The graphic features a light grey background with several overlapping red and yellow 3D spheres. Two white plus signs are placed on the spheres. The text 'Microbiological assessment of' is in white, while 'INFECTED' is in large, textured yellow 3D letters and 'WOUNDS' is in large, textured red 3D letters. There are also red splatters and a thin red line across the bottom of the letters.

Microbiological assessment of

INFECTED WOUNDS

When to take a swab and how to interpret the results

Identifying and managing infection in wounds is an important aspect of primary care practice. However, many issues relating to the aetiology of infection and the sampling of wounds remain controversial, with limited expert consensus. Most wound infection is diagnosed clinically, with laboratory testing used to provide further information to guide management. It is only necessary to swab a wound if there are clinical signs of infection and the wound is deteriorating, increasing in size or failing to heal. Swabbing a wound that is not infected results in the unnecessary identification and analysis of organisms which are colonising the wound, rather than causing an infection.

Characteristics of a wound

A wound is defined as any injury that damages the skin and therefore compromises its protective function. An acute wound is generally caused by external damage to the skin, including abrasions, minor cuts, lacerations, puncture wounds, bites, burns (heat, cold, friction, chemical) and surgical incisions. A wound is defined as being chronic if it has failed to heal (i.e. achieved anatomical and functional integrity) within three months.¹ The most common type of chronic wound is an ulcer, usually on the lower leg, and usually associated with underlying diabetes or vascular causes.

The aim of good wound care is to promote healing, prevent infection and ideally to achieve a good cosmetic result for the patient.² The immediate treatment of wounds, including dressings and follow-up care, is a crucial aspect of wound management, and is usually undertaken by the Practice Nurse team. The focus of this article is on identifying wound infection and interpreting the results of microbiological analysis of a wound swab.

Wound healing

Wounds heal by either primary closure, as in the case of a clean, fresh wound, with well-approximated edges which are sutured together, or by contraction and epithelialisation, such as for a wound left open due to loss of skin or contamination.² Normal wound healing requires a sufficient supply of blood to the affected tissues. A delay in healing can be caused by a number of factors, both local (related to the wound itself) and systemic (related to the patient and their clinical condition). Many of these factors not only delay healing but increase the likelihood of infection developing in the wound.

Local factors which may delay wound healing include:^{3,4}

- The underlying cause and severity of the wound
- A delay in the patient presenting for medical attention
- The presence of necrotic tissue in the wound – this can promote the growth of bacteria, especially anaerobes
- The presence of foreign bodies in the wound
- Impairment of the local circulation
- The site of the wound, e.g. wounds near the anal area are at increased risk of contamination
- A haematoma or any “dead space” in a wound – this can provide a ideal environment for bacterial growth
- Oedema in the tissues surrounding the wound
- Continued trauma or pressure to the wound site

Systemic factors which may delay wound healing include:^{1,3}

- Predisposing medical condition, e.g. diabetes, which compromises the health of the skin and increases the risk of infection
- Older age
- Obesity
- Smoking
- Poor nutrition
- Immunosuppression associated with either an illness, e.g. AIDS, or medicine, e.g. chemotherapy, corticosteroids.

Colonisation versus infection

All open skin wounds are colonised by bacteria, however, this does not mean that all wounds are infected. Inflammation occurs in all wounds during healing, regardless of whether they are infected, and a certain level of swelling, erythema and increased warmth at the site is normal and should not be confused with clinical infection. When skin is broken, its protective defence mechanisms are impaired, and the environment becomes more conducive for bacteria, which increase in number. These bacteria come from three main sources; the environment (e.g. dust, foreign bodies, bacteria on hands, clothing and equipment), the surrounding skin (normal skin contains colonising bacteria, referred to as commensals) and from the mucous membranes (gastrointestinal, oral and genitourinary).

The significance of biofilms

Several factors determine the progression of a wound from contamination to infection, including the bacterial load, the types of bacteria present and their synergistic action and virulence.^{1,5} Wounds are initially colonised with skin commensals (bacteria which reside symbiotically on the skin). If the wound does not heal, over time it will be colonised by different pathogenic species. The polymicrobial populations then interact synergistically, making it difficult to isolate a particular causative organism for a wound infection.⁵

Biofilms are communities of bacteria, embedded in an extracellular polysaccharide matrix. A biofilm forms when bacteria attach to a wound and form a micro-colony over time. Bacteria within a biofilm are physically protected from the host environment and can communicate with each other (quorum sensing). This leads to bacteria changing their phenotypes, resulting in increased virulence and greater likelihood of causing infection. The biofilm becomes an impediment to the healing of chronic wounds, and bacteria in a biofilm are 50 – 1000 times more resistant to conventional antimicrobial treatment than unattached bacteria.⁵ However, this is an area of controversy as more recent research suggests that the significance of biofilms is not fully understood, and they may also have a beneficial effect in wound healing.⁶ Biofilms can be physically removed through debridement of the wound, e.g. as part of the management of a chronic diabetic foot wound.

Wound infection can be classified on a spectrum of five progressively more severe stages:^{1,5}

1. **Contamination** occurs when non-replicating bacteria enter the wound.
2. **Colonisation** occurs when the bacteria begin replicating and adhere to the wound site, but do not cause tissue damage. The healing process of the wound is not delayed by colonisation alone, and in some cases, colonisation can enhance the healing process.
3. **Local infection or critical colonisation** occurs when the number of bacteria is greatly increased and begins to overwhelm the host immune system. The wound does not heal, but tissue invasion has not yet occurred. During this stage, the granulation bed in the wound appears unhealthy, e.g. atrophied, deep red or grey discolouration, with increased discharge, but there is no sign of invasion of the surrounding tissues. Delayed healing may be the only clinical sign.
4. **Spreading invasive infection** occurs when the bacteria overwhelm the patient's immune system and begin to invade and damage the surrounding tissue. Signs and symptoms of infection occur, such as erythema, pain and purulent discharge.
5. **Septicaemia** occurs when the infection spreads throughout the body via the blood stream and causes systemic symptoms such as fever, chills and tachycardia.

Red flags for wound care

Specific wound features or patient factors greatly increase the risk of infection or other complications. Referral for hospital assessment should be considered if a patient presents with high risk features, such as:^{7,8,9}

- Rapidly developing tissue necrosis or gangrene
- Extensive cellulitis, or cellulitis of the face, hands, over joints or periorbital area
- Systemic illness without another obvious cause
- Clinical signs suggestive of osteomyelitis, e.g. deep bone pain, fever or chills
- Pain unrelieved by analgesics such as paracetamol or codeine
- A non-healing or worsening wound in a patient with diabetes
- Suspected malignancy of the wound

There should be a lower threshold for both referral and treatment in patients with co-morbidities such as diabetes or vascular disease, or if psychosocial factors are present that may increase the risk of infection, e.g. inability to adequately care for a wound at home, unsanitary living conditions.

Tetanus immunisation status should be established in all patients who present with a wound, and vaccination given where necessary.

When and how should a wound be swabbed?

Microbiological assessment can be important in the management of infected wounds. Information on the microbiological species present in the wound is useful for determining antibiotic choice and predicting response to treatment. However, these results are only significant if interpreted in the context of a wound that is infected, as non-pathogenic, colonising bacteria will also be detected.

A wound should only be swabbed if there are clinical signs of infection and the wound is deteriorating, increasing in size or failing to heal.¹⁰

The classic clinical signs of infection in an acute wound include:¹⁰

- New or increased pain
- Swelling
- Erythema
- Purulent exudate (or serous exudate with inflammation)
- Malodour
- Localised warmth around the site of the wound

Signs of spread of a localised wound infection include extension of erythema (and development of cellulitis), abscess formation, lymphangitis, crepitus in the soft tissues and breakdown or dehiscence (splitting open) of the wound.

In people with diabetes or with other conditions where perfusion and immune response are diminished, classical clinical signs of infection are not always present,⁹ so the threshold for suspecting infection should be lower. In addition, the classical clinical signs of infection in acute wounds may not always be obvious in patients with chronic

wounds, and more subtle signs of infection can help indicate whether a chronic wound is infected. These signs include discolouration of the granulation tissue, “foamy” granulation tissue, contact bleeding, tissue breakdown (particularly new tissue) and epithelial bridging (where there is incomplete epithelialisation).¹¹

How do you swab a wound?

In primary care, a swab is the most common method used for sampling a wound. Although biopsy or aspirates of pus are the “gold standard” techniques, wound swabs can provide acceptable samples for bacterial culture provided that the correct technique is used.

If the wound is not purulent it should be cleaned prior to swabbing. Some literature suggests that cleaning the wound before sampling is unnecessary, however, if the wound is not clean it often leads to the isolation of multiple organisms which may not be relevant and can generate laboratory results reporting “mixed bacterial flora” rather than individual species.¹² Cleaning removes the organisms present in the surface material, which are often different from those responsible for the pathology, and allows for more accurate culture results. Wounds should be washed with sterile saline and then superficially debrided with a cotton, alginate or rayon-tipped swab.^{1, 4} Ideally, the patient should not have received recent antibiotic treatment before swabbing a wound as this can affect the microbiological results.

The recommended swabbing procedure is as follows:¹

1. Apply sterile saline to moisten the head of the swab to increase the adherence of bacteria
2. Pass the swab over the wound area in a zigzag motion while twisting the swab so that the entire head of the swab comes into contact with the wound surface
3. Swab from the centre of the wound outward to the edge of the wound
4. The swab should be pressed firmly enough that fluid is expressed from the wound tissue (this may be painful for the patient)
5. Repeat the process with a separate swab if a pocket or sinus is present in the wound

Once the sample has been collected it should be labelled with the patient identification details, date and time of the sample and wound site. On the request form record relevant clinical information such as the site and type of wound, the indication for taking a swab and any medication that the patient is taking that may affect the result, e.g. systemic antibiotics, topical antibacterials applied to the wound, corticosteroids. It is also important to make it clear on the request form that the sample is from a wound rather than a superficial skin lesion (this will alert the laboratory to select the appropriate culture media).

The sample should be transported as quickly as possible to the laboratory; ideally it should be processed within 48 hours. The swab should be stored at room temperature if same-day processing is not possible.

When should empiric antibiotics be prescribed?

Immediate treatment with empiric antibiotics is usually necessary for patients with acute wounds, where the risk of infection and complications is increased, e.g. a mammalian bite or a contaminated wound.¹³ In addition, the threshold for empiric antibiotic treatment may be lower if there are medical conditions, e.g. diabetes, or psychosocial factors present which may increase the risk of infection and complications. Depending on the patient and clinical circumstances, a wound swab may still be required in addition to empiric antibiotics and the antibiotic choice altered if necessary once the results become available.

In some situations, antibiotics should not be prescribed to a patient with a suspected infected wound until the results of the laboratory assessment are available so that the appropriate antibiotic can be prescribed, e.g. in a patient with a chronic leg ulcer where there is likely to be a large number and variety of bacteria present.

 For information on recommended antibiotic regimens for common wounds and complications, including diabetic foot infection, bites, abscesses and cellulitis, see “Antibiotic guide for common infections”; available from: www.bpac.org.nz

Interpreting the results of a wound swab analysis

Most laboratories will provide information on the bacteria cultured from a wound swab, the number of organisms grown (either quantitatively or semi-quantitatively), and the antibiotic susceptibility of the grown organisms, which should guide treatment.

The flora of wounds

Approximately half of all infections in soft-tissue, community-acquired wounds are polymicrobial, and approximately one-quarter of infections in these type of wounds are caused by *Staphylococcus aureus*.¹⁰ Bacterial infection with multiple species produces a synergistic effect, leading to increased production of virulence factors and greater delays in healing (see “The significance of biofilms”). The presence of an organism in an infected wound does not necessarily mean that it has caused the infection, and in practice it is not possible to differentiate between pathogenic and non-pathogenic organisms.

Certain kinds of wounds have characteristic bacterial flora, for example:

Superficial burns do not usually become infected, unless other systemic factors are present. When infection does occur, the most commonly reported microbes from a burn wound in the days immediately following the injury are *S. aureus* and other Gram-positive organisms. Later, Gram-negative organisms such as *Pseudomonas aeruginosa* or coliforms, e.g. *E. coli* may be implicated.^{13, 14}

Bite wounds often contain more exotic flora, reflecting the source of the bite.¹⁰ They are commonly polymicrobial, with very high microbial loads. *Staphylococcus spp*, *Peptostreptococcus spp* and *Bacteroides spp* are the most common microorganisms in wounds from human and animal bites.¹³ Less commonly, organisms such as *Pasteurella multocida*, *Capnocytophaga canimorsus*, *Bartonella henselae* and *Eikenella corrodens* will be present.¹³

Surgical wounds from a “clean” surgery, i.e. non-emergency surgery that does not enter the gastrointestinal or genitourinary tract, do not usually become infected. However, when infection does occur, antibiotic-resistant organisms, such as methicillin resistant *Staphylococcus*

aureus (MRSA) and vancomycin resistant enterococci, are more commonly encountered, reflecting hospital-acquired flora.¹⁰

Diabetic foot infections are frequently associated with *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus spp.*, *P. aeruginosa*, *Enterococcus spp.* and coliform bacteria.¹³ With good laboratory technique, anaerobes can be isolated in up to 95% of people with severe diabetic lower leg infections, most commonly *Peptostreptococcus*, *Bacteroides* and *Prevotella spp.* However, the clinical significance of the type of microorganism present is reduced if there are limited signs of infection, which is common in people with infected diabetic ulcers.¹³ Delayed healing is more likely to occur in people with diabetic foot infections, even when less pathogenic microorganisms are present.¹⁵

Deeper penetrating wounds are associated with a wider range of bacteria, representing the increased likelihood of foreign bodies in the wound. Referral is often necessary for exploration of the wound if it fails to heal.

Is species or number of organisms more significant?

There is some debate as to whether the type of bacteria or the overall density of the bacteria affects healing rates more significantly. It is likely that both factors play a role, however, the more widespread opinion is that organism type has the greater effect on wound healing. It is thought that aerobic or facultative pathogens in particular, such as *S. aureus*, *P. aeruginosa*, and beta-haemolytic streptococcus are the primary causes of delayed healing and infection in both acute and chronic wounds.¹³

Laboratories may provide either a quantitative or semi-quantitative result for bacterial load. A quantitative result gives the estimated number of organisms per gram of tissue or per mm³. Organism load above 100 000 per gram of tissue or per mm³ is considered significant, and is likely to reduce healing times significantly. Semi-quantitative analysis is based on grading bacterial growth as scant, light, moderate or heavy (or 1+, 2+, 3+ or 4+), of which moderate and heavy usually indicate significant bacterial counts (i.e. greater than 100 000 per gram).¹³

Antibiotic choice and susceptibility

Susceptibility testing is performed for all of the potential pathogens isolated from the swab. A “susceptible” report

means that the organism should respond to treatment with the recommended antibiotic as long as there is a good blood supply to ensure adequate tissue levels of the antibiotic. This may not always be the case, e.g. if necrotic tissue is present. When an organism is reported as resistant to a particular antibiotic it is important to assess the clinical response, if treatment has already commenced, with consideration given to changing the antibiotic if necessary.

In slower-developing infections or wounds that have failed to resolve over time, antibiotic choice should be directed by the relevant susceptibilities provided by the laboratory analysis.

If empiric antibiotic treatment is prescribed, i.e. without swabbing, or before receiving the results of the wound culture, it is important to be aware of local antibiotic susceptibility, to guide treatment choice. Susceptibility differs by geographical area, as well as in different rest homes or long-term care facilities, e.g. MRSA is more common in some locations.

 For information on nationwide susceptibilities and resistance, see: www.surv.esr.cri.nz (search antimicrobial resistance)

 In addition to antibiotic treatment, wound cleansing, surgical debridement and correct dressing is essential to reduce the microbial load, and likelihood of infection.

Think twice before using mupirocin (Bactroban)

Mupirocin 2% (Bactroban) is a topical antibacterial treatment. When it first became available in New Zealand, it could be purchased in pharmacies (as a pharmacy-only “restricted” medicine). Its frequent use led to increased bacterial resistance to mupirocin, and as a result, mupirocin became a prescription-only medicine. Mupirocin remains active against some MRSA strains and as such, it is recommended that it should be reserved for use only when susceptibility testing shows MRSA to be present.

What should you do if a wound infection does not resolve?

If signs of infection are not reduced 48 – 72 hours after initiation of antibiotic treatment for an acute wound, a swab should be taken to reassess the wound flora and relevant susceptibilities. If a wound fails to heal within four to six weeks following treatment, particularly if antibiotics were used, discussion with a wound specialist is recommended.

In some cases, a non-healing wound may raise the suspicion of malignancy and this should be investigated.

Is this wound malignant?

Chronic wounds can degenerate into malignancy, and conversely a malignancy may present as, or be mistaken for, a chronic wound.

It is estimated that approximately 3% of malignant lesions masquerade as a chronic wound. Primary malignancy should be considered in a patient with an ulcer which has developed over a relatively short time.¹⁸ The typical

example of this type of malignancy is a basal cell carcinoma, normally caused by sun exposure. Presentation varies, but the classic appearance is a “rodent ulcer”, which has raised pearly edges and central atrophy or ulceration. A pearly, shiny nodule with prominent capillary networks is also common. A basal cell carcinoma may also present as an eczema-like patch. Only advanced cancers appear as wound-like, having outgrown their blood supply and eroded.

A chronic wound that develops into a malignancy is referred to as a Marjolin’s ulcer.¹⁹ The incidence varies, but it is estimated that approximately 2% of chronic wounds undergo malignant transformation.¹⁸ Marjolin’s ulcer is most commonly associated with burn wounds, but has been reported in various other types of chronic, non-healing wounds, such as lower leg ulcers. The ulcer is usually present for more than six months, but may be present for up to several decades, as it slowly undergoes malignant change. The most common resulting malignancy is a squamous cell carcinoma,¹⁹ which is a slow-growing cancer derived from the epithelial cells.

MRSA: the super-villain of the 21st Century

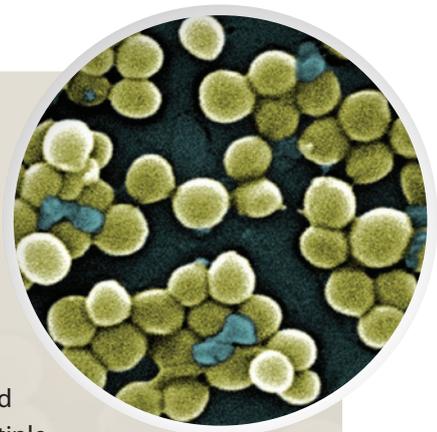
Staphylococcus aureus is the most frequently isolated bacterial pathogen in wounds. Although non-pathogenic colonisation is common,¹⁶ *S. aureus* is an important cause of both acute and chronic wound infection. Methicillin-resistant *S. aureus* (MRSA) are a subclass of *S. aureus* that are resistant to all classes of penicillins and cephalosporins.

There appears to be limited biological or clinical difference between MRSA and non-resistant staphylococcus with the exception of resistance. Adhesion ability, colonisation and infectivity, modes of transmission and survivability are all similar.¹⁶ However, the difficulty in treating infections caused by MRSA mean that the MRSA infections are associated with higher mortality.

MRSA was first seen in New Zealand in 1975.¹⁶ Traditionally, MRSA was almost exclusively hospital-acquired; however, since the 1990s community-

acquired MRSA has been increasing in prevalence. Infections caused by MRSA are most common in hospitals, prisons, residential care and other areas where multiple people, often with lowered immune response live in close proximity.

Depending on the severity of the infection and the clinical situation, patients with MRSA infection in a wound may require referral to hospital for IV antibiotic treatment, usually with vancomycin. Patients with soft tissue infections that can be treated in the community are usually prescribed oral co-trimoxazole or clindamycin, but discussion with a clinical microbiologist or infectious disease specialist may be useful.¹⁷



Other factors that may indicate a malignant wound include:¹⁸

- Excessive granulation tissue that extends beyond the wound margin
- Wounds with an irregular base or margins
- Wounds with a change in discharge, bleeding or with outward (exophytic) growth

A punch biopsy of the wound should be taken if there is a suspicion of any malignancy; particularly if the wound has been present for longer than three months or developed rapidly and has not responded to treatment or is increasing in size.¹⁸

The biopsy site is important. If malignancy is suspected, the biopsy site should be on the wound margin and must include tissue from the wound bed and surrounding, non-damaged skin.^{18, 20}



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