Review

Bacteriology of pressure ulcers in individuals with spinal cord injury: What we know and what we should know

Ali N. Dana^{1,2}, William A. Bauman^{3,4,5}

¹Dermatology Service, James J. Peters VA Medical Center, Bronx, NY, USA, ²Department of Dermatology, Columbia University School of Medicine, New York, NY, USA, ³Department of Veterans Affairs Rehabilitation Research & Development Service's National Center of Excellence for the Medical Consequences of Spinal Cord Injury, James J. Peters Veterans Affairs Medical Center, Bronx, NY, USA, ⁴Medical Service, James J. Peters VA Medical Center, Bronx, NY, USA, ⁵Department of Medicine and Rehabilitation Medicine, Ichan School of Medicine at Mount Sinai, New York, NY, USA

Individuals with spinal cord injury (SCI) are at increased risk for the development of pressure ulcers. These chronic wounds are debilitating and contribute to prolonged hospitalization and worse medical outcome. However, the species of bacteria and the role that specific species may play in delaying the healing of chronic pressure ulcers in the SCI population has not been well characterized. This study will review the literature regarding what is known currently about the bacteriology of pressure ulcers in individuals with SCI. An electronic literature search of MEDLINE (1966 to February 2014) was performed. Eleven studies detailing bacterial cultures of pressure ulcers in the SCI population met inclusion criteria and were selected for review. Among these studies, bacterial cultures were often polymicrobial with both aerobic and anaerobic bacteria identified with culture techniques that varied significantly. The most common organisms identified in pressure ulcers were *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*. In general, wounds were poorly characterized with minimal to no physical description and/or location provided. Our present understanding of factors that may alter the microbiome of pressure ulcers in individuals with SCI is quite rudimentary, at best. Well-designed studies are needed to assess appropriate wound culture technique, the impact of bacterial composition on wound healing, development of infection, and the optimum medical and surgical approaches to wound care.

Keywords: Pressure ulcer, Spinal cord injury, Bacteria, Bacteriology, Wound care

Introduction

Individuals with paralysis following spinal cord injury (SCI), as well as others who have medical or neurological conditions that predispose to prolonged immobilization, are at significant risk for the development of skin breakdown. The cause of these localized wounds (e.g. pressure sores, pressure ulcers, decubitus ulcers, or bedsores) may be due to pressure over a bony prominence that results in shearing and/or ischemia of the overlying skin, leading to tissue breakdown.¹ However, it is now generally accepted that external pressure compromises skeletal muscle, as well as cutaneous

microvascular blood flow, resulting in the development of pressure ulcers that actually arise from deeper tissue beds. As such, many wounds initially result from deep tissue ischemia and necrosis prior to the appearance of skin breakdown.² Skin breakdown in patients with SCI will be referred to as pressure ulcers in this review because the defect typically occurs at a site of prolonged and unrelieved pressure.

Compared with the general population, individuals with SCI are at significant risk of developing a pressure ulcer. In recent years, the prevalence of pressure ulcers in individuals with SCI has increased.³ In the literature, prevalence rates of pressure ulcers vary due to differences in study methodology, but more recent studies suggest that between 25 and 50% of veterans with SCI

Correspondence to: Ali N. Dana, Dermatology Service, Suite 2F, James J. Peters Veterans Affairs Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468, USA. Email: Ali.Dana@va.gov.

are estimated to receive treatment for pressure ulcers.^{3–5} Pressure ulcers not only pose a significant medical burden, but they also are associated with high costs of care. The annual cost of treating pressure ulcers in the SCI population has been projected to be \$1.3 billion.⁶ Healthcare costs for individuals with SCI and pressure ulcers significantly exceed the costs for individuals without pressure ulcers.⁷ The average cost per hospitalization is estimated to be about \$150 000 for the treatment of a pressure ulcer in an individual with SCI.⁴

Complications arising from pressure ulcers are associated with significant morbidity and mortality. Bacterial infection is the most common complication associated with pressure ulcers. Infection of a pressure ulcer may result in soft tissue and bone infections: cellulitis, abscess formation, bursitis, and osteomyelitis of bone underlying the wound bed.⁸ Pressure ulcers are a common source of bacteremia in individuals with SCI.⁹ However, in general, the relationship between bacterial colonization, infection, and wound healing is not well characterized.

To begin to understand the influence of bacteria on the development of disease states, significant effort has focused on identifying the mutualistic microorganisms that reside in the human microbiome. Using standard culture methods, *Corynebacterium* species, *Propionibacterium acnes*, and coagulase-negative *staphylococcus* have been identified as resident flora of the skin.¹⁰ Newer molecular studies have demonstrated that in addition to these commensals, there is a vast complexity of organisms that comprise the cutaneous microflora. Studies using 16sRNA sequencing have shown that the majority of these bacteria belong to the four phyla: Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria.^{11–16} Many of these species had not been associated with the resident flora of the skin and have typically accounted for <1% of the total flora in any distinct area. In a normal host, commensal bacteria are considered non-pathogenic and may beneficially influence the host immune response to serve to decrease colonization of potentially more pathogenic species. For instance, the presence of commensal *Staphylococcus epidermidis* prevents nasal colonization with the more pathogenic species *Staphylococcus aureus*.¹⁷ But if the epithelium is compromised, depending on the type and number of organisms, resident flora may contribute to delayed wound healing or the development of local or systemic infection.^{18,19} Recent areas of research have focused on understanding how communities of bacteria positively and negatively affect wound healing. In diabetic mice with wounds, the temporal shift in

composition of microflora is accompanied by changes in local host immunoregulation.²⁰ This review focuses on the bacteriology of pressure ulcers in individuals with SCI. The aim of this review is to compile available bacteriological data of chronic pressure ulcers in the spinal cord injured. A better understanding of bacterial colonization and wound infection of chronic pressure ulcers may be expected to provide insight into new therapeutic interventions that may prevent or reduce morbidity and mortality.

Methods

Identification of articles

An electronic search of MEDLINE (1966 to February 2014) was performed using the following combination of keywords (“pressure ulcer*” OR “pressure sore*” OR “bedsore*” “decubitus”) AND (“bacter*” OR “culture” OR “micro*”) AND (“spinal cord injur*” OR “?plegia”). Studies chosen were limited to the English language. All titles and abstracts identified by the above search strategy were screened for relevance to the inclusion criteria. If relevance could not be ascertained from the title and abstract alone, the full text of the article was reviewed for possible inclusion. Full texts of articles that were initially considered to be potentially relevant were reviewed for possible inclusion. In addition, cited literature of relevant articles was examined for further meaningful content.

Studies were included if the patient cohort had SCI and if bacteriological data for more than one pressure ulcer was available. Studies with mixed populations were included only if data for >80% subjects with SCI could be identified. Similarly, studies with bacteriological data for treated or manipulated pressure ulcers were included only if culture data were unequivocally from chronic pressure ulcers and not from post-operative wounds in individuals with SCI. Studies were not included if results of bacterial cultures were not adequately specified. Case reports and reviews were excluded from our analysis.

For the purpose of this review, “pressure ulcer” will be term that will be used for other common terms to identify this condition—that is, for pressure sores, pressure ulcers, decubitus ulcers, or bedsores. In addition, the relationship between bacteria and the wound substrate is complex, and considered an ill-defined continuum. This review focuses on bacterial presence within chronic pressure ulcers, and not the identification of bacterial species that are recognized to be potentially pathologic. Studies were included even if they did not present all data on bacterial culture by the willful exclusion of the reporting of the presence of skin commensals.

Study characteristics

From the articles that met the inclusion and exclusion criteria, available data were extracted, including patient number and sex, number of pressure ulcers included in the study, number of pressure ulcers from which bacterial samples were obtained, number of bacterial cultures obtained from pressure ulcers, type of bacterial culture performed, the number of polymicrobial cultures, and type and incidence of bacterial species identified. Information about the pressure ulcers studied was also extracted, such as ulcer stage and size, and whether or not the patient or the pressure ulcer was treated prior to bacterial sampling of the pressure ulcer. In addition, data concerning physical characteristics of pressure ulcers were collected including the presence of osteomyelitis, malodor, tissue necrosis, and exudate.

Due to inconsistencies in the level of speciation within each article, the bacterial species recorded in each study were sorted into broad categories based on the characteristics of cell wall structure, morphology, and cellular metabolism. Using the incidences of bacterial species identified, the relative frequency of each category (aerobic vs. anaerobic, Gram positive vs. negative, cocci vs. rods) was calculated and graphed for each study. Similarly, bacterial species were classified into genera and the frequencies of unique genera were charted. The anatomic sites and frequency of pressure ulcers affecting these sites were recorded; anatomic sites were classified into four main categories: pelvis, buttock/thigh/leg, ankle/foot, and other) and frequencies of pressure ulcers affecting these sites were calculated if the number and physical

location of cultured pressure ulcers were reported in the study.

Results

Literature search

The electronic literature search returned 51 articles. We screened the titles and abstracts of all of these articles for relevance. If an article was considered relevant, the literature cited within the study was also screened for relevance. The eligibility of relevant articles for inclusion in the review was determined by evaluating the full text to ascertain if inclusion or exclusion criteria were satisfied (Fig. 1). A total of 11 studies were identified for inclusion in this review.

Study characteristics

We identified 11 articles with data on species of bacteria cultured from pressure ulcers in the spinal cord injured.^{9,21–30} The characteristics of these studies were tabulated (Table 1), including authors and date of publication, purpose of study, number of patients and ulcers included in the study, number and type of bacterial cultures obtained from pressure ulcers, and number of polymicrobial cultures. Among the articles included in this review, the number of patients with SCI and pressure ulcers that were sampled ranged from 12 to 101 (average 32 ± 26). The number of pressure ulcers sampled ranged from 5 to 47 (average 23 ± 13), and the number of bacterial cultures obtained ranged from 12 to 168 (average 51 ± 59). Among the 11 studies, bacterial sampling methods were not standardized and included needle aspiration, surgical drainage, cotton swab, tissue biopsy, or a combination of these

Figure 1 Flow diagram of the literature review.

Table 1 Characteristics of the 11 studies included in our review that examined the bacteriology of pressure ulcers in individuals with SCI

	Galpin <i>et al.</i> ²³	Vaziri <i>et al.</i> ²⁹	Sugarman <i>et al.</i> ²⁷	Sapico <i>et al.</i> ²⁶	Thornhill-Joynes <i>et al.</i> ²⁸	Montgomerie <i>et al.</i> ²⁵	Biering-Sorensen <i>et al.</i> ²¹	Waites <i>et al.</i> ³⁰	Wall <i>et al.</i> ⁹	Heym <i>et al.</i> ²⁴	Biglari <i>et al.</i> ²²
Study focus	Bacteriology of PU associated with sepsis	Infections in patients undergoing hemodialysis	Osteomyelitis beneath pressure sores	Microbiology of PU in different stages of healing	Evaluation of the frequency of osteomyelitis in patients with SCI and PU	Mortality among patients with bacteremia	Bacterial contamination of bath water	Bacteremia after SCI during hospitalization	Risk factors for mortality from bacteremia in individuals with SCI	Bacteriology of PU and impact on antibiotic therapy	Use of Medihoney as non-surgical therapy for PU
Number of patients	21 (14 M, 7 F)	43 (43 M)	19 (NA)	25 (21 M, 4 F)	40 (35 M, 5 F)	93 (74 M, 19 F)	18 (NA)	59 (48 M, 11 F)	63 (62 M, 1 F)	101 (68 M, 33 F)	20 (13 M, 7 F)
Number of patients with PU	21	27	19	25	40	18	12	NA	36	101	20
Number of PU cultured	47	NA	22	25	35	NA	12	5	21	NA	20
Number of cultures	NA	NA	NA	49	38	20	12	NA	NA	168	20
Sampling method	Needle aspiration, surgical drainage, or cotton swab	Needle aspirate or cotton swab	NA	Cotton swab or tissue biopsy; debridement prior to sampling	Cotton swab	NA	Cotton swab culture	NA	NA	Tissue biopsy, needle aspiration, surgical drainage, or cotton swab; debridement prior to sampling	Cotton swab
Type of cultures	Aerobic and anaerobic	Aerobic and anaerobic	NA	Aerobic and anaerobic	Aerobic and anaerobic	Aerobic and anaerobic	Aerobic	NA	NA	Aerobic and anaerobic	NA
Percentage polymicrobial cultures	NA	55% (≥2 spp.)	16% (1 spp.) 16% (2 spp.) 21% (3 spp.) 31% (4 spp.) 15% (≥5 spp.)	NA	NA	NA	75% (1 spp.) 25% (2 spp.)	NA	NA	69% (≤ 2 spp.) 21% (3 spp.) 8% (≥ 4 spp.)	70% (1 spp.) 15% (2 spp.) 10% (3 spp.) 5% (4 spp.)
Predominant organism (percentage of bacterial isolates)	<i>P. mirabilis</i> (18%)	<i>P. mirabilis</i> (17%)	<i>P. mirabilis</i> (17%), <i>P. aeruginosa</i> (17%)	<i>S. aureus</i> (11%) <i>P. aeruginosa</i> (11%)	<i>S. aureus</i> (16%)	<i>S. aureus</i> (20%)	<i>S. aureus</i> (30%), <i>E. faecalis</i> (30%)	<i>S. aureus</i> (50%)	<i>S. aureus</i> (57%)	<i>S. aureus</i> (23%)	<i>S. aureus</i> (30%)

F, female; M, male; NA, not available; PU, pressure ulcer; spp., species.

techniques. At least seven studies indicated whether aerobic and anaerobic cultures were performed (Table 1).^{21,23–26,28,29}

Bacteriology of pressure ulcers

The anaerobic and aerobic bacterial composition of wounds also seems to be similar among studies that provided bacteriological data for pressure ulcers in patients with SCI. A non-redundant list of unique aerobic and anaerobic bacteria identified in the 11 articles was compiled (Table 2). More unique species of aerobic bacteria (30 unique species) were identified when compared with anaerobic species (22 unique species). The aerobic Gram-negative rod category contained the greatest number of unique bacterial genera (18 unique genera). The relative frequency of isolation of each of the bacterial categories for each study reviewed herein has been provided (Fig. 2). Aerobic Gram-negative rods and aerobic Gram-positive cocci were the bacterial categories most commonly isolated. Anaerobes typically accounted for less than one-third of bacterial isolates (Fig. 2). The percentage of aerobic organisms isolated appears greater for studies performed after the year

2000, but this may merely reflect sampling methods. The predominant bacterial species and frequency for each of the 11 studies have been provided (Table 1). In the earlier studies, *Proteus mirabilis* was the most frequently identified bacterium, but there is a trend for the predominance of *S. aureus* to be increasingly identified from bacterial cultures in later studies.^{9,21–30} A composite of all bacterial genera and the frequency of their identification among all of the 11 studies have been presented (Fig. 3). The most common genus of bacteria identified was *Staphylococcus* (23% of all genera identified) followed by *Proteus* (14% of all genera identified) (Fig. 3).^{9,21–30} Eight studies identified *S. aureus* as the most predominant organism, one of which also isolated an equal number of *Enterococcus faecalis* isolates and another study had an equivalent frequency of *Pseudomonas aeruginosa* (Table 1).^{9,21,22,24–26,28,30} Three other studies had identified *P. mirabilis* as the most common organism, with one report identifying an equal number of *P. aeruginosa*.^{23,27,29} Thus, it appears that the predominant organism identified in pressure ulcers is highly variable, and largely dependent upon the study population.

Table 2 Composite list of unique bacterial isolates identified in chronic pressure ulcers in individuals with SCI among the 11 studies included in our review

	Aerobic bacterial organisms	Anaerobic bacterial organisms
Gram-negative rod	<i>Acinetobacter anitratus</i> <i>Acinetobacter baumannii</i> <i>Acinetobacter calcoaceticus</i> <i>Citrobacter diversus</i> <i>Enterobacter cloacae</i> <i>Escherichia coli</i> <i>Flavobacterium</i> spp. <i>Haemophilus</i> spp. <i>Klebsiella oxytoca</i> <i>Klebsiella pneumoniae</i> <i>Morganella morganii</i> <i>Proteus mirabilis</i> <i>Proteus rettgeri</i> <i>Proteus vulgaris</i> <i>Proteus</i> spp., other <i>Providencia stuarti</i> <i>Pseudomonas aeruginosa</i> <i>Pseudomonas</i> spp., other	<i>Bacteroides assacharalyticus</i> <i>Bacteroides corrodans</i> <i>Bacteroides distasonis</i> <i>Bacteroides fragilis</i> <i>Bacteroides Group 3452A</i> <i>Bacteroides mealaninogenicus</i> <i>Bacteroides oralis</i> <i>Bacteroides thetaiotaomicron</i> <i>Bacteroides uniformis</i> <i>Bacteroides urealyticus</i> <i>Bacteroides vulgatus</i> <i>Eggerthella lentum</i> <i>Fusobacterium necophorum</i> <i>Fusobacterium nucleatum</i>
Gram-positive cocci	<i>Enterococcus faecalis</i> <i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i> <i>Streptococcus</i> Group A <i>Streptococcus</i> Group B <i>Streptococcus</i> Group D (non-enterococcal) <i>Streptococcus</i> Group G <i>Streptococcus</i> Group F <i>Streptococcus viridans</i> <i>Streptococcus intermedius</i>	<i>Clostridium bifermentans</i> <i>Clostridium clostridiiforme</i> <i>Clostridium difficile</i> <i>Microaerophilic streptococcus</i> spp. <i>Peptostreptococcus assacharolyticus</i> <i>Peptostreptococcus magnus</i> <i>Peptostreptococcus prevotii</i>
Gram-positive rod	<i>Corynebacterium</i> spp. <i>Bacillus</i> spp.	<i>Lactobacillus</i> spp.

*Other organisms isolated included *Candida* spp.

Figure 2 Percentages of bacterial type identified in chronic pressure ulcers in individuals with SCI for each study of the 11 publications included in our review. GPC, Gram-positive cocci; GPR, Gram-positive rod; GNR, Gram-negative rod.

Only five studies indicated whether or not bacterial cultures grew more than one species of bacteria, or were polymicrobial (Table 1).^{21,22,24,27,29} Of the limited number of studies that provided detailed data on the microbial complexity of pressure ulcers, 10–21% of bacterial cultures grew more than three bacterial species per culture.^{22,24,27} In this review, three studies provided data from which the average number of organisms cultured per wound could be calculated. Biglari *et al.*²² identified 1.5 bacterial species per wound, Biering-Sorensen *et al.*²¹ cultured 1.25 bacterial species per wound, and Sugarman *et al.*²⁷ identified 3.3 bacterial species per wound.

Pressure ulcer characteristics

The percentages of pressure ulcers sampled from a particular anatomic region have been provided (Fig. 4). Of the six studies that were reviewed which included data of the anatomic location of the pressure ulcer, more than half of all pressure ulcers were located in the pelvic region (i.e. sacral, ischial, perineal, and trochanteric regions).^{21–23,26–28} The next most common area was the buttock and/or legs. Data concerning the physical characteristics of the sampled pressure ulcers have been provided (Table 3). There is a paucity of data concerning the physical characteristics of the pressure ulcers sampled among the studies included in this review. Six

Figure 3 Bacterial species as a percentage of total bacteria identified in chronic pressure ulcers in individuals with SCI among the 11 studies included in our review.

Figure 4 Frequency of anatomic site affected by chronic pressure ulcers in individuals with SCI from six studies that detailed anatomic location among the 11 studies included in our review.

studies included at least one wound characteristic for the pressure ulcers sampled,^{21–23,26–28} with only three studies reporting pressure ulcer size and^{21,22,26} one study reporting pressure ulcer staging.²²

Discussion

This study reviews the available bacterial culture data available for pressure ulcers in the SCI population. Bacterial constituents of pressure ulcers in individuals with SCI are quite varied and can include both aerobic and anaerobic bacteria. Fifty-two unique species of bacteria were identified (22 unique genera), of which almost half were anaerobic (Table 2).^{9,21–30} This number of genera may underestimate the quantity of unique bacteria due to variations in anaerobic culture methods and inability to grow fastidious organisms. Many organisms have complex nutritional growth requirements, and often are not identified using traditional culture methods. Fewer than half of studies in this review performed both aerobic and anaerobic cultures, and the majority did not detail specific methods of the culture technique employed. A prior study that identified bacteria in chronic pressure ulcers in individuals without SCI using traditional culture methods isolated an average of 1.2–5.8 organisms per culture.³¹ This is similar to the average of 1.25–3.3 organisms per culture identified from pressure ulcers in the spinal cord injured. More recently, studies of chronic wounds employing molecular techniques have demonstrated that microbiomes of wounds are more complex than previously determined.^{32–34} Using molecular techniques, Dowd *et al.*³⁵ found that the bacterial

population in pressure ulcers in individuals without SCI is represented by 28 distinct genera and mainly comprised by obligate anaerobes (62%). Price *et al.*³² used large-scale sequencing to identify an average of 10 bacterial families that had residence in a chronic wound, which is several times greater than estimated by traditional culture methods. Compared with traditional culture methods, molecular methods may permit more comprehensive analysis of bacterial populations in wounds.

Although the most predominant organism was found to be different among reports, it should be appreciated that the most prevalent bacterial species actually varied relatively little among the bacteria isolated from chronic pressure ulcers. Prior studies evaluating the microbiology of pressure ulcers in the general population have identified *S. aureus*, *S. epidermidis*, *P. mirabilis*, and *P. aeruginosa* as common bacterial species identified from ulcers.^{36–38} In an extensive clinical compilation of 2500 cases of chronic pressure ulcers in patients with SCI, El-Toraei *et al.*³⁹ identified the following bacteria and their prevalence: *S. aureus* (35%), *Proteus* spp. (19%), *P. aeruginosa* (13%), *Escherichia coli* (10%), *Acinetobacter* spp. (8%), *Providencia* spp. (6%), *Klebsiella* spp. (4%), and other species (5%). These findings are reflected in all of the studies reviewed herein that examined the bacteriology of pressure ulcers in individuals with SCI. Resident bacterial flora may account for the predominance of enterobacteria in chronic pressure ulcers of individuals with SCI. Due to skin breakdown, chronic wounds can be colonized by microbial flora that may originate from the external

Table 3 Wound characteristics among studies examining pressure ulcers in individuals with SCI from the 11 studies included in our review

	Galpin <i>et al.</i> ²³	Vaziri <i>et al.</i> ²⁹	Sugarman <i>et al.</i> ²⁷	Sapico <i>et al.</i> ²⁶	Thornhill-Joynes <i>et al.</i> ²⁸	Montgomerie <i>et al.</i> ²⁵	Biering-Sorensen <i>et al.</i> ²⁸	Waites <i>et al.</i> ³⁰	Wall <i>et al.</i> ⁹	Heym <i>et al.</i> ²⁴	Biglari <i>et al.</i> ²²
Presence of osteomyelitis	8 individuals (38%)	NA	3 individuals (16%)	8 individuals (32%)	25 PU of 38 sampled in 24 patients (66%)	NA	1 individual (8%)	NA	NA	NA	NA
Presence of malodor	13 individuals (62%)	NA	NA	8 individuals (16%)	NA	NA	NA	NA	NA	NA	NA
Presence of tissue necrosis	18 individuals (86%)	NA	NA	17 individuals (68%)	NA	NA	NA	NA	NA	NA	5 individuals (25%)
Presence of exudate	NA	NA	NA	NA	13 PU sampled in 24 patients (62–68%)	NA	5 individuals (42%)	NA	NA	NA	NA
Ulcer staging performed	NA	NA	NA	Arbitrary categorization of healing stages	NA	NA	NA	NA	NA	NA	5 individuals (25%) with grade IV
											15 individuals (75%) with grade III
Ulcer size	NA	NA	NA	Ave 50 cm ²	NA	NA	5 mm to 6 cm undermining	NA	NA	NA	Average 21.7 cm ²
				Range 4–225 cm ²							Range 8–80 cm ²
Treatment of PU before sampling	62% of patients receiving antibiotics	NA	26% of patients received antibiotics prior to sampling	38% of tissue biopsies obtained while receiving antibiotics; 47% of tissue biopsies obtained while receiving local wound care	NA	NA	NA	NA	NA	Patient received antibiotics after PU sampling	Patient received local wound care with Medihoney after PU sampling

NA, not available; PU, pressure ulcer.

environment, contiguous skin, gastrointestinal tract, and/or the urogenital mucosa.

In general, chronic pressure ulcers are colonized and infected with normal cutaneous and enteric commensal bacteria, but the bacterial constituents of microbial flora can change depending on host immunity and environmental factors. Individuals with SCI are more likely to develop Gram-negative bacteria as part of their normal flora as compared to individuals without SCI. Factors that may influence Gram-negative bacteria colonization of the perineum include the presence of neurogenic bladder dysfunction, external condom catheter use, changes in skin pH, and bacteriuria.⁴⁰⁻⁴³ Studies of skin flora in the male genital region have identified several bacterial isolates: *Pseudomonas* spp., *Klebsiella* spp., *E. coli*, *Acinetobacter* spp., *Proteus* spp., *Enterobacter* spp., and *Citrobacter freundii*.⁴⁰ In a study of genital skin flora, Gram-positive cocci and diphtheroids accounted for 93.7% of bacterial isolates from the skin in controls, whereas Gram-negative enteric organisms and enterococci accounted for 54% of bacterial isolates from males with SCI.⁴³ In particular, the perineal region in individuals with SCI is more likely to become colonized with *Pseudomonas* and *Klebsiella*, with rates of colonization ranging from 20 to 80%.^{40,44-48} Colonization with *Klebsiella* has been shown to persist as long as 55 days and for *Pseudomonas*, 120 days during hospitalization.^{45,49} These prior described changes in skin flora may be anticipated to affect the bacterial composition of pressure ulcers in individuals with SCI.

Overall changes in the bacteria isolated from chronic pressure ulcers may reflect changes in the microbiome of the skin. In the earlier studies in this review, *P. mirabilis* was more commonly identified, but studies after 1986 started to identify *S. aureus* more often. The predominance of *S. aureus* may be due to the increasing prevalence of methicillin-resistant *S. aureus* (MRSA) in the community. An emerging pathogen, MRSA has become ubiquitous in the hospital and community settings. MRSA has been identified as the most common multi-drug-resistant organism colonizing patients with SCI.⁵⁰ Individuals with SCI are at increased risk of becoming colonized or infected with MRSA because they often require lengthy hospital stays and are routinely transferred between skilled nursing facilities and rehabilitation centers where nosocomial transmission more frequently occurs.^{51,52} Community-acquired MRSA is becoming more prevalent than MRSA acquisition from nosocomial transmission.^{53,54} Previously, rates of colonization varied widely, and ranged from <5 to >70% of individuals with SCI in a hospital

setting or specialized SCI center.^{53,55} In 2013, MRSA was identified in 38.6% of individuals with SCI within 48 hours of admission to an acute care facility.⁵⁶ In those with SCI and MRSA, the bacterium may typically be isolated from three or more body sites, but most frequently from pressure ulcers.^{50,51,54} Wounds that are colonized with MRSA may remain colonized despite antibacterial treatment and other decolonization measures.^{54,57} Not only has MRSA colonization been associated with prolonged hospital stays for individuals with SCI, but also the predisposition to the development of pressure ulcers and nosocomial infections.^{9,50,54,58} Because of the prevalence of MRSA in the community and among individuals with SCI, the identification of MRSA in pressure ulcers will likely only increase over time.

Chronic wounds are complex, and typically contain more than one bacterial species. Three of the studies reviewed herein identified >10% of bacterial cultures that grew more than three bacterial species per culture. Thus, molecular techniques may also identify larger numbers of distinct bacteria from wounds as compared to conventional culture methods and, as such, application of these more sophisticated techniques may provide a useful clinical tool in the analysis of wound flora of chronic pressure ulcers in the SCI population. However, standardization is lacking in how bacterial cultures are obtained from wounds, and differences in bacterial sampling may occur as a result. In general, the methods of bacterial identification vary and include superficial and deep tissue biopsy, curette, superficial and deep swab culture, needle aspiration, surgical drainage, and molecular studies. Traditionally, aerobic and anaerobic bacterial cultures of deep tissue biopsies have been considered the gold standard for the identification of wound bacteria. Several studies have suggested that the swab technique, such as the Levine technique, in which a cotton Q-tip twirled for 5 seconds on an area of an open wound measuring at least 1 cm² in size with sufficient pressure to incite minimal bleeding of the underlying wound bed, may be considered a viable sampling alternative to tissue biopsy.⁵⁹ In a prior review of five studies in which swab technique was described and compared with tissue biopsy, the swab technique yielded a sensitivity of 93.5–100%, and a specificity of 76.3–94.2%.⁶⁰ The studies reviewed herein mostly used swab cultures, although several researchers used a combination of sampling methods, including needle aspiration, surgical drainage, and/or tissue biopsy. Only two of the studies of SCI cohorts that we reviewed addressed rates of concordance between the two sampling methods.^{24,26} In a study that examined 25 chronic

pressure ulcers in 25 patients with SCI during different stages of healing, the mean quantitative concordance of swab cultures to biopsy cultures was 74.5%.²⁶ Whereas in a comparison of bacterial cultures from chronic pressure ulcers in individuals with SCI obtained by swab culture on admission and tissue sampling at the end of surgical intervention, only 28 (36%) of the 77 patients had similar bacterial cultures.²⁴ In both of these studies, the ulcer bed was debrided prior to tissue biopsies, a technique that would be anticipated to decrease the number of unique bacterial isolates due to removal of bacterial colonizers.

There appears to be a trend for less anaerobic organisms being cultured over time, but this may merely be a reflection of variability in culture methods employed. Another possibility to consider is that differences exist in the location and physical characteristics of pressure ulcers being sampled among the different studies comprising our review. The association of enteric bacteria with pelvic wounds would suggest that the presence of particular microbes in a pressure ulcer depends on the locale of the affected site. Previous studies that have examined the bacterial composition on normal intact skin have shown that the presence of certain bacteria differs widely among different sites of the body.^{61,62} A recent 16S rRNA gene sequence analysis of the microbial diversity among 20 distinct skin sites of healthy humans demonstrated that physiologically similar sites (sebaceous, moist, and dry) retain comparable bacterial communities but that these communities demonstrated significant variation in composite species.^{15,16,63} How these bacterial communities influence the bacterial colonization of chronic wounds is yet to be determined.

Although an individual with an SCI may develop an ulcer in any location, chronic wounds have a predilection for certain sites, which tend to depend upon the duration of injury. Early after becoming paralyzed, the anatomic areas involved are often the sacrum, trochanters, ischia, heads of fibula, and calcaneal prominences. In those with chronic SCI, the most common region for pressure ulcers is the pelvis; however, in those individuals with increased spasticity, ulcers will also develop with increased frequency in the areas of patellae, medial tibial prominence, and medial malleoli.⁶⁴ Of the six studies discussed in this review that provided the locations of the pressure ulcers, only one study provided bacterial data and site of location for pressure ulcers, but the limited data in this one study does not allow for any conclusion to be drawn concerning prevalence of bacteria based on anatomic location (data not shown).²² Thus, the extent to which normal

flora of adjacent sites impact the microbiota within a wound based on pressure ulcer location has not been established.

Regardless of the location of the pressure ulcer, microbial diversity within an individual wound is also largely unknown. In an evaluation of tissue culture from opposing rims in 11 chronic wounds and from the center of 8 of these wounds, sampling yielded the same organisms in 90–94.7% of the wounds.³³ In a study of chronic venous leg ulcers from 14 skin graft operations, quantitative polymerase chain reaction demonstrated large variations in bacterial numbers, and molecular methods demonstrated differences in bacterial species identification based on spatial distribution within a wound, especially with regard to anaerobic pathogens, which were not detected by routine anaerobic culture methods.⁶⁵ Only one study involving pressure ulcer healing in individuals with SCI examined spatial differences in bacterial composition by location within a wound; sampling a pressure ulcer from the periphery and the center had a mean quantitative concordance of 63%.²⁶ The difference in culture findings demonstrates that variability exists in cultures obtained from different areas of the same ulcer. This is not surprising given that fluorescent *in situ* hybridization techniques have determined that structural organization of bacteria within the center of a chronic wound is non-random and heterogeneous: *P. aeruginosa* aggregates as microcolonies in a polymicrobial biofilm, whereas *S. aureus* localizes to the surface of the wound.⁶⁶ These findings would suggest that there are spatial differences and microenvironmental factors that influence microbial diversity.

Other factors, in addition to wound location and associated microflora, may have the potential to affect bacterial composition of pressure ulcers. For instance, location of a pressure ulcer may not have as much impact on the bacterial colonizers as the physical factors of the wound. Epidemiological studies have shown that bacterial species in a wound differ as a function of the type of chronic wound. Depending on whether the etiology of a wound is due to underlying venous disease, diabetes, or pressure, the ratio of aerobic to anaerobic bacteria varies.³⁵ Sapico *et al.*²⁶ demonstrated that the ratio of anaerobic to aerobic bacteria in 25 patients with SCI who had chronic pressure ulcers was correlated with the amount of tissue necrosis in the wound; although comparable numbers of aerobic and anaerobic bacteria were isolated from pressure ulcers with tissue necrosis, the presence of anaerobes significantly decreased as the ulcer healed and malodor abated. *Bacteroides* spp., *E. coli*, *Proteus* spp., group D

streptococci, and anaerobic streptococci were the most prominent organisms in pressure ulcers with necrotic tissue, whereas *P. aeruginosa* and *S. aureus* were most frequently isolated from healing wounds.²⁶

Other than tissue necrosis, specific wound characteristics that may be predictive of certain microbial organisms or communities have not been identified but may include the moistness or aridity of a wound. For instance, Gram-negative bacilli do not typically colonize intact epithelium due to the aridity of the skin.⁶⁷ In all of these studies examining microbial species in pressure ulcers affecting individuals with SCI, the wounds are typically poorly characterized with no physical description (i.e. dimensions, the presence of necrosis, etc.) provided. In addition, the impact of local and systemic antibiotics in conjunction with local wound care on bacterial composition has not been determined. Thus, future studies are needed to assess how wound characteristics, the potential development of resistant pathologic strains with systemic antibiotic usage, and routine or more experimental approach to wound care impact the microbiome of pressure ulcers in patients with SCI.

Bacterial composition of pressure ulcers may vary depending on concomitant soft tissue or bone infections. In a study of infected pressure ulcers (purulent exudate present), the most common organisms identified were Enterobacteriaceae, *Pseudomonas*, *Bacteroides*, and *Streptococcus faecalis*, with *Staphylococcus* less frequently identified.⁶⁸ Gram-negative bacilli and anaerobes (fecal flora) were most commonly identified in infected pressure ulcers with concurrent soft tissue abscesses and osteomyelitis.^{69,70} Necrotizing soft tissue infections (i.e. myonecrosis, Fournier's gangrene, necrotizing fasciitis) are characterized by fulminant and progressive necrosis of fascia, subcutaneous tissue, and/or muscle often with systemic signs of sepsis. Common characteristics between necrotizing soft tissue infections in individuals with and without SCI are that the perineal region is most often affected, and the infection is typically polymicrobial with as many as four different species of bacteria identified by routine bacterial methods.⁷¹⁻⁷⁴ In individuals with necrotizing fasciitis without SCI, streptococcal species was the most common bacteria identified, which were followed by *Bacteroides*, staphylococci, and enterococci.⁷³ Similarly, in studies of individuals with SCI with necrotizing fasciitis or Fournier's gangrene, streptococci was most commonly identified.⁷⁵ However, *P. aeruginosa* was also commonly identified in individuals with necrotizing fasciitis and SCI, an organism not typically identified in individuals without SCI.^{71,76} Individuals with grade III and grade IV pressure ulcers, characterized

by full thickness tissue loss and the presence of non-viable tissue, are at increased risk of developing necrotizing soft tissue infections.^{75,76}

Often, pressure ulcers associated with osteomyelitis are cultured positive for Gram-negative bacilli and anaerobes.^{27,70} However, bone cultures may differ significantly from wound cultures obtained from the overlying pressure ulcers. In another study of pressure ulcers associated with osteomyelitis, Sugarman⁷⁷ found that bone cultures often contained fewer organisms than wound culture; the organisms identified from bone cultures were frequently different than those identified from cultures obtained from the surface of the pressure ulcer, with these cultures found to be concordant in only 25% of the cases. This work strongly indicates the need for bone biopsies to be performed surgically under sterile conditions to differentiate skin flora and/or the more superficial infection from the offending organism that is responsible for the osteomyelitis, which will then ensure initiation of the appropriate antibiotic coverage.

There are many factors that may influence the microbial ecology of chronic wounds. The impact of each of these factors is difficult to assess given that each study has varying aims and methodologies. The ability to compare the studies presented in this review was limited due to lack of wound description, including those of size, location, presence of necrosis and/or malodor, underlying osteomyelitis, and staging. In addition, sampling, bacteriological, and reporting methods were not standardized. These studies also differed in the number of cultures per ulcer procured and the number of wounds studied per patient. The data were, in part, skewed due to differences in the aims of the studies, and thus differences in methodology employed (i.e. bacteriology of pressure ulcers were reported only if congruent with blood cultures in bacteremic patients, exclusion of the reporting of skin commensals, lack of anaerobic data, as well as other factors). However, despite these limitations, it is apparent that our understanding of the wound microbiome in pressure ulcers of individuals with SCI is rudimentary.

Conclusions

In general, the characterization of bacterial presence as colonizing bystanders vs. invasive pathogens that impede wound healing in chronic pressure ulcers is not well defined. Depending on the bacterial species present in a wound, both beneficial and detrimental effects have been associated with wound colonization.¹⁹ In addition, the concept of bacterial pathogenicity is evolving from identification of a single species of bacteria that cause disease to defining microbial ecologies

that influence health states, although the clinical value of this more global microbiological approach to pressure ulcer care has not been proven. Previously standard culture methods have been used to define the microbial environment of wounds, but typically only several species of bacteria are identified using this methodology due to inherent limitations of growing bacteria in cultures. Unfortunately, <1% of bacterial species can be identified by culture technique using routine laboratory conditions.⁷⁸ Recent molecular studies have shown that the microbiome of wounds is far more complex than previously thought.^{32,35,79–81} With the advent of molecular analysis, vast numbers of different bacterial species are being identified within a single wound.

There are many questions that still remain to be addressed. Does the diversity of the cutaneous microbiome influence the microbial ecology and pathogenicity of a wound? Do certain populations of bacteria provide any benefit to the healing of a wound, i.e. probiotics? How does the complexity of bacterial environment influence the local host immune responses? Are the physical characteristics of a wound and/or its anatomic location predictive of a predominant organism, or vice versa? Can identification of a wound's microflora predict concomitant or future development of soft tissue or bone infection? Studies using molecular methods to identify the microbial diversity of pressure ulcers in correlation with wound physical characteristics, anatomic location, and stage of healing are needed to understand the impact of different species of bacterial colonization.

The role of routine bacterial culture is unclear given the lack of standard methods of collection and interpretation of culture data. Understanding changes in the microbiome of a wound before and after systemic antibiotics or surgical debridement will help to identify successful treatment approaches to wound healing. Does the administration of topical and/or systemic antibiotics adversely affect the microbial flora in a wound with the emergence of antibiotic-resistant organisms that then have deleterious impact on wound healing? For instance, Price *et al.*³² demonstrated that recent antibiotic use was associated with increased wound colonization with *Pseudomonas* bacteria. Depending on the answers to these questions, the clinical approach to chronic wounds may change concerning appropriateness of the use of topical anti-microbial agents and/or systemic or local antibiotic therapy, aggressiveness of debridement (available evidence would suggest the vigorous removal of necrotic tissue), and the application of various physical adjunctive therapies, such as negative pressure, electrical stimulation, ultraviolet or laser, hyperbaric oxygen, or ultrasound.

A more comprehensive evaluation of pressure ulcers and the bacteria that inhabit the wounds in individuals with SCI is needed to improve our insight into the effect of colonization or pathogenic bacteria as it relates to the rate of wound healing and associated complications, as well as consideration of the effect of current approaches to wound care on microflora and the generally recognized pathogenic species of organisms.

Disclaimer statements

Contributors All authors contributed equally.

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