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Bacteriological Profile and Antibiogram of Aerobic Burn Wound Isolates At A Tertiary Care Institute in Northern India

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Abstract:
Background: *Infection is the commonest and the most serious problem in patients who survive the initial shock phase of major thermal injury. It is an important cause of mortality in burns. Rapidly emerging nosocomial pathogens and the problem of multi-drug resistance in burn patients providing a important periodic review of isolation patterns and Antibiograms in the burn ward.*
Aim and Objective: *The present retrospective study from wounds of patients admitted to burns unit was undertaken to determine the bacteriological profile and the resistance pattern from the burn ward over a period of one year and four months (January 2012 to April 2013).*
Materials and Methods*: 450 wound swabs were taken from burn patients and were cultured on Blood-agar and MacConkey-agar .Out of 450 samples only 400 showed growth and so its isolates were identified by conventional biochemical methods and antimicrobial susceptibility was performed. Isolates were tested for susceptibility to antimicrobials using the Kirby Bauer Disc diffusion method.*
Result: *During the period from January2012 to April 2013 study is done.450 aerobic bacterial isolates were recovered from 400 patients. Pseudomonasaeruginosa was the commonest pathogen isolated (35.55%) followed by Klebsiellaspecies (23.34%),Acinetobacter species (22.22%), Staph. aureus (9.11%), (5.56%), Proteus species (1.78%), Citrobacter species (1.33%) and E.coli (1.11%). Ampicillin, Piperacillin was most resistant drugs in case of Gram-negative bacteria.Piperacillin-tazobactum was (100%) sensitive antibiotic in all Gram negative bacteria. Amoxicillin and Penicillin G were the resistant antibiotics in case of Gram –positive bacteria.*
Conclusion: *The growth of multidrug-resistant organisms should be considered as a serious risk in burn units. Aggressive infection control measures should be applied to limit the emergence and spread of multidrug-resistant pathogens.*
Keywords:

I. Introduction

Burns may be caused by scalding or flammable liquids, fires and other sources of heat, chemicals, sunlight, electricity, and very rarely by nuclear radiation (1).Burn injury is a major problem in many parts of the world. It has been estimated that 75% of all deaths following burns are related to infection. Thermal injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients (2).Infection of burns is common because the skin, a physical barrier against microbes, has been compromised. First degree burns involve the epidermis. Second-degree burns penetrate to the dermis. Third-degree burns penetrate through all of the layers of the skin and frequently damage the tissues below it(1, 3). The risk of infection increases proportionately with the size, site, depth of the burn (4).Burn wound infection is problematic because it delays healing, encourages scarring and may result in bacteraemia, sepsis or multiple-organ dysfunction syndrome whereby organs from several systems are unable to maintain homeostasis on their own, requiring immediate medical attention(3).

Bacteria and fungi are the most common pathogens of burn wounds. These microbes form multi-species bio films on burn wounds within 48 – 72 hours of injury (3). Organisms originate from the patient's own skin, gut and respiratory flora, as well as from contact with contaminated health care environments and workers (5, 6, 7). Gram- positive bacteria in the depth of sweat glands and hair follicles heavily colonize the wounds within 48 h of the injuryfollowed quickly by Gram-negative(8,9). Fungal infection tends to occur in the later stages after the majority of bacteria have been eliminated by topical antibiotics (3, 10). Infection is a major cause of morbidity and mortality in hospitalized burn patients(2). Staphylococcus aureus was frequently isolated pathogen in both community and hospital practices (3, 11).Two bacterial species, MethicillinResistantStaphylococcusaureus (MRSA) and Pseudomonas aeruginosaare most prevalent infective agents. These have proven particularly difficult to treat because they possess a large number of virulence factors and antimicrobial resistance genes (3).However, different studies have shown that Staphylococcus aureus is one of the greatest causes of nosocomial infection in burn patients. The resistance of the hospital strains of S. aureus

to methicillin remains a global problem (11). This study was done with the objective to study the aerobic bacterial burn wound isolates in burn patients admitted in burn unit of Govt. Medical College Hospital, Jammu (J&K) and to study the Antibiotic resistance and sensitivity pattern of aerobic bacterial isolates recovered from burn wounds.

II. Material and Methods

The present study was a retrospective study conducted in bacteriology section of Department of Microbiology, Government Medical College and Hospital, Jammu(Jammu and Kashmir). A total of 450 pus samples were collected from patients admitted in burn unit of hospital .Specimens were received from patients hospitalized from January 2012 to April 2013 and were processed for isolation and identification of bacterial pathogens, according to the standard microbiological techniques. Bacterial isolates from wound swabs taken from burn patients were identified by conventional biochemical methods and antimicrobial susceptibility was performed. Inclusion criteria were more than 48hrs of stay in hospital with total burn surface area (TBSA) >10% and age more than 10 years. Exclusion criteria were chronic burn wounds with burn wound contracture and burn surface area more than 90%. Thereafter, detailed history of the patients with reference to present illness, past illness and other associated features was taken as per tested Performa. Complete general physical and systemic examination was then performed. Burn index was assessed and recorded as per guidelines.

2.1 Sample collection and Processing

Under aseptic precautions, clinical specimens were collected from open burn wounds preferably from upper and lower extremities avoiding oral, genital, scalp, and anal regions.These were taken initially on admission before dressing changes and before administration of antibiotics. Wound swabs were taken whenever there were clinical signs of grafted skin infection.Samples to be processed were pus and burn wound swabs and were inoculated on Blood agar and MacConkeyagar. Incubationwas done at 37^0C for 24 hrs.Culture plates were observed for the growth of bacteria and Colony Characteristics noted.For conformation of the particular bacteria, Biochemical tests were performedand Antibiotic sensitivity was done on Muller Hinton agar withparticular antibiotic discs.Plates were incubated at 37^0C for 24 hrs.

III. Observation And Results

This study was conducted in Department of Microbiology and burn unit of Government Medical College & Hospital, Jammu. A total of 450 patients were studied in this study. Among these, 400 showed growth and their wound bacterial isolates were studied whereas 50 had no evidence of bacterial contamination.

Table 1: Total sample size (showing percentage of growth and no growth)

Sno.	Sample Size	Number (n=450)	Percentage (%)
1	Positive Samples with Bacterial Growth	400	89%
2	No growth	50	11%

Total sample size is 450 burn patients, out of 450, 400 (89%) are showing growth in culturing media while 50 (11%) are showing no growth.

Table 2: Showing sex wise distribution in burn patient

S no	Sex	Number (n=400)	Percentage (%)
1	Female	250	62%
2	Male	150	38%

Out of 400 patients, females were 250(62%) and males were 150 (38%).

Table 3:Burn patients are categorisedaccording to Age

Sno.	Age	Number (n=400)	Percentage (%)
1.	10-20	50	12.5 %
2.	21-30	165	41.25 %
3.	31-40	120	30 %
4.	41-50	45	11.25 %
5.	51-60	15	3.75 %
6.	61-70	5	1.25 %
7.	71+	0	

Maximum patient susceptible in case ofburn patients belong to Age group: 21 to 30 years that is 165 patients.

Table 4: Gram Positive and Gram negative bacteria isolated in burn patients

Gram positive	Gram negative
Staphylococcus aureus	Pseudomonas aeruginosa
MRSA	Klebsiella spp.

	Acinetobacter
	Proteus
	Citrobacter
	E.coli

Gram positive bacteria isolated were Staphylococcus aureus and MRSA .Gram negative bacteria isolated were Pseudomonas aeruginosa followed by Klebsiellaspp.,Acinetobacter, Proteus, Citrobacter and E.coli.

Table 5: List of isolated bacteria in burn patients

Sno.	Bacterial isolates	Number (n=450)	Percentage (%)
1.	Pseudomonas aeruginosa	160	35.55 %
2.	Klebsiella sp.	105	23.34 %
3.	Acinetobacterspp	100	22.22 %
4.	Staphylococcus aureus	41	9.11 %
5.	MRSA	25	5.56 %
6.	Proteus spp.	8	1.78 %
7.	Citrobacter spp.	6	1.33 %
8.	E.coli	5	1.11 %

In 400 burn patients, 450 bacterial isolates are recovered. There is also recovery of single and multiple bacteria in patients due to large exposure to hospitalisation, contamination by environment or by attack of nosocomial bacteria. Pseudomonasaeruginosa is leading bacterial isolatei.e160 isolates (35.55%) followed by Klebsiella spp.105isolates (23.34 %), Acinetobacter spp. 100 isolates (22.22%), Staphylococcusaureus 41 isolates (9.11 %), MRSA 25 isolates (5.56%), Proteus spp. 8 isolates (1.78 %), Citrobacter spp. 6 isolates(1.33 %) andE.coli only 5 isolate (1.11%) respectively**.**Out of 400 patients, 355 patients are showing only one bacterium and 45 patients are showing isolation of multiple bacteria.

Table 6: AntibioticResistant pattern of Pseudomonas aeruginosa

Sno.	Antimicrobial agent	No. of Resistant isolates (n=160)	Percentage of resistant isolates (%)
1.	Ampicillin	160	100%
2.	Piperacillin	160	100%
3.	Cefepime	140	87.5 %
4.	Tobramycin	138	86.25 %
5.	Cotrimoxazole	138	86.25 %
6.	Gentamycin	130	81.25%
7.	Colistin	125	78.12%
8.	Ciprofloxacin	120	75 %
9.	Ceftazidime	114	71.25%
10.	Amikacin	100	62.5%
11.	Cefotaxime	96	60%
12.	Imipenem	90	56.25 %
13.	Polymyxin B	40	25 %
14.	Cefoperazone-sulbactum	45	28.12 %

In pseudomonas, Ampicillin andpiperacillin are 100% resistant.

Table 7: Antibiotic sensitivity pattern in case of Pseudomonas aeruginosa

Sno.	Antimicrobial agent	No. of sensitive (n=160)	Percentage of sensitive(%)
1.	Piperacillin-tazobactum	160	100 %
2.	Cefoperazone-sulbactum	115	71.88 %
3.	Polymyxin B	120	75 %
4.	Amikacin	60	37.5 %
5.	Ceftazidime	46	28.8 %
6.	Ciprofloxacin	40	25 %
7.	Gentamycin	30	18.75 %
8.	Tobramycin	22	13.75 %
9.	Cefepime	20	12.5 %

Piperacillin-tazobactum is 100% sensitive in case of pseudomonas aeruginosa

Table 8: AntibioticResistance pattern of Klebsiella spp.

Sno.	Antimicrobial agent	Number of resistant isolates (n=105)	Percentage of resistant isolates (%)
1.	Ampicillin	104	99.04 %
2.	Amoxyclavulanic acid	100	95.23 %
3.	Imipenem	96	91.42 %
4.	Cefuroxime sodium	86	81.90 %
5.	Cefotaxime	55	52.38 %

6.	Tobramycin	47	44.76 %
7.	Amikacin	40	38.09 %
8.	Ciprofloxacin	25	23.80 %
9.	Tetracycline	20	19.04 %
10.	Cotrimoxazole	17	16.19 %
11.	Chloramphenicol	15	14.28 %
12.	Piperacillin-tazobactum	5	4.76 %

Ampicillin (99.04%), Amoxyclavulanic acid (95.23%) and Imipenem (91.42%) are most resistant drugs.

Table 9: Antibiotic Sensitivity pattern of Klebsiella spp**.**

Sno.	Antimicrobial agent	Number of sensitive isolate (n=105)	Percentage of sensitive isolate (%)
1.	Piperacillin-tazobactum	100	95.23%
2.	Chloramphenicol	90	85.71 %
3.	Cotrimoxazole	88	83.80 %
4.	Tetracycline	85	80.96 %
5.	Ciprofloxacin	80	76.19 %
6.	Amikacin	65	61.9 %

Piperacillin-tazobactum (95.23%) is sensitive drug followed by Chloramphenicol (85.71%).

Table 10: AntibioticResistance pattern of Acinetobacter spp**.**

Sno.	Antimicrobial agent	No. of resistant isolates (n=100)	Percentage of resistant isolates (%)
1.	Piperacillin	100	100 %
2.	Imipenem	90	90 %
3.	Cotrimoxazole	80	80 %
4.	Ceftazidime	80	80 %
5.	Ciprofloxacin	60	60 %
6.	Cefepime	51	51 %
7.	Tobramycin	18	18 %
8.	Gentamycin	8	8 %
9.	Tetracycline	5	5 %

Piperacillin is 100% resistant drug followed by Imipenem (90%)

Table 11: AntibioticSensitivity pattern of Acinetobacterspp.

Sno.	Antimicrobial agent	No. of sensitive (n=100)	Percentage of sensitive (%)
1.	Piperacillin-tazobactum	100	100 %
2.	Gentamycin	92	92 %
3.	Polymyxin B	90	90 %
4.	Colistin	85	85 %
5.	Tobramycin	82	82 %
6.	Cotrimoxazole	20	20 %
7.	Cefoperazone-sulbactum	13	13 %

Piperacillin- tazobactum is 100% sensitive drug, followed by Gentamycin (92%).

Table 12:Antibiograms of S.aureus isolates (n = 41) from burn wound patients showing resistance patterns

Sno.	Antimicrobial agent	Number of resistant isolates(n=41)	Percentage of resistant isolates (%)
1.	Penicillin G	39	95.12 %
2.	Amoxicillin	36	87.80 %
3.	Clindamycin	28	68.23 %
4.	Cotrimoxazole	25	60.98 %
5.	Erythromycin	20	48.78 %
6.	Gentamycin	14	34.14 %
7.	Ciprofloxacin	13	31.70 %
8.	Tetracycline	10	24.39 %
9.	Linezolid	9	21.95 %

Pencillin G is (95.12%) resistant followed by Amoxicillin (87.80%)

Table 13: Antibiograms of S.aureus isolates (n = 41) from burn wound patients showing sensitivity patterns

Sno.	Antimicrobial agent	Number of sensitive isolates (n=41)	Percentage of sensitive isolates (%)
1.	Vancomycin	41	100%
2.	Tetracycline	31	75.60 %
3.	Oxacillin	30	73.17 %
4.	Ciprofloxacin	28	68.29 %

5	Gentamycin	27	65.86%
6.	Erythromycin	21	51.21 %
7.	Cotrimoxazole	16	39.02 %
8.	Clindamycin	13	31.70%

Vancomycin is (100 %) sensitive drug followed by Tetracyclin (75.60%) and Oxacillin (73.17%)

Table 14: Antibiograms of MRSA isolates (n = 25) from burn wound patients showing resistivity& sensitivity patterns

	Antimicrobial agent	**Number of resistant isolates (n=25)**	**Percentage of resistant isolates (%)**	**Number of sensitive isolates**	**Percentage of sensitive isolates (%)**
1.	Oxacillin	25	100%	0	0
2.	Penicillin G	25	100%	0	0
3.	Erythromycin	23	92 %	2	8 %
4.	Tetracycline	13	52 %	12	48 %
5.	Gentamycin	8	32 %	17	68 %
6.	Clindamycin	7	28 %	18	72 %
7.	Vancomycin	0	0	25	100%

Oxacillin and Pencillin G is 100 % resistant drug. Vancomycin is 100% sensitive drug

Table 15: Antibiograms of Proteus isolates (n = 8) from burn wound patients showing resistance patterns

Sno.	**Antimicrobial agent**	**Number of resistant isolates(n=8)**	**Percentage of resistant isolates (%)**
1.	Ampicillin	8	100%
2.	Amoxyclavulanic acid	8	100%
3.	Imipenem	8	100%
4.	Tetracycline	6	75 %
5.	Tobramycin	4	50 %
6	Gentamycin	4	50 %

Ampicillin, Amoxyclavulanic acid and Imipenem is 100 % resistant drugs.

Table 16: Antibiograms of Proteus isolates (n = 8) from burn wound patients showing Sensitive patterns .

Sno.	**Antimicrobial agent**	**Number of sensitive isolates(n=3)**	**Percentage of sensitive isolates(%)**
1.	Piperacillin	8	100%
2.	Amikacin	6	75 %
3.	Gentamycin	4	50 %
4.	Tobramycin	4	50 %
5.	Cefotaxime	2	25 %
6.	Tetracycline	2	25 %

Piperacillin is (100 %) sensitive drug followed by Amikacin (75%).

Table 17: Antibiograms of Citrobacter isolates (n = 6) from burn wound patients showing resistance and sensitive patterns**.**

Sno.	**Antimicrobial agent**	**Number of resistant isolates(n=6)**	**Number of sensitive isolates(n=6)**
1.	Piperacillin	6 (100%)	0
2.	Ceftazidime	6(100%)	0
3.	Cefotaxime	6(100%)	0
4.	Cefepime	6(100%)	0
5.	Cefoperazone-sulbactum	6(100%)	0
6.	Tobramycin	6(100%)	0
7.	Cotrimoxazole	6(100%)	0
8.	Colistin	6(100%)	0
9.	Tetracycline	0	6(100%)
10.	Piperacillin-sulbactum	0	6(100%)

Piperacillin, Ceftazidime, Cefotaxime ,Cefepime, Cefoperazone-sulbactum, Tobramycin, Cotrimoxazole and Colistin show 100% resistance whereas Tetracycline and Piperacillin-sulbactum are 100% sensitive drugs.

Table 18: Antibiograms of E.coli isolates (n = 5) from burn wound patients showing resistance and sensitive patterns

Sno.	Antimicrobial agent	Number of resistant isolates(n=5)	Number of sensitive isolates(n=5)
1.	Ampicillin	5 (100%)	0
2.	Amoxyclavulanic acid	5 (100%)	0
3.	Amikacin	5 (100%)	0
4.	Tobramycin	5 (100%	0
5.	Ciprofloxacin	5 (100%)	0
6.	Cotrimoxazole	5 (100%)	0
7.	Tetracycline	5 (100%)	0
8.	Piperacillin-tazobactum	0	5 (100%)
9.	Cefepime	0	5 (100%)

Ampicillin,Amoxyclavulanicacid,Amikacin,Tobramycin,Ciprofloxacin,Cotrimoxazole and Tetracycline show 100% resistance whereas Piperacillin-tazobactum and Cefepime are 100% sensitive drugs.

IV. Discussion

Colonisation of burn wounds with microorganisms is almost certain to occur in patients with major burns. Colonisation may occur initially from normal resident flora in skin and throat, like staphylococci andstreptococci; later organisms from the gastrointestinal tract, likeE. coli, Klebsiella, Proteus, etc., may also become involved(12, 13). In addition, infection may also be transmitted by fomites or the hands of personnel (12). Such initial colonisation always carries the potential to cause overt burn wound infection and subsequent invasion of the Bloodstream. Therefore, identifying the burn wound isolates and testing their susceptibility to antimicrobial agents are important in the management of the burn wound sepsis (13). Female preponderance in burn patients in India may be due to their confinement to the Home, their loose outfits and unfortunate practice of bride-burning for dowry .We found that to start with, the burn wounds were mostly sterile as surface microbial are destroyed by heat. But they get rapidly colonised within 24 hours because of which a spurt was noted in positive cultures on third day. Burn wound sepsis occurs most frequently in cases of extensive deep burns but it is also a complication of partial thickness burn and may convert superficial burns into deeper ones by causing further destruction of surviving tissue. According to Karyoute et.al, history indicates that the relative importance and cyclical pathogen city of various microbial has changed and may be expected to change as systemic and topical treatment evolves. Initially staphylococcus was the commonest isolate with gram negatives a somewhat distant second (14). Youngobserved progressively changing profile of wound flora (15). Tahlan et alfound pseudomonas as the most common organism followed by staphylococci in their study(16). Lawrencereported higher incidence of gram negative bacteria than gram positive ones as noted inpresentstudy (17). Gupta et al also noted staphylococcus as the single most frequent isolate but gram positive ones being surpassed by gram negative(18). Sharmafound that pseudomonas was the common most bacteria that were isolated in burnWounds (19). Agniho trial so noted pseudomonas as predominant bacteria isolated from burn wounds (20).Burn injuries remain a huge public health issue in terms of morbidity and long-term disability throughout the world (2,21,22). Thermal injury impairs the skin its normal barrier function, thus allowing microbial colonization of the burn wounds. Severe dysfunction of the immune system, a large cutaneous colonization, the possibility of gastrointestinal translocation, a prolonged hospitalization and invasive diagnostic and therapeutic procedures, all contribute to infections (21,23).

Patient factors such as age, extent of injury, and depth of burn in combination with microbial factors such as type and number of organisms, enzyme and toxin production, and motility determine the likelihood of invasive burn wound infection (24). Although any organism is a potential pathogen in burned patients, S. aureus and Methicillin resistant S.aureus were the most common gram positive pathogens and P. aeruginosa, Klebsiella,Acinetobacter, Proteus spp. ,Citrobacter spp. , E. coli, were the most common gram negative microorganisms(3,25,26) .The most common pathogen isolated from burn wounds in our study was Pseudomonas aeruginosa. In our study, out of 400 patients, 250 were Females and 150 were Males. The major age group involved is between 21-30 years that is 165 patients. Pseudomonas aeruginosa (35.55%) was major burn bacterial wound isolates. Klebsiella was second main isolate of our study followed by Acinetobacter spp. S. aureus and MRSA was the fourth in the list of microbial isolates recovered in our study. This is contrary to many previous reports indicating a much higher frequency of isolation of this organism. In our study,Proteus spp., Citrobacterspp.,E.coli was the other frequently recovered organisms. Pseudomonasaeruginosa was the commonest pathogen isolated (35.55 %) followed by Klebsiellaspecies (23.34%),Acinetobacter species (22.22%), Staph. aureus (9.11%), Methicillin Resistant Staphylococcus aureus (5.56%), Proteus species (1.78%), Citrobacter (1.33%) and E.coli (1.11%). Piperacillin-tazobactum was (100%) sensitive antibiotic in all gram negative bacteria .Ampicillin, Piperacillin was the most resistant antibiotics followed by Imipenem,Amoxyclavulanic acid, Amikacin in gram negative bacteria. Amoxicillin and Penicillin G were the resistant antibiotics in case of S.aureus. Vancomycin is most sensitive drug used in case of S.aureus, gram

positive bacteria.The pattern of bacterial resistance is important for epidemiological and clinical purposes. The results of the antimicrobial resistance pattern give serious cause for concern because the predominant bacterial isolates were highly resistant to the commonly available antimicrobial agents. Pseudomonas aeruginosa and Acinetobacterspp.were found to be multidrug-resistantorganisms(27,28).

Limitations

The main limitations of this study are the retrospective design and use of only a single burn centre's data. Culture isolates were unavailable for additional testing or molecular analysis to determine if isolates were acquired through nosocomial transmission. Additionally data obtained from electronic patient records makes it difficult to distinguish infection from colonization. Although it is known that the widespread use of broad spectrum antimicrobials in burn units would provide a fertile ground acquisition of resistance and transformation to form new strains, detailed treatment regimens were unavailable in our study and it is unknown what impact antibiotic use had on culture data.

V. Conclusion

Multidrug-resistant microbial infections are becoming increasingly common and difficult to treat. Composition of bacterial flora in burns is dependent not only on the depth and extent of the burn but also on the site of burn, the duration of burn, the age of the patient and his/her co-morbidities. The development of resistance to a particular antibiotic is dependent on the use of that antibiotic in society at large scale. Overuse of any antibiotic predisposes to development of resistance. In conclusion, the growth of multidrug-resistant organisms should be considered as a serious risk in burn units. Aggressive infection control measures should be applied to limit the emergence and spread of multidrug-resistant pathogens.

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