Infected Surface Wound: an Experimental Model and a Method for the Quantitation of Bacteria in Infected Tissues

DENNIS G. SAYMEN, PAUL NATHAN, IAN A. HOLDER, E. O. HILL AND BRUCE G. MACMILLAN

Departments of Physiology, Surgery, and Microbiology, University of Cincinnati College of Medicine, and the Shriner’s Burns Institute, Cincinnati, Ohio 45219

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Methods for the quantitation of bacteria in infected tissues must be rigidly standardized to insure uniformity of results. In this communication we report on a laboratory animal model for the study of surface wound infection and the development of a standardized method for the quantitative estimation of bacteria in infected surface wound tissue by mechanical tissue homogenization and serial dilution. Parallel comparative studies demonstrated that a moist-swab sampling procedure detected only 10% of the bacteria recoverable by a surface-wash procedure. Either tissue homogenization or surface-wash procedures recovered significantly more bacteria from contaminated surface wounds than were obtained by surface-swab sampling techniques.

Bacterial infection of traumatic and burn wounds continues to plague clinicians. To determine the need for therapy, or to evaluate the success of such treatment, several quantitative methods have been reported to estimate the number of organisms growing on the surface or within infected tissues. Eade (3) devised a method to elute bacteria from the surface of infected burn wounds by agitation of a small volume of sterile saline over a standard area of wound surface and quantitated the number of viable organisms in the saline wash. Brentano and Gravens (1) quantitated sterile, saline-soaked, gauze pads placed on wound surfaces for 5-min periods. The number of viable bacteria per unit area sampled was counted after eluting these from the gauze into sterile media. Serafinska et al. (9) reported the use of a similar technique but placed gauze pads on wound surfaces for 1-hr periods. Georgiade et al. (5, 6) compared the gauze-capillary method with their own moist-swab technique for bacterial quantitation of wound surfaces of experimental animals and hospitalized patients (4). They reported no difference between these two methods in the numbers of bacteria recovered from surface wounds of experimental animals. In clinical trials, however, the moist-swab technique was reported to be the better method. Douglas (2) demonstrated that the varied surfaces presented by traumatized wounds could influence bacterial recovery by swabbing procedures.

The present report describes a reproducible laboratory model, using the rat, for the investigation of surface wound infection. A mechanical method of homogenizing tissue is presented which provided an objective and reproducible method of sample preparation for bacterial quantitation. The application of tissue homogenization to bacterial quantitation has been used by others (8, 10). However, its systematic application to the study of surface wound infection is not widespread. In the present study, a modification of Eade’s technique (3) for eluting bacteria from wound surfaces was developed and a combination of the surface-wash plus homogenization was compared with the moist-swab technique described by Georgiade et al. (4).

MATERIALS AND METHODS

Animals. Adult female Lewis or Sprague Dawley rats weighing 200 to 300 g were maintained on Purina rat chow and tap water, ad lib., for at least 1 week prior to use. The rats were anesthetized and sheared dorsally. The sheared dorsum was shaved by using pHisoHex and a stainless-steel surgical prep blade.

Experimental surgical wound. Under aseptic conditions, a 1-inch (2.54 cm) square was outlined by an incision into the skin, dorsolaterally just anterior to the pelvis, with a no. 11 surgical blade. The depth
of the incision extended through the full thickness of skin but did not include the underlying muscle, panniculus carnosus. The anteriomedial corner of the incised skin was lifted with a toothed forceps and carefully dissected away from the panniculus by using the index finger wrapped in a gauze pad (Fig. 1). The resulting full-thickness surface wound provided the basic surgical preparation for all experiments. The panniculus muscle was inoculated with microorganisms and reproducibly removed for evaluation of the resulting infection. Figure 2 shows a cross section of skin from the back of a Lewis rat including the attached underlying muscle layer and the panniculus carnosus (P).

**Bacterial culture.** A strain of *Pseudomonas aeruginosa* from a patient with septicemia was used for these studies. The culture was maintained as a frozen suspension in sterile skim milk. Portions (0.5 ml) of the suspension were sealed in sterile ampoules, quick-frozen in a dry ice-acetone bath and stored at –112 °F (–80 °C). As needed, an ampoule of suspension was quick-thawed in warm water, and the entire 0.5-ml portion was transferred to 10 ml of a basal salt medium of the following composition (per liter of water): NaCl, 5.0 g; K2HPO4, 1.0 g; KH2PO4, 1.0 g; MgSO4·7H2O, 0.2 g; (NH4)2SO4, 0.1 g; l-asparagine, 1.0 g; glycerol, 2.0 ml. The inoculated tube of medium was incubated for 15 to 18 hr at 35 C on a reciprocating shaker. One milliliter of this culture was inoculated into 100 ml of the same basal salt medium and then was incubated on a shaker for 5 to 6 hr at 35 C. A 10-ml sample of the culture was centrifuged at 800 × g for 10 min at 4 C. One milliliter of the supernatant culture prepared in this manner contained 10⁴ viable *P. aeruginosa*.

**Infected wound.** The surface of the surgical wound was seeded with 10⁴ *P. aeruginosa* by pipetting 0.1 ml of the supernatant culture described above onto the panniculus carnosus. Care was taken to prevent run-off from the wound surface.

The skin grafts were then applied to the exposed pannicular surface either immediately after seeding with *P. aeruginosa* or 24 hr after seeding, when the panniculus muscle contained an established infection. Grafts were covered with Telfa pads held in place with adhesive tape. When the contaminated panniculus remained ungrafted it was protected from abrasion by a perforated polystyrene weighing tare inverted over the wound surface. These shields were secured with collodion and swabbed with tincture of benzoin to discourage the animals from chewing them. Animals were housed individually in clean cages and were supplied with food and water ad lib.

**Tissue homogenization.** By using aseptic technique, grafts and underlying muscle tissues were removed, individually weighed, diced, and placed in sterile 50-ml Duali tissue grinders (Kontes Glass Co.) containing 10 ml of sterile iced saline. The panniculus muscle, less than 1 mm in thickness, separates cleanly, but care must be exercised in identifying and removing this muscle. The loaded grinders

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**Fig. 1.** Technique for removal of the full-thickness skin flap leaving the panniculus carnosus intact by gently pressing the muscle away from the dermal surface of the skin.
were placed in an ice bath, and the samples were homogenized for 5 min at 720 rev/min by using the constant torque power of a one-half-horsepower motor on a 15.5-inch (ca. 39 cm) drill press.

**Surface washing technique.** Figure 3 shows a schematic of a device designed for extraction of bacterial cells from the wound surface. The delivery ends of 30-ml plastic syringes were cut off to provide cylinders of uniform bore. The syringes were gas-sterilized with ethylene oxide prior to use. After graft removal, the sterile cylinder was tightly pressed against the exposed panniculus surface and held in place with a clamp attached to a ringstand. Ten milliliters of sterile saline pipetted into the cylinder was mechanically mixed with a glass T-rod driven by a Tri-R variable speed motor (Tri-R Instruments, Inc.). The fluid was agitated for 5 min at a speed sufficient to produce a vortex, removed with a sterile pipette, transferred to a test tube, and kept on ice until quantitated. After the wash procedure, the animal was sacrificed and the panniculus was removed as described above. In these experiments, the area of the panniculus biopsied was only the 0.6-inch² (3.8 cm²) portion outlined by the impression of the plastic syringe on the wound surface.

**Moist-swab technique.** As in the method described by Georgiade et al. (4), a sterile cotton-tipped applicator moistened with sterile saline was rubbed over the panniculus, across and back 10 times, advancing and rotating the applicator with each stroke. The animal was rotated 90°, and the procedure was repeated with the same applicator. The applicator was then placed in a tube containing 10 ml of sterile saline and agitated on a Vortex mixer for 30 sec to elute the bacterial cells contained in the applicator. The animal was sacrificed, and the entire 1-inch (2.54 cm)-square area of panniculus was surgically removed for homogenization and bacterial quantitation. To compare this technique with the combined surface-wash plus homogenization method, the individual data from these experiments were recalculated from the total *P. aeruginosa* per square inch to the total per 0.6 inch².

**Bacterial counts.** The bacteria in homogenates, surface-wash fluids, and moist-swab samples were estimated by standard plate count methods, substituting a selective growth medium, acetamide agar (7), for the usual agar medium. Colonies were counted after 48 hr of incubation at 35°C. The bacterial count was expressed as the number of cells per unit surface area of tissue biopsied, washed, or swabbed. The total viable count of the tissue biopsied divided by its wet weight provided the bacterial density or concentration. The bacterial concentration was transformed to the log₁₀ scale to normalize the data and reduce inequality of variances. The data were expressed as the mean log₁₀ ± the standard error of the mean (SEM). Significance of individual treatment modes was determined by the Student’s *t* test. Data were considered significant
for this comparative evaluation. After surface sampling, the panniculus muscles were excised for homogenization and quantitation of the residual bacterial content. In control animals, the wounds were neither washed nor swabbed prior to biopsy of the infected muscle. These animals were included to insure that at least as many bacteria could be recovered by the combined quantitative sampling methods as could be obtained by homogenization alone. The data presented in Fig. 6 illustrate the log₁₀ total bacteria recovered with each technique. In controls, which were homogenized only, the panniculus contained a log₁₀ of 9.0 P. aeruginosa in a 0.60-inch² area. In 25 rats, the 10 ml of wash fluid contained a log₁₀ of 9.22 bacteria. The corresponding 0.6-inch² area of panniculus contained a residual mean of 8.48. After surface sampling by the moist-swab technique, in six rats, the 10 ml of saline eluate from the swab applied to the muscle surface contained a log₁₀ of 8.28 and the panniculus a mean of 8.72 (both values corrected to an area of 0.6 inch²). Statistically there was no difference in the numbers of bacteria remaining in the panniculus after either surface sampling method was used. However, the bacterial content of the wash fluid was significantly higher than could be detected in the saline eluate of the moist-swab applicator (P < 0.001) and was approximately 40% greater than that recovered from the infected panniculus by homogenization alone. If the bacterial content of the wash fluid is assumed to represent 100% recovery of bacteria extractable from the wound surface, the

RESULTS

Standardization of homogenization technique. The Duall tissue grinder (Kontes Glass Co.) provided the most uniformly prepared homogenate upon visual examination compared with several other homogenizers evaluated. Tissues were homogenized for 5 min. Beyond this time no additional increase in bacterial extraction occurred. Figure 4 presents data showing the ability of the Duall grinders to extract P. aeruginosa from halves of rat skin autografts. These grafts had been applied for 24 hr to 15 rats with freshly contaminated panniculus wounds. There was no significant difference between the mean log₁₀ (log₁₀ P. aeruginosa concentrations (per milligram of tissue wet weight) of 3.6 and 3.7 for halves of grafts A and B, respectively.

The Duall tissue grinder did not kill the bacteria during homogenization. Figure 5 shows the viable cell counts in 1.0-ml portions in two pure cultures of P. aeruginosa in samples obtained prior to and during homogenization for periods of up to 30 min. In one of the tests (dotted line), the increase and stabilization in cell counts suggested that the grinders were dispersing cells growing in clumps.

Comparison of surface-wash with moist-swab. Animals with ungrafted panniculus muscles 24 hr postcontamination were chosen

Fig. 3. Apparatus for quantitative extraction of bacteria from a surface wound.

when the means differed at a probability equal to or less than 0.05.

Fig. 4. Comparison of mean P. aeruginosa content from halves of autografts (A and B) 24 hr after application to freshly contaminated wound surfaces; homogenized in Duall tissue grinders for 5 min.
moist-swab only recovers about 10% of the total bacteria present. The residual *P. aeruginosa* content of the panniculus after moist-swab sampling was significantly higher than that demonstrated in the saline eluate of the applicator (*P* < 0.05).

**DISCUSSION**

Several methods are currently in use (1, 4, 8) to provide estimates of bacteria recovered from a wound surface. Our data demonstrate the advantage of a surface-wash procedure compared with the moist-swab technique for extracting bacteria from such surfaces. The results show that, 24 hr after contaminating the panniculus carnosus with $10^7$ *P. aeruginosa*, 90% of the bacterial cells in the resulting infected tissue are extractable from the wound surface by washing, and an additional 10% can be recovered by tissue homogenization of the washed muscle. Homogenization of the whole infected tissue without washing permits recovery of about 60% compared with the wash procedure. Possibly, better dispersion of the extracted bacteria by the wash procedure may account for this difference. In 25 animals, the extremely low standard error of 0.092 for the bacteria extractable into 10 ml of wash fluid demonstrates the reproducibility of this procedure as a tool for the study of wound infection. The gauze-capillary and moist-swab tech-
niques certainly are useful and can be helpful for estimating the degree of wound contamination. During the development of wound sepsis (10), however, when bacteria begin to penetrate below the wound surface to invade the tissues and lymphatics, a knowledge of surface contamination alone may not provide sufficient information to evaluate the success or failure of a particular therapy. From our data, it is apparent that this information can be obtained by means of actual tissue biopsies which are homogenized and quantitated under rigid experimental conditions. In addition, the data indicate that the degree of wound infection can be estimated by the surface-wash procedures. Further, the application of a mechanical homogenization technique to the bacterial quantitation of infected tissues removes the subjectivity of the individual investigator as a factor influencing the uniformity or reproducibility of experimental sampling procedures.

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