A New Semiquantitative Culture Method for Early Detection of Surgical Incisional Wound Infection

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A semiquantitative culture technique for early detection of surgical wound infection was done by rolling a segment of a plastic intravenous catheter across a blood agar plate after insertion into the most inflamed part of the wound on postoperative day 3. Patients were monitored daily for purulent discharge until healing. Of the 53 wounds studied, 44 (83%) had no growth or low-density superficial colonization on the blood agar (generally <15 colony-forming units and within the upper 1.5 cm of the catheter). None of these 44 wounds was subsequently infected; therefore, these colonies represented colonization. Of the 9 wounds (17%) that yielded >15 colony-forming units and a diffuse subcutaneous pattern (colonies below the upper 1.5 cm of the catheter), all developed purulent discharge with a positive culture of the same organisms found by semiquantitative culture. This result differed significantly ($P < .01$) from the 44 wounds without subsequent infection. This semiquantitative technique has the potential to distinguish infection from colonization and may be useful in diagnosing surgical wound infection.

More than 7% of all surgical incisions are complicated by wound infection [1], usually the second largest category of nosocomial infections [2]. There is no simple and conclusive way of diagnosing wound infection until purulent discharge occurs [3]. Clinical signs of inflammation are inadequate because they are neither objective nor unifactorial in establishing the cause, and superifical wound swab cultures do not distinguish colonization from infection. Quantitative culture by tissue biopsy [4] is laborious and invasive. An early method for detecting wound infection before the purulent stage would improve our understanding of the pathogenesis of wound infection and the formulation of therapeutic strategy. This may be significant in decreasing morbidity, mortality, and economic loss. We report a semiquantitative culture method for the early detection of wound infection and an evaluation of this method in the investigation of 53 surgical wounds.

Materials and Methods

Patients. Within a 6-month period (July–December 1988) 116 patients who underwent operations in the University Department of Surgery were seen on postoperative day 3. Patients with the following signs of wound inflammation were included in this study: redness, increased warmth, swelling, and lymphangitis. Also included were patients with pain but without signs of inflammation. Three patients who had wounds with purulent discharge were excluded. Clinical information on all patients, including demographic data, major underlying illness, type of operation, local signs of wound inflammation, body temperature, peripheral white blood cell count, and antimicrobial therapy, was obtained at the time of the semiquantitative culture. Surgical wounds were classified into four categories: clean, clean-contaminated, contaminated, and dirty [5].

Cultures. Superficial swabs for culture (Culturette and Anaerobic Culturette, Marion Laboratories, Kansas City, MO) were taken from the most tender or inflamed spot of the wound. The culture swabs were repeated after the wound was aseptically prepared with 0.5% Hibitane (Stuart Pharmaceuticals, Wilmington, DE). After the wound was completely dry, a 20-gauge intravenous (iv) catheter (Angiocath, Du Pont Pharmaceuticals, Wilmington, DE) fitted with a 5-ml syringe on a Cameco handle was inserted into the incision at the chosen site vertically or obliquely to maximize contact. Once past the dermal layer, the needle was withdrawn so that its tip was located just within the outer plastic catheter opening. The catheter was then gently and fully inserted through the subcutaneous layer until resistance of the muscle layer was felt.

While in place, the plastic catheter was marked by a scalpel blade ~1 cm above the surface of the wound. With continuous gentle suction, the whole catheter with the inner needle was withdrawn immediately. The catheter was then severed at the marked site and rolled over the blood agar plate at least four times back and forth in the same direction using sterile forceps. The segment was then implanted in the center of the agar (figure 1). A smear of the aspirate (if not a dry tap) from the inner needle was Gram stained for microscopic examination. The procedure was repeated with another iv catheter on prereduced media for anaerobic culture. The anaerobic specimen was transported to the laboratory by GasPak anaerobic jar (BBL Microbiology Systems, Cockeysville, MD).

The wound was examined daily for purulent discharge until postoperative day 8, when the patient was sent home. The wound was reexamined for evidence of infection on postoperative day 14 and 4 weeks later at the outpatient clinic. Samples of discharge from infected wounds were sent for aerobic and anaerobic bacterial cultures.

The blood agar plate (a 5% horse blood agar in Columbia base) with the catheter segment was incubated at 37°C and processed according to standard culture procedures [6]. The aerobic cultures were

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All patients gave informed consent for wound aspiration.
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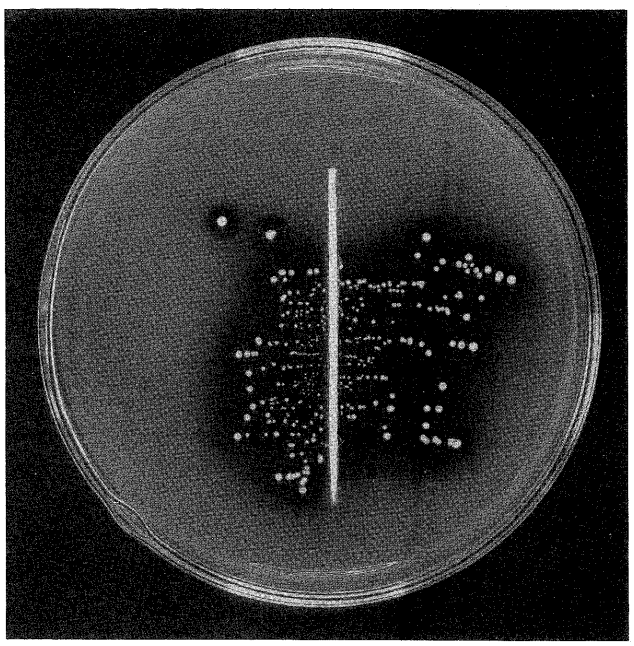

Figure 1. Diffuse subcutaneous growth pattern that positively predicted wound infection.

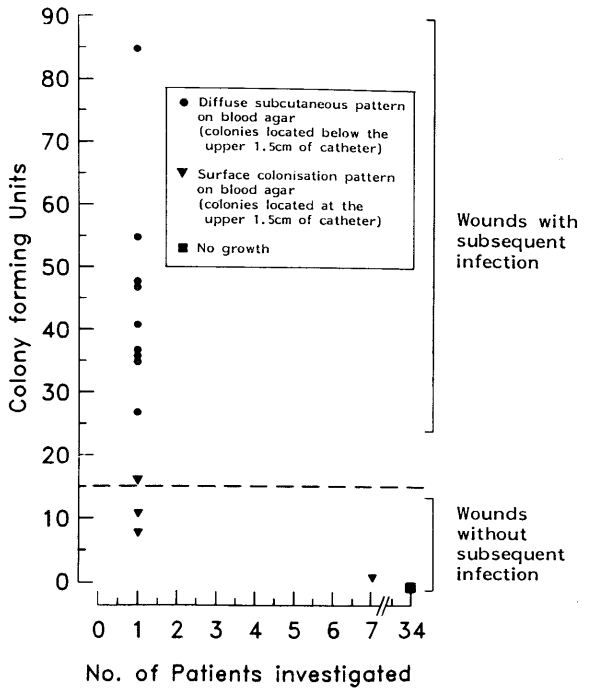

Figure 2. Bimodal distribution of counts of colony-forming units by semiquantitative culture in the 53 cases.

examined at 24 and 48 h. The pattern of growth and number of colony-forming units (cfu) were recorded. The bacterial isolates were identified by conventional methods and confirmed with the Vitek Automicrobic System. The anaerobic cultures were examined and processed at 48 h and on day 5 before being discarded. Gram stained aspirate smears were examined for bacteria and white and red blood cells.

Statistical analysis. Data for infected and uninfected cases were compared by χ^2 test with Yates's correction or Fisher's exact test. The numbers of cfu on semiquantitative culture of the two groups were compared by calculating the means and standard errors by Student's *t* test. Days to onset of wound discharge were compared using the Wilcoxon rank sum test.

Results

The surgical wounds of 53 patients were examined by the semiquantitative culture technique. All catheter segments were 4–5 cm long. Colony counts on the blood agar plates were bimodal in distribution (figure 2). No growth was found on 34 catheters; 7 catheters grew only one bacterial colony, primarily *Staphylococcus epidermidis*. Three cases yielded ≤16 cfu; all were situated within the upper 1.5 cm of the catheter with a wedge-shaped pattern, suggesting superficial colonization (figure 3). Of the 10 cases with positive growth within the upper 1.5 cm of the catheter, there were 7 *Staphylococcus epidermidis*, 1 *Corynebacterium* species, 1 *Micrococcus* species, and 1 *Acinetobacter calcoaceticus lwoffi*, not a well-recognized skin flora. None of these 44 cases became infected. Nine catheters yielded >20 colonies that were found mainly below the upper 1.5 cm of the catheter, suggesting a diffuse subcutaneous pattern of growth (figure 1). These 9 cases were designated as positive semiquantitative cultures, and all developed purulent discharge before postoperative day 8 (table 1; $P < .01$). Using the criterion of 15 cfu below the upper 1.5 cm of the catheters, wound infections would have been accurately predicted without any false positives or negatives.

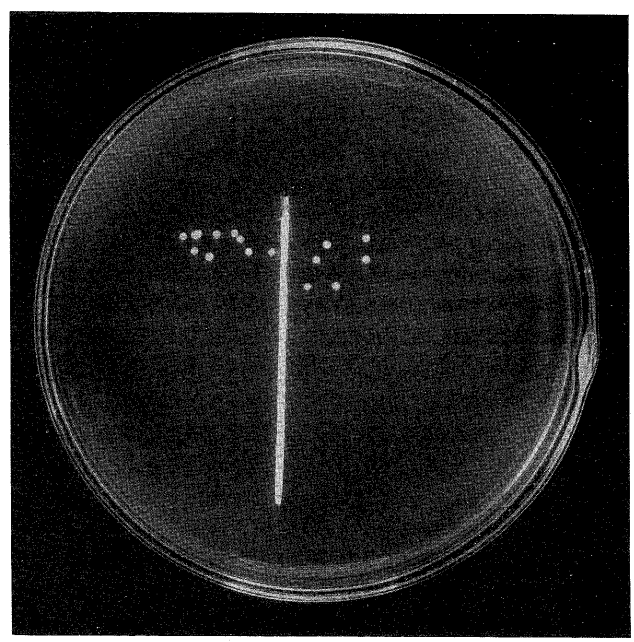

Figure 3. Wedge-shaped growth pattern of superficial colonization.

Table 1. Clinical data from the nine patients with positive semiquantitative culture and subsequent wound infection.

Type of operation	Bacteria isolated from the positive semiquantitative culture and pus	Antibiotic, regimen	Antibiotic susceptibility or resistance of bacteria	Postoperative days to wound discharge
Cholecystectomy, repair of duodenum	*Escherichia coli*	Cefuroxime (P), 0.75 g iv every 8 h	S	8
Appendectomy	*E. coli*	Gentamicin (P), 60 mg iv every 8 h; metronidazole (P), 0.5 g iv every 8 h	S	7
	Streptococcus anginosus	Ampicillin (P), 2 g iv every 6 h; sulbactam (P), 1 g every 6 h	S	8
Abdominoperineal resection	*Acinetobacter calcoaceticus anitratus*	Cefuroxime (P), 0.75 g iv every 8 h; metronidazole (P), 0.5 g iv every 8 h	R	4
Transgastric plication of esophageal varices	*Flavobacterium meningosepticum*	Cefuroxime (P), 0.75 g iv every 8 h	R	4
Cholecystectomy, exploration of common bile duct	*Bacillus cereus*	Cefotaxime (T), 1 g iv every 8 h	R	5
Laryngectomy, block dissection of neck	*Staphylococcus aureus*	Ampicillin (P), 2 g iv every 6 h	R	4
Ileoileostomy, enterolysis	*Enterococcus faecalis**	Cefuroxime (P), 0.75 g iv every 8 h; metronidazole (P), 0.5 g iv every 8 h	R	4
Repair of ileal perforation	*Enterococcus faecalis*,* *Candida albicans*	Cefuroxime (T), 0.75 g iv every 8 h; metronidazole (T), 0.5 g iv every 8 h	R	4

NOTE. (P) = prophylactic, three doses of antibiotic given before surgery; (T) = therapeutic course with 7 days of treatment; S = susceptible on break-point susceptibility testing by agar dilution method; R = resistant.

* Formerly *Streptococcus faecalis*.

Of the nine patients with subsequently infected wounds, six had organisms that were resistant by in vitro susceptibility testing to the prophylactic or therapeutic antibiotics (table 1). All six wounds started to discharge on or before day 5. The other three patients had organisms that were sensitive to the prophylactic regimens of three doses, and all developed discharge on postoperative day 7 or 8. Using the Wilcoxon rank sum test, there was significant delay to onset of wound discharge in the group treated with the correct antibiotics ($P < .01$, $Z = 2.67$).

No significant difference ($P > .05$) was found between wounds with and without subsequent infection with respect to gender ratio (1.25 vs. 1.09), underlying illnesses, signs of inflammation, or type of wound (table 2). Patients who developed subsequent wound infection were older than noninfected patients (mean age, 66 and 54, respectively). The mean and standard error of the number of cfu from cultures of the subsequently infected wounds were 45.78 and 5.63; those of the subsequently noninfected wounds were 0.95 and 0.46. Thus, the means also differed significantly ($P < .01$, $t = 7.93$).

The number of patients receiving antibiotics in both groups did not differ significantly. Patients with clean-contaminated wounds were given a three-dose regimen of prophylactic antibiotic; a therapeutic course of 7–10 days was given to patients with contaminated and dirty wounds. The type of operations performed, the doses and type of antibiotics given, and the postoperative day of subsequent purulent wound discharge in the group with positive semiquantitative culture are shown in table 1. In the group without subsequent wound infection, the type and number of operations performed comprised appendectomy (11), colectomy (6), colostomy closure (1), Hartmann's procedure (1), cholecystectomy (5), exploration of common bile duct (3), choledochojejunostomy (1), gastrectomy (2), truncal vagotomy and pyloroplasty (3), patch repair of perforated peptic ulcer (4), peritoneal toilet and laparotomy (2), total cystectomy (1), ileosigmoid bypass (1), and herniorraphy (3).

The antibiotics and numbers of patients who received prophylactic therapy with three iv doses included gentamicin and metronidazole (6), cefuroxime and metronidazole (10), cefuroxime (9), and ampicillin and sulbactam (4). The antibiotics and numbers of patients who received therapeutic treatment for 7–10 days comprised cefuroxime and metronidazole (7), netilmicin and metronidazole (1), gentamicin and metronidazole (1), cefotaxime and metronidazole (1), cefaperazone (1), and cefaperazone and metronidazole (1).

Cultures of the predisinfection superficial wound swabs predicted the correct organisms recovered from pus in only 4 cases and all were mixed with normal skin flora; cultures of the postdisinfection wound swabs yielded the correct pathogens in 3 wounds without mixing with commensals. Dry aspirates were obtained from 15 wounds (28%). However,

Table 2. Clinical and laboratory data for 53 surgical patients with and without subsequent wound infection.

	Wounds with subsequent infection (n = 9)	Wounds without subsequent infection (n = 44)
Underlying illnesses*	4	13
Local signs of inflammation	5	10
Type of wound		
I, clean	0	3
II, clean-contaminated	6	27
III and IV, contaminated and dirty	3	14
Fever (>37.4°C)	4	14
White blood cells (>11 × 10^9/l)	1	1
Positive superficial swab culture†		
Before disinfection	7 (4)	17 (0)
After disinfection	4 (3)	2 (0)
Smear of wound aspirate		
Bacteria	3	0
White blood cells‡	9	11
Red blood cells§	4	9

* Include malignancies (13), liver cirrhosis (2), and neutropenia, chronic renal failure, and diabetes mellitus (1 each).

† Number of swabs with organisms similar to those isolated from pus are in parentheses.

‡ >10/high power field (×400).

§ >20/high power field (×400).

all 9 cases that proved to be infected yielded significant numbers of white blood cells on Gram-stained smears, whereas 11 (25%) of 44 uninfected wounds also yielded white blood cells and 9 of those contained red blood cells. Only 5 (33%) of 15 inflamed wounds and 4 (11%) of 38 noninflamed wounds became infected (table 2).

The spectrum of pathogens and commensals obtained by semiquantitative cultures and superficial swabs was not remarkable. No anaerobes were found. The organisms isolated from the cases with positive semiquantitative culture and pus are shown in table 1. Other organisms isolated from the pre- and postdisinfection swabs were *Staphylococcus epidermidis* (20 cases), *Corynebacterium* species (5), *Micrococcus* species (2), *Acinetobacter calcoaceticus lwoffi* (2), and *Pseudomonas aeruginosa* (3). No complications were recorded, pain was minimal, and analgesic was not required with the procedure.

Discussion

Wound infection results when there is an imbalance between the number and virulence of the attacking bacteria and the efficiency of the host defenses [7]. The importance of quantitation must be emphasized because it has been found that a dose of 10^6 bacteria/g of tissue was needed in experimental wound infection [8, 9]. In diagnostic microbiology, quantitation of bacteria is often used to differentiate colonization from infection. Maki et al. [10] were the first to use a semiquantitative method for the diagnosis of infection or colonization in intravascular catheters. Similar to the results of Maki et al., all wounds with a positive semiquantitative culture (>15 cfu below the upper 1.5 cm of the catheter) subsequently became infected.

Another advantage of this semiquantitative culture technique is its unique ability to map out the density and pattern of bacterial population at the cutaneous layer (the upper 1.5 cm of the catheter, which included 1 cm of catheter above the skin) and the subcutaneous layer (the rest of the catheter). During wound infection, the septic process is usually most severe in the subcutaneous fat layer, which is relatively avascular and prone to hematoma development during surgery [11]. The semiquantitative technique directly samples this area (reflected by the catheter segment below the upper 1.5 cm). Colonies found at the upper 1.5 cm of the catheter, including one case of gram-negative bacilli, may reflect colonization at the cutaneous layer rather than actual infection. Therefore, besides counting the colonies, the position of the colonies in relation to the catheter was useful in differentiating surface colonization from infection.

The semiquantitative culture technique was simple and quick to perform. Patient acceptability was good because of the minimal discomfort experienced when the catheter was inserted through the plane of fibrin adhesion loosely formed at the line of incision during the early stage of wound healing. The procedure was done on postoperative day 3, as most infected wounds started to discharge by 4–8 days [1]. We did not attempt to culture the aspirate, as many of the wounds gave a dry aspiration. Nevertheless, in wounds with a positive aspirate, the amount was sufficient for doing a Gram-stained smear for bacteria and white blood cells, an additional monitor to our culture results.

A large proportion (94%) of patients in the study were given antibiotics, but there was no evidence that this affected the diagnostic value of the test. Of the nine subsequently infected wounds, the diagnostic criterion of 15 cfu below the upper 1.5 cm of the catheter was valid, even for the three wounds infected with organisms that were sensitive to the antibiotics given.

Other studies have utilized needle aspiration to obtain deep tissue for culture [12, 13]. However, this requires the injection of isotonic saline before aspiration. Such a procedure without quantitation still could not distinguish infection from exogenous contamination or colonization, especially when performed adjacent to surgical wounds; even a single bacterium in the deep tissue aspirate could produce a positive culture. Thus, this procedure might not correctly predict the outcome of the surgical wounds. Even if laborious quantitation were done, it might underestimate the actual number of bacteria in the deep tissue because of the saline injection. Potential hazards would be spread of infection due to saline injection, bleeding, and hematoma formation.

In assessing a new diagnostic procedure, it is prudent to review the potential problems. There is always a theoretical

risk of introducing infection when the catheter is inserted into the wound. In practice, the risk is minimal if the procedure is done aseptically. In this study, the nine cases of wound infection were unlikely to be iatrogenic because a significant number of white blood cells were observed in the Gram-stained smear of the needle aspirate obtained during the semiquantitation procedure. Another problem is the possibility of missing the infected area, especially when the procedure is performed on a long incision. Therefore, we inserted the catheter at the most inflamed site of the wound; multiple sites of the incision could also be sampled.

The data indicate that prophylactic antibiotics may affect the outcome of wound infection. The causative organisms of most of the infections were found to be resistant to the antibiotics given; for the three that were sensitive, the time to onset of wound discharge was significantly delayed. Future studies of the effects of antibiotics on wounds with positive semiquantitative culture results before the development of purulent discharge would be interesting. Nevertheless, this semiquantitative culture technique may be useful in research on the pathogenesis of wound infection in addition to its clinical use.

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