



Supporting Documentation  
On Wound Irrigation Methods

## **Quantitative Bacterial Analysis of Comparative Wound Irrigations**

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# Quantitative Bacterial Analysis of Comparative Wound Irrigations

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It is a biologic fact that all open wounds contain bacteria and remain contaminated with varying levels of bacteria until successful wound closure has been accomplished. The sine qua non in the management of the contaminated wound has been and remains adequate sharp debridement. In a standardized experimental model, three methods of irrigation were compared employing quantitative bacteriology of tissue to evaluate their effectiveness at decreasing bacterial levels and reducing wound infection. The pulsating jet lavage was found to be significantly better than gravity flow irrigation or wound irrigation with a bulb syringe. These data on tissue biopsies support previous reports using surface and wound exudate cultures.

**T**RAUMATIC wounds, whether produced in military or civilian surroundings, are uniformly contaminated.<sup>1,16</sup> In addition to the large inoculum of bacteria, these wounds are often complicated by debris, blood clot, and necrotic tissue. Proper mechanical debridement and delayed closure of contaminated wounds have markedly reduced the incidence of infection.<sup>5,14,19</sup> The value of irrigation as an adjunct to mechanical debridement is less clear. Singleton, et al.<sup>20</sup> and Peterson<sup>15</sup> have shown that saline irrigation decreases the incidence of wound infection and that the decrease was proportional to the amount of irrigating solution used. Conversely, several investigators have reported that low pressure saline irrigation is ineffective in preventing wound infections.<sup>6,7,12</sup>

Recently a great deal of data has emerged suggesting that a pulsating irrigation stream delivered at high pressure and with a high flow effectively decreases bacteria, foreign bodies, and necrotic crushed tissue in wounds and decreases the incidence of resultant wound infection.<sup>3,4,7,10</sup> These data have been based on a semi-quantitative technique of smear cultures obtained from the surface of wounds or from pyogenic exudates from the wounds. Multiple studies over the past 10 years have

shown that infection rates are best correlated with the tissue level of bacteria and that surface cultures or cultures of wound discharges are often misleading.<sup>19</sup>

The present study was undertaken to evaluate the effectiveness of various types of wound irrigation techniques in decreasing the tissue bacterial level in experimental wounds.

## Materials and Methods

One hundred male Sprague-Dawley rats weighing 200 to 250 grams were divided in two major groups. The animals were individually caged and given unrestricted amounts of food and water. The animals were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg). Skin preparation consisted of clipping the hair from the back of each animal followed by skin cleansing with Betadine® solution.\* The experimental model consisted of a standard incision 4 cm in length and 5 mm lateral and parallel to the vertebral column. The incision was carried through the skin, panniculus carnosus, and 4 mm into the paraspinal muscles. When necessary, hemostasis was accomplished by sterile gauze pressure.

### Group I—Wounds Contaminated with Bacteria Only

The wounds in the first major group were topically inoculated with  $5 \times 10^7$  *Staphylococcus aureus* organisms and  $5 \times 10^7$  *Escherichia coli* organisms from fresh 18 hour broth cultures. After a 30 minute contamination period, the animals were divided into four sub-groups and tissue biopsies taken for quantitative and qualitative bacterial analyses.<sup>13,17</sup> The sub-groups were treated in one of the following ways.

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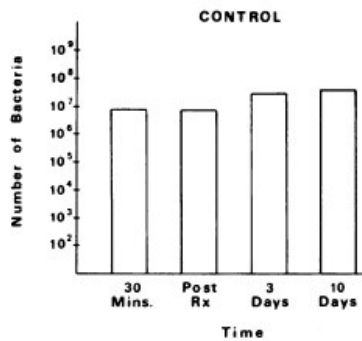


FIG. 1. Graph of geometric means of animals in control sub-group inoculated with bacteria alone showing persistently high bacterial counts throughout experimental period.

**A. Control.** The wounds in the control sub-group underwent closure with interrupted monofilament nylon sutures.

**B. Gravity flow irrigation.** The wounds in the gravity flow sub-group were irrigated with 300 ml of normal saline from a standard intravenous bottle and tubing with the bottle being suspended at a constant height 60-65 cm above the wound. The tip of the intravenous tubing was held 2 to 3 cm from the wound. Following irrigation a second tissue biopsy was obtained and the wounds closed.

**C. Bulb syringe irrigation.** The wounds in the bulb syringe sub-group were irrigated with 300 ml of normal saline using a conventional 50 ml Dakin's syringe. The bulb was squeezed firmly and rapidly and moved back and forth over the wound with the tip 4 to 5 cm above the wound. Following irrigation a repeat biopsy was obtained and the wounds closed.

**D. Pulsating jet lavage.** Wounds in the pulsating jet lavage sub-group underwent irrigation with 300 ml of normal saline delivered in a pulsating stream through a four hole tip at 50 pounds psi (approximately three grams of pressure per square millimeter of surface) over a period of 20 seconds. The tip was held 4 to 5 cm from the wound surface. The device used for this purpose pulsates at 1200 cycles per minute, delivering 800 to 1000 ml per minute at a variable pressure of 2 to 75 psi using a four-hole tip. Immediately following lavage, a second biopsy was taken and the wounds closed.

Three days after wounding, each of the wounds was inspected for gross infection and was opened under aseptic conditions. Tissue biopsies were again obtained for quantitative and qualitative bacterial analyses. The wounds were reclosed with monofilament nylon sutures. At ten days, the surviving animals were sacrificed, their wounds grossly inspected, and tissue cultures performed.

#### Group II—Wounds Contaminated with Bacteria and Foreign Body

Because bacteria is rarely present alone in clinically contaminated wounds, the second major group of ani-

mals was contaminated with bacteria and a foreign body. In addition to  $5 \times 10^7$  *Staphylococcus aureus* and  $5 \times 10^7$  *Escherichia coli* organisms, Group II animals had 0.5 grams of sterile sand introduced into their wounds. After a 30 minute contamination period, repeat bacterial analyses were performed and the animals divided into four sub-groups and treated as before. **A. Control, B. Gravity flow irrigation, C. Bulb syringe irrigation, D. Pulsating jet lavage:** Each of the animals in these sub-groups was treated as described in Group I with the exception of the addition of sand as a foreign body in their wounds.

### Results

#### Group I—Wounds Contaminated with Bacteria Only

**A. Control.** The results for the control sub-group are diagrammed in Fig. 1 which shows the geometric means of the bacterial counts of the animals. The control animals obviously had no treatment and, therefore, the bacterial count listed in the post-treatment column is the same as in the pre-treatment biopsy. However, the bacterial counts increased over the initial pre-treatment level at 3 and 10 days, from  $8 \times 10^6$  organisms per gram of tissue to  $3 \times 10^7$  and  $4 \times 10^7$  organisms per gram of tissue respectively. All wounds were grossly infected.

**B. Gravity flow irrigation.** Gravity flow was ineffective in lowering the bacterial counts after treatment and also allowed an increased bacterial growth above the pre-treatment levels of  $1 \times 10^6$  organisms per gram of tissue to  $4 \times 10^7$  organisms per gram of tissue at three days (Fig. 2). Approximately 85% of these wounds were grossly infected.

**C. Bulb syringe irrigation.** The bulb syringe did lower the bacterial count to  $9 \times 10^5$  organisms per gram of tissue from a pre-treatment level of  $6 \times 10^6$  organisms per gram

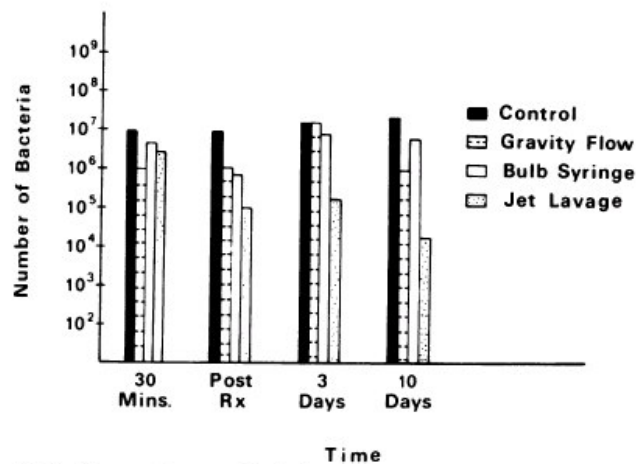


FIG. 2. Comparative wound irrigation in Group I animals whose wounds were contaminated with  $5 \times 10^7$  *Staphylococcus aureus* organisms and  $5 \times 10^7$  *Escherichia coli* organisms.

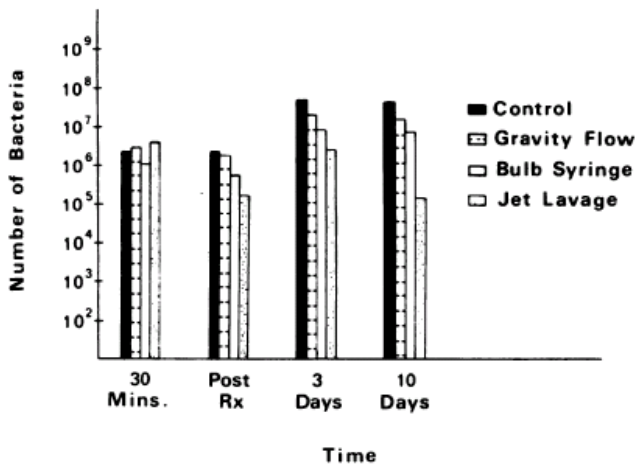


FIG. 3. Comparative wound irrigation in Group II animals whose wounds were contaminated with bacteria and foreign body.

of tissue. This decrease was statistically significant ( $P < 0.01$ ). However, it did not prevent a rebound effect at 3 and 10 days (Fig. 2). Although 85% of these wounds were grossly infected at 3 days, the number had decreased to 60% at 10 days.

**D. Pulsating jet lavage.** The pulsating jet lavage significantly decreased the bacterial count from  $4 \times 10^6$  to  $1 \times 10^5$  ( $P < 0.01$ ). It was the only technique which kept the bacterial count below pre-treatment levels throughout the experiment (Fig. 2). At 3 days and at 10 days the pulsating jet lavage was not only significantly better than the control sub-group but also significantly better than the bulb syringe sub-group. At 10 days the control wounds had bacterial counts of  $4 \times 10^7$  organisms per gram of tissue, while the bulb syringe and pulsating jet lavage wounds had  $9 \times 10^6$  and  $3 \times 10^4$  organisms per gram of tissue respectively. Only 20% of the wounds were grossly infected.

Summarizing the Group I animals, the bulb syringe and the pulsating jet lavage significantly decreased the bacterial counts immediately after treatment. At 3 days only the pulsating jet lavage was significantly better than the control and it was also significantly better than the bulb syringe. At ten days, again only the lavage was better than the control and also better than the bulb syringe. Only the pulsating jet lavage consistently lowered the bacterial count to  $10^5$  or fewer bacteria per gram of tissue, and this resulted in significantly fewer infections.

#### Group II—Wounds Contaminated with Bacteria and Foreign Body

**A. Control.** Wounds in the control sub-group had bacterial counts whose geometric means increased from  $4 \times 10^6$  in the pre-treatment stage to  $9 \times 10^7$  organisms per

gram of tissue at 3 days and  $6 \times 10^7$  organisms per gram of tissue at 10 days (Fig. 3). All wounds were grossly infected at 3 and 10 days.

**B. Gravity flow irrigation.** The gravity flow irrigation was again ineffective in decreasing the bacterial count. The post-treatment count of  $3 \times 10^6$  organisms per gram of tissue was not significantly different from the pre-treatment level of  $6 \times 10^6$  (Fig. 3). All wounds became grossly infected.

**C. Bulb syringe irrigation.** As compared to the Group I animals without the foreign body addition, the bulb syringe irrigation did not significantly reduce the bacterial count in the animals in Group II and by 10 days all of these wounds became infected. The pre-treatment levels of  $1 \times 10^6$  organisms per gram of tissue were only lowered to  $7 \times 10^5$  organisms per gram of tissue and by 3 days the counts had increased to  $1 \times 10^7$  (Fig. 3).

**D. Pulsating jet lavage.** Only the pulsating jet lavage decreased the bacterial count by a significant amount ( $P < 0.01$ ). The post-treatment level was  $3 \times 10^5$  organisms per gram of tissue compared to a pre-treatment level of  $6 \times 10^6$  organisms per gram of tissue (Fig. 4). Although at 3 and 10 days the bacterial counts hovered around  $10^6$  organisms per gram of tissue, only 50% of the wounds were grossly infected at 10 days.

To summarize the group of animals contaminated with both bacteria and foreign body, only the pulsating jet lavage significantly reduced the bacterial count in the post-treatment period. By 3 days, and again at 10 days, this significance was much less, being only at the 95% confidence level.

#### Discussion

In any traumatic wound the ability of the host to combat infection is greatly influenced by the level of bacteria, foreign body contamination, necrotic tissue, and blood clots.<sup>2,5,11,14</sup> All surgical therapy must be directed toward rendering the local wound environment and the bacterial flora suitable for closure. Meticulous mechanical surgical removal of debris and contused or compromised tissue remains the single most important step in managing the

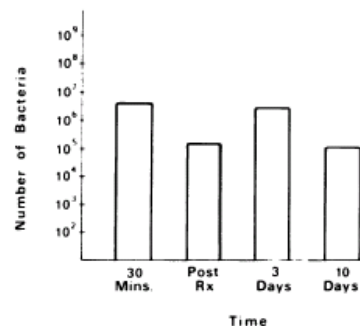


FIG. 4. Effect of the pulsating jet lavage on the tissue bacterial level in wounds contaminated with high levels of bacteria and foreign body.

contaminated wound. Irrigation may be an adjunct to debridement. Edlich, et al.<sup>6</sup> Hopson, et al.<sup>12</sup> and Gross, et al.<sup>7</sup> have demonstrated that the standard low pressure saline irrigation is ineffective at significantly reducing wound infections. Bhaskar and various of his associates in a series of publications have reported the use of a pulsating jet lavage operating at 70 pounds psi, and found it 7 times more effective than the bulb syringe in reducing infection rates;<sup>7</sup> 3 to 7 times more effective in removal of foreign contaminants;<sup>4,9</sup> and 40 times more effective in the removal of tissue fragments from crush wounds.<sup>8,10</sup>

All studies previously reported employing the pulsating jet lavage were performed using semi-quantitative techniques. Bacterial studies were performed on surface swabs and wound exudates. As shown by Robson and Heggors,<sup>18</sup> there is little correlation between the number and species of bacteria found in wound exudates and those reaching tissue levels. Wound infection is correlated with a bacterial count in tissue of greater than  $10^6$  bacteria per gram of tissue.<sup>13,17,19</sup> Therefore, a comparison of irrigating techniques was performed to see which, if any, techniques available decreased bacterial counts to  $10^6$  or fewer bacteria per gram of tissue and thus resulted in fewer wound infections.

The fact that the gravity flow irrigation was ineffective at lowering the bacterial counts was not surprising. Previous authors have reported failure of low pressure irrigations.<sup>6,12</sup> The bulb syringe did remove a great deal of bacteria, but was less effective than the pulsating jet lavage. This difference was even more marked when foreign body was added to the wound contamination. It is thought that the advantage of the pulsating jet lavage is its ability to deliver a pulsating stream producing rapid compression of the tissue and an interpulse decompression phase which allows the soft tissue with its inherent elasticity to rebound thus loosening the contamination particles. Also, once the particles are dislodged from the tissue the high flow rate of 800 to 1000 ml per minute removes them from the wound. The pulsating jet lavage was the only one of the 3 irrigating methods evaluated in this study which reduced the

bacterial level to the critical level of  $10^6$  or fewer bacteria per gram of tissue.

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