



DIFFERENT METHODS FOR ISOLATION OF BACTERIA IN BURN WOUND INFECTIONS WITH ITS ANTIMICROBIAL SENSITIVITY PATTERN: A COMPARATIVE STUDY IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

Background: Burn wound sepsis is major cause of mortality.

Objective: To explore method of isolation of bacteriological profile and antibiotic sensitivity for burn wound associated infection.

Materials and Methods: A descriptive cohort study was conducted in 2018 involving 124 patients admitted in BSMCH with >20% total body surface area burn. Information was collected via interview, clinical examination and scrutinizing records using questionnaire. Wound swab, tissue biopsy and blood specimens were obtained from each patient for comparison efficacy in isolating bacteriae and their antibiotic sensitivity.

Results: Majority (59.7%) were female patients belonged to the age group 21-30 years age sustaining burn between 20-40% TBSA with higher surface swab and tissue biopsy culture isolates. Blood culture positivity mostly was in 41-60% of TBSA burn. Swab and tissue culture positivity was significantly higher in each TBSA group compared to blood culture with significantly higher tissue biopsy and blood culture isolates across TBSA groups. High, moderate was noted between swab and tissue cultures for *Pseudomonas aeruginosa*; for *Staphylococcus aureus*, *E. coli*, *Citrobacter freundii* and *Acinetobacter* spp. Gram negative enterobacteriaceae were highly sensitive to Imipenem, piperacillin-tazobactam with high resistance towards cephalosporins and fluoroquinolones. *S. aureus* exhibited highest (96%) sensitivity to vancomycin and linezolid whereas CoNS showed 100% sensitivity towards these two.

Conclusion: This study suggested good correlation between surface swab and tissue biopsy for identifying pathogens on and within burn wounds

KEYWORDS

total body surface area, antimicrobial sensitivity

INTRODUCTION

It is estimated that 50-75% of mortality among resuscitated burn patients pertains to infection.^[1] The colonizing flora of the burn wound also influence the risk of infection and its invasive potential^[2]. Age of patients, extent and depth of burn wound coupled with microbial factors such as type, their infective dose, enzyme and toxin production and motility of organisms determine the likelihood of invasive burn wound infection.^[3] Skin flora, dominantly gram-positive bacteria colonize immediately after burn and remain the predominant flora throughout but with passing time, gram-negative bacteria become more prevalent inhabitants of the burn wound.^[4]

Cultures from the surface area is useful for identifying the possible colonizing bacteria present within the burn wound but this method of sample collection is incapable of differentiating between burn wound colonization from invasion & infection.^[5] It is now accepted that quantitative cultures of tissue biopsy specimen along with its histopathological features are required to differentiate between colonization (low count) and actual invasive infection (colony count 10^5 or greater).^[6] Tissue biopsy and its quantitative bacterial culture for verification of microbial invasion have been the "gold standard" to confirm invasive burn wound infection.^[7] As it is a quite laborious procedure, many burn centres have shifted to procure burn wound surface swabs for qualitative or semi-quantitative culture for monitoring the burn wound infection.^[8] The procedure of superficial swab sampling is simple, inexpensive, non-invasive and convenient for the majority of wounds. Moreover, tissue biopsy and blood cultures yield a positive culture report only at a later date.^[9]

Bankura Sammilani Medical College & Hospital (BSMCH), Bankura, a tertiary care teaching hospital in Rural West Bengal caters a huge turnover of patients including burn patients belonging to the poor socio-economic strata from Bankura and adjacent districts. Successful treatment of burn patients needs a reliable data base for drawing a strategy for choosing cost-effective method for detecting wound infection as well as an antibiotic policy for their management. The

present study aimed at, to compare the effectiveness of surface swab and quantitative biopsy from burn wounds and blood culture samples taken simultaneously for evaluating the true infective status of the burn patients along with the antimicrobial sensitivity pattern existing in the burn unit of BSMCH.

OBJECTIVES

1. To find out the bacteriological profile causing colonization and deep infection in burn wounds.
2. To evaluate the significance of surface swab, biopsy specimen & blood sample in detecting burn wound associated infection.
3. To determine resistogram of common organisms associated with burn wound infection

MATERIALS AND METHODS:

An institution based descriptive cohort study was conducted from March 2017- February, 2018 involving one hundred and twenty four patients admitted in burn ward, Department of Surgery of BSMC&H

Inclusion criteria:

1. Patients with burn injury of more than 20% total body surface area (TBSA),
2. Who were willing to participate in the study, were included in the study

Exclusion criteria:

1. Patients either suffering from Immunocompromised state long before the burn or from any other systemic illness,
2. Pregnancy

Baseline information like age, sex, percentage and type of burn (to extent of second degree) were collected via interview of patient and/or party as well as clinical examination of patient after obtaining informed consent and examining the hospital records of patients using predesigned questionnaire. Three types of clinical samples i.e. surface wound swab, biopsy of tissue, and blood for Culture and sensitivity (C/S) were collected simultaneously aseptically from each of the 124

patients for examining in laboratory, Department of Microbiology, BSMCH as per standard protocol using existing laboratory set up. Wound swab and tissue biopsy specimens were collected from leading edge of wound sites showing signs of infection such as discoloration, bad odour, presence of pus or eschar.

Surface samples of wounds swabs (two) were collected from an area measuring 4 cm² after removing topical agents. For dry wounds, swab was moistened with sterile saline before swabbing and put into sterile normal saline to make organisms in suspension.^[5] The blood samples were processed as per standard guidelines.

For quantitative cultures of biopsy samples, approximately 20-50 mg of tissue from wound along with underlying fat was removed using 5 mm punch biopsy forceps. The tissue was then suspended in 2 ml of physiological sterile saline and homogenized by a glass homogeniser. After making serial dilutions, 0.1 ml of undiluted or diluted sample was inoculated on blood and MacConkey Agar.^[10] Surface viable colony counts were determined, and the number of organisms per gram of tissue was calculated. Preliminary identification i.e. Direct Microscopy, culture, and Anti-Microbial Susceptibility Testing was done for all bacterial isolates as per Clinical & Laboratory Standards Institute (CLSI) guidelines, 2017 also by using commercial identification kits.^[11,12]

The colony count per gram of tissue was obtained by the formula developed by Miles and Misra.^[17] Colony forming units (CFU)/gm. of tissue = C × D × V/W × 0.01, where C =total number of CFU, D = dilution factor, W =weight of tissue, V = volume of normal saline and 0.01 = volume of the inoculums. No growth up to 48 hours was considered negative.

Control strains used with each batch were: Escherichia coli - ATCC 25922, Pseudomonas aeruginosa- ATCC 27853, Staphylococcus aureus- ATCC 25923.

Detection of methicillin resistant staphylococcus aureus (MRSA) was done by Cefoxitin disk diffusion test.

Analysis of information was done by calculating proportions and displaying data with charts and tables. Chi-square test was used for drawing inference about categorical variables. P value of < 0.05 was considered as statistically significant. Statistical analyses were performed using Statistical package for Social Sciences (SPSS) 20 version.

The present research work was done after obtaining approval of the Institutional Ethics Committee, BSMC, and Bankura.

RESULTS:

The most common age group was 21-30 years (36.3%), followed by age group 31-40 years (21%).[Fig.1] There was female preponderance among the cases (59.7% Vs 40.3%).

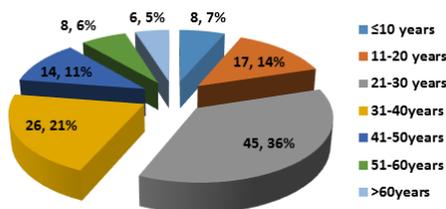


Fig-1: Distribution of participants according to their age

Majority (60.5%) of participants burn victims got discharged, however, 39.5% died.

Table-1: Distribution of burn victims as per the relation between TBSA and culture positivity-

TBSA (%)	Swab culture No. (%)	Tissue Biopsy No. (%)	Blood Culture No. (%)	*2, P at df 3
20-40 [n1=52]	39 (75.0)	36 (69.0)	6 (11.5)	51.31, 0.000
41-60 [n2=29]	24 (82.7)	20 (69.0)	12 (41.4)	11.23, 0.003
61-80 [n3=22]	19 (86.4)	22 (100.0)	4 (18.8)	30.49, 0.000
>80 [n4=21]	18 (86.7)	16 (76.2)	5(24.0)	19.79, 0.00
Total[n=124]	100(80.6)	94(75.8)	27(21.8)	-----

* Test across the methods

On the whole, majority (42.0%) persons were between 20-40% TBSA burns followed by 29 (23.4%) cases between 40-60% TBSA burns. Majority of surface swab and tissue biopsy culture isolates were found to sustain TBSA burn of 20-40%, whereas blood culture positive cases were seen mostly in 41-60% of TBSA burn wound.

Maximum culture positivity in surface swab and tissue biopsy was found among patients with TBSA more than 60% whereas blood culture showed maximum culture positivity in TBSA burns of 41-60%. Across the method analysis revealed that the proportion of swab and tissue culture positivity was significantly higher in each group of TBSA than that of blood culture. However, across the TBSA group test wasn't shown to be statistically significant for swab culture isolates ($\chi^2=1.95, p = 0.583$ at $df=3$) whereas it was significant for Tissue biopsy and blood culture isolates ($\chi^2 = 8.99$ & $9.96, p = 0.0294$ & 0.0189 at $df=3$).[Table-1]

In tissue biopsy samples, out of 20 samples taken on Day 1, 9 (45.0%) showed culture positivity but 14(70.0%) cases were positive in case swab culture. On day 5, out of 26 samples taken tissue biopsy was positive for 15 (57.69%) cases but 19 (73.08%) cases were positive for surface swab. On day 10, out of 16 samples all (100%) were positive for tissue biopsy but 14 (87.5%) in case of swabs. Beyond 14th day, all (100%) cases were positive in both swabs and tissue biopsy.

While comparing both the swab, tissue biopsy and blood cultures, all showed predominance of Pseudomonas aeruginosa followed by coagulase negative staphylococcus (CoNS) and staphylococcus aureus in case of surface swab culture but Acinetobacter spp in case of tissue biopsy culture and Staphylococcus aureus for blood culture. On comparison of surface swab cultures and tissue biopsy cultures, there was high concordance for Pseudomonas aeruginosa, moderate concordance for Staphylococcus aureus, E.coli, Citrobacter freundii and Acinetobacter spp but low concordance for CoNS, Klebsiella pneumoniae and Proteus mirabilis.[Table-2]

Table-2: Distribution of isolates in various methods (n=124)

Organism	Swabs No. (%)	Tissue Biopsy No. (%)	Blood Culture No. (%)
P. aeruginosa	57(38.5)	57(38.0)	13(46.4)
CONS	21(14.2)	02(1.33)	---
K. pneumoniae	10(6.76)	30(20.0)	02(7.1)
E. coli	16(10.82)	13(8.67)	---
P. mirabilis	02(1.3)	06(4.0)	---
C. freundii	05(3.41)	03(2.0)	---
S. aureus	18(12.17)	13(8.67)	09(32.14)
Acinetobacter spp	15(10.14)	19(12.67)	04(14.28)
E. Cloacae	04(2.7)	07(4.67)	---
Total	148 (100.0)	150(100)	28(100.0)

P. aeruginosa showed highest sensitivity to piperacillin /tazobactam (88.9%) and imipenem (87%), moderate sensitivity to aztreonam (78.6%), amikacin (72.8%) and tobramycin (60%). K. pneumoniae showed high sensitivity of 94.5% to imipenem, 92% to piperacillin tazobactam, 83.7% to amikacin and 75.6% to gentamicin; moderate sensitivity towards ceftazidime (48.6%) and ceftriaxone (32.4%) and totally resistant towards cefuroxime, cotrimoxazole and cefepime. All gram negative bacilli of enterobacteriaceae group were highly sensitive to Imipenem, piperacillin tazobactam and amikacin along with high resistance towards cephalosporins and fluoroquinolones.

Antibiotic sensitivity pattern of gram positive isolates: S. aureus showed highest sensitivity to vancomycin and linezolid (96%) whereas CONS showed 100% sensitivity towards these two. S. aureus and CoNS showed moderate sensitivity towards piperacillin/ tazobactam (88%, 91.3%) and clindamycin (79.6%, 78.2%) respectively. CONS showed 60.8% sensitivity but S. aureus exhibited only 50% sensitivity to erythromycin and least sensitivity to penicillin 30% and 13%, respectively.

DISCUSSION:

As per present study the most common age group affected was 21-30 years (36.3%), and females were predominant (59.7%). Chakraborty S et al. who reported 56.6% cases were of 20-39 years age.^[13] However, Jaiswal AK et al. stated 21 – 30 years as most common age group.^[14]

Female predominance was reported by Kaur H et al., Rajput A et al. and Ganesamoni S et al.^[15-17] in contrast to higher male incidence reported by Ramakrishnan MK et al.^[18]

Branski et al. reported a mortality rate of 50% close to 39.5% revealed in present study.^[19] However, lower mortality were found by Sadeghi-Bazargani H et al. (18%) and Alaghebandan R et al (10.3%).^[20]

Flame burn attribution of 76.6% of all cases had concurrence to what reported by most of similar studies.^[21]

Swab, tissue and blood culture positivity of 80.64%, 75.8% and 20.97%, respectively found in this study was comparable to the findings of Srinivasan S et al. (86.3%).^[22] Others reported higher isolation rates such as 93% by Ramakrishnan MK et al., 95% by Kaur H et al.^[18,23] Among tissue biopsy specimens 38% isolates were single and 62% multiple but in swab culture 52% single and rest were multiples whereas in blood culture 96% were monoclonal isolates. This is contrary to observation made by Ramakrishnan MK et al., and Kaushik R et al. reporting solitary isolation in 89.3%, 84% and 78%, respectively.^[18,24] This study finding was close to the isolation rate of 58.42% observed by Dhar S et al.^[25]

Among Swab culture, tissue biopsy and blood culture 26.0%, 11.1% and 30.0% isolates were gram positive and rest were gram negative. Surface swabbing showed predominance of gram positive organisms. This was in contrast to the observation of gram positive predominance made by VG Bhat et al.^[26] But studies carried out by Macedo JLS et al. reported *S. aureus* as predominant organism.^[27]

Here, *P. aeruginosa* was the most common isolate in burn patients having concurrence with results obtained from other study.^[24] It has been suggested that with the success of antibiotics against Gram positive bacteria a significant rise in *Pseudomonas* infection of burn patients had occurred.^[15] Prevalence of *Pseudomonas* species in the burn wards might be due to fact that the organism thrives in moist environment.^[16] The finding of *S. aureus* being the second most common isolate was similar to reports from other studies.^[16, 18, 24] However, some studies reported *S. aureus* as the most predominant organism in burn patients.^[28] *Staphylococcus* was predominant cause of burn wound infection in preantibiotic era and remains an important pathogen at present.^[29] Srinivasan S et al. stated that incidence of staphylococci is declining from 2002-2005.^[22]

Present finding reflected CoNS as colonizer in agreement with CoNS isolate rates reported by other investigators and Altoparlak U et al. reporting CoNS to be the most prevalent isolate in admission cultures decreasing over time.^[23,30] *K. pneumoniae* accounted for 6.76% isolates in surface swab culture, 20% in tissue biopsy and 7.1% in blood culture as revealed in this study and might be suggestive of endogenous invasive infection. With similar rate of isolates of this organism in tissue biopsy other researchers considered *K. pneumoniae* as among one of the most common organisms isolated from burn patients.^[22]

Proportions of isolates in swab, tissue biopsy and blood culture (10.14%, 12.67% & 14.28 %) indicated *Acinetobacter baumannii* is an emerging nosocomial pathogen of burn wounds and a cause of concern because its rapidly developing resistance to antimicrobial agents.^[31]

Comparison of swab, tissue biopsy and blood culture showed predominance of *P. aeruginosa* followed by CoNS and staphylococcus in case of swab culture but *Acinetobacter* spp in tissue biopsy culture and *S. aureus* for blood culture. By its least isolation in tissue and none in blood culture CoNS proved those two as better predictors of infection and invasion. Moreover, *K. pneumoniae* predominantly isolated from tissue biopsy cultures and blood culture indicated its endogenous route, which was better isolated from tissue and blood as compared to surface swab. On comparison of swab and tissue biopsy cultures there was high concordance for *P. aeruginosa*, moderate concordance for *S. aureus*, *E. coli*, *C. freundii* and *Acinetobacter* spp but low concordance for CoNS, *K. pneumoniae* and *P. mirabilis*. Bill et al. reported a correlation of 79% between quantitative swab culture growing and tissue biopsy culture with chronic wounds of various etiologies.^[32] Steer et al. reported a correlation of 54% between biopsy and swab growing the same set of organisms.^[33] According to the study carried out by Basak et al., swab cultures correlated with biopsy specimens in 72% of cases.^[34] The investigators believe that there is a considerable similarity between the methods. It might be due to higher quality of swab sampling which was not contaminated or was exactly taken from the source of infection.

Till day 7 of the present study, positive isolates were more in swabbing

and after that tissue biopsy positive isolates were on a rise indicating more precision in detecting invasion.

There was significantly lower level of TBSA in patients with positive surface swab cultures compared with that biopsy cultures ($p < 0.05$). Steer et al. in their study made a comparison between qualitative results and quantitative bacterial counts and concluded that use of quantitative microbiology in burns is limited by the unreliability of a single surface swab or biopsy sample to represent the whole burn wound.^[35] Through their study Steer and coworkers demonstrated that quantitative bacteriology by burn wound biopsy or surface swab sample does not aid the prediction of sepsis or graft loss.^[35] Loebel and colleagues demonstrated that recovery of bacterial flora from un-excised burn wound surface showed poor correlation with that from tissue biopsy samples taken from deep sites beneath the eschar.^[36] Tahlan and colleagues in a study comparing surface swabs and wound biopsy cultures found no difference in types of microorganisms.^[37] Sjoberg and colleagues reported that quantitative tissue biopsies gave better prediction of sepsis than surface swabs but concluded that amount of labour involved in collection and analysis of multiple biopsy samples limited clinical relevance of this approach.^[38] Bharadwaj et al. also assessed value of blood cultures in diagnosis of burn wound sepsis compared to burn wound cultures by either swab or tissue biopsy concluded that blood cultures were found to be of only prognostic value.^[27] Blood cultures are shown to be a late sign of invasive burn wound infection even when they are positive.^[39] McManus and colleagues found that quantitative cultures of tissue biopsy samples provided a better determination of predominant bacterial types present in the burn wound.^[8]

Among gram negative isolates the most effective drug was imipenem showing 98.7% sensitivity in accordance with observation of Guggenheim M et al.^[40] The most common isolate *P. aeruginosa* showed moderate sensitivity with amikacin (72.8%) and gentamicin (60%) as also reported by Rajput A et al.^[16] *K. pneumoniae* isolates showed maximum sensitivity to Piperacillin/tazobactam (92%) and Imipenem (94.5%) followed by Amikacin (83.7), Gentamicin (75.6%) and Ceftazidime (48.6%) with resistance to Cotrimoxazole. Mehta M et al. saw a significantly high percentage of resistance among gram negative bacilli to aminoglycosides, ciprofloxacin, carbenicillin, and ceftriaxone but imipenem and combination drugs like cefoperazone/sulbactam were reported to be effective.^[41] Singh NP et al. also reported a high degree of resistance to antimicrobial agents.^[31]

Among Gram positive isolates, CoNS showed 100% sensitivity to vancomycin and linezolid whereas *Staphylococcus* showed 96% sensitivity to above drugs. This was followed by 88% and 91.3% sensitivity for piperacillin/tazobactam and 79.6% and 78.2% sensitivity for clindamycin, respectively for CoNS and *Staphylococcus*. Similar observations were made by Kaushik R et al.^[24] Present study reflected that 33% of *S. aureus* were methicillin resistant in accordance with 40% incidence of MRSA observed by Rajput A et al.^[16]

The resulting antibiograms gave some cause for concern because the predominant bacterial isolates were relatively resistant to commonly available, economical antimicrobials. However, this was not entirely unexpected as hospitals are an important breeding ground for development and spread of antibiotic resistant strains arising out of high patients density coupled with unrestricted visitors and heavy use of antibiotics. Study revealed that most of organisms causing infection in burn patients are highly resistant to routinely used antibiotics.^[28]

CONCLUSION:

This study suggested good correlation between surface swab and tissue biopsy for identifying the pathogens on and within the burn wounds without having enough predictive value to assess clinical outcome. There is a considerable similarity between the methods for identification of infection. Quantitative swabbing seems to be better than expensive, invasive, labour intensive, painful tissue biopsy which disrupts the wound bed from healing and requires trained personnel. Moreover, repeat culture at intervals required for accurate identification of infection in burn wound is feasible with swabbing method. MDR organisms in burn wound isolates were identified necessitating formulation of an effective infection control and rational antibiotic policy for health care facilities. Judicious use of third generation Cephalosporins with appropriate MIC for Ceftazidime, Ceftriaxone, Cefotaxime would take care of MDR organisms. Increase degrees of susceptibility to Cotrimoxazole among the gram

positive isolates including MRSA and some gram negative isolates suggested adequate “antibiotic holidays” can be allocated to those drugs which suffer extensive resistance like Ampicillin, Erythromycin and Cephalexin for a short course of time.

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