Assessing bacterial burden in wounds: comparing clinical observation and wound swabs

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ABSTRACT

A randomised controlled trial (RCT) was conducted to compare the efficacy of nanocrystalline silver and cadexomer iodine dressings in healing chronic lower leg ulcers. The relationships between wound swab culture results and nurses' clinical assessments of critical colonisation, and between bacterial burden and healing rate, were also examined. There were 281 individuals with leg ulcers recruited. The bacterial burden of wounds was assessed using semi-quantitative wound swabs collected at baseline and intervals during the study. The study found no relationship between the nurses' clinical assessments and bacterial burden as identified from wound swabs in the wounds. A significant difference in wound healing was found with the use of nanocrystalline silver as compared to cadexomer iodine in the first 2 weeks of treatment when nil or low levels of leukocytes, gram positive bacilli, gram positive cocci or gram negative cocci were reported. This study has raised a number of questions regarding the need for further investigation into methods of assessing wound bacterial burden as well as the impact of wound biofilms on wound assessment and treatment.

Key words: Antimicrobial • Bacterial burden • Biofilm • Critical colonisation • Semi-quantitative bacteriology

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INTRODUCTION

In Australia, leg ulcers were found to be 1·1 per 1000 population (0·11% point prevalence) (1) with an age-related ulcer prevalence for females and males at 1:1·9. It was also reported that 24% of ulcers were present for 1 year, 35% of individuals had a problem of ulceration for 5 years, 20% had experienced ten or more episodes of ulceration and 45% were housebound (2). Moreover, the number of elderly Australians with leg ulcers is estimated to double over the next 20 years (3). Leg ulceration has a profound impact on individuals' health and social aspects of quality of life (4–6) as well as having considerable financial implications for these individuals and their health providers. The cost of leg ulcers to the

Key Points

- inconsistencies were found between clinical signs of bacterial burden as assessed by nurses and the results of wound swabs
- the validity and reliability of methods for assessing bacterial burden require further investigation
- the presence and impact of biofilms on the assessment of bacterial burden, wound assessment and treatment require further investigation

Australian health care system was estimated in 2005 as AUD $3 billion per annum (7).

Most chronic wounds such as leg ulcers are managed in the community setting and 22–50% of community nursing time involves wound management (8–10). Best practice treatment of venous leg ulcers is considered to be in control of wound exudate and lower leg oedema using compression therapy (11), with multilayer compression therapy regarded to be the gold standard (12). Wound healing is impaired in the presence of covert or overt infection (13). Therefore, the bacterial burden must be managed to prevent delayed healing, the development of infection and complications such as cellulitis, sepsis or even death.

It is generally accepted that the use of primary dressings with antimicrobial properties are clinically indicated when a wound becomes critically colonised (14–16) or infected and bacterial burden is unlikely to be managed by drainage, debridement or cleansing alone (13). However, there is little consensus regarding what constitutes best practice in antimicrobial preparations to treat bacterial colonisation and wound infection.

Silver and iodine are antimicrobial agents commonly used in the treatment of critically colonised or infected leg ulcers (17). Although the clinical efficacy has been demonstrated for both these antimicrobial dressings (17), prior to the current study there were no reported clinical trials directly comparing these treatments to determine their relative effectiveness in the treatment of leg ulcers.

The study

A randomised controlled trial (RCT) was conducted in 2006 and 2007 to compare the clinical efficacy and cost effectiveness of two antimicrobial dressings used to treat infection in lower leg ulcers (18). The RCT received philanthropic funding and was undertaken independently of any commercial interests. Nanocrystalline silver (Acticoat™) and cadexomer iodine (Iodosorb™) dressings were compared for their efficacy in controlling bacterial burden and facilitating wound healing among individuals with venous or mixed aetiology leg ulcers. Study participants were recruited from two not-for-profit community nursing services in Australia.

Individuals with leg ulcers were assessed and recruited to the study if they presented with the classical criteria for infection or clinical signs identified for chronic wounds with localised infection (19–21). Localised infection is also referred to as 'critical colonisation', 'covert' or 'occult' infection (13) and for the purposes of this study we used the term 'critical colonisation'.

In addition to a comparison of healing rates and costs, the study also examined the relationship between nurses' clinical assessments of signs and symptoms of critical colonisation and wound swab culture findings and between the type of dressing used and wound healing in the presence or apparent absence of different bacterial colonies and degrees of bacterial burden. It is these aspects of the study that are reported here.

METHODS

Details of the methodology used within the RCT are described elsewhere (18). Only those aspects of the method that are specifically related to the research questions examined in this paper have been provided here. Ethics approval was received from the two community nursing organisation's Human Research Ethics Committees and the trial was registered with the Australian and New Zealand Clinical Trials Registry. All study participants provided written, informed consent prior to commencing their participation in the study.

Study design

The RCT used an open label, parallel-group design in which the 281 participants were randomly allocated to receive either nanocrystalline silver (Acticoat™) or cadexomer iodine (Iodosorb™) dressings.

Inclusion criteria

Individuals were eligible for the study if they

1. Had a lower leg ulcer (not pressure ulcer) with ankle brachial pressure index (ABPI) of 0·6 or above
2. The wound was 15 cm or less in diameter
3. Were 18 years or older
4. Had not been on a course of topical antiseptic treatment 1 week prior to recruitment

5. Were not using any antibiotics 48 hours prior to recruitment
6. Were not using systemic steroids
7. Did not have a diagnosis of diabetes
8. Did not have a diagnosis of malignancy related to the leg ulcer
9. Were not receiving palliative care
10. Had no known contraindication to the treatment products

Participants' wounds also had to have at least one of the following clinical signs of infection or critical colonisation identified in the literature (13,19–21):

1. Cellulitis (pain, heat, erythema and swelling of surrounding tissues)
2. Suppuration (purulence or the presence of pus)
3. Lymphangitis
4. Sepsis (requiring confirmation by blood test)
5. Bacteraemia
6. Changes in granulation tissue (change in colour of granulation tissue, which may appear dusky, darker or bright red, hypergranulation tissue, friable or fragile granulation tissue that is prone to bleeding)
7. Increased or malodourous exudate
8. New areas of slough or wound breakdown
9. Impaired or delayed wound healing (epidermis fails to migrate across the wound bed, static, rolled or undermined wound edges or bridging segments of epithelial closure and breakdown in the tissues)
10. Increased or new pain

Nurses employed by the two community nursing organisations were provided with the eligibility criteria and received education on the assessment of these clinical signs of critical colonisation or infection. Where individuals presented with multiple wounds, the wound which showed the greatest signs of critical colonisation or infection was included. However, the same randomised treatment was applied to all leg ulcers if a participant had more than one, in order to avoid any confounding from variations in treatment.

Data collection and measures

All study participants were observed for 12 weeks from their recruitment to the study or less if their wound healed before 12 weeks. Data were collected at recruitment and every 2 weeks by one of the nurses trained for the study.

The primary outcome measures were wound healing rates (% change in wound size) and the number of healed wounds (100% closure) over a 12-week period. Wound size was measured using the Advanced Medical Wound Imaging System V2.2 (AMWIS™) software (22). Inter-rater reliability was demonstrated to be high for measurement of the total wound area (intraclass correlation coefficient = 0·958, $P < 0{\cdot}001$) undertaken by our data collection team (23). A daily healing rate was generated by determining the percentage change in the total surface area between two wound size measures and dividing by the number of days between wound measures. The healing rate was expressed as a daily rate to accommodate slight variations in the time of the data collection visit.

The presence or absence of the designated study signs of critical colonisation and infection was also assessed at baseline and every 2 weeks for 12 weeks or less if the wound healed.

Wound swabs were obtained during the study at recruitment, when ceasing or recommencing the antimicrobial, and at 6 weeks and 12 weeks if still using either antimicrobial dressing. The swabs were obtained by nurses using a zigzag technique and semi-quantitative bacteriological analysis was conducted (24,25) in wounds which had been irrigated and cleaned with sterile water prior to the collection of the specimen. The zigzag swab technique involves rotating a swab in a 10-point zigzag across a thoroughly cleaned wound bed while avoiding any necrotic tissue (24,26–28). A standardised protocol was followed to ensure the swabs were collected in a consistent manner and with all due care to sample 'healthy' granulation tissue and minimise risk of contamination from wound edges or necrotic tissue. Two swabs were collected for culture and microscopy; one swab was placed in the Amies Transport Media and the second was used to prepare a glass slide by placing the tip of the swab in the centre of the glass then rolling the side of the swab over the middle one third of the slide. The air-dried slide was

then placed in a plastic slide carrier. Specimens were stored in insulated boxes or with cold packs if the outside temperature was greater than room temperature. Arrangements were made for collection of the specimens or they were delivered to a laboratory within 24 hours of collection.

The swab specimens were cultured by independent pathology companies in Victoria and Western Australia. The pathology agencies liaised with each other to ensure swabs were managed and tests conducted consistently in accordance with industry standards. Gram stain and semi-quantitative analysis were conducted to identify the species of bacteria present and the level of bacterial burden. The bacterial burden was classified as either nil/scant, low, moderate or heavy. Sensitivity and resistance data were also gathered. The results were reported according to an agreed, standardised format across the two sites.

Treatment protocols

Study participants received their randomised silver or iodine dressings until all signs of critical colonisation and infection (according to the study eligibility criteria) were absent for 1 week. The nurses then continued the management of the wound with a non antimicrobial dressing regimen. If one or more signs of critical colonisation or infection were observed after this time, the antimicrobial treatment to which the client was originally randomised was recommenced. Changes in treatment were recorded. In accordance with the intention to treat principle, clients were analysed in the treatment group to which they were originally randomised and included in the sample irrespective of whether the antimicrobial treatment was ceased or recommenced.

The treatment protocol also required the use of compression bandaging, which was standardised to either a three (Profore™ Lite) or four layer (Profore compression system) compression bandaging system subject to lower leg assessment and (ABPI) as assessed by hand-held Doppler at recruitment. Adherence to compression bandaging was monitored to enable adherence to be examined as a covariate to healing.

Statistical analysis

The statistical package for the social sciences (SPSS) for MS Windows Release 16·0 (SPSS Inc., Chicago, IL) was used to analyse data. The statistical analysis used for the investigations described in this paper included Spearman's correlation and Mann–Whitney *U* tests. In assessing the semi-quantitative bacteriology results for each swab, the highest level of growth for either bacilli positive, bacilli negative, cocci positive or cocci negative was used regardless of which organism was isolated from the wound culture. This approach acknowledges the fact that a high degree of bacterial growth could be determined even when a specific organism could not be isolated. When assessing the growth associated with a particular organism, the level of growth indicated for the corresponding bacterial colony for each organism was used. The leukocytes colony data were also considered given that increased white bloods cells are representative of an immune response associated with infection.

RESULTS

The trial had 281 participants: 180 from one study site and 101 from the other. An equivalent number of participants were allocated to the silver ($n = 140$) and iodine ($n = 141$) treatments. Participants were 80 years of age on average (SD = 11·8) and slightly more likely to be female (58·6%). Ulcers were located on the lower leg (97·0%) with the remainder on the ankle or foot. Most ulcers were diagnosed as 'venous' (73·7%) with the remainder 'mixed' in aetiology. The average wound size was 704·66 (mm^2) (SD = 880·71) and median wound duration was 12 weeks at recruitment.

Data from 278 study participants were included in the swab culture analyses as two participants missed having a baseline swab taken and there were missing data for one participant regarding which the signs of critical colonisation and infection were present on recruitment. For analyses of healing rate, a sample of 266 participants applies as there were 15 participants for whom a healing rate could not be calculated for reasons of loss to follow up, withdrawal or death. The sample represented across the two weekly assessments declines as wounds healed and for which, therefore, a measure of wound size or bacterial burden was applicable.

Wound culture results

In addition to the wound swabs collected on all participants at recruitment, a further 301 swabs were obtained during the study. Most swab specimens were obtained when the individual was still receiving a silver or iodine dressing at 6 weeks (41·2%) and 12 weeks (21·6%) of study participation. Few swabs were collected because the participant was ceasing the randomised treatment (17·9%) and only one was collected because the randomised treatment was recommenced.

Of the swabs collected at baseline (the first swab), when all ulcers had to have demonstrated clinical signs of critical colonisation or infection to be eligible for recruitment, 37·8% had a nil or scant level of bacterial growth and 65·5% of swabs had nil or scant growth of leukocytes reported (Table 1). Remaining baseline swabs presented with a relatively even distribution of low, moderate and heavy growth.

The swabs gathered after baseline (second–fifth) were obtained for a multitude of reasons and subsequently reclassified accordingly to the reason they were gathered for analysis. A criterion for ceasing the randomised dressing was the absence of clinical signs of critical colonisation or infection for 1 week. As shown in Table 2, over half of the swabs collected for this reason had no bacterial growth (51·4%) or growth of leukocytes (84·7%). Of wounds swabbed after 6 and 12 weeks because signs of increased bacterial burden remained, over a quarter of wounds registered no bacterial burden (26·2% and 31·6%, respectively) and three quarters registered no growth of leukocytes (73·1% and 70·5%, respectively).

Table 1 Degree of bacterial burden and leukocytes growth at each swab

%	*n*	Nil/scant	Low	Moderate	Heavy
Bacterial burden					
First swab	278	37·8	18·7	18·0	25·5
Second swab	171	34·9	27·3	16·3	21·5
Third swab	85	34·9	24·4	20·9	19·8
Fourth swab	23	47·8	17·4	8·7	26·1
Fifth swab	5	20·0	60·0	20·0	–
Leukocytes growth					
First swab	278	65·5	22·7	9·4	2·5
Second swab	189	72·0	18·0	7·9	2·1
Third swab	97	74·2	18·6	7·2	–
Fourth swab	26	84·6	7·7	7·7	–
Fifth swab	5	80·0	20·0	–	–

Table 2 Degree of bacterial burden and leukocytes growth by swab indication

%	Nil/scant	Low	Moderate	Heavy
Bacterial burden				
Ceasing Antimicrobial Treatment swab ($n = 37$)	51·4	18·9	24·3	5·4
6th-week swab ($n = 107$)	26·2	29·9	15·0	29·0
12th-week swab ($n = 57$)	31·6	26·3	24·6	17·5
Leukocytes growth				
Ceasing Antimicrobial Treatment swab ($n = 46$)	84·7	6·5	6·5	2·2
6th-week swab ($n = 115$)	73·1	17·4	8·7	0·9
12th-week swab ($n = 61$)	70·5	18·0	11·5	–

When an organism could be isolated from the wound, *Staphylococcus aureus* was most common with almost nine in ten wounds in which an organism was identified being burdened with this organism. While *Pseudomonas* organisms and *Streptococcus* were also detected, their numbers were very low. Only 16 swabs were identified with methicillin-resistant *Staphylococcus aureus* (MRSA). Of these, 15 were community-derived MRSA as determined by their sensitivity to methicillin only, with one instance of MRSA classified as being of hospital origin (resistant to methicillin and gentamycin).

Signs of critical colonisation and infection

Table 3 provides descriptive information about the presence of the designated signs of critical colonisation and infection identified at baseline and every 2 weeks. On recruitment to the study most wounds were judged to have impaired or delayed healing (88·1%) and many had new areas of slough or wound breakdown (69·1%). Changes in granulation tissue (51·8%) and malodorous exudate (45·0%) were common and pain was reported by one third of participants (34·2%). While 66·3% of participants had one or more signs of critical colonisation, one third

Table 3 Presence of signs of infection or critical colonisation

	Baseline ($n = 278$)	Week 2 ($n = 272$)	Week 4 ($n = 248$)	Week 6 ($n = 194$)	Week 8 ($n = 158$)	Week 10 ($n = 124$)	Week 12 ($n = 98$)
% with no signs of bacterial burden	0·0	27·6	41·1	47·4	45·6	43·5	43·9
Number of signs of bacterial burden (Ave)	3·28	1·46	1·14	1·06	1·00	1·07	0·88
% of signs of bacterial burden							
Impaired/delayed healing	88·1	53·3	38·6	38·5	40·3	44·0	40·4
New areas of slough/wound breakdown	69·1	14·0	13·3	11·3	11·3	9·6	7·1
Changes in granulation tissue	51·8	33·1	25·3	22·6	18·9	22·4	24·2
Increased/malodorous exudate	45·0	12·1	12·9	12·8	13·2	8·0	8·1
Increased/new pain	34·2	12·1	8·4	6·2	5·0	4·8	1·0
Cellulitis	24·8	6·2	6·0	4·6	1·9	4·8	3·0
Suppuration	9·4	5·9	4·4	2·6	3·1	4·8	6·1
Lymphangitis	0·0	0·7	0·4	0·0	0·0	0·0	0·0
Sepsis	5·8	10·3	6·0	7·7	6·3	8·8	8·1
Bacteraemia	0·4	0·0	0·0	0·0	0·0	0·0	0·0

of them (33·7%) also had one or more signs of infection (cellulitis, suppuration, lymphangitis, sepsis and bacteraemia). Almost three quarters of participants (71·8%) had multiple signs of infection or critical colonisation at baseline with an average of 3·28 signs.

Relationship of wound culture results to signs of bacterial burden

The relationship between the semi-quantitative bacteriology results and the observed clinical signs of critical colonisation and infection was examined at baseline. Table 4 cross-tabulates the number of signs of bacterial burden and the level of bacterial growth identified by the wound culture obtained at recruitment. A χ^2 test of the association found no significant association between clinician observations and bacterial burden [$\chi^2(6) = 9{\cdot}41$, $P > 0{\cdot}05$] or the presence of leukocytes [$\chi^2(3) = 5{\cdot}93$, $P > 0{\cdot}05$]. This finding is consistent with a Spearman's correlation which also found a very low, non significant relationship between the number of characteristics of critical colonisation observed or infection reported and the swab culture ($r = -0{\cdot}081$, $P > 0{\cdot}05$) and leukocytes ($r = -0.011$, $P > 0{\cdot}05$).

Relationship between wound culture results and wound healing rates

The bacterial burden, both type and amount of colonisation, as identified by the baseline wound swab results was examined in relation to the healing rate achieved in the first fortnight by each treatment arm. Table 5 presents the results of Mann–Whitney *U* tests used to compare the healing rates of the two antimicrobial treatment groups in the first 2

Table 4 Number of eligibility criteria identified and degree of bacterial growth

	Degree of bacterial burden				Degree of leukocytes growth			
% Number of wound characteristic criteria met	Nil/scant ($n = 105$)	Low ($n = 52$)	Moderate ($n = 50$)	Heavy ($n = 71$)	Nil/scant ($n = 182$)	Low ($n = 63$)	Moderate ($n = 26$)	Heavy ($n = 7$)
1 criterion	8·6	7·7	4·0	9·9	9·3	4·8	3·8	14·3
2 criteria	15·2	13·5	30·0	25·4	19·8	23·8	15·4	14·3
3 criteria	37·1	19·2	20·0	38·0	30·8	36·5	26·9	–
4 criteria	23·8	25·0	26·0	12·7	20·9	20·6	26·9	28·6
5 criteria	13·3	25·0	12·0	11·3	17·0	9·5	11·5	14·3
6 criteria	1·9	9·6	6·0	2·8	1·6	4·8	15·4	28·6
7 criteria	–	–	2·0	–	0·5	–	–	–

Table 5 Comparing the healing rate for the first 2 weeks for the treatment groups and swab results

		n		Mean rank		
Colony	Degree of growth	Silver	Iodine	Silver	Iodine	Mann–Whitney *U* test
Leukocytes	Nil/scant/low	116	110	124·02	102·40	5159·50**
	Mod/high	16	17	18·69	15·41	109·00
Gram + bacilli	Nil/scant/low	90	90	99·53	81·47	3237·00*
	Mod/high	5	1	3·80	2·00	†
Gram – bacilli	Nil/scant/low	62	64	67·97	59·17	1707·00
	Mod/high	43	32	40·60	34·50	576·00
Gram + cocci	Nil/scant/low	72	73	82·62	63·51	1935·00**
	Mod/high	41	35	40·56	36·09	633·00
Gram – cocci	Nil/scant/low	87	83	93·88	76·72	2881·50*
	Mod/high	–	–	–	–	–

*Significant at $< 0{\cdot}05$,

**Significant at $< 0{\cdot}01$,

†Sample too small for analysis.

weeks when bacterial growth was categorised as nil/scant/low or moderate/heavy growth. Where moderate-to-heavy growth was identified, there were no differences in healing rates between the silver- or iodine-treatment groups. In contrast, where nil/scant or low bacterial growth was identified, silver had a significantly faster healing rate compared to iodine for the growth of leukocytes [Mann–Whitney U $(224) = 5159{\cdot}50$, $P < 0{\cdot}01$], gram positive bacilli [Mann–Whitney U $(178) = 3237{\cdot}00$, $P < 0{\cdot}05$], gram positive cocci [Mann–Whitney U $(143) = 1935{\cdot}00$, $P < 0{\cdot}01$] and gram negative cocci [Mann–Whiney U $(168) = 2881{\cdot}50$, $P < 0{\cdot}05$] within the first 2 weeks. These results suggest that silver is more effective than iodine in the first 2 weeks when there is nil or only low levels of bacterial colonisation for all bacterial colonies except for gram negative bacilli.

The overwhelming dominance of *Staphylococcus aureus* restricted the capacity to examine the healing performance of both silver and iodine given different types of organisms, as well as the influence of organisms on the specific signs of bacterial burden being observed within the wound.

DISCUSSION

This paper examined the relationship between nurses' clinical assessments of signs and symptoms of critical colonisation and infection and wound swab culture findings, and the relationship between the type of wound dressing (nanocrystalline silver and cadexomer iodine) and healing rate in the presence of certain colonies and degree of bacterial burden.

Signs of critical colonisation and infection

The results reveal that the nurses' assessment of the clinical signs of critical colonisation or infection has little relationship with the bacterial burden detected by semi-quantitative bacteriology. Given that all wounds were recruited to the study with one or more clinical signs of critical colonisation or infection (an average of 3·28 signs was identified), it is noteworthy that microbiology results revealed almost four in ten of the wounds had nil or scant levels of any bacterial growth and two thirds demonstrated no growth of leukocytes.

This study used a list of signs and symptoms of critical colonisation and infection as informed by the literature (19–21) as the benchmark to determine the presence of bacterial burden and indication for topical antimicrobial treatment. Although wound swabs were obtained at recruitment, these results did not influence eligibility for the study nor their treatment. This approach was adopted as it was thought to best reflect the prevailing practice of commencing a broad spectrum topical antiseptic dressing in the presence of clinically assessed signs of critical colonisation or infection (29). In the case of suspected infection, clinically assessed signs are generally used to initiate antibiotic treatment, although ideally a diagnosis of infection would take into account the clinical signs and symptoms as

well as microbiology findings (13,25,30). The classic signs and symptoms of infection tend to be more evident in acute surgical or traumatic wounds. However, in chronic wounds and especially when the host response is compromised, the signs and symptoms may be more subtle or differ according to wound type and aetiology (13,20,21,30,31). This discrepancy in clinical signs and symptoms between different wound types and different aetiologies raises important issues for clinical practice, including the potential for errors in clinical assessment, the need to determine the most efficacious method for specimen collection, and reporting accuracy.

The discrepancies between the clinical observations of critical colonisation and infection and semi-quantitative bacteriology findings in this study may suggest that clinical alterations in wound characteristics develop in advance of increases in bacterial burden that can be accurately detected on a standard wound swab and culture (32–35). Alternatively, signs and symptoms aligned to critical colonisation may actually represent chronic inflammatory changes rather than a host-pathogen interaction (31). Regardless of references in the literature to the concept of critical colonisation and the myriad of other descriptors which are used interchangeably, there remains a lack of international consensus as to the condition and what precipitates it (13). This shortfall highlights the need for expedient and dedicated research to address the debate.

The 'gold standard' for specimen collection and diagnosis of infection is reported to be a quantitative tissue biopsy (25,33,36). However, for practical reasons related to technical skill required, potential sampling errors, potential risk of infection or healing delays and increased costs, they are infrequently performed in the community setting (25,35). Instead, the semi-quantitative swab technique is more frequently performed, presumably because it is simple, non invasive and relatively inexpensive (37). Furthermore, it has been reported by several authors to correlate significantly with quantitative biopsy results (25,30,35,36,38). While not all studies have observed a correlation between swab and biopsy, although both swab and biopsy independently correlated with the results obtained using absorbent polyvinyl acetate foam discs, the surface sampling methods were found to recover more species than biopsy (39). This method was chosen in this study because of these reports and the fact that at the time of planning and commencement of the study this was the standard method employed for collection of wound swabs by both community nursing agencies.

Another possible explanation for the study results relates to a lack of consensus in the literature as to which wound swab technique achieves a more representative sample of bacteria in a wound. Some reports indicate better wound sampling results with the Levine method (33,40). The Levine swab technique involves rotating a swab over a 1 cm^2 area with sufficient pressure to express fluid from within the wound tissue and then agitating it in 1 ml of transport media before it is then serially diluted and cultured on pour plates (25,41). More research is needed into swabbing methods in order to ascertain whether there are significant differences in what is cultured depending on the method used, rather than on the presence or absence of organisms.

A third possible explanation for the discrepancies between clinically observed signs and symptoms of critical colonisation and infection and the microbiology results could be assessor error. Gardner and colleagues (33) examined the reliability of a tool that listed the clinical signs and symptoms of localised infection in chronic wounds as proposed by Cutting and Harding (19). Gardner and colleagues (21) found acceptable reliability estimates among assessment parameters and favourable comparisons between other similar research (40,42). However, Lorentzen and Gottrup found the opposite to be the case and reported 'great variability and low reliability' when they tested six wound management specialists' clinical assessment of infection in chronic wounds (43). Although the nurses involved in our study were provided with comprehensive education on assessment of the clinical signs and symptoms, there was no specific inter-rater reliability testing of their clinical assessment of infection. Future research could compare best practice, non antimicrobial treatments to antimicrobial treatments to further examine the effectiveness of topical antimicrobials when signs of critical colonisation have been assessed. By utilising varied methods of assessing critical colonisation, comment would be possible as to the significance of each methods determination of critical colonisation

and subsequent antimicrobial treatment on healing.

Wound biofilms

A further possible explanation for the discrepancies between the nurses' assessment and swab findings is the presence of wound biofilms and the barrier they may pose to sampling bacteria in a wound (44,45). Although long recognised in other domains such as dentistry and engineering, the concept of biofilms in wounds is a relatively recent phenomenon (45) and one that is attracting increasing interest as researchers endeavour to define reasons for wound chronicity and impaired healing (46). The wound environment is conducive to biofilm development given the moist conditions (47) and it has been suggested that 60% of chronic wounds, as compared to 6% of acute wounds, contain a biofilm (48).

Biofilms are complex communities of bacteria which evolve when a planktonic bacterium attaches itself to the exposed extracellular matrix proteins found on the surface of the wound (48). Microcolonies are formed rapidly within an extracellular matrix of extrapolymeric substances and mature into an encased biofilm (47,49) which are suggested to have some capacity to resist host-defence mechanisms and topical antimicrobial assault (45). The emergent role biofilms play in wound colonisation and infection is of increasing interest to wound clinicians and scientists alike (44,50).

Although most planktonic bacterium (single-celled non attached) are capable of being collected on a swab and cultured (45), this is not the case when a biofilm phenotype exists (44,45). As may be the case with this study, there is potential for the wound culture results to be distorted and be an unreliable indicator of critical colonisation or infection. Furthermore, these findings challenge the practice and reliability of collecting wound swab specimens in chronic wounds, especially if as reported as many as 60% of chronic wounds contain biofilms (48). The literature reports wound biopsy and more sophisticated light microscopy, scanning electron microscopy and epifluorescence microscopy techniques, and molecular analysis needs to be used to identify pathogenic biofilms in wounds (48,50). These sophisticated diagnostic tools require a high degree of technical skill and would add to the expense, and both factors would present considerable challenges in community practice. Nevertheless, research into both the presence of biofilms and techniques for their effective and safe removal in a community setting, such as through debridement, is very much needed (47,49,51).

Antimicrobial efficacy

The efficacy of the silver and iodine wound dressings was ascertained in the presence of leukocytes and gram positive and negative bacilli and cocci. Improved healing rates were achieved with the use of silver as compared to iodine dressings when there was a low level of bacterial growth in the wound, with the exception of gram negative bacilli, during the first 2 weeks of treatment. Examination of healing rates in light of bacterial colony and degree of bacterial burden was not examined for the entire 12-week study period due to variations in the timing of swabs; the baseline swab being the only consistent time when all study participants were swabbed. Healing rate was the principle outcome measure for the study and as reported in the main project findings (18), the silver antimicrobial had a significant quicker healing rate in the first 2 weeks compared to iodine but at no other two weekly assessment nor overall in the 12-week study period. Similar initial healing advantages within the first 4 weeks of treatment were found in a Cochrane Collaboration review (52) of three studies that investigated the use of silver alginate versus non silver alginate dressings, although no comparisons with swab results were reported in the literature. Planktonic bacteria are destroyed with appropriate topical antimicrobial use (53), while biofilms have high resistance to topical antimicrobials as well as other control mechanisms such as antibodies, phagocytic inflammatory cells and systemic antibiotics (49,53,54). The findings of this study and those reviewed by Cochrane (52) could be indicative of early planktonic bacterial kill of low levels of bacterial burden, but failure to control established biofilm populations. More work is required to advance the understanding of biofilm resistance to topical antimicrobial treatments.

Some limitations of this study include the capacity to consider antimicrobial effectiveness only for the first 2 weeks using baseline swab information as the study protocol for swabbing thereafter resulted in variation in the timing

of wound swabs. The reliability of clinician assessment of the signs and symptoms of bacterial burden is another area which would have provided useful information to help resolve the potential reasons for the discrepancies observed among these data. As the data were generated from a study for which a number of eligibility criteria applied, the population cannot be considered representative of all individuals with leg ulcers. There was no statistical adjustment for power used in the analyses. As such, any result which was marginally significant at an alpha of 0·05 did not reconcile with other data or present as a consistent pattern of effect was qualified in the text.

Study recommendations and conclusion

This study provides the first direct comparison of two commonly used antimicrobial dressings, nanocrystalline silver and cadexomer iodine, which were used in the treatment of critically colonised or infected venous or mixed aetiology leg ulcers.

Wound swab results were compared with the clinical assessment findings and discrepancies were found to exist. A strong case has been demonstrated for further examination of the validity and reliability of wound swab techniques as well as assessment using signs and symptoms of critical colonisation and infection. Furthermore, there is a need for studies which advance reliable diagnostic methods to assess bacterial burden in the presence of biofilms and those that lead to a greater understanding of the impact of biofilms on treating chronic wounds. Similar dedicated research is encouraged into advancing the understanding of critical colonisation, its aetiology and some consensus regarding terminology.

This study has yielded results which prompt considerable discussion with regard to bacterial burden assessment and intervention. The use of multiple measures of critical colonisation and infection including wound culture specimens and clinical assessment outcomes need to be considered jointly until methods of sampling and assessing bacteria and wound biofilms are better understood.

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