounds is Bacillus sp. There were 8 (66.7%) bacteria and 4 (33.3%) Staphylococcus epidermis. Based on the results of laboratory examinations, all patients with grade II open fracture at lower extremity were contaminated with bacteria prior to wound irrigation. A total of 7 subjects (58.4%) were found to have 1–10,000 CFU/ml of bacterial colonies, 1 subject (3.3%) 10,001–20,000 CFU/ml, and 4 subjects (33.3%) 30,000 CFU/ml.

After 3 L of wound irrigation, the number of bacterial colonies on each subject's wound had begun to decrease. A total of 2 subjects (16.7%) had no bacterial colony or 0 CFU/ml, 6 subjects (50%) had 1–10,000 CFU/ml of bacterial colonies, 3 subjects (25%) had 10,001–20,000 CFU/ml, and 1 subject (8.3%) had 20,001–30,000 CFU/ml, and none had > 30,000 CFU/ml. Furthermore, 6 L of wound irrigation was performed, showing a decrease in the number of bacterial colonies, where 6 subjects (50%) had no bacterial colony or 0 CFU/ml, 6 subjects (50%) had 1–10,000 CFU/ml of bacterial colonies, and none had > 10,000 CFU/ml. Wound irrigation was performed again with 9 L of physiological saline solution. The number of subjects without bacteria was greater where 15 subjects (91.7%) had no bacterial colony or 0 CFU/ml, 1 subject (8.3%) had 1–10,000 CFU/ml of bacterial colonies, and none had > 10,000 CFU/ml.

Table 1. Sample characteristics (categorical data)

Characteristics	Category	Frequency	Percentage
Gender	Male	12	66.7%
	Female	4	33.3%
Fracture Location	Tibia	3	25%
	Tibia-fibula	6	50%
	Femur	3	25%
The number of patients with the type of bacterial colony	*Bacillus sp.*	8	66.7%
	Staphylococcus epidermidis	4	33.3%

Table 2. Sample characteristics

Variable	Mean	Elementary school	Min.	Max.
Age (year)	31.7	15.22	12	53

3.2 Bivariate Analysis

There was a significant difference in the number of bacteria with 3, 6, and 9 liters of wound irrigation, where the smallest number of bacterial colonies was found in 9 L wound irrigation; thus, 9 L irrigation is more effective for wound irrigation in grade II open fracture at lower extremity in reducing the number of bacterial colonies ($p < 0.05$).

From the Fig. 1. Shown the comparison of the number of bacterial colonies before and after irrigation. (A) Before irrigation there was 150300 CFU/mL, (B) 3 L irrigation there was 80000 CFU/mL, (C) 6 L irrigation there was 24000 CFU/mL, and (D) 9 L irrigation there was 100 CFU/mL.

4. DISCUSSION

Physiological saline wound irrigation plays an important role in the management of open fracture to reduce the incidence of infection [6]. Open fracture is a fracture characterized by direct contact between the part of the bone and the outside of the body, accompanied by trauma from the overlying soft tissue with varying degrees of damage [7]. Due to wounds and skin damage, bacteria from outside can enter and cause wound contamination. The degree of contamination in open fracture is strongly influenced by the extent of soft tissue damage and the length of time since occurrence. If an open fracture has occurred for more than six hours, the degree of wound contamination will increase [8].

The main goal of treating open fractures is to prevent infection, in addition to treating the fractured bone. Therefore, the initial treatment of people with open fracture will determine the outcome of trauma to the life and function of their extremity [9]. Adequate management will restore normal function and life while inadequate treatment will lead to complications such as infection, osteoporotic disuse, or non-union [8,9]. Wound irrigation is important in the management of open fracture. Although this procedure seems simple, if it is done properly and correctly, it is believed to be the most important factor in reducing the prevalence of infection due to open fracture. Unfortunately, there is not much scientific information and research results regarding the parameters of wound management, especially on wound irrigation. The common irrigation fluid is physiological saline (NaCl 0.9%) [10].

The results of this study showed a significant difference in the number of bacteria between 3, 6, and 9 liters of wound irrigation, where the lowest number of bacterial colonies was in 9 L wound irrigation, no bacterial colony was found in 15 samples, and 100 CFU/ml was seen in one sample. Thus, 9 liters of irrigation is more effective for wound irrigation in grade II open fracture at lower extremity in reducing the number of bacterial colonies. It should be emphasized that in discussing debridement, it is generally recognized that wound irrigation is the most important step of the whole debridement procedure. Although this statement is not entirely true because removing all avital and

Table 3. Differences in the number of bacterial colonies before and after wound irrigation in grade II open fracture at lower extremity

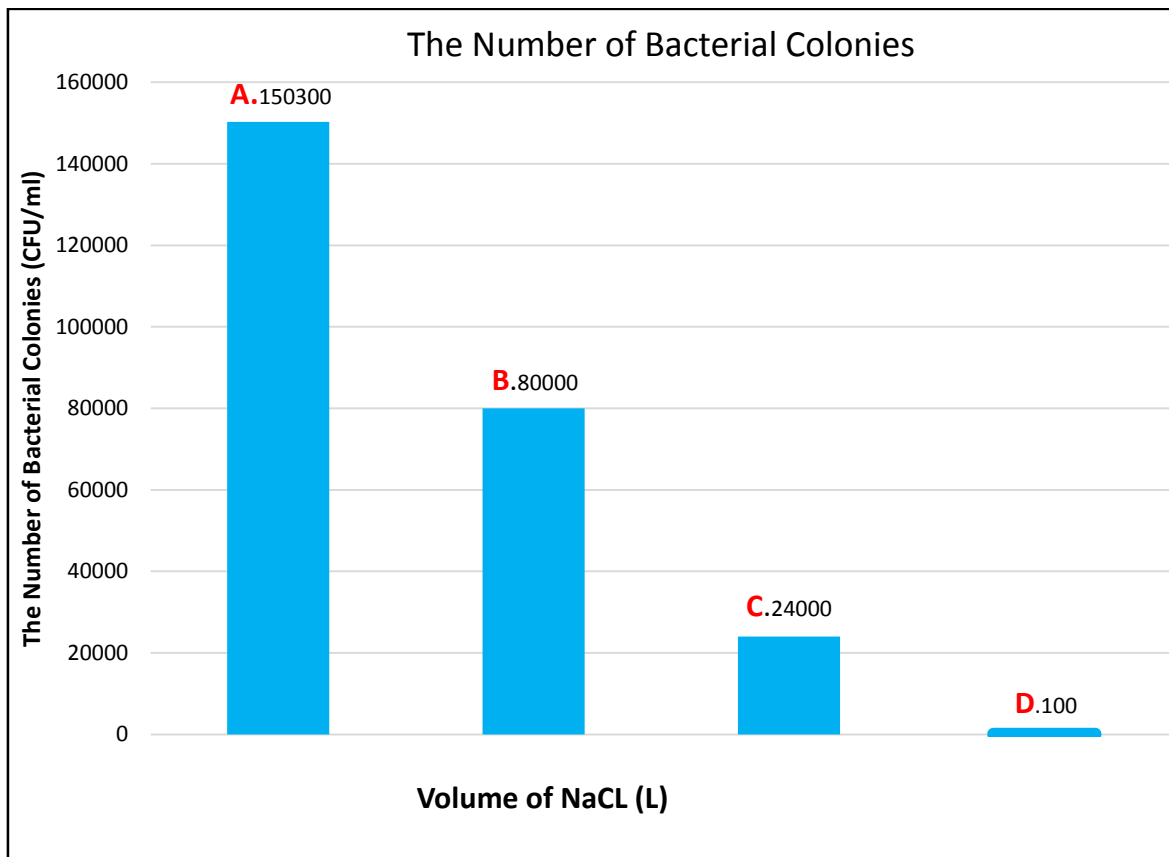| Volume of NaCl	Types of Bacteria	Mean	Elementary school	T
Before irrigation	Bacillus sp. Staphylococcus epidermidis	9.393.75	12.675.82	0.000
NaCl 3 liters	Bacillus sp. Staphylococcus epidermidis	13.043.75	35.547.78	0.007
NaCl 6 liters	Bacillus sp. Staphylococcus epidermidis	1.500.00	2.859.83	0.000
NaCl 9 liters	Bacillus sp. Staphylococcus epidermidis	6.25	25.00	0.000

Fig. 1. Comparison of the number of bacterial colonies before and after irrigation *(A:before irrigation, B: 3 L irrigation, C: 6 L irrigation, D: 9 L irrigation)*

contaminated tissue is equally important, it proves that wound irrigation should be considered important [10].

Wound debridement is to reduce the number of bacteria, remove avital tissue which can be a medium for bacterial growth, and prevent further contamination to facilitate an effective defense mechanism for the body [11]. There are two proper bywords regarding the volume of irrigation fluid for open fracture wounds; they are, "If a little does some good, a lot will do a great deal more" and "A solution to pollution is dilution". Most of the literature states that the volume of irrigation fluid to be given is copious, ample, adequate, or "arbitrary" without mentioning the specific volume recommended. Some other researchers recommend that the volume of irrigation fluid ranges from 3 to 14 liters without reference to the results of certain clinical studies [6,11].

The results of this study showed the best results for wound irrigation were found in 9 L of physiological saline where 15 subjects (91.7%) had no bacterial colony or 0 CFU/ml, 1 subject

(8.3%) had 100 CFU/ml of bacterial colonies, and none had > 10,000 CFU/ml. Thus, wound irrigation with 9 L of physiological saline is effective in reducing the number of bacterial colonies. The most common bacteria found in grade II open fracture wounds were Bacillus sp. as many as 8 (66.7%) and Staphylococcus epidermidis as many as 4 (33.3%). The results of the study by Lawrence et al. revealed that various species of contaminant bacteria were found in open fracture wounds. The bacteria that are often found in open fracture wounds are Bacillus sp., S. epidermidis, Corynebacterium spp, Micrococcus spp, Propionibacterium spp, and S. aureus [12]. These bacteria are normal flora on the skin. The presence of pathogenic Gram-negative stem bacteria or even more pathogenic bacteria such as Pseudomonas will significantly increase the risk of clinical infection [12].

Based on the study by Steven M et al., there was a decrease in the average colony of bacteria after washing wounds using 3 liters of physiological saline (NaCl 0.9%) by 52.0%, 6

liters 64.0%, and 9 liters by 70.0% [6]. Another study showed that the group of open fracture patients irrigated with physiological saline less than 10 liters had a higher incidence rate of infection than those who received irrigation of more than 10 liters [7]. In 2001, Anglen recommended the volume of irrigation fluids, namely 3 liters for grade I open fracture, 6 liters for grade II open fracture, and 9 liters for grade III open fracture [5]. Unfortunately, no human research data has been reported to support the recommendation of Anglen.

The limitation in this study is no examination of the type and number of bacteria after debridement, so the type and number of bacteria after debridement is unknown. From this study, it can be concluded that irrigation using 9 liters of physiological saline in grade II open fracture at lower extremity is more effective than 6 liters of physiological saline; besides, it can reduce the number of bacterial colonies in grade II open fracture wounds at lower extremity ($p < 0.05$), and the volume more than 9 liters is not required.

4. CONCLUSION

Wound irrigation with 3, 6, and 9 liters of normal saline will significantly reduce the number of bacteria. However, irrigation with 9 liters of physiological saline dilution in grade II open fracture at the lower extremity is the most effective.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Ethics committee approval was obtained.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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