

## Original Research Article

# A pilot study to assess superficial versus deep tissue culture in acute wounds

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## ABSTRACT

**Background:** To determine whether concordance exists between superficial and deep tissue culture in isolating organisms in acute wounds.

**Methods:** In acute wounds presenting to surgical department a superficial swab was taken and after debridement of the wound a deep tissue biopsy was taken to isolate organisms for sensitivity. Results were analyzed using z-score at  $p < 0.05$ .

**Results:** Sample size of 50 cases was studied for isolates. 28 cases were non diabetic and the rest diabetics. There was a significant discordance of isolates in diabetics (81.8%) as compared to 21.4% in non-diabetics.

**Conclusions:** Simultaneous swabbing and deep tissue biopsy improves the isolate detection rate in ulcers for diabetics and minimizes the under treatment risk in this group of patients. This is also true for non-diabetic ulcers as there is a possibility of discordance in this group too, though infrequent.

**Keywords:** Swab culture, Deep tissue culture, Sensitivity

## INTRODUCTION

There is still an on-going debate of whether superficial swabs or deep tissue cultures are more beneficial for organism identification, particularly so in diabetic wounds.<sup>1</sup> Swabs, for taking a culture are almost universally available in healthcare settings and are quick and easy to use. But, they may be susceptible to collecting contaminants and the inability to grow some pathogens.<sup>2,3</sup> This is due to the fact that the humid environment of an ulcer is likely to promote the overgrowth of skin opportunistic flora. Wound infections are a substantial healthcare burden, contributing to increased morbidity and mortality, prolonged hospitalization, limb loss, loss of working days and

higher medical costs. They also pose a potential risk of sepsis for patients.

For wound care providers, the aim is to eliminate infection before adverse consequences arise. It was our endeavour to study whether there is any significant difference in identifying organisms by the superficial swabbing method and deep tissue biopsy culture. We undertook this study as a pilot study in our rural medical college. For a patient with a suspected wound infection, cultures are important in diagnosing infection, identifying the specific organism and its susceptibility to antibiotics. This information guides appropriate antibiotic treatment and is crucial in preventing antibiotic-resistant infections and reducing morbidity and mortality in a cost effective

way more so in a rural set up. The aim of this study was to assess the concordance between superficial and deep culture results.

## METHODS

This study was undertaken for acute limb ulcers. In superficial swabbing technique the Levine's technique was used wherein wound was first cleaned with normal saline in order to remove all exudates and then a swab was twirled over the wound with enough pressure to cause minimal bleeding and transported to the microbiology department at the earliest.

Immediately after the cotton swab has been collected, a tissue sample was removed from the same area of the ulcer bed. This procedure was done using aseptic precautions. It involved the biopsy of a small piece of wound tissue at the base of the wound by scraping or scooping using a scalpel blade. The tissue was transported in a plain sterile bulb. The deep tissue was minced in sterile pestle and mortar before inoculation. All the samples were inoculated on Blood and MacConkey agar. A primary smear was prepared for gram staining and results were noted. The plates were incubated overnight and isolates were identified by conventional methods. All the isolates were subjected to antibiotic sensitivity using Kirby Bauer Disc Diffusion method as per CLSI guidelines. Muller Hinton agar was used for antibiotic sensitivity testing. The inoculum of the test strain to a density visually equivalent to 0.5% McFarland turbidity standard was prepared by growing the strain in peptone water. The entire surface of the plate of medium was inoculated. The surface was allowed to dry,

antibiotic discs were placed and the plates were incubated at 37 degrees for 24 hours. No attempt was made to use anaerobic culture.

## RESULTS

This study included 50 patients. 24 cases showed discordance of isolates on culture and 26 indicated concordance. This observation indicates that majority of the patients had no different isolates on superficial and deep cultures which was proved by the Z-Score which was -0.4. The p-value was 0.68916 indicating no significance at  $p > 0.05$ .

**Table 1: Case distribution.**

	Frequency	Percent
Non Diabetic	28	56
Diabetic	22	44
Total	50	100

However on dividing these two groups into subgroups of diabetics and non-diabetics it was observed that 28 were non diabetic and the rest diabetic (Table 1). Amongst the non-diabetic cases the culture was discordant in 6 cases (21.4%) and 22 were concordant, whereas in diabetic group culture was discordant in 18 cases and concordant in only 4. On applying the z score in non-diabetic group it was -5.8 which was significant at  $p < 0.05$  and 6.4 in diabetic group which too was significant at  $p < 0.05$  (Table 2). Significantly, concordant pairs are more in non-diabetics and discordant pairs are more in diabetics.

**Table 2: Percentage of concordance and discordance.**

Concordance	Non-diabetic		Diabetic	
	Frequency	Percent	Frequency	Percent
Discordant pairs	6	21.4	18	81.8
Concordant pairs	22	78.6	4	18.2
<b>Total</b>	<b>28</b>	<b>100</b>	<b>22</b>	<b>100</b>

The Z-score is 5.8; The result is significant at  $p < 0.05$ ; The Z-score is 6.4; The result is significant at  $p < 0.05$ .

## DISCUSSION

Limb ulcers are highly prevalent in rural population and cause considerable morbidity and socioeconomic strain. This is more relevant in diabetics as a combination of increased blood sugar with reduced immune system, neuropathy increase their susceptibility to life threatening infections leading to a cascade of complications that varies from sepsis to amputations and death. But, identifying the responsible organism is of paramount importance. This is compounded by the presence of both, pathogens and colonizers in majority of foot wounds.

Hence, the culturing technique becomes crucial in identifying the true pathogens. Collection of samples for isolating the organisms can be done by various techniques viz. swab, aspiration, curettage, and tissue biopsy. Some authors have suggested qualitative instead of quantitative analysis of the wound swab, whereby specific strains of bacteria are identified.<sup>4</sup>

A deep-tissue biopsy after initial debridement and cleaning of superficial slough with normal saline solution is the most appropriate way to detect invasive organisms. Unfortunately quantitative biopsies are hard to perform,

painful, expensive and not available in all settings. Necrotic tissues particularly in deeper planes in a wound should be removed as it interferes with proper assessment of the wound bed, and also can be a source of bacterial growth. The deep bacterial colonies can potentially produce unwanted metalloproteinase that adversely affect extracellular matrix (ECM) components during the healing process, and form biofilm in wound beds. Biofilm is bacterial colonization of the wound surface that is highly resistant to antibiotic treatment, including standard treatments such as systemic antibiotics.

We decided to undertake a pilot study in our rural medical college wherein, in an acute ulcer two specimens viz. one superficial swab and the second, deep tissue biopsy for culture were taken irrespective of their diabetic status or bony involvement (osteomyelitis). Why do we need to do this? It is because we feel that awareness regarding discordance of organisms in wounds is important for prevention of chronicity of wounds and to avoid potential complications. Conventionally many surgeons obtain cultures from the surface of wound beds as the initial step in the wound evaluation process. This allows for appropriate antibiotic treatment in the initial phase of treatment.<sup>5</sup> It has been found that majority of organisms can be identified using a superficial swab technique but an argument is put forth that in doing so the wounds will be over treated, as the superficial swab is likely to contain contaminants, which could be minimized by opting for a deep tissue culture thus preventing the broadening of antibiotic coverage, in turn preventing antibiotic resistant strains from developing as also obviating effects of biofilm. However our study revealed that in non-diabetics, chances of discordance is less as compared to diabetic wounds. We feel that taking a superficial swab and deep biopsy culture in diabetics would be prudent because chronicity of wounds is common in diabetics leading to bone infections thus making it imperative to treat diabetic wounds promptly to avoid prolonged hospital stay and avoid the dreaded risk of amputations.

However some studies suggest that taking a superficial swab is erroneous as it may be contaminated by normal skin flora and might give up a wrong sensitivity result.<sup>6</sup> Newer techniques for rapid identification of causative pathogens include the polymerase chain reaction (PCR) assay for organisms and the oligonucleotide assay for virulent gene identification for treating diabetic foot infections.

Regardless of the many studies that compare whether swab cultures are beneficial or not, it seems prudent that swabbing wounds when one initially encounters an acute ulcer will only aid in beginning the treatment process.<sup>7</sup> Some metaanalysis also suggest that the sensitivity and specificity of superficial swabbing is a dismal 49% and 62%.<sup>8</sup>

Factors that increase the risk for diabetic foot infections are presence of an ulceration for >30 days; recurrent foot ulcers, traumatic wound, peripheral vascular disease in the affected limb; a past history of lower extremity amputation, neuropathy and nephropathy. *Staphylococcus aureus* are the predominant organisms isolated from wounds particularly of diabetic aetiology.<sup>9</sup>

The concept of deep tissue biopsy culture may be more relevant in cases of chronic wounds with osteomyelitis because the humid environment of a chronic ulcer is likely to facilitate overgrowth of skin or opportunistic flora. Deep tissue biopsy has yielded better results and might be more sensitive than superficial swabbing for monitoring ulcers that are still active after 30 days of treatment.

Our study revealed majority of the patients in the non-diabetic category had similar isolates whereas the diabetic patients showed a more propensity towards discordance. We did not attempt to isolate MRSA (methicillin-resistant *Staphylococcus aureus*) which also constitutes a substantial part of the isolate that would require surgical intervention.<sup>10</sup> It is necessary to distinguish diabetic and non-diabetic ulcers as the risks associated with the former tax the patient's resources with a looming risk of amputation.

## CONCLUSION

Control of infection and prevention of complications in limb ulcers are important goals to be achieved more so in diabetic patients. In a rural setup the least that could be done to prevent chronicity or non-healing of ulcers is to have a superficial swab for culture followed in the same sitting by a deep tissue culture after debridement. It would be prudent to demonstrate concordance or discordance of isolates for proper use of antibiotics apart from other measures to control diabetes.

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