



International Journal of Surgery Science

E-ISSN: 2616-3470

P-ISSN: 2616-3462

© Surgery Science

www.surgeryscience.com

2020; 4(2): 146-148

Received: 04-02-2020

Accepted: 06-03-2020

N Sankar

Department of General Surgery,
Vinayaka missions medical college
and hospital, Karaikal,
Puducherry, India

S Khaja Moinuddin

Department of Microbiology,
Vinayaka Missions Medical College
and Hospital, Karaikal,
Puducherry, India

S Mohan

Department of Microbiology,
Vinayaka missions medical college
and hospital, Karaikal,
Puducherry, India

Microbial etiology of diabetic foot ulcers: Swab versus tissue culture

N Sankar, S Khaja Moinuddin and S Mohan

DOI: <https://doi.org/10.33545/surgery.2020.v4.i2c.408>

Abstract

Introduction: Diabetic foot infection is limb threatening condition among patients suffering from diabetes. Choosing suitable antibiotic against particular pathogen to treat diabetic foot infection is most critical step in preventing further consequences such as amputation. The present study aimed to determine consensus and degree of difference between the two methods of collecting wound specimens by comparing the pathogens isolated from the same wound from each method.

Materials and Methods: This was a prospective, observational study in which a total of 77 patients with infected diabetic wound seen both inpatient and outpatient department of surgery. Wagner's method was used to classify diabetic foot ulcers. Samples were collected by two methods, superficial swab (Levine's method) and tissue sample.

Results: A total of 79 bacteria were isolated from 77 patients. Out of 79 bacteria, 68 were isolated from swabs and 77 were from tissue samples. Isolation rate of Gram positive bacteria was differed in type of sample. Tissue specimens showed high yield than swab. But no difference was observed in isolation rate of Gram negative bacteria based on type of sample collected.

Conclusion: In our study, tissue culture was found to be the most acceptable technique in identifying the microbial etiology of limb threatening diabetic foot infections.

Keywords: Diabetic foot infection, levine's method, tissue sample

Introduction

Diabetes is the primary cause of artery atherosclerosis end-stage renal impairment, adult-onset blindness, and nontraumatic lower extremity amputations. In 2011, there were 366 million people living with diabetes worldwide and this is projected to rise to 552 million by 2030 ^[1]. India with approximately 42 million cases is ranked first in the list of the ten nations most affected with diabetes ^[2]. Various factors such as barefoot walking, inadequate facilities for diabetes care and education, and poor socioeconomic conditions are responsible for diabetes mellitus related complications. Foot ulceration can be avoided and relatively straight forward treatments will reduce amputations by up to 80%. Good regulation of hemoglobin, blood pressure and lipid levels is well known as key elements in raising the risk of diabetes complications ^[3].

Diabetic foot infection is a clinical condition made using criteria for classification to aid surgeons in assessing seriousness of infection. Antibiotics are usually administered immediately (empirical treatment) and the findings of samples obtained for the detection and exposure of wound pathogens are then used to tailor the antibiotic dosage, preventing excessive wide-spectrum therapy and antibiotic resistance ^[4].

Precise effects of cultivation rely on obtaining samples of infected tissue which is less likely to be contaminated with colonizing flora. Swabs samples are readily available, simple and easy to use, and can be obtained by most forms of healthcare workers. Alternatively, specimens may be obtained by collecting tissue from the wound base; this requires much more expertise and time, which can uncover more contaminants and be less vulnerable to non-pathogens contamination.

Researchers have believed that tissue collections are the 'gold standard' for sampling but this approach could still be lacking wound flora ^[5]. We therefore aimed to determine consensus and degree of difference between the two methods of collecting wound specimens by comparing the pathogens isolated from the same wound from each method. `

Corresponding Author:

S Khaja Moinuddin

Department of Microbiology,
Vinayaka Missions Medical College
and Hospital, Karaikal,
Puducherry, India

Materials and Methods

This was a prospective, observational study in which a total of 77 patients with infected diabetic wound seen both inpatient and outpatient department of surgery, Vinayaka Mission's Medical College and Hospital. All patients gave written informed consent for their participation in the study. All wounds were assessed for the presence and severity of infection based on Wagner's classification.⁶ Demographic details of patients such as age, sex, duration of diabetes and duration of ulcer were collected. HbA1c levels were also determined.

Swab sample: Wound beds were prepared before specimen collection, where the wound immediate surface exudates and contaminants were cleansed off with moistened sterile gauze and sterile normal saline solution. Dressed wounds were cleansed with non bacteriostatic sterile normal saline after removing the dressing. Aseptically the end of a sterile cotton-tipped applicator was rotated over 1 cm² area for 5 seconds with sufficient pressure to express fluid and bacteria to surface from within the wound tissue^[7].

Tissue sample: A deep tissue specimen of 4mm in diameter was obtained from the base of the ulcer via punch biopsy^[8].

By maintaining proper sterile conditions, collected specimens were immediately transported to microbiology department for culture. Cultures were processed following the same standard procedures for the swab and tissue samples^[9].

Statistical analysis was done by simple percentage method.

Results

A total of 77 patients were included in the present study. Age was ranged between 40-81. Majority of patients belongs to the age group 51-60 which accounted for 48.05% followed by >70, accounted for 27.27%. Majority of patients were males (70.13%). Occurrence of diabetic foot infection was high among the patients who had diabetes for more than 6 years (72.72%). Majority of patients presented to the hospital when the duration of ulcer was between 1 week to 1 month. Higher number of patients with diabetic foot infection had HbA1c levels >7 (89.61%). All the ulcers were graded as per Wagner's grading and it was observed that more number of patients had Grade III (36.36%) followed by Grade IV (31.17%). Table. I.

Table 1: Demographic and clinical characteristics of patients

| Variables | Number (%) |
|-------------------------------------|------------------|
| Age | |
| 40-50 | 19 (24.67) |
| 51-60 | 37(48.05) |
| 61-70 | 18(23.38) |
| >70 | 21(27.27) |
| Sex | |
| Male | 54(70.13) |
| Female | 23(29.87) |
| Duration of diabetes (Years) | |
| <1 | 3 (3.90) |
| 1-5 | 18(23.28) |
| >6 | 56(72.72) |
| Duration of ulcer | |
| <1 Week | 11(14.29) |
| 1 Week- 1 Month | 53(68.83) |
| > Month | 13(16.89) |
| HbA1c levels | |
| ≤7 | 8(10.39) |
| >7 | 69(89.61) |
| Wagner's grade | |
| Grade II | 18(23.28) |
| Grade III | 28(36.36) |
| Grade IV | 24(31.17) |
| Grade V | 7(9.09) |

Samples were collected by two different techniques from the diabetic foot ulcer and subjected for microbiological methods to isolate the etiology of diabetic foot infection. A total of 79 bacteria were isolated from 77 patients. Out of 79 bacteria, 68 were isolated from swabs and 77 were from tissue samples. *Staphylococcus aureus* (29) was found to be the frequently isolated pathogen. Only 22 strains of *S.aureus* were isolated from swab culture. But tissue samples yielded the growth of 29 strains of *S.aureus*. Second common pathogen isolated was Coagulase negative *Staphylococci* (17). All 17 strains of CONS were grown in swab culture but tissue culture yielded 15 strains of CONS. (Table.II).

Table 2: Comparison of swab and tissue culture techniques.

| Bacteria (number) | Swab(+) | Tissue(+) |
|-----------------------------------|-------------|-----------|
| <i>Staphylococcus aureus</i> (29) | 22 | 29 |
| CONS (17) | 17 | 15 |
| Enterococci (3) | 1 | 3 |
| Streptococci(3) | 3 | 3 |
| <i>Klebsiella species</i> (11) | 9 | 11 |
| <i>Pseudomonas aeruginosa</i> (9) | 9 | 9 |
| NFGNB (3) | 3 | 3 |
| <i>Proteus</i> (2) | 2 | 2 |
| <i>E.coli</i> (2) | 2 | 2 |
| Total | 68 (86.07%) | 77(97.47) |

CONS: Coagulase negative staphylococci

NFGNB: Non fermenting Gram negative bacilli

Discussion

The present study was carried out to analyze two various methods of sample collection in identifying the etiology of diabetic foot infections. A sum of 77 patients were included in the present study. From 77 patients, a total of 79 bacterial isolates were identified from diabetic foot infections. Samples collected by using swab sample, 68 (86.07%) bacteria were isolated. Tissue sample yielded 77(97.47%) bacterial growth.

In our study it was observed that tissue samples showed higher yield than wound swab specimens, hence providing more information on wound flora. These findings are in agreement with the study conducted by Nelson *et al.*^[5] He observed that tissue sampling had a better yield than wound swab tests, offering more knowledge about wound flora as a result. Although total tissue sampling identified more species than wound swabs, certain species were left out on both techniques. They thus provide similar knowledge to an extent and both methods can be useful.

However, yield was differed from bacteria to bacteria. In our study, total number of *S.aureus* isolated were 29. Swab samples yielded only 22 strains of *S.aureus* but tissue sample yielded 29 strains of *S.aureus*. In case of CONS, total number was 17. Swab samples yielded 17 strains of CONS and tissue samples yielded only 15 strains. Higher number of CONS isolation in superficial swab samples could be due to contamination of the skin flora. It was noticed that Enterococcal growth was predominally seen in tissue samples than swab specimen. Surprisingly, no disparities were noticed in isolation of Gram negative bacilli which is not in agreement with the study conducted by Ying Huang *et al.*^[10] As per their study, swab culturing is associated with a high risk of missing pathogens, especially Gram-negative bacteria.

According to the study conducted by Mutluoglu *et al.* the findings of swab culture-taken specimens did not compare well with those collected for tissue culture. Which indicates that swab samples may be less accurate than tissue samples to direct antimicrobial therapy^[11].

Another study conducted by Demetriou *et al.* assessed the diagnostic efficiency of swabs versus tissue cultures, and the discrepancies in bacterial isolates between neuropathic and neuroischemic patients. Swab cultures are highly sensitive but less specific, and have an outstanding negative predictive value in both neuropathic and neuroischemic foot ulcer patients with diabetes. There are no variations in microbial load between the types.^[12] According to Macías *et al.* report, swab culture is a sensible option for assessing the microbiology of diabetic foot which is not in line with our research findings^[13].

All Gram positive bacterial isolates from Grade II ulcer yielded similar results in both sampling techniques compared to grade 3 and 4. Therefore, for grade 2 wounds, ulcer swabbing, which is easier to conduct and comparatively non-invasive, could be a suitable sampling procedure compared to deep tissue biopsy, which could cause skin, blood vessels and nerves to sustain damage^[14].

Our findings show improved tissue yield relative to wound swab specimens; the full details will be possible as data are collected by using both sampling techniques. This, in conjunction with the literature currently available, supports the suggestions that tissue samples should be chosen over swab specimens when choosing one form.

Our study has few limitations. Sample size was limited to analyse the data. Obtained results were analysed with the aid of simple percentage method. In addition, fungal microbiota and anaerobic bacteria culture was not performed.

Conclusion

In our study, tissue culture was found to be the most acceptable technique in identifying the microbial etiology of limb threatening diabetic foot infections. Appropriate specimen collection helps to overcome the unnecessary usage of antibiotic therapy.

References

- Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract.* 2011; 94:311-21.
- Ramachandran A, Ma RCW, Snehalatha C. Diabetes in Asia. *Lancet.* 2010; 375:408-418.
- Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet.* 2005; 365:1333-1346.
- Lipsky BA. Empirical therapy for diabetic foot infections: are there clinical clues to guide antibiotic selection? *Clin Microbiol Infect.* 2007; 13:351-3.
- Nelson A, Wright-Hughes A, Backhouse MR *et al.* CODIFI (Concordance in Diabetic Foot Ulcer Infection): a cross-sectional study of wound swab versus tissue sampling in infected diabetic foot ulcers in England. *BMJ Open.* 2018; 8:e019437. doi:10.1136/bmjopen-2017-019437
- Oyibo SO, Jude EB, Tarawneh I, Nguyen HC, Harkless LB, Boulton AJ *et al.* A comparison of two diabetic foot ulcer classification systems: The Wagner and the University of Texas wound classification systems. *Diabetes Care.* 2001; 24:84-8.
- Levine NS, Lindberg RB, Mason AD, Pruitt BA. The quantitative swab culture and smear: a quick, simple method for determining the number of viable aerobic bacteria on open wounds. *Journal of Trauma—Injury, Infection and Critical Care.* 1976; 16(2):89-94.
- Demetriou M, Papanas N, Panopoulou M, Papatheodorou K, Bounovas A, Maltezos E. Tissue and swab culture in

diabetic foot infections: neuropathic versus neuroischemic ulcers. *International Journal of Lower Extremity Wounds.* 2013; 12(2):87-93.

- Forbes BA, Sahm DF, Weissfeld AS. Chapter 13, Overview of bacterial identification methods and strategies. *Bailey and Scott's diagnostic Microbiology*, 12th ed. St.Louis: Mosby, 2007, 216-47.
- Huang Y, Cao Y, Zou M *et al.* A comparison of tissue versus swab culturing of infected diabetic foot wounds. *Int J Endocrinol.* 2016, 1-6.
- Mutluoglu M, Uzun G, Turhan V *et al.* How reliable are cultures of specimens from superficial swabs compared with those of deep tissue in patients with diabetic foot ulcers? *J Diabetes Complications.* 2012; 26:225-9.
- Demetriou M, Papanas N, Panopoulou M *et al.* Tissue and swab culture in diabetic foot infections: neuropathic versus neuroischemic ulcers. *Int J Low Extrem Wounds.* 2013; 12:87-93.
- Macías HAE, Álvarez JA, Cabeza VF *et al.* Microbiology of the diabetic foot: is the swab culture useful?. *Gac Med Mex.* 2011; 147(2):117-124.
- Gjødsbøl K, Skindersoe ME, Christensen JJ *et al.* No need for biopsies: Comparison of three sample techniques for wound microbiota determination, *International Wound Journal.* 2012; 9(3):295-302.