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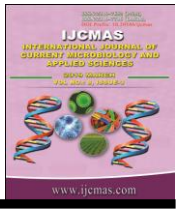

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Study of Microbial Profile, Antimicrobial Susceptibility of Burns Wound Infection with Specific Reference to Phenotypic Detection of MRSA and ESBL Producing Isolates in a Tertiary Care Hospital

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A B S T R A C T

Burn wound infections are most serious and often lead to life- threatening complications which are the major cause of morbidity and mortality in burns patients. Generally burn wound has high susceptibility rate of bacterial colonization and infection, this is because of destroyed skin barrier due to burns and which leads to direct bacterimic spread. To detect the prevalence of MRSA producers among Gram positive cocci and ESBL producers among Gram negative bacilli from the burn wound isolates. This Prospective study was conducted for 3 months (May, June July 2017) at Govt. Mohan Kumaramangalam Medical College Hospital, Salem. Under standard protocol 2 Pus samples were collected from each burns patient after 4-5 days of admission irrespective of their age and gender. The isolates were screened for MRSA & ESBL. A total of 76 samples were collected from patients with burns wound infection. Out of that 40 (52.6%) were females and 36 (47.3%) were male patients. Among the total patients, 42% were 20-30yrs, 35.5% were 30-40yrs, 7.8% were 40-50% yrs and 14.4% more than 50 yrs. Out of total samples, 74 (97.3%) were culture positive. 13 (17.1%) were showed poly microbial growth. Among total GPC, 9(40.9%) were MRSA isolates (confirmed by Cefoxitin sensitivity), of which 6(27.2%) were *Staphylococcus aureus* and 3 (13.6%) were CONS. Among total GNB, 32 (49.2%) were screened as ESBL producers by Ceftazidime sensitivity. The number of isolates confirmed as ESBL producers were 28 (43%) by Combined disc test. So screening of all the isolates in burn wound infection is very much essential. Regular monitoring of burn wound infection is an important tool for infection control practice. Even though the mortality is in-evitable in >75% burns patients, by doing proper surveillance activity in all aspects, treatment failure and morbidity can be prevented.

Introduction

Burns are one of the most common and devastating forms of trauma. Since the burns patients are ideal hosts for opportunistic infections, they are most frequently prone to get infection. Burn wound infections are most serious and often lead to life- threatening complications which are the major cause of morbidity and mortality in burns patients [(1)]. Generally burn wound has high susceptibility rate of bacterial colonization and infection,

this is because of destroyed skin barrier due to burns and which leads to direct bacterimic spread. The next significant precipitating factors for complications are necrotic tissue and protein-rich exudates that provide a rich growth medium for microorganisms and release of toxic substances leading to septic shock [(2)]. Since burn units are common places where explosive and prolonged outbreaks of infections caused by resistant organisms, periodical surveillance of potential pathogensandtheirsusceptibilitypatternwillhelp inearlymanagementofburnwound infection and thereby reduce the morbidity and mortality of burn patients with septicemia to some extent[(3)]. Patterns of antimicrobial agent usage and bacterial resistance in each community should be known and considered when prescribing antimicrobial agents and it has a significant role in infection control practices[(3)]. Inappropriate and prolonged empirical treatment of severe infections caused by resistant pathogens like MRSA (Methicillin resistant *Staphylococcus aureus*)/ ESBL (Extended Spectrum Beta Lactamase) producers has been associated with increased morbidity and the spread of resistant organisms in the community[(2)].

MRSA is prevalent in hospitals, prisons and nursing homes, where people with open wounds, invasive devices such as catheters are common. Patients in Immuno- compromised status are highly susceptible to get nosocomial infection (hospital- acquired infection). MRSA began as a hospital-acquired infection, but has developed limited endemic status and is now community-acquired as well as livestock-acquired. Extended Spectrum Beta-lactamase (ESBL) isolates incidence have been increasing sharply and have become recognized as a major worldwide problem. ESBL producing organisms have been detected in every inhabited continent. Several works have been done by Tsegayesuvanet *et al.,* Yekatit burn centre, Ethiopia[(12)] and in India, by Rajeshwar Rao *et al.,* in Hyderabad, Vimal Rathod *et al.,* and Vishaka Shikhare *et al.,* in Maharashtra, Sukumar nirmala *et al.,* in Salem, Lakshmi *et al.,* in Vishakapatnam, Jyothi Bhat *et al.,* on antimicrobial susceptibility pattern of burns wound infections. Similarly, this study exhibits the prevalence of MRSA and ESBL isolates among the burns wound patients.

The main aim of this study includes to detect the aerobic bacterial profile and their antimicrobial susceptibility pattern in patients with burn wound infection. To detect the prevalence of MRSA producers among Gram positive cocci and ESBL producers among Gram negative bacilli from the burn wound isolates. And also to determine the antimicrobial susceptibility pattern for MRSA and ESBL isolates.

Materials and Methods

This Prospective study was conducted for 3 months (May, June July 2017) at Govt. Mohan Kumaramangalam Medical College Hospital, Salem. Under standard protocol 2 Pus samples were collected (by using sterile cotton swabs) from each burns patient after 4-5 days (first week) of admission irrespective of the their age and gender. The samples were collected before the wounds cleaned with antiseptic lotions. The pus swabs were transported to Microbiology laboratory without any delay for further processing [(4)].

After receiving the pus swabs in duplicate, the first swabs were used for Gram's staining and second swabs were inoculated in Nutrient agar, MacConkey agar and 5% sheepblood agar for culture. The inoculated culture plates were incubated aerobically at 37ºC for 24-48 hours. After incubation, the bacterial isolates were correlated with Direct Gram's smear and the isolates were further Identified and spceiated by standard biochemical tests[(4,5)].
The isolates were tested for Antimicrobial

sensitivity by using Kirby Bauer disk diffusion method according to the CLSI guidelines with commercially available antimicrobial discs (Hi-Media) on Mueller Hinton agar plates. The *Staphylococcus aureus* ATCC25923 strain and Escherichia coli ATCC25922 were used as control strains for Gram positive cocci and Gram negative bacilli respectively [4,5,6]. The panel of antibiotic discs used was as follows;

Gram positive cocci –Ampicillin (10mcg), Amoxyclav (30/10mcg), Erythromycin (15mcg), Cefotaxime (30mcg), Amikacin (30mcg), Ciprofloxacin (5mcg), Doxycycline (10mcg), Cefaperazone/ Sulbactum (75/10mcg), Linezolid and Vancomycin (30mcg).

Gram negative bacilli-Amoxyclav (30/10mcg), Gentamicin (10mcg), Amikacin (30mcg), Ciprofloxacin (5mcg), Cotrimoxazole (23.75/1.25mcg), Doxycycline (10mcg), Cefotaxime (30mcg), Ceftazidime (30mcg) Cefaperazone/ Sulbactum (75/10mcg), Piperacillin/ Tazobactum (100/10mcg) and Imipenem (10mcg).

Phenotypic Detection of MRSA

According to the CLSI guidelines (2016) for screening and confirmation of Methicillin resistance (MRSA isolates) Cefoxitin (30 µg) disc was used. After over night incubation the zone of inhibition was measured and the zone size ≤21mm considered as a MRSA isolate and >21mm zone size considered as a MSSA isolate [6,7,8]

Phenotypic Detection of ESBL

Screening of ESBL

After antimicrobial susceptibility test, ESBL producers were screened presumptively by measuring the zone of inhibition to Ceftazidime. The zone of inhibition ≤ 22mm for Ceftazidime was considered as ESBL producers and these isolates were confirmed by phenotypic methods such as Combined Disc Test (CDT).

Phenotypic confirmation of ESBL (Combined Disc Test-CDT)

According to CLSI guidelines, *E. coli* ATCC 25922 as a negative control and *Klebsiella pneumoniae* ATCC700603 as a positive control was used for confirmation of ESBL. Discs containing Ceftazidime (30µg) alone and Ceftazidime combined with Clavulanic acid (CAC - 10µg) are placed on MHA plate at a distance (edge to edge) of 20mm. After overnight incubation the zone of inhibition around the Ceftazidime/Clavulanic acid disc ≥5mm than the zone around the Ceftazidime disc alone was confirmed as an ESBL producing isolate [6,9,10] (Figure 2).

Results and Discussion

A total of 76 samples were collected from patients with burns wound infection in a tertiary care hospital. Out of that 40 (52.6%) were females and 36 (47.3%) were male patients. Among the total patients, the frequency of age group is as follows;42%were 20-30 yrs, 35.5% were 30-40 yrs, 7.8% were 40-50% yrs and 14.4% more than 50 yrs (Table 1). Out of total samples, 74 (97.3%) were culture positive. Among total positive culture 87 isolates were recovered as pathogens because 13(17.1%) were showed poly microbial growth.

Among 87 isolates, 22(25.2%) were found to be Gram positive cocci and 65 (74.7%) were found to be Gram negative bacilli. Among the total isolates, the frequency of isolates is as follows: *Staphylococcus aureus* 18(20.6%), CONS 4(4.5%), *Klebsiella* spp., 42(48.2%), *Citrobacter* spp., 7(8%), *E. coli* 4(4.5%), *Proteus* spp., 3(3.4%), *Providencia* spp., 2(2.2%), *Acinetobacter* spp., 3(3.4%),

Pseudomonas aeruginosa 4(4.5%) [Chart-1]. The antimicrobial sensitivity pattern for the isolates were as follows: GPC showed sensitivity to Ampicillin (36%), Amikacin (73%), Erythromycin (23%), Ciprofloxacin (27%), Cotrimoxazole (23%), Doxycycline (27%), Amox/clav (27%), Cefotaxime (45%), Cefoperazone/ Sulbactum (73%), Linezolid (100%) and Vancomycin (100%) (Table 2 and Fig. 1).

GNB showed sensitivity to Amikacin (51%), Gentamicin (25%), Ciprofloxacin (17%), Cotrimoxazole (12%), Doxycycline (29%), Amox/clav (14%), Ceftazidime (50.7%) Cefotaxime (22%), Cefoperazone/Sulbactum (97%), Piperacillin/ Tazobactum (92%) and Imipenem (100%) (Table 3).

Among total GPC, 9(40.9%) were MRSA isolates (confirmed by Cefoxitin sensitivity), of which 6 (27.2%) were *Staphylococcus aureus* and 3(13.6%) were CONS. Among total GNB, 32(49.2%) were screened as ESBL producers by Ceftazidime sensitivity. They were subjected to confirmation test by Combined Disc Test, after which 28 (43%) were confirmed as ESBL producers. So the number of isolates confirmed as ESBL producers were 28(43%) (Table 4).

The sensitivity pattern for MRSA isolates were as follows; 66% to Amikacin, 22% to Erythromycin, 44% to Ciprofloxacin and Cotrimoxazole, 33% to Doxycycline, 55%to Cefotaxime, 22%to Amox/clav and 100%to sensitivity towards Linezolid and Vancomycin and all the isolates were resistant to Ampicillin.

The sensitivity pattern for the ESBL isolates were, 92% to Amikacin, 53% to Ciprofloxacin, 35% to Cotrimoxazole, 25% to Doxycycline, 42% to Cefotaxime, 46% to Amox/clav, 92% to Pip/Taz and 100% sensitive to Cefoperazone/ sulbactum and Imipenem. (Chart-2). In our study out of 76 samples 74(97.3%) samples were culture positive and it is supported by Vishakha *et al.,* (96%)[11], Herjinder *et al.,* (95%) and contradicted by Rajeshwar Rao *et al.,* (69.8%) study. In our study 13(17.1%) samples showed mixed growth. This is supported by Muhammed *et al.,* (29%) and contradicted by Rajeshwar *et al.,* (4.6%). This present study showed incidence towards female patients (52.6%) more than male (47.3%). This supported by Lakshmi *et al.,* [12] and Rajeshwar *et al.,* (59.3% and 56.2% respectively). Females are more prone to get accidental burns in kitchen.

Our study showed the most commonly affected age group as 20-40yrs. This is supported by Herjinder kaur *et al.,* and Rajeshwar *et al.,* studies (20-40yrs) and contradicted by Muhammed Naveed *et al.,* (20-30yrs) study[13].

The present study showed 22(25.2%) GPC and 65 (74.7%) GNB out of total isolates. This is similar to the results of Sukumar nirmala *et al.,*[14] study in which GNB 35%, and GPC 18% and also with Herjinder et al, Muhammed *et al.,* studies. But the study by Rajeshwar Rao *et al.,* showed GPC is 48.95% and GNB is 51.05% which is almost equal.

The present study showed that, *Klebsiella* spp., (48.2%) was the predominant isolate, followed by *Staphylococcus aureus* (20.6%) among total isolates. This is supported by Sukumar Nirmala *et al.,* study in which *Klebsiella* spp., (35%) followed by *S. aureus* (18%) were found to the predominant isolates. In contrast S. Rajeshwar Rao *et al.,* showed S aureus (42%) followed by *Klebsiella* spp., (31%) as predominant. But in Vishakha *et al.,* study, *Pseudomonas* spp (29%) followed by *S. aureus* (15%) and in Lakshmi *et al.,* study *Pseudomonas* aeruginosa (34%) followed by *S. aureus* (22%) were the predominant isolates.

Table.1 Age wise distribution of burn wound infection

S.No	Age group	Percentage
1	20-30	42%
2	30-40	35.5%
3	40-50	7.8%
4	>50	14.4%

Table.2 Antibiotic sensitivity pattern for GPC in Burn wound infection

Name of the drug	S aureus (n=18)	CONS (n=4)	Total (%) (n=22)
Ampicillin	7(39%)	1(25%)	8(36%)
Amikacin	12(67%)	4(100%)	16(73%)
Erythromycin	422%)	1(25%)	5(23%)
Ciprofloxacin	5(28%)	1 (25%)	6(27%)
Cotrimoxazole	4(22%)	1(25%)	5(23%)
Doxycycline	5(28%)	1(25%)	6(27%)
Cefotaxime	8(44%)	2(50%)	10(45%)
Cefoperazone/Salbactum	14(78%)	2(50%)	16(73%)
Amox/clav	5(28%)	1(25%)	6(27%)
Linezolid	18(100%)	4(100%)	22(100%)
Vancomycin	18(100%)	4(100%)	22(100%)

Table.3 Antibiotic sensitivity pattern for GNB in burns wound infection

Name of the Antibiotic	*Klebsiella* spp., (n=42)	E.coli (n=4)	*Proteus* spp., (n=3)	*Citrobacter* spp., (n=7)	Providen n cia (n=2)	Pseudo (n=4)	Acineto (n=3)	Total GNB (n=65)
AK	16(38%)	4(100%)	2(67%)	4(57%)	2(100%)	3(75%)	2(100%)	33(51%)
G	5(12%)	3(75%)	1(33%)	3(43%)	1(50%)	2(50%)	1(33%)	16(25%)
CIP	4(10%)	1(25%)	1(33%)	2(29%)	150%)	1(25%)	1(33%)	11(17%)
COT	4(10%)	1(25%)	0	1(14%)	0	0	2(67%)	8(12%)
DO	11(24%)	4(100%)	1(33%)	3(43%)	0	0	0	19(29%)
CTX	7(17%)	1(25%)	1(33%)	2(29%)	1(50%)	1(25%)	1(33%)	14(22%)
CAZ	17(40.4%)	1(25%)	3(100%)	6(85.7%)	0	3(75%)	3(100%)	33 (50.7%)
CFS	36(86%)	3(75%)	3(100%)	5(72%)	2(100%)	3(75%)	2(67%)	63(97%)
AMC	5(12%)	0	0	2(29%)	0	1(25%)	1(33%)	9(14%)
PIT	**32(76%)**	**3(75%)**	**3(100%)**	**7(100%)**	**2(100%)**	**4(100%)**	**3(100%)**	**60(92%)**
IMP	42(100%)	4(100%)	3(100%)	7(100%)	2(100%)	4(100%)	3(100%)	65(100%)

AK – Amikacin, G- Gentamicin, CIP - Ciprofloxacin, COT- Cotrimoxazloe, DO – Doxycycline, CTX- Cefotaxime, CAZ– Cetazidime ,CFS- Cefoperazone/Sulbactum, AMC-Amoxyclav, PIT- Piperacillin/Tazobactum, IMP-Imipenem

Table.4 Prevalence of MRSA and ESBL among burn wound isolates

% of MRSA (GPC)		% of ESBL(GNB)	
S aureus	CONS	By CAZ – S	By CDT
27.2%	13.6%	49.2%	43%

Chart.1

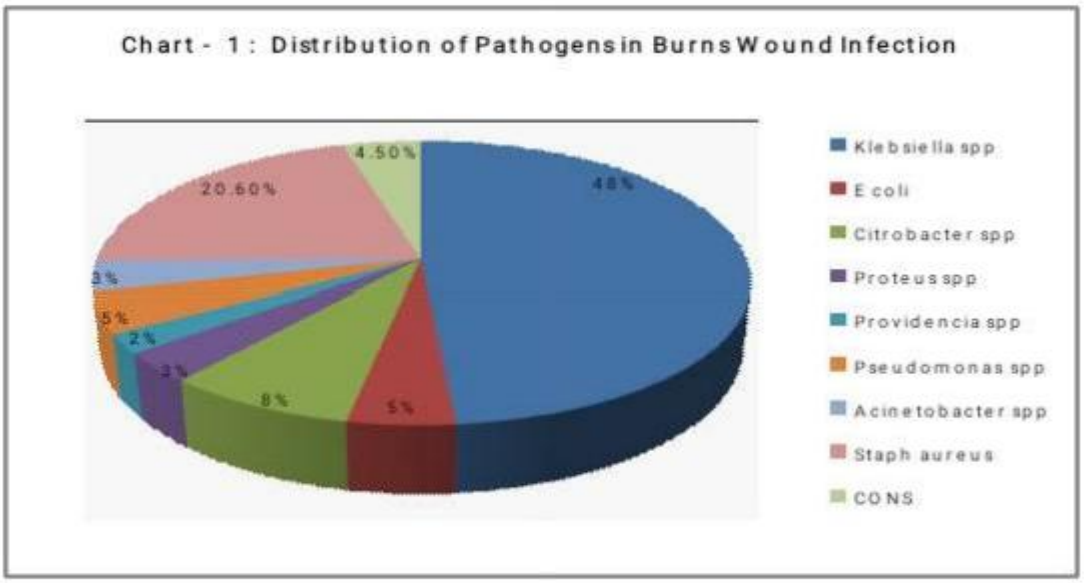


Chart.2 AST pattern for MRSA and ESBL isolates

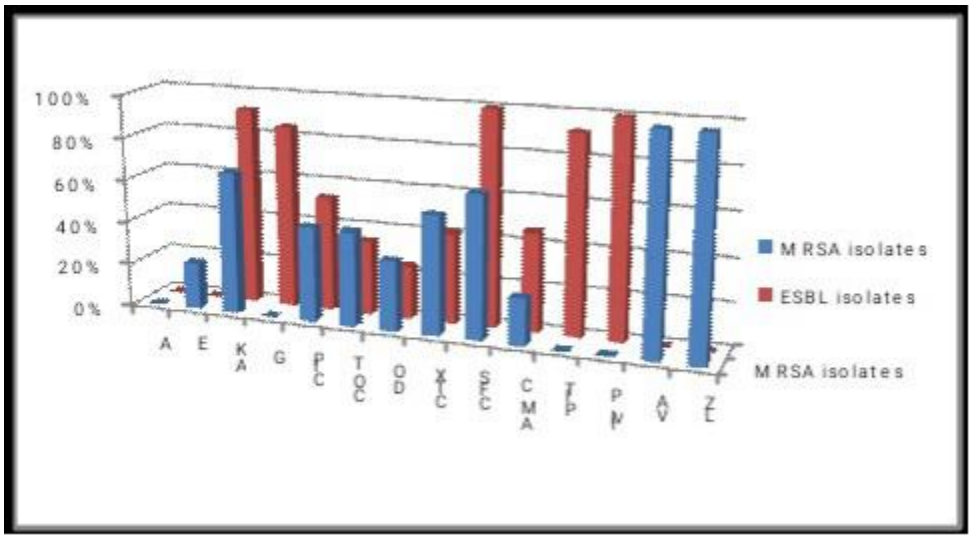
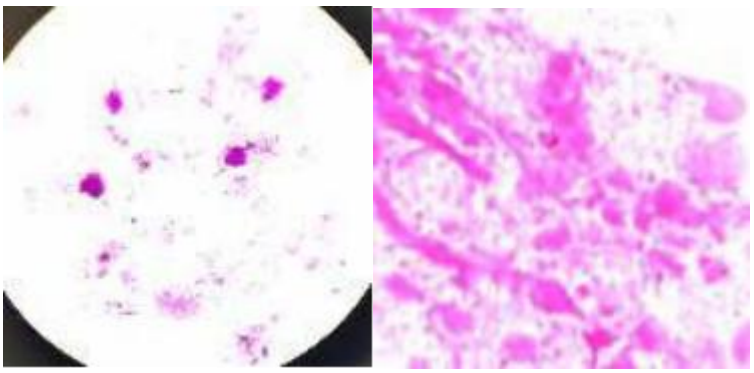


Fig.1 Direct Gram's stain picture shows GNB (a) and GPC (b) with pus cells



(a) (b)

Fig.2 Shows ESBL producer in CDT (a) and in AST(b) – shows the difference in zone of inhibition

(a) (b)

In Sanjay *et al.,* [16] study also showed *S.epidermidis* (56%) and Sulaiman *et al.,* [17] study showed *Pseudomonas* aeruginosa (36%) was the predominant isolate. This may be due to variability in the prevalence and environmental distribution of pathogens.

The present study showed that all GPC were 100 % sensitivity to Linezolid and Vancomycin followed by Cefoperazone/ sulbactum, Amikacin and Cefotaxime (73%, 73% and 45%). This almost correlates with the results of Sukumar Nirmala *et al.,* i.e. Vancomycin (100%) followed by 60% to Betactum drugs and 59% to Aminoglycosides. In contrast the Rajeshwar *et al.,* showed 100% sensitivity showed to Vancomycin and followed by Ofloxacin and Amoxyclav and Amikacin (79%, 68% and 52%) All the GNB isolates were 100% susceptible to Imipenem followed by Cefperazone/ Sulbactum, Piperacillin/ Tazobactum, and Amikacin (97%, 92% and 51%). This is almost similar to the results of Vimal Rathod *et al.,*[15] (IMP-100%, AK-94% and PIT- 90%), Lakshmi et al (Meropenem-100%, AK-92% and PIT-86%) and Vishakha *et al.,* (IMP-100%, AK-71% and Ceftriaxone –54%). In contrast, Rajeshwar *et al.,* study showed IMP-100%, Ciprofloxacin -86% and PIT-71% sensitivity.

Our study showed 40.9% MRSA isolates. This is similar to the results of Lakshmi *et al.,* study (39%) and contrast with Vimalrathod *et al.,* Vishakha *et al.,* and Rajeshwar *et al.,*

studies were 59%, 54% and 45.9% respectively. But the MRSA isolates were only 7.3% in the study of Tsegayesewvnet *et al.,*

In our study the prevalence of ESBL was 43% (CDT). This is similar and almost coincides with Rajeshwar *et al.,* and Vishaka *et al.,* (30.9% and 37%) and but is in contrast with Vimal Rathod *et al.,* study which showed a higher rate (61.4%) and with Lakshmi *et al.,* study which showed a lower rate (28%). Our study showed the treatment choice for MRSA were Vancomycin and Linezolid and for ESBL Betalactum/clav combinations like Piperacillin/ Tazobactum, Cefperozone/ sulbactum and Carbapenems. This supported various studies like Rajeshwar *et al.,* and Sukumar Nirmala *et al.,* Always we prefer Vancomycin and Carbapenemsare as reserve drugs for GPC and GNB respectively.

Resistant pathogens (in GPC and GNB) cause major life threatening infections in hospitalized patients now days. The prevalence of high percentage of Multidrug resistant isolates are probably due to prolonged empirical use of broad- spectrum antibiotics, prolonged hospitalization, colonization of normal flora and non-adherence to hospital antibiotic policy[(11)]. Regarding burns wound infection, ensuring strict infection control practices reduce the incidence of infections due to these Multidrug resistant organisms[(15)].

In conclusion, burn wound infection is one of the most common serious complication following burn injury. Infection is a major cause of morbidity about 75% in hospitalized burns patients in developing countries. Over crowding is an important cause of cross infection in burns ward[(1,10)]. Regular monitoring of burn wound infection is an important tool for infection control practice[(11)]. Since the frequency of isolates and their sensitivity patterns are varied from one area to another and emerging of many resistant isolates, detection of MRSA and ESBL producing isolates and their antimicrobial susceptibility pattern will help the clinician to select appropriate antibiotics for treatment and thereby prevent he secondary infections in burns[(1,15)]. Even though the mortality is in-evitable in >75% burns patients, by doing proper surveillance activity in all aspects and will decrease the morbidity of patients and most importantly prevents the spread of resistant isolates from one to another and thereby we can prevent the Hospital acquired infection in hospitalized patients.

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