

Original Research Article

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Bacteriological Profile (Aerobic) of Burn Wound Infection with Its Antibiotic Sensitivity Testing in Silchar Medical College and Hospital

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ABSTRACT

Burn wounds are highly susceptible to colonization and infection which creates obstacle in proper management of burn victims. Since burn wound infection shows changing trends in pathogenicity of microorganisms as well as their antibiotic sensitivity, hence, it is crucial to perform frequent evaluation of the burn wound to ensure early and appropriate therapy in burn patients. The study was conducted to find out the common organisms in infected burn wound samples and their antibiotic sensitivity pattern. The study was conducted in department of Microbiology and Surgery of Silchar Medical College and Hospital between July, 2015 and June, 2016. Pus samples and wound swabs collected from the hospitalized burn patients were processed according to standard microbiological techniques and Antibiotic sensitivity testing was done using Kirby Bauer's Disc diffusion technique according to C.L.S.I guideline. Out of 100 pus samples collected from patients admitted in burn unit, 79(79%) cases were culture positive, while 21(21%) were sterile. Out of 79 organisms isolated, 31 (39.24%) were *Pseudomonas aeruginosa*, 21 (26.58%) were *Staphylococcus aureus*, 14 (17.72%) were *Klebsiella pneumoniae*, 8 (10.13%) were *Klebsiella oxytoca* and 5(6.33%) were *Proteus mirabilis*. The Gram positive organism showed maximum sensitivity towards Vancomycin and Linezolid (100%) and minimum towards Ampicillin (28.57%) while gram negative isolates showed maximum sensitivity to Imipenem (100%) and minimum towards Ampicillin(17.24%). The high prevalence of antimicrobial resistance emphasizes the need to strengthen the infection control practices along with regular and periodical monitoring and surveillance activities to restrict emerging trend of antimicrobial resistance.

Keywords

Burn wound infection,
Pseudomonas aeruginosa,
Antibiotic sensitivity testing,
Infection control practices.

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Introduction

Infected burn wounds are not only associated with a delay in epidermal maturation and deep scar formation but also prolongs the hospital stay of the patient and increases the chances of mortality due to sepsis, when compared to

non-infected patients.¹ Most of the burn victims, who survive including the initial 24 hours after burns, succumb to burn infection and its complications. Immediately following the thermal injury, the burn wounds are sterile; but eventually get Colonized with microorganisms.² Various factors responsible

are disruption of the skin barrier, a large cutaneous bacterial load, the possibility of the normal bacterial flora becoming opportunistic pathogens and severe depression of the immune system. All these factors contribute towards the sepsis in a burn victim.³ The pattern of infection differs from hospital to hospital; the bacterial flora of infected wound may change considerably during the healing period.^{4,5}

Microorganisms are transmitted to the burn wound surfaces by the hands of personnel, by fomites and possibly by hydrotherapy.⁶ The gastrointestinal tract is a potential reservoir for organisms that infect burn wounds, and it is likely that endogenous microbes are transmitted to burn wound surfaces by faecal contamination.⁷ Earlier, *Streptococcus pyogenes* was the most frequent isolate from infected burn wounds. Currently, the common pathogens isolated from burn wounds are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, coliforms, *Acinetobacter* spp., and others like anaerobic bacteria and fungi.^{8,9}

Good infection control practices have a great impact on survival rate of burn patients. Emerging antimicrobial resistance in burn wound bacterial pathogens represent a serious therapeutic challenge for clinicians treating these patients. In order to overcome this problem continuous microbiological surveillance is needed.¹⁰

So this study is conducted to isolate and identify the common organisms causing burn wound infection and to determine their antibiotic sensitivity pattern to provide empirical treatment for favourable outcome.

Materials and Methods

Study design: Observational study (Cross-sectional study).

Study area: Silchar Medical College & Hospital (Microbiology & Surgery Department)

Study Period: One year from July 2015 to June 2016.

Inclusion criteria: All pus samples/ wound swabs collected from the hospitalized burn patients.

Exclusion criteria: 1) Patients on antibiotic therapy.

2) Patient with wounds caused by other than burns

Study population: Pus samples in the form of wound swabs were collected from patients admitted in burn unit of Department of Surgery, Silchar Medical College & Hospital. Patients of any age and both the sexes were included in this study.

Number of specimen: A total of 100 pus samples were collected.

A detailed history was taken with reference to name, age, sex, religion, hospital number, chief complaints, past history, underlying disease, antibiotic history etc. and all these informations were recorded in a pre-designed proforma.

The collected samples were processed for identification of organisms using standard microbiological techniques and biochemical test. All strains were tested for antimicrobial susceptibility testing using C.L.S.I guidelines. Under strict aseptic condition pus samples from burn wounds were collected in the form of swabs in sterile test tubes. The collected samples were immediately transferred to Bacteriology section of Department of Microbiology, Silchar Medical College & Hospital for processing. The samples were at

first inoculated into culture media and then direct smears were prepared. The direct smears were then subjected to Gram staining.

Smear were prepared from the specimen in clean grease free glass slides, dried and then heat fixed. Gram staining of the smear was done according to the methods described by Duguid JP(2006).¹¹ It was examined for the presence of any bacteria and pus cells.

For primary isolation of bacteria the specimens were inoculated into the following media:

- 1)5% sheep blood agar media
- 2)MacConkey agar media

The media were prepared as per methods described by Collee *et al.*, (2006).¹²

The inoculated blood agar and MacConkey agar media were incubated aerobically at 37⁰C for 24 hours. If no growth was observed after 24 hours incubation then it was reincubated for another 24 hours after which if there was no growth it was considered sterile.

After incubation, identification of bacterium from positive cultures was done with a standard microbiological technique which includes motility testing by hanging drop preparation, gram staining and biochemical reactions such as catalase, coagulase, indole, methylred, Voges-Proskauer, citrate, urease, Phenyl pyruvic acid test and oxidase test. Further biochemical tests done were carbohydrate fermentation test using Lactose, sucrose, mannitol and Maltose, Triple sugar Iron test, Nitrate reduction test, Arginine dihydrolase production, lysine and ornithine decarboxylase test, Hugh and leifson test.

The antimicrobial susceptibility testing were done by Kirby Bauer's Disk Diffusion method and interpreted as per Clinical Laboratory

Standard Institution (CLSI) guidelines. Mueller Hinton agar (MHA) was used as media, it was inoculated with a suspension of organisms equivalent to 0.5 McFarland turbidity standard and discs were applied. Maximum six (6) antimicrobial discs were put in the 100 mm diameter MHA plate and plates were incubated at 37⁰C overnight¹³.

The antibiotic discs used were purchased from HiMedia Lab Pvt. Ltd. Inhibition zones were measured and reported as sensitive or resistant according to manufacturer's literature.

Klebsiella pneumoniae ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 were used as quality control strain. Antibiotic discs were used for determination of sensitivity by Kirby- Bauer disc diffusion test.

For gram positive organisms Vancomycin, Linezolid, Cefoxitin, Cefotaxime and Penicillin and for gram negative organisms Imipenem, Piperacillin/Tazobactam, Aztreonam, Ceftazidime and Cefuroxime were exclusively used in this study.

Antibiotic- concentration/disc	Antibiotic- concentration/disc
IMIPENEM(IPM)-10mcg	CEFTRIAZONE(CTR)-30mcg
AMPICILLIN(AMP)-10mcg	AMIKACIN(AK)-30mcg
LINEZOLID(LZ)-30mcg	AZTREONAM(AT)- 30mcg
VANCOMYCIN(VA)-30 mcg	GENTAMICIN(GEN)-10mcg
AMOXYCLAV(AMC)-30mcg	LEVOFLOXACIN(LE)-5mcg
PIPERACILLIN/TAZOBACTAM (PIT)100/10 mcg	CIPROFLOXACIN(CIP)-5mcg
CEFOTAXIME(CTX)-30mcg	CEFUROXIME(CXM)-30mcg
CEFOXITIN(CX)-30mcg	PENICILLIN(PE)-10 units
CEFTAZIDIME (CAZ)-30mcg	

Results and Discussion

Out of 100 samples collected, 79(79%) cases were culture positive, while 21(21%) were sterile. Out of 100 patients 30 burn patients

(30%) are in the age group of 20-29 years which is the most common age group found in this study followed by 22 patients between 30-39 years, 19 patients between 0-9 years, 13 patients between 40-49 years, 10 patients between 10-19 years, 4 patients above 59 years and 2 patients between 50-59 years respectively. Out of 79 culture positive cases, 21 (26.58%) cases were caused by gram positive organisms, while 58 (73.42%) cases were caused by gram negative organisms. Out of 79 culture positive cases, *Pseudomonas aeruginosa* 31(39.24%), *Staphylococcus aureus* 21(26.58%), *Klebsiella pneumoniae* 14 (17.72%), *Klebsiella oxytoca* 8 (10.13%) and *Proteus mirabilis* 5(6.33%). Gram positive isolate showed maximum sensitivity towards Vancomycin and Linezolid (100%) followed by Ciprofloxacin (85.71%), Cefoxitin (80.95%), Gentamicin (76.19%), Levofloxacin (71.43%), Amikacin (71.43%), Amoxicillin-Clavulanic acid (66.67%), Ceftriaxone (61.9%), Penicillin (42.85%), Cefotaxime (38.09%) and Ampicillin (28.57%). MRSA detected was 19.05%. The gram negative isolates showed maximum sensitivity to Imipenem (100%) followed by Ciprofloxacin (84.48%), Levofloxacin (81.03%), Ceftriaxone (67.24%), Aztreonam (67.24%), Piperacillin/Tazobactam (65.52%), Amikacin

(62.06%), Ceftazidime (58.62%), Cefuroxime (56.89%), Gentamicin (53.45%), Amoxicillin-Clavulanic acid (41.38%) and Ampicillin (17.24%).

Infection is the most important problem in the treatment of burns. Burns become infected because the environment at the site of the wound is ideal for the multiplication of infecting organisms. The immune-suppressive status of the patient, immediate lack of antibodies, plentiful supply of moisture and nutrients in the physical environment; the temperature and gaseous requirements etc. are ideal for the growth of microorganisms.^{6,7}

Burn wound infections are showing changing trends in the relative importance and cyclic pathogenicity of microorganisms as well as their antimicrobial sensitivities. To ensure early and appropriate therapy in burn patients, a frequent evaluation of the wound is necessary. Thus, a continuous surveillance of microorganisms and their antibiotic susceptibility patterns is essential to maintain good infection control programmes in the burn unit, thus improving the overall infection related morbidity and mortality.¹⁴

Table.1 Age wise distribution of burn patients

AGE GROUP	NO. OF CASES	CULTURE POSITIVE
0-9 YEARS	19	12
10-19 YEARS	10	9
20-29 YEARS	30	21
30-39 YEARS	22	20
40-49 YEARS	13	11
50-59 YEARS	2	2
>59 YEARS	4	4

Table.2 Distribution of isolates based on gram staining

Isolates	Number	Percentage
Gram positive	21	26.58%
Gram negative	58	73.42%
Total	79	100%

Table.3 Different organisms isolated

ORGANISM	NUMBER	PERCENTAGE
<i>Pseudomonas aeruginosa</i>	31	39.24%
<i>Staphylococcus aureus</i>	21	26.58%
<i>Klebsiella pneumoniae</i>	14	17.72%
<i>Klebsiella oxytoca</i>	8	10.13%
<i>Proteus mirabilis</i>	5	6.33%
TOTAL	79	100%

Table.4 Distribution of patients based on total burn surface area

Total Burn Surface Area(TBSA)	NO. OF CASES	PERCENTAGE
<25%	44	44%
25- 50 %	38	38%
51- 75%	11	11%
>75%	7	7%
TOTAL	100	100%

Table.5- Distribution of patients based on type of burn

TYPE OF BURN	NO. OF PATIENTS	PERCENTAGE
FLAME BURN	71	71%
SCALD BURN	14	14%
ELECTRIC BURN	15	15%
TOTAL	100	100%

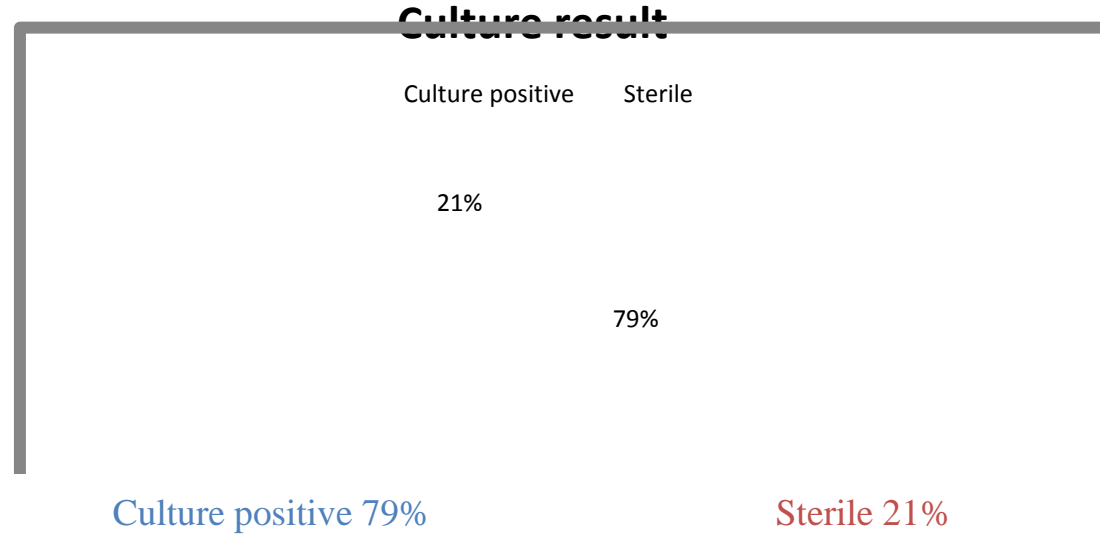
Table.6 Sensitivity pattern of Gram positive isolate (*Staphylococcus aureus*)

ORGANISM	TOTAL NO	CIP	LE	GEN	AMP	CTR	CTX	CX	AMC	PE	AK	LZ	VA
S.aureus	21	18	15	16	6	13	8	17	14	9	15	21	21
PERCENTAGE		85.71 %	71.43 %	76.19 %	28.57 %	61.9% %	38.09 %	80.95 %	66.67 %	42.85 %	71.43 %	100 %	100 %

Table.7 Sensitivity pattern of Gram negative isolates

Organism	Total No	CIP	LE	GEN	AMP	AK	CTR	PIT	CAZ	CXM	AMC	AT	IMP
<i>P.aeruginosa</i>	31	27	25	15	5	16	22	19	18	17	10	20	31
<i>K.pneumoniae</i>	14	11	12	9	2	11	9	10	8	8	8	10	14
<i>K.oxytoca</i>	8	7	6	5	2	6	5	6	5	5	4	6	8
<i>P.mirabilis</i>	5	4	4	2	1	3	3	3	3	3	2	3	5
Total no	58	49	47	31	10	36	39	38	34	33	24	39	58
Percentage		84.48%	81.03%	53.45%	17.24%	62.06%	67.24%	65.52%	58.62%	56.89%	41.38%	67.24%	100%

Fig.1 Pie diagram showing culture results



In this study out of 100 samples from burn patients 79(79%) samples were culture positive. This finding is comparable to findings of Kaushik *et al.*,¹⁵, AL –Bdour *et al.*,¹⁶, Idomir *et al.*,¹⁷, Dash *et al.*,¹⁸, Saxena *et al.*,⁵, Modi *et al.*,¹⁴, Magnet *et al.*,¹⁹ and Sharma *et al.*,²⁰. In Kaushik *et al.*,¹⁵ culture positivity was 293 out of 336 samples i.e. (87.2%). Culture positivity of AL-B dour MN *et al.*,¹⁶ was 84.6%, Idomir *et al.*,¹⁷ was 86.2%, Dash *et al.*,¹⁸ was 88.6%, Saxena *et al.*,⁵ was 70.33% i.e. 147 out of 209 samples showed culture positivity, Modi *et al.*,¹⁴ was 85.7%, Magnet *et al.*,¹⁹ showed 66.66% i.e. 100 out of 150 samples showed positive growth and Sharma *et al.*,²⁰ showed 87.96% positive culture.

In other studies, conducted by Agnihotri *et al.*,²¹, Begum *et al.*,²², Mamani *et al.*,²³, Kulkarni *et al.*,²⁴, Shrivastava *et al.*,²⁵, rate of culture positivity were high compared to present study. Culture positivity showed by Agnihotri *et al.*,²¹ was 96%, Begum *et al.*,²² was 92.85%, Mamani *et al.*,²³ was 93.3%. In Kulkarni *et al.*,²⁴ 83 out of 91 samples i.e. 91.2% showed positive growth and in Shrivastava *et al.*,²⁵ 109 out of 118 samples i.e. 92.37% showed positive culture.

While studies conducted by Vaez *et al.*,²⁶ and Mohamed *et al.*,²⁷ found comparatively low rate of culture positivity of 31% and 60 % respectively.

In the present study, gram negative organisms were the predominant pathogens constituting 73.42% case.

This finding is in concordance with Kulkarni *et al.*,²⁴, Vaez *et al.*,²⁶ and Sharma *et al.*,²⁰. However studies conducted by Komolafe *et al.*,²⁸ and Idomir *et al.*,¹⁷ found higher percentage of gram positive organisms compared to gram negative organisms.

In the present study the most common organism isolated was *Pseudomonas aeruginosa* which constituted 39.24% of total organisms followed by *Staphylococcus aureus* (26.58%). This finding correlates with studies conducted by Kaushik *et al.*,¹⁵, Agnihotri *et al.*,²¹, Rajput *et al.*,⁴, Dash M *et al.*,¹⁸, Saxena *et al.*,⁵ and Magnet *et al.*,¹⁹.

However in study conducted by Srinivasan *et al.*,²⁹ the most common organism was *Klebsiella* (33.91%), in Vindenes *et al.*,³⁰ the most common organism was Coagulase – negative *Staphylococcus* (21.5%) and in study conducted by Bayram Y *et al.*,³¹ the most common organism was *Acinetobacter baumannii* (23.6%) which is dissimilar to present study.

Among the gram positive isolates, Linezolid and Vancomycin are found to be most effective drugs showing 100% sensitivity to all isolates. Similar observation was made by Sharma *et al.*,²⁰ where gram positive isolates were 100 % sensitive to Vancomycin and Linezolid.

In studies conducted by Ahsan *et al.*,³² and Bhamra *et al.*,³³ *Pseudomonas aeruginosa* was 100% sensitive to Imipenem which is similar to present study. However studies like Dash *et al.*,¹⁸, Saxena *et al.*,⁵ and Behesti *et al.*,³⁴ low rate of sensitivity was found which were 90.8%, 95.77% and 38.9% respectively. Since burn wound infection shows changing trends in pathogenicity of microorganisms as well as their antibiotic sensitivity, hence, it is crucial to perform frequent evaluation of the burn wound to ensure early and appropriate therapy in burn patients. Also, the high prevalence of antimicrobial resistance emphasizes the need to strengthen the infection control practices along with regular and periodical monitoring and surveillance activities to restrict emerging trend of antimicrobial resistance.

This study concludes that in vitro testing of antibiotics prior to its use may help to prevent multidrug resistant organisms in burn infection which will help in reducing morbidity and mortality of burn patients.

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