The clinical efficacy of two semi-quantitative wound-swabbing techniques in identifying the causative organism(s) in infected cutaneous wounds

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ABSTRACT

A prospective randomised controlled trial of two paired wound-swabbing techniques (Levine versus Z) was conducted to establish which method was more effective in determining the presence of bacteria in clinically infected wounds. The Levine technique involves rotating the wound swab over a 1-cm^2 area of the wound; the Z technique involves rotating the swab between the fingers in a zigzag fashion across the wound without touching the wound edge. Fifty patients were recruited into the study with acute (42%) and chronic wounds (58%). Overall, the Levine technique detected significantly more organisms than the Z technique ($P \leq 0{\cdot}001$). When acute and chronic wounds were analysed separately, the Levine technique again detected more organisms in both acute ($P \leq 0{\cdot}001$) and chronic wounds ($P \leq 0{\cdot}001$). We conclude that the Levine technique is superior to the Z technique and this result may be because of the Levine technique's ability to express fluid from the wound bed and thereby sampling a greater concentration of microorganisms from both the surface and slightly below the surface of the wound.

Key words: Wound swab • Wound-swabbing techniques

Key Points

- the aim of this study was to compare two wound-swabbing techniques, to determine which method is more effective in determining the causative organism(s) in infected cutaneous wounds
- it is necessary to culture a wound for a number of reasons, first to identify the causative organism(s) and for the provision of an antibiogram for pathogens to guide antimicrobial therapy

INTRODUCTION

The aim of this study was to compare two wound-swabbing techniques, to determine which method is more effective in determining the causative organism(s) in infected cutaneous wounds. Wound infection occurs when there is a replication of one or more microorganisms in a wound, which provokes a series of local and systemic host responses that leads to a delay in wound healing (1–3). It is necessary to culture a wound for a number of reasons, first to identify the causative organism(s) and for the provision of an antibiogram for pathogens to guide antimicrobial therapy (1,4). The three methods for collecting a wound sample are: tissue biopsy, wound fluid aspirate and wound

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swab. Tissue biopsy is considered the 'gold standard' for determining the species and the number of organisms, which penetrate the soft tissue (5–12). Tissue biopsy is expensive, invasive, labour intensive, painful, disrupts the wound bed from healing, and requires trained personnel to perform the procedure (13). It is not standard practice in the majority of settings and is usually reserved for clinical research. Across settings, wound swabbing is the most frequently used method of collecting a wound sample, it is simple, non invasive and inexpensive (14). However, controversy exists in the literature as to how best to carry out this procedure (1,14). Some practitioners clean the wound prior to taking the sample and some do not, others take the sample from one place on the wound bed, and some wipe the swab all over the wound in a random fashion. Wound swabs that are collected incorrectly can identify bacteria on the wound surface alone and not those that penetrate the soft tissue, giving false positive results (14). To date there is no single universally accepted method of collecting a wound swab (1,15–17). Regardless of this lack of consensus, wound swabbing remains the most frequent method of sampling wounds for microbiological analysis (14).

CLINICAL STUDIES COMPARING PUNCH BIOPSY WITH WOUND SWAB

Gardner *et al*. compared the Levine technique, with the Z and a sample of viable tissue (18). This group recruited 83 patients with chronic wounds excluding arterial leg ulcers. Interestingly only 30 patients had clinically infected wounds. The mean concordance between the Levine technique and tissue specimen was 78%. Their findings suggest that the Levine technique provided acceptable accuracy of wound bioburden.

Levine *et al*. showed that bacteria counts from wounds are linearly related to biopsy quantification in open burn wounds (19). Their sample size was small with 24 patients. They collected 41 wound swabs using the Levine technique and tissue biopsy from 41 granulating wounds. Uppal *et al*. undertook of a larger study of 100 patients with burns. In 95% of cases, both wound swab and biopsy identified the same organisms (20). Basak *et al*. found concordance of 72% between wound swab and biopsy in a larger study of 171 patients with wounds of various aetiology. They also found that wound swab was reliable in 95% for both assessment of wounds as well as monitoring response to treatment (21). In a small study of 38 patients with chronic wounds, Bill *et al*. found a correlation of 79% between biopsy and wound swab (22). A prospective study to evaluate wound-healing outcomes was undertaken by Davies *et al*. Biopsy versus wound swab was compared in 70 patients with chronic venous leg ulcers. None of the wounds were infected. Logistic regression showed that the biopsy offered no predictive information in terms of wound-healing outcomes when compared with a wound swab ($P = 0{\cdot}27$). The authors recommend that biopsy should be discouraged in clinically non infected wounds (23). Sapico *et al*. undertook a study of 25 pressure ulcers, this group found concordance of 75% between biopsy and wound swab (24). Kelkar and Kagal recruited 50 patients with diabetic foot ulcers into their study and comparing biopsy with wound swab. Biopsies identified higher numbers of bacteria than wound swabs. The authors concluded that although wound swabs may provide useful information, they argue that certain organisms might be missed on wound swabbing (25). Slater *et al*. collected a wound swab prior to debridement of each wound and a tissue sample post-debridement in 60 patients with diabetic foot wounds. In 62% of cases, the results were the same and in 20% of cases the swab identified more organisms. Further analysis showed that in wounds not extending to bone (90% of cases), the swab identified all organisms isolated from the tissue sample. In wounds with bone exposed the correlation was poor at only 65% (26). Biopsy was not warranted in a recent study of the microbiological profile of 20 'locally' infected leg ulcers in a study undertaken by Cooper *et al*. This group compared biopsy, wound swab and polyvinyl acetate (PVA) foam disc. They found greatest agreement of the bacterial bioburden between swab and PVA foam disc, and also between PVA disc and biopsies (27). There is mounting evidence to indicate that wound swab cultures are a useful alternative to invasive tissue biopsy; however, the question remains as to what is the most effective technique for taking a wound swab.

Key Points

- the three methods for collecting a wound sample are: tissue biopsy, wound fluid aspirate and wound swab
- across settings, wound swabbing is the most frequently used method of collecting a wound sample, it is simple, non invasive and inexpensive
- wound swabs that are collected incorrectly can identify bacteria on the wound surface alone and not those that penetrate the soft tissue, giving false positive results
- there is mounting evidence to indicate that wound swab cultures are a useful alternative to invasive tissue biopsy; however, the question remains as to what is the most effective technique for taking a wound swab

Key Points

- physiological responses to microbial pathogens vary greatly in acute and chronic wounds
- an important consideration is that it is the interaction between the host and the bacteria that will determine the organisms' influence on wound healing, not the presence of bacteria alone
- fifty patients were recruited from both an inpatient and outpatient setting of an 855-bed university teaching hospital in Perth, Western Australia

MICROBIOLOGICAL PROFILE OF ACUTE AND CHRONIC WOUNDS

Optimal diagnosis and management of wound infection is crucial if healing is to be promoted and associated morbidity and mortality reduced (28). All open wounds are colonised with bacterial, and the progression of wound healing can still occur in their presence (28–30). Bacterial involvement in a wound can be defined in four ways: contamination, colonisation, critical colonisation or 'locally infected' and wound infection (3,27–30).

A wide diversity of aerobic and anaerobic organisms contaminates and colonise acute and chronic wounds. Bowler and Davies cultured 367 isolates from 61 acute wounds and 45 chronic wounds (31). However, it is the bacterial species, not the number of organisms present that is significant (32). Wright *et al*. were the first to report that regardless of the quantity of organisms, surgical wounds would not heal if haemolytic *Streptococcus pyogenes* strain was present (33). Since then other pathogens have also been identified as impairing wound healing and causing infection regardless of quantity (32). Gram-positive organisms are usually present in higher numbers in infected wounds that have been present for less than a month. For example acute wounds such as traumatic, surgical or burn wounds *Staphylococcus aureus* is considered the main culprit in causing wound infection (34–39). Chronic wounds are likely to be polymicrobial in nature, including gram-negatives and anaerobes in addition to Gram-positive bacteria (32). *S. aureus, Pseudomonas aeruginosa* and beta haemolytic streptococci are the most commonly cited pathogens causing delayed healing and wound infection (40–49). Physiological responses to microbial pathogens vary greatly in acute and chronic wounds.

An important consideration is that it is the interaction between the host and the bacteria that will determine the organisms' influence on wound healing, not the presence of bacteria alone.

The formation of biofilms on the exposed extracellular matrix is also problematic in chronic wounds. The wound bed may appear healthy in appearance while playing host to colonies of bacteria enchased within a biofilm (50) attached to the wound bed. These replicating bacteria secrete an extracellular polymeric substance which provides protection against topical antimicrobial agents such as antibiotics and antiseptics and host defences (3,29,51,52). Biofilms have been reported to exhibit the ability to mutate and to alter their sensitivity to antibacterial agents. Planktonic bacteria are released from the biofilm onto the wound bed forming new colonies, leading to local infection or weakening of the collagen matrix in healed wounds (53–55). Without appropriate scanning electron microscopy or confocal laser scanning microscopy, it could be postulated that wound swabbing alone will not detect the bacterial contained within biofilms because of the inability of the swab to penetrate the protective film afforded by the biofilm and to sample the actual bacterial cells.

RESEARCH AIM

To compare two wound-swabbing techniques (Levine versus Z technique) to establish which method is more effective in determining the causative organism(s) in infected cutaneous wounds.

MATERIALS AND METHODS

Patient recruitment

Fifty patients were recruited from both an inpatient and outpatient setting of an 855-bed university teaching hospital in Perth, Western Australia. There were 28 males and 22 females, with a mean age of 62·46 years. Human Research Ethics Committee approval for the study was granted by the hospital prior to any data collection. Of the recruited patient cohort, acute wounds accounted for 42% and the remaining 58% were chronic. Table 1 provides a break down of the wounds by aetiology. The wounds in the 'other' category were comprised of four traumatic wounds and one invasive squamous cell carcinoma.

Inclusion criteria for the study comprised; patients with a wound of any aetiology greater than 1 cm^2, showing clinical signs of infection. Acute wound infection was defined as: inflammation present for longer than 5 days, purulent drainage, elevated temperature >38°C, spontaneous dehiscence or presence of an abscess (30,56). The criteria for chronic wound infection was: increased exudate, presence of odour, erythema >1–2 cm, warmth around the wound, poor quality granulation tissue,

Table 1 Wound aetiology

Wound aetiology	*N*	%
Arterial ulcers	5	10
Venous ulcers	13	26
Mixed arterial/venous ulcers	1	2
Neuropathic ulcers	7	14
Neuro-ischaemic ulcers	6	12
Pressure ulcer	5	10
Surgical	8	16
Other	5	10
Total	50	100

pain or tenderness at the wound site or no improvement in wound healing in the preceding 2 weeks in a clean wound (6,30,57,58).

Data collection

Two consecutive semi-quantitative wound swabs were collected using the Levine technique and Z technique from the same wound not more than 5 minutes apart. The order of the swab collection was randomised by the flip of a coin. The wound was cleansed once with sterile 0·9% normal saline, prior to the collection of the wounds swabs. The wound was not cleansed again between each method. The Z technique involves rotating the swab between the fingers as the swab is manipulated in a 10-point zigzag fashion (side to side across the wound without touching the wound edges or the peri-wound skin from one edge to the other). With the Levine technique, the specimen is obtained from a limited area within the wound, excluding the wound edge or peri-wound skin. The swab is rotated over a 1 cm^2 area with sufficient pressure to express fluid within the wound. Two sterile cotton-tipped swabs were used for each method. The swabs were pre-moistened with sterile 0·9% normal saline. One swab was used to obtain a gram stain and the other was placed in Stuart's medium to identify the species of organisms present. All swabs were collected by two nurses who had been trained and certified in each technique prior to data collection in order to minimise the potential for swab collection technique error.

Microbiological analysis

All specimens were analysed by the same scientist on the day of specimen collection. Gram stain procedure was performed by heat fixing the smear. The smear was then flooded with methanol fixative. The specimen was examined under low power (×100 objective lens) to quantify leucocytes and oil immersion lens 9 × 100 objective). Anaerobic culture was performed by inoculating a pre-reduced blood agar plate, at the same time as the rest of the plates were inoculated. A metronidazole disc was then added. The blood agar plate was placed in an anaerobic holding chamber, until there were enough plates to fill an anaerobic jar (normally up to an hour in the holding chamber). The anaerobic jars are Oxoid brand, 2·5-l airtight jars, to which Oxoid brand anaerobic indicator was added, as well as a blood agar plate pre-inoculated with three anaerobic organisms. The plates were incubated in the anaerobic jar, which was placed in an incubator for 48 hours, at 35°C. Before opening the jars, the colour of the indicator was noted, if it was white, then anaerobic conditions had been reached, if it was pink the sachet did not work, or the jar could have leaked or had been opened. Upon opening the jar, growth of the three anaerobic organisms (*Clostridium difficile, Bacteroides fragilis* and *Fusobacterium nucleatum*) was noted. Only if the indicator was white and all three anaerobes grew, was anaerobe culture successful. If these criteria were not fulfilled, then culture was repeated. The metronidazole disc was added to the blood agar plate, as almost all anaerobes are metronidazole sensitive, and thus any zone of inhibition around the disc would signal the possibility of anaerobes. This is an accredited method for culturing anaerobes.

Aerobic culture was performed by inoculating the swab onto horse blood agar medium and cysteine lactose electrolyte deficient agar. Both plates were then stored at 35°C in a carbon dioxide holding chamber. The holding chamber is a top opening box with airtight sides, with a constant injection of CO_2 at a rate of 1·2 l/minute. As CO_2 is heavier than air, the chamber fills up with CO_2, and any air that enters when the chamber is pushed out through the lid by the heavier CO_2 constantly injected into the chamber. The holding chamber was not used for incubation, only for holding the plates for a short time until there was enough to set up a jar, which holds 12 plates. This chamber was not the same as an anaerobic culture chamber. The plates were initially examined after 24 hours and then re-incubated

for a further 24 hours if there was no growth. Microscopy was reported as leucocytes not seen, few, moderate, or abundant. All potential pathogens were reported and growth quantified as; + (scant growth), ++ (small growth), +++ (moderate growth) and ++++ abundant growth. Anaerobic culture was performed on all wounds.

Data analysis

All statistical procedures were carried out with Statistical Package for the Social Sciences (SPSS) windows version 16. For all continuous variables, descriptive statistics including means and standard deviation were calculated. Frequencies and proportions were determined for all categorical variables. Differences between the detected microbiological burden values were analysed with *t* test for paired samples. It was determined that 50 paired observations were adequate to provide 80% power at a *P* value of 0·05.

RESULTS

Microbiological profile

Tables 2 and 3 identify the bacteria isolated from both acute and chronic wounds using the Levine and Z technique. Overall the Levine detected more organisms in both acute and chronic wounds. In acute wounds, the Levine detected 25 different species of organisms compared with 20 organisms in chronic wounds. By comparison, the Z technique identified 18 organisms in acute wounds compared with 23 in chronic wounds. In summary, more bacteria were isolated in chronic wounds than those wounds that were classed as acute. Using a one-sample *t* test there was a statistically significant difference in the number of organisms detected in acute and chronic wounds. In acute wounds, the Levine technique detected more organisms ($t = 9{\cdot}55$, $P \leq 0{\cdot}001$). In chronic wounds, the Levine also detected more organisms ($t = 12{\cdot}04$, $P < 0{\cdot}001$). There was also a difference in the species of organisms detected between the Levine and the Z technique in both acute and chronic wounds (Tables 2 and 3). When both groups were combined there was still a statistically significant difference in the number of organisms detected between the Levine and the Z technique in the study population. The Levine detected more organisms ($t = 15{\cdot}46$, $P \leq 0{\cdot}001$) than the Z technique (Table 4).

Table 2 Organisms identified in acute and chronic wounds Levine technique

Acute wounds Levine technique	Chronic wounds Levine technique
Not detected	*Acineobacter baumannii*
Acinetobacter haemolyticus	*Acinetobacter haemolyticus*
Alcaligenes faecalis	Not detected
Alpha-haemolytic streptococcus	*Alpha-haemolytic streptococcus*
Not detected	*Anaerobic organisms*
Bacillus species	Not detected
Diptheroid bacillus	*Diptheroid bacillus*
Enterobacter aerogenes	Not detected
Enterobacter cloacae	Not detected
Enterococcus aerogenes	Not detected
Enterococcus species	*Enterococcus species*
Escherichia coli	*Escherichia coli*
Klebsiella pneumoniae	Not detected
Klebsiella oxytoca	*Klebsiella oxytoca*
Morganella morganii	*Morganella morganii*
Non epidemic methicillin-resistant *Staphylococcus aureus* (MRSA)	Non epidemic methicillin-resistant *Staphylococcus aureus* (MRSA)
Proteus mirabilis	*Proteus mirabilis*
Not detected	*Proteus species*
Not detected	*Proteus vulgaris*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
Serratia marcescens	*Serratia marcescens*
Serratia odorifens	*Serratia odorifens*
Staphylococcus aureus	*Staphylococcus aureus*
Staphylococcus coagulase negative	*Staphylococcus coagulase negative*
Stenotrophomanas maltophilia (Xanthomonas maltoph)	Not detected
Streptococcus agalactiae (group B)	*Streptococcus agalactiae* (group B)
Streptococcus milleri group	*Streptococcus milleri* group
Streptococcus pyogenes (group A)	*Streptococcus pyogenes* (group A)

Organisms identified by aetiology of wounds

Altogether there was 31 different species of microorganisms isolated in the study population. Further analysis was undertaken to determine if there was a difference in the bacteria species in the various wound types identified between the Levine and Z technique. Pressure ulcers accounted for 10% ($n = 5$) of the

Table 3 Organisms identified in acute and chronic wounds with the Z technique

Acute wounds Z technique	Chronic wounds Z technique
Not detected	*Acineobacter baumannii*
Not detected	*Acinetobacter haemolyticus*
Alpha-haemolytic streptococcus	*Alpha-haemolytic streptococcus*
Not detected	*Anaerobic organisms*
Bacillus species	Not detected
Not detected	*Clostridium perfringens*
Cedecae species	Not detected
Corynebacteruim jeikeium	*Corynebacteruim jeikeium*
Diptheriod bacillus	*Diptheriod bacillus*
Enterobacter aerogenes	Not detected
Enterobacter cloacae	Not detected
Escherichia coli	*Escherichia coli*
Enterococcus species	*Enterococcus species*
Klebsiella pneumoniae	Not detected
Not detected	*Morganella morganii*
Non epidemic methicillin-resistant *Staphylococcus aureus* (MRSA)	Non epidemic methicillin-resistant *Staphylococcus aureus* (MRSA)
Proteus mirabilis	*Proteus mirabilis*
Not detected	*Proteus species*
Not detected	*Proteus vulgaris*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
Staphylococcus aureus	*Staphylococcus aureus*
Staphylococcus coagulase negative	*Staphylococcus coagulase negative*
Stenotrophomanas maltophillia (Xanthomonas maltoph)	Not detected
Streptococcus agalactiae (group B)	*Streptococcus agalactiae* (group B)
Not detected	*Streptococcus milleri* group
Streptococcus pyogenes (group A)	*Streptococcus pyogenes* (group A)
Not detected	*Serratia marcescens*
Not detected	*Serratia odoriferans*

Table 4 Identified organisms Levine versus Z

Levine technique	Z technique
Acineobacter baumannii	*Acineobacter baumannii*
Acinetobacter haemolyticus	*Acinetobacter haemolyticus*
Alcaligenes faecalis	Not detected
Alpha-haemolytic Streptococcus	*Alpha-haemolytic Streptococcus*
Anaerobic organisms	*Anaerobic organisms*
Bacillus species	*Bacillus species*
Not detected	*Cedecea species*
Not detected	*Clostridium perfringens*
Not detected	*Corynebacterium jeikeium*
Diptheroid bacillus	*Diptheroid bacillus*
Enterobacter aerogenes	*Enterobacter aerogenes*
Enterobacter cloacae	*Enterobacter cloacae*
Enterococcus species	*Enterococcus species*
Escherichia coli	*Escherichia coli*
Klebsiella pneumoniae	*Klebsiella pneumoniae*
Klebsiella oxytoca	Not detected
Morganella morganii	*Morganella morganii*
Non epidemic methicillin *Staphylococcus aureus* (MRSA)	Non epidemic methicillin *Staphylococcus aureus* (MRSA)
Proteus mirabilis	*Proteus mirabilis*
Proteus species	*Proteus species*
Proteus vulgaris	*Proteus vulgaris*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
Serratia marcescens	*Serratia marcescens*
Serratia odoriferans	*Serratia odoriferans*
Staphylococcus aureus	*Staphylococcus aureus*
Staphylococcus coagulase negative	*Staphylococcus coagulase negative*
Stenotrophomanas maltophila (Xanthomonas maltoph)	*Stenotrophomanas maltophila (Xanthomonas maltoph)*
Strep. agalactiae (group B)	*Strep. agalactiae* (group B)
Streptococcus milleri group	*Streptococcus milleri* group
Streptococcus pyogenes (group A)	*Streptococcus pyogenes* (group A)
Streptococcus pyogenes (group A)	*Streptococcus pyogenes* (group A)

study population and this was the only wound group where 11 identical species of organisms were isolated by each technique. No bacteria were isolated by either technique from the one patient with a mixed arterial/venous leg ulcer.

The most common species of bacteria isolated from all wound types were *Enterococcus species* with both the Levine and Z technique. *S. aureus*, *Staphylococcus coagulase* negative and *P. aeruginosa* were isolated from arterial leg ulcers, venous leg ulcers, neuropathic, neuro-ischaemic foot ulcers, pressure ulcers and the wounds in the 'other category'. *Non epidemic methicillin Staphylococcus aureus* (MRSA) was isolated from arterial leg ulcers, venous leg ulcers, neuro-ischaemic foot ulcers, and pressure ulcers. The remaining organisms as shown in Table 4 were isolated in one or more of the wound categories. Interestingly *Clostridium perfringens* was only isolated in the venous leg ulcer group. *Corynebacterium jeikeium* was only isolated in neuropathic foot ulcers and surgical wounds. *Cedecea species* was isolated in surgical wounds only, with the Z technique. Anaerobic organisms were only detected in the venous leg ulcer group.

Key Points

- in this study, there was a statistically significant difference between the Levine and the Z technique, in the wounds in our study population
- the findings of this study show that a wide diversity of organisms colonise acute and chronic wounds, this is consistent with the literature
- the majority of chronic wounds in the study cohort were polymicrobial, making it difficult to determine which of the organisms were pathogenic and caused the wound infections
- as the majority of wounds in this study were chronic (58% versus 42% of acute wounds), it is reasonable to assume that biofilms were present in the chronic wounds swabbed
- it could be postulated that with the Levine technique, the pressure exerted may also be releasing organisms within the soft tissue that the Z technique, thorough the method of collecting the sample does not

DISCUSSION

In this study, there was a statistically significant difference between the Levine and the Z technique, in the wounds in our study population. Despite tissue biopsy being considered the 'gold standard' for collecting a wound sample for microbiological analysis, there is a growing body of evidence to support the use of wound swab cultures as a safer alternative to invasive tissue biopsy (19–22,24,26,59,60). However these studies do not detail which method was used to collect the wound swab samples. Based on the results from this study and that of Gardner *et al*. (18), one could hypothesise that the Levine technique is a safer alternative than tissue biopsy at detecting organisms within an infected wound.

Different species of organisms were detected in both acute and chronic wounds with both the Levine and Z technique in this study. Anaerobic organisms were only detected in chronic wounds (venous leg ulcers), by both methods of specimen collection. The laboratory did report the species of anaerobic organisms in one case *(Clostridium perfingens),* with the Z technique. However with the Levine technique, the laboratory reported the presence of anaerobes but not the species. Identification of the species of anaerobic bacteria is generally considered to be expensive and labour intensive. Many clinicians argue that anaerobic organisms are not harmful to wound healing (48,61–63). Anaerobes are generally associated with leg ulcers, as was the case in this study (34). Anaerobic organisms are not generally associated with acute wounds (31,64), this is thought to account for the lack of anaerobes identified in acute wounds in this study population, as the majority of wounds were chronic 58% versus 42% of acute wounds.

The findings of this study show that a wide diversity of organisms colonise acute and chronic wounds, this is consistent with the literature (31,34,64–66). The majority of chronic wounds in the study cohort were polymicrobial, making it difficult to determine which of the organisms were pathogenic and caused the wound infections. Gram-positive organisms, such as *S. aureus* and *E. species*, are usually present in acute wounds and considered the main contributor to cause infections; this was also the case in this study. The majority of chronic wounds were polymicrobial. *S. aureus* and *P. aeruginosa* are two commonly cited pathogens (41,43,44), as was also the case in this study.

Biofilms and wound swabs

The presence of biofilms on the exposed extracellular matrix of the wound bed may have contributed to a difference between the organisms detected with the Levine and Z technique. Biofilms are commonly associated with chronic wounds rather than acute wounds (51,52). As the majority of wounds in this study were chronic, it is reasonable to assume that biofilms were present in the chronic wounds swabbed. It is also reasonable to suspect that biofilm formation may have had a role to play in the sensitivity of the swabbing techniques, in detecting the presence of microorganisms. However, it has been reported that antimicrobial agents and antiseptics cannot penetrate a biofilm (52), therefore it is unlikely that a wound swab would penetrate a biofilm. More plausible is the difference between the two wound-swabbing techniques. The pressure exerted on the wound bed required to collect the wound swab with the Levine technique may be responsible for collecting more planktonic bacteria on the exposed extracellular matrix, than the Z technique. With the Z technique, the wound swab is rotated across the wound bed in a zigzag fashion, it is difficult for the person collecting the sample to maintain this technique and apply any pressure on the wound bed. It could be postulated that with the Levine technique, the pressure exerted may also be releasing organisms within the soft tissue that the Z technique, through the method of collecting the sample does not.

LIMITATIONS OF THE STUDY

The sample size in this study was small and the study did not compare the Levine and the Z techniques with tissue biopsy. Therefore the authors were unable to determine the accuracy of the organisms detected between the two techniques used to what may be referred to as the 'gold standard'.

Both the Levine and the Z technique are only suitable for open wounds healing by secondary intention. For the Levine technique the wound has to be greater that 1 cm^2. The 'Z' technique requires a wound large enough to swab the wound in a 10-point zigzag fashion across the wound bed. For wounds that are approximated

such as surgical wounds that display clinical signs of infection, which may or may not have a wound dehiscence, it is not possible to use either method. It is also not achievable to use either method in cavity wounds where the base cannot be identified. Therefore this study only provides guidance in swabbing in superficial or partial thickness wounds.

RECOMMENDATIONS

The study should be replicated with a larger study size population, comparing the effectiveness of tissue biopsy with both methods of wound swab collection. As biofilms are problematic in chronic wounds future studies should include the identification of biofilms and organisms contained within them.

CONCLUSION

The findings of this study demonstrate that a wide diversity of organisms colonise acute and chronic wounds. Consistent with the literature that chronic wounds are polymicrobial, this study also found that more organisms were isolated from chronic wounds than acute wounds, highlighting the need for accurate specimen collection to support subsequent antimicrobial therapy. Anaerobic organisms were only detected in the venous leg ulcer group. This study goes some way in validating that they are less likely to be cultured from acute wounds.

We believe that our study illustrates the clinical efficacy of the Levine and the Z methods of wound swab collection and semi-quantitative microscopy. The results suggest that the Levine method is more reliable in determining the organisms in acute and chronic wounds when wound swabbing is the selected method for sample and culture.

Wound biofilms are difficult to identify and are believed to significantly contribute to delayed wound healing. The difference that we detected between the results from the Levine and Z techniques in identifying microorganisms may be partly accounted for by the potential presence of biofilm on the exposed extracellular matrix of the chronic wounds in this study. We speculate that the pressure applied to the wound bed when using the Levine technique may be responsible for collecting planktonic bacteria released from the biofilm if present.

However we do not have definitive evidence of the presence of biofilm in the wounds in our study and therefore our thoughts remain speculation at this time, yet we believe this area warrants further investigation.

Overall, the study provides the clinician with evidence of the superiority of the Levine technique over the Z technique, when wound swabbing is clinically indicated.

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Key Points

- this study only provides guidance in swabbing in superficial or partial thickness wounds
- the study should be replicated with a larger size population comparing the effectiveness of tissue biopsy with both methods of wound swab collection
- as biofilms are problematic in chronic wounds future studies should include the identification of biofilms and organisms contained within them
- the findings of this study show that a wide diversity of organisms colonise acute and chronic wounds
- we believe that our study illustrates the clinical efficacy of the Levine and the Z methods of wound swab collection and semi quantitative microscopy
- the results suggest that the Levine method is more reliable in determining the organisms in acute and chronic wounds when wound swabbing is the selected method for sample and culture
- we do not have definitive evidence of the presence of biofilm in the wounds in our study and therefore our thoughts remain speculation at this time, yet we believe this area warrants further investigation
- overall, the study provides the clinician with evidence of the superiority of the Levine technique over the Z technique, when wound swabbing is clinically indicated

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