https://doi.org/10.48047/AFJBS.2.1.2020.55-73

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Evaluate hisological changes and bacterial infections in skin burns at tertiary teaching hospital, Tirupati,Andhrapradesh, India.

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Volume 2, Issue 1,Feb 2020

Received: 15 Jan 2020

Accepted: 05 Feb 2020

Published: 29 Feb 2020

doi:10.48047/AFJBS.2.1.2020.55-73

ABSTRACT
This study analyzed bacterial infections and histological changes in skinburn wound infections, in order to evaluate their histological change and antimicrobial susceptibilities. Out of 100 burn wound cases admitted to surgery department, Pseudomonas aeruginosae as the most common organism followed by Staphylococcus aureus, Escherichia coli and Klebsiellapneumoniae etc. However, all the staphylococci were susceptible to Vancomycin and the gram negatives were susceptible to Imipenam. For empiric treatment, Vancomycin and Imipenem appear to be a good combination in this hospital. The histopathological changes during the distinctive phases of burn wound is correlates the bacterial infections and their antibiotic susceptibilities. Although medical centers have devoted intensive resources to improving the survival rates of burn patients with bacterial infections. This study may contribute to the establishment of a nationwide burn database and the elaboration of strategies to control burns wound infections.

Key Words: Skin Burns, Histopathological evaluation, Infection, Common Pathogens, Antimicrobial Susceptibility pattern.

INTRODUCTION

A burn is an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals. Burns are a global public health problem, accounting for an estimated 180 000 deaths annually. In our country, the most common manner of sustaining flame burns is accidental (Sawhney CP *et al.,* 1993).

The majority of these occur in low- and middle-income countries and almost two thirds occur in the WHO African and South-East Asia regions. Non-fatal burns are a leading cause of morbidity, including prolonged hospitalization, disfigurement and disability, often with resulting stigma and rejection. Burns are among the leading causes of disability-adjusted life-years (DALYs) lost in low- and middle-income countries. In 2004, nearly 11 million people worldwide were burned severely enough to require medical attention.

The cutaneous reaction to heat and flame leading to vital reaction (red-flare/red-line), vesication/blisters and microscopic examination of the tissues from the burnt area has been considered very important (Vij K.*et al.,* 2002). In severe burn injuries (i.e. >30% TBSA), complex reactions occur both at the burn and away from the burn (Schaefer TJ *et al.,* 2013). This results in extensive inflammatory reactions within a few hours of the burn injury (Jeschke MG *et al.,* 2010).The inflammatory response leads to rapid edema formation. This is caused by 1. Increased microvascular permeability 2. Increased hydrostatic microvascular pressure 3. Vasodilation 4. Increased extravascular osmotic activity. These reactions are caused by the direct effect of heat on the microvasculature and the chemical mediators of inflammation (Arturson G *et al.,* 1980).

Contamination in the burn injury stretches the therapeutic of the wound in all phases of healing; for this reason, it is important, that the treatment of the burn infection includes antibiotic therapy, deletion of necrotic tissues in time, ensuring the blood and oxygen source to the wound, the augmentation of the resistance of the organism, and the adequate diet (Bowler P.G *et al.,* 2001). Bacterial contamination is one of the greatest severe difficulties in burn basis serious complications and death following thermal injury. P. aeruginosa and S.

aureus are the furthermost chief contagious and risky bacteria in injury patient.P. aeruginosa is one of the maximum significant and greatest reasons of grave contamination in injury patients (Bowler P.G 2001, Oncul O 2002).Treatment of these infections is problematical by antibiotic resistance (Xue B1999, .Rastegar L.A.R 2005, Alaghehbandan R 2001, Rastegar L.A 2000, Rastegar L.A 1998, Karimi E.H 2002).

AIM AND OBJECTIVE

To evaluate histological changes and bacterial infections in addition to antimicrobial susceptibilities in skin burns.

PLAN OF WORK, METHODS AND TECHNIQUES:

1. STUDY DESIGN: Prospective analytical study
2. STUDY SETTING: Study subjects were patients admitted with thermal burns and scalds categories of skin burns.
3. METHOD:

   Skin tissue was taken from the burnt and junctional area of burnt and unburnt region and was subjected to histopathological examination as described by Culling *et al.,* 1985.

   Informed consent was obtained from the patients or their relatives for participation in the study.

   Patients with thermal burns and scalds categories were included and assessed for the severity of burn injury by calculating the percentage of total burnt body surface area (%TBSA)(Coleman DJ 2004).Clinical evaluation of burn wound extent was by Wallace's rule of nine. A detailed clinical history was taken at the time of admission and physical examination was carried out. The post burn period was divided into 4 stages of healing for the study purpose, which was as follows, 1. Hemostasis stage (0 – 12 hours) 2. Inflammatory stage (12hours -3 days), 3. Proliferation stage (3-14 days) and 4. Remodeling stage (15-28 days).

TECHNIQUES WERE USED: Histopathology, Standard bacterial isolation technique and antibiotic susceptibility.

Method of Slide Preparation for histopathological examination

The slide preparation procedure started with fixation of tissue. For that, it was done in 10% formalin solution for 6-12 hours at room temperature. After that, Dehydration of the tissue was done in the ascending series of alcohol i.e. 50%, 70%, 90% absolute alcohol. The tissue was cleared by two changes in xylene. Then the tissue was impregnated with paraffin wax for 10-12 hours and block was made. Tissue was cut in 3-5 microns thick section with the help of rotatory microtome floated in water with a Petri dish and subsequently transferred to a clean glass slide. For removal of paraffin, the slides were placed on the hot plate for melting of wax and then were given three changes in xylene for 5 minutes each. Slides should be hydrated by bringing them to descending series of alcohol i.e. absolute alcohol 90%, 70% and 50% and then by placing them under running water for 2 minutes for complete hydration. Then the slides were stained with haematoxylin and eosin. For haematoxylin and eosin staining, section from distilled water dipped in haematoxylin solution for 15 minutes. Then the slide with section was removed and was thoroughly wash with tap water for half minute. One % of acid alcohol was kept on the slide for 15 seconds and then washed with tap water. For Bluing of the section was done by keeping the slide in tap water for 10 minutes. 1% aqueous eosin used for 1-3 minutes for counter staining and excess of stain was removed by washing with tap water. Finally before mounting, the slide was dehydrated again with ascending series of 50%, 70%, 90% alcohol and absolute alcohol for 2-3 minutes duration each and then mounting was carried out by putting few drops of DPX (Distrene Plasticizer Xylene) on dried slide (slide was dried by placing it over the heater). Cover slips were placed with precaution taken to avoid collection of air bubbles. Then the slides were examined under the light microscope to get the information in relation to different histological changes.

Microbiologic sampling procedures

Diagnosis of burn wound infection was based on clinical signs and microbiologic surveillance by surface swabs taken routinely twice a week. Microbial colonization of all wounds was studied from the time of admission to discharge.

At admission, the sampling procedure included swabbing clinically deep areas of the burn wound before any cleansing. Later swabbing was performed twice per week in the morning, while dressing burn wounds. Swab samples were taken from the wound area where the degree of burn was highest. When samples were collected, special attention was paid to the areas where infection was most evident before the dressings were changed. Wound samples were collected for culture after several clinical assessments from the leading edge of wound sites showing signs of infection, such as discoloration, bad odor and rapid separation of the eschar or the presence of pus. The oral, genital, scalp and anal regions were never used for sample collection. Bandages were removed, the remnants of the previous day's ointment were washed away and the wounds were swabbed and cultured as follows. A sterile cotton swab was moistened with sterile normal saline, and the centre was swabbed for 30 seconds according to Levine's technique. After ensuring that the swabs were saturated with wound exudates, the swabs were placed in appropriate containers. The swabs were transported to the laboratory for immediate processing. Upon arrival at the laboratory, the wound swab samples were immediately cultured using nutrient agar, Blood agar and MacConkey agar (Himedia Laboratories Pvt Ltd., Mumbai), then incubated at 37°C for 18 to 72 hours. After incubation, the plates were examined for bacterial growth. Further identification and confirmation of organisms was done by the standard identification technique, which include studying the colonial morphology, Gram's stain and Biochemical reactions. After identification of the bacterial strains, the antibiotic susceptibility of all cultured pathogens was determined.

Antibacterial Susceptibility Testing

Susceptibility testing was performed by Kirby-Bauer disk diffusion technique according to criteria set by CLSI 2009. The drugs tested for gram-positive cocci were Penicillin G (10U), Ampicillin (10 µg), Oxacillin (1µg), Ciprofloxacin (5µg), Gentamicin (10µg), Amikacin (30µg), Clindamycin (2µg), Erythromycin (5µg), Novobiocin (30µg), Co-trimoxazole (1.25/23.75 µg) and Vancomycin (30µg). The drugs tested for Enterobacteriacea were Gentamicin (10µg,) Amikacin (30µg), Ampicillin (10 µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime(30µg), Ciprofloxacin (5µg), Co-trimoxazole (1.25/23.75 µg), Amoxycillin/clavulanate (20/10µg), Imipenam (10µg), Piperacillin/tazobactam (100/10µg) and Ticarcillin/clavulanic acid (75/10µg). The drugs tested for Pseudomonas aeruginosa were Gentamicin (10µg), Amikacin (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime

(30µg), Ciprofloxacin (5µg), Co-trimoxazole (1.25/23.75 µg), Amoxycillin/clavulanate (20/10µg), Imipenam (10µg), Piperacillin/tazobactam (100/10µg), and Ticarcillin (75 µg).

INCLUSION CRITERIA:

1. Patients admitted with definite history of thermal and scalds skin burns.
2. The cases with different duration and survival times were studied.

EXCLUSION CRITERIA:Patients with obvious infections at other sites, patients with a recent history of diseases such as malaria/typhoid/viral hepatitis/other infectious diseases, and patients with chronic diseases such as diabetes mellitus / tuberculosis / chronic obstructive pulmonary disease / malignancies etc and burn patients with less than 24 hours' hospitalization were not included.Burn injuries due to electrocution, chemical and corrosives were not included.

ETHICAL CLEARANCE:BySri Venkateswara Medical College, Multi-Disciplinary Research Unit (MDRU- ICMR- DHR- Government of India), Tirupati, Andhrapradesh, India.

STATISTICAL ANALYSIS**:**

Allthestudy data wereenterdintothecomputer data base using standard format, checkedforerrors and verified. Data maintained in thecomputersheetswereorganisedby SPSS version 20 software for Windows. Data will be peresented in aprropriateTablesbycaliculating of percentage, rate etc.

RESULTS:

A total number of 100 patients Skin burnwoundsamplesfromvariousbodyareaswereincluded in thisProspectiveanalyticalstudy. Theages of studygroupsrangedfrom 20 years to 60 years.

Table- 1: Age Distribution of the Skin Burns Patients

	Adults 20 – 40 years	OlderAdults 40 - 60 years	Total
Number	68	32	100

Percentage	68%	32%	100%

Theoccurrence of the Skin Burns in adults (68%) wasfound to be highercompared to elderly cases (32%) respectively. (Table -1).

Table2: Demographic Data of patients and types of burns

I. Gender: Number (%)

a. Male: 46 (46%)
b. Female 54 (54%)

II. Kind of Burn:Number (%)

a. Flame: 88 (88%)
b. Scald: 12 (12%)

III. Percentage of Burn- (%)

a. 10- 30 58 (58%)
b. 30 - 50 36 (36%)
c. >50 6 (6%)

IV. Out come: Number (%)

d. Recovery: 56 (56%)
e. Partial recovery: 38 (38%)
f. Death: 06 (6%)

V. Histopathological findings of burnt skin

g. Separation epidermis and dermis 72
h. Vacuolisation 58
i. Petechial Haemorrhage 58
j. Flattened and elongated epithelial cells 70

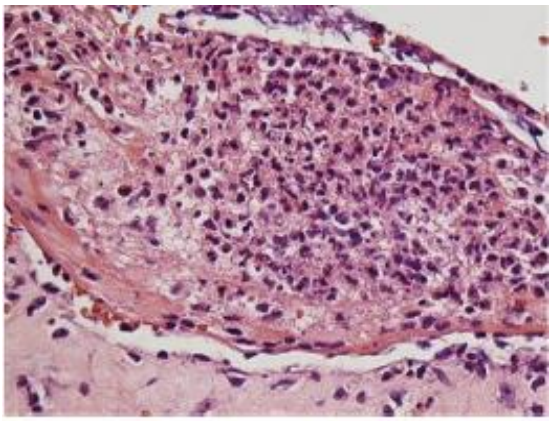
VI. Histopathological Changes Between The Healthy And Burnt Skin (Junctional Skin) In Burn Cases

k. Capillary dilatation 60
l. Oedema 59
m. Congestion 67
n. Leucocytic infiltration 67

The Prospective analytical study of 100 patients admitted to the Burns Unit, 46 (46%) were male and 54 (54%) were female, 88 (88%) flame burns and 12 (12%) were scalds burns (Table 2). Percentage of body surface area burns 10% -30% is 58% , 30% - 50% is 36% and >50% is 6% , the patients 56% (*N* = 56) were discharged after full recovery and 36% (*N* = 36) after partial recovery; 6% (*N* = 06) died (Table 2). Most of the patients who died

were >50% body surface burns and elderly patients. Most of the patients who died had flame burns, followed by scald skin burns. Death is mainly due to infection, hypovolaemic shock and acute renal failure.

HISTOPATHOLOGICAL EXAMINATION:



During the inflammatory stage, the most prominent changes were infiltration of neutrophils and extensive edema and necrosis (Figure. 2).

Figure. 1. Burned skin during inflammatory stage (24 h post burn) showing acute inflammation, extensive edema and necrosis

In the proliferation stage: by the day 3, macroscopically, crust was formed from the necrotic tissues and microscopically this crust was rejected from the underlying viable tissues along with the zone of neutrophils infiltration. By the day7, neutrophils were replaced largely by macrophages with the early formation of granulation tissue and new blood vessels. By the day 9, the neovascularization reaches its peak with started scab formation. By the end of inflammatory and starting of proliferative stage, decrease in the neovascularization with started deposition of few collagen fibers from accompanied fibroblasts was noticed. The

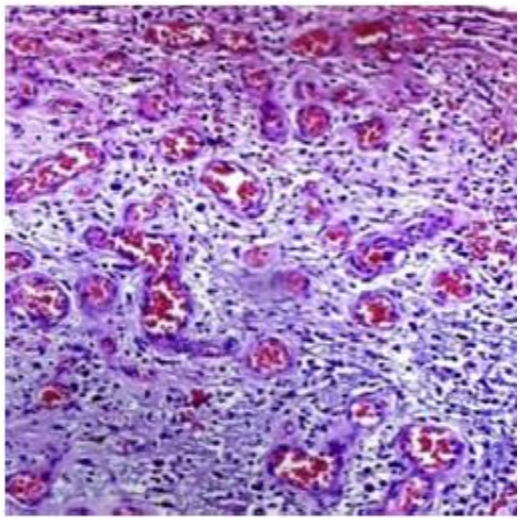


Figure. 2. Burned skin during proliferation stage -day 7, showing extensive acute inflammation, neovascularization

edema fluid started to decrease with increased infiltration with histiocytes, lymphocytes and plasma cells

In the remodeling stage -14 - 28 days, the number of inflammatory cells decreased and collagen accumulation and fibroblast proliferation was increased till all the burnt area started to be replaced by collagen at the end of the stage - Figure. 3.

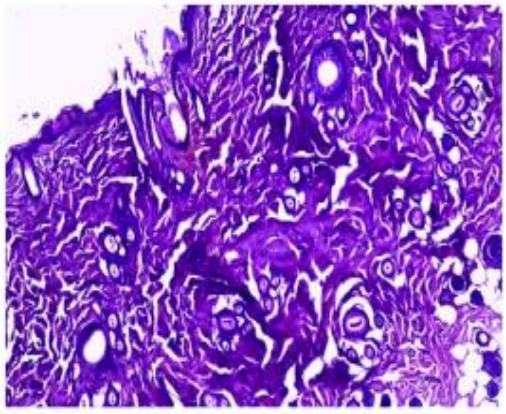


Figure. 3. Burned skin during remodeling stage –day 15, showing dense collagen deposition

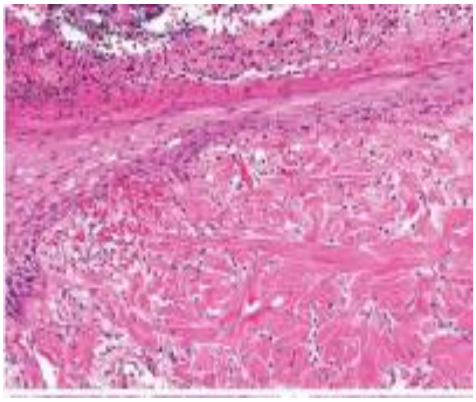


Figure.4. Epithelial responses to infection

Reepithelialized sections in infected wounds contained minimal amounts of compact keratotic and parakeratotic hyperkeratosis and moderate to marked rete-peg formation with significantly delayed wound healing.

Infected wounds retained necrotic tissue and were slower to infiltrate with granulation tissue at days 7 and 14.

Table- 3: Overall results of the cases studied

Details of Isolation	**Number**	**Percentage**
Monomicrobial	36	36%
Polymicrobial	46	46%
Sterile	18	18%
Total	100	100%

Of the 100 swabs, 36 (36%) were monomicrobial pathogens, 46 (46%) were Polymicrobial pathogens and 18(18%) were sterile. The presence of more than one species isolated from the sample was the most frequent (46%) while, one species were isolated (36%) and sterile were 18% from 100 samples. (Table 3).

Table - 4: Distributions of Isolates in various Skin Burns

Details of Isolation	**Flames N %**	**Scalds N%**
Monomicrobial	32 (36.1%)	4(33.33%)
Polymicrobial	44 (52.27%)	2 (16.67%)
Sterile	12 (13.3%)	6 (50%)
Total	88	12

Highest infection rate was observed in Skin burns due to Flames (52.27%) and lowest rate was in Scalds skin burns (16.67%) (Table - 4).

Table - 5: Aerobic bacterial pathogens isolated from Skin Burn cases

Organisms	**Monomicrobial**	**Polymicrobial**	**Number of Isolates**	**Percent Among the Isolates**
Staphylococcus aureus	9	15	26	21.67%
Staphylococcus epidermidis	5	7	12	10%
Enterococcus faecalis	0	2	2	0.01%
Klebsiellapneumoniae	8	10	18	15%
Escherichia coli	7	13	20	16.67%
Pseudomonas spp.	14	26	40	33.34%

Total Number of Specimens – 100, Total Number of Isolates – 120

A total of 120 bacterial isolates were obtained, 78(65%) were aerobic gram negative bacilli. While 40 (33.33%) were aerobic gram positive cocci. Staphylococcus aureus was the predominant organism isolated 26 (21.67%). Pseudomonas aeruginosae was the predominant gram negative bacilli isolated 40 (33.34%), followed by Eschericia coli 20 (16.67%) and Klebsiellapneumoniae 18 (15%) (Table–5). A study conducted by Rajput et al. also showed that P. aeruginosa (55%) was the most common isolate in burn wound infections, followed by S.aureus (19.29%) (Backstein R *et al.*, 1993).

Table-7: Antibiotic sensitivity pattern of aerobic Gram-positive cocci isolated from Skin Burn cases.

Antibiotic	**Staphylococcus aureus**		**Staphylococcus epidermidis**		**Enterococcus fecalis**	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Penicillin	0	100	14	86	-	100
Erythromycin	75	25	76	24	68	32
Clindamycin	82	18	74	26	-	-
Amikacin	84	16	74	26	-	-
Gentamicin	65	35	75	25	72	28
Ciprofloxacin	35	65	20	80	-	-
Co-trimoxazole	53	47	20	80	-	-
Oxacillin	82	18	82	18	-	-
Vancomycin	100	0	-	-	100	-
Novobiocin	-	-	100	-	-	-

In our study, all Gram-positive cocci showed 100% susceptibility to Vancomycin. Followed by Amikacin (84%), Clindamycin (82%) and Oxacillin (82%), Erythromycin (75%), Gentamicin (65%) and the least effective drug was Ciprofloxacin. In addition, 18% of the isolates of Staphylococcus aureus were resistant to Oxacillin. The Gram-positive bacterial isolates were found to be 100% resistant towards Penicillin (Table- 7).

Table-8: Antibiotic sensitivity pattern of aerobic Gram-negative bacilli isolated from Skin Burncases.

Antibiotic	**Klebsiellapneumoniae**		**Escherichia coli**		**Pseudomonas Aeruginosae**	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ampicillin	10	90	8	92	-	-
Amikacin	96	4	92	8	84	16
Gentamicin	82	18	94	6	78	22
Cefotaxime	26	74	18	82	16	84
Ceftriaxone	35	65	28	72	28	72
Ceftazadime	22	78	26	74	65	35
Ciprofloxacin	46	54	21	79	25	75
Co-trimoxazole	48	52	32	68	14	86
Amoxyclav	30	70	25	75	15	85
Piperacillin/ tazobactam	72	28	76	34	82	18
Ticarcillin/ clavulanate	26	74	39	61	-	-
Ticarcillin	-	-	-	-	78	22
Imipenam	100	-	100	-	100	-

Among all Gram negative isolates showed 100% susceptibility to Imipenam. Klebsiellapneumoniae showed 96% Sensitivity to Amikacin, Escherichia coli showed 92% sensitivity to Amikacin, Pseudomonas aeruginosae Showed 100% sensitivity to Imipenem& (84%) sensitivity to Amikacin.

DISCUSSION

Burns continue to be a major environmental factor responsible for significant morbidity and mortality in developing Countries. In our study, a positive correlation was also found between type and degree of burn, scalds usually being more superficial and flame burns deeper. Care of patients with full-thickness burns is more difficult and takes longer than care of those with less severe burns. Infection may be caused by factors such as a delay in admission to emergency service, introduction of non-sterile materials to the wound, covering the patient with non-sterile sheets or blankets, or increased susceptibity to NCIs because of nutritional deficits (Lari AR 2000, Gomez-Cia T 1999, Bu¨ttemeyer R 2004).

This study was carried out to evaluate histopathological features corresponding, different bacterial aetiological agents of burn wound infections with their antimicrobial susceptibility.

These differences, which included epithelial migration and proliferation, stromal necrosis, fluid accumulation and inflammatory cell responses, were identified in infected wounds. We observed that the predominance of vacuolated macrophages and multinucleated giant cells in wounds might correlate to the bacterial infections. Mono-species infected wounds developed a hyper-proliferative wound edge. Coinfected wounds had the largest wound sizes; increased amounts of neutrophilic inflammation, immaturity of the wound bed, retention of necrotic tissue and inhibited wound contracture were evaluated (Gordon S 2003, McWhorter FY 2013). As wounds heal, the removal of necrotic tissue is a pivotal event to aide in the clearance of inflammatory cells, nidi for bacterial infection, granulation tissue formation and epithelial migration. Retention of necrotic tissue is associated with delayed wound healing and chronic wound formation (Schultz GS *et al.,* 2003).

In the present study, monomicrobial infection occurred in 36 (36%) cases. Polymicrobialinfection occurred in 46 (46%) cases. Among the monomicrobial infections, P. aeruginosa 40 (33.34%) was the most common organism obtained. A study conducted by Rajput et al. also showed that P. aeruginosa (33.34%) was the most common isolate in burn wound

infections, followed by S. aureus (21.67%) (Rajput A *et al.*, 2008). Among the polymicrobial infections, the combination of P. aeruginosa and S. aureus was the commonest. This is in accordance with the study conducted by Nagoba*et al.*, 1999.

Antibiotic sensitivity pattern

Pseudomonas aeruginosae

In the present study, among the isolates of *P. aeruginosa* were sensitive to Imipenem (100%), Amikacin (84%) and Piperacillin-Tazobactam (82%). A study by Agnihotri*et al.* showed Imipenem and Piperacillin-Tazobactam was the most effective drug against *P. aeruginosa.* In the present study sensitive to Gentamicin (78%) and Amikacin (84%). This is quite alarming as aminoglycosides are the mainstay of treating *Pseudomonas* sepsis. This finding is similar to the study finding of Branski*et al*.

Staphylococcus aureus

In the present study, all the MSSA were sensitive to Amikacin and Vancomycin. This finding is similar to the study findings of Sarma et al. [20] A high degree of Penicillin resistance was noted in our study. In the present study, all MRSA isolates were sensitive to Vancomycin, This finding is similar to the study findings of Shehab et al.[21] and Revathy et al. [22] All the resistant isolates of E. coli and K. pneumoniae were extended spectrum beta-lactamase (ESBL) producers. The percentage of multidrug-resistant (MDR) isolates is probably due to empirical use of broad-spectrum antibiotics and nonadherence to hospital antibiotic policy. Therefore, careful microbiological surveillance and in vitro testing before the start of antibiotic therapy and strict antibiotic policy may be of great help in prevention and treatment of MDR isolates in burn units and, thus, reduction of overall infection related morbidity and mortality.

CONCLUSION:

Further studies are needed to understand the significance of mono-species infected wounds and polymicrobial-infected wounds with their correlation by histopathological findings. This study will allow researchers to study novel treatment modalities with host and bacterial responses.

ACKNOWLEDGMENTS

We acknowledge the cooperation rendered by the staffs of the Microbiology department of Sri Venkateswara Medical College, Tirupati, India, and like to acknowledge our second and third authors for carrying out manual statistical analyses of the data.

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