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# COMPARATIVE STUDY BETWEEN SWAB AND TISSUE MICROBIAL CULTURE TECHNIQUES IN THE MANAGEMENT OF DIABETIC WOUND INFECTION

Surgery

**Ganesh kumar P** Assistant Professor, Department of General Surgery, Government Villupuram Medical College and Hospital, Villupuram, Tamilnadu, India.

**Rajapandi P*** MS, Associate Professor, Department of General Surgery, Government Villupuram Medical College and Hospital, Villupuram, Tamilnadu, India.*Corresponding Author

**Hepsika Sofia T** Undergraduate student, Government Villupuram Medical College and Hospital, Villupuram, Tamilnadu, India.

**Saravanakumari A D** Assistant Professor, Department of Community Medicine, Government Villupuram Medical College and Hospital, Villupuram, Tamilnadu, India.

**Vasanthi S** Tutor, Department of Microbiology, Government Villupuram Medical College and Hospital, Villupuram, Tamilnadu, India.

## ABSTRACT

**Background and Aims:** Diabetic ulcers are a major cause of morbidity and mortality. Identifying and isolating the causative microbe helps in control of infection. The aim of the study is to compare swab culture and tissue culture for the routine identification of pathogens in diabetic wound infection.
**Materials and Methods:** This was a prospective observational study conducted in patients admitted at Surgery ward with diabetic ulcers in the months of June and July 2019. The patients were classified according to the PEDIS grading. Wound swab and tissue specimen were obtained by Levine technique and punch biopsy respectively and processed in the microbiology lab to identify the causative pathogen in the wound.
**Results:** Of the 72 patients observed, males were affected more (72.2%) and many had grade 3 ulcers (86.1%). While Klebsiella was the predominant organism (42.9%) in tissue isolates, Pseudomonas was predominant in swab isolates (41.2%). Swab cultures were more effective in detecting microbes (40%) in grade 2 wounds, while tissue cultures were more effective in grade 3 wounds (70%). The mean of swab culture and tissue culture were 4.25 and 4.00 respectively in student's paired t-test.
**Conclusion:** Swab culture was more reliable for detecting microbes in grade 2 diabetic ulcers while tissue culture along with swab culture are needed for ulcers of grade 3 and above to aid in appropriate wound healing.

## KEYWORDS

## INTRODUCTION:

Diabetes Mellitus with infected ulcers is a major cause of morbidity and mortality which is responsible for prolonging the hospital stay than any other complication.[1] It has a larger impact on the health status of the patient. Diabetic ulcer is probably a major burden for the (non-paid) individuals from the patient's family and friends, as these caregivers frequently assist in wound care and support the patient in coping with the physical disabilities and emotional distress.[2] Diabetic wound will prolong the bed occupancy in the health care set up. Inappropriate treatment will also lead to unnecessary resource utilization and emergence of drug resistant pathogens.

Identifying and isolating the causative microbe by swab, curettage or tissue biopsy helps in the treatment of wound infection. While swab cultures have advantages like being non-invasive and ease of sample collection by any health care worker, they also have disadvantages like contamination by skin commensals, difficulty in identifying deep tissue pathogens, suboptimal for fastidious and obligate anaerobes and is often less reliable as it often lacks true pathogen.[3,4] Swab cultures are preferred for grade 1 and 2 category of wounds as per PEDIS system. Even though tissue biopsy cultures are the standard method to detect deeper infections, they carry risks like spreading of infection, ischemia and injury to adjacent blood vessels, nerves and surrounding tissues.[5,6] But identifying the incriminating organism helps in managing wound infection in the setting of immunosuppression seen in Diabetes and microbial resistance to antimicrobials.[7]

As both Swab and tissue specimen helps in the identification of causative organisms, it will guide in the antimicrobial treatment based on antibiotic susceptibilities. Hence a comparison on culture results from swab and tissue specimens is essential to identify superiority. This study aimed to identify the reliability of swab and tissue culture by comparing the two microbiological sampling techniques for routine identification of pathogens in diabetic wound infection.

## MATERIALS AND METHODS

This was a prospective observational study conducted at the Department of General Surgery and Department of Microbiology at Government Villupuram Medical College and Hospital, Villupuram, Tamilnadu, India in the months of June and July 2019. Patients of age 18 and above who attended the surgery outpatient department with complaints of diabetes and symptomatic infected ulcers (any clinical sign of inflammation) were included in the study. Patients with ulcer due to non-diabetic causes, ulcers which were gangrenous or with dry eschar and those who were on prior antibiotic use were excluded from the study. Institutional ethical committee clearance was obtained before data collection. All patients were enrolled only after obtaining written informed consent. Demographics and clinical data were entered in a structured questionnaire. Clinical diagnosis of infection was defined by the presence of at least 2 of the following indicators: local swelling or induration, erythema of size > 0.5 cm around the ulcer, local tenderness or pain, local warmth and purulent discharge.[8,9,10] Clinical severity of the infection was quantified according to the infection category of Perfusion, Extent, Depth, Infection and Sensation (PEDIS) system proposed by International Working Group of the Diabetic Foot (IWGDF). Grade 1 ulcers were uninfected: grade 2 ulcers were mildly infected, grade 3 ulcers were moderately infected, involving tissues deeper than skin and subcutis (e.g., bone, joint, tendon or muscle) or with erythema extending > 2 cm from the wound margin; and grade 4 ulcers were severely infected with systemic toxicity or metabolic instability.[9]

Two specimens were collected from each of the wound after the wound had been cleansed (using sterile saline and gauze) and debrided (removal of necrotic tissue, foreign material, calluses, and undermined wound edges).[11] No antiseptic agent was introduced into the wound before specimen collection. Each wound was swabbed using the Levine technique, involving rotation of a wound swab over a 1 $cm^2$ area of the wound for 5 seconds, using sufficient pressure to extract fluid from the inner part of the wound.[12,13] A deep tissue specimen of 4 mm in diameter was obtained from the base of the ulcer via punch biopsy.[14] The specimens were placed into a sterile transport medium and sent to Microbiology laboratory, for aerobic culturing within 20 minutes. Biopsy samples were homogenized with PBS in a petri dish.

100 microliters of homogenate was used for serial dilutions with PBS. For Swab samples 100 µL volume of the suspension was used for serial dilution in PBS. Quantification was performed using the 10-fold serial dilution method, and 100 µL of each dilution was inoculated into MacConkey agar with 5% sheep blood and the plates were incubated under aerobic conditions at 35.8 degrees. Additionally, the samples were inoculated in Brain Heart Infusion Broth to allow recovery of fastidious or low concentration organisms. Isolates were identified by the standard methods.[15] Anaerobic culture was not performed. Any culture in the media was evaluated with Gram's stain and special stains when needed.

Data entry and analysis were done using SPSS software version 20.0 (IBM, USA). Statistical analysis was done with mean, standard deviation and student's t-test for paired samples. $p < 0.05$ was considered significant.

## RESULTS

A total of 72 patients were enrolled and data taken for final analysis. All the patients were suffering from Type 2 Diabetes Mellitus and its complications (72/72) (100%). Most of the patients were of age more than 50 years (48/72) (66.7%). Males were predominant in the study group (52/72) (72.2%). The most common reason for the infected ulcer was gradual progression (28/72) (38.9%) followed by trauma (16/72) (22.2%). Most of the patients in the study group were teetotalers (44/72) (61.1%) while 18.1% (13/72) had history of alcohol abuse. 25% of the patients (18/72) had previous history of amputation for non-healing ulcers and other complications of diabetes.

The most common site of the infected ulcer was lower limb (66/72) (91.7%). 41.6% of the ulcers were less than 20 cm in size (30/72). While signs of inflammation were observed surrounding ulcers in all patients (100%), slough overlying the ulcer was observed in 44.4% (32/72) of the patients and no granulation was observed in 36.1% (26/72). Edge of the ulcer was irregular in most of the patients (69.4%) (50/72). Base of the ulcer was formed by muscle in 41.7% (30/72) followed by necrotic tissue in 25% (18/72). Purulent discharge was observed in 47.2% of ulcers (34/72) while 25% did not show any discharge (18/72). The surrounding skin was edematous in 75% (54/72) while blackening and scaling were also observed in few cases. Most of the ulcers belonged to grade 3 in PEDIS grading system (86.1%) (62/72) and the remaining patients had grade 2 ulcers (10/72) (13.9%).

All the patients were subjected to swab cultures and tissue cultures (100%). While results were obtained for all swab cultures (100%), 2 tissue cultures could not be completed and hence the final percentage was 97.2% (70/72). While swab cultures yielded 47.3% isolates in culture (34/72), tissue cultures had a better yield of 60% isolates in culture (42/70) (Table 1.)

**Table 1. Comparison between positive isolates in swab and tissue cultures based on PEDIS grading**

| | GRADE 2 | | GRADE 3 | |
|---|---|---|---|---|
| | n = 10 | | n = 62 | |
| | SWAB | TISSUE | SWAB | TISSUE |
| No. of samples taken | 10 | 10 | 62 | 60 |
| Cultures with positive isolates | 4 | 0 | 30 | 42 |

More than half of the swab cultures did not showed any growth (52.7%) (38/72), but Pseudomonas and Klebsiella species were predominant in cultures with growth (Fig. 1).

**Fig 1. Distribution of microbes identified in swab cultures (n = 72)**

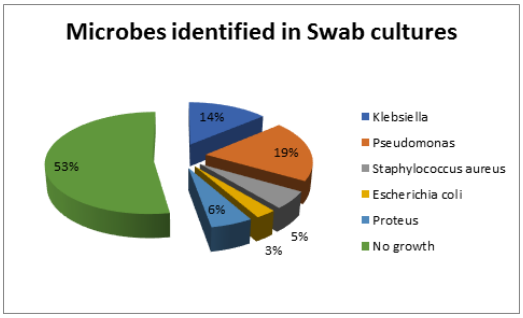
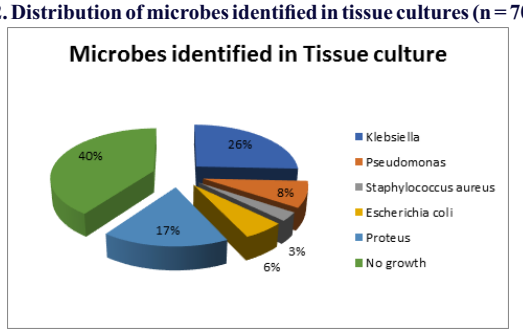



**Fig 2. Distribution of microbes identified in tissue cultures (n = 70)**




Klebsiella and Proteus species were observed more in tissue cultures. Polymicrobial infection was not noted. Student's paired t-test showed a mean of 4.25 for swab culture and a mean of 4.00 for tissue culture.

## DISCUSSION

India is home to 69.1 million patients with diabetes mellitus with an overall prevalence of 9.3%.[16] The annual incidence of diabetic foot ulcer in population-based studies is 1.0 to 4.1% and prevalence of 4.5 to 10%, with an overall lifetime incidence of up to 25%.[17] Diabetic ulcer is a heavy burden to the patient and the health care system. Nearly 600 million people worldwide are expected to have diabetes mellitus in 2035.[18] Diabetic wound occurs as a result of poor glycemic control. A commonly accepted definition of foot infection is the presence of systemic signs of infection (e.g., fever, leukocytosis) or purulent secretions, or two or more local symptoms or signs (redness, warmth, induration, pain, or tenderness).[19]

More recently, an increase in the incidence of multi-drug resistant organisms, namely methicillin-resistant Staphylococcus aureus and extended-spectrum β-lactamase (ESBL)-producing gram negative bacteria, is threatening the outcome of anti-microbial therapy in the community and in hospitalized patients.[20] Hence, it is necessary to obtain specimens for culture before initiating empirical antibiotic therapy to help with the selection of a definitive therapy. The required specimens are wound swab and the tissue biopsy.

Chakraborti *et al*. conducted a meta-analysis by comparing superficial and deeper cultures from various types of lower extremity wounds. Their results showed that the sensitivity for swabs culture was only 45% and the specificity was 62%, with a non-significantly higher mean number of isolates from superficial than deep specimens (2.7 and 2.2, respectively).[21] Lipsky *et al*. found that in 33% of cases of diabetic foot infections in an outpatient setting, the swab culture missed at least one pathogen found in the specimen taken by wound biopsy.[22]

Pellizzer *et al*. found that swab cultures and tissue cultures were equally reliable for the initial monitoring of antimicrobial treatment in severe diabetic wound infection. However, deep tissue cultures were more sensitive than swabbing for monitoring antibiotic resistance cases, i.e. those with active ulcers even after 30 days of treatment.[23] Slater *et al.* in their study of 60 ulcers from 56 patients found that 62 percent of the results are identical in both swab and tissue culture. Swab cultures were valuable in identifying pathogens in diabetic ulcers when bone was not involved.[4]

According to many studies, Staphylococcus aureus was the predominant organism isolated.[24] But in our study, Klebsiella was the predominant organism (42.9%) (n = 18/42) in tissue culture followed by Proteus species (28.6%) (n = 12/42). In Swab culture, Pseudomonas was the predominant organism (41.2%) (n= 14/34) followed by Klebsiella (29.4%) (n = 10/34). Since most of the ulcers were deeper, gram negative microbes were the predominantly isolated pathogens.

In several studies, the number of pathogens identified by swab culture was significantly higher than those identified by tissue biopsy.[25] This discrepancy may be related to the cleansing or debridement that is performed before the collection of the specimen in the study. It may also be related to Levine swab technique that we did. In Levine technique, swab is rotated with sufficient pressure to extract fluid from the inner part of the wound. This may reduce superficial contamination and also omits the deeper pathogens. Poly microbial infection increases with increase in PEDIS grading. But in our study, we found only mono microbial infection of the wounds. In our study, majority of the cases belonged to PEDIS grade 3 ulcers. Tissue culture didn't

identify any of the pathogens from PEDIS grade 2 ulcers. But in grade 3 ulcers, tissue culture was more reliable as number of isolates was greater than swab cultures.

According to our paired sample tests, swab culture had a statistically significant mean of 4.25 which was greater than tissue culture. Hence the patients with grade 2 ulcers can be treated with antibiotics from the result of swab culture alone. It is also easy and noninvasive to the patient. But in grade 3 ulcers, pathogens identified from swab and tissue culture might be different. Our data suggests that it is necessary to do a tissue biopsy along with swab, for ulcers of grade $\geq 3$, to obtain an adequate microbiological diagnosis of diabetic wound infection and to guide the antibiotic therapy.

To conclude, Swab culture is a reliable technique for grade 2 ulcers. For ulcers of grade $\geq 3$, both tissue culture and swab culture are necessary to guide the appropriate antibiotic therapy and also to prevent drug resistance. Follow up of patients to find their wound healing process, based on the antibiotic therapy guided by our swab culture and tissue culture reports, is the next step of research on this topic.

PR conceptualized and designed the study. PG approved and analyzed data. HS collected and interpreted data.

## ABBREVIATIONS

PEDIS - Perfusion, Extent, Depth, Infection and Sensation system
IWGDF - International Working Group of the Diabetic Foot

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## DECLARATIONS:

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**Conflict of interest:** ICMR STS program

**Ethics approval:** Obtained from Institutional Ethical Committee, Government Villupuram Medical College and Hospital, Villupuram, Tamilnadu, India.

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