Quantitative Microbiology of Traumatic Orthopedic Wounds

RUTH M. LAWRENCE,[1]* PAUL D. HOEPRICH,[1] ALICE C. HUSTON,[1] DANIEL R. BENSON,[2] AND RICHARD S. RIGGINS[2]

Departments of Internal Medicine[1] and Orthopaedic Surgery,[2] University of California, Davis, Sacramento Medical Center, Sacramento, California 95817

Quantitative bacterial and fungal cultures carried out on 116 specimens of tissue debrided from traumatic orthopedic wounds yielded 118 bacterial and 8 fungal isolates. The organisms obtained reflected primarily the resident flora of the skin.

Infection at the site of traumatic wounds is a common complication of orthopedic injuries. With open fractures, bacterial contamination of the wound has been reported to be present in 65% of the cases (3). Because of the high rate of contamination, use of antimicrobics has been suggested to reduce rates of clinical infection. In the study referred to above, administration of a cephalosporin was associated with a reduction in the rate of wound infection from 13.9% to 2.9% in the treated group. In 73% of the infections the bacteria responsible were cultured from the wound before any surgical or antimicrobial therapy. Complete bacteriological data regarding the kinds of microorganisms encountered were not provided (3). Because the density of contamination might also be a determinant of subsequent clinical infection, we carried out quantitative as well as qualitative bacterial and fungal cultures of tissue obtained at the time of initial surgical debridement of orthopedic wounds.

MATERIALS AND METHODS

Patients and specimens. Specimens were obtained from adult patients admitted to the University of California, Davis, Medical Center, Sacramento, for therapy of severe orthopedic trauma of one or more extremities. Wounds which were grossly contaminated or dirty at the time of entry were excluded. Shortly after admission, before scrubbing of the area of injury, 1 to 5 g of traumatized tissues was excised and placed in a sterile tube which was loosely capped and then put into a miniature anaerobic jar of the type described by Attebery and Finegold (1) for transport to the laboratory. The jars were kept at room temperature before tissue processing. All specimens were processed within 24 h of collection—23% of them were processed within 6 h of collection, 44% were processed in 7 to 12 h after collection, 25% were processed in 13 to 18 h after collection, and 8% were processed in 19 to 24 h after collection. Miniature anaerobic jars of this type have been shown to provide an anaerobic environment capable of maintaining even fragile anaerobes for 24 h or longer (1). Because no diluting transport medium is added, the tissues can be utilized for quantitative cultures.

After each specimen was weighed, it was ground in a sterile tissue grinder with 1 to 5 ml of phosphate-buffered saline (PBS). The resulting suspension was then serially diluted in PBS, and 0.1 ml of each dilution was used for inoculation. Blood agar, MacConkey agar, and chocolate agar plates were streaked for aerobic culture. For anaerobic cultures, 0.1 ml of suspension was added to (i) brucella agar (Difco Laboratories, Detroit, Mich.) with 5% sheep blood (vol/vol) and supplemented with vitamin K and hemin, (ii) chopped meat medium (Difco), and (iii) thioglycolate medium (Difco), also supplemented with vitamin K and hemin. A Gram stain was performed on material left in the tissue grinder.

Identification of organisms was begun 48 h after inoculation, and standard methods were used.

RESULTS

A total of 116 specimens from 95 patients were analyzed. These 116 specimens yielded a total of 118 bacterial and 8 fungal isolates. Sixty specimens (52%) yielded only aerobes, 3 (2.5%) yielded only anaerobes, 11 (9.5%) yielded both aerobes and anaerobes, and 42 (36%) yielded no bacteria. Fungi were cultured only in the presence of bacteria. The microorganisms isolated are listed in Table 1.

The most common aerobes were *Staphylococcus epidermidis* (33 isolates, 2.10×10^7 to 6×10^7 colony-forming units [CFU] per g of tissue); *Corynebacterium* spp. (21 isolates, 3×10^1 to 7×10^7 CFU per g of tissue); *Micrococcus* spp. (15 isolates, 1×10^1 to 4×10^5 CFU per g of tissue); and *Bacillus* spp. (13 isolates, 2×10^1 to 6.6×10^7 CFU per g of tissue). *Staphylococcus aureus* was isolated only five times, and the CFU ranged from 1×10^1 to 5×10^6 per g of tissue. A variety of aerobic gram-negative bacilli were encountered. The most common were *Escherichia coli* (four isolates, 2×10^3 to 1×10^6 CFU per g of tissue) and *Enterobacter agglomerans* (four isolates, 6×10^2 to 4×10^7 CFU per g of tissue).

Propionibacterium spp. was the most common anaerobe and was found seven times $(1 \times 10^1$ to 5×10^3 CFU per g of tissue). The other anaerobes found on primary isolation were *Propioni-*

TABLE 1. *Bacteria and fungi obtained from traumatic orthopedic wounds at the time of initial surgical debridement*

Organism	No. of isolates	Range of CFU/g of tissue
Aerobes		
Gram-positive cocci		
S. epidermidis	33	2×10^1–6×10^7
Micrococcus spp.	15	1×10^1–4×10^5
S. aureus	5	1×10^1–5×10^6
Streptococcus spp. *viridans* group	3	5×10^0–7×10^1
Streptococcus spp. (nonhemolytic)	1	5×10^2
Gram-positive bacilli		
Corynebacterium spp.	21	3×10^1–7×10^7
Bacillus spp.	13	2×10^1–6.6×10^7
Streptomyces spp.	3	2×10^1–7×10^2
Lactobacillus spp.	2	7×10^3–9×10^6
Bacillus cereus	1	9×10^4
Gram-negative bacilli		
E. coli	4	2×10^3–1×10^6
E. agglomerans	4	6×10^2–4×10^7
Acinetobacter spp.	2	2×10^4–5×10^4
Pseudomonas fluorescens	2	2×10^2–1×10^7
Serratia spp.	2	6×10^3–9×10^6
Klebsiella spp.	1	6×10^3
Moraxella spp.	1	2×10^6
Citrobacter diversus	1	9×10^4
Pseudomonas stutzeri	1	1×10^7
Pseudomonas cepacia	1	9×10^4
Aeromonas hydrophila	1	1 + (secondary isolate)
Pectobacterium spp.	1	2×10^7
Anaerobes		
Propionibacterium spp.	7	1×10^1–5×10^3
Propionibacterium acnes	1	5×10^2
C. perfringens	1	9×10^4
C. bifermentans	1	1 + (secondary isolate)
Microaerophilic *Streptococcus* spp.	1	1 + (secondary isolate)
Fungi		
Candida spp. (non-*albicans*)	3	2×10^1–2×10^7
Trichosporon spp.	1	8×10^1
Rhodotorula spp.	1	2×10^2
Penicillium spp.	1	2×10^4
Mucor spp.	1	2×10^1
Aspergillus spp.	1	1.5×10^1

bacterium acnes and *Clostridium perfringens* (one isolate each). *Clostridium bifermentans* and microaerophilic *Streptococcus* spp. were each encountered once as secondary isolates.

The fungi included three isolates of Candida spp. (non-*albicans*) and one isolate each of *Trichosporon* spp., *Rhodotorula* spp., *Penicillium* spp., *Mucor* spp., and *Aspergillus* spp.

DISCUSSION

In this group of traumatic orthopedic wounds of the extremities, bacterial and fungal contamination of the injured tissue at the time of the initial debridement was common (64%). Thirty-four different species of microorganisms were cultured; from 47 of the 116 specimens (40%) more than one organism was isolated. The colony counts varied widely, but counts of 10^6 and 10^7 were encountered regularly. The bacteria most commonly isolated were members of the resident flora regularly present on normal skin (2) (*S. epidermidis*, *Corynebacterium* spp., *Micrococcus* spp., and *Propionibacterium* spp.), suggesting that the wounds were contaminated by the skin rather than by the external environment. Organisms frequently responsible for wound infections and osteomyelitis (*S. aureus*, beta-hemolytic streptococci, and enteric gram-negative bacilli) were less common, perhaps because most individuals carry these organisms on the skin only transiently (4).

Despite the frequency of wound contamination, the incidence of infection was low. After debridement, irrigation of the wound with copious amounts of 0.9% sodium chloride solution, and closure, all patients received a 5-day course of either cefazolin (provided by Smith Kline & French Laboratories) or clindamycin (provided by The Upjohn Co.) as prophylaxis. Three of the 95 patients developed infections requiring systemic antimicrobial therapy. All three infections were caused by enteric gram-negative bacilli—two infections were caused by *Serratia* spp. and one was caused by *E. coli*. In each instance, the organism responsible for the infection was isolated from the material obtained at initial debridement and was resistant to the antimicrobic used for prophylaxis. All were present in high numbers (*E. coli*, 1×10^5 CFU per g; *Serratia* spp., 6×10^3 CFU per g and 9×10^6 CFU per g) and were isolated in mixed culture. Both of the infections caused by *Serratia* spp. occurred in wounds contaminated with the greatest variety of microorganisms (six and nine other species isolated, respectively.)

We conclude from this study that: (i) even though not obviously soiled, traumatic orthopedic wounds are frequently contaminated by bacteria and fungi inoculated from the skin;

(ii) initial, thorough mechanical cleaning of the wound at the time of surgery is important to reduce the number of bacteria already present; and (iii) cultures and susceptibility testing performed at the time of debridement can provide the clinician with helpful information regarding the likely etiology of wound infections occurring after closure. This may be especially true if enteric gram-negative bacilli resistant to one or more antimicrobics are present. From our limited observations, it appears that the common skin inhabitants were suppressed by the antimicrobics, leaving the resistant enteric bacilli to cause infection. Such use of antimicrobics in this group of patients is not truly prophylaxis, but is actually therapy in view of the high likelihood of wound contamination at the time of injury. However, because of the variety of microorganisms involved, it is unlikely that antimicrobial therapy will prevent the development of infection in all instances.

LITERATURE CITED

1. Attebery, H. R., and S. M. Finegold. 1970. A miniature anaerobic jar for tissue transport or for cultivation of anaerobes. Am. J. Clin. Pathol. 53:383–388.
2. Kligman, A. M. 1965. The bacteriology of normal skin, p. 13–31. In H. E. Maibach and G. Hildick-Smith (ed.), Skin bacteria and their role in infection. McGraw Hill Book Co., New York.
3. Patzakis, M. J., J. P. Harvey, and D. Ivler. 1974. The role of antibiotics in the management of open fractures. J. Bone Jt. Surg. 56-A:532–541.
4. Rothman, S., and A. L. Lorincz. 1963. Defense mechanisms of the skin. Annu. Rev. Med. 14:215–242.