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Original Research Article

A Study on Time Related Changes in Bacterial Pattern in Burn Wound Infections and Their Antibiogram in a Tertiary Care Hospital

A. Renuka Devi[1], J. Vijayalakshmi[2], S. Shanthi Kumari[3], A. Praveen Kumar[4]

[1]Professor & Head, Department of Microbiology, Kurnool Medical College Kurnool
[2]Associate Professor, Department of Microbiology, Kurnool Medical College Kurnool
[3]Assistant Professor, Department of Microbiology, Kurnool Medical College Kurnool
[4]Senior Resident, Department of Microbiology, Kurnool Medical College Kurnool

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Corresponding Author: Dr. A.Praveen Kumar
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Abstract:

Background: Burns remain a significant health problem in terms of morbidity, long term disability & mortality throughout the world, especially in economically developing countries.

Objectives: To identify the bacterial agents responsible for burn wound infections from admission day 0 to day 21 and to study the antibiogram of bacterial isolates for effective infection control. And also to detect various resistant organisms like MRSA, ESBL, AmpC β-lactamase and MBL producers phenotypically.

Results: 112 burn patients were included in the present study. A total of 448 swabs were collected. Most frequent isolate was Pseudomonas aeruginosa 30.15%, followed by Staphylococcus aureus 23.71%, Klebsiella pneumoniae 12.3%, Coagulase negative Staphylococci 10.82%, Escherichia coli 9.27%, Acinetobacter baumanii 6.95%. MRSA was isolated in 30.43% cases. ESBL, AmpC, MBL production was seen in 16%, 8% and 10% cases respectively. Studying the time related changes of bacteria in burns wound showed that on day 0, most of the samples were sterile and an initial predominance of Gram positive cocci in the first week. From day 14, Gram negative bacilli began to predominate. Antimicrobial sensitivity testing showed Colistin to be very effective drugs for Gram negative bacilli while Linezolid very effective for Gram positive isolates.

Conclusion: The antimicrobial treatment must be changed as microbial flora of the burn wound is an ever-changing entity. Constant analysis of the wound cultures will help the treating physicians to keep abreast with the pathogens and their antimicrobial susceptibility. Early detection of the ESBL, AmpC, and MBL producing isolates in a diagnostic laboratory could help to avoid treatment failure. Aggressive infection control measures should be applied to limit the emergence and spread of these pathogens.

Keywords: Antibiogram, MRSA (Methicillin resistant Staphylococcus aureus), ESBL (Extended spectrum beta lactamases), MBL (Metallo Beta lactamase), AmpC Beta lactamase.

Introduction

Burns remain a major health problem in terms of morbidity and mortality throughout the world. Globally about 1,95,000 deaths occur annually. In India, over10 lakh people are moderately or severely burnt every year.

The leading cause of morbidity and mortality after burn injury is infection as open and large wounds make burn patients more susceptible to infection. [1]

In particular, immunosuppression caused by impaired neutrophil function and impaired immune system can facilitate colonization of burn wounds by different organisms. 75% of all deaths are related to sepsis from burn wounds infections or other complications due to infections. [2] The risk of infection is directly proportional to the extent of injury, age of the patients, colonizing organisms and their invasive potential. Spectrum of bacterial isolates in burn wound varies with time. The problem of multidrug resistant organisms is becoming a serious threat. This necessitates periodic review of organisms isolated i.e. on day 0,7,14,21. Antibiogram of the bacterial isolates forms the basis for modification of drug regimen.

Etiology of burn injury: Cutaneous burns are caused by the application of heat, cold or caustic chemicals to the skin. Thermal Burns are the very common type of burn injury and may be industrial, domestic or environmental in origin. Ex: Scalds, Electrical burns and Chemical burns.

Classification of burn wounds:

1. Based on area of burn wound: Various

methods are available to determine the percentage of body surface that is burnt. The simplest method is the 'rule of nines' which was advocated by Wallace (1951).

2. Depth of burn wound: Depth of the burn is another primary determinant of mortality. Burns are classified according to increasing depth as epidermal (first degree), superficial and deep partial thickness (second degree), full thickness (third degree) and fourth degree burns.
3. Manifestations: Burn wounds can be classified as Impetigo, Surgical wound infection, Cellulitis and Invasive infection. [3]

Pathogenesis: Thermal damage breaches skin. Intercellular edema and dermo-epidermal junction separation cause partial-thickness burn and vesiculation. Blisters are transudates from the well-developed dermal capillary plexus. Transudation of fluid and protein from wound vasculature causes oedema. [4]

Immunology in burn wound infections: Significant thermal injuries cause immunosuppression, predisposing burn patients to infections. In the initial reaction to severe burn injury, proinflammatory cytokines including IL-18 and TNF-α are elevated, leading to increased production of IL-6, platelet activating factors, and gamma-interferon. Gamma-interferon activates macrophages and differentiates CD4 into Th1 cells.

Proinflammatory reaction becomes anti-inflammatory to preserve homeostasis. T helper cells develop into Th-2 cells that generate IL-4 and IL-10. Major burns reduce NK cell activity, neutrophil chemotaxis, complement levels, and macrophage phagocytosis.

These phenomena make burn victims more susceptible to wound infections, severe sepsis, and multi-organ failure.[5].

Infection of Burn Wounds: The types of microorganisms colonizing the burn wound may affect its risk of invasive wound infection. Burnt surfaces are sterile at first, but within 48 hours, skin commensals colonize the wound, and after one week, respiratory, gastrointestinal, or hospital organisms invade it.

Collagenase, elastase, protease, lipase, and exotoxin help organisms penetrate wound.[6] Gram negative organisms' wide repertoire of virulence factors and antibiotic resistance features has made them the most common cause of invasive infections in recent decades.

Bacteremia, sepsis, and multi-organ failure can result from microorganism invasion into tissue layers under the dermis.

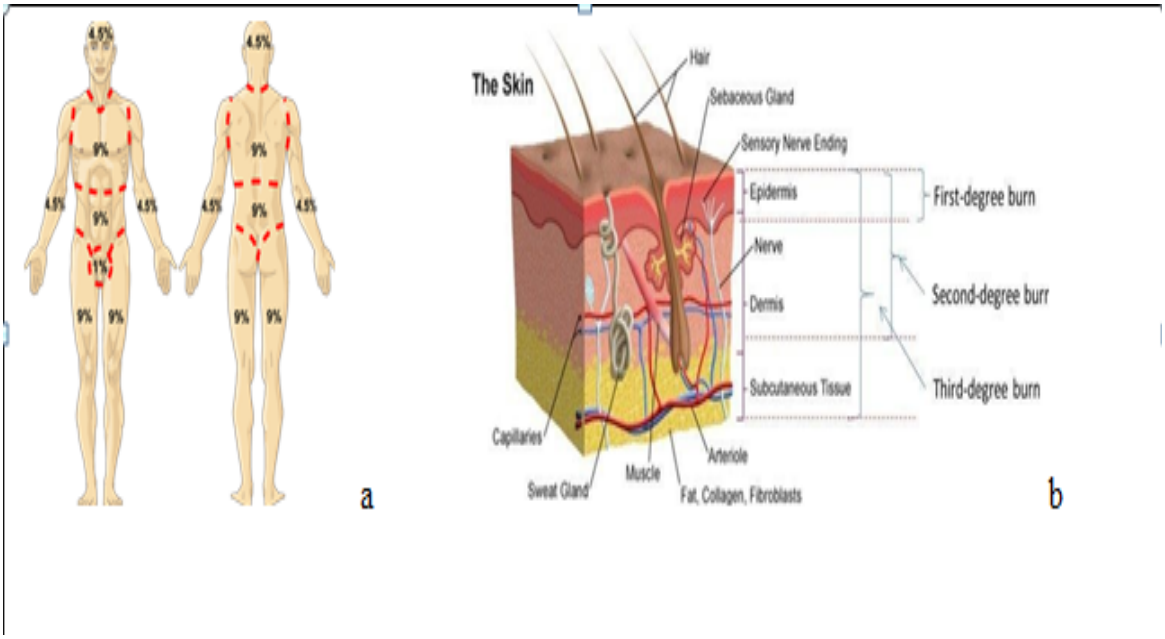

Figure 1: a. Body diagram used for estimating TBSA in adults using 'rule of nines' b. Showing depth of injury for various degree of burns

Etiology of burn wound infection: Bacterial causes of burn wound infections [6]:

Gram positive bacteria	Gram negative bacteria
Staphylococcus aureus. Coagulase negative Staphylococci. Enterococcus species	Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Acinetobacter baumanii, Proteus species, Citrobacter species, Bacteroides species, Serratia marcescens, Enterobacter species.

Keeping this in mind, the present study is planned to determine the bacteriological profile

and resistance pattern in burn wound infections at tertiary care hospital.

Objectives: To identify the aerobic bacterial agents responsible for burn wound infections from admission day 0 to day 21.

To study the antibiogram of bacterial isolates for effective infection control.

To detect various resistant organisms like MRSA, ESBL, MBL, AmpC β-lactamase producers phenotypically, if any.

Materials and Methods

The present study was carried out in the Department of Microbiology, Kurnool Medical College, Kurnool over a period of 23 months from Jan 2020 to November 2021. A total of 158 cases of burn wounds were admitted in our Burn Care Unit.

Among them, 46 patients were discharged within 10 days and 112 patients stayed upto 21 days during the study period and samples were collected only from these 112 patients. Informed consent was obtained from all the patients enrolled.

Sample Collection and Transport: The area around the burn wounds was cleaned with 70% ethyl alcohol & the sample was collected from the depth of the wound using sterile cotton swabs (4 swabs from each patient). Samples were collected immediately after the patients were admitted to the burns unit and every week (i.e. on day 0,7,14 and 21).

Methods:

The samples were processed immediately in the following manner:

a. Direct microscopic examination

b. Inoculation on NA, MAC and BA culture media

c. Preliminary identification of the growth by Gram stain, motility, catalase test, oxidase test,

d. Bio-chemical tests: Tube coagulase test, nitrate reduction test, OF test, sugar fermentation tests, IMViC tests, Urease and TSI

e. Antimicrobial susceptibility test by Kirby Bauer disc diffusion method

Phenotypic detection of MRSA, ESBL, MBL and AmpC β lactamases was carried out on Mueller Hinton agar as follows:

I) MRSA detection: Cefoxitin disc was used. Zone of Inhibition < 21mm was considered as Methicillin Resistant Staphylococcus aureus (MRSA) and > 22mm was considered as MSSA.

II) ESBL detection: Ceftazidime disc (30µg) and ceftazidime+clavulanic acid disc (30µg/10µg) were used. An increase in Inhibition zone diameter of ≥ 5mm in the presence of clavulanic acid than ceftazidime alone was interpreted as ESBL producers.

III) MBL detection: Imepenem disc (10µg) and imepenem+EDTA (10µg/750mcg) were used. An increase in Inhibition zone diameter of ≥ 7mm in the presence of EDTA than Imepenem alone was interpreted as MBL producers.

IV) Amp C detection: Cefoxitin 30µg disc was used. Isolates with zone of inhibition diameter less than 18mm were considered as Amp C producers

Results

Majority of the cases were between the ages of 20-30 years. Children less than 10 years contributed to 17.14% and elderly patients contributed to 3.81% of the total cases. Out of the 112 patients, 42 (37.5%) were males and 70 (62.5%) females. Of the 448 swabs taken, 96 (21.42%) samples were sterile which were obtained immediately after admission on day 0. Pseudomonas aeruginosa was the most common isolate 117 (30.15%) followed by Staphylococcus aureus 92 (23.71%) and Coagulase negative Staphylococci 42 (10.82%). The other isolates included Klebsiella pneumoniae (12.3%), Escherichia coli (9.27%), Acinetobacter spp. (6.95%), Proteus mirabilis (4.63%), Citrobacter freundii (2.06%). Overall, Gram negative organisms were predominantly accounting for 254 (65.46%) of the total isolates. On day 7 Gram positive cocci were more predominant whereas on day 14 and day 21, frequency of isolation of Gram negative organisms increased.

The antimicrobial sensitivity pattern of the organisms to different antimicrobials varied depending on the isolate.

The drugs very effective against P.aeruginosa were Colistin (100%), Imipenem and Meropenem (96.18%) and Piperacillin/tazobactam (89.21%).

K.pneumoniae showed 100% sensitivity to colistin, 97.1% to Imipenem and Meropenem and 86.2% to Piperacillin/tazobactam. The other drugs showed high level of resistance.

Vancomycin and Linezolid remained the most effective in Gram positive bacteria, followed by Piperacillin/tazobactam (93.94%), Clindamycin (75.71%) and Doxycycline (72.86%). Methicillin resistance was seen in 30.43% of Staphylococcus aureus and 26.19% of CONS.

ESBL producing Pseudomonas was 38.33%, Klebsiella 27.08%, E.coli 19.4%.

Amp C producing Pseudomonas was 9.40%, Klebsiella 10.41% and E.Coli 11.11%.

MBL producing Pseudomonas was 3.41%, Klebsiella 4.16% and Acinetobacter 18.51%.

MRSA, ESBL, Amp C & MBL phenotypically detected.

Table 1: Frequency of organisms isolated by burn wounds on day 0,7,14&21

Organism	Day0	Day7	Day14	Day21	Total
P. aeruginosa	0	41	59	17	117
S.aureus	0	66	23	3	92
CONS	6	32	4	0	42
K.pneumoniae	0	9	37	2	48
E.coli	0	7	26	3	36
Acinetobacter	0	5	18	4	27
Proteus mirabilis	0	4	14	0	18
C.freundii	0	2	6	0	8

Table 2: MRSA, ESBL, AmpC& MBL phenotypically detected

Organism	MRSA	ESBL	Amp C	MBL
P.aeruginosa (117)	----------	44 (38.33%)	11(9.40%)	4(3.41%)
S.aureus (92)	28(30.43%)	----------	----------	---------
CONS (42)	----------	----------	----------	----------
K.pneumoniae(48)	----------	13(27.08%)	5(10.41%)	2(4.16%)
E.coli (36)	----------	7(19.4%)	4(11.11%)	----------
Acinetobacter (27)	----------	2(7.40%)	1(3.70%)	5(18.51%)
P.mirabilis (18)	----------	5(27.7%)	1(5.55%)	----------
C.fruendii (8)	----------	----------	----------	----------

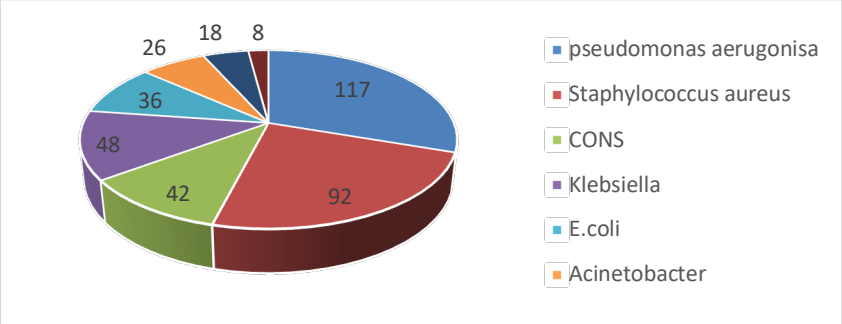

Figure 2: Distribution of the isolates

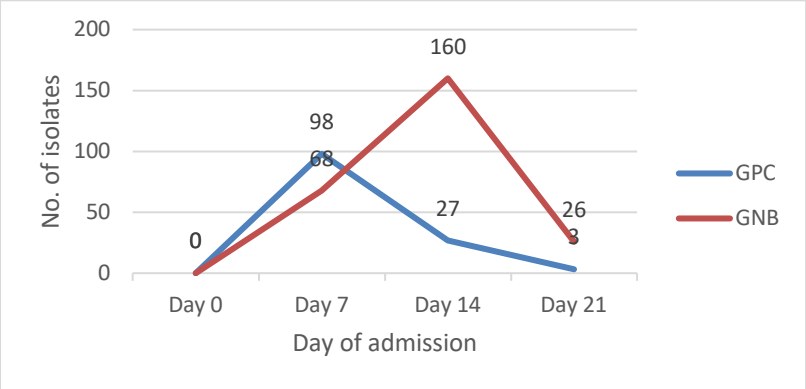

Figure 3: Time related changes in the bacterial isolates

Table 3: Antibiotic sensitivity pattern of Gram Negative isolates

Organism		CXM.	CTR.	COT.	CIP.	AK	GEN	CPM	IPM	PIT	CAZ	AT	TOB	CL
P.aeruginosa	%	-----	-----	-----	52.7	60	65.5	27.3	96.2	89.2	30.9	76.4	52.7	98.1
K.pneumoniae	%	26.2	36.4	6.25	18.1	18.8	18.1	22.9	97.1	86.2	62.6	-----	-----	100
E.coli	%	32.3	78.3	16.7	39.3	53.7	50	41.7	100	88.8	53.7	-----	-----	100
Acinetobacter	%	29.6	29.6	20.1	26.3	38.2	33.3	33.3	88.8	85.2	38.3	----	-----	96.3
P.mirabilis	%	44.4	44.4	11.1	61.1	55.6	50	33.3	100	73.3	66.7	-----	-----	100
C.fruendii	%	50	25	0	50	0	0	0	100	87.5	75	-----	-----	100

Table 4: Antibiotic sensitivity pattern of Gram positive isolates

Organism	P	E	CD	LZ	VA	DO	CIP	PIT	COT	AK	GEN
S.aureus	16.67	61.1	77.78	100	92	72.22	57.2	86.4	58.33	55.56	52.17
CONS	33.33	55.4	75.76	100	100	75.76	48.48	93.94	50.52	60.16	60.16

Discussion

In this study 55.23% of the patients were in the age group of 20 to 40 years. According to Sadeghi-Bazargani H et al, average age of the patients varies from 19 to 35 in different studies they reviewed. Incidence was more in females than males. This is similar to findings of Kaur Rajput A et al [7].

Pseudomonas aeruginosa was the most common isolate. This is comparable to studies by Jefferson Lessa Soares de Macedo et al [9], Ramakrishnan MK et al [8]. Klebsiella pneumoniae accounted for 7.48% of all the total isolates. Our results are comparable with those of Kaur et al [7].

Escherichia coli accounted for 4.08% of the total isolates. Nasser S et al however, reported a higher incidence of E. coli (13.6%). In this study, on day 0, samples were sterile and few of them showed CONS, Gram positive organisms predominated on day 7 after burn injury. From day 14, the Gram negative organisms were more prevalent. This is similar to study by Sonia Mehta et al 2017.

In the antibiotic sensitivity testing, Colistin (100%) was effective against all Gram Negative bacteria. This is in accordance with a study by Jiaping Zhang et al 2009. Mehta M et al saw a significantly high percentage of resistance among Gram negative bacilli to Aminoglycosides, Ciprofloxacin and Ceftriaxone [10]. But in comparison, Colistin, Imipenem & combination drugs like Piperacillin/tazobactum were found to be effective as in our study.

The Gram positive isolates showed 100% sensitivity to Linezolid, followed by Vancomycin. Similar findings were seen by Kaushik R et al in 2006 [11]. 30.43% of the isolates of S. aureus were Methicillin resistant. This is in accordance with study on MRSA in burn patients by Rajput A et al [12].

In our study prevalence of ESBL producing pathogens was prominent in pseudomonas aeruginosa (44 isolates of 117) followed by Klebsiella pneumonia (13 isolates of 48), E.coli (7 isolates of 36), Proteus mirabilis (3 isolates of 18), Acinetobacter baumanii (2 isolates of 27). This is similar to study conducted by Angus Nnamdi et al 2017 [13]. In a study conducted by Gupta et al 2017 MBL activity was maximum exhibited by Acinetobacter (25%), which is similar to our result 18.51% (5 isolates out of 27) [14].

Conclusion

The present study has given us the knowledge regarding incidence of time related changes of bacterial infection of burn wounds in our hospital. If the patients host defense is inadequate and therapeutic measures delayed, microbial invasion of viable tissue occurs resulting in invasive burn wound infection. The emergence of multidrug resistance organisms is a real threat and the detection of ESBL, MBL, AmpC producers and MRSA is absolutely necessary. Our results are helpful for clinicians in changing antibiotics during first, second and third week in burn wound infections and help in formulating effective guidelines for therapy, thus improving overall infection related morbidity and mortality.

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