Research Article

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COMPARISON OF MICROBIAL FLORA BY DEEP TISSUE BIOPSY AND SUPERFICIAL SWAB CULTURE OF SPECIMEN COLLECTED FROM VARIOUS ANATOMICAL SITES IN EARLY WOUND INFECTIONS

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ABSTRACT

The aim of this study is to evaluate and to compare the microorganisms isolated by two different methods of specimens collected from different anatomical sites in early wound infection, further to study the type of organisms grown in those sites. A total of 62 specimens were collected from 32 patients treated in Karpaga Vinayaga Institute of Medical sciences and Research Centre , during the period of six months from 15 .09 .13 to 15.03.14.Specimens were collected from gluteal, thigh, knee, leg, foot, breast, umbilical and genital regions. Initially a specimen was collected aseptically by common swab method from the wound and then the wound was cleaned, deep tissue biopsy was taken by scraping from the base of the same site of the wound to compare the two different methods of collection of specimens. Specimens were cultured by standard microbiological methods and the isolates were identified by standard biochemical methods. Among the 32 cases 7 were culture negative and the remaining 25 were culture positive. Most of the wound infections were presented in the foot 59.3% followed by leg 9.3 %. Knee, umbilical, gluteal and breast were presented with 6.2% of infection rate. Thigh and genital regions showed only 3.1% each. The commonest isolate was *Staphylococcus aureus* 41.2%. Out of the 14 isolates 12 were isolated from swab culture (35.3%). The next predominant organism isolated was *E. coli* which was 20.6 %, out of which 11.8% were isolated from deep tissue culture. Other isolates were *Klebsiella* 11.8%, *Proteus* and *Pseudomonas* spp were 8.8% each, *Streptococcus* 5.9% and *Acinetobactor* 2.9%. On comparison with the rate of isolation of organisms, it was more in swab culture (73.5%) than in the deep tissue culture (26.5%). *Staphylococcus aureus* found to be the most common Gram positive organism in the swab culture and *E.coli* was the more common Gram negative organism isolated in deep tissue culture. Out of 25 culture positive cases, 2 of them showed the same organisms both in swab culture and in deep tissue culture (8%). However the organisms isolated in swab culture were highly differ with deep tissue culture (28%). This study results reveal that the wound infections commonly seen in foot followed by leg. The common organism seen in swab culture is *Staphylococcus aureus* and the common Gram negative organism isolated was *E.coli* which is more predominate in deep tissue culture.

Key Words: Wound infections, Deep tissue culture, Superficial swab culture

INTRODUCTION

Skin as protect layer which prevent the underlying vulnerable tissues from the attack of various microorganisms. A major non specific protection as a physical barrier with salt and sebaceous secretions, does not allow the action of environmental microbial flora. Any damage or loss to the intact skin will prove the colonization, invasion and the pathogenesis of verity of microbial flora, best observed in burn wound infection.

In spite of careful preventive measures, human got wounded in the modern mode of life either by cut wound, burn wound or by infections. However the wound or ulcer easily get complicated by patients won flora or by environment contamination. Open wound provide moisture and nutrition, microbes colonize easily and proliferate. Therefore it is important to identify the etiological agents and its antibiotic susceptibility pattern for proper treatment. Previous study reports revealed that opportunistic pathogens of patients won flora and the immune

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status of the patient plus the depth and the location of the lesion are the major contributive factors.(1) Chronic wounds are mainly caused by endogenous mechanisms (2).Acute wounds usually caused by external damage to intact skin such as bites, cuts or burn wounds. It was observed the microbial flora differ with acute wounds and the chronic ones.

In acute wounds most frequently the normal skin flora of Gram positive cocci are common latter, the gram negative bacilli will be predominate and are involved with deeper tissues. Anaerobic organisms will occur in very later stage (3). Studies revealed that deep tissue biopsy is the gold standard in culture technique (4). Swab cultures are regarded as the least reliable (5-6), they often lack the true pathogens, however they cannot completely ignored.It is useful in acute wound infections. Therefore results from swab cultures also used in the identification of pathogens in wound infections (7-8). Thus a wide range of diverse claims were made by different researchers. This study is planned to compare the microbial flora in deep tissue culture and the superficial swab culture in early wound infection to study the efficacy of these methods. Further to study the type of organism isolated from the wound occur in different parts of human body.

MATERIALS AND METHODS

A total number of 62 samples were collected from gluteal region, thigh, knee, leg, foot, breast umbilical and genital area in which, 28 were from deep tissue biopsy and the remaining 34 samples included wound swabs, pus and aspirates. Initially pus samples/ aspirates/ wound swabs or the scrapings from the base of ulcer were collected. For superficial swab culture the wound was cleaned with sterile saline and a sterile swab was being rotated directly on to the base of the ulcer. Following that, the deep tissue biopsy was collected. For deep tissue biopsy, the wound was cleaned well using sterile curette and forceps by an experienced clinician and the tissue materials were collected and sent to laboratory for immediate processing .Both swab culture and deep tissue were collected from the same site for each patient. All the samples were collected aseptically from patients treated in Karpaga Vinayaga Institute of Medical Sciences. Informed consent was obtained from those who underwent the test procedure in this study. Ethics committee of Dr MGR University approved this study.

METHOD

Collected specimens were inoculated onto appropriate culture media (5% blood agar and macConkey agar) and incubated aerobically at 37 ° C for 24 to 48 hours. Organisms grown on culture media were identified by standard microbiological methods. Only qualitative bacteriology was performed. A direct Gram stained smear was examined from each sample. Presence of bacteria and pus cells were regarded. The Gram stain results were studied in comparison with isolation of organisms.

As the study designed for acute and the early infectious period the exclusion criteria for the study were the gangrenous wound, patients with dry Escher and antibiotic use before hospitalization. As per the localization of the wound / ulcer /abscess they were classified in to three groups. Breast and umbilical region as group 1, gluteal, genital and thigh as group 2, Knee, leg and foot as group3. Wound formation occurred less than a month considered as acute infection.

RESULTS

Out of 32 patients included in this study 19 were males (59.4%) and 13 were females (40.6%).The age group ranges from 20 to70 years (Table 1). Wound infections were more common in foot 59.4% followed by leg (9.3%). Wound infection in the foot in males is 46.9% and12.5% in females. Age groups between 31-40 years and again 61 -70 years are prone for the wound infection on foot (Table 2). Among the 32 cases studied 7 were culture negative and the remaining 25 were culture positive (Table 3). Out of the 25 culture positive cases in superficial swab culture(SSC) all the 25 samples were showed growth in culture whereas, in deep tissue culture(DTC) only 16 samples were showed growth and the remaining 9 samples were culture negative (Table 4). Wound in the breast and umbilical region showed 6.2%. Wound infection on genital region and knee also showed 6.2% whereas thigh and gluteal region showed only 3.1% each (Table: 5).Commonest isolate was *Staphylococcus aureus* (41.2%). Out of the 14 Staphylococcus isolates 12 were isolated from swab culture (35.3%). The next predominant organism isolated was E. coli which was 20.6 %, out of which 11.8% were isolated from deep tissue culture. Other isolates were *Klebsiella* 11.8%, Proteus and *Pseudomonas spp* were 8.8% each *Streptococcus* 5.9% and *Acinetobacter* 2.9%. On comparison of organisms isolated, it was more in swab culture (73.5%) than in the deep tissue culture (26.5%) *Staphylococcus aureus* found to be the most common Gram positive organism in the swab culture and *E.coli* was more common Gram negative organism isolated in deep tissue culture (Table:6).Out of 25 culture positive cases 2 of them showed the same organisms both in swab culture and in deep tissue culture (8%). However the organisms isolated in swab culture were highly differ with deep tissue culture (28%).

Direct Gram stain smear report showed Gram positive cocci in clusters, and in chains and Gram negative rods along with pus cells. It was observed that lot of pus cells

were seen when there were more no of bacteria and less number of pus cells when no bacteria. In most of the culture positive cases the direct Gram stain report correlate with many pus cells and bacteria.

DISCUSSION

Usually open wounds are polymicrobial, however the superficial wounds and in the early infective phase are monomicrobial (9-10). The source of infection being the patients won flora of skin and mucous membranes, those organisms generally aerobic Gram positive cocci. If the wound occur near the genital and perineum the pathogens generally Gram negative bacilli like *E.coli.* Present study report showed that the foot infection is predominate (59.3%) and the most common isolate is *Staphylococcus aureus* 41.2% and most of the wounds showed monomicrobial growth. Our results correlates with Ana kaftandzieva et al.(2012)(11).

In wound infection exogenous pathogens include Gram negative bacilli present in the nearby environment. In early acute wound, normal skin flora predominates. After about four weeks facultative aerobic Gram negative rods *colonize* the wound. *E.coli, Proteus* and *Klebsiella* are most commonly observed. Present study report showed 52.9% of Gram negative bacilli isolates from wound infections. The most common Gram negative isolate was *E.coli* (20.6%), among which 11.8% were isolated from deep tissue culture. The other isolates were *Klebsiella* 11.2% *Proteus* and *Pseudomonas spp* were 8.8% each. Similar findings were observed in studies done previously (12). Isolation of *Staphylococcus aureus* when compared to the isolation of any single Gram negative bacteria (except *E.coli*) the difference is statistically significant. (P=0.01).

Different studies on wound infection with different conditions generate a verity of results, surface swab culture by the easy collection and reduce laboratory processing cost have attracted much attention as a potential alternate to the gold standard histology and quantitative culture method for microbiological wound monitoring. Gram stain examination was used for Gram stain affinity, morphology and arrangement of organisms however there were few reports suggested that direct Gram stain microscopy and the correlation of the culture report (13). In our study the organisms present in direct smear were generally isolated in culture too however, to prove this it requires further elaborate investigations.

CONCLUSION

In early wound infection the commonest pathogen isolated was *Staphylococcus aureus*, and the common Gram negative bacilli isolated was *E. coli* followed by *Klebsiella*. Swab culture is probably the most commonly used method to determine the resistant pattern of skin pathogens in clinical practice. Based on this study report surface swab culture may be considered as a tool for monitoring the surface wound with in the first few weeks of treatment. Patients who remain in the ward for a prolonged period deep tissue samples are justified for monitoring the bacteriological activity in wound infection. Superficial swab culture could be valuable to identify the pathogens in infected diabetic wounds without osteomyelitis, and deep tissue culture will be more sensitive and reliable in osteomyelitis cases.

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Table 1: Age and sex wise distribution in wound infection

Years	20-30	31-40	41-50	51-60	61-70	Total	(%)
Male	1	5	5	3	5	19	59.4
Female	3	3	--	4	3	13	40.6
Total	4	8	5	7	8	32	100

Table 2: Prevalence of wound infection in foot age and sex wise distribution

Years	20-30	31-40	41-50	51-60	61-70	Total	(%)
Male	--	5	3	2	5	15	46.9
Female	--	2	--	--	2	4	12.5
Total	--	7	3	2	7	19	59.4

Table 3: No of Culture positive and culture negative Cases in wound infection

		(%)
Culture negative	7	21.9
Culture positive	25	78.1
Total	32	100

Table 4: Culture positive and culture negative samples

			(%)
1	No of samples Positive by SSC	25	50
2	No of samples Positive by DTC	9	18
3	No of samples negative by DTC	16	32
Total		50	100

SSC –superficial swab culture DTC-deep tissue culture

Table 5: Site of specimen collection and no of organisms isolated

	Breast	Umblical	Gluteal	Genital	Thigh	Knee	Leg	Foot
Male	-	-	-	1	1	1	2	15
Female	2	2	1	1	-	1	1	4
Total	2 (6.2 %)	2 (6.2%)	1 (3.1%)	2 (6.2 %)	1 (3.1 %)	2 (6.2%)	3 (9.3%)	19 (59.4%)

Table 6: No of Gram positive and Gram negative Organisms isolated from wound infection

Gram positive			**Gram negative**					**Total**
	Staph	**Strept**	**E.coli**	**Kleb**	**Proteus**	**Pseudo**	**Acenetobact**	
SSC	12 (35.3%)	2 (5.9%)	3 (8.8%)	3 (8.8%)	2 (5.9%)	2 (5.9%)	1 (2.9%)	25 (73.5%)
DTC	2 (5.9%)	0 -	4 (11.8%)	1 (2.9%)	1 (2.9%)	1 (2.9%)	0 -	9 (26.5%)
Total	14 (41.2%)	2 (5.9%)	7 (20.6%)	4 (11.7%)	3 (8.8%)	3 (8.8%)	1 (2.9%)	34 (100%)

SSC –superficial swab culture DTC-deep tissue culture