# Wound Microbiology and Associated Approaches to Wound Management

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INTRODUCTION

From a microbiological perspective, the primary function of normal, intact skin is to control microbial populations that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens. Exposure of subcutaneous tissue following a loss of skin integrity (i.e., a wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. However, the abundance and diversity of microorganisms in any wound will be influenced by factors such as wound type, depth, location, and quality, the level of tissue perfusion, and the antimicrobial efficacy of the host immune response. Whereas the microflora associated with clean, surgical wounds would be expected to be minimal, the presence of foreign material and devitalized tissue in a traumatic wound is likely to facilitate microbial proliferation unless early prophylactic antibiotic treatment and surgical debridement is implemented (201).

Since wound colonization is most frequently polymicrobial (25, 27, 44, 166, 226), involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected. In the event of infection, a wound fails to heal, the patient suffers increased trauma, treatment costs rise, and general wound management practices become more resource demanding. An analysis of postsurgical wound infections following head and neck surgery demonstrated an increase in the average hospitalization period from 14 days when wounds healed without complication to 24 days when the wounds became infected (118). In a similar analysis of 108 postsurgical wounds, Zoutman et al. (249) concluded that 10.2 days per case was directly attributable to wound infection and that the associated hospital cost was $3,937 per infected patient.

Thus, concern among health care practitioners regarding the risk of wound infection is justifiable not only in terms of increased trauma to the patient but also in view of its burden on financial resources and the increasing requirement for cost-effective management within the health care system. From a clinical perspective, fears associated with wound infection have paralleled the increasing use of occlusive dressings since the 1960s. The primary function of dressings such as polyurethane films, polyurethane foams, and hydrocolloids is to maintain a moist and optimal environment for wound healing. Although they have been reported to encourage microbial proliferation in wounds (95, 128), the infection rate is lower under occlusive dressings than under conventional dry dressings (24, 113) and wound healing is not impaired (95).

Although microorganisms are responsible for wound infection, widespread controversy still exists regarding the exact mechanisms by which they cause infection and also their significance in nonhealing wounds that do not exhibit clinical signs of infection. One school of thought is that the density of microorganisms is the critical factor in determining whether a wound is likely to heal (100, 102, 151, 196, 202). However, a second school of thought argues that the presence of specific pathogens is of primary importance in delayed healing (59, 130, 149, 181, 216, 217), while yet others have reported microorganisms to be of minimal importance in delayed healing (4, 70, 80, 95, 98, 214, 237).

There is also debate about whether a wound should be sampled for culture, the value of wound sampling in determining the cause of infection and subsequent treatment, and the sampling technique required to provide the most meaningful data. Regarding the role of the microbiology laboratory, consideration must be given to the relevance of culturing microbial specimens, the value of identifying one or more microorganisms, and which microorganisms (if any) should be assayed for antibiotic susceptibility. By questioning and justifying the need to sample and perform microbiological analyses on any problematic wound, long-term savings in cost, labor, and time to both the wound management team and the microbiology laboratory could be considerable. In this respect, the value of the Gram stain as a quick and inexpensive additional or alternative test is also worthy of consideration.

Although appropriate systemic antibiotics are considered essential for the treatment of nonhealing, clinically infected wounds, there is debate about the relevance and use of systemic and topical antibiotics and of topical antiseptics in the treatment of nonhealing, noninfected wounds. Other, nonmicrobiological approaches to controlling potentially pathogenic microbial populations in wounds must also be considered part of a multidisciplinary wound management effort.

In view of the fears, uncertainties, and controversies regarding the role of microorganisms in wounds, this review aims to capture current opinion, evaluate the role of the microbiologist and the microbiology laboratory in wound management, and clarify the relevance of treatment and treatment options in controlling microbial colonization and infection in wounds.

WOUND TYPES

Wounds can be broadly categorized as having either an acute or a chronic etiology. Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries (60). Irrespective of the nature of the cutaneous injury, acute wounds are expected to heal within a predictable time frame, although the treatment required to facilitate healing will vary according to the type, site, and depth of a wound. The primary closure of a clean, surgical wound would be expected to require minimal intervention to enable healing to progress naturally and quickly. However, in a more severe traumatic injury such as a burn wound or gunshot wound, the presence of devitalized tissue and contamination with viable (e.g., bacterial) and nonviable foreign material is likely to require surgical debridement and antimicrobial therapy to enable healing to progress through a natural series of processes, including inflammation and granulation, to final reepithelialization and remodeling.

In marked contrast, chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue (60). Pathophysiological abnormalities that may predispose to the formation of chronic wounds such as leg ulcers, foot ulcers, and pressure sores include compromised tissue perfusion as a consequence of impaired arterial supply (peripheral vascular disease) or impaired venous drainage (venous hypertension) and metabolic...
diseases such as diabetes mellitus. Advancing age, obesity, smoking, poor nutrition, and immunosuppression associated with disease (e.g., AIDS) or drugs (e.g., chemotherapy or radiation therapy) may also exacerbate chronic ulceration. Pressure or decubitus ulcers have a different etiology from other chronic wounds in that they are caused by sustained external skin pressure, most commonly on the buttocks, sacrum, and heels. However, the underlying pathology often contributes to chronicity, and in this situation, pressure sores, like all chronic wound types, heal slowly and in an unpredictable manner.

WOUND MICROBIOLOGY

Microbial Colonization

Exposed subcutaneous tissue provides a favourable substratum for a wide variety of microorganisms to contaminate and colonize, and if the involved tissue is devitalized (e.g., ischemic, hypoxic, or necrotic) and the host immune response is compromised, the conditions become optimal for microbial growth. Wound contaminants are likely to originate from three main sources: (i) the environment (exogenous microorganisms in the air or those introduced by traumatic injury), (ii) the surrounding skin (involving members of the normal skin microflora such as Staphylococcus epidermidis, micrococci, skin diphtheroids, and propionibacteria), and (iii) endogenous sources involving mucous membranes (primarily the gastrointestinal, oropharyngeal, and genitourinary mucosae) (65). The normal microflora of the gut, the oral cavity, and the vagina are both diverse and abundant, and these sources (particularly the oral and gastrointestinal mucosae) supply the vast majority of microorganisms that colonize wounds. Detailed microbiological analyses of wounds demonstrate close correlations between the species found in the normal flora of the gut or oral cavity and microorganisms present in wounds in close proximity to those sites (33–35, 43, 46). Whereas a minor, healing wound may allow sufficient time for a relatively small number of skin contaminants to take residence, the continued exposure of devitalized tissue associated with a slowly healing chronic wound is likely to facilitate the colonization and establishment of a wide variety of endogenous microorganisms. Dental plaque, the gingival crevice, and the contents of the colon contain approximately $10^{11}$ to $10^{12}$ microorganisms/g of tissue, of which, up to 90% of the oral microflora (16) and up to 99.9% of the colonic microflora (105) are anaerobes. In view of this situation, it is reasonable to predict that wounds with a sufficiently hypoxic and reduced environment are susceptible to colonization by a wide variety of endogenous anaerobic bacteria. However, to date, widespread opinion among wound care practitioners is that aerobic or facultative pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, and beta-hemolytic streptococci are the primary causes of delayed healing and infection in both acute and chronic wounds. Such opinion has been formed on the basis of referenced comments and studies performed largely during the last two decades that have investigated the role of microorganisms in wound healing (58, 59, 81, 94, 146, 182, 216, 217, 238; D. J. Leaper, Editorial, J. Wound Care 7:373, 1998). A common oversight in these and other studies and opinions is that the culture and isolation of anaerobic bacteria was minimal or omitted, whereas when wounds are investigated by appropriate microbiological techniques anaerobes are found to form a significant proportion of the microbial population in both acute and chronic wounds (25, 27, 28, 33–38, 41–45, 64, 80, 98, 143, 166, 185, 213, 226). On the basis of the studies reviewed in Table 1, which involved detailed microbiological analyses of clinically noninfected (i.e., colonized) wounds of varied etiology, anaerobes constituted, on average, 38% of the total number of microbial isolates per study. It should be emphasized that the studies reported did not investigate specifically the effect of microorganisms on wound healing.

Recognition of the fact that anaerobes are too often overlooked, although many are potentially highly virulent, has led experts in the field to define members of this group of bacteria as being “the secret pathogens” (74) and “invisible villains” (18). Nichols and Smith (175) reported that endogenous anaerobic bacteria were the likely cause of postoperative infections when wound specimens failed to yield bacterial growth on routine culture.

The failure to recognize the prevalence of anaerobic bacteria in wounds may be due to several reasons. (i) Anaerobes are not regarded as being detrimental to normal wound healing (70, 80, 150, 217). (ii) Compared with aerobic and facultative microorganisms, the culture, isolation, and identification of anaerobic bacteria is more time-consuming, labor-intensive, and expensive and is often deemed to be too demanding for many diagnostic microbiology laboratories. The relevance of culturing specimens for anaerobic bacteria is discussed in “Microbiological analysis of wounds” below. (iii) Since anaerobes are often perceived to die rapidly in air, the method of specimen collection and transportation to the laboratory is assumed to be critical for maintaining viability and for effective culture. However, many of the frequent wound colonizers, including Bacteroides, Prevotella, Porphyromonas, and Peptostreptococcus spp., will survive for several days in the presence of air (17, 26, 99, 142). Consequently, the methods for sampling and transportation are probably less critical than the microbiological methods used to ensure effective isolation of anaerobic bacteria. However, this does not imply that specimen collection and transport should be performed without the utmost care and meticulous procedures.

Both acute and chronic wounds are susceptible to contamination and colonization by a wide variety of aerobic and anaerobic microorganisms, as indicated in Table 2.

Factors Predisposing to Microbial Proliferation

Surgical wounds will heal rapidly if blood perfusion is maximized, thus delivering oxygen, nutrients, and cells of the immune system to the site of injury and providing minimal opportunity for microorganisms to colonize and proliferate (110). This situation is exemplified by wounds in the anus, which, despite being susceptible to gross microbial contamination, are very well perfused and rarely become infected (110). The probability of wound healing is extremely high if the tissue oxygen tension ($p_{O_2}$) is >40 mm Hg, but healing is unlikely to occur at levels of <20 mm Hg (110). In well-perfused periwound tissue, reported oxygen tensions of 60 to 90 mm Hg compare with levels of near zero in central dead wound space (176).

In contrast, chronic, nonhealing wounds are frequently hypoxic (218) as a consequence of poor blood perfusion (ischemia), and host and microbial cell metabolism contributes to a hypoxic environment (110, 201) in which microorganisms and especially anaerobes thrive (110). Typically, $p_{O_2}$ in chronic wounds is <10 mm Hg (218). This situation provides the ideal environment for microbial proliferation (110, 201).


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Further to a lowering of the local pO₂, oxygen tensions of between 5 and 20 mm Hg have been recorded in nonhealing wounds (218), and values of less than 30 mm Hg have been recorded in infected and traumatized tissue (164); this correlates with a reported pO₂ requirement of approximately 30 mm Hg for active cell division (111). Thus, cell death and tissue necrosis caused by tissue hypoxia or anoxia are likely to create ideal growth conditions for members of the wound microflora, including fastidious anaerobes that will proliferate as residual oxygen is consumed by facultative bacteria. Such interaction between microorganisms was recognized as long ago as 1915 by Alexander Fleming in his studies on gunshot wounds during the First World War (63). As well as being essential for cell growth and wound healing, oxygen is a critical component of the respiratory burst activity in polymorphonuclear leukocytes (PMNs), resulting in the intracellular production of highly potent antimicrobial metabolites. A significant reduction in the killing capacity of PMNs at a pO₂ of ~30 mm Hg has been reported (107), and in this respect, poorly perfused wound tissue is considered to be far more susceptible to infection than are wounds involving well-perfused tissue (176).

Although many endogenous anaerobes survive prolonged periods of exposure to air (17, 26, 99, 142, 230) and tolerate oxygen tensions up to 60 mm Hg (8% oxygen) (184), the redox (oxidation-reduction) potential (Eₚ) of tissue is also important for their survival (11). Generally, a low Eₚ (measured in millivolts) favors the growth of anaerobic bacteria, as demonstrated in the colon, where values can be as low as −250 mV, compared with approximately +150 mV in normal tissue and up to +250 mV in circulating blood. Although some anaerobes have been reported to survive in an aerated broth culture medium that was maintained at low Eₚ (73), other investigations have demonstrated that several intestinal pathogens (Clostridium perfringens, Bacteroides fragilis, and Pseudomonas aeruginosa) were inhibited in the presence of oxygen at an Eh of 150 mV in normal tissue and 30 mV at pH 7.8 but can survive at 250 mV in circulating blood. Although some anaerobes have been reported to survive in an aerated broth culture medium that was maintained at low Eₚ (73), other investigations have demonstrated that several intestinal pathogens (Clostridium perfringens, Bacteroides fragilis, and Pseudomonas aeruginosa) were inhibited in the presence of oxygen at an Eh of −50 mV (73). Also, the tolerance of anaerobes to Eₚ is influenced by pH, as demonstrated by C. perfringens, which has a growth-limiting Eₚ of +30 mV at pH 7.8 but can survive at +250 mV at pH 6.0 (73).

Although opinion is conflicting regarding the importance of the redox potential in supporting the growth of anaerobic bacteria, a wound environment that has a low oxygen tension (hypoxia or anoxia) and a low redox potential will facilitate the development of polymicrobial aerobic-anaerobic populations.

### Wound Infection

Infection occurs when virulence factors expressed by one or more microorganisms in a wound outcompete the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses. Characteristic local responses are a purulent discharge or painful spreading erythema indicative of cellulitis around a wound (186). The progression of a wound to

### TABLE 1. Studies involving a detailed analysis of the aerobic and anaerobic microbiology of noninfected wounds without specifically investigating the role of microorganisms in wound healing

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Study description</th>
<th>No. of microbial isolates (% that were anaerobes)</th>
<th>Predominant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brook et al. 1998 (44)</td>
<td>432 specimens taken from burn sites in 180 children</td>
<td>580 (23)</td>
<td>S. epidermidis, S. aureus, alpha-hemolytic streptococci, Propionibacterium acnes, Peptostreptococcus spp., Bacteroides spp.</td>
</tr>
<tr>
<td>Hansson et al. 1995 (98)</td>
<td>Analysis of leg ulcers without clinical signs of infection in 58 patients</td>
<td>325 (22)</td>
<td>S. aureus, Enterococcus faecalis, Enterobacter cloacae, Peptostreptococcus magnus</td>
</tr>
<tr>
<td>Brook et al. 1990 (42)</td>
<td>Analysis of 584 wounds from many sites</td>
<td>1,470 (62)</td>
<td>Bacteroides spp., Peptostreptococcus spp., S. aureus, Clostridium spp., Fusobacterium spp.</td>
</tr>
<tr>
<td>Brook 1987 (33)</td>
<td>Aspirates from human and animal bite wounds in 39 children</td>
<td>59 from animal bites (37); 97 from human bites (55)</td>
<td>S. aureus, Peptostreptococcus spp., Bacteroides spp. (in both wound groups)</td>
</tr>
<tr>
<td>Brook et al. 1991 (37)</td>
<td>Analysis of decubitus ulcers in 58 children</td>
<td>132 (40)</td>
<td>S. aureus, Peptostreptococcus spp., B. fragilis group, P. aeruginosa</td>
</tr>
<tr>
<td>Hansson et al. 1995 (98)</td>
<td>Analysis of leg ulcers without clinical signs of infection in 58 patients</td>
<td>325 (22)</td>
<td>S. aureus, Enterococcus faecalis, Enterobacter cloacae, Peptostreptococcus magnus</td>
</tr>
<tr>
<td>Brook et al. 1998 (44)</td>
<td>43 swab specimens from chronic leg ulcers in 41 patients</td>
<td>97 (34)</td>
<td>S. aureus, Peptostreptococcus spp., B. fragilis group</td>
</tr>
<tr>
<td>Bowler et al. 1999 (28)</td>
<td>Swab specimens from 30 noninfected leg ulcers without clinical signs of infection</td>
<td>110 (36)</td>
<td>S. aureus, coliforms, coagulase-negative staphylococci, fecal streptococci, Peptostreptococcus spp.</td>
</tr>
</tbody>
</table>
an infected state is likely to involve a multitude of microbial and host factors, including the type, site, size, and depth of the wound, the extent of nonviable exogenous contamination, the level of blood perfusion to the wound, the general health and immune status of the host, the microbial load, and the combined level of virulence expressed by the types of microorganisms involved. Most acute and chronic wound infections involve mixed populations of both aerobic and anaerobic microorganisms, and this is demonstrated in Table 3, which collates some of the published literature regarding the microbiology of a variety of infected wound types.

Surgical wound infections. The risk of infection is generally based on the susceptibility of a surgical wound to microbial contamination (196). Clean surgery carries a 1 to 5% risk of postoperative wound infection, and in dirty procedures that are significantly more susceptible to endogenous contamination, a 27% risk of infection has been estimated (174). The Guideline for Prevention of Surgical Site Infection, 1999 issued by the Centers for Disease Control and Prevention classified surgical wound infections as being either incisional (involving skin, subcutaneous tissue, or deeper fascia and muscle tissue) or organ/space, involving any internal organs or anatomical spaces (151). Examples of the latter include surgery associated with the large intestine and the head and neck, where extensive

<table>
<thead>
<tr>
<th>Aerobic and facultative microorganisms</th>
<th>Type of wound</th>
<th>Anaerobic bacteria</th>
<th>Type of wound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>A, C</td>
<td>Peptostreptococcus asaccharolyticus</td>
<td>A, C</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>C</td>
<td>Peptostreptococcus anaerobius</td>
<td>A, C</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>A, C</td>
<td>Peptostreptococcus magnus</td>
<td>A, C</td>
</tr>
<tr>
<td>Beta-hemolytic streptococcus (group C)</td>
<td>A</td>
<td>Peptostreptococcus prevotii</td>
<td>A, C</td>
</tr>
<tr>
<td>Beta-hemolytic streptococcus (group G)</td>
<td>C</td>
<td>Peptostreptococcus indolicus</td>
<td>C</td>
</tr>
<tr>
<td>Streptococcus spp. (facial)</td>
<td>A, C</td>
<td>Peptostreptococcus sp.</td>
<td>A, C</td>
</tr>
<tr>
<td>Streptococcus spp. (viridans)</td>
<td>A, C</td>
<td>Streptococcus intermedius</td>
<td>C</td>
</tr>
<tr>
<td>Corynebacterium xerosis</td>
<td>C</td>
<td>Clostridium perfringens</td>
<td>A, C</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>A, C</td>
<td>Clostridium clostridiiforme</td>
<td>A, C</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>A</td>
<td>Clostridium cadaveris</td>
<td>A, C</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>A, C</td>
<td>Clostridium baratti</td>
<td>C</td>
</tr>
<tr>
<td>Escherichia hermanii</td>
<td>A</td>
<td>Clostridium septicum</td>
<td>A</td>
</tr>
<tr>
<td>Serratia liquefaciens</td>
<td>C</td>
<td>Clostridium histolyticum</td>
<td>A, C</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>A, C</td>
<td>Clostridium tertium</td>
<td>A</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>A, C</td>
<td>Clostridium ramosum</td>
<td>C</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>A, C</td>
<td>Clostridium sphenogenes</td>
<td>A, C</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>C</td>
<td>Clostridium difficile</td>
<td>C</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>C</td>
<td>Clostridium bifermantans</td>
<td>A</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>A, C</td>
<td>Clostridium limosum</td>
<td>A</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>C</td>
<td>Eubacterium limosum</td>
<td>C</td>
</tr>
<tr>
<td>Providencia stuartii</td>
<td>A</td>
<td>Propionibacterium acne</td>
<td>A, C</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>C</td>
<td>Bacteroides fragilis</td>
<td>A, C</td>
</tr>
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<td>Acinetobacter calcoaceticus</td>
<td>A, C</td>
<td>Bacteroides ureolyticus</td>
<td>A, C</td>
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<td>Pseudomonas aeruginosa</td>
<td>A, C</td>
<td>Bacteroides ovatus</td>
<td>A</td>
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<td>Stenotrophomonas maltophilia</td>
<td>A</td>
<td>Bacteroides uniformis</td>
<td>A, C</td>
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<td>Sphingobacterium multivorax</td>
<td>C</td>
<td>Bacteroides stercoris</td>
<td>C</td>
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<td>Candida parapsilosis</td>
<td>A</td>
<td>Bacteroides capillosus</td>
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<td>Candida krusei</td>
<td>A</td>
<td>Bacteroides thetaiotaomicron</td>
<td>C</td>
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<td></td>
<td></td>
<td>Bacteroides cacaee</td>
<td>C</td>
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<tr>
<td></td>
<td></td>
<td>Prevotella oralis</td>
<td>A, C</td>
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<td></td>
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<td>Prevotella oris</td>
<td>A, C</td>
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<td></td>
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<td>Prevotella disiens</td>
<td>A</td>
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<td></td>
<td></td>
<td>Prevotella bivia</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prevotella buccae</td>
<td>C</td>
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<tr>
<td></td>
<td></td>
<td>Prevotella sp.</td>
<td>A</td>
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<td></td>
<td></td>
<td>Prevotella corporis</td>
<td>A, C</td>
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<tr>
<td></td>
<td></td>
<td>Prevotella intermedia</td>
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<td></td>
<td></td>
<td>Prevotella melaninogenica</td>
<td>C</td>
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<tr>
<td></td>
<td></td>
<td>Porphyromonas asaccharolytica</td>
<td>A, C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gram-negative pigmented bacillus</td>
<td>A, C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fusobacterium necrophorum</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Veillonella spp.</td>
<td>A</td>
</tr>
</tbody>
</table>

*Adapted from reference 27 with permission of the publisher.

Acute wounds (A) included primarily cutaneous abscesses and postsurgical wounds; chronic wounds (C) included primarily leg ulcers, foot ulcers and pressure sores. A total of 367 isolates were cultured from the 106 wounds (61 acute wounds and 45 chronic wounds).
endogenous wound contamination, and hence a higher probability of wound infection, is likely.

With the exception of clean operative procedures, surgical wound infections are recognized as having a polymicrobial etiology, involving both aerobic and anaerobic microorganisms (Table 3), and intra-abdominal infections normally reflect the microflora of the resected organ (34, 175). Reported wound infection rates following orthopedic surgery are relatively low (2 to 6.8%) (20, 61, 223), and similar studies, involving a large number of generalized postoperative wound types, have reported overall infection rates of 3.4% in 5,129 operations (1), 4.7% in 62,939 operations (57), and 9.4% in 1,770 operations (238). In the last two studies, the infection rates ranged from 1.5% (57) and 5.9% (238) following clean surgery to 40% (57) and 52.9% (238) following contaminated surgery. Despite the frequency and prevalence of endogenous anaerobes in surgical wound infections (Table 3), the Centers for Disease Control and Prevention guideline for the prevention of surgical site infection has recognized S. aureus, coagulase-negative staphylococci, Enterococcus spp., Escherichia coli, P. aeruginosa, and Enterobacter spp. as the most frequently isolated pathogens (151). Unfortunately, this view has been based on only two published reports that provided no indication of the inclusion of anaerobic bacteriology.

### Table 3. Studies involving a detailed microbiological analysis of the aerobic and anaerobic microbiology of infected wounds

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Study description and no. of wounds</th>
<th>No. of microbial isolates (% that were anaerobes)</th>
<th>Predominant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanderson et al. 1979 (212)</td>
<td>Anaerobes in 65 purulent postappendectomy wounds (swab samples)</td>
<td>179 (54)</td>
<td>E. coli, Bacteroides spp., Peptostreptococcus spp.</td>
</tr>
<tr>
<td>Brook 1989 (34)</td>
<td>89 specimens from postsurgical abdominal wound infections</td>
<td>235 (55)</td>
<td>E. coli, Bacteroides spp., Peptostreptococcus spp., Clostridium spp.</td>
</tr>
<tr>
<td>Brook 1989 (36)</td>
<td>Specimens from 74 patients with postthoracotomy sternal wound infections</td>
<td>87 (22)</td>
<td>S. epidermidis, S. aureus, coliforms, Peptostreptococcus spp.</td>
</tr>
<tr>
<td>Brook 1989 (35)</td>
<td>Analysis of pus from a Bartholin’s abscess in 28 patients</td>
<td>67 (64)</td>
<td>Bacteroides spp., Peptostreptococcus spp., E. coli</td>
</tr>
<tr>
<td>Brook et al. 1990 (42)</td>
<td>Analysis of 676 cutaneous abscesses</td>
<td>1,702 (65)</td>
<td>Bacteroides spp., Peptostreptococcus spp., S. aureus, Clostridium spp., Fusobacterium spp.</td>
</tr>
<tr>
<td>Johnson et al. 1995 (116)</td>
<td>Swab samples from 43 diabetic foot ulcers (46 infected sites)</td>
<td>285 (36)</td>
<td>Peptostreptococcus spp., Prevotella spp., Bacteroides spp. (emphasis on anaerobes)</td>
</tr>
<tr>
<td>Brook 1995 (38)</td>
<td>Analysis of pus from gastrostomy site wound infections in 22 children</td>
<td>102 (44)</td>
<td>E. coli, Peptostreptococcus spp., Enterococcus spp., Bacteroides spp., S. aureus</td>
</tr>
<tr>
<td>Di Rosa et al. 1996 (64)</td>
<td>Role of anaerobes in 300 postoperative wound infections</td>
<td>639 (23)</td>
<td>Clostridium spp., Bacteroides spp., Peptostreptococcus spp. (emphasis on anaerobes)</td>
</tr>
<tr>
<td>Mousa 1997 (166)</td>
<td>Swab samples of burn wounds from 127 patients</td>
<td>377 (31)</td>
<td>P. aeruginosa, S. aureus, Bacteroides spp., Peptostreptococcus spp., Klebsiella spp.</td>
</tr>
<tr>
<td>Brook et al. 1997 (43)</td>
<td>Analysis of perirectal abscesses in 44 patients</td>
<td>456 (72)</td>
<td>B. fragilis group, Peptostreptococcus spp., Prevotella spp., S. aureus, Streptococcus spp.</td>
</tr>
<tr>
<td>Brook et al. 1998 (45)</td>
<td>Analysis of 368 specimens from 340 trauma patients with wound infection</td>
<td>711 (63)</td>
<td>B. fragilis group, Peptostreptococcus spp., Clostridium spp., S. aureus, Prevotella spp.</td>
</tr>
<tr>
<td>Brook 1998 (40)</td>
<td>Analysis of 175 specimens from 166 children with infected traumatic wounds</td>
<td>521 (70)</td>
<td>Peptostreptococcus spp., Prevotella spp., Fusobacterium spp., S. aureus, B. fragilis group</td>
</tr>
<tr>
<td>Pathare et al. 1998 (185)</td>
<td>Pus or tissue specimens from 252 diabetic foot infections</td>
<td>775 isolates (29)</td>
<td>Staphylococcus spp., Streptococcus spp., Peptostreptococcus spp.</td>
</tr>
<tr>
<td>Bowler et al. 1999 (28)</td>
<td>Swab samples of 44 infected leg ulcers (based on clinical signs)</td>
<td>220 isolates (49)</td>
<td>Peptostreptococcus spp., coliforms, coagulase-negative staphylococci, pigmented and nonpigmented gram-negative bacteria (anaerobes), fecal streptococci</td>
</tr>
</tbody>
</table>

*IVDU, intravenous drug user.*
in the associated studies, and hence the data may have been biased in favor of aerobic and facultative microorganisms (50, 154). In contrast, Rotstein et al. (207) emphasized the polymicrobial nature of almost all surgical infections and commented that the critical importance of aerobic-anaerobic mixtures in these infections had received relatively little attention.

Minimizing the incidence of postoperative wound infection relies on asepsis and antisepsis and preservation of the local host defenses (109). Asepsis involves the utilization of effective infection control procedures (e.g., air filtration, skin barrier garments, disinfection) to minimize exogenous microbial contamination during surgery. Antisepsis involves the use of skin antiseptics on the operative site and also, in the case of dirty surgical procedures, administration of prophylactic antibiotics at a time point just prior to surgery that will ensure adequate tissue levels of antibiotic during surgery. As part of the surgical procedure, endogenous and exogenous microbial contamination must be minimized by ensuring good aseptic, skilled surgical techniques and minimizing the duration of surgery, while also optimizing the local wound conditions (97). This primarily involves removing any devitalized tissue to re-establish blood flow to the wound area (2), thereby maintaining adequate perfusion to enable the delivery of immune cells, oxygen, and nutrients and reducing the microbial load.

Acute soft tissue infections. Acute soft tissue infections include cutaneous abscesses, traumatic wounds, and necrotizing infection. Microbiological investigations have shown that *S. aureus* is the single causative bacterium in approximately 25 to 30% of cutaneous abscesses (41, 158), and the same organism has also been recognized as being the most frequent isolate in superficial infections seen in hospital Accident and Emergency Departments (180). However, other studies have demonstrated that approximately 30 to 50% of cutaneous abscesses (41, 158, 226), 50% of traumatic injuries of varied etiology (40, 45), and 47% of necrotizing soft tissue infections (69) have a polymicrobial aerobic-anaerobic microflora.

Necrotizing soft tissue infections occur with different degrees of severity and speed of progression; they involve the skin (e.g., clostridial and nonclostridial anaerobic cellulitis), subcutaneous tissue to the muscle fascia (necrotizing fasciitis), and muscle tissue (streptococcal myositis and clostridial myonecrosis). *S. aureus* has been described as being the single pathogen in two patients with rapidly progressing necrotizing fasciitis of the lower extremity (199), and in a study of necrotizing fasciitis in eight children, Brook (40) reported the presence of pure *Streptococcus pyogenes* in two patients and a mixed predominance of *Peptostreptococcus* spp., *S. pyogenes*, *B. fragilis*, *C. perfringens*, *E. coli*, and *Prevotella* spp. in the others. Potentiation of infection by microbial synergistic partnerships between aerobes, such as *S. aureus* and *S. pyogenes*, and nonsporing anaerobes has been recognized in various types of nonclostridial cellulitis and necrotizing fasciitis (40, 122).

The classification of necrotizing soft tissue infections is complex and is based on (i) the assumed causative microorganism(s), (ii) the initial clinical findings, (iii) the type and level of tissue involved, (iv) the rate of progression, and (v) the type of therapy required (12). However, Elliot et al. (69) argued that the classification of such infections serves little clinical purpose because the prognosis and treatment are the same and, consequently, differentiation is required only between pure clostridial myonecrosis (since it involves muscle invasion and is associated with a higher mortality rate) and other non-muscle-associated soft tissue infections.

The management of necrotizing soft tissue infections requires early diagnosis, aggressive and, if necessary, repeated debridement, and antibiotic therapy (12, 69, 191). Hyperbaric oxygen (HBO) therapy is also believed by many to facilitate wound healing (12, 69), although its use is controversial (53, 69, 165). HBO therapy is discussed in more detail in “Control of microbial populations in wounds” (below). The pilonidal sinus is another type of acute wound that is susceptible to fecal contamination and infection; Bascom (18) reported that anaerobes were the true and invasive causative microorganisms. Surgical reshaping of the wound to provide improved oxygenation is often required (18).

Bite wound infections. The reported infection rate for human bite wounds ranges from 10 to 50% depending on the severity and location of the bite, and up to 20% of dog bites and 30 to 50% of cat bites become infected (92). Brook (33) reported that 74% of 39 human and animal bite wounds contained a polymicrobial aerobic-anaerobic microflora, with *S. aureus*, *Peptostreptococcus* spp., and *Bacteroides* spp. being the predominant isolates in both wound types.

Due to the complex nature of the oral microflora in humans and animals, the majority of bite wounds harbor potential pathogens, many of which are anaerobes. As well as the common anaerobes in both human and animal bite wounds, such as *Bacteroides*, *Prevotella*, *Porphyromonas*, and *Peptostreptococcus* spp. (83), less common potential pathogens such as *Pasteurella multocida*, *Capnocytophaga canimorsus*, *Bartonella henselae*, and *Eikenella corrodens* may also be involved (75).

Management of bite wounds is likely to involve high-pressure irrigation to reduce the microbial load, debridement of devitalized tissue, and antibiotic treatment for high-risk wounds such as punctures (75, 82).

Burn wound infections. Infection is a major complication in burn wounds, and it is estimated that up to 75% of deaths following burn injury are related to infection (200, 239). Although exposed burned tissue is susceptible to contamination by microorganisms from the gastrointestinal and upper respiratory tracts (239), many studies have reported the prevalence of aerobes such as *P. aeruginosa*, *S. aureus*, *E. coli*, *Klebsiella* spp., *Enterococcus* spp., and *Candida* spp. (13, 132, 154, 200, 239). In other studies involving more stringent microbiological techniques, anaerobic bacteria have been shown to represent between 11 and 31% of the total number of microbial isolates from burn wounds (46, 166, 197). While the aerobes isolated in the latter studies were similar to those reported previously, predominant anaerobic burn wound isolates were *Peptostreptococcus* spp., *Bacteroides* spp., and *Propionibacterium acnes* (46, 166). Mousa (166) also reported the presence of *Bacteroides* spp. in the wounds of 82% of patients who developed septic shock and concluded that such microorganisms may play a significant role in burn wound sepsis.

Management of infection in burn wounds involves the use of topical and systemic antimicrobial agents, aggressive debridement of dead tissue, maximization of the immune response, and provision of adequate nutrition (147).

Diabetic foot ulcer infections. Plantar ulcers associated with diabetes mellitus are susceptible to infection due to the high
incidence of mixed wound microflora (62) and the inability of the PMNs to deal with invading microorganisms effectively (8). However, with optimal treatment involving debridement of devitalized tissue, the use of appropriate dressings, and pressure relief, wound infection can be minimized. Boulton et al. (24) reported an infection rate of 2.5% in diabetic wounds treated with a moisture-retentive hydrocolloid dressing, compared with a 6% infection rate under a traditional gauze dressing. Laing (127) also observed a similar infection rate (2%) in diabetic foot ulcers treated with a hydrocolloid dressing, despite the number of species increasing during treatment.

As in most wound types, S. aureus is a prevalent isolate in diabetic foot ulcers, together with other aerobes including S. epidermidis, Streptococcus spp., P. aeruginosa, Enterococcus spp., and coliform bacteria (8, 121, 185). With good microbiological techniques, anaerobes have been isolated from up to 95% of diabetic wounds (78), the predominant isolates being Peptostreptococcus, Bacteroides, and Prevotella spp. (62, 78, 116, 121, 243). In view of the polymicrobial nature of diabetic foot ulcers, Karchmer and Gibbons (121) questioned the need for precisely defining the causative microorganism(s) and suggested that the treatment of infection could be based on a better understanding of the general microbiology of these wounds. Armstrong et al. (8) supported this view by commenting that repetitive cultures following initial culture and subsequent treatment do not confirm or rule out the presence of infection and, consequently, that the foot infection must be diagnosed primarily on clinical grounds.

**Leg and decubitus (pressure) ulcer infections.** The microflora of chronic venous leg ulcers is frequently polymicrobial, and anaerobes have been reported to constitute approximately 30% of the total number of isolates in noninfected wounds (28, 42, 98). Although S. aureus is the most prevalent potential pathogen in leg ulcers (28, 42, 98), Bowler and Davies (28) reported a significantly greater frequency of anaerobes (particularly Peptostreptococcus spp. and pigmented and nonpigmenting gram-negative bacilli) in clinically infected leg ulcers than in noninfected leg ulcers (49 versus 36% of the total numbers of microbial isolates, respectively). The same investigators also suggested that aerobic-anaerobic synergistic interactions are likely to be more important than specific microorganisms in the pathogenesis of leg ulcer infection; this mechanism is not widely recognized in the management of surgical (207) and chronic wound infections.

Decubitus ulcers develop as a consequence of continued skin pressure over bony prominences; they lead to skin erosion, local tissue ischemia, and necrosis, and those in the sacral region are particularly susceptible to fecal contamination. Approximately 25% of decubitus ulcers have underlying osteomyelitis (47), and bacteraemia is also common (128). One of the few reported acknowledgments of the role of polymicrobial synergy in chronic wound infection was made by Kingston and Seal (122), who commented that since the bacteriology of decubitus ulcers is similar to that of some of the acute necrotizing soft tissue infections, the anaerobic and aerobic bacteria involved are likely to contribute to the deterioration of a lesion. The opportunity for microbial synergy in many decubitus ulcers was demonstrated by Brook (37), who reported mixed aerobic and anaerobic microflora in 41% of 58 ulcers in children; S. aureus, Peptostreptococcus spp., Bacteroides spp. (formerly members of the B. fragilis group), and P. aeruginosa were the predominant isolates. Although localized wound care is normally sufficient to facilitate primary healing in decubitus ulcers, occasional necrosis of adjacent soft tissues leading to necrotizing fasciitis has been reported (120).

Initial management of infected decubitus ulcers normally involves aggressive surgical debridement and broad-spectrum antimicrobial coverage (128). Although leg ulcers frequently display a dense microflora, the occurrence of infection is relatively low (<5%) (113); however, again, treatment normally includes topical and systemic antimicrobial agents and surgical debridement as necessary.

**Significance of Microorganisms in Wounds**

**Quantitative microbiology: significance of microbial numbers.** The clinical significance of the microbial load in delaying wound healing was described in 1964 by Bendy et al. (19), who reported that healing in decubitus ulcers progressed only when the bacterial load was $<10^4$ CFU/ml of wound fluid. In this study, quantification was determined by using superficial wound swab samples. Similar observations, placing emphasis on counts in tissue biopsy specimens, were reported in studies involving skin graft survival in experimental wounds inoculated with various types of bacteria (126), pressure ulcer healing (203), and delayed closure of surgical wounds (204, 205). Aligned with this early work and in recognition of the fact that quantitative culture of tissue biopsy specimens was demanding on counts in tissue biopsy specimens, were reported in studies involving skin graft survival in experimental wounds inoculated with various types of bacteria (126), pressure ulcer healing (203), and delayed closure of surgical wounds (204, 205). Aligned with this early work and in recognition of the fact that quantitative culture of tissue biopsy specimens was demanding on counts in tissue biopsy specimens, were reported in studies involving skin graft survival in experimental wounds inoculated with various types of bacteria (126), pressure ulcer healing (203), and delayed closure of surgical wounds (204, 205). Aligned with this early work and in recognition of the fact that quantitative culture of tissue biopsy specimens was demanding on counts in tissue biopsy specimens, were reported in studies involving skin graft survival in experimental wounds inoculated with various types of bacteria (126), pressure ulcer healing (203), and delayed closure of surgical wounds (204, 205). Aligned with this early work and in recognition of the fact that quantitative culture of tissue biopsy specimens was demanding on counts in tissue biopsy specimens, were reported in studies involving skin graft survival in experimental wounds inoculated with various types of bacteria (126), pressure ulcer healing (203), and delayed closure of surgical wounds (204, 205). Aligned with this early work and in recognition of the fact that quantitative culture of tissue biopsy specimens was demanding on counts in tissue biopsy specimens, were reported in studies involving skin graft survival in experimental wounds inoculated with various types of bacteria (126), pressure ulcer healing (203), and delayed closure of surgical wounds (204, 205).
sample procedure (181). However, the technique requires specialized equipment, and the work of Pallua et al. (181) excluded investigation for anaerobic bacteria.

The quantitative studies described in the literature can be broadly differentiated on the basis of those that used surface sampling techniques and those that used deep-tissue biopsy techniques. Robson and Heggars argue strongly that deep-tissue biopsies are essential to quantify and determine the causative (invasive) microorganisms in wound infection. However, noninvasive techniques have also been shown to be beneficial in determining the numbers of microorganisms that are likely to interfere with wound healing or cause infection. The value of superficial cultures in wound assessment has been questioned, and Robson (201) stated that purulent wound fluid may fail to yield microorganism growth whereas biopsied tissue may yield significant numbers of bacteria if such cultures were performed routinely. However, it should be borne in mind that with the exception of deep surgical wounds involving internal organs, wound contamination by members of the endogenous microflora will occur from sources external to the wound. Thus, superficial tissue is likely to harbor a diversity of aerobic and anaerobic microorganisms, one or more of which may invade deeper tissue, and it is highly unlikely that superficial tissue will be “sterile” while deeper tissue is “infected.” Most wounds are colonized with microorganisms, and a failure to isolate them is more likely to be a consequence of poor microbiological technique, particularly in the case of anaerobes.

Thus, quantitative analysis of superficial tissue may also have a role to play in predicting the risk of wound infection (150, 196), and several studies have demonstrated a correlation between surface cultures and tissue biopsy cultures. Levine et al. (139) demonstrated a close correlation between quantitative swab and tissue biopsy specimen counts in open burn wounds, and Armstrong et al. (8) observed no difference in the isolation rate of microorganisms from deep tissue and superficial curettage in 112 diabetic foot ulcer infections. In an experimental rat model, Bornside and Bornside (23) demonstrated that a tissue count of $10^5$ CFU/g was equivalent to a $10^5$ CFU/ml count obtained from a moist swab and concluded that the moist swab provides a direct and simple method for ascertaining infection. Similarly, Thomson (235) demonstrated a correlation between a semiquantitative surface swab count (1+ to 4+) and a fully quantitative biopsy specimen count in burn wounds; 1+ growth from a swab correlated with a tissue count of $10^2$ to $10^3$ CFU/g, and 4+ correlated with a tissue count of approximately $10^7$ CFU/g. Lawrence (132) also commented that quantitative bacteriology for burn wounds provides little information beyond that obtained from a surface swab, and swabs that yield more than 30 CFU reliably indicate a tissue count of >$10^5$ CFU/g. Also, Vindenes and Bjerknes (239) concluded that invasive microorganisms in burn wounds mirror those found in swab cultures of superficial tissue. A comparison of studies reported by Bowler and Davies (28) and Sapico et al. (214) also demonstrates a close correlation between the isolation of microorganisms in superficial and deep tissue. The microbiology of infected leg ulcers investigated by a surface-swabbing procedure demonstrated mean isolation rates of 2.6 aerobes and 2.5 anaerobes per wound (28), which compares with deep-tissue biopsy specimen mean isolation rates of 2.3 aerobes and 2.4 anaerobes per infected diabetic ulcer (214).

In another study, no relationship was shown between the density of microorganisms in deep tissue and the eventual outcome of myocutaneous rotation flap surgery in pressure sores (213), and Hansson (98) concluded that the number of microorganisms or number of species present in 58 non-clinically infected leg ulcers had no effect on wound healing.

Another factor that must be taken into consideration when relying on quantitative cultures to determine the likelihood of wound healing is the distribution of microorganisms within wound tissue. In an evaluation of the microbial distribution in tissue taken from seven decubitus ulcers, Schneider et al. (215) concluded that on the basis of the variability of counts obtained from a single tissue biopsy specimen, a bacterial count at a single location has limited value in determining the optimal time required to perform a wound closure. Similarly, Sapico et al. (213) reported only a 63% concordance between quantitative results from biopsy specimens taken from the periphery and center of 25 pressure sores. Quantitative microbiology clearly has a valid role to play in wound management since numerous studies have shown that it can reliably predict the risk of infection and the probability of wound healing. However, the need to quantify bacteria by performing tissue biopsy, which is invasive, potentially traumatic to the patient, and nonroutine, requires expert manipulation, and creates an increased workload for the microbiology laboratory, is debatable. Noninvasive procedures such as the velvet pad technique and the quantitative swab are also able to provide similar information, and, whether the investigation is quantitative or semiquantitative, a correlation with deep tissue biopsy results has been demonstrated.

**Qualitative microbiology: significance of specific microorganisms.** The effect of specific types of microorganisms on wound healing has been widely published, and although the majority of wounds are polymicrobial, involving both aerobes and anaerobes, aerobic pathogens such as *S. aureus*, *P. aeruginosa*, and beta-hemolytic streptococci have been most frequently cited as the cause of delayed wound healing and infection (39, 58, 59, 81, 146, 216, 217, 238). As a specific example, *S. aureus* is considered to be the most problematic bacterium in traumatic, surgical, and burn wound infections (96, 123, 154, 175, 180), primarily based on the knowledge that its incidence is high in these, and other, types of wound (25, 27, 180). However, although polymicrobial wounds are frequently colonized with *S. aureus*, a correlation between the presence of this particular pathogen and wound infection is lacking (25, 28, 98).

As long ago as 1918, the prestigious team of Almroth Wright, Alexander Fleming, and Leonard Colebrook (247) reported that a surgical wound could not be successfully closed if a hemolytic *Streptococcus pyogenes* strain was present. More recently, Robson and Heggars (204) singled out the beta-hemolytic streptococcus as being the only bacterium that is capable of causing infection at levels significantly lower than $10^5$ CFU/g of tissue. Similarly, at a consensus meeting of the European Tissue Repair Society and the European Wound Management Association in 1998, a general opinion was that the presence of beta-hemolytic (group A) streptococci or *P. aeruginosa* in a chronic wound was an indicator of the need for antimicrobial therapy (135). Although the presence of such microorganisms frequently raises concern among health care
practitioners, their identity as the etiological agent in wound infection or delayed healing can be confirmed only if they are present as a pure monomicrobial flora. In two studies involving the microbiology of cutaneous abscesses, *S. aureus* was present as a pure culture in 24 to 29% of the infections (41, 158). Elsewhere, *S. pyogenes* and *S. aureus* have been reported as being the sole pathogens in some cases of necrotizing fasciitis (199), and pure isolates of unusual pathogens have also been isolated from bite wound infections (67). Although other studies have identified specific microorganisms as being responsible for delayed wound healing or wound infection, clarity regarding their existence as a mono- or polymicrobial population is unclear (59, 179), and some studies used selective culture media to isolate specific microorganisms, which is likely to have biased the results (198, 216). Contrary to widespread published references to the involvement of specific microorganisms in wound infection, other investigators have demonstrated that the resident microflora has little effect on the outcome of wound healing (4, 70, 80, 95, 98, 213). Based on this collective evidence, the role of specific microorganisms in many types of infected wound is still uncertain.

It is not possible to differentiate between pathogenic (causative) and nonpathogenic species in polymicrobiologically infected wounds (97), and Armstrong et al. (8) commented that the presence of a pathogen in a mixed-culture infection does not necessarily establish that particular microorganism as the etiologic agent. Consequently, diagnosis of infection in polymicrobiologically infected wounds should be based primarily on clinical signs, such as heat, pain, erythema, edema, suppuration, and fever; microbiological results may be helpful but can often be misleading, especially with polymicrobioculturally infected wounds containing numerous potential pathogens. However, when clinical signs of infection are less evident, as is often the case in diabetic foot ulcers, greater emphasis may have to be placed on microbiological results. In a study of the bacteriology of chronic leg ulcers in 52 patients, Trengove (237) reported that no single microorganism or group of microorganisms was more detrimental to wound healing than any other (inclusive of *S. aureus*, *P. aeruginosa*, beta-hemolytic streptococci, anaerobes, and coliform bacteria). However, a significantly lower probability of healing was observed if four or more bacterial groups were present in any ulcer (237), and this indicates that microbial interactions may have induced an enhanced pathogenic effect. Similarly, Bowler and Davies (28) reported a greater diversity of microorganisms in infected leg ulcers than in noninfected leg ulcers (means of 5.1 and 3.6 isolates per wound, respectively). These observations support an earlier view of Kingston and Seal (122), who argued that all species associated with a microbial disease should be considered potentially synergistic, rather than a single species being causative, as is commonly perceived.

Aerobic pathogens such as *S. aureus*, *P. aeruginosa*, and beta-hemolytic streptococci are recognized for their ability to produce potentially destructive virulence factors (101), and the clinical effects associated with clostridial exotoxins (65) are also widely acknowledged. However, many of the nonsporing gram-negative anaerobes that are often abundant (yet often “invisible”) in wounds also possess a wide variety of virulence factors that may impair wound healing. *Bacteroides*, *Prevotella*, and *Porphyromonas* species are capable of expressing adhesion factors (e.g., capsular polysaccharide, fimbriae, and hemagglutinin), tissue-damaging exoenzymes (e.g., proteases, collagenase, hyaluronidase, fibrinolysin, gelatinase, elastase, and chondroitin sulfatase), and antiphagocytic factors (e.g., capsule, short-chain fatty acids, and immunoglobulin A [IgA], IgM, and IgG proteases) (65), all of which may contribute to the impairment of wound-healing processes. In association with aerobic microorganisms, the pathogenic potential of some gram-negative anaerobes is often increased, and, consequently, the combined effects of aerobes and anaerobes in wounds may produce a pathogenic effect that cannot be achieved by one type of microorganism alone.

Microbial synergy may increase the net pathogenic effect and hence the severity of infection in several ways: (i) oxygen consumption by aerobic bacteria induces tissue hypoxia and a lowering of the redox potential, which favors the growth of anaerobic bacteria; (ii) specific nutrients produced by one bacterium may encourage the growth of fastidious and potentially pathogenic cohabiting microorganisms; and (iii) some anaerobes are able to impair host immune cell function and thus provide a competitive advantage to themselves and other, cohabiting microorganisms.

Many investigators have recognized that some fastidious anaerobes require specific nutrients for growth and that this has been indirectly associated with enhanced virulence. *S. aureus* promotes the growth of a vitamin K-dependent strain of *Prevotella melaninogena* in vitro (155), and the pathogenicity of *P. melaninogena* in gingivitis has been related to the presence of vitamin K produced by a cohabiting bacterium (145). Similarly, Ingham et al. (114) observed that the antiphagocytic activity in *P. melaninogena* was expressed only in the presence of hemin and vitamin K provided by cohabiting microorganisms. In the same study, the incorporation of metronidazole in an in vitro phagocyte-polymicrobial model led to the elimination of anaerobes and subsequent increased uptake of aerobic bacteria by phagocytes. Consequently, the loss of antiphagocytic activity following metronidazole treatment facilitated the exclusion of aerobic bacteria also. These observations indicate that infection may be resolved by eliminating a critical component of a microbial population and hence disrupting aerobic-anaerobic interactions.

Hemin derived from hemoglobin is an essential growth factor for black-pigmented gram-negative anaerobes, and a correlation between virulence in *Porphyromonas gingivalis* and the degree of pigmentation of the bacterium on blood agar has been recognised (157). Black colonies grown in the presence of excess hemin were pathogenic for mice, but in the absence of hemin they were nonpathogenic. Similarly, Bowler (26) demonstrated the ability of *S. aureus* to enhance the growth and pigmentation of *Prevotella loescheii* and *P. gingivalis* in vitro (Fig. 1). The aerobe-anaerobe combinations were isolated from the same infected leg ulcers, and it is likely that the *S. aureus* induced growth and virulence in the pigmented anaerobe by providing an essential growth factor. Sterile fluid culture filtrates of the aerobe were not able to induce a similar effect. In the absence of *S. aureus*, the anaerobes either failed to grow or grew as pinpoint, nonpigmented colonies on an enriched blood agar medium. Other facultative bacteria such as *E. coli* and *Klebsiella pneumoniae* were shown to be capable of inducing a similar effect, but to a lesser extent than *S. aureus*. 
Elsewhere, K. pneumoniae was reported to be instrumental in enhancing virulence in P. melaninogena by providing succinate as an essential growth factor (138, 155). Succinate is an important short-chain fatty acid (SCFA) that is produced by both aerobic and anaerobic bacteria (e.g., E. coli, K. pneumoniae, Bacteroides spp., and Prevotella spp.); it accumulates in anaerobe-dominated infected sites (236) and inhibits the chemotaxis and phagocytosis of E. coli (208) and S. aureus (190). Mayrand and McBride (155) also demonstrated that succinate production by K. pneumoniae is enhanced in the presence of glucose. This observation may be indirectly related to the close correlation between blood glucose levels and wound infection rates in diabetic patients, whose probability of infection is five times greater than in nondiabetic patients (210). Neutrophil chemotaxis, phagocytosis, intracellular killing mechanisms, and serum opsonic activity are impaired in diabetic patients (9, 177) and oxidative and nonoxidative antimicrobial mechanisms in neutrophils are also impaired by SCFAs (178). Thus, it can be hypothesized that high levels of glucose in chronic wound fluid will stimulate the production of succinate in gram-negative bacteria, which will subsequently impair host cell function and render the host more susceptible to infection. Furthermore, since subcutaneous insulin infusion has been associated with improved wound healing, meticulous blood glucose control is considered to be essential in treating diabetic wounds (209). High levels of succinic acid (>30 mmol) have been measured in clinical abscesses (87), and neutrophil migration and activity have been impaired in vitro in the presence of >20 mmol of succinate. Succinate is also more active at acidic pH, and therefore the hypoxia and low pH associated with many chronic wounds will facilitate succinate activity and hence exacerbate impaired neutrophil function.

Thus, the quantitative and qualitative aspects of wound microbiology are critical determinants in the development of infection. Assuming that the qualitative microbiology remains constant, the probability of wound infection increases as the microbial load increases, up to a critical level where infection or a failure to heal is considered by some to be almost inevitable. In theory, the presence of S. aureus and a pigmented gram-negative anaerobe in a wound is likely to be more detrimental than the presence of a similar quantity of S. aureus alone. In the absence of facultative bacteria that can provide the necessary growth factor, a pigmented gram-negative anaerobe may be unable to proliferate and thus to express its full pathogenic potential. Therefore, the composition of the polymicrobial wound flora is likely to be more important than the presence of specific pathogens, since this will determine whether pathogenic interactions are likely to occur.

A third critical factor in wound healing and infection is the efficacy of the host immune response in dealing with wound microflora. Local environmental factors such as tissue necrosis, hypoxia, and ischemia impair immune cell activity in a wound, as do diabetes mellitus, chronic granulomatous disease, and other immune deficiencies. As discussed above, microorganisms can also participate in compromising the immune response, and in these situations, the patient is at greater risk of infection. Only by assessing the host and microbial factors collectively can the probability of wound infection be addressed.

**MICROBIOLOGICAL ANALYSIS OF WOUNDS**

In clinical practice, the presentation of a devitalized acute or chronic wound or a clinically infected wound is likely to prompt a practitioner to sample the wound for microbiological analysis. However, from a wound management perspective, there is little consensus regarding whether sampling is relevant, when and how a wound should be sampled, how a specimen should be transported to the laboratory, and what analyses should be requested. Confusion also exists in view of the fact that health care practitioners often consider a microbiological report to provide definitive information on whether a wound is infected (76, 173), and the provision of an antibiogram for a particular pathogen can often be misleading and prompt unnecessary treatment.

The aim of the following sections is to clarify current controversies in wound sampling and discuss the role of the health care practitioner and the microbiology laboratory in achieving clinically relevant outcomes.

**Wound-Sampling Methods**

In generalized terms, the quantitative and qualitative microbiology of wounds can be investigated by sampling either wound tissue or wound fluid.

**Wound tissue sampling.** The acquisition of deep tissue during biopsy following initial debridement and cleansing of superficial debris is recognized as being the most useful method for determining the microbial load and the presence of invasive pathogens (76, 173, 235). Tissue is obtained aseptically and is then weighed, homogenized, serially diluted, and cultured on selective and nonselective agar media under aerobic and anaerobic conditions to provide quantitative and qualitative information. Superficial, devitalized tissue removed by curettage, which is often used in the management of diabetic foot ulcers, may also be investigated for microbial content. Another technique involving dermabrasion has recently been described that enables the acquisition of deeper tissue without being as invasive as the biopsy method (181).

**Wound fluid sampling.** When a copious volume of wound fluid exists, sampling by needle aspiration can be employed. This technique may also be used to sample deeper pockets of...
fluid beneath superficial debris and is the most useful procedure for sampling purulent fluid from intact cutaneous abscesses. If the technique is performed with strict aseptic techniques, the target site can be located in a relatively nontraumatic way and without any significant exogenous contamination. In cavity wounds such as some pressure sores, irrigation with sterile saline and gentle massaging may be performed to provide fluid for aspiration.

Wound swabbing most frequently involves the use of a cotton-tipped swab to sample superficial wound fluid and tissue debris, and this enables a semiquantitative and qualitative analysis of the wound microflora. An alginate-tipped swab can also be used to perform a fully quantitative analysis, since the swab will dissolve and release all associated microorganisms when transferred to an appropriate diluent. Despite its widespread use, there is debate over the value of the swab sampling technique and the value of cleansing a wound before swabbing is performed.

A variety of other techniques, including the dry and pre-soaked velvet pad, filter paper disks, and cylinder scrubbing, have also been used to sample superficial wound fluid for microbiological analysis.

The plethora of sampling methods available to the practitioner creates a considerable problem in the microbiological management of wounds, since all are reputed to have benefits and there is no single, universally accepted method. Consequently, debate and controversy continue regarding the type of sample required to provide the most meaningful data, the superiority of one sampling procedure compared with another in terms of microbial recovery (116, 188, 211, 214), and the relevance of wound cleansing prior to sampling (98). The lack of consensus regarding the correct way to prepare and sample a wound is a point of concern within the wound care profession, and results generated by the microbiology laboratory may be misinterpreted and encourage inappropriate treatment.

Without question, tissue biopsy or excision is considered the most appropriate sampling method for identifying wound infection and the causative pathogens. From a quantitative perspective, this method is perhaps most beneficial in determining the optimal time point for skin grafting and surgical wound closure (202). From a qualitative perspective, excised, uncontaminated tissue from infected sites or exudate aspirated from a closed or open lesion is considered to be most appropriate for isolating causative microorganisms. However, the validity and value of a single biopsy specimen, particularly in chronic wounds, is debatable. Although variability in both tissue counts and microbial isolates obtained from different parts of chronic wounds has been observed (215), comparable counts were reported in wound fluid samples of known volume taken from central and peripheral parts of leg ulcers in another study (99).

Although the merits of acquiring deep tissue for microbiological analysis are widely acknowledged, the procedure is not routinely available for the majority of wound types (68) and its use is restricted primarily to acute and surgical situations where excision, biopsy, or aspiration is urgently required. Particularly in slow-healing or nonhealing chronic wounds that require frequent and long-term care, tissue biopsy is of minimal value since it requires expert surgical technique, is nonroutine, and is potentially traumatic to the patient. For these reasons, the use of more conventional and readily available sampling methods must be considered.

Aspirates of purulent fluid should always be used for analysis if possible, since the clinical presentation is indicative of a focus of infection and a specimen can be obtained without trauma to the patient and with minimal invasion. However, Johnson et al. (116) demonstrated superior isolation of anaerobic bacteria from infected diabetic foot ulcers by a swab technique than by a needle aspiration technique (83 and 41% isolation, respectively, of a total of 103 isolates from 43 wounds).

Although the value of acquiring superficial swab samples has been seriously questioned, the procedure is simple, inexpensive, noninvasive and convenient for the majority of wounds. Swab sampling has been challenged on the basis that the superficial microbiology does not reflect that of deeper tissue (88, 188) and that subsequent cultures do not correlate with the presence of pathogenic bacteria (47). Also, if a swab sample is taken inappropriately (i.e., prior to wound cleansing and removal of devitalized superficial debris), the resulting culture has been considered to reflect only surface contamination (211) and provide misleading or useless information (76). Papasin and Kragel (183) suggested that if vital tissue could not be sampled, a specimen should not be collected at all and the patient should be treated empirically. A misconception regarding superficial sampling is that surface swabs will isolate only a small number of anaerobes and that deeper specimens are required to demonstrate the true microflora (127). However, since the majority of wounds are contaminated with endogenous microorganisms from the external environment, any microorganisms present in deeper tissue are also likely to be present in the superficial debris. Consequently, it is most likely that superficial wound fluid and tissue debris display a full spectrum of the wound aerobic and anaerobic microflora, some of which may be involved in pathogenesis and some of which may not be. Also, since many endogenous anaerobes can withstand prolonged periods of exposure to air (17, 26, 99, 142), they will survive in superficial tissue and multiply in devitalized and hypoxic or anoxic tissue. For this reason, and because anaerobes constitute a significant proportion of the microflora in both acute and chronic wounds, investigation for the presence of anaerobes is as important as (although more difficult than) investigation for aerobes. Studies by Bowler and Davies (27) have demonstrated the efficacy of the swab sample in isolating anaerobes from a variety of acute and chronic wounds.

Thus, there are arguments to support the use of the swab sample as a useful method for routinely assessing the microbiology of appropriate wounds. It is the opinion of the authors that for routine management, only wounds that are clinically infected or those that have no clinical signs of infection but are deteriorating (e.g., diabetic foot ulcers) or have a long history of failure to heal (primarily chronic wounds) should be sampled for microbiological analysis. In these situations, the surface swab sample can provide useful data regarding the presence of potential pathogens, the diversity of microorganisms involved, and, consequently, an indication of the probability of microbial synergy. However, indiscriminate swabbing of wounds that do not require sampling causes an unnecessary drain on labor and financial resources, and consequently selective application for wounds that are likely to benefit is a
prerequisite. A swab sample can also provide a semiquantitative estimation of the microbial load (e.g., light growth to heavy growth, or >10^5 CFU/ml), which is considerably easier to perform than a fully quantitative analysis. A correlation between semiquantitative swab data and quantitative biopsy data has previously been demonstrated (8, 23, 132, 139, 235, 239).

Although wound cleansing is considered necessary to avoid the pointless exercise of sampling superficial devitalized tissue (47, 88, 188, 211), Hansson et al. (98) observed no difference in the qualitative and quantitative microbiology of leg ulcers, whether or not they were cleansed prior to sampling with absorbent disks. Additionally, if vital tissue at the wound base cannot be obtained, swab sampling of an uncleansed wound surface will comprehensively demonstrate the aerobic and anaerobic microflora, any component(s) of which may be involved in wound infection. However, notification that a specimen had been obtained from an uncleansed wound by a swab procedure would be beneficial to the microbiology laboratory, since the diversity of microflora in such a sample is likely to render the isolation and identification procedures more problematic and complex. The subsequent reporting of a “mixed culture” is often interpreted as being associated with a poorly sampled or dirty wound with no pathogens, when a more detailed explanation of the microorganisms involved may provide clues to potential polymicrobial interactions. This aspect is discussed in more detail in “Reporting of microbiological results” (below).

An important factor to consider when sampling a wound for microorganisms is the administration and route of antimicrobial therapy. If a patient is receiving treatment, microbial isolation from swab samples is likely to be significantly influenced by topical antimicrobial agents (particularly in chronic, ischemic wounds) whereas the microflora of deep tissue is more likely to be influenced by systemic antibiotic therapy. In summary, superficial swabbing can be justified as a simple procedure for assessing the microflora of wounds that are clinically infected or failing to heal. Semiquantitative analysis will provide a good indication of the microbial load, and the qualitative analysis, although complex, will provide an indication of the diversity of microorganisms and potential for microbial synergy. A request for both aerobic and anaerobic microbiological investigation is an important aspect of obtaining the information required to guide appropriate antibiotic therapy, although a broad categorization for anaerobic bacteria (e.g., pigmented anaerobes, anaerobic streptococci) is likely to be adequate in most cases.

Specimen Transport

Following the acquisition of wound fluid or tissue for microbiological analysis, prompt delivery of the specimen to the laboratory is considered to be of utmost importance (32), particularly if anaerobic bacteria are being investigated. Aspirates of purulent fluid and tissue samples are considered to be preferable to swabs (32, 119) because they will maintain the conditions required to sustain microbial viability (i.e., a moist and reduced environment) if processed promptly. However, prereduced commercially available transport media offer advantages if specimen culture is delayed beyond 1 to 2 h after isolation. Since swab samples are susceptible to desiccation and oxygen exposure, a prereduced, nonnutritive transport medium is essential to maintain the viability of both aerobic and anaerobic microorganisms on cotton swabs.

Although anaerobic bacteria are commonly perceived to die in air, they have been shown to survive in mixed cultures over extended periods (24 h) (99, 142) and various anaerobes have been shown to survive in clinical specimens despite delayed processing (17). Also, pure cultures of various anaerobic bacteria (including sporing and nonsporing gram-positive and gram-negative bacteria) can survive in air for up to 72 h and can resume growth when reintroduced into an anaerobic environment (26). These observations correlate with those of Bowler and Davies (27), who isolated 157 anaerobes from 106 acute and chronic wounds, the majority of which had been swab sampled and the swabs had been transferred to the laboratory in a standard transport medium. Similarly, in a study that compared the efficacy of three commercially available transport media in facilitating microbial recovery from purulent wounds, a time delay between collection and plating did not affect the isolation of aerobic and anaerobic microorganisms in any of the systems (156). Thus, despite the obvious merits of specialized anaerobic transport media (116), transportation of a moist swab sample to the laboratory in a prereduced transport medium offers a cheap and effective method to enable the culture of both aerobic and anaerobic microorganisms (provided that the isolation and identification methods are adequate). For specimens that cannot be transferred to the laboratory within 1 to 2 h, storage at room temperature is considered to be appropriate for the maintenance of aerobic and anaerobic microorganisms; elevated temperatures may cause differential growth or death of some microorganisms, and lower temperatures will cause increased oxygen diffusion (225).

Analysis of Wound Specimens

On arrival at the laboratory, a specimen will be presented to the microbiologist as a tissue, aspirate, fluid, or swab sample that may or may not be accompanied by a clinical description of the wound. Information regarding the type of wound (e.g., surgical, traumatic, leg ulcer, or pressure ulcer), the position of the wound, clinical signs of infection, presence of necrosis, associated malodor, and antimicrobial therapy will greatly assist the microbiologist in predicting the microorganisms that are most likely to be involved and therefore the types of culture media and complementary analyses that should be used. For example, a purulent and malodorous wound fluid aspirate would be indicative of the presence of anaerobic bacteria and gas liquid chromatography (GLC) analysis would most probably provide rapid confirmation of potentially pathogenic anaerobes and facilitate early and appropriate antibiotic treatment. Similarly, the description of a wound associated with colorectal surgery or a sacral pressure ulcer is indicative that the wound is likely to be soiled with fecal microorganisms and therefore that mixed aerobes and anaerobes are likely to be present. If a wound displays clinical signs of infection, the microbiologist is expected to identify the microorganism, or group of microorganisms, most likely to be involved. Also, the provision of information regarding current antibiotic treatment may assist the microbiologist in determining which microorganisms are
most likely to persist in a wound and therefore guide appropriate culturing procedures.

For the practitioner caring for a patient's wound, an early indication of the microorganisms present and guidance about the most appropriate antibiotic treatment (if considered to be necessary) are of primary importance. With the benefit of knowledge of the wound status, the role of the microbiologist is to perform a series of investigations that will yield prompt and meaningful data. Since microbial culture and antibiotic sensitivity results cannot be generated in less than 48 h (and may, on occasion, take considerably longer), a number of rapid investigations must be considered at the outset.

Gas-liquid chromatography for malodorous specimens. As previously discussed, an offensively malodorous wound specimen is indicative of the presence of anaerobic bacteria (29). Since these microorganisms can be characterized on the basis of the SCFAs produced as end products of metabolism, rapid determination of the presence of potentially pathogenic anaerobic bacteria can be performed by direct GLC analysis on the specimen (87). However, specimens that can benefit from this type of analysis are normally restricted to purulent drainage from enclosed abscesses.

Although GLC is a valuable method for rapidly identifying anaerobic bacteria in clinical specimens, the costs involved with such equipment have prevented its use as a routine procedure in microbiology laboratories.

Gram stain. Despite being used for over a century, Gram’s stain is still the most important stain in microbiology (192) and is widely used as a rapid technique for guiding antibiotic therapy in life-threatening infections such as bacterial meningitis. However, the value of the Gram stain as a diagnostic tool is debatable. Although its use in the evaluation of tracheal aspirate samples has been reported as insufficiently reliable to guide antibiotic selection (172), the value of the Gram stain has been recognized in diagnosing the cause of peritonitis in continuous ambulatory peritoneal dialysis patients (21), in predicting skin catheter-related bacteremia (137), and in detecting bacterial vaginosis in pregnant women (231).

In wound management, Gram staining of a known volume of tissue biopsy specimen homogenate has been used to rapidly estimate the microbial load of a wound and thus facilitate successful closure of surgical wounds (102). Also, the presence of microorganisms in a Gram-stained smear prepared from a wound swab has been shown to consistently reflect a microbial load of $\geq 10^6$ organisms isolated by a quantitative swab technique from open burn wounds (139). However, in diabetic foot infections and burn wounds, both of which involve complex microbial ecosystems, a poor correlation between Gram stain and culture results from deep tissue biopsy specimens has been reported (229).

The value of Gram’s stain in facilitating early and appropriate treatment of a wound infection by the clinician is questionable and is primarily dependent on the type of wound. Meislin et al. (159) reported that the Gram stain reliably indicates sterile and mixed abscesses, as well as those containing pure S. aureus. Similarly, this procedure may also facilitate identification of the etiological agent of wound infection following clean surgery, where there is a higher probability of one microorganism being involved (e.g., clusters of gram-positive cocci). However, in most other wound types that are characterized by a complex aerobic-anaerobic microflora, the Gram stain has little value, although the combined presence of leukocytes and bacteria is likely to be a good indicator of infection, as reported by Hussey et al. (112) in studying rapid diagnostic tests for intra-amniotic infection. With the exception of gram-positive spore-forming anaerobes such as Clostridium perfringens, differentiation between aerobic and anaerobic bacteria is difficult and is further complicated by the fact that many gram-positive anaerobes become gram variable on exposure to oxygen (117).

**Culture of wound specimens.** Routine analysis of wound specimens normally involves the use of selective and nonselective agar media to culture aerobic bacteria and yeasts and, if a specimen is purulent and/or malodorous, anaerobic bacteria also. Although anaerobic bacteria often constitute a significant proportion of the total microflora in wounds, their culture and isolation is prolonged and more resource demanding than investigations of aerobic bacteria, and consequently, anaerobic microbiology is often excluded from a routine analysis.

Following incubation under aerobic or anaerobic conditions for 24 to 48 h, qualitative and semiquantitative assessments of the cultures are normally made. S. aureus, P. aeruginosa, and beta-hemolytic streptococci are generally singled out as potential aerobic pathogens, and their growth is often reported as being light (1+) to profuse (4+). With the exception of Clostridium spp., anaerobes (if investigated) are likely to be reported as being “mixed” with aerobic microflora. Antibiograms are most frequently performed for the aerobic pathogens, particularly if they are cultured in abundance and with minimal cohabiting microflora. If aerobes are absent but the wound is reported as being clinically infected, anaerobes should be suspected and investigated more thoroughly.

Since many of the endogenous anaerobic bacteria require between 2 and 5 days to grow on selective culture media before they can be investigated further, they may not be detected after a routine 48-h culture. Also, the time delay is problematic to the clinician, who needs to administer appropriate treatment to a patient with an infected wound. However, anaerobes should not be overlooked, because they are frequently present and many are potentially highly virulent. Whereas C. perfringens will often grow as a characteristic colony on a nonselective agar within 2 days, some of the more fastidious pigmented gram-negative bacilli and fusobacteria, which also have highly pathogenic capabilities, may require up to 5 days to grow on a suitable culture medium. However, in mixed aerobic-anaerobic cultures that reflect polymicrobial wounds, the fastidious anaerobes often grow more readily due to the availability of essential growth factors provided by cohabiting facultative bacteria. To enable the microbiologist to address many of these problems in a standardized way and provide the clinician with meaningful data within an acceptable time frame, consideration should be given to the following analyses. (i) A Gram stain should always be performed on a wound specimen. If numerous leukocytes are observed, together with a single bacterial morphology (e.g., gram-positive cocci in chains), an early indication of the probable pathogen can be provided. (ii) If available, GLC analysis should be performed on appropriate specimens (purulent aspirates) to enable the early detection of the presence of anaerobes. A specimen characterized by a foul odor usually indicates the presence of anaerobic bacteria. (iii) Wound specimens should be cultured for both aerobic and
anaerobic microorganisms (although limited investigation for anaerobic bacteria will be adequate in most cases). At least one selective and one nonselective culture medium should be used for the isolation of anaerobic bacteria; a 5-μg metronidazole disk placed on the agar surface is useful in detecting anaerobic bacteria. After incubation for 48 to 72 h under anaerobic conditions, the presence of anaerobic bacteria is shown by a zone of inhibition around the metronidazole disk. Black-pigmented species will often grow and produce pigment only in the presence of some facultative bacteria (e.g., S. aureus); this may indicate the presence of Prevotella or Porphyromonas spp. Unless it is specifically requested, little benefit is likely to be achieved by isolating and the anaerobes and determining their species. From the colony types that are sensitive to the metronidazole disk, Gram stains can be performed to quickly identify other groups of anaerobes (Peptostreptococcus spp., Clostridium spp., and nonpigmented gram-negative bacilli belonging primarily to Bacteroides, Prevotella, and Fusobacterium spp.). A foul odor from a mixed plate culture usually indicates the presence of pigmented gram-negative anaerobes and facultative bacteria, a combination that is likely to involve synergy (29) and an increased level of virulence in vivo. (iv) If one microorganism is clearly prevalent (i.e., in pure culture or in abundance with minimal involvement of other microorganisms), an antibiogram should be performed. (v) Antibiograms should also be performed on prevalent microorganisms in mixed cultures, particularly if large numbers of leukocytes are observed in the Gram stain.

Reporting of microbiological results. The abrupt onset and rapid progression of acute wound infections such as necrotizing fasciitis usually requires therapeutic intervention (in terms of surgical debridement and empiric antibiotic therapy) long before the microbiology laboratory can generate results, and consequently the role of the laboratory in this situation is limited (183). In contrast, the laboratory has a key role to play in providing information about wounds that are slowly deteriorating or failing to heal. From a microbiological perspective, the main pathogens or groups of microorganisms that the microbiology laboratory should routinely detect and report (with antibiograms being provided when appropriate) are as follows: S. aureus, P. aeruginosa, beta-hemolytic streptococci, coliform bacteria, pigmented gram-negative anaerobes (Prevotella and Porphyromonas spp.), nonpigmented gram-negative anaerobes (primarily Bacteroides, Prevotella, and Fusobacterium spp., Peptostreptococcus spp., and Clostridium spp.).

With respect to the anaerobes and the fact that they are often relatively difficult to isolate and identify, simple observations and tests such as pigmentation on blood agar, Gram stain, and sensitivity to metronidazole (5-g disk) is generally adequate for broad categorization.

In some wounds, other, more unusual microorganisms should be sought (e.g., in animal bite wounds), and yeasts should be specifically sought in wounds of patients receiving immunosuppressive therapy.

In order that the microbiology laboratory can provide the wound care practitioner with clinically relevant information, it is essential that the microbiology results (e.g., Gram stain, culture, and antibiograms) be interpreted in association with clinical information provided by the practitioner (e.g., wound type, location, condition, signs of infection, and sampling method). In this respect, the medical microbiologist may need to interpret the significance of a particular wound pathogen in the light of the clinical presentation. Table 4 provides examples of clinical and microbiological observations that, collectively, will guide the provision of clinically relevant information.

In summary, a continuous dialogue between the microbiology department and the wound care practitioner is essential to ensure that (i) only wounds that are likely to benefit from a microbiological investigation are sampled (i.e., those with clinical signs of infection or those that are failing to heal); (ii) the microbiologist has a thorough understanding of the clinical presentation of the wound; (iii) the microbiologist has an understanding of the method of wound sampling; (iv) the microbiologist is aware of the requirements of the practitioner and the urgency of the results; and (v) the practitioner understands the rationale for advice given by the microbiologist (e.g., the reporting of a mixed aerobic-anaerobic culture from an infected sacral pressure ulcer may not merely indicate a “dirty” wound but may emphasize the significance of microbial synergy; an antibiogram for S. aureus isolated from a mixed culture may not be provided if clinical signs of infection are not evident and if no inflammatory cells are seen in the Gram stain).

By adopting a collaborative approach to the microbiological management of wound complications, significant savings in cost and time (i.e., nursing, medical, and microbiological) may be achieved while providing prompt and appropriate treatment for the patient.

CONTROL OF MICROBIAL POPULATIONS IN WOUNDS

As discussed above, the reporting by the microbiology laboratory of specific microorganisms isolated from a wound and the associated antibiograms may be interpreted by the practitioner as a diagnosis of wound infection that requires antimicrobial treatment. However, without clinical signs of infection and careful consideration, a wound should not be treated with systemic antibiotics, and it is for this reason that all clinical observations and microbiological findings should be taken into consideration before the medical microbiologist provides an expert opinion.

Irrespective of the value of a microbiology report to the wound care practitioner, there is widespread debate regarding when and how infected wounds should be treated, whether noninfected, nonhealing wounds should be treated with antimicrobial agents, what agents should be used, and whether topical or systemic antimicrobial agents should be administered. Although systemic antibiotic therapy is essential for advancing cutaneous infections and those that involve deeper tissues, wounds that exhibit only localized signs of infection or are failing to heal but do not have clinical signs of infection (i.e., heavy colonization) may initially be treated with topical agents. Topical antimicrobial agents include both antiseptics and antibiotics, and the wide choice available creates a further problem to the wound care practitioner. Other treatment options such as HBO therapy, which facilitates the host immune response and may also have a direct antimicrobial effect against some anaerobic bacteria (e.g., C. perfringens), antimicrobial peptides, and botanical extracts may also have roles to play in wound management and are worthy of consideration.
showed to be of greatest therapeutic value in these circum-
stances (206). Synergy between cohabiting bacteria in
frequently polymicrobial, and the role of both aerobic and
anaerobic bacteria is important in reducing infection as well
when prophylaxis is effective in reducing infection and is
heavily contaminated with exogenous microorganisms, anti-
microbial treatment, although the involvement of foreign ma-
rerials such as sutures, dirt, grafts, or prosthetic devices may
increase the risk of infection in clean wounds (187). However,
in surgical wounds that are potentially heavily contaminated
with endogenous aerobic and anaerobic bacteria derived from
the gastrointestinal and urinary tracts, facultative and anaerobic
intestinal bacteria. The aim is to achieve high concentra-
tions at the time of surgery and throughout the surgical pro-
dure (187). In established mixed infections, failure to target both facultative and anaerobic bacteria
often leads to a poor clinical outcome (86). Poor success rates
have been demonstrated with the use of metronidazole or clindamycin alone, targeting only the anaerobic components,
in the treatment of serious abdominal infections (86). Combi-
nation therapy with an aminoglycoside (e.g., gentamicin) or a
cephalosporin (e.g., cefuroxime or cefotaxime) plus clindamycin
or metronidazole has proved to be very effective. The cephaplexin agent cefoxitin has been widely used as a single
agent for prophylaxis in the United States and for the treat-
ment of mixed aerobes and anaerobes, including beta-
hemolytic streptococci; leukocytes indicate early signs of infection; topical antiseptic (e.g., PVP-I or cadexomer iodine) recommended

Infected and noninfected, nonhealing wounds can also ben-
efit considerably from surgical debridement, since devitalized
tissue both obstructs the healing process and often forms the
focus for microbial proliferation. As a consequence, surgical
debridement will significantly reduce the microbial load as well
as exposing healthy tissue required for wound healing.

Approaches to controlling microbial populations in acute
and chronic wounds by use of antimicrobial agents and other
means are discussed in more detail below.

**Antimicrobial Methods of Treatment**

**Antibiotics.** (i) Acute wounds. Although the primary pur-
pose of antibiotics is to treat infection, prophylaxis associated
with surgical practice accounts for up to half of all antibiotics
prescribed (187). Most uncomplicated surgical or traumatic
wounds heal normally without the need for prophylactic anti-
microbial treatment, although the involvement of foreign ma-
rerials such as sutures, dirt, grafts, or prosthetic devices may
increase the risk of infection in clean wounds (187). However,
in surgical wounds that are potentially heavily contaminated
with endogenous aerobic and anaerobic bacteria derived from
the disruption of mucosal surfaces (e.g., by gastrointestinal and
gynecological surgery) or in severe traumatic wounds that are
heavily contaminated with exogenous microorganisms, anti-
microbial prophylaxis is effective in reducing infection and is
recommended as a routine procedure. Surgical infections are
frequently polymicrobial, and the role of both aerobic and
anaerobic bacteria in the pathogenesis of these infections is
well recognized (206). Synergy between cohabiting bacteria in
intra-abdominal infections has also been demonstrated, and the
use of broad-spectrum antimicrobial therapy has been shown to be of greatest therapeutic value in these circum-
stances (86, 206). In such situations, perioperative antibiotic
prophylaxis is often administered because the potential bene-
fits exceed the perceived risks of excessive antibiotic usage
(187). It is recommended that prophylaxis be restricted to a
single dose of the antibiotic(s) at the beginning of the opera-
tion (usually given at the induction of anesthesia) with a max-
umum of one further dose if surgery is prolonged over 3 to 4 h
(222). The choice of prophylactic antibiotics should cover both
facultative and anaerobic intestinal bacteria. The aim is to
achieve high concentrations at the time of surgery and through-
out the surgical procedure (187). In established mixed infec-
tions, failure to target both facultative and anaerobic bacteria
often leads to a poor clinical outcome (86). Poor success rates
have been demonstrated with the use of metronidazole or clindamycin alone, targeting only the anaerobic components,
in the treatment of serious abdominal infections (86). Combi-
nation therapy with an aminoglycoside (e.g., gentamicin) or a
cephalosporin (e.g., cefuroxime or cefotaxime) plus clindamycin
or metronidazole has proved to be very effective. The cephaplexin agent cefoxitin has been widely used as a single
agent for prophylaxis in the United States and for the treat-
ment of established infection. The subsequent development of
new classes of antibiotics such as the ureidopenicillins, the
carbapenems, and the β-lactam/β-lactamase inhibitor combi-
inations has expanded the choice for both prophylactic and therapeutical treatment.

Particularly in contaminated surgery, where excessive pop-
ulations of gram-negative bacteria are likely to be present,
careful selection of antibiotics is required since some are
known to influence endotoxin liberation and hence septic
shock (187). Antibiotics that target the bacterial cell wall liber-
ate larger amounts of endotoxin than do other classes of
antibiotics, such as those that inhibit protein synthesis (187).
Conversely, polymyxin B and the glycopeptide teicoplanin have

---

TABLE 4. Examples of observations that guide the provision of relevant information

<table>
<thead>
<tr>
<th>Clinical and microbiological observation</th>
<th>Example 1</th>
<th>Example 2</th>
<th>Example 3</th>
<th>Example 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical signs of infection reported</td>
<td>None</td>
<td>Pain, inflammation, green exudate</td>
<td>Pain, purulent exudate, pyrexia</td>
<td>None</td>
</tr>
<tr>
<td>Leukocytes in Gram stain</td>
<td>–</td>
<td>2+</td>
<td>3+</td>
<td>1+</td>
</tr>
<tr>
<td>Wound malodor</td>
<td>1+</td>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td>–</td>
<td>1+</td>
<td>–</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>1+</td>
<td>2+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Beta-hemolytic streptococci</td>
<td></td>
<td>–</td>
<td>–</td>
<td>1+</td>
</tr>
<tr>
<td>Coliform bacteria</td>
<td>3+</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td>Peptostreptococcus spp.</td>
<td></td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td>Clostridium spp</td>
<td></td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pigmented gram-negative anaerobes</td>
<td></td>
<td></td>
<td>3+</td>
<td>–</td>
</tr>
<tr>
<td>Nonpigmented gram-negative anaerobes</td>
<td>1+</td>
<td></td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Information provided on microbiology report</td>
<td>Moderate growth of mixed aerobes and anaerobes, including S. aureus; no antibiogram provided unless requested</td>
<td>Moderate growth of S. aureus and P. aeruginosa; antibiograms provided</td>
<td>Moderate to heavy growth of mixed aerobes and anaerobes; antibiotic coverage for both aerobes and anaerobes required</td>
<td>Light growth of mixed aerobes and anaerobes, including beta-hemolytic streptococci; leukocytes indicate early signs of infection; topical antiseptic (e.g., PVP-I or cadexomer iodine) recommended</td>
</tr>
</tbody>
</table>

* 1+, light growth/minimal malodor or leukocytes; 4+, heavy growth/offensive odor or numerous leukocytes.
the ability to neutralize endotoxin, and vancomycin has been shown to down-regulate lipopolysaccharide-induced tumor necrosis factor alpha production from monocytes and is thus beneficial in the treatment of sepsis (187).

Since *S. aureus* is considered to be the most problematic pathogen associated with infected traumatic wounds (71, 187), cephalosporins, macrolides, clindamycin, and semisynthetic penicillins such as flucloxacillin and oxacillin are often treatments of choice (71). If methicillin-resistant strains are involved, the glycopeptide antibiotics vancomycin and teicoplanin are alternatives (187).

(ii) Chronic wounds. Like contaminated surgical wounds, the majority of chronic wounds (e.g., leg ulcers, foot ulcers, and pressure ulcers) are characterized by a polymicrobial anaerobic-microaerobic microflora. Consequently, the careful use of broad-spectrum antimicrobial agents is likely to be the most successful treatment in the management of clinically infected chronic wounds. The presence of anaerobic bacteria in foot ulcers of diabetic patients has been associated with a greater likelihood of the patient becoming febrile, developing a more serious deep-wound infection, and requiring amputation (84). Goldstein (84) emphasized the importance of broad-spectrum antibiotics in the treatment of nonsurgical infections and suggested the use of cefoxitin, imipenem, or ticarcillin-clavulanate as sole agents, with other agents being used in combination, including metronidazole or clindamycin for the anaerobic component. Although the use of metronidazole alone to treat abdominal infections is unsuccessful, in vitro studies have demonstrated that protection of facultative bacteria from phagocytosis was prevented when the anaerobic component was eliminated following treatment with metronidazole (114). Consequently, in theory, an alternative prophylactic approach to disrupting synergy and enhanced pathogenicity in polymicrobial wounds may be to treat the patient with an anaerobicidal antibiotic such as metronidazole and depend on the disruption of synergy and the up-regulation of the host defense mechanisms to eliminate the facultative bacteria. However, this mechanism of action has not been clinically proven, and studies are required to evaluate the overall antimicrobial effect of metronidazole treatment and its role in wound healing.

Recent guidelines on the treatment of pressure ulcers issued by the European Pressure Ulcer Advisory Panel recommended that systemic antibiotics not be required for pressure ulcers that exhibit only clinical signs of local infection (72). Since leg ulcers and foot ulcers often exhibit a similar microflora to pressure ulcers, such advice could probably be extended to cover a wider variety of chronic wound types. In the absence of advancing cellulitis, bacteremia, fever, or pain, topical antimicrobial agents (antibiotics or antiseptics) may offer the most useful first line of treatment. If a wound fails to improve after an initial course of topical treatment (e.g., 2 weeks), continued use is not likely to be of benefit. Alternative topical agents may be considered at this point unless clinical signs of infection are progressing, in which case systemic antibiotic therapy should be considered. At this stage, microbiological data are likely to be available to guide systemic therapy.

The use of broad-spectrum topical antibiotics to treat wounds that are failing to heal or wounds that are at risk of infection is justified on the basis that they provide a high concentration at the local site, they avoid systemic allergic reactions (22), and they also avoid more-widespread effects on endogenous bacteria (e.g., disturbance of the normal commensal microflora or the action of induced resistance). However, the probability of a successful outcome in chronic wounds may be compromised by the presence of ischemic and necrotic tissue which may impair tissue distribution and therapeutic efficacy of the drug. Antibiotics used topically are generally restricted to those that are toxic when administered systemically. Bacitracin, polymyxin B, and neomycin are used as double or triple topical antibiotic combinations to provide an appropriate spectrum of activity. Other topical agents such as mupirocin and fusidic acid are rarely used in wounds but are most frequently used to treat superficial skin infections caused by *S. aureus* or in the elimination of staphylococcal carriage. Metronidazole in a gel or solution formulation at a concentration of approximately 8,000 μg/ml is specifically used to eliminate anaerobic bacteria responsible for the offensive malodor in many chronic wound types, including ulcerated malignant tumors. Also, anecdotal evidence indicates that metronidazole may have a direct effect in improving wound healing (10, 85, 228, 246).

(iii) Complementary therapy. In addition to antibiotic therapy, wound cleansing and surgical debridement may assist antibiotic treatment by reducing the microbial load, and hence the opportunity for infection, and enabling better penetration of antibiotics to where they are needed. Delayed wound closure may also be considered to allow time for antimicrobial therapy to reduce the microbial load, without which healing may not progress, and also to avoid the accumulation of a blood clot in tissue debris, which is ideal for microbial growth (204, 205).

(iv) Role of the microbiology laboratory in guiding antibiotic treatment in wound management. As discussed above, in rapidly progressing soft tissue infections such as necrotizing fasciitis, empirical therapy is essential and consequently the microbiology laboratory has a minimal role to play in assisting clinicians in their immediate choice of early treatment (183). However, microbiological data are important in confirming subsequently that the chosen regimen is appropriate. In contrast, in wounds that are failing to heal or have a more prolonged chronic infection, the microbiologist can have an important role to play in advising on whether to treat a wound and, if so, on the choice of antibiotic treatment. Most clinicians prescribe broad-spectrum antimicrobial agents before reviewing a microbiology report, and in many cases the treatment may be inappropriate or may not be necessary, and this can have a serious impact on hospital budgets. Furthermore, broad-spectrum antibiotics can adversely affect the normal gastrointestinal microflora, possibly predisposing patients to *Clostridium difficile* colitis and selecting for resistance in some bacterial strains (e.g., vancomycin resistant *Enterococcus*) (183).

Based on information received regarding the site of wound infection and clinical symptoms, the role of the microbiology laboratory is to determine the clinically significant isolates, perform antimicrobial susceptibility testing, and subsequently provide guidance on the most appropriate treatment (242). Not only will this assistance facilitate successful wound management but also it will assist in the control of antibiotic usage and hence stem the spread of antibiotic-resistant bacteria.

Antibiotic susceptibility of wound isolates observed in the laboratory cannot always be related directly to the clinical
situation since the in vitro and in vivo conditions vary considerably. The number of organisms at the infected site may be significantly different from the standard inoculum size used in vitro, the wound pH is likely to differ from test pH, and the pharmacokinetics and conditions at the infected site are also likely to influence microbial susceptibility in vivo (242). It is therefore important to consider that laboratory data may not always translate to therapeutic success.

**Antiseptics.** Unlike antibiotics, antiseptics are chemical agents that are potentially toxic to both microbial cells and host cells. Therefore, their use is limited to topical application to wounds and intact skin. Wounds that are most likely to benefit from topical antiseptic treatment are primarily those of a traumatic or chronic nature that are heavily contaminated with a variety of microorganisms and are failing to heal, with or without clinical signs of infection. Commonly used topical antiseptic agents include iodine-releasing agents (e.g., povidone iodine [PVP-I] and cadexomer iodine), chlorine-releasing solutions (e.g., Dakin's solution and sodium hypochlorite solution), hydrogen peroxide, chlorhexidine, silver-releasing agents, and acetic acid. In terms of efficacy, acetic acid (1%) has limited activity but has been used with greatest success in the management of wounds heavily colonized with *P. aeruginosa* (189). Silver-releasing agents, particularly silver sulfadiazine (1%), have historically been used to treat *P. aeruginosa* infection in patients with burn wounds, although the spectrum of activity is capable of controlling a variety of common wound pathogens (152). Hadjiiannis (93) reported that a 1% silver sulphadiazine cream (Flamazine) was more effective than PVP-I in preventing infection in burn wounds prior to skin grafting.

Although iodine is perhaps the most potent and broad-spectrum antiseptic agent, its use in wound management is controversial because some iodine formulations (e.g., PVP-I) have been shown, in vitro, to impair the functioning of cells involved in normal wound healing (48). However, this effect has not been observed in vivo when it was used at concentrations lower than 1% (48). Despite the highly efficacious antimicrobial properties of PVP-I and hence the potential to significantly reduce the microbial load in wounds, accelerated healing has not been observed in experimental and clinical studies (48). This implies either that the PVP-I does not demonstrate in vivo antimicrobial efficacy (possibly due to inactivation by organic material) or that the beneficial effects are counterbalanced by the cytotoxic effects (48). A recent review of the literature that specifically addressed in vivo animal and human studies, concluded that the use of PVP-I was associated with poor wound healing in the majority of cases (125).

In recent years, improved formulations that are able to release low levels of iodine over a sustained period have been shown to be highly effective against wound pathogens while not impairing wound healing (161). The sustained release of iodine also overcomes the neutralizing effect that organic material in wounds has on iodine activity. Similarly, a recently described enzyme-catalyzed iodine formulation, capable of generating high concentrations of molecular iodine while forming relatively low levels of total iodine, has been shown to elicit greater microbicidal activity than a PVP-I solution and has an effect comparable to normal saline on the rate of epithelialization in superficial wounds in pigs (106). An extensive review of the literature supports the use of cadexomer iodine as a safe, effective, and economical treatment in a variety of chronic wound types (227), and the same agent has been shown to specifically prevent the proliferation of methicillin-resistant *S. aureus* in partial-thickness experimental wounds (161). Experts in the field consider iodine to have an important role to play in wound management, as concluded at a recent consensus meeting sponsored by the European Tissue Repair Society (79). Although the prophylactic role of iodine in acute surgical wounds is widely supported in the literature, there is minimal evidence to support its use in chronic-wound prophylaxis (79). However, topical iodine antisepsis should be considered for nonhealing wounds, with or without clinical signs of infection (79). The primary concern regarding the use of iodine-containing formulations is toxicity and its associated effects on wound healing. However, most of these concerns are based on in vitro data that may not be relevant to the clinical situation, and the newer, slow-release iodine formulations appear to be both safe and effective (79).

**Alternative antimicrobial therapies.** As the development of bacterial resistance to antibiotics continues and controversy regarding the use of topical antiseptics persists, the need for identification and development of new antimicrobial agents that are safe and broadly effective and have a low propensity to induce resistance becomes increasingly critical. In recent years, widespread interest has focused on a class of naturally occurring peptides that protect a variety of animals from infection. These peptides are found in a variety of cell types and operate by attaching to microbial cells, perforating the cell wall, and inducing leakage of cell contents. Such pore-forming antimicrobial peptides are widespread throughout nature: human neutrophils produce defensins, magainins have been isolated from the skin of the African clawed frog, and cecropins have a similar function in the giant silkworm moth (55). The opportunity to synthesize more potent and broad-spectrum analogues of the natural endogenous peptides has been recognized by pharmaceutical companies, and topical formulations are now in development for indications such as infected diabetic foot ulcers (90).

Concern over the use of antibiotics and the search for new antimicrobial agents has also led to the reemergence of therapies that have been used for centuries but have become less fashionable during the antibiotic era. The use of fly maggots to treat infected and necrotic wounds is being increasingly acknowledged and will be discussed as a biosurgical form of wound debridement in a later section. Other resurgent topical therapies include botanical extracts and honey.

Modern-day recognition of the therapeutic value of essential oils extracted from plants (aromatherapy) dates back to 1928, when a French chemist observed the healing capacity of lavender oil following a burn injury to his hand (14). Many essential oils possess antimicrobial properties, and tea tree oil in particular (derived from the Australian native plant *Melaleuca alternifolia*) has been recognized for its efficacy against methicillin-resistant *S. aureus* and has consequently been considered as an alternative treatment for mupirocin-resistant methicillin-resistant *S. aureus* (49). However, despite the potential for novel agents such as tea tree oil, their acceptance and use in wound management will be limited until adequate safety and clinical efficacy data have been generated.

Honey is another ancient remedy that is gaining renewed
popularity as alternative treatments for antibiotic-resistant bacteria are pursued. Both honey and sugar paste are considered useful as topical antimicrobial agents, primarily as a consequence of their high osmolarity and ability to minimize water availability to bacteria (163). Although the dilution of honey in the presence of wound fluid is likely to reduce the efficacy of its osmotic effect, the slow and sustained production of hydrogen peroxide by some types of honey (e.g., manuka honey) is capable of maintaining an antimicrobial effect at a concentration approximately 1,000-fold lower than that commonly used in antiseptics (i.e., 3%) (163). Also, components of manuka honey such as flavonoids and aromatic acids, demonstrate antimicrobial properties (163). Honey is also an effective wound deodorizing agent, and this effect has been attributed to the rich supply of glucose that is metabolized by bacteria in preference to proteinaceous necrotic tissue, resulting in the production of lactic acid and not the malodorous compounds generated by protein degradation (163). The observed benefits of honey in infected wounds may also be attributed to the high glucose content and low pH, both of which are stimulatory to macrophages (56).

Despite the multifactorial benefits of certain types of honey in the management of many wound types, widespread acceptability is likely to be slow at best. This assumption is based on the fact that such therapy is ancient and therefore represents a regressive step rather than advancing toward new and innovative therapies, and it is also based on the wide variation in potency that exists in honeys derived from different floral sources (163).

**Hyperbaric oxygen therapy.** Wounds often fail to heal because the tissue is ischemic and consequently starved of oxygen, nutrients, and host defense cells that are essential to the healing process. Combined with impaired oxygen delivery, microbial and host cell metabolism within the wound space rapidly leads to the formation of a hypoxic or anoxic environment. Aerobic respiration yields approximately 17 times more energy per molecule metabolized than does anaerobic respiration (219), and consequently cellular responses to tissue injury (e.g., leukocyte function, fibroblast proliferation, granulation, angiogenesis, and collagen deposition) are enhanced in the presence of oxygen (244). Furthermore, a hypoxic environment will facilitate the growth of anaerobic bacteria and impair the PMN oxygen-dependent antimicrobial mechanisms that are essential in reducing the opportunity for infection (244). Sheffield (218) suggested that the severity of the problem in a nonhealing wound is partially proportional to the degree of tissue hypoxia.

During normal wound healing, the deposition of a collagen matrix forms a scaffold that supports new blood vessels (angiogenesis), and these, in turn, enable the delivery of oxygen and nutrients required to maintain the healing process (248). Angiogenesis is driven by an oxygen gradient between peripheral normal tissue and the wound space (124), and some treatments capable of exaggerating the gradient have been shown to enhance the angiogenic response. In a porcine full-thickness wound model, Lydon et al. (144) demonstrated increased blood vessel counts and perfusion in wounds that were treated with an occlusive dressing (i.e., one that excluded environmental oxygen and thus increased the oxygen gradient) compared with dressings that were exposed to air.

HBO therapy, involving the intermittent inhalation of pure oxygen (100%) at a pressure greater than 1 atm (136), has also been used in wound management to increase tissue oxygen tension and hence promote angiogenesis, fibroblast proliferation, and PMN microbicidal efficacy (248). However, the value of HBO therapy in wound healing is controversial, and in order to prevent indiscriminate use, the Committee on Hyperbaric Oxygenation (established in 1976) restricted HBO therapy to four wound categories, namely, acute traumatic ischémias (e.g., crush injuries), clostridial myonecrosis, necrotizing soft tissue infections, and selected nonhealing problem wounds (53).

In a study involving 10 patients with lower-extremity diabetic wounds, HBO therapy was shown to increase the peri-wound oxygen tension from an initial mean of 12 mm Hg to 560 mm Hg, which resulted in a more rapid reduction in the wound area (248). Similarly, it has been suggested that HBO therapy improves healing in diabetic foot ulcers when it is used as an adjunct to broad-spectrum antibiotics, aggressive surgical debridement (to preserve the peripheral circulation), and adequate metabolic control (136). However, in general, HBO therapy has not been widely accepted as a standard treatment for chronic nonhealing wounds, and this is due, in no small part, to the lack of a body of evidence from structured randomized clinical trials in this area. In a retrospective evaluation of 54 patients with lower-extremity nonhealing wounds of varied etiology, 80% showed no improvement after 30 treatment sessions (53). From this study, Ciavarino et al. (53) concluded that because of the expense (average cost of $14,000 per complete treatment), poor efficacy, and potential complications, HBO therapy was difficult to justify. To date, the majority of studies involving nonhealing wounds have been performed without good scientific rationale (53), precise wound-healing measures, or well-defined wound-healing end points (245). Also, since adequate tissue perfusion is a prerequisite for HBO therapy to have any beneficial effect in the treatment of lower-extremity wounds, HBO therapy cannot be used as a substitute for surgical revascularisation in patients with advanced arterial insufficiency (53). Well-perfused wounds that are deteriorating or failing to heal and acute, rapidly advancing soft tissue infections such as necrotizing fasciitis meet these criteria and are thus likely to gain most benefit from HBO therapy.

Polymicrobial aerobic-anaerobic interactions are known to contribute significantly to the progression and severity of disease in acute soft tissue infections (12). In such situations, HBO therapy is likely to be of benefit by both directly inhibiting anaerobic bacteria (and possibly thereby reducing the level of virulence caused by aerobic-anaerobic synergistic interactions) and optimizing the antimicrobial efficacy of PMNs (11). In rapidly progressing soft tissue infections, aggressive surgical debridement, together with broad-spectrum antibiotic therapy, is critical. Although no large prospective clinical trials have been performed to evaluate the role of HBO therapy, several studies have demonstrated that such adjunctive therapy can significantly reduce mortality and wound morbidity (12, 153, 191). In particular, the value of HBO therapy has been recognized in cases of clostridial myonecrosis (gas gangrene), since the potent alpha-toxin (lecithinase) produced by *C. perfringens* (type A) is inactivated at a tissue oxygen tension of greater than 33 kPa (11). However, the acceptance of HBO therapy in the treatment of necrotizing soft tissue infections is not universal, and Elliot et al. (69) concluded, from a retro-
spective study, that it provided no survival benefit although it may hasten wound closure. Most recently, Clark and Moon (54) formed the opinion that on the basis of in vitro data and growing evidence from retrospective clinical studies, HBO therapy is a valuable adjunct to surgical debridement and antibiotic therapy in the treatment of life-threatening soft tissue infections.

Surgical wounds may also benefit from supplemental oxygen. Elevation of the oxygen tension in perfused tissue during colorectal surgery by the administration of 80% inspired oxygen has been shown to significantly reduce the incidence of postoperative wound infection (91).

### Nonantimicrobial Methods of Treatment

While the pharmacologic maintenance of a manageable bacterial burden may prove to be of central importance in wound healing, it is an adjunct in an overall regimen that includes debridement and appropriate protection or pressure off-loading. This section will evaluate nonantimicrobial methods of treatment, including surgical, enzymatic, autolytic, and biological debridement, as well as the importance of pressure reduction and off-loading in wounds whose prime etiology is repetitive stress (as occurs with the diabetic foot ulcer).

#### Debridement

Debridement involves the removal of devitalized and contaminated tissue from wounds to expose healthier tissue and facilitate wound healing (240). Devitalized tissue provides a favorable environment for microbial growth, and thus its removal will also reduce the microbial load and microbially induced malodor and will minimize the opportunity for wound infection. Debridement can be achieved in a variety of ways.

(i) **Surgical debridement.** Surgical (sharp) debridement has been the mainstay of treatment for wounds of various etiologies. The rationale behind the necessity for periodic surgical debridement has been largely anecdotal but centers around the limitation of undermining, the promotion of an adherent wound margin and subsequently a favorable environment for epithelial cell migration, and the reduction of the bacterial burden (5, 224). In a survey of two diabetic foot specialty clinics, Frykberg (77) reported a shorter healing time in the surgically oriented center. Steed et al. (224), in a subanalysis of a multicenter randomized clinical trial of recombinant platelet-derived growth factor, reported that centers that regularly debrided wounds had a higher proportion of subjects heal in both the active growth factor and placebo gel vehicle arms.

(ii) **Autolytic and enzymatic debridement.** A variety of autolytic and enzymatic dressings and topical modalities have been in use for over a generation. While a body of work exists to support their use as an adjunct, there is little objective clinical evidence to support their use as a primary means of debriding wounds. The theory surrounding autolytic debridement stems from the concept that some dressings tend to provide the moist environment needed to activate endogenous enzymes that promote the autolysis of fibrin and facilitate extracellular matrix turnover and maintenance (52, 168, 169). In a clinical trial of 30 patients with pressure ulcers, Barr et al. (15) found that wounds that were surgically debrided prior to the use of autolytic debridement (delivered via a hydrocolloid-alginate dressing) showed a significantly better clinical response than if they received autolytic debridement alone. There are additional reports to suggest that the risk for infection may be reduced through the use of a barrier-type dressing with autolytic properties in contrast to a nonocclusive dressing such as gauze (148, 221). Clearly, however, there is a need for more objective study in this area to determine the clinical utility of autolytic and enzymatic debridement both combined with and independent of standard surgical debridement (3). From a microbiological perspective, since autologous debridement does not involve the physical removal of devitalized tissue (until cleansing at dressing change), this method is unlikely to be as effective as surgical debridement in reducing the microbial load and hence the risk of wound infection.

(iii) **Biosurgical debridement.** Prior to the advent of antibiotic therapy, the use of larvae (maggots) as an effective method of wound debridement (162, 170) was routine. Much of the early work detailing the breeding and application of the larvae was performed by one man, Stanton Livingston, at and around the time of the Great War (89, 140, 141). Although biosurgical debridement has played a minor role in wound management during the last 50 years, its popularity has gradually increased again during the 1990s as alternative treatments have been sought in an attempt to combat the surge in infections caused by antibiotic-resistant bacteria. Larval therapy is currently being used in the treatment of a variety of infected acute and chronic wounds, including those colonized by resistant bacteria such as methicillin-resistant S. aureus (233, 234). The fly maggots of the common greenbottle, Lucilia sericata, are capable of physically and enzymatically degrading devitalized tissue in a safe and effective way. During this process, potentially pathogenic bacteria may be destroyed as part of the natural feeding process, but endogenous antimicrobial secretions are also considered to play an important role in microbial elimination (233, 234). Additional data suggest that fly larvae may stimulate fibroblast proliferation in vitro (193).

As with autolytic and enzymatic debridement, the medical literature is replete with isolated case reports, and again, there are few data to concisely compare outcomes to other modes of therapy.

**Pressure reduction in wounds.** (i) **Off-loading.** With the exception of burn wounds, pressure and duration of pressure application are prime factors in the pathogenesis of the majority of wounds treated today. Therefore, it would stand to reason that mitigation of these stresses is important in the healing of these wounds. While seemingly disconnected from the microbiology of wound care, a closer inspection may prove that mechanical care of the wound and infection may be more intimately related. Certainly, wounds in which pressure is not off-loaded appropriately may take longer to heal and therefore may be exposed to a greater risk of infection (167). The literature describing areas in which the pressure reduction component of treatment is relevant is quite extensive. In this review, we will focus on one very challenging clinical situation, off-loading the diabetic foot ulcer to reduce pressure stresses.

While many methods have been used to protect the lower extremity from high repetitive pressures (e.g., bed rest, non-weight bearing, crutch assistance, and prostheses), many of these approaches have proven impractical. Most ambulatory patients with wounds in weight-bearing regions, such as the sole of the foot, developed those wounds because of neurolog-
physical sensory damage. Therefore, the normal nociceptive feedback that would deter activity in the sensitive individual is absent, thereby removing perhaps the biggest ally of the clinician attempting to prevent further damage to the wound—pain.

Based on the above-mentioned frequent lack of painful feedback, comprehensive off-loading strategies for persons with wounds in weight-bearing areas have faced a conundrum: the desire to ensure frequent surveillance and care of the wound (e.g., ease of removal of the off-loading device) and the desire to ensure compliance with off-loading (e.g., making the device difficult to remove). This has led to two major categories of off-loading devices: removable and nonremovable devices. While there is a paucity of comparative trials evaluating these devices in a randomized fashion, a guide to the results of various reports is given in Table 5. While one cannot draw sharp conclusions from these reports (primarily due to significant differences in methods and populations), the consistency in the duration to healing with the total-contact cast has led to its being considered the “gold standard” in terms of its quality of off-loading (131) and duration to healing (3, 7, 51, 103, 104, 108, 129, 167, 171, 220, 241).

(ii) Vacuum-assisted wound closure. Another recently introduced perspective to pressure reduction in wounds involves the application of subatmospheric pressure. Essentially, a sterile open foam dressing is applied to a wound, which is then closed to the external environment to enable the application of a low-level vacuum (125 mm Hg) (164). This type of system has been used to good effect in the treatment of a variety of wound types and has been shown to reduce interstitial pressure, restore blood flow, and remove cell-inhibitory factors within chronic wound fluid. Additionally, vacuum-assisted wound closure has been shown to reduce the bacterial load in tissue 1,000-fold after 4 days of treatment in an experimental infected wound model (164) and also to be of benefit in the management of deep sternal wound infection (232).

Infection Control. As well as the need to control microbial populations that exist in wounds in order to minimize the likelihood of wound infection, there is a need to control the dissemination of potentially pathogenic members of the wound microflora into the surrounding environment in order to minimize the opportunity for cross-infection. In this respect, wound dressings should be considered an important infection control tool, since many are able to physically prevent the transmission of pathogenic bacteria and blood-borne viruses (30, 66, 115, 134, 160). Dressings that are capable of maintaining a moist wound environment have also been shown to minimize airborne dispersal of microorganisms from burn wounds at the time the dressing is removed (133) and also to reduce the wound infection rate (24, 113, 127).

**CONCLUSIONS**

Dermal wounds involve exposed tissue, which, under normal circumstances would be sterile, i.e., free from microbial contamination. However, like normal intact skin, a newly formed wound will naturally become colonized by microorganisms and compromised tissue will encourage their proliferation. In addition to the warm, moist, and nutritious conditions, ischemic wounds (usually chronic) are often characterized by tissue hypoxia, necrosis, and an impaired immune response. Consequently, commensal aerobic and anaerobic microfloras of the human body (with primary sources being the skin, the oral cavity, the gut, and the genitourinary tract) are presented with an opportunity to colonize a common but unnatural habitat, microbial interactions unique to this particular environment may significantly influence wound pathogenesis and healing.

The majority of open wounds are polymicrobial. A review of the literature indicates that anaerobic bacteria constitute, on average, one-third of the total number of microbial species in colonized wounds, and this number increases to approximately 50% in infected wounds. Therefore, antimicrobial treatment of clinically infected and/or nonhealing polymicrobial wounds should cover a variety of potentially synergistic aerobic or facultative and anaerobic microorganisms and should not simply target specific pathogens that are often perceived to be the causative agents (e.g., *S. aureus* and *P. aeruginosa*).

The progression of a colonized wound to an infected state cannot be predicted by the presence of a specific type of bacterium or a specific pathophysiological condition, because a multitude of factors are likely to simultaneously influence wound pathogenesis. Microbiological factors such as the population density, the types of microorganisms present, and microbial interactions and host factors such as the efficacy of the immune response and the condition of the tissue are all critical and must be considered collectively as factors predisposing to infection.

**TABLE 5. Healing times by off-loading modality**

<table>
<thead>
<tr>
<th>Modality</th>
<th>Author (reference)</th>
<th>Type of ulcer</th>
<th>Mean healing time (days)</th>
<th>% Healed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total-contact cast</td>
<td>Myerson (171)</td>
<td>Wagner grade I and II</td>
<td>30 (forefoot ulcers), 63 (midfoot and rearfoot ulcers)</td>
<td>90</td>
</tr>
<tr>
<td>Total-contact cast</td>
<td>Helm (105)</td>
<td>Wagner grade I, II, and III</td>
<td>38.3</td>
<td>72.7</td>
</tr>
<tr>
<td>Total-contact cast</td>
<td>Mueller (167)</td>
<td>Wagner grade I and II</td>
<td>42</td>
<td>90</td>
</tr>
<tr>
<td>Total-contact cast</td>
<td>Walker (241)</td>
<td>Wagner grade I, II, and III</td>
<td>30.57 (forefoot ulcers), 42.08 (nonforefoot ulcers)</td>
<td>NR</td>
</tr>
<tr>
<td>Total-contact cast</td>
<td>Armstrong (7)</td>
<td>UT grade Ia</td>
<td>38.8</td>
<td>100</td>
</tr>
<tr>
<td>Total-contact cast</td>
<td>Lavery (129)</td>
<td>UT grade Ia</td>
<td>28.4 (midfoot ulcers)</td>
<td>100</td>
</tr>
<tr>
<td>Half-shoe</td>
<td>Chantelau (51)</td>
<td>Apelqvist grade I, II, III, and IV</td>
<td>70 (median healing time)</td>
<td>96</td>
</tr>
<tr>
<td>Insoles</td>
<td>Holstein (108)</td>
<td>NR</td>
<td>108</td>
<td>97</td>
</tr>
</tbody>
</table>

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NR, not reported.
Clinical studies have demonstrated that a measure of the tissue microbial load in a wound can predict delayed healing or infection. The quantitative tissue biopsy specimen technique is probably most useful in traumatic or surgical wounds to determine the correct time for wound closure or grafting, but its value in the routine management of chronic, nonhealing wounds is less practical. The swab sampling technique is also considered to have limitations, in particular the fact that it collects superficial contaminants that do not reflect the deeper, infecting microorganisms. However, since synergy undoubtedly occurs in polymicrobial wounds, superficial microorganisms may well contribute to wound pathogenesis. Superficial sampling can provide qualitative and quantitative or semiquantitative data, and correlations between tissue counts and superficial swab counts have been demonstrated. Although superficial sampling can be performed by personnel without surgical expertise and does not involve tissue invasion or trauma to the patient, its widespread and unconditional use should be challenged. In this respect, it is the opinion of the authors that only wounds that are clinically infected or those that have no clinical signs of infection but are deteriorating or have a long history of failing to heal merit superficial swab sampling for microbiological analysis.

In the laboratory, microbiological analysis must be guided by the clinical information provided (e.g., type and site of wound, associated malodor, signs of infection, antibiotic therapy) in order to ensure that meaningful results can be provided in as short a time as possible. As an example, an antibiogram for *S. aureus* isolated from a mixed culture should not be provided if clinical signs of infection are not evident and if no inflammatory cells are seen in the Gram stain. Similarly, the reporting of a mixed aerobic-anaerobic culture from an infected sacral pressure ulcer may not merely indicate a “dirty,” fecally-contaminated, and poorly sampled wound but may, in fact, indicate the potential for enhanced pathogenicity caused by microbial synergy. Consequently, to minimize the opportunity for wound infection and exclude microorganisms as a factor in delayed healing in noninfected wounds, a multidisciplinary approach to wound management, involving a continuous dialogue between laboratory and clinical staff, is vital. From a practical perspective, controlling the microbial load in wounds is a vital factor in minimizing infection, and this can be achieved in several ways. Antimicrobial agents (antibiotics) are primarily used either prophylactically in the treatment of wounds that are likely to be heavily contaminated following surgery or therapeutically in the treatment of clinically infected wounds. Since both aerobic and anaerobic pathogens may contribute to infection in polymicrobial wounds (often via synergistic interactions), broad-spectrum antibiotics provide the most successful treatment. Wounds that are heavily contaminated (e.g., chronic or acute traumatic), are failing to heal and possibly deteriorating, but have only local or no clinical signs of infection may benefit from topical antibiotic or antiseptic therapy. Although topical antibiotics are selectively toxic to bacteria rather than to host tissue, they are likely to induce bacterial resistance, and for this reason antiseptics are more frequently used topically. However, prolonged antiseptic use may compromise host tissue viability and hence counteract the promotion of healing induced by the antimicrobial effect.

HBO therapy has also been used to treat a variety of oxygen-compromised (hypoxic) acute and chronic wound types. In theory, HBO therapy stimulates cellular processes involved in wound healing, directly impairs the growth of anaerobic pathogens, and enhances the potency of the oxygen-dependent antimicrobial mechanisms in PMNs. Although there is currently a paucity of prospective, controlled clinical data regarding the efficacy of HBO therapy, its usefulness as an adjunct to surgical debridement and antibiotic therapy has been demonstrated, particularly in some of the aggressive necrotizing soft tissue infections.

Surgical debridement of compromised (nonviable) tissue not only exposes the healthy, perfused tissue required to initiate wound healing but also effectively removes the majority of microbial contaminants and any associated malodor, thus reducing the risk of infection. Biosurgical debridement, involving the use of fly larvae (maggots), has also regained popularity in recent years and is proving efficacious in the treatment of both infected and necrotic wounds.

Although the microbiology of wounds has been actively researched in recent years, there is still much to be learned about the microbial mechanisms that induce infection and prevent wound healing. Consequently, debate regarding microbial involvement in wound healing is likely to persist. In providing a detailed analysis of wound microbiology, together with current opinion and controversies regarding wound assessment and treatment, this review has attempted to capture and address microbiological aspects that are critical to the successful management of wounds and their microbial floras.


228. Wright, A. E., A. Fleming, and L. Colebrook. 1918. The conditions under which the sterilisation of wounds by physiological agency can be obtained. Lancet 1851–1853.
