Scholars Bulletin
(A Multidisciplinary Bi-weekly Journal)
An Official Publication of "Scholars Middle East Publishers",
Dubai, United Arab Emirates
Website: http://scholarsbulletin.com/

ISSN 2412-9771 (Print)
ISSN 2412-897X (Online)

Comparing swab culture, tissue culture to identify the infecting organism in diabetic foot ulcers

Prof. Dr. D. Premkumar Daivasikamani
Professor in Surgery, Department of Surgery, Karpaga Vinayaga Institute of Medical Sciences and Research, Maduranthagam, Kanchepuram, India.

***Corresponding Author:**
Prof. Dr. D. Premkumar Daivasikamani
Email: dr.premkumar52@gmail.com

Abstract: A study was conducted in 112 diabetic foot ulcers to identify the ideal method were the infecting organisms can be identified. Two methods were followed, one by taking the specimen by swab and another by a small piece tissue using punch biopsy forceps. It is found that more positive culture was obtained by tissue culture method and surface contamination was more in the swab method.so it is better to obtain the specimen from the tissues is better than swab method.

Keywords: diabetic foot infection, organisms, swab method, tissue culture method, Staphylococcus

INTRODUCTION

A wound culture is a diagnostic laboratory test in which microorganisms like bacteria or fungi from an infected wound are grown in the laboratory on nutrient-enriched media and then identified. Wound cultures always include aerobic culture. The culture is done to identify the causative organism and to guide antibiotic therapy, systematically and topically.

There are several methods of obtaining a wound drainage culture: tissue biopsy, needle aspiration, or the swab technique. The swab technique is most commonly used, but contains the least amount of specimen. A biopsy sample is usually preferred by clinicians, but this is a moderately invasive procedure and may not always be feasible. There is a very slight risk of spreading some infections if a biopsy is done. Needle aspiration is less invasive and is a good technique to use in wounds where there is little loss of skin. A sterile swab may be used to collect cells or pus from a superficial wound site. From deeper wounds, aspirations of fluid into a syringe and/or a tissue biopsy are the optimal specimens to allow for the recovery of bacteria.

A normal culture may be contaminated by a mixture of microorganisms normally found on a person's skin (normal flora).Necrotic tissue does support microorganism growth and should be debrided with gauze piece before taking the material for culture in managing a non-healing wound. Before taking material for culture the affected area is cleaned with a sterile solution, such as saline. Antiseptics such as ethyl alcohol are not recommended, because they kill bacteria and cause the culture results to be negative. Some literature suggests that cleaning the wound before sampling is unnecessary, however, if the wound is not clean it often leads to the isolation of multiple organisms which may not be relevant and can generate laboratory results reporting "mixed bacterial flora" rather than individual species[1]. Cleaning removes the organisms present in the surface material, which are often different from those responsible for the pathology, and allows for more accurate culture results. Ideally, the patient should not have received recent antibiotic treatment before swabbing a wound as this can affect the microbiological results. Siddiqui A has recommended a swabbing procedure[2].

The aim of study is to compare various methods of collecting the specimen for culture of organisms in the diabetic foot infection with objective to identify the best method of specimen collection for culture to identify the infecting organism in diabetic foot ulcers.

MATERIAL AND METHODS

The specimens were collected in two different methods. First by swab method, next by taking a bit of tissue.

A. Swab method: Apply sterile saline to moisten the head of the swab to increase the adherence of bacteria

- Pass the swab over the wound area in a zigzag motion while twisting the swab so that the entire head of the swab comes into contact with the wound surface.
- Swab from the centre of the wound outward to the edge of the wound.

- The swab should be pressed firmly enough that fluid is expressed from the wound tissue (this may be painful for the patient).
- Repeat the process with a separate swab if a pocket or sinus is present in the wound.

B: Tissue culture method: After cleaning the wound with normal saline a bit of tissue is taken by using punch biopsy forceps and cultured by usual method. The necrotic material is not taken for culture.

RESULTS

From 112 patients with diabetic foot ulcer samples were collected by both swab method and also by using punch biopsy forceps for tissue culture. Out of 112 cultures, organisms were grown in 91 in swab culture and 107 in tissue culture. (Table 1) Staphylococcus was the common organisms by both methods followed by E coli, Klebsiela, Pseudomonas, Proteus and others. Commensals were grown more in swab method 14 and 5 in the tissue culture. (Table 2) Mixed organisms are more in swab 11 and 9 in tissue culture.(Table 3)

Table-1: Organisms identified by different methods

Total No	Swab culture	Tissue culture
112	91	107

Table-2: Infecting organisms identified by different methods

	Swab culture	Tissue culture
Staphylococcus	31	37
Streptococcus	7	11
E coli	13	18
Pseudomonas	12	15
Klebsiela	12	17
Proteus	9	13
Commensals	12	5
Total	102	116

Table-3: Culture of mixed organisms.

No	Mixed Organism	Swab culture	Tissue culture
1	Staphylococcus aureus+E.coli	3	3
2	Staphylococcus aureus+Klebsilla	2	2
3	Pseudomonas+E.coli	3	2
4	Klebsiella+E.coli	1	1
5	Proteus+E.coli	2	1
Total		11	9

DISCUSSION

Wound specimens are cultured on both nonselective enriched and selective media. Cultures are examined each day for growth and any colonies are Gram stained and sub cultured to appropriate media. The sub cultured isolates are tested via appropriate biochemical identification panels to identify the species present. Organisms are also tested for antibiotic susceptibility. The initial Gram stain result is available the same day, or in less than an hour if requested by the physician.

An early report, known as a preliminary report, is usually available after one day. This report will tell if any microorganisms have yet been found, and, if so, their Gram stain appearance. The final report, usually available in three days, includes complete identification, an estimate of the quantity of the microorganisms, plus a list of the antibiotics to which they are sensitive. Cultures for fungi and anaerobic bacteria may take two to three weeks. Typically, there is only one kind of bacteria found or one type predominates in a wound. In some cases, however, there may be several types of bacteria present that have different requirements for growth. Some bacteria infecting a wound may be aerobic while others areanaerobic or microaerophilic. Wound infections often contain multiple organisms, including both aerobic and anaerobic gram-positive cocci and gram-negative bacilli and yeast. The most common pathogens isolated from wounds are Streptococcus group A, Staphylococcus aureus, Escherichia coli, Proteus, Klebsiella, Pseudomonas, Enterobacter, Enterococci, Bacteroides, Clostridium, Candida, Peptostreptococcus, Fusobacterium, and Aeromonas.Culture that does not grow any bacteria may not mean that you do not have an infection.

Sometimes the amount of sample collected, the age of the wound, the type of culture done, and previous use of antibiotics can prevent the growth of bacteria in the culture. For some infections, such as postoperative wound infections, use of fine-needle aspiration is superior to the use of wound swabs for obtaining specimens .The tissue Gram stain is an insensitive test for detecting bacteria. For example, staphylococci may not form clusters, and streptococci may not form chains. Tissue or aspirated fluid samples should be submitted for culture; there is no role for the use of swabs in collecting specimens for culture other than for viral cultures[3].

This study explores the absorptive capacity of standard cotton wool-tipped swabs in vitro and in vivo. An alternative wound exudate sampling technique using small filter paper discs is also briefly examined. Results showed a poor uniformity of fluid absorption by swabs. It was found, however, that swabs reliably removed material from a wound surface. The filter paper technique appeared to offer no advantages Swabs and other sampling techniques[4]. In primary care, a swab is the most common method used for sampling a wound. Although biopsy or aspirates of pus are the "gold standard" techniques, wound swabs can provide acceptable samples for bacterial culture provided that the correct technique is used. If the wound is not purulent it should be cleaned prior to swabbing[5]. Swab systems are not the best way to collect patient samples for either aerobic or anaerobic specimens[6]. Pus and soft tissue had predominantly polymicrobial flora, whereas bone infections were mono-microbial. The isolates from soft tissue specimens were different from those from bone and pus in 57% and 54% of cases, respectively. The common bacterial isolates from 117 specimens included Escherichia coli (21%) and Proteus species (15.9%). Nearly 70% of Staphylococcus aureus isolates were methicillin sensitive. All S. aureus and Enterococcus isolates were sensitive to vancomycin. Susceptibility of Gram-negative organisms to ciprofloxacin was 50%.Diabetic foot infections are mostly polymicrobial with Gram-negative predominance[7]. The results of bone culture or culture of other deep-tissue biopsy specimens should not be used as the sole criterion for infection without supporting clinical or histopathological evidence[8]. The fine-needle aspiration microbiology (FNAM) approach allows antibiotic sensitivities to be obtained enabling specific antimicrobial therapy to be implemented early. FNAM also has a higher yield of cultures than wound swabs. Cellulitic areas can be sampled even when use of wound swabs is not possible[9]. With aspirated specimens from clinical infections, they evaluated the recovery of anaerobic, aerobic, and facultative bacteria in three widely used transport systems: (i) aspirated fluid in a gassed-out tube (FGT), (ii) swab in modified Cary and Blair transport medium (SCB), and (iii) swab in a gassed-out tube (SGT). The FGT method was clearly superior at 48 h to the SCB and SGT systems in this study and is recommended as the preferred method for transporting specimens for anaerobic culture[10]. Aspirates of fluids and exudates from suspected infected sites are superior to samples collected on swabs. However, because of the ease of using swabs, clinical microbiology laboratories continue to receive patient samples in swab transport systems. Swab transport systems with semi gel-stabilizing ingredients are effective methods for specimen collection and transport in the event that aspirates cannot be collected. Moreover, these swabs have been shown to protect and maintain the viability of both fastidious aerobic and anaerobic organisms[11]. The Copan Vi-Pak system outperformed the other s by maintaining the viabilities of both anaerobic and fastidious aerobic bacteria for 24 h for the majority of the organisms evaluated. This time period should be sufficient for transport of specimens to the clinical microbiology laboratory without compromising organism recovery[12]. Bone and deep soft tissue specimens were obtained from all patients by open surgical procedures under aseptic conditions during debridement or amputation. The results of bone and soft-tissue cultures were identical in 49% (n = 22) of cases. In 11% (n = 5) of cases there were no common pathogens. In 29% (n = 13) of cases there were more pathogens in the soft-tissue specimens; these microorganisms included microbes isolated from bone cultures[13]. Histopathologic and cyto-pathologic examination, including Gram staining or examination of a frozen section of fresh tissue, can provide preliminary (and often definitive) diagnoses more rapidly than can culture, but they also may be necessary for assessing the clinical relevance of culture results, particularly when cultures yield bacteria that could be either pathogenic or a contaminant[14]. Swab cultures are probably the most commonly used method to determine the resistance pattern of skin pathogens treated in nursing home residents. Wound swabbing is most widely used, but may mislead by detecting surface colonizing microorganisms rather than more deeply sited pathogens. When very rapid identification is required, e.g. in sepsis, microscopic examination by a Gram stain may be useful in guiding early antimicrobial therapy. If quantitative microbiological analysis is available, the Levine technique(A swab is rotated over a 1cm2 area of the wound with sufficient pressure to express fluid from within the wound tissue may be the most useful.) In general, sampling should take place after wound cleansing (and, if appropriate, debridement), and should concentrate on areas of the wound of greatest clinical concern[15]. With pressure ulcers superficial swab cultures generally reflect bacterial colonizationrather than overt infection[16]. Although tissue biopsy is considered the gold standard to diagnose infection, it is rarely used in clinical settings. Swab culture is the most frequently employed method of confirming wound infection in the United States[17].

This study aimed to re-evaluate the accuracy of swab cultures vs. deep tissue cultures in diabetic wounds of varying depth and severity. Analysis according to the depth of the wound, demonstrated that swabs identified all micro-organisms isolated from the deep tissue specimens in 36/40 wounds (90%) that did not extend to bone as opposed to 13/20 wounds (65%) that extended to bone. Swab cultures are valuable in identifying pathogens in diabetic foot wounds when bone is not involved. When surgical debridement is contraindicated or delayed, swab cultures can be used to select appropriate antibiotic therapy[18]. Ulcer swabbing and needle puncture were performed and the drop of fluid obtained by aspiration was used for both aerobic and anaerobic bacterial culture. Three bacterial species were isolated by needle puncture only in one patient while three or more bacterial isolates were obtained by superficial swabbing in six patients. No bacterial isolate was detected in five patients by needle puncture and in two patients by superficial swabbing. Staphylococcus aureus accounted for 70% of cases (seven patients) when a single bacterial species was obtained by needle puncture. Invasive needle puncture diagnostic technique should be considered for deep direct sampling in diabetic patients with osteomyelitis related to foot ulcer when surgical debridement is contraindicated or delayed[19]. Transcutaneous bone biopsy specimens, needle puncture specimens, and swab samples were collected on the same day for each patient. Twenty-one bone biopsy specimens (67.7%), 18 needle puncture specimens (58%), and 30 swab samples (96.7%) had positive culture results. Staphylococcus aureus was the most common type of bacteria that grew from bone samples, followed by Proteus mirabilis and Morganellamorganii. The mean number of bacteria types per positive sample were 1.35, 1.32, and 2.51 for bone biopsy specimens, needle puncture specimens, and swab samples, respectively. The study results suggest that needle punctures, compared with transcutaneous bone biopsies, do not identify bone bacteria reliably in patients with diabetes who have low-grade infection of the foot and suspected osteomyelitis.Needle puncture and transcutaneous bone biopsy cultures are inconsistent in patients with diabetes and suspected osteomyelitis of the foot[20]. The deep tissue biopsy is regarded as the gold standard for identification of wound bio burden and clinical infection[21].

CONCLUSION

The best method to collect the specimen for culture is by tissue method. The swab method grows more commensals than the tissue method. Mixed organisms are grown more in swab method. Comparing swab culture, tissue culture identify the infecting organism in diabetic foot ulcers.

REFERENCES

1. Starr, S., & MacLeod, T. (2003). Wound swabbing technique. *Nurs Times*, *99*(5), 57-59.
2. Siddiqui, A. R., & Bernstein, J. M. (2010). Chronic wound infection: facts and controversies. *Clinics in dermatology*, *28*(5), 519-526.
3. Wilson, M. L., & Winn, W. (2008). Laboratory diagnosis of bone, joint, soft-tissue, and skin infections. *Clinical infectious diseases*, *46*(3), 453-457.
4. Lawrence, J. C., & Ameen, H. (1998). Swabs and other sampling techniques. *Journal of wound care*, *7*(5), 232-233.
5. Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical microbiology reviews*, *14*(2), 244-269.
6. Perry, J. L., Ballou, D. R., & Salyer, J. L. (1997). Inhibitory properties of a swab transport device. *Journal of clinical microbiology*, *35*(12), 3367.
7. Parvez, N., Dutta, P., Ray, P., Shah, V. N., Prakash, M., Khandelwal, N., ... & Bhansali, A. (2012). Microbial profile and utility of soft tissue, pus, and bone cultures in diagnosing diabetic foot infections. *Diabetes technology & therapeutics*, *14*(8), 669-674.
8. Bowler, P. G. (2003). The 10 (5) bacterial growth guideline: reassessing its clinical relevance in wound healing. *Ostomy/wound management*, *49*(1), 44-53.
9. Parikh, A. R., Hamilton, S., Sivarajan, V., Withey, S., & Butler, P. E. M. (2007). Diagnostic fine-needle aspiration in postoperative wound infections is more accurate at predicting causative organisms than wound swabs. *Annals of the Royal College of Surgeons of England*, *89*(2), 166.
10. Helstad, A. G., Kimball, J. L., & Maki, D. G. (1977). Recovery of anaerobic, facultative, and aerobic bacteria from clinical specimens in three anaerobic transport systems. *Journal of clinical microbiology*, *5*(6), 564-569.
11. Rishmawi, N., Ghneim, R., Kattan, R., Ghneim, R., Zoughbi, M., Abu-Diab, A., ... & Hindiyeh, M. Y. (2007). Survival of fastidious and nonfastidious aerobic bacteria in three bacterial transport swab systems. *Journal of clinical microbiology*, *45*(4), 1278-1283.
12. Hindiyeh, M., Acevedo, V., & Carroll, K. C. (2001). Comparison of three transport systems (Starplex StarSwab II, the new Copan Vi-Pak Amies Agar Gel collection and transport swabs, and BBL Port-A-Cul) for maintenance of anaerobic and fastidious aerobic organisms. *Journal of clinical microbiology*, *39*(1), 377-380.
13. Ertugrul, M. B., Baktiroglu, S., Salman, S., Unal, S., Aksoy, M., Berberoglu, K., & Calangu, S. (2008). Pathogens isolated from deep soft tissue and bone in patients with diabetic foot infections. *Journal of the American Podiatric Medical Association*, *98*(4), 290-295.
14. Procop, G. W., & Wilson, M. (2001). Infectious disease pathology. *Clinical infectious diseases*, 1589-1601.

15. Drinka, P., Bonham, P., & Crnich, C. J. (2012). Swab culture of purulent skin infection to detect infection or colonization with antibiotic-resistant bacteria. *Journal of the American Medical Directors Association*, *13*(1), 75-79.
16. Nicolle, L. E., Orr, P., Duckworth, H., Brunka, J., Kennedy, J., Urias, B., ... & Harding, G. K. (1993). Prospective study of decubitus ulcers in two long term care facilities. *The Canadian journal of infection control: the official journal of the Community & Hospital Infection Control Association-Canada= Revue canadienne de prevention des infections/Association pour la prevention des infections a l'hopita..*, *9*(2), 35-38.
17. Bonham, P. A. (2009). Swab cultures for diagnosing wound infections: a literature review and clinical guideline. *Journal of Wound Ostomy & Continence Nursing*, *36*(4), 389-395.
18. Slater, R. A., Lazarovitch, T., Boldur, I., Ramot, Y., Buchs, A., Weiss, M., ... & Rapoport, M. J. (2004). Swab cultures accurately identify bacterial pathogens in diabetic foot wounds not involving bone. *Diabetic medicine*, *21*(7), 705-709.
19. Kessler, L., Piemont, Y., Ortega, F., Lesens, O., Boeri, C., Averous, C., ... & Pinget, M. (2006). Comparison of microbiological results of needle puncture vs. superficial swab in infected diabetic foot ulcer with osteomyelitis. *Diabetic Medicine*, *23*(1), 99-102.
20. Senneville, E., Morant, H., Descamps, D., Dekeyser, S., Beltrand, E., Singer, B., ... & Yazdanpanah, Y. (2009). Needle puncture and transcutaneous bone biopsy cultures are inconsistent in patients with diabetes and suspected osteomyelitis of the foot. *Clinical Infectious Diseases*, *48*(7), 888-893.
21. Spear, M. (2012). Best technique for obtaining wound cultures. *Plastic Surgical Nursing*, *32*(1), 34-36.