Bacteriology of diabetic ulcers: effect of sample collection method

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ARTICLE POINTS

1 Foot ulcers are an important cause of morbidity in people with diabetes.

2 Polymicrobial infections are common in the devitalised tissues.

3 Treatment and fate of the extremity depends upon the culture report.

4 Sampling by sterile swabs misses important pathogens.

5 True bacteriological yield is obtained from the deep tissue samples.

6 Rational therapy should be based on culture report from tissue samples.

KEY WORDS
- **Diabetes**
- **Foot ulcers**
- **Swab samples**
- **Deep tissue samples**
- **Bacterial yield**

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Introduction

Most diabetic foot lesions have a polymicrobial aetiology; however, bacterial yield varies with the method of sample collection. This study compared the bacteriology of swab samples and deep tissue samples obtained at the same time from 50 diabetic foot ulcers. The bacterial yield from the deep tissue samples was significantly higher than from the swabs, and provided a comprehensive picture of the pathogens present in the ulcer. As the fate of the lower extremity is dependent on the treatment regimen, which is based on the culture report, it is crucial that no pathogens are missed. Although swabs may be a useful adjunct, they must be accompanied by deep tissue samples to enable health professionals to decide on the most appropriate therapy.

Foot infections are a major cause of morbidity in people with diabetes. Devitalised tissue is the site where the bacteria responsible for the non-healing ulcers inflict damage. The bacteriology of diabetic foot ulcers has been studied by numerous investigators (Sharp et al, 1979; Wheat et al, 1986; Bamberger et al, 1987; Peterson et al, 1989; Lipsky et al, 1990; Gerding, 1995). Most of these lesions have been found to have a polymicrobial aetiology (Louie et al, 1976; Sapico et al, 1984; Wheat et al, 1986.). However, there are several techniques of sample collection, and the bacterial yield may vary with the technique.

Aim of the study

A study was undertaken to assess the influence of sample collection methods on the yield of aerobic and anaerobic bacteria from diabetic foot ulcers.

Method

This study was carried out in a large general hospital at Pune, India, over a one-year period.

Fifty people with diabetes and a foot ulcer of grade II or more (Wagner, 1981) attending surgery clinics were enrolled in the study. Wagner (1981) defines a grade II ulcer as a deep ulcer, often infected, but with no bony involvement. Patients with superficial ulcers or mere abrasions were excluded.

Sample collection and processing

The ulcer site and size were examined with the patient laying supine on an examination table. Superficial dead tissue or eschar was removed with sterile scissors and a scalpel blade. After local debridement of devitalised tissue, the wound was cleaned with sterile saline.

Samples were then obtained from each ulcer, using two different techniques:

- Swab samples were collected by rubbing the deepest accessible area of the lesion with a cotton-wool tipped swab moistened with saline.
- Samples of devitalised tissue were obtained from the depth of the wound, taking aseptic precautions.

Both samples were transferred to the laboratory in transport media, and processed immediately.

The swab samples were inoculated on culture media. The tissue samples were ground in a sterile mortar and pestle with sterile peptone water. The resulting homogenate was used immediately for inoculation of culture media and smear preparation. The organisms were identified by routine tests using standard procedures (Collee et al, 1989; Koneman et al, 1992).

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1 In this study the yield of organisms from the deep tissue samples was significantly higher than from the surface swab samples.

2 Swab samples always contain organisms that normally colonise the skin and so may not be reliable.

3 Needle aspiration of deep tissue is probably the ideal method of collecting samples, as aspirate contains only the organisms colonising the inflamed tissues.

4 This method excludes surface contaminants, but may miss pathogens if the needle is not inserted into the infected portion of the deep tissue.

5 Needle aspirates therefore have a high specificity but low sensitivity for pathogenic bacteria within the deep tissue of the lesion.

Table 1. Method of sample collection and numbers of aerobes and anaerobes isolated

	Swab (n=50)		Tissue (n=50)	
	Total	Average*	Total	Average*
Organisms isolated	150	3.0	185	3.7
Aerobes	125	2.5	145	2.9
Anaerobes	25	0.5	40	0.8

*organisms per sample

Results

Swab samples and deep tissue samples obtained from the diabetic foot ulcers of 50 patients were processed.

The swab samples yielded a total of 150 organisms, comprising 125 aerobes and 25 anaerobes (average 3.0 organisms per sample). The deep tissue samples yielded a total of 185 organisms, comprising 145 aerobes and 40 anaerobes – average 3.7 organisms per sample (*Table 1*; *Figure 1*). The yield from the deep tissue samples was significantly higher than the yield from the surface swab samples ($p<0.01$).

Among the aerobic organisms cultured, *Staphylococcus aureus* was the most common, followed by *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* species and *Enterococcus* species, in descending order.

Among the anaerobic organisms cultured, *Peptostreptococcus anaerobius* was the most common, followed by *Prevotella melaninogenica* and *Prevotella intermedia*. *Clostridium perfringens* was isolated from the deep tissue sample in two patients, but not from the surface swab sample.

Discussion

In diabetic foot ulcers, samples for bacteriological analysis can been obtained by several methods:

- sterile swabs
- needle aspiration
- tissue samples.

Swab samples are obtained from the base of the ulcer after cleaning with saline and rubbing the swab over the lesion. They can also be taken directly from the purulent exudate.

Specimens obtained by swabs almost always contain organisms that normally colonise the skin and so may not be reliable. Some of the isolates obtained by this method have been labelled 'false positives' by Bamberger et al (1987). However, Wheat et al (1986) have demonstrated that these isolates, although commensals, cannot be ignored and may have a role in the aetiology of the ulcer.

Needle aspiration of deep tissue is probably the ideal method of collecting samples, as aspirate contains only the organisms that are colonising the inflamed tissues. Specimens obtained by this method almost certainly exclude the surface contaminants, but pathogens may be missed if the needle is not inserted into the infected portion of the deep tissue.

Furthermore, Gerding (1995) noted that needle aspirates tend to yield fewer isolates than are actually present in the deep tissue. Thus, although aspirates are highly specific for pathogenic bacteria within the deep tissue of the lesion, they have a low sensitivity. Peterson et al (1989) compared the needle aspiration and swabbing methods, and found no significant difference in quantitative concordance between the two techniques.

In our study, significantly more organisms were isolated from deep tissue samples (185; average 3.7) than from properly collected swabs (150; average 3.0) ($p<0.01$), indicating that deep tissue sampling is a more sensitive method. Jones et al (1985) and Lipsky et al (1990) also found that

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1 Obtaining samples from diabetic foot ulcers using a sterile swab in a meticulous manner is a useful screening method but may miss important pathogens.

2 Treatment regimen is based on the culture report, so this has to be accurate and include all pathogens.

3 Unlike swab samples, deep tissue samples from the devitalised tissue within a foot ulcer provide a comprehensive picture of the pathogens involved.

4 This enables more rational, and hence more effective, therapy to be commenced.

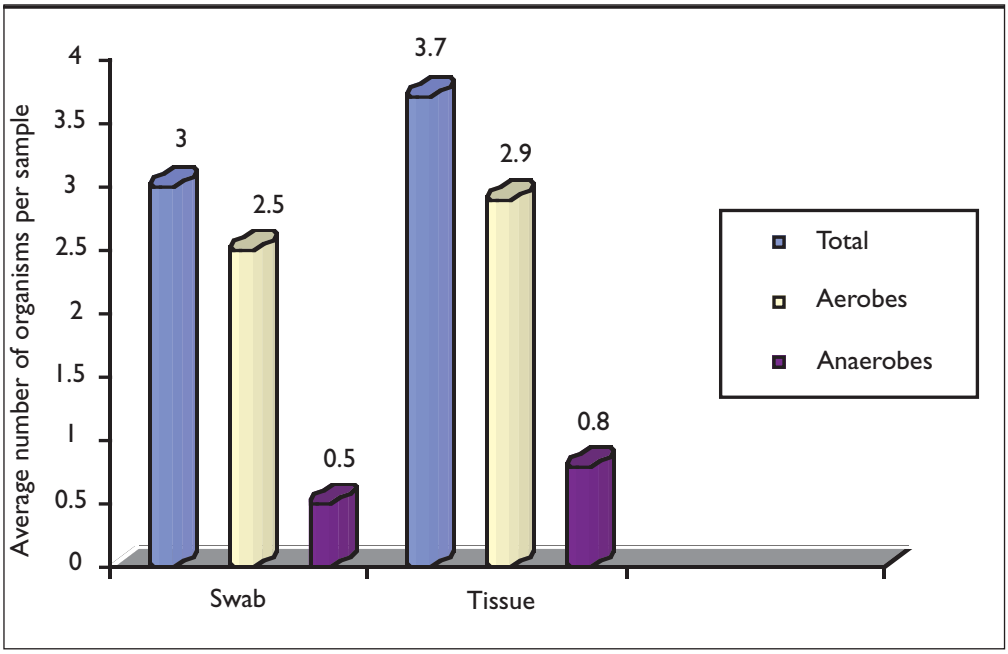

Figure 1. Bar chart showing the average total number of organisms, and average numbers of aerobic and anaerobic organisms, isolated per sample obtained from swab and deep tissue samples from the diabetic ulcers (n=50) in the study.

culture of specimens obtained by deep tissue sampling is the most sensitive method for detecting pathogens in diabetic foot ulcers.

Conclusion

Obtaining samples from diabetic foot ulcers using sterile swabs in a meticulous manner, although a useful screening method, may miss important pathogens. In a patient with a diabetic foot ulcer, the treatment regimen is based on the culture report, on which the fate of the lower extremity in question may be decided. It is therefore of paramount importance that the culture report does not miss any pathogens.

Deep tissue samples collected from the devitalised tissue provide a comprehensive picture of the pathogens involved, unlike swab samples. Although swabs can be a useful adjunct, they must always be followed by deep tissue samples, so that more rational – and hence more effective – therapy can be commenced. ■

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