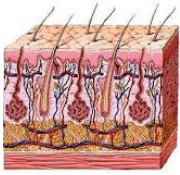




Skin and Soft Tissue Infections (SSTI) Panel



The skin is the largest organ of the body, creating a protective barrier to prevent entry of pathogenic particles. While this physical barrier serves as the first line of immunologic defense, the skin also supports a complex microbial ecosystem comprised of a number of bacterial species, primarily the Gram-positive Staphylococci, Micrococci, Corynebacteria, and Propionibacteria, and the Gram-negative

Acinetobacter (1, 2). These microbial species are referred to as commensal organisms because they can exist harmoniously and non-pathogenically on the skin so long as the integrity of the skin is maintained. These resident bacteria also serve to protect their host by competing out other, more pathogenic bacteria through a number of methods (2). Any breach of this physical barrier could alter this relationship, allowing these commensal and other pathogenic bacteria to gain entry to and serve as pathogenic agents within the underlying tissue. Skin damage results in a number of ways, including dermatologic irritations, burns, lacerations, abrasions, infection, blunt force trauma and surgery.

All wounds have the potential to become infected. In large part the chances increase with anatomic site. The closer the wound is to the anatomic sites known to serve as reservoirs of both aerobic and anaerobic bacteria, namely the respiratory and gastrointestinal tracts, the greater the chance of infection onset. The SENTRY Antimicrobial Surveillance Program has been recording information on and ranking pathogens isolated from skin and soft tissue infections (SSTIs) serious enough to require hospitalization. Within the United States the causative agents have been fairly static since this program was initiated, with *Staphylococcus aureus* accounting for almost 50% of all analyzed cases (3). A summary of seven years of analyses (1998-2004) ranks the leading ten pathogens detected in 5,837 cases of SSTIs (Table 1). A subsequent report compared molecular techniques with those of culture with regard to identifying pathogens associated with various wounds. They identified limitations associated with culturing methodologies that may skew the detection rates of some bacterial species, most notably the strict anaerobes, thus adversely affecting treatment regimens (4). In this analysis, which used sequencing to identify pathogens within a number of samples, a host of difficult-to-culture, bio-film associated anaerobic genera were identified from three different forms of skin ulcers (4, 5, 6). Medical Diagnostic Laboratories (MDL) has developed a panel of tests including the most frequently detected pathogens associated with surgical site infections (SSIs) and SSTIs to aid physicians in the diagnosis and proper treatment of a host of wound types.

Table 1. Incidence rates of pathogens isolated from SSTI and SSI within the United States as reported by the SENTRY Antimicrobial Surveillance Program and the CDC's Guidelines for Prevention of Surgical Site Infection. Both reports summarize infectious rates over a seven year time frame. ND indicates "not determined" in that study (3, 7).

Pathogenic Agent	Detection in SSTIs (N = 5,873)	Detection in SSI (N = 17,671)
<i>S. aureus</i>	2,602	3,534
<i>P. aeruginosa</i>	648	1,414
<i>Enterococcus</i> species	542	2,121
<i>E. coli</i>	422	2,121
<i>Enterobacter</i> species	282	1,237
<i>Klebsiella</i> species	248	530
Streptococci	237	884
<i>P. mirabilis</i>	166	530
CoNS	161	2,474
<i>Serratia</i> species	125	ND
<i>Bacteroides fragilis</i>	ND	353
Other Gram Positive Aerobes	ND	353

Staging of Wounds

Wounds are classified according to their degree of severity on a four point scale (8). **Stage 1** is typified by alteration in skin color, texture and temperature with no overt lesion present and does not involve the dermis. **Stage 2** describes instances whereby the epidermal and dermal layers of skin are breached as a result of a tear in the epidermal layer that can include infection and tissue necrosis; drainage at the site is not an uncommon characteristic. **Stage 3** wounds are distinguished from Stage 2 wounds based on the depth of the trauma. In these instances, the damage extends to, but no further than, the subcutaneous fat layer of the skin. **Stage 4** wounds are by far the most serious, affecting both bone and muscle as well as supporting structures. Necrosis and drainage are extensive.

Ulcers

The term ulcer is ascribed to any slow healing, open sore on the surface of the skin or mucous membrane that is accompanied by tissue loss, disintegration and necrosis. Diabetic foot, venous and pressure ulcers are three common ulcer types routinely seen clinically.

Diabetic Foot Ulcers

According to the 2011 Diabetes Fact Sheet published by the American Diabetes Association (9), there are currently 25.8 million Americans, 8.3% of the current population, suffering from Diabetes Mellitus. Within this population approximately 15%, or 4 million individuals, will develop foot ulcers, 6% of which will require hospitalization for infection and complication (10). Statistical analysis of medical costs within the United States in 2007 report a total of \$116 billion dollars in direct costs spent for the treatment of diabetes, with 33% (\$38 billion) of this amount used specifically for the care and treatment of foot ulcers (11). These numbers are staggering when you consider the number of newly diagnosed cases continues to rise.

Diabetic foot ulcers are multifactorial in origin, the result of a host of biological issues brought about by uncontrolled blood sugar levels. First, diabetics are more prone to infection as a result of changes in their skin, leaving it dry and susceptible to cracking and fissuring. Diabetics also experience atrophy of the small muscles of the foot leading to structural deformity which results in increased amounts of pressure on the ball and heel of the foot leading to accelerated callous formation. The diabetic callous differs from that of the non-diabetic population in that they are often much thicker and more prone to cracking, infection and ulcer formation (Figure 1) (12). This increased pressure leads to accelerated callous formation which, if not properly attended, can readily become ulcers in the diabetic patient. Compounding matters are two other anomalies associated with diabetes, sensory neuropathy and vascular disease. Diabetic neuropathy describes the sensory nerve damage that hinders a person's ability to feel their extremities, allowing them to unknowingly continue on with their daily routines despite being injured. Poor circulation to the extremities as a result of narrowing and hardening of blood vessels complicates matters further by increasing the level of cell death and decreasing the numbers of immune mediators capable of trafficking to the affected areas. Ulcers detected early are highly treatable and curative as they are often infected with aerobic, Gram-negative cocci. As the infection progresses and the ulcer grows deeper, the infections predominantly become polymicrobial in nature and often limb threatening (12). Prompt and proper treatment is essential in order to save the affected toe, foot or leg from amputation. Despite physicians' best efforts, almost 66,000 lower-limb amputations were performed in 2006 (9).





Figure 1. An image of a diabetic foot ulcer that initiated from a thickened callous. (13)

Pressure Ulcers

Pressure ulcers, also known as bed sores, typically arise as a result of prolonged and persistent pressure applied to the skin, usually developing at bony areas like the hips, heels, elbows, shoulders, back and back of the head. Friction and shear forces also play parts in wound development as the skin is often pulled unnaturally either as the result of a caregiver repositioning an individual by dragging them across a surface or the repositioning of a mechanical bed that allows the individual to slide along a surface (14,15). Sores develop as a result of decreased blood flow to the compressed tissue, leading to increased cellular death and often develop quickly, making them difficult to manage. Those at greatest risk of developing pressure sores have limited mobility and are often unable to shift their position to alleviate the pressure; this includes those confined to a wheelchair or a bed (15). The incidence of developing such sores increases with age, as the skin becomes more fragile, regenerates more slowly and sensory and mental perception begin to wane (15). Diabetics, as a result of their associated vascular issues, are also at greater risk of developing pressure sores. If not treated promptly and properly, serious complications including cellulitis, bone/joint infection, sepsis and even melanoma can occur (15). A joint study performed by the Centers for Disease Control and Prevention (CDC) and National Center for Health Statistics (NCHS) evaluated the incidence rate of pressure sores within nursing home facilities. Their study found 11% of 159,000 nursing home residents had pressure sores and that half of them were classified as stage 2 sores on the Shea Pressure Sore Scale (14).

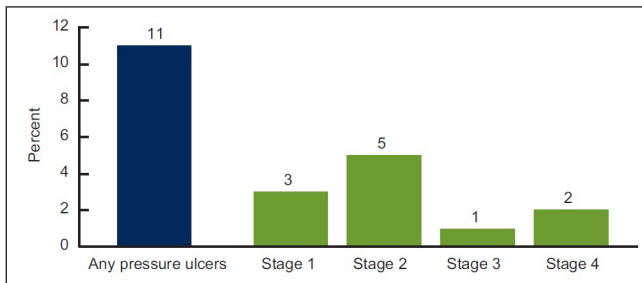


Figure 2. Percentage of nursing home residents with pressure ulcers by stage. (CDC/NCHS National Nursing Home Survey). These values were reported for the year 2004 whereby approximately 159,000 (11%) of nursing home residents were reported to have some form of pressure ulcer. (14)

Venous Stasis Ulcers

Venous stasis ulcers arise as a result of the natural aging process. Bicuspid valves within leg veins begin to lose their elasticity, allowing blood to pool. Individuals with deep vein thrombosis and edema have a sedentary lifestyle or who are obese are at greater risk of developing these valvular issues. The loss of valvular function leads to increased venous pressure which puts more strain upon the affected valves, damaging them further, and elicits an immune response that affects tissues within these regions.

Compression of the affected area to minimize edema and swelling is the primary form of treatment (16).

Wound Repair

What makes the skin unique is its ability to repair itself and recover from these various insults. Tissue repair and remodeling is a multistep process that is orchestrated by a number of key cell types and factors working in concert with one another to seal-off the site in order to localize infection, decrease blood loss and initiate cellular replication to regenerate the damaged tissue. Typically, these events occur as a continuum of overlapping stages, some lasting only a few hours and others potentially for several years. Initiation begins with the process of Hemostasis, typically not considered an actual stage in the process, then to Inflammation (early), transitioning into the Proliferation Stage (intermediate) and culminating with Maturation (late) (Figure 3) (17).

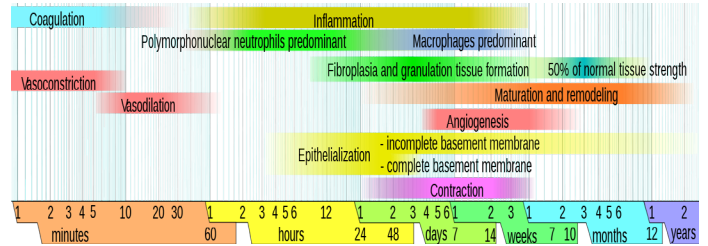


Figure 3. The stages and duration of the wound repair process. Adapted from (17).

Pathogens Often Isolated from SSTIs and SSIs

Pathogen association with various SSTI and SSI is often dictated by the anatomic location; the infectious agent is typically considered normal flora within that region. For the first phase MDL has selected the following pathogenic agents for inclusion in the Skin and Soft Tissue Infection panel.

Bacteroides fragilis: Bacteroides are Gram-negative, anaerobic bacilli associated with a number of different types of infections that are typically polymicrobial in nature. Anatomic sites affected include the central nervous system, head, neck, chest, abdomen, pelvis, skin and soft tissue. Due to their fastidious growth requirements, *Bacteroides* species are extremely hard to identify by culturing methodologies and, as a result, are believed to be under reported pathogenic agents (18). *B. fragilis* is considered normal flora of the gastrointestinal tract and is commonly associated with SSI of the abdomen and abscesses.

E. coli: *E. coli* are Gram-negative, facultative, rod-shaped bacteria that naturally inhabit the gastrointestinal tract. Outside their normal environment, *E. coli* can cause infection, particularly within the urinary tract. They are also associated with skin infections in regions in close proximity to the rectum, particularly with incontinent individuals. Individuals undergoing surgical procedures associated with the gastrointestinal tract and lower regions of the spine are also at risk of contracting infection (19, 20, 21).

Klebsiella oxytoca & Klebsiella pneumoniae: *Klebsiella* species are Gram-negative, facultative rods that colonize the skin and gastrointestinal tract. These opportunistic pathogens are a leading cause of nosocomial infections second only to *E. coli*, and account for 8% of all hospital-acquired infections (19, 21, 22). Such infections typically arise within the respiratory, biliary and urinary tracts as well as surgical sites. The ubiquitous nature of these bacteria, in combination with increased treatment with broad-spectrum antibiotics, has led to the development of resistant strains. Two species, *K. pneumoniae* and *K. oxytoca*, account for the majority of infections with *K. pneumoniae* serving as an important cause of community-acquired pneumonia in the elderly and *K. oxytoca* more commonly associated with UTIs.

Prevotella species: *Prevotella* species are Gram-negative, anaerobic bacilli that colonize the vaginal and oral cavities. Depending on their anatomic location, these bacteria cause a wide-range of infections. Oral

cavity colonization is associated with sinus and periodontal infections, peritonsillar abscess and pneumonia, while those colonizing the GI tract have been isolated from cases of peritonitis, intra-abdominal abscess, postoperative wound infections, pelvic inflammatory disease, vulvovaginal and perianal infections. Infections of the soft tissue include gangrene and necrotizing fasciitis (23).

Proteus mirabilis: *Proteus* species are a Gram-negative, facultative bacilli that colonize the gastrointestinal tract and are a source of nosocomial infection within hospitals and long-term care facilities (19, 24). Usually associated with UTI, *Proteus mirabilis* has also been isolated from abscesses, SSI, decubitus ulcers and burns (24).

Pseudomonas aeruginosa: *Pseudomonas aeruginosa* is a Gram-negative bacillus associated with a number of different opportunistic infections and is particularly problematic for ventilated patients, burn patients and those with chronic debilities (19, 21, 26). Infections of the skin include those affecting the feet and toenails (tinea), hot tub/swimming pool infections (folliculitis) and burn wound sepsis (27). Recently, the ability of *P. aeruginosa* to form bio-films has been postulated as a mechanism for long standing wounds that will not heal. (28).

Streptococcus pyogenes (GAS): GAS is a Gram-negative, coccus that resides harmlessly on the skin as a commensal until the protective skin barrier is breached and it becomes pathogenic. GAS is a causative factor, along with *Staphylococcus aureus*, for impetigo. While impetigo itself is not life-threatening, it can lead to more serious complications, including cellulitis and MRSA affecting the skin and poststreptococcal glomerulonephritis affecting the kidney (29).

Streptococcus agalactiae (GBS): GBS is a Gram-positive coccus that causes a number of serious infections in both pregnant women and adults with underlying health issues, like diabetes mellitus, heart disease and malignancy. Aside from its role in neonatal sepsis, GBS has been associated with infections within the over-seventy years of age group, particularly the bedridden and those afflicted with congestive heart failure, where UTI, pneumonia and soft tissue infections are the most frequent manifestations (19, 21, 30). Streptococci, along with Staphylococci, are the leading causative agents associated with the potentially life-threatening skin infection, cellulitis (30).

Staphylococcus aureus with methicillin resistance screening: *Staphylococcus aureus* is a Gram-positive coccus that is largely considered to be normal flora of the skin. However, upon breach of this protective barrier, Staph can become highly pathogenic, particularly within individuals having chronic disorders such as diabetes, cancer, vascular and lung disease, eczema and individuals with weakened immune systems. Infections of the skin often go untreated as initial infections resemble pimples or spider bites, allowing the infection to progress to greater degrees of severity. Infections are further complicated by the emergence and circulation of methicillin-resistant strains (19, 21, 32).

Clinical Benefits of Testing

MDL offers highly sensitive and specific quantitative Real-Time PCR (qPCR) based assays for the detection of skin and soft tissue infection associated pathogens utilizing the **OneSwab®** platform:

366 **Skin & Soft Tissue Infections (SSTI) Panel by Real-Time PCR** [*Aspergillus fumigatus*, *Bacteroides fragilis*, *Enterococcus faecalis*, *Escherichia coli*, *Fusobacterium* species, Group A Streptococcus, Group B Streptococcus, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, Prevotella Groups 1 & 2, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), Community Associated MRSA (CA-MRSA)]

367 **SSTI Panel Antibiotic Resistance** [*Enterococcus faecalis*, *Escherichia coli*, Group A Streptococcus, Group B Streptococcus, *K. oxytoca*, *K. pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, Community Associated MRSA (CA-MRSA): amoxicillin-clavulanic acid, ampicillin (for *E. faecalis*), cephalothin (cephalexin), clindamycin, doxycycline, trimethoprim-sulfamethoxazole, ciprofloxacin, cefepime, piperacillin-tazobactam, imipenem, gentamicin]

Benefits of this system include:

- Real-Time PCR.
- Simple and convenient sample collection.
- No refrigeration is required before or after collection.
- Specimen stable for up to five days.
- Test additions are available up to 30 days after receipt of the specimen.
- High diagnostic specificity and sensitivity.
- One vial, multiple pathogens.

Antibiotic Treatment for Skin and Soft Tissue Infections.

Skin and soft tissue infections (SSTI), surgical site infections (SSI), and wounds are a major issue of morbidity and mortality in the community and healthcare system (reviewed in 33, 34, 35). Skin or our epidermal layer provides us with a protective barrier between the microbial environment and our sub-dermal tissue, organs, and blood stream. Whenever that barrier is breached by trauma, surgery, or infectious abscess, a strong immune response is triggered to the infecting organism. SSTI, SSI, and wound infections can be caused by a single bacterium or can be polymicrobial depending on the site and length of time of the infection (33). The initial stages of these infections usually involve the Gram-positive *Staphylococcus* and occasionally *Streptococcus*, especially for those infections above the waist. The major cause and ever increasing in number are SSTIs due to methicillin-resistant *Staphylococcus aureus* (MRSA), which are resistant to the β -lactam class of antibiotics (36). Other bacteria in addition to *Staphylococci* and *Streptococci* can cause infections, such as the facultative anaerobic Gram-negative rods *Escherichia coli*, *Klebsiella* species, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, or Gram-positive *Enterococcus*, especially for those infections below the waist although less frequently (33). The enterics, *Pseudomonas*, *Enterococcus*, Gram-positive and Gram-negative anaerobic bacteria are usually present in SSTIs and wounds, such as animal or human bites, lasting for weeks or SSIs, especially those associated with the gut or OB/GYN surgeries (33,34). Chronic wounds lasting weeks can be due to facultative anaerobic bacteria such as *Bacteroides fragilis*, *Peptostreptococcus*, and facultative anaerobic Gram-negative rods such as *Pseudomonas aeruginosa* and *Enterobacter* species. Drainage of the abscess is recommended for uncomplicated SSTI often associated with *Staphylococcus*. Topical, oral (PO), intramuscular (IM), and intravenous (IV) antibiotics are used to treat these infections depending on the clinical presentation and severity of the infections (Table 2) (36). Antibiotics suggested by the Infectious Disease Society of America (IDSA, provider survey, and the Clinical and Laboratory Standards Institute (CLSI) were selected for this antibiotic susceptibility assay (37). Other antibiotics recommended to treat MRSA (non-community-associated MRSA) infections include vancomycin (IV), linezolid (PO, IV), and daptomycin (IV). These antibiotics are not included in the antibiotic susceptibility panel due to very low levels of resistance.

Table 2.



Table 2. Summary of Treatment Options.

Antibiotic	Routes of Administration	Effective	Not Effective
Amoxicillin-clavulanic acid	PO	Staphylococcus Streptococcus ^c Enterococcus Enterobacteriaceae Anaerobes ^d	MRSA ^a CA-MRSA ^b Pseudomonas
Cephalothin Cephalexin	IV PO	Staphylococcus Streptococcus ^c Enterobacteriaceae	MRSA ^a CA-MRSA ^b Pseudomonas Enterococcus Anaerobes
Clindamycin	PO, IM, IV	Staphylococcus CA-MRSA ^b Streptococcus ^c Anaerobes ^d	MRSA ^a Enterobacteriaceae Pseudomonas Enterococcus
Doxycycline	PO	Staphylococcus MRSA ^a CA-MRSA ^b Streptococcus ^c Enterococcus Enterobacteriaceae Anaerobes ^d	Pseudomonas Not for pregnancy or children < 8yrs
Trimethoprim-sulfamethoxazole	PO, IV	Staphylococcus MRSA ^a CA-MRSA ^b Streptococcus ^c Enterobacteriaceae	Enterococcus Anaerobes Pseudomonas
Ciprofloxacin	PO, IV	Staphylococcus Streptococcus ^c (Levofloxacin for Strep.) Enterococcus Enterobacteriaceae Anaerobes ^d Pseudomonas	MRSA ^a CA-MRSA ^b Not for pregnancy
Cefepime	IM, IV	Staphylococcus Streptococcus ^c Enterobacteriaceae Pseudomonas	MRSA ^a CA-MRSA ^b Enterococcus Anaerobes
Piperacillin-tazobactam	IV	Staphylococcus Streptococcus ^c Enterococcus Enterobacteriaceae Anaerobes ^d Pseudomonas	MRSA ^a CA-MRSA ^b
Imipenem	IM, IV	Staphylococcus Streptococcus ^c Enterococcus Enterobacteriaceae Anaerobes ^d Pseudomonas	MRSA ^a CA-MRSA ^b
Gentamicin	IM, IV	Combination antibiotic ^e	

Abbreviations: PO (per OS oral) IM (intramuscular); IV (intravenous); MRSA (Methicillin-Resistant *Staphylococcus aureus*) and CA-MRSA (Community-associated MRSA). Enterobacteriaceae includes enterics such as *E. coli*, *Klebsiella* species, and *Proteus* species.

^a Methicillin-Resistant *Staphylococcus aureus* isolates are resistant to the β-lactam class of antibiotics (e.g. penicillin, ampicillin, amoxicillin, piperacillin, cephalosporins, carbapenems). For hospital-associated MRSA (HA-MRSA or MRSA), vancomycin (IV), linezolid (PO, IV), and daptomycin (IV) are effective. These antibiotics are not included in the panel due to very low levels of resistance.

^b Community-associated MRSA (CA-MRSA), Type IV SCC and Pantone-Valentine Leukocidin positive, unlike HA-MRSA, are often susceptible to other non-β-lactam common antibiotics such as trimethoprim-sulfamethoxazole, doxycycline, and clindamycin.

^c *Streptococcus* species are susceptible to the β-lactam class of antibiotics (e.g. penicillin, ampicillin, cephalosporins, carbapenems). For penicillin allergic patients at risk for anaphylaxis, clindamycin, vancomycin, doxycycline, fluoroquinolones can be alternatives.

^d For anaerobic bacteria, metronidazole is used, often in combination with ampicillin, amoxicillin, cephalosporins with anaerobic activity, and/or fluoroquinolones.

^e For *Enterococcus* spp., combination therapy of ampicillin, penicillin, or vancomycin (for susceptible strains), plus an aminoglycoside, is usually indicated for serious Enterococcal infections, unless high-level resistance to both gentamicin and streptomycin is documented; such combinations are predicted to result in synergistic killing of the *Enterococcus*. High-level gentamicin resistance screening is not performed in this antibiotic susceptibility assay

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