Original article

Bacteriological profile of burn patients at Yekatit 12 Hospital Burn Center, Ethiopia: A longitudinal study

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Abstract

Introduction: Burn is one of the most common devastating and a very painful form of trauma. Significant thermal injuries induce a state of immune-suppression that predisposes burn patients to infection complications.

Materials and methods: A prospective hospital based study was carried out from December 2010 to February 2011 at Yekatit 12 hospital burn center. Periodic wound swabs and blood samples were collected on 1st, 7th, and 14th days of hospital stay and processed with conventional culture and biochemical tests. Isolates were tested against commonly used antibiotics by Modified Kirby-Bauer disc diffusion methods. Data were analyzed by SPSS version 17.0 for Windows.

Results: From the total of 104 pus cultures, 101 isolates were identified. At the 1st day of pus culture the dominant isolate was *Staphylococcus aureus* 15(46.9%). On the 7th day of pus culture *S. aureus* 21 (46.1%) and *Pseudomonas spp* 20 (44.4%) were isolated. Similarly, at the 14th day the most frequent isolates were *S. aureus* 12 (50%) *and Pseudomonas spp*11 (45.8 %). There was no significant change on time regarding blood culture isolates. Of 92 blood cultures, 15 gram positive isolates were identified the majority being coagulase negative staphylococci (CoNS), 8 (53.3%). Gram negative isolates, mainly *Pseudomonas spp* were found resistant for most of antibiotics used in the hospital.

Conclusions: The nature of periodic microbial wound colonization, flora changes and their antibiotic susceptibility pattern should be taken into consideration in empirical antimicrobial treatment of burned patients. [*Ethiop. J. Health Dev*. 2014;28(1):40-44]

Introduction

An intact human skin is vital to the preservation of body fluid homeostasis, thermoregulation, and the host's protection against infection**.** Breaches in this protective barrier thus represent a form of immune-compromisation that predisposes the patient to infection (1).

Thermal burns are burns to the skin caused by any external heat source. This may be in the form of a naked flame from an open fireplace or house fire, a scald from steam, hot or molten liquid, or via direct contact with a hot object such as a hot oven rack or hot cooking pan. Other types of burns include radiation, chemical and electrical burns (1, 2).

Developing countries have a high incidence of burn injuries, creating a formidable public health problem. (3). Despite major advances in the care of burned patients, multi-organ failure and infection complications remain an important cause of morbidity and mortality (4-7).

The causative bacterial pathogens in any burn facility change with time. Thus, to have an in-depth knowledge of the organisms that are predominant in that particular treatment facility during the particular period along with their drug susceptibility pattern is vital as many burn patients need to be treated with antibiotics before culture results (5).

The fact that there has not been longitudinal blood culture study of burn patients in Ethiopia has prompted the conduct of this study.

Methods

Study Setting and Period:

This study was carried out among burn patients attending at Yekatit 12 hospital burn center from December 2010 to February 2011.

Study Design:

Hospital based prospective longitudinal study.

Study Subjects:

Patients with open burn wound visiting Yekatit 12 hospital burn center.

Sample Size Determination and Sampling Technique:

All burn patients visiting burn OPD and burn unit (BU) within the data collection period were included in the study using convenient sampling technique.

Specimen Collection and Handling:

Periodic wound swab and blood samples were collected

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on 1st, 7th and 14th days of hospital stay by experienced and trained nurses who have been working in the hospital burn center. All samples were collected aseptically following standard operating procedures (8, 9) and processed immediately in clinical Microbiology laboratory unit of Yekatit 12 hospital burn center.

Processing of Specimens and Bacterial Identification:
All wound swabs were inoculated on Blood Agar (BA) plate, Manitol Salt Agar (MSA) and MacConkey Agar (McA) and then incubated at 37ºC for 24 hours. Similarly, blood samples were inoculated and incubated aerobically on Tryptone soya (tryptic soy) broth for up to a week. Each bottle was inspected for growth every day. After a week all bottles were sub-cultured on BA plate, MSA and McA.

Preliminary identification of the isolates was made based on macroscopic characteristics. Gram-negative rods were identified by series of biochemical tests (Oxoid, LTD): Indole, Simon's citrate agar, Kligler Iron Agar (KIA), lysine iron agar, and motility. Gram-positives were identified based on their preference of growth on BA plate and MSA followed by Coagulase test (8, 9).

Antimicrobial Susceptibility Testing*:*
Bacterial suspensions were prepared in nutrient broth by picking colonies of similar test organisms with a sterile wire loop. The density of suspension to be inoculated was determined through comparison with opacity standard on McFarland 0.5 Barium sulphate solution (8). A sterile swab dipped into the suspension of the isolate in broth, and then speeded over Muller-Hinton agar plate (Oxoid, LTD). Grades of susceptibility pattern were determined after incubation at 37^0C for 24hs as 'Sensitive' and 'Resistant' by comparison of zone of inhibition against standard chart (8, 9). Intermediately susceptible isolates were merged with sensitive category as the infection they might cause is likely to respond to treatment when the drug is used in larger doses (8).

Quality Control:
The reliability of the study findings were guaranteed by implementing quality assurance (QA) measures throughout the whole laboratory work. Proper specimen collection was done by experienced nurses. Staining reagents, culture media and antibiotic discs were checked for their normal shelf life before use. All culture plates and antibiotic discs were stored at recommended refrigeration temperature (2-8^0C) after had prepared and sterilized by autoclaving at 121 ^{0}C for 15 minutes. The standard reference strains S. aureus ATCC 25923 and P. aeruginosa ATCC 27853 were used as controls. All laboratory procedures were done based on recommended Standard Operating Procedures (SOPs).

Ethical considerations*:*
Ethical approval was obtained from Institutional Review Board of College of Health Science, Addis Ababa University. Written informed consent was obtained from participants of the study. The procedure of specimen collection was also explained for all participants. Patients directly benefited from the laboratory result through communicating the findings with the physicians in charge of attending them.

Results

Socio-demographic and Clinical Data*:*
A total of 41 patients (24 male and 17 female) were included in the study. The mean age of the study participants was 25.7 years old (range: 2-65 years). Greater than half of the patients, 32 (78.0%), came to the burn center from urban, while the rest were from rural setting. The most common cause of burn was exposure to hot liquid or steam scald 27 (65.9%).The total burned surface area (TBSA) was ranged from 2.0% to 59.0% with a mean of 11.9%. Those patients who get admission in burn unit (BU) were relatively with larger TBSA even though TBSA had no statistical association with admission type (p=<0.56) (Table1 and 2).

Table 1: **Socio-demographic and clinical data of burn patients at Yekatit 12 burn center. Addis Ababa- Ethiopia, 2011**

Variables	Frequency n (%)
Age	
Mean (SD)	25.7 (13.1)
Range	2-65
Sex	
Female	17 (41.5)
Male	24 (58.5)
Address	
Urban	32 (7)
Rural	9 (22)
Cause of burn	
Scald	27 (65.9)
Flame	10 (24.4)
Electricity	4 (9.7)
Anatomical location of the burn	
Extremities	21 (51.2)
Trunk	1 (2.4)
Perineum	1 (2.4)
Extremities, head and neck	8 (19.5)
Extremities and trunk	4 (9.6)
Extremities and perineum	3 (7.3)
Extremities, trunk, head and neck	2 (4.9)
All body parts	1 (2.4)
Depth of the burn	
Partial thickness	33 (80.5)
Full thickness	8 (19.5)
%TBSA*	
Mean (SD)	11.9(7.3)
Range	2-59
Admission type	
In patient (BU)	16 (39)
Outpatient (OPD)	25 (61)

*TBSA= Total Burned Surface Area

Table 2: **Total Burned Surface Area (TBSA) distribution of burn patients at Yekatit 12 burn center. Addis Ababa- Ethiopia, 2011**

	% TBSA	
Range	**Frequency N (%)**	
1-10	26 (65)	
11-20	8 (20)	**Mean %TBSA of all patients= 11.9**
21-30	2 (5)	**Mean %TBSA of BU patients= 15.3**
31-40	3 (7.5)	**Mean %TBSA of OPD patients=9.7**
41-50	0	
>50	1 (2.5)	
Total	40 (100)	

***Pattern of Bacterial Colonization of Burn Wound**:*

A total of 101 bacterial isolates were identified from 104 wound swab cultures. Of the total 101 isolates, S. aureus and Pseudomonas spp together accounted 88 (87.1%). S. aureus 15 (46.9%) and Pseudomonas spp 9 (28.1%) were the most prevalent isolates on day1 cultures (pus 1). At the 7th day (pus 2), the most frequent organisms isolated were S. aureus 21 (46.7%), and Pseudomonas spp 20 (44.4%) similarly at the 14th day S. aureus 12 (50%), and Pseudomonas spp 11 (45.8%) have predominated.

There was a gradual increment in the number of S. aureus and Pseudomonas spp isolates from the 1st day to the 14th day. While 15 (36.6%) wound swabs were sterile on day 1, microbial colonization reached 94.7% within the first week. Level of bacterial isolation from burn wound culture was found significantly associated with degree of TBSA ($P<0.05$). About 19 (18.3%) wound swabs were observed with mixed growth of which 15 (78.9%), accounted for Pseudomonas spp and S. aureus co-growth (Table 3).

Table 3: **Isolation pattern of bacteria from burn wound swabs of patients at Yekatit 12 hospital burn center. Addis Ababa-Ethiopia, 2011.**

Isolated Bacteria	Pus 1 isolates n (%)	Pus 2 isolates n (%)	Pus 3 isolates n (%)	Total
S. aureus	15 (46.9)	21 (46.7)	12 (50)	48 (47.5%)
Pseudomonas spp	9 (28.1)	20 (44.4)	11 (45.8)	40 (39.6%)
Proteus spp	3 (9.3)	1 (2.2)	1 (4.2)	5 (5%)
Klebsiellaspp	2(6.3)	-	-	2 (2)
E. colli	1(3.1)	1 (2.2)	-	2(2)
Citrobacterspp	1(3.1)	1(2.2)	-	2(2)
S. pyogens	1(1.1)	-	-	1(1)
Providenciaspp	-	1(2.2)	-	1(1)
Total isolates	**32 (100)**	**45 (100)**	**24 (100)**	***101(100)***

***Antibiotic Susceptibility Pattern of Bacterial Isolates from Burn Wound**:*

Each identified isolates were tested for antibiotic susceptibility pattern against commonly used antibiotics at the hospital burn center. A high level of drug resistance was observed among gram negative isolates. All isolates of Pseudomonas spp were completely resistant for ampicillin, augmentin, amoxicillin and ceftazidime and 39 (97.5%) and 38 (95%) isolates were also resistant for Doxycycline and Nalidixic acid respectively. In contrast, only 6 (15%) isolates of Pseudomonas spp found resistant to norfloxacin.

The antibiotic susceptibility pattern of S. aureus showed that most isolates were susceptible for commonly used antibiotics. However, all isolates were observed to be resistant for penicillin G while 15 (31.3%) were resistance for methicillin (Table 4).

Pattern of Blood Culture Isolates from Burn Patients:

Periodic blood cultures were also performed, starting from the time of first day of hospital visit to 14th day of patient's hospital stay. The number of blood cultures done has been decreasing starting from the first blood sample as those with growth on the first blood sample were excluded in the later consecutive blood culture tests and some of the patients got discharged from the burn center or transferred to other health centers near to their place of residence when they get better prognosis. Out of the 92 blood cultures done in aerobic condition only 15 (16.3%) yielded 15 isolates. There was no significant change on the isolation pattern from each periodic blood samples. All of the isolates were gram positive; of which CoNS constituted with the largest percentage 8 (53.3%) closely followed by *S. aureus* 6 (40%).

Two of the eight isolates of CoNS were resistant for penicillin G and kanamycin. The remaining isolates were susceptible for the rest of antibiotics.

Table 4: **Antibiotic resistance pattern of bacterial isolates from burn wound at Yekatit 12 hospital burn center. Addis Ababa- Ethiopia, 2011.**

Antibiotics	Bacterial isolates from burn wound							
	A n (%)	B n (%)	C n (%)	D n (%)	E n (%)	F n (%)	G n (%)	H n (%)
Ampicillin	40 (100)		5(100)	2(100)	2(100)	1(50)	1 (100)	
Agumentin	40 (100)	10 (20.8)	1(20)	2(100)	2(100)	0	1 (100)	0
Amoxycillin	40 (100)		5(100)	2(100)	2(100)	1(50)	1 (100)	
CAF	35 (87.5)	19 (39.6)	2(40)	2(100)	1(50)	0	1 (100)	1(100)
Ceftazidime	40 (100)		3(60)	2(100)	2(100)	2(100)	1 (100)	
Ceftriaxone	15 (37.5)		1(20)	2(100)	1(50)	0	0	
Doxycycline	39 (97.5)		5(100)	2(100)	2(100)	1(50)	1 (100)	
Nalidixic Acid	38 (95)		0	1(50)	0	0	0	
Norfloxacine	6 (15)		0	1(50)	0	0	0	
Cepalothin		18(37.5)						0
Methicillin		15 (31.3						0
Penicillin G		48 (100)						0
Amikacin		9 (18.8)						0
Clindamycine		2 (4.2)						1(100)
Vancomycin		4 (8.3)						0
Kanamycine		12 (25)						0
Total isolate	40	48	5	2	2	2	1	1

A: *Pseudomonas Spp*, B: *S. aureus*, C: *Proteus Spp*, D: *Citrobacter Spp*, E: *Klebsiella Spp*, F: *E. coli*, G: *Providentia Spp*, H: *S. pyogenes*

Discussion

Knowledge of a burn center microbial flora and the current antibiotic susceptibility pattern of isolates are important for the better management of burn patients (10). In the present study, of the total 41 patients, 3 (7.3%) of them died during the study period. A study conducted in Nepal by Chalise *et al.* (1) reported 14% death among 50 burn patients whose mean TBSA was 33.9%. In this study, the low mortality rate (7.3%) was probably due to the low TBSA (mean=11.9%).

A total of 101 isolates were identified from 104 pus cultures processed during the study period. *Pseudomonas spp* and *S. aureus* together accounted 88 (87.1%). This finding is comparable with a study by Sewnet *et al.* (13) in the same study area. There was a gradual increment in the number of isolates of *S. aureus* and *Pseudomonas spp* from admission to 14th day. This finding was comparable with the studies done in Turkey and Nepal (1, 11) where gradual increment in the number of isolates of *S. aureus* and *Pseudomonas spp* observed.

Out of 27 wound swabs without growth during the study period, 21(77.8%) were from burn OPD which can be attributed to relatively low (mean=9.7) TBSA than BU patients (mean=15.3). It was also observed that TBSA had significant association ($p<0.05$) with the level of bacterial isolation. This is also demonstrated by another study (12).

Regarding antibiotic resistance pattern, all isolates of *Pseudomonas spp* were resistant for ampicillin, augmentin, amoxicillin and ceftazidime. Comparable resistance pattern for these drugs have also demonstrated by another study (13) in the same study area.

Concerning the antibiotic susceptibility pattern of *S. aureus*, most isolates were susceptible for commonly used antibiotics. However, all isolates were found resistant for penicillin G and methicillin, 31.3% resistance was seen. In this study, methicillin resistance is higher than another study by Negeri (12) where all of the isolates (n=26) of *S. aureus* were sensitive to methicillin and 96.2% were resistant to penicillin G. This disparity with the present study may be attributed probably that methicillin and penicillin resistance has been increasing from time to time as it was also established by Erol S *et al*. (11).

Along with pus culture periodic blood culture was also performed to demonstrate etiologies of septicemia (if any) on burn patients. Most of blood samples 77 (83.7%) studied for blood culture were negative. No attempt was made to grow anaerobes as it is not common to isolate them in burn patients (14).

Only 15 gram positive isolates were identified. There was no mixed growth. Since there was no regular blood culture surveillance in the burn center, the presence or absence of these isolates was unnoticed by the clinicians who were attending the patients. This reflects the importance of periodic surveillance of blood cultures in addition to clinical follow up.

Total burned surface area (TBSA) was marginally associated with degree of bacterial isolation from blood

($p<0.057$). The majority of the patients (65%) had less than 10 %TBSA. This may contribute for the low level of bacterial isolation from blood. A false negative result is more likely if there were few microorganisms in the blood stream during the time of blood collection. Number of blood samples drawn at a time also affects the isolation rate (8, 14).This study had focused exclusively on the microbiological profile i.e. blood culture has been performed periodically regardless of the patient's clinical septicemic status as the fact that blood culture gives best result when specimen is drawn while the patient is under indicative sign and symptoms of blood infection (14, 15).

Conclusions and Recommendations:

The degree of bacterial colonization in burns is dependent on various factors such as the extent of the burn. Burn wound monitoring requires the study of changing bacterial flora and the antibiotic sensitivity reports. Every treatment facility has microorganisms unique to it and these change with time. It is, therefore, of a paramount importance to have an in-depth knowledge of the resident organisms and their antibiotic susceptibility pattern in burn centers.

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