CHAPTER 2

INTRA-OPERATIVE CULTURE SWABS VERSUS DEEP TISSUE SPECIMENS: Assessing Concordance of Isolates Collected From Diabetic Foot Infections

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INTRODUCTION

Diabetes mellitus affects 12 million individuals in the Unites States.[1] This astounding number of people who are at risk of neuropathy, peripheral vascular disease, and infection has truly overwhelmed the medical community while placing urgency on proper treatment. Unfortunately, by the year 2025, there will be an estimated 300 million people affected with diabetes worldwide.[1] From this, one can gather the importance of treating diabetic foot infections properly, as foot disorders account for 1 in 5 of all diabetes-related admissions.[1] To do this, physicians must accurately culture sources of pedal infections. While this seems simplistic, the literature has been controversial for over 2 decades.

Research has supported poor concordance of swabs and deep tissue specimens[2] as it has also documented that swabs may be reliable markers of infection.[3,4] Sapico, in 1984 related poor concordance of swabs as compared with deep tissue. In return, Pelizzer found superficial swabs to be consistent with deep tissue isolates in the first 2 weeks of an ulcer's existence. Recently, Slater et al determined 90% of swabs identified the same bacterial isolates as deep tissue samples when bone was not exposed. With bone exposure, they concluded 65% of swabs identified the same isolates as deep tissue. This controversy continues to exist.

Identifying the responsible pathogens is complicated by the presence of both pathogens and colonizers in most pedal wounds.[3] It is vital that patients are placed on the proper antimicrobial agents for resolution of infection. Improper identification of microbes, or even neglecting to isolate various microorganisms could be catastrophic for the patient and expensive for the hospital. Therefore, the aim of this study was to determine if there was a difference in intra-operative culture swabs versus deep tissue specimens taken from University of Texas (UT) grade 2 and 3 ulcerations via sterile technique. This study was also designed to determine aerobes, anaerobes, acid fast bacilli, and fungi most commonly isolated from each technique (Figure 1).

PATIENTS AND METHODS

This prospective study included 32 patients who had a University of Texas grade 2 or 3 ulceration with infection. All wounds were graded according to the University of Texas Wound Classification System.[3] Grade 1 wounds indicate a superficial wound, while grade 2 wounds involved subcutaneous tissue up to, but not including, tendon or capsule. Grade 3 wounds extended to bone or joint. The authors diagnosed extension to bone by probing bone with a sterile steel probe.[5] The majority of patients in this study had UT grade 2 or 3 ulcerations. This was because an inclusion criterion for this study was surgical debridement by means of incision and drainage or amputation. Cultures were only taken in a sterile surgical environment. Hence, the probability of a wound needing surgical intervention was greater with a deeper level of infection or osteomyelitis.

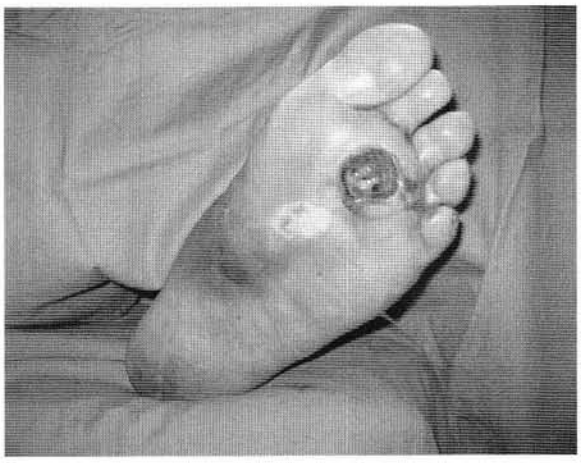

Figure 1.

Determination of infection was clinically diagnosed prior to culturing. Infection criteria included open wound or abscess, with any of the following signs of inflammation: erythema, edema, calor, or dolor. Infection was also diagnosed when purulent drainage, malodor, or bone exposure was present. Osteomyelitis was confirmed in patients upon receipt of a bone biopsy yet suspected when bone was soft/ exposed, and had radiographic changes on plain film or MRI. The final inclusion criterion was the patient had to be medically cleared for surgical intervention with anesthesia.

Patients not requiring surgical debridement for treatment of infection were excluded. Likewise, patients with clinical signs of infection but not medically cleared for surgery were excluded as well. Patients with vascular compromise received a vascular consult and reperfusion as needed prior to surgical debridement. Therefore all patients who met inclusion criteria were fully medically managed and consented for surgery at Scripps Mercy Hospital in San Diego, California.

SPECIMEN COLLECTION

The foot was prepped and draped via sterile technique under the surgical guidelines of Scripps Mercy Hospital. Consequently the infected foot received both application of a betadine scrub and betadine solution up to the level of the malleoli. Surgeons remained sterile throughout the surgical incision and drainage or amputation. All surgical procedures and cultures were conducted under sterile technique in an operating room.

The wound was debrided via both blunt and sharp dissection to the interface of nonviable and viable pedal tissue. Cultures were taken only at this deep interface to minimize colonizers. Care was taken to avoid all superficial ulcerative tissue. The following 4 individual culture swabs were taken of each wound; aerobe, anaerobe, acid fast bacillus, and fungal. Hence each culture tube was sent for 1 of the 4 studies rendering 4 culture tubes per wound. Each swab was in contact with tissue for at least 5 seconds. Cultures were sealed, labeled and sent to microbiology.

The deep tissue sample was surgically excised via a scalpel or rongeur. This sample was excised after each of the 4 swabs were complete and then placed into a sterile empty tissue specimen cup. The sample was placed in the cup under sterile conditions. Finally the lid was tightened and labeled appropriately. No transport media or liquid was present in the specimen containers. Each tissue specimen was evaluated per microbiology for the following isolates; aerobes, anaerobes, acid fast bacillus, and fungus. All cultures were sent to microbiology and received within 30 minutes.

Upon receipt of specimen, the microbiology lab plated each culture tube based upon the media required for bacterial growth. The tissue specimens were ground and plated on identical culture media according to the protocol per Scripps Mercy Department of Microbiology.

The majority of ulcerations in this study had visibly exposed bone. These wounds were each probed via a steel probe and assessed intraoperatively for soft consistency of bone. Beyond this study, bone specimens were sent to pathology, as well, for documentation of osteomyelitis. Bone cultures were not included in the results of this study. Deep tissue specimens cannot replace a bone culture when diagnosing osteomyelitis.

RESULTS

Thirty-two patients with diabetes were included in this study. Each patient had a UT grade 2 or 3 infected foot ulceration requiring surgical intervention. The mean number of isolates cultured from each UT Grade 2 or 3 diabetic foot ulceration per swabbing and tissue biopsy sampling was 2.06 and 1.88 respectively (Table 1). The most common isolates were staphylococcus species including MRSA, as well as Beta hemolytic Streptococcus, Peptostreptococcus, Enterococcus, Pseudomonas aerugenosa, and Prevotella (Table 2). Isolates were found to not favor a specific culturing technique. The difference in yield of bacterial isolates is attributed to a swab's inability in capturing microenvironments of deep ulcerations.

Table 1

MEAN NUMBER OF ISOLATES TAKEN FROM UT GRADE 2 AND 3 DIABETIC FOOT ULCERATIONS

Culturing Technique	Isolates (mean)
Swab	2.06
Tissue Specimen	1.88

Swabs and tissue specimens were found to neither inhibit bacterial growth nor favor specific colonies in growth. Both culturing techniques allow for growth of aerobes, anaerobes, AFB and fungal species. Swabs were incapable in capturing a chronic wound's microenvironment. This accounts for the pathenogenic bacteria missed via the swab technique. No pathogenic microorganisms were missed per tissue biopsy sampling (Table 3).

Colonizers have been defined as both harmless bacteria and normal flora. Microorganisms such as coagulase- negative staphylococcus, enterococcus, alpha streptococcus, and diptheriods have all been defined as colonizers.[6] As for severe diabetic foot infections, chronic wounds develop flora similar to those of feces, with aerobic gram negative rods, anaerobes, and enterococci, in addition to gram positive aerobes.[6] In this study, Swabs identified additional colonizers as compared with corresponding tissue 33.3% of the time. Two of those swabs missed VRE and beta hemolytic streptococcus group B.

Our results demonstrated a poor concordance between swab cultures and deep tissue biopsy results. Swabs identified the same isolates as deep tissue biopsy sampling 37.5% (12/32) of the time (Table 4). In deep ulcerations with bone exposure, swabs isolated different species as compared with the corresponding tissue specimen 62.5% (20/32) of the time. This study indicates a poor concordance between isolates cultured from swabs and deep tissue biopsy sampling of deep ulcerations with bone exposure. Deep Tissue specimens

Table 2

YIELD OF BACTERIAL ISOLATES BY CULTURE TECHNIQUE

	Swab	Tissue
MRSA	8	9
Coagulase negative Staphylococci	9	5
Beta hemolytic Streptococci group B	3	4
Peptostreptococci	3	4
Enterococci	5	3
Diptheriods	5	3
Pseudomonas	5	7
Prevotella	4	3
Enterobacter	2	2
Serratia	2	2
Alpha hemolytic streptoccoci	2	2
Staphyloccus aureus	3	5
Streptococcus group D	2	1
Yeast	2	2
Streptococcus group G	1	1
Beta hemolytic Streptococci group A	0	1
Proteus	2	2
E. Coli	2	1
Proprionibacterium	0	1
VRE	0	1
Moganella	1	0
Cornybacterium	0	1
Acinetobacter	1	0
Bacteroides fragilis	1	1

Table 3

PATHOGENIC ISOLATES MISSED BY SWABS AND CULTURED PER TISSUE SPECIMEN

Patient	Microorganisms
1	Beta hemolytic group B Streptococcus
	Pseudomonas aeruginosa
2	Staphylococcus aureus
3	Beta hemolytic group A Streptococcus
4	Proprionobacterium
9	Candida
15	Pseudomonas aeruginosa
22	Peptostreptococcus
25	Vancomycin resistant enterococcus
27	Peptostreptococcus
29	MRSA

Table 4

A total of 37.5% (12/32) of swabs isolated more colonizers than corresponding tissue samples: This table represents the additional number of colonizers found per swabs

Isolate	Swab
Coagulase Negative Staphylococcus	5
Diptheriods	2
Enterococcus	2
Morganella	1
Acinetobacter	1
Prevotella	1

Table 5

CONCORDANCE BETWEEN INTRA-OPERATIVE SWABS AND DEEP TISSUE CULTURES

Swabs and deep tissue cultures identical	37.5% (12/32)
Swabs contained all organisms cultured from deep tissue	43.8% (14/32)
Swabs contained all isolates found in deep tissue plus additional growth	28.1% (9 /32)
Swabs lacked isolates found in deep tissue	37.5% (12/32)

isolated fewer colonizers and did not fail to isolate pathenogenic microorganisms.

Table 5 presents the poor concordance which exists between intra-operative swabs and deep tissue cultures. Only 37.5% (12/32) of swabs and deep tissue cultures were identical. These results conclude swabs are an unreliable culturing technique when evaluating UT grade 2 or 3 diabetic foot ulcerations with infection.

DISCUSSION

By 2025, 300 million people worldwide will be affected by diabetes mellitus. Even more concerning, over six million of those people will develop a lower extremity ulceration placing them at significant risk of infection, deformity and death.[1] With this growing statistic it is essential to collect accurate wound cultures to assist in adequate antibiotic treatment. Inaccurate cultures consumed by colonizers are misleading and can lead to a superinfection, amputation, or death. Colonization accounts for the harmless bacteria that covers the body surface and produces overgrowth on wound beds.[6] The dominant flora of normal skin consists of gram positive aerobes, such as low virulence coagulase negative staphylococcus, alpha hemolytic streptococcus and corynebacteria.[6] As for infection, this process may either follow colonization or occur as a primary event.[6] All in all, there should be great importance placed on proper culturing techniques. This study focused on the need to determine if a culture swab identified the same isolates as a deep tissue specimen in diabetic foot ulcerations primarily with bone exposure.

A wound is the terrain in which most bacterial growth flourishes. As each individual requires different components to survive, microbes do exactly the same. For example, strict aerobes require oxygen while anaerobes can only survive in a wound which is depleted of oxygen. While these two extremes exist, facultative microorganisms have adapted to live successfully in an oxygenated or non-oxygenated environment. Hence, each microorganism prefers a different location or depth within a wound. This should enforce the importance of deep tissue specimens. The inevitable colonization of the ulcer surface means that meaningful samples for microbial culture must be obtained by deeper tissue.[6] A swab simply fails to capture varying microenvironments and tissue composition. A swab may accurately isolate bacterial, fungal, and AFB growth taken from acute superficial ulcers since most tend to be monomicrobial, and caused by gram positive pathogens.[1,6]

Our results indicate a poor concordance of isolates obtained from swabbing as without deep tissue biopsy sampling. Only 37.5% of swabs and deep tissue cultures were identical. In addition, 12 out of 32 swabs lacked isolates found in deep tissue. Swabs were found to lack pathenogenic organisms such as beta hemolytic group b streptococcus whereas deep tissue specimens captured all gram positive pathenogenic bacteria. This data renders deep tissue biopsy sampling superior as compared with the swab technique when culturing deep chronic ulcerations. We attribute this to the heterogeneity of chronic and or deep ulcerations. A deep ulceration is a wound with varying terrain and micro-environments. Therefore, different micro-organisms may be growing in these isolated micro-environments.[3,7-9] A swab is not capable of capturing the vast terrain of a deep ulceration.

It is true that one must account for the microenvironments of a deep chronic wound and question whether a swab is fully capable of capturing pathologic growth.[3] Although various reports have supported use of swabs, this study

suggests inaccuracy lies within swabs of UT Grade 2 or 3 ulcerations. This is important to realize since one-third of diabetic patients who present with foot infections are found to have osteomyelitis,[1,10,11] and over three-quarters of amputations are preceded by a foot ulcer.[1,2]

In summary, our data indicates a poor concordance between isolates of swabs as compared with deep tissue specimens in UT Grade 2 or 3 ulcerations. While the mean number of isolates is not statistically different, swabs failed to isolate infectious micro-organisms. Swabs likewise captured additional colonizers as compared with deep tissue specimens. Therefore, these results should stress the importance of taking deep tissue specimens of deep ulcerations with capsule, tendon, joint, or bone exposure. One may use the swabbing technique as an adjunctive procedure but this technique should not be the primary culturing method of a deep wound. As for superficial or acute ulcerations, the swabbing technique may be accurate due to the lack of microenvironments and microorganisms. This study did not include superficial ulcerations. In conclusion, swabbing is an inaccurate culturing technique when capturing growth from deep UT Grade 2 or 3 ulcerations.

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