Research Article

Bacteriology of infected burn wounds in hospitals in and around Davangere

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Abstract

Introduction: Study was conducted to isolate and identify the aerobic bacterial flora in burn wound patients admitted in and around hospitals of Davanagere. It also includes knowing common microbial pathogen involved in causing infection & antibiotic resistance pattern of isolates. **Methods:** The study was done on 100 patients admitted to burns wards in the following 2 Hospitals in Davangere: Chigateri Government Hospital and Bapuji Hospital. Sample is collected from patients in 2 different swabs, one for smear and another for culture. Each pair of swabs from single patient is analysed for 3 days of thorough investigations like Day 1: Examining sample under Gram's stain and inoculating for sample for culture. Day 2: Noting the morphological characteristics of growth and further for microscopy. Day 3: Final identification of organism and biochemical tests for gram negative organisms. **Result:** According to the study results have been following in different categories like: Most common organisms found: Staphylococcus aureus and Pseudomonas aeruginosa. Staphylococcus aureus is most resistant to Penicillin G and 60% resistant to Methicillin whereas it is most sensitive to Chloramphenicol. It has various pattern of sensitivity for others various antibiotics. **Conclusion**: As we get results from the study, it can concluded that staphylococcus aureus and Pseudomonas aeroginosa are the most common organisms causing infections in burn patients, and these organisms are becoming resistant to most of the present day antibiotics like Penicillin G and Methicillin, treating resistant strains is the challenging job now a days.

Keywords: Burns, Infection, Methicillin Resistant Staph Aureus, Staphylococcus.

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Introduction

A burn is a wound in which there is a coagulation necrosis of the tissue, majority of which are caused by heat. The leading cause of morbidity and mortality in burn wound patients is infection. Bacterial infection is inevitable complication in burns and most frequent cause of death because the large low area with its (burn) serous exudates may act as huge culture plate on which organisms mostly bacteria can establish and multiply [1]. So, burn injury with infection is a major public health problem in many areas of world especially in India where scientific treatment of burns is still in its infancy. It has been estimated that as many as 75% of all deaths following burn injuries are related to infection in India. A burn wound has a much higher incidence of sepsis as compared to other forms of trauma because of extensive skin barrier disruption and an alteration in cellular and humeral immune response. In all centers where burn cases are isolated, given best of care with an almost microscopic monitoring of patients states, still the same pattern of infection and the same virulence has been noticed [2, 3]. In high infection rate units, periodic monitoring of microbial species and of this respective susceptibility of antibiotics is important, because significant shifts in these data may be correlated with changes in clinical management and useful indications on drug effectiveness at given time can be obtained [4].

With all newer antibiotics and facilities to tackle infection, there has been almost no difference with results between best equipped and worst of burn centers [1]. Treatment of burn wounds infection is complicated by emergence of antibiotic resistance. In this series, therefore an attempt has been made to study pattern of infection in burn patients with possible methods of trying to curb lavages.

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Methods

The present study will be carried out in Department of Microbiology, J J M Medical College, Davanagere. A total number of 100 patients admitted with different degree of burns will be studied. The cases are from Chigateri General Hospital and Bapuji hospital in davanagere.

Criteria for inclusion in study: All cases of thermal burns who are hospitalized for treatment.

Criteria for exclusion from study: All cases of thermal burns on OPD basis. All cases of acid burns, electric burns and wounds other than thermal burns were excluded from study. The sample is collected under aseptic precautions from burn wound with help of dry sterile cotton swab sticks for bacteriological examination.

Two culture swabs each are obtained, one for smear study and other for aerobic culture. Culture swabs are taken from each patient and immediately brought to laboratory for investigation.

Bacteriological investigation:-

Day-1: Direct smear examination by Gram's stain to look for presence of pus cells and bacteria. All smears are made by rolling the swab stick on clean glass slides, which are heat fixed.

Day-2: The antibiotic susceptibility testing of the isolates are done on Muller-Hinton agar using Kirby-Bauer disc diffusion method.

The antibiotic discs (obtained from Hi-media laboratory) used differed depending on the organisms isolated that is whether gram positive or gram negative.

All strains of staphylococcus aureus are tested for Methicillin resistance on Mueller-Hinton agar with 4% sodium chloride using oxacillin (1mg) disc. They are incubated at 30*c and reading is taken after 48 hours.

Day-3: The final identification of the organisms and the antibiotic susceptibility pattern are reported. All the gram negative bacilli are identified by the media are prepared as per the standard procedures.

Results and Discussion

A total of 100 cases of burns wound infection were included in the present study. The study included 31 males and 69 females. The age ranged from 3-90 years. Most number of burns was seen between the age group of 22-45 years and most commonly in females. The male to female ratio was 0.4:1. In the 100 cases of the present study, a total of 127 organisms were isolated, accounting for an average of 1.3 organisms per case. Out of these 100 cases, 3 cases (3%) showed no growth on culture, 69 cases (69%) were monomicrobial in nature and remaining 28 cases (28%) were polymicrobial.

Out of these 127 isolates, all were aerobes and facultative anaerobes. The gram stain of all direct smears correlated well with the growth on culture. Of these 127 isolates, 57 (44.9%) were gram positive cocci and 70 (55.1%) were gram negative bacilli. Staphylococcus aureus was isolated most frequently (39.4%) followed by Pseudomonas aeroginosa (14.2%), Klebsiella pneumoniae (13.4%), Escherichia coli (8.7%) and Acinetobacter (7.9%).

Table-1: Aerobes isolated in the study group.

Organisms	Number of cases	Percentage
Staphylococci aureus	50	39.4
Pseudomonas aeroginosa	18	14.2
Klebsiella pneumoniae	17	13.4
Escherichia coli	11	8.7
Acinetobacter	10	7.9
Coagulase negative Staphylococci	5	3.9
Proteus mirabilis	5	3.9
Klebsiellaoxytoca	3	2.4
Proteus vulgaris	3	2.4
Providencia stuartii	3	2.4
Enterococci	2	1.6

Burn Wound Infections Related To Pure And Mixed Flora: The most common organism isolated in both pure and mixed flora is Staphylococcus aureus (50% and 27.1% respectively). In mixed flora, this was followed by Klebsiella pneumoniae (15.3%), Pseudomonas aeroginosa (11.9%) and Acinetobacter (11.9%). Likewise in pure flora, Staphylococcus aureus was followed by Pseudomonas aeroginosa (16.2%), Klebsiella pneumoniae (11.8%) and Proteus mirabilis (5.9%).

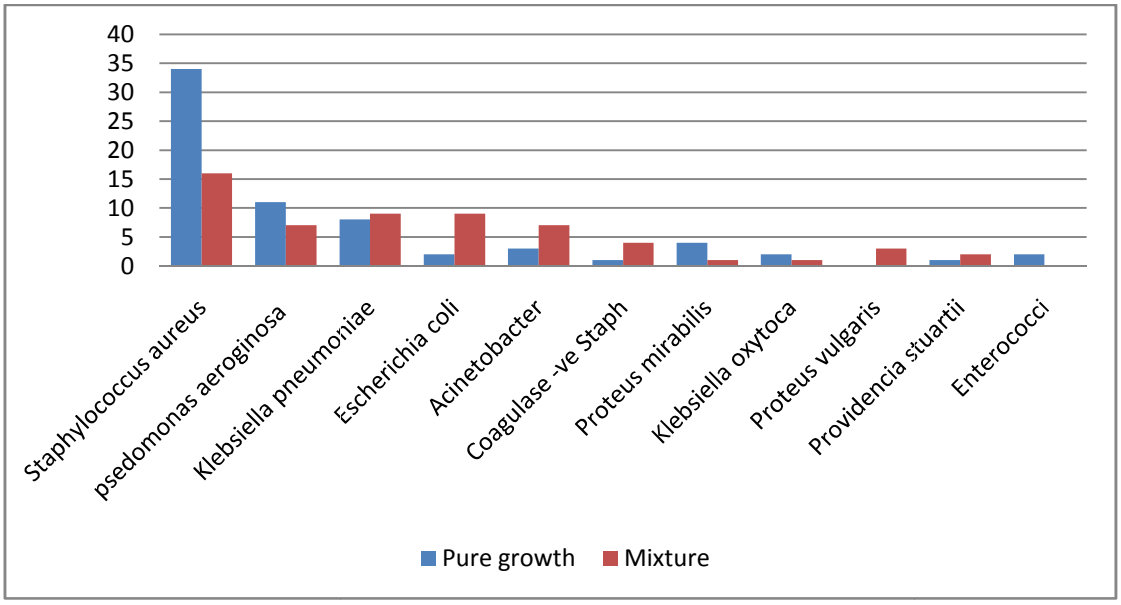

Fig-1: Bacterial flora in pure growth and mixtures in burn wounds

Burn Wound Infection In Relation To Depth: In relation to the depth of the burns wound, the most common organism isolated both in superficial and deep burns was Staphylococcus aureus (41.3% and 37.5% respectively). This is followed by Pseudomonas aeroginosa (15.9%) in superficial burn wounds and Klebsiella pneumoniae (15.6%) in deep burns.

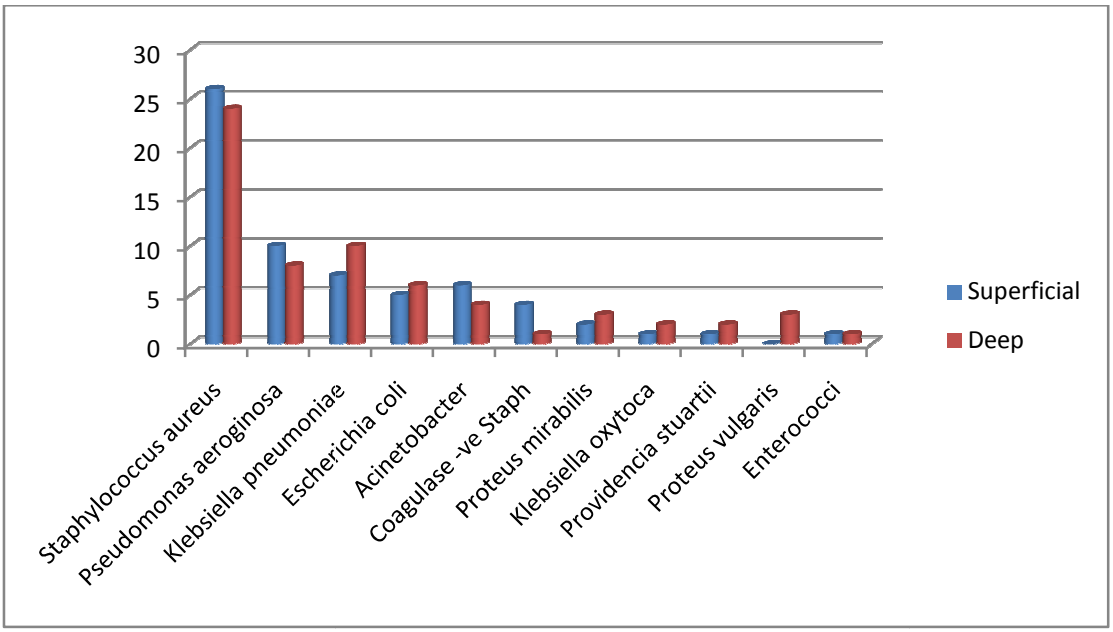

Fig.-2: The following table shows the percentage of different bacterial organisms in both superficial and deep wounds

Out of the 100 cases, 45 cases (45%) had 25-50% degree of burns which was followed by 40 cases (40%) having less than 25% degree of burns. The number of cases in relation to the degree of burns is given in pie chart below:-

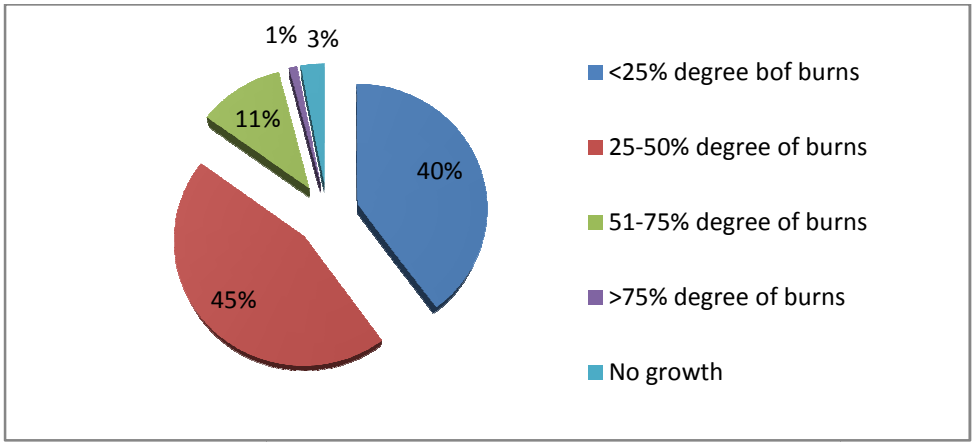

Fig-3: Burn wound infections in relation to degree of burns

Antibiotic Susceptibility Pattern in Isolates: Staphylococcus aureus was most sensitive to Chloramphemicol (100%), Amikacin (92%) and Cefuroxime (68%). Out of the total of 50 isolates of Staphylococcus aureus 31 (62%) were Methicillin resistant Staphylococcus aureus (MRSA). All the MRSA strains were 100% sensitive to Vancomycin and Linezolid. Sensitivity pattern of the organism in shown in below table:

Table-2: Antibiotic susceptibility pattern in MRSA and MSSA isolates.

Total (50)	Methicillin resistant R	SA (31) S (62%)	Methicillin sensitive	SA (19%) S (38%)
Penicillin	32 (100%)	0	19 (100%)	0
Amoxicillin	30 (97%)	1 (3.22%)	16 (84.2%)	3 (15.79%)
Erythromycin	28(91.42%)	3 (9.67%)	7 (36.84%)	12(63.16%)
Roxithromycin	27 (87.1%)	4 (12.9%)	5 (26.31%)	14(73.68%)
Ciprofloxacin	29(93.54%)	2 (6.45%)	7 (36.84%)	12(63.16%)
Ofloxacin	23(74.19%)	8 (25.81%)	6 (31.58%)	13(68.42%)
Gentamycin	31 (100%)	0	14(73.68%)	5 (26.31%)
Amikacin	18(58.06%)	13(41.93%)	1 (5.26%)	18(94.74%)
Chloramphenicol	0	31 (100%)	0	19 (100%)
Cefotaxime	16 (51.6%)	15(48.39%)	2 (10.52%)	17(89.47%)
Linezolid	0	31 (100%)	0	19 (100%)
Vancomycin	0	31 (100%)	0	19 (100%)

None of the cases of Staphylococcus aureus isolated was sensitive to Penicillin G. 4 cases (8%) of the Staphylococcus aureus isolated were sensitive to Gentamycin, while the rest (92%) was resistant. 49 cases (98%) were resistant to Netilimycin, Enterococcus species showed maximum susceptibility to Amikacin (92%), Cloxacillin (92%), Cefuroxime (68%) and Ofloxacin (52%), coagulase negative Staphylococcus were 100% sensitive to Chloramphenicol and Ceftriaxone. The gram negative enteric pathogens showed maximum sensitivity to Fluroquinolones (Ofloxacin), aminoglycosides (Amikacin) and 3rd generation Cephalosporin (cefotaxime). Pseudomonas aeroginosa isolates were most sensitive to Amikacin (94.4%), Cefotaxime (77.8%), Amoxycillin (77.8%), Cephalexin (66.7%) and Pfloxacin (61.1%).

Escherichia coli were most sensitive to Amikacin (100%), Ceftriaxone (81.8%), Ceftazidime (72.7%) and Ciprofloxacin (54.5%). Klebsiella pneumoniae isolates were susceptible to Amikacin (100%), Cotrimazole (94.1%), Ofloxacin (64.7%) and Ceftriaxone (58.8%). Acinetobacter isolates were most sensitive to Amikacin (100%), Carbenicillin (90%), Polymixin B (80%) and Ceftazidime (60%). Proteus mirabilis showed 80% sensitivity to Tetracycline, Amoxycillin, Ofloxacin and Ceftazidime.

Table-3: Antibiotic susceptibility pattern in Gm negative organism.

		Acinetobacter		Proteus mirabilis		Proteus vulgaris		Providencia Stuartii	
Total		10	%	5	%	3	%	3	%
Gentamycin	S	3	30	-	-	-	-	1	33.3
	R	7	70	5	100	3	100	2	66.7
Ciprofloxacin	S	1	10	3	60	2	66.7	-	-
	R	9	90	2	40	1	33.3	3	100
Cotrimoxale	S	-	-	4	80	1	33.3	3	100
	R	-	-	1	20	2	66.7	-	-
Ampicillin	S	-	-	3	60	-	-	-	-
	R	-	-	2	40	3	100	3	100
Chloramphenicol	S	2	20	2	40	1	33.3	2	66.7
	R	8	80	3	60	2	66.7	1	33.3
Amikacin	S	10	10	4	80	3	100	3	100
	R	-	90	1	20	-	-	-	-
Cefotaxime	S	2	20	3	60	2	66.7	1	33.3
	R	8	80	2	40	1	33.3	2	66.7
Polymixin B	S	8	80	-	-	-	-	-	-
	R	2	20	-	-	-	-	-	-
Carbenicillin	S	9	90	-	-	-	-	-	-
	R	1	10	-	-	-	-	-	-
Tetracycline	S	-	-	4	80	3	100	2	66.7
	R	-	-	1	20	-	-	1	33.3
Cefuroxime	S	5	50	1	20	-	-	-	-
	R	5	50	4	80	3	100	3	100
Amoxicillin	S	4	40	4	80	2	66.7	3	100
	R	6	60	1	20	1	33.3	-	-
Ofloxacin	S	2	20	4	80	3	100	-	-
	R	8	80	1	20	-	-	3	100
Netilymicin	S	2	20	1	20	-	-	-	-
	R	8	80	4	80	3	100	3	100
Ceftriaxone	S	3	30	3	60	2	66.7	1	33.3
	R	7	70	2	40	1	33.3	2	66.7
Ceftizidime	S	6	60	4	80	3	100	1	33.3
	R	4	40	1	20	-	-	2	66.7
Cephalexin	S	4	40	1	20	-	-	1	33.3
	R	6	60	4	80	3	100	2	66.7

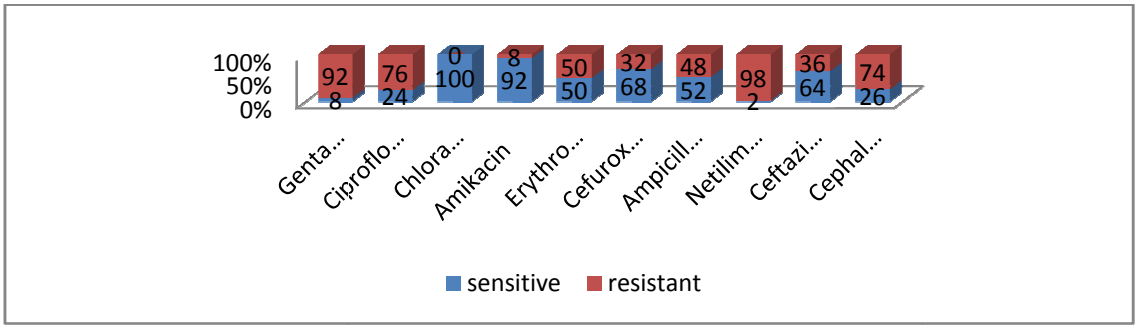

Fig-4: Sensitivity pattern of Staphylococcus aureus in burn wound infection.

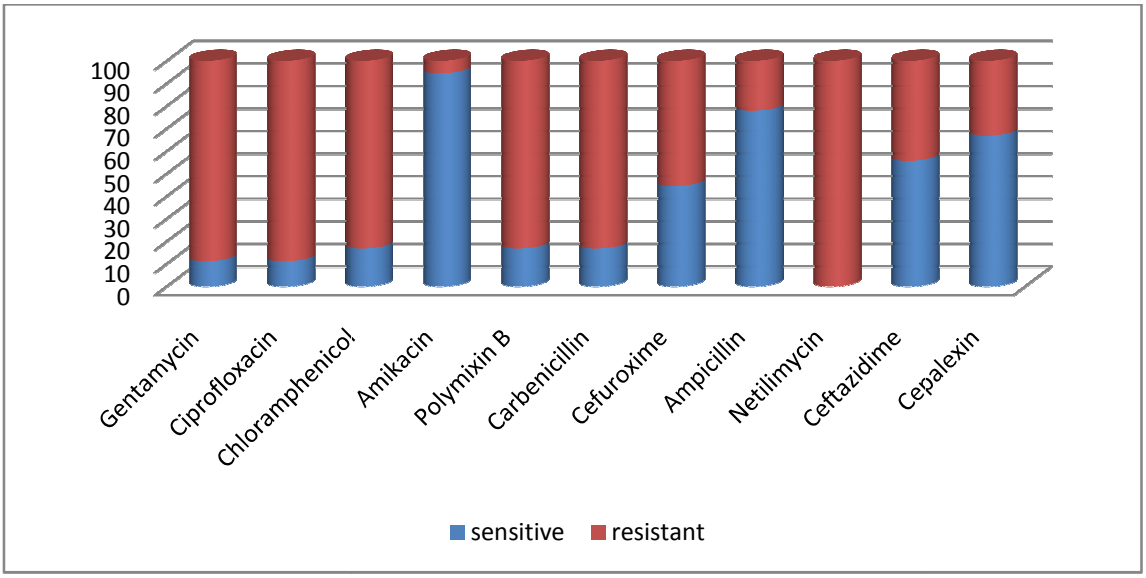

Fig. -5: Sensitivity pattern of Pseudomonas aeroginosa in burn infections

Discussion

The burn wound is particularly susceptible to bacterial colonization and infection due to physical disruption of the normal skin barrier and the accompanying reduction in cell mediated immunity.

Infection in burns continues to be a great problem which is not yet solved and poses a challenge to the microbiologist and the surgeon in particular. In the present work an effort has been made to study the pattern of microbial infection in the burn wounds, with particular reference to the identification of the organism which is the most predominant cause of infection.

In present study, the majority of the population studied were within 20-45 years of age (62%) and the burned TBSA ranged within 25-50% (45%). This was similar to the study by Zorgani A et al (30 years and 50% respectively) [5]. The male to female ratio was 0.4:1 which was similar to a study done by Raj Kumar Gang et al (1:1.5) [6]. This was probably due to fact that the majority of severe flame burns occurred in females at home, either due to cooking gas accident or to clothes catching fire.

Out of the 100 cases studied, a total of 127 isolates were seen of which gram positive organisms composed of 57 (44.9%) and gram negative organisms 70 (55.1%). A single type of organisms either gram positive or gram negative were isolated from 69 cases according for 69% and more than one organism was isolated in 28 cases (28%) in the present study. Staphylococcus aureus was the most predominant organism in burn patients accounting for 39.4% of the total isolates. This is similar to the study by M.I. Lesseva et al (36.9%) and V.L. Yemul et al (41.5%) [7,8]. This is in contrast to the study conducted by A. Rastegar Lari et al, which reported Pseudomonas aeroginosa as the most predominant organism in burn patients [9].

According to our study, the frequency of Staphylococcal infection was almost the same both in superficial (41.3%) as well as deep burns (37.5%). This is similar to the work done by Lesseva et al who states that the frequency of staphylococcal infection did not seem to depend on the burn wound area and its colonization. Among the other gram positive organisms isolated, coagulase negative Staphylococci was the next common isolate accounting for 3.9% this was followed by Enterococci (1.6%). This is less compared to the study by H. Vindenes et al (21.5% and 11.3% respectively) and G Revathi et al and R L Bang et al where Enterococcus isolates accounted for 8.5% and 3.4% respectively [10-12].

Out of 50 strains of Staphylococcus aureus studied 31 (62%) were resistant to Oxacillin (MRSA) and the remaining 19 (38%) were sensitive (MSSA). All the MRSA isolates were sensitive to Vancomycin and Linezolid. The prevalence of MRSA is lower than that of W. Song et al and Sanyal et al but it is much higher than other reports.

Table-4: Prevalence of MRSA in different places.

Author and place	Year	Total studied	% MRSA
Lesseva et al, [13] Scientific Institute of Emergency Medicine, Sofia, Bulgaria	1994	4552	23.8
R.L. Bang et al, [14] Department of Microbiology, Ibn Sina Hospital, Kuwait	1996	79	41
Sanyal et al, [15] Ibn Sina Hospital, Kuwait	1996	943	92
W. Song et al, [10] Department of Clinical Pathology, South Korea	1996	2190	98
Present study, Davangere, Karnataka	2014	100	62

Gram negative bacilli were the next, common organisms to follow Staphylococcus aureus. Among the 70 (55.1%) of gram negative pathogens, Pseudomonas aeroginosa was the commonest isolate accounting for 14.2% of total isolates which is in contrast to studies done by Barier et al [1]. Pseudomonas aeroginosa is a well organized cause of nosocomial infections among the patients with burns usually spread from patient to patient by direct contact, via staff.

The next common isolate in the present study was Klebsiella pneumoniae (13.4%). The relatively high incidence of Klebsiella pneumoniae may be due to frequent cross infections. The next commonest gram negative bacilli isolated were Escherichia coli (8.7%) and Acinetobacter (7.9%) which was more compared to study by G. Revathi et al [5] (5.1% and 1.1% respectively).

The other gram negative bacilli isolated in the present study were Proteus mirabilis (3.9%), Klebsiellaoxytoca (2.4%), Proteus vulgaris (2.4%) and Providencia stuartii (2.4%). All these isolates have been reported by most of the workers.

The pattern of antibiotic sensitivity is a source of serious concern as many of the isolates are resistant to newly available antibiotics.

In the present study, Staphylococcus aureus was most sensitive to Chloramphenicol, Amikacin, Cloxacillin, Piperacillin, Cefuroxime, Ceftriaxone and Ofloxacin with a sensitivity rate of 100%, 92%, 92%, 78%, 68%, 68% and 52% respectively. None of the Staphylococcus aureus isolates were sensitive to Penicillin. About 62% of the Staphylococcus aureus isolates were Methicillin resistant Staphylococcus aureus (MRSA). This was more compared to Lesseva et al [13] (23.8%) but less compared to W. Song et al [10] (98%). A marked increase in the number of hospital infections due to MRSA has been recently reported in many countries. Lesseva et al [13] reported that 79.9% of the MRSA strains were isolated from the patients admitted for some time in the hospital.

In contrast to the relatively high sensitivity of MSSA strains to antimicrobial agents, multi drug resistance is common among the MRSA isolates. In our study, a great proportion of them were resistant to Gentamycin (100%), Penicillin (100%), Erythromycin (91.4%) and Ciprofloxacin (93.5%). This was similar to Lesseva et al [13] (82.2%, 100% and 71.1% respectively) but in contrast to their study concerning Ciprofloxacin (33.3%). All the MRSA isolates were fully sensitive to Vancomycin and Cloramphenicol which was again similar to Lesseva et al [13] (100%) and 66.7% respectively. Over 94.7% of MSSA were susceptible to Amikacin and 63.2% to Erythromycin. This was similar to Lesseva et al [13] (88% and 63.9% respectively). The higher incidence of MRSA strains in our study may probably due to the fact that MRSA either develops in the wounds during antibiotic therapy or enters the burns ward already resident in such patient.

Coagulase negative Staphylococci showed maximum susceptibility to Cloxacillin (100%), Amikacin (80%), Erythromycin (100%) and Cefuroxime (100%). They were resistant to Gentamycin (60%). Enterococcus species isolated

in the present study were sensitive to Amikacin (100%), Cloxacillin (100%) and Ceftriaxone (50%). They showed resistance to Gentamycin (100%).

The gram negative bacilli isolated in the present study were more sensitive to Amikacin, Cefotaxime, Amoxycillin, Ofloxacin and Ceftazidime. Pseudomonas aeroginosa which was the second commonest isolate (14.2%) of all the isolates was most susceptible to Amikacin (94.4%), Cephalaxin (66.7%) and Ceftazidime (55.6%). Of the 17 isolates of Klebsiella pneumoniae, 100% were susceptible to Amikacin, Ofloxacin (64.7%), Ceftriaxone (58.8%) and Tetracycline (52.9%).

Escherichia coli isolates demonstrated 63.6% sensitivity to Cefotaxime, 72.7% to Ceftazidime and 100% to Amikacin. Acinetobacter showed 100% susceptibility to Amikacin while Proteus mirabilis showed 80% susceptibility to Amikacin and 60% to Ciprofloxacin. The above mentioned organisms showed maximum resistance to antibiotics such as Gentamycin, Carbencillin, Co-trimoxazole and Cloramphenicol. This was similar to study done by G Revathi et al. [5].

Selective pressure of antimicrobials is thought to be a risk factor for the emergence of resistant organisms. Since the pharmacokinetics of the most antimicrobials is greatly altered in burn patients, it is suggested that the concentration of these drugs should be monitored in such patients and dosages should be adopted to avoid treatment failures and selection of resistant isolates.

Adequate bacteriological surveillance and monitorization from the moment of admission into the burns care unit, in order to diagnose any infection and study the colonization flora, is an important measure in the assessment of the more pathogenic or multi resistant organism.

To control hospital acquired infection in burn wards, over-crowding must be avoided as this is an important cause for cross infection in the burns ward. Strict hand washing is both before and after handling patients and restriction of the movement within the burns ward should be implemented.

Conclusion

The present study observed that the Staphylococcus aureus and Pseudomonas aeruginosa are the most common organisms causing infections in burn patients, and these organisms are becoming resistant to most of the present day antibiotics like Penicillin G and Methicillin.

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Permission from IRB: Yes

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