Journal of Clinical and Biomedical Sciences

Original Article

Evaluation of Superficial and Deep Specimens for Isolation and Identification of Bacterial Isolates from Diabetic Foot Infections.

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Introduction

Worldwide, Diabetic foot infections (DFIs) are a major medical, social, and economic problem reaching epidemic proportions carrying the increased risk of complications.[1,2] About 25% of the diabetics have the risk of developing foot ulceration which is one of the leading cause of mortality and morbidity in developing countries.[2,3,4]The most feared complication of infected diabetic foot ulcers is gangrene which results in amputations and occurs 10-30 times more often in diabetics. About one major amputation in 30 seconds worldwide in diabetics and the elevated mortality at follow up, ranging from 13% to 40% at 1 year to 39% – 80% at 5 years requires urgent strategies towards prevention of foot ulceration and amputations. [3,5] Once the protective layer of skin is broken, the deep tissues are exposed to bacterial infection that progresses rapidly.

India having more diabetics than any other country is alarming.[4,6 and 7] Barefoot walking, inadequate facilities for diabetic care, low socioeconomic status and illiteracy are even now the major reasons for foot problems and amputations in India.[4, 8] In most Indian hospitals the collection of sample from diabetic foot ulcer is often a superficial swab specimen, the deeper tissue is generally collected only when osteomyelitis is suspected. The superficial swabs mostly yield surface contaminants which may not be actual pathogens. The deeper tissues actually harbour the real pathogens. Deep tissue cultures obtained by punch biopsy, ulcer curettage, or aspiration of pus, is reported to provide the most reliable bacteriologic information which reflect the actual pathogens in DFIs.[9] A comparative study of the clinical specimens of the superficial swabs and biopsy/ pus aspiration would exclude the environmental contaminants and will help in the isolation of the infecting pathogen in the deeper tissues. This to a great extent will help in appropriate use of antibiotics, targeting the specific pathogen instead of their indiscriminate use to treat the surface contaminants or the colonizers. This also is crucial in reduction in selection of multi drug resistant mutants. This study was conducted to isolate the specific bacterial pathogens causing the diabetic foot infections and to compare the bacterial isolates of superficial swab and punch biopsy/pus specimens in diabetic foot infections. We tried to evaluate and assess the antimicrobial sensitivity pattern of the infecting and colonizing organisms from same patients and to help the treating consultant to choose an appropriate antibiotic and to assess the response.

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Conflict of Interest: None
Financial Aid: Nil

Materials and methods

This was a prospective study carried out at R.L Jalappa Hospital & Research Centre, attached to Sri Devaraj Urs Medical College, Tamaka, Kolar, between February 2010 to March 2011. The study protocol was approved

by the Institutional ethics committee and written informed consent was taken from all the 50 patients recruited. The specimens collected were superficial wound swabs, punch biopsy tissues & aspirated pus. The inclusion criteria were patients with clinically diagnosed infected diabetic foot with ulcer/wound/ osteomyelitis or previous amputated stump reinfected. The exclusion criteria was cellulitis, clinically non infected ulcers. The diabetic foot ulcers were graded using IWGDF–PEDIS (Perfusion, Extent, Depth, Infection, Sensation) classification and Grade III and Grade IV were included in this study. Two surface swab specimens and 4 to5 bits of deep tissue samples from punch biopsy were simultaneously obtained from each foot ulcer. Tissue samples were immediately smeared on to the Blood agar and inoculated into the liquid media. The samples from superficial swab and deep tissue were subjected to gram staining and inoculated on to Brucella blood agar, Anaerobic Hi veg agar and Anaerobic basal broth(Himedia laboratories), Robertson's cooked meat broth and incubated at 35^0c in a gaspak jar for 5-7 days for anaerobic study. Colonies on the Blood agar, Mac conkey's agar were processed and monitored daily according to standard methods and Thioglycollate broth was subcultured onto Blood agar and Mac Conkey's agar. Bacterial colonies were identified by standard methods and were then studied and categorized as cocci and rods. Cocci that fermented Mannitol were considered Staphylococci and confirmed as Staphylococcus aureus by the isolate's ability to produce coagulase both on slide and test tubes using human pooled plasma. Those Staphylococci that did not produce coagulase were deemed coagulase negative (CONS). Antibiotic susceptibility of the identified organism was carried out according to the CLSI guidelines Anaerobes were identified using the Anident discs (Oxoid, USA).[10,12]

Results

Fifty patients of type II diabetes mellitus, with diabetic foot ulcers were recruited.

Table 1. Clinical characteristics of the patients with diabetic foot ulcer

Parameters		Results
Age Range		35 – 76 yrs
Gender	Males	43 (86)
Number (%)	Females	07 (14)
Residence		Kolar & Chikballapur districs
Total No. of Inpatients (%)		42 (84)
Total No. of Out patients (%)		08 (16)
Duration of diabetes mellitus		2 Days To 20 Yrs
Duration of foot infection		2 Days To 1 Year
No. of patients with good glycemic control (%)		12 (24) To 38 (76)
No. of patients with poor glycemic control (%)		

Table 2. Statistical analysis of the superficial and the deep tissue samples

	Aerobes	AnaeroStudy site bes	Mean No. Of Organisms	Standard Deviation	p value
Superficial	89(25.57)	0	1.78	0.89	**<0.001****
Deep	**135**(38.79)	**124**(35.63)	**5.18**	**1.57**	

** Highly significant

Table 3. Comparison of organisms isolated from the superficial and the deep samples

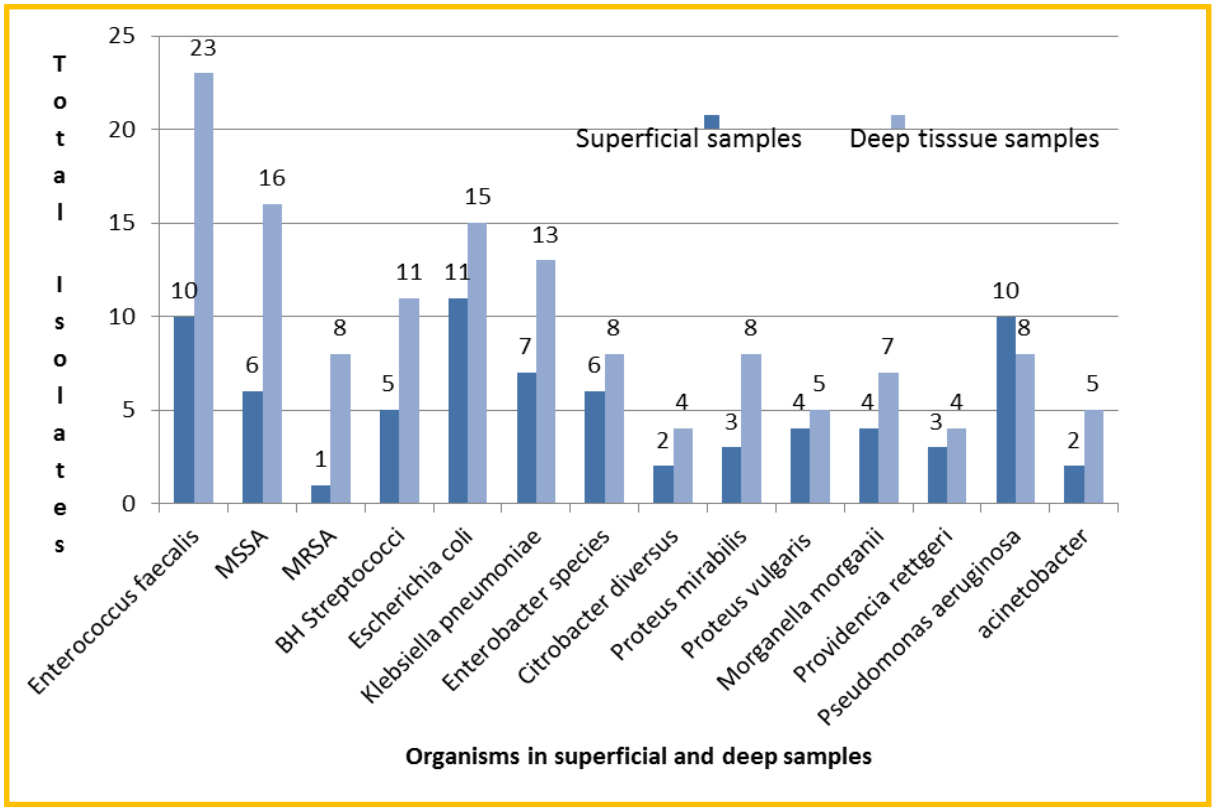

Table 4. Statistical analysis of the superficial and the deep tissue samples

Total aerobic isolates from superficial and deep samples			
Organisms	**Superficial samples** (% per 50 cases)	**Deep samples** (%per 50 cases)	**P value**
Enterococcus faecalis	10(20%)	23(46%)	**0.03***
MSSA	06(12%)	16(32%)	0.07
MRSA	01(2%)	08(16%)	**0.023***
Streptococci	05(10%)	11(22%)	**<0.001****
CONS	09(18%)	00	-
Diphtheroids	06(12%)	00	-
Escherichia coli	11(22%)	15(30%)	**0.01***
Klebsiella pneumoniae	07(14%)	13(26%)	**<0.001****
Enterobacter Species	06(12%)	08(16%)	**0.04***
Citrobacter diversus	02(4%)	04(8%)	0.15
Proteus mirabilis	03(6%)	08(16%)	**<0.001****
Proteus vulgaris	04(8%)	05(10%)	**0.002****
Morganella morganii	04(8%)	07(14%)	**<0.001****
Providencia rettgeri	03(6%)	04(8%)	**<0.001****
Pseudomonas aeruginosa	10(20%)	08(16%)	**<0.001****
Acinetobacter species	02(4%)	05(10%)	**0.008***

* Significant , ** Highly significant

Table 5. Total anerobes in the deep tissue (% per 50 cases)

Total anerobes in the deep tissue(% per 50 cases)
Peptococci 47 (94%)
Peptostreptococc 47(94%)
Bacteroides sp. 13(26%)
Propionibacterium sp.9 (18%)
Fusobacterium sp. 5 (10%)
Prevotella melaninogenica 2(4%)
Clostridium novyii 1 (2%

DISCUSSION

This study highlights the importance of appropriate samples to be collected from infected diabetic foot ulcers to isolate the pathogens. Diabetic foot infections are commonly multimicrobial.[1,2] Most commonly in most of the hospitals in India, just a swab is collected from the superficial aspect of the foot ulcer and sent for microbiological study to isolate organisms and frequently empiric antibiotics are started and if necessary altered according to those culture results. However this superficial sample may not show the actual pathogen or pathogens and the antibiotic therapy may not be appropriate.[3,5,6]

Many studies on diabetic foot ulcer state that superficial samples are insufficient for treating a diabetic foot ulcer and only deep tissue isolates the real pathogen infecting the ulcer.[3,7] However the study in South-western Nigeria found no difference in the microorganisms of the superficial swab and Deep tissue. As surface swabs of decubitous ulcers, swab samples of encrusted walls of abscesses, mucosal linings, and eschars are not the samples to be processed for anaerobes, according to the standard text books and references, we did not proceed to look for anaerobes in the superficial samples. Superficial swabs which are usually collected for microbiological diagnosis of diabetic foot infections usually shows only surface contaminants.[8,9] This study though small in number has brought out additional organisms & anaerobes, isolated from deeper tissues in diabetic foot infections. Superficial swabs are not useful for isolation of anaerobes.[10,11,12] Totally six patients in our study were admitted for amputation with very badly infected limbs. With immense cooperation from the surgery and the medicine departments, timely collection of the deep tissue samples and the meticulous culture of all the organisms that infected the foot were studied and the patient was treated according to the sensitivity report. The patients were discharged without amputation, saving their limbs. Out of 50 badly infected diabetic foot infections, most cases altered the treatment after deep tissue report was given with appropriate antibiotics, with patients responding well to the treatment and the level of amputations were lowered or avoided and mostly discharged with well healed lesions.

However, it is possible that the superficial colonizing or contaminating organisms may be recovered from the deep tissues also while inappropriate collection. This can be avoided to a large extent by careful sampling after thorough cleaning of the superficial aspect, debridement and then taking a punch biopsy under strict aseptic precautions. ESBLs and Amp C were detected by phenotypic methods according to CLSI 2010 guidelines.[6] Among Enterobacteriaceae 18 isolates were found to be ESBL positive, with 5 Escherichia coli, 5 Klebsiella pneumoniae, 4 Enterobacter species, 2 Proteus species, 1 Citrobacter species & I Morganella morganii. We did not find resistance to Carbapenems in Enterobacteriaceae isolates. However we found few isolates of Acinetobacter showing Carbapenem resistance. 9 isolates were Amp C producing with 2 Escherichia coli, 2 Enterobacter species,2 Providencia rettgeri, 1Citrobacter freundii, 1 Morganella morganii, 1 Klebsiella pneumoniae. All patients in our study were treated according to the sensitivity of the isolates from deep tissue. Patients received a change in treatment after the sensitivity report was given. Treatment based on superficial swab isolates may not be effective since the actual pathogens are deeper in the tissues which are identified by processing deep tissue biopsy specimen/ aspirated pus.

Deep tissue cultures obtained by punch biopsy or aspiration provides the most reliable bacteriologic information in diabetic foot infections. However deep tissue isolates may be

contaminated with colonizers during collection but still, deep tissue gives better knowledge on the infecting microorganisms and avoids antibiotics to be directed only against superficial contaminants. Hence we recommend that there should be a uniform policy to collect deeper tissue for microbiological study of DFIs. Collection of superficial or surface swabs from the ulcers or wounds should be discouraged or totally avoided and in every hospital this should be communicated to the treating consultant and the clinical microbiologist.

Depending upon the microbiological data from deep tissue samples in DFIs an appropriate empiric therapy of antibiotic policy could be developed in each hospital or health care facility where DFIs are routinely treated. In this study we found that empirically a combination of an Aminoglycoside, a Fluoroquinolone or Linezolid and Metrogyl or Clindamycin proved useful in the treatment of DFIs. Depending upon the organisms isolated from the deep tissues and their antibiotic sensitivity patterns, the therapy can be de-escalated or changed to the sensitivity of the etiological agents.

This in some cases may avoid un necessary amputations which has happened in 6 of our cases. Needless to say that this is a great benefit to the patient with DFIs. Furthermore early identification of the microorganisms and appropriate therapy promptly will reduce the further complications of DFIs. We do not recommend the use of Carbapenems routinely, unless there is an overwhelming systemic infections such as septicemia or septic shock.

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