



Supporting Documentation
On Wound Irrigation Methods

Evaluation of Wound Irrigation by Pulsatile Jet and Conventional Methods

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Irrigation of wounds to remove bacteria and foreign material is an essential of wound management along with debridement. The effectiveness of saline lavage by high pressure (50 psi) pulsatile jet irrigation has been compared with conventional gravity flow and bulb syringe procedures. Experimental paravertebral incisional surface wounds in 234 randomized rats were either clean or traumatized and soiled. Wounds in 200 of the rats were seeded with *E. coli* (log 8.80). Swab specimens of each wound were taken at incision, after seeding, after irrigation, and at three, seven, and ten days after closure. Eluates of more than 1600 specimens were cultured. No anaerobes were found. Irrigation diminished bacterial counts in all wounds, but only pulsatile jet irrigation brought about significant ($P < 0.05$) reduction of bacteria in each type of wound. After three days *E. coli* was significantly diminished in all wounds, regardless of irrigation or none, owing to host defense mechanisms. Nevertheless, clean contaminated wounds were infected at three days but not at seven days after lavage, while traumatized wounds remained infected at ten days except for those initially irrigated by pulsatile jet. Thus, pulsatile jet irrigation removed bacteria from experimental wounds more efficiently than conventional procedures.

IRRIGATION AND DEBRIDEMENT are the essentials of wound management. Traumatic wounds are irrigated with copious amounts of saline solution to remove bacteria and particles of foreign material. Madden et al.⁴ showed that the efficiency of irrigation increases directly as the pressure at which a solution is delivered to the wound. Other recent studies show the effectiveness of high pressure irrigation (continuous, as well as pulsating) from eight to 70 psi in removing bacteria and decreasing the incidence of wound infection.^{1,2,6} Wheeler et al.⁹ showed that high pressure lavage can also cause damage by injecting irrigant into tissue adjacent to the wound. Since this tissue damage increases susceptibility to infection, they recommend that high pressure irrigation be reserved for use in heavily contaminated wounds.

We wished to examine the validity of claims that the innovative pulsatile jet lavage was indeed better

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than routine conventional procedures, such as gravity flow and bulb syringe irrigation. Accordingly, we have employed these three procedures with two types of experimental incisional wounds in rats: 1) clean contaminated wounds (without crushed tissue or sterile soil), and 2) devitalized contaminated wounds with added sterile soil. The effectiveness of wound irrigation has been evaluated in relation to removal of bacteria and subsequent lowered bacterial counts within wounds during healing.

Materials and Methods

Standardized Wound

Two hundred thirty-four female white rats (mean weight 249 g) were studied. Each was entered randomly into the study, and given food and water *ad libitum* in its individual cage. Each was anesthetized with 30 mg pentobarbital/kg body weight injected intraperitoneally. The skin over the back was prepared by removing the hair with barber clippers, applying povidoneiodine aerosol spray, and allowing it to remain in contact with the skin for 10 min before making the incision. One standard incision 4 cm long and 5 mm lateral and parallel to the vertebral column was made into the underlying paraspinal muscles (approximately 4 mm deep). Hemostasis was achieved by direct pressure with sterile gauze. Skin edges were closed with interrupted sutures of polypropylene (5-0 Prolene). Sterile surgical gloves and instruments were used for each rat.

Bacteriologic Procedures

A strain of *E. coli*, previously isolated from a human wound infection, was used to seed incisions. It was grown for 24 hrs in 50 ml of Trypticase soy broth (BBL)

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in flasks incubated at 37° on a rotary platform shaker, and was prepared for each workday. Each incision was seeded with 1 ml of broth containing 8.80 ± 0.13 bacteria (mean \pm standard deviation). Incisions were sampled at several time intervals with a sterile cotton tipped swab freshly moistened with 0.1 ml of sterile saline solution. The swab specimens were obtained from each wound immediately after incision, 30 min after the incision had been seeded with *E. coli* (the wound was covered with a sterile gauze pad during this interval), and immediately after irrigation.

Wounds were examined and cultured on postoperative days three, seven, and ten to correlate bacterial counts during healing with clinical diagnosis of wound infection. To obtain these specimens the rat was anesthetized, the wound was reopened, cultured, and then resutured. In addition, on the tenth postoperative day wound tissue itself was cultured. Approximately 0.1 g of wound tissue was excised, weighed, and homogenized with saline solution in a ground glass grinder to obtain a 1:10 suspension. Clinical infection was evaluated on the basis of inflammatory reaction, dehiscence, pus, necrosis, and serous wound fluids.

Swab specimens were eluted in 0.9 ml saline solution. From each eluate, two plates of blood agar medium (5% human blood in brain-heart infusion agar) were inoculated by the single plate serial dilution method of Lindsey.³ One plate was incubated aerobically at 37°; the other anaerobically at 37° in an atmosphere of 95% H₂ and 5% CO₂ within a Brewer jar. The eluate was also used to inoculate a tube of fluid thioglycollate medium which was subsequently subcultured if no growth occurred on agar plates (*i.e.*, less than 1000 bacteria/ml). If bacteria other than *E. coli* grew on the agar plates, they were identified and the fluid thioglycollate was streaked for isolation to confirm the presence of the other species in the wound. The same protocol was employed with tissue homogenates.

Experimental Design

The first experimental group (102 rats) had clean incisional wounds seeded with *E. coli* (but with no devitalized tissue or added sterile soil), and was subdivided into four treatment groups: 1) No irrigation (26 rats)—Wounds seeded with *E. coli*; control. 2) Gravity flow irrigation (25 rats)—Seeded wound irrigated with 300 ml sterile saline solution delivered through sterile intravenous tubing. The tip of the tubing was held 4 cm above the wound; the reservoir was suspended 60 cm above the rat. 3) Bulb syringe irrigation (27 rats)—Seeded wound irrigated with 300 ml sterile saline solution delivered by a conventional bulbed Asepto 60 ml glass irrigating syringe held 4 cm

above the wound. 4) Pulsatile jet irrigation (24 rats)—Seeded wound irrigated with 300 ml sterile saline solution delivered by a sterile single hole, tip nozzle held 4 cm above the wound. The nozzle was attached to a *Water Pik*® unit (Teledyne Aquatec Corp., Fort Collins, Colorado) calibrated to deliver irrigant to the wound at a pressure of 50 psi. The unit was sterilized by gas after use each day.

The second experimental group (98 rats) had incisional wounds complicated with devitalized tissue and added foreign material (sterile garden soil). After the incision, the paraspinous muscles were tented and serially crushed with a sterile hemostat; 10 mg sterile garden soil were placed in the wound; then the wound was seeded with *E. coli*. Organic and inorganic components of soil damage tissue, and combined with mechanical trauma insure massive local destruction of tissue.⁶ These rates were subdivided into the same treatment groups studied in the first experiment: 1) No irrigation (20 rats); control. 2) Gravity flow irrigation (26 rats). 3) Bulb syringe irrigation (26 rats). 4) Pulsatile jet irrigation (26 rats).

A control group of 34 rats comprised four (in two instances five) rats assigned to each of the above eight treatment groups. Wounds were not seeded with *E. coli*, but were otherwise prepared and irrigated as described.

Statistical Methods

Bacterial counts were analyzed as a randomized experiment involving a two-way classification of treatments (lavage methods) and time periods. Results of these preliminary two-way analyses of variance showed significant interactions, and invalidated tests for main effects. Therefore, treatment effects were examined separately at each time period and time effects separately for each treatment employing individual one-way analyses of variance and Newman-Keuls tests for multiple comparisons of means.⁸ The *t* test was used to examine differences between controls in the two experimental groups. Chi-square values for bacterial counts obtained from the same rats by different sampling procedures (moist swabs versus tissue homogenates) were calculated by McNemar's test for correlated proportions since the basic assumption of the usual chi-square test that the observations be independent was invalid.

Results

Two hundred thirty-four rats completed the ten days of the experimental protocol. No anaerobic bacteria were isolated from the more than 1600 specimens cultured. Initial cultures of all incisions before seeding were sterile.

TABLE 1. Clean Incisional Wounds Seeded with *Escherichia coli*

Type of Irrigation	Number of Rats	Log Bacterial Count (Mean \pm Standard Deviation)				
		After Seeding	After Lavage	3 Days	7 Days	10 Days
None; control	26	7.19 \pm 1.52	Not done	5.04 \pm 1.59*	3.81 \pm 1.78*	3.10 \pm 2.28
Gravity flow	25	7.44 \pm 0.44	6.84 \pm 0.49	4.42 \pm 1.44*	2.68 \pm 1.63*	1.24 \pm 1.73*
Bulb syringe	27	6.87 \pm 2.01	6.19 \pm 1.82	4.70 \pm 1.78*	2.80 \pm 2.08*	1.78 \pm 2.39
Pulsatile jet	24	7.35 \pm 0.34	5.85 \pm 0.91*	4.02 \pm 0.67*	1.21 \pm 1.61*	0.83 \pm 1.49

* Indicates a statistically significant difference ($p < 0.05$) from the preceding mean as determined by the Newman-Keuls test of multiple comparisons.

Evaluation of Irrigation Procedures

The numbers of *E. coli* in all clean contaminated and devitalized contaminated wounds were diminished by lavage. However, only pulsatile jet irrigation brought about a statistically significant reduction of *E. coli* in each type of wound (Tables 1 and 2).

Bacterial Clearance During Healing

Three days after seeding and lavage numbers of *E. coli* in each type of wound were significantly diminished regardless of irrigation by gravity flow, bulb syringe, pulsatile jet, or no lavage. Intragroup analysis at three days revealed no significant differences in numbers of *E. coli* among any of the four treatment groups with clean contaminated wounds (Table 1), but significantly fewer *E. coli* in the lavage groups with devitalized contaminated wounds as compared to the controls (Table 2).

Throughout the study, control unirrigated devitalized contaminated wounds harbored significantly more *E. coli* than control clean contaminated wounds. Regardless of the type of wound, those which were irrigated by pulsatile jet retained fewer *E. coli* and consequently maintained lower numbers during healing. Thus, in each category of wound the more effective the lavage, the fewer the bacteria in the wound during healing.

Clinical Evaluation of Infection

The therapeutic effectiveness of irrigation is assayed by its contribution to fewer infections. This can be

evaluated by clinical observation and also by bacterial content of the wound during healing. Baseline information was obtained from the 34 control wounds not seeded with *E. coli*. Infection was diagnosed in 14 of these 34 control rats (infection rate of 41.2%). At three days postoperatively there were four infections among the 34 rats; at seven days, there were four infections among the 30 remaining rats; at ten days, there were six infections among the remaining 26 rats. These proportions are not statistically different. Thus, clinical infection was not promoted as a consequence of aseptically reopening healing wounds to monitor bacterial content. Moreover, in the absence of seeding with *E. coli*, there was no significant difference between numbers of clinical infections diagnosed in either type of experimental wound. The bacteria isolated (irrespective of observed infection) were staphylococci (16 rats), diphtheroids (6 rats), streptococci (3 rats), and lactobacilli (2 rats). Among the 200 rats seeded with *E. coli*, the following additional bacteria were also isolated during healing: staphylococci (23 rats), diphtheroids (21 rats), and streptococci (3 rats).

During this work we came to realize that our clinical evaluation of pure culture *E. coli* wound infections was neither accurate nor consistent throughout the study because the signs of infection were not striking. Therefore, correlation between clinical diagnosis and numbers of *E. coli* is not valid here.

Quantitation of Wound Infections

The course of wound infection, however, was followed through changes in the population dynamics of

TABLE 2. Devitalized Incisional Wounds (Plus Sterile Soil) Seeded with *Escherichia coli*

Type of Irrigation	Number of Rats	Log Bacterial Count (Mean \pm Standard Deviation)				
		After Seeding	After Lavage	3 Days	7 Days	10 Days
None; control	20	7.38 \pm 0.39	Not done	5.98 \pm 1.06*	5.53 \pm 1.71	5.48 \pm 2.12
Gravity flow	26	6.90 \pm 1.46	6.23 \pm 1.39	5.19 \pm 1.54*	4.54 \pm 1.41*	3.83 \pm 2.00
Bulb syringe	26	6.87 \pm 2.03	6.81 \pm 0.40	4.88 \pm 1.56*	4.21 \pm 1.67*	3.83 \pm 1.89
Pulsatile jet	26	7.23 \pm 0.43	6.02 \pm 0.88*	4.81 \pm 1.05*	3.21 \pm 1.48*	2.13 \pm 2.16*

* Indicates a statistically significant difference ($p < 0.05$) from the preceding mean as determined by the Newman-Keuls test of multiple comparisons.

E. coli. The bacterial population of wounds was determined at ten days by both routine moist swab culture of the reopened wound and by assay of homogenized wound tissue. Homogenized tissue yielded counts of *E. coli* approximately 2 logs higher than did moist swabs. Higher counts are to be expected in tissue samples since both surface and tissue-associated organisms were assayed in contrast to only surface and fluid-associated organisms sampled by the moist swab procedure. The coefficient of correlation for numbers of *E. coli* by the two techniques was 0.68. The highest mean bacterial counts by each method occurred among rats with control (unirrigated) devitalized contaminated wounds.

Bacterial quantitation of wounds by tissue homogenization procedures has shown that counts of $\geq 10^5/g$ of tissue are associated with sepsis, breakdown of wounds and failure of skin grafts.^{5,7} Accordingly, we have reasoned that since a 2 log differential exists between bacterial levels as determined by tissue homogenization and moist swab procedures of assay, it should be possible to relate infection as defined by tissue homogenization counts to a corresponding 2 log unit lower count obtained from moist swab specimens of the wound. The proportions of rats having bacterial counts by each of the methods were tabulated and examined for homogeneity by McNemar's test. Identity was found to exist only between counts of $\geq 10^5/g$ of homogenized wound tissue and $\geq 10^3/ml$ of tissue fluid by moist swab of the open incision. (A detailed analysis of this relationship is being prepared for publication elsewhere.)

Employing the above criterion, re-examination of bacterial counts obtained from swab specimens (Table 1) reveals that only incisions which had been lavaged were no longer infected at seven and ten days. Devitalized contaminated wounds, however, all remained infected at seven days, and only those initially irrigated by pulsatile jet were not infected at ten days (Table 2).

Discussion

These results demonstrate the greater effectiveness of pulsatile jet irrigation, as compared with conventional gravity flow and bulb syringe procedures, in removing bacteria from experimental wounds. The pulsatile jet was exceptionally effective in our experimental traumatic wounds (devitalized, contaminated and with added garden soil). However, regardless of a

clean contaminated or a devitalized contaminated wound, fewer *E. coli* remained after pulsatile jet irrigation, and fewer were present during healing. This work confirms the findings and conclusions of Gross et al.¹ and Hamer et al.²

These results also demonstrate the activity of non-specific host defenses in combating bacterial infection during healing, and emphasize the magnitude of the problem of cleansing and managing heavily contaminated traumatic wounds. Thus, despite removal of some contaminants and debris by conventional low pressure irrigation with gravity flow and bulb syringe methods, only high pressure pulsatile jet irrigation lowered the numbers of *E. coli* significantly. Wheeler et al.⁹ emphasize that the benefits of pulsatile jet irrigation in cleansing and promoting healing without infection outweigh concern with possible tissue trauma provided that the procedure is used only in heavily contaminated wounds. Mindful of this proviso, we propose that clinical studies be conducted with severely traumatized, heavily contaminated wounds cleansed by pulsatile jet irrigation.

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