Original article:

ESBL producing GNBs in burn wound infections – An alarming situation

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Abstract:

Burns are one of the most common form of trauma. These patients are always at higher risk of infection because of destruction of normal skin barrier, suppressed immunity, prolonged hospitalization and invasive procedures. Even with the availability of the newer antimicrobial agents, burn wound infections still remain major cause of morbidity and mortality. As the antimicrobial susceptibility pattern varies from region to region, and antimicrobial susceptibility profile of organisms from burn unit may not necessarily correlate with identical pathogens recovered from other units in the hospital, it is very essential for every hospital to formulate its own data and profile of common organisms causing burn wound infection with their antimicrobial sensitivity pattern.

We had collected 181 samples from a total of 158 burn patients. A total of 157 aerobic isolates were identified which were further subjected to antimicrobial sensitivity and ESBL detection. *Pseudomonas aeruginosa* was the commonest organism (29.93%) followed by *Staphylococcus aureus (15.28%), Klebsiella pneumoniae* and Coagulase negative staphylococcus (12.73%). All gram negative bacilli were sensitive to imipenem and all methicilin resistant staphylococcal isolates were sensitive to vancomycin. We observed (37.16% of GNBs) amongst ESBL producers commonest was *K. pneumoniae* (30.95%), followed by *E.coli* and *P. aeuginosa* (23.80%).

Key words: ESBL

Introduction:

At the dawn of history of medicine, burns were regarded as an accidental injury that would be complicated by suppuration. [1] Although the incidences of mortality and morbidity resulting from burns have declined over the years, burn wound infections pose serious threat to burn victims. The burn surfaces are sterile immediately following injury but later on colonized by different microorganisms.

The most common colonizers are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The treatment of burns was first described in Beers papyrus in 1500 BC. Hippocrates had followed the principle i. e. simple cleanliness and keep them dry to avoid suppuration. Colebrook and other in 1950's stressed that the burn patients should be treated in separate unit to prevent cross infections. Today most of the hospitals have adopted this policy by establishing burn care units. After the many researches of medical experts from different countries a great amount of experimental data is available which advanced the field of burns treatment. [2, 3]

The diagnosis of burn wound infection can be made clinically, however additional microbiological evidence is needed for instillation of proper antimicrobial therapy. Over last few decades, gram negative organisms have emerged as the most common aetiological agent by virtue of their

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virulence factors and antimicrobial resistance traits. The incidence of extended spectrum beta lactamases producing strains is also steadily increasing. The emergence of antimicrobial resistance among bacterial pathogens limits the available therapeutic options for effective treatment. Thus it is necessary to know the bacterial profile of burn unit, their resistance pattern and mechanism of resistance so as to formulate a policy of empirical therapy and to take preventive measures. In the view of this study was undertaken in the department of Microbiology at a tertiary care teaching hospital from Jan 2013 to Dec 2013.

Material and Methods:

A total of 181 burn wound swab samples of all age groups and both sexes admitted to burn care unit were collected under aseptic precautions and processed immediately. These samples were subjected to microscopy using Gram staining, aerobic bacterial culture by standard microbiological procedures. The organisms grown were identified by standard biochemical tests. All isolates were subjected to antimicrobial susceptibility testing by Modified Kirby Bauer disc diffusion method as per CLSI guidelines. All staphylococci were screened for methicillin resistance by cefoxitin disc diffusion method. [4,5] All the Gram negative bacilli (GNB) were further tested for ESBL production by predictor disc approximation method [6]

Results:

Out of 181 samples collected 134 were culture positive while 24 samples yeilded no growth. The overall isolation rate in the present study was 86.74%. From 134 culture positive swabs, 157 strains were isolated. Amongst these 113 were identified as gram negative bacilli and 44 staphylococci. *Pseudomonas aeruginosa* was the most predominant organism followed by *Staphylococcus aureus* (Table No. 1). Table No 2, 3 and 4 show the antimicrobial resistance pattern of the isolates. ESBL production among gram negative bacilli was observed in 37.16% as depicted in Table no. 5.

Table No.1 Distribution **of organisms isolated.**

Organism	**No.**	%
P. aeruginosa	47	29.93
S. aureus	24	15.28
K. pneumoniae	20	12.73
CoNS	20	12.73
E.coli	16	10.19
P. mirabilis	6	3.82
P. vulgaris	5	3.18
C. diversus	5	3.18
Enterobacter *spp.*	4	2.54
K.a oxytoca	3	1.91
Nonfermenter	3	1.91
Acinetobacter *spp*	2	1.27
C. freundii	2	1.27
TOTAL	157	

Table no.2: Antibiotic resistance among Nonfermenters

ANTIBIOTIC	***P. aeruginosa* n= 47**	***Nonfermenter* n = 3**	***Acinetobacter* n = 2**	**Total**
CAZ(30µg)	30(63.8%)	1(33.3%)	2(100%)	63.46%
GEN (10µg)	20(42.5%)	2(66.7%)	1(50.0%)	44.23%
PIP(100µg)	24(51.06%	1(33.3%)	1(50.0%)	50.00%
CIP(5µg)	23(48.7%)	0(0.0%)	0(0.0%)	44.23%
IPM(10µg)	0 (0.0%)	0(0.0%)	0(0.0%)	0.00%
CPM(30µg)	38(80.8%)	1(33.3%)	2(100%)	78.84%
AK(30µg)	19(40.4%)	2(66.7%)	2(100%)	44.23%

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Table no.3 : Antibiotic resistance among *Enterobacteriaceae*

Antibiotic	*Kleb. pneumoniae (20)*	*E.coli (16)*	*Proteus mirabilis (6)*	*Proteus vulgaris (5)*	*Enterobacter Spp (4)*	*Citrobacter diversus (5)*	*Citrobacter freundii (2)*	*Kleb. oxytoca (3)*	**Total %**
AMP (10µg)	20 (100%)	15 (93%)	6 (100%)	5 (100%)	3 (75%)	3 (60%)	2 (100%)	1 (33.3%)	90.16%
CZ (30µg)	12 (60%)	7 (43.7%)	2 (33.3%)	3 (60%)	2 (50%)	3 (60%)	2 (100%)	2 (66.7%)	54.09%
CXM (30µg)	20 (100%)	10 (62.5%)	3 (50%)	5 (100%)	2 (50%)	5 (100%)	1 (50%)	3 (100%)	80.32%
CTR (30µg)	12 (60%)	6 (37.5%)	2 (33.3%)	5 (100%)	1 (25%)	5 (100%)	2 (100%)	1 (33.3%)	55.73%
COT (1.25/23.75)	13 (65%)	11 (68.7%)	6 (100%)	3 (60%)	2 (50%)	2 (40%)	1 (50%)	1 (33.3%)	63.93%
AK (30µg)	9 (45%)	4 (25%)	1 (16.6%)	0 (0%)	2 (50%)	0 (0%)	1 (50%)	1 (33.3%)	29.50%
GEN(10µg)	17(82%)	10 (62.5%)	6 (0%)	2 (40%)	2 (50%)	1 (20%)	2 (100%)	1 (33.3%)	67.21%
CIP (30 µg)	16 (80%)	10 (62.5%)	2 (33.3%)	2 (40%)	1 (25%)	3 (60%)	2 (100%)	2 (66.7%)	62.29%
IPM (10 µg)	0 (0%)	0 (0%)	0 (0%)	0 0(0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0%
CX (30 µg)	12 (60%)	11 (68.8%)	4 (66.7%)	2 (40%)	0 (0%)	3 (60%)	2 (100%)	1 (33.3%)	55.73%
CAZ (30 µg)	12 (60%)	12 (75%)	3 (50%)	3 (60%)	0 (0%)	1 (20%)	2 (100%)	1 (33.3%)	55.73%

Abbreviations used – AMP-Ampicillin , CZ-Cefazolin , CXM-Cefuroxime , CTR-Ceftriaxone ,COT – Cotrimoxazole , AK-Amikacin , GEN- Gentamicin , CIP –Ciprofloxacin , IPM –Imipenem , CX-Cefoxitin , CAZ-Ceftazidime .

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Table no. 4: Percentage of antiobiotic resistance in Staphylococci

Antibiotic	*S .aureus(24)*	CONS(20)	*TOTAL*
P(10U)	20(83.3%)	12(60%)	72.72%
CX(30µg)	13(54.1%)	7(35%)	45.45%
E(15µg)	9(37.5%)	10(50%)	43.18%
COT (1.25/23.75)	8(33.3%)	10(50%)	40.9%
CIP(5µg)	13(54.1%)	9(45%)	50%
GEN(10µg)	15(62.5)%	10(50%)	56.81%
AMP (10µg)	10(41.6%)	6(30%)	36.36%
TE(30µg)	10(41.6%)	6(30%)	36.36%

Abbreviations used – P- Penicillin, CX – Cefoxitin, E – Erythromycin, COT–Cotrimoxazole, CIP –Ciprofloxacin, GEN –Gentamicin, AMP- Ampicillin, TE- Tetracycilne.

Table no. 5: Production of ESBL among gram negative bacilli

GRAM NEGATIVE BACILLI	ESBL PRODUCERS
P. aeruginosa (n=47)	10 (21.2%)
K.pneumoniae (n=20)	13 (65.0%)
E.coli (n=16)	10 (62.5%)
Proteus spp *(n=11)*	4 (36.3%)
Citrobacter spp*(n=7)*	2 (28.5 %)
Enterobacter Spp. *(n=4)*	0 (0.0%)
K.oxytoca (n=3)	0 (0.0%)
Nonfermenter *(n=3)*	1 (33.3%)
Acinetobacter *(n =2)*	2 (100%)
TOTAL = 113	42 (37.16%)

Discussion:

Burn wound infection is one of the most common and serious complication following burn injury. This makes the burn wound susceptible to infection. Immediately following thermal injury, burn wound surfaces are sterile, eventually these become colonized with gram positive bacteria which survive the thermal insult and heavily colonize the surfaces. These wounds are colonized by other bacteria derived from host's normal gastrointestinal and respiratory tract flora and / or from hospital environment or health care worker's hands. An extensive surface

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with a large mass of dead tissue and free exudation of serum in these patients are favorable for bacterial growth. The character of microbial flora of burn wound changes with time. Gram positive organisms predominate in the initial period, later on replaced by gram negative organisms.[7] Table No 1 shows distribution of organisms in burn wound infection samples in the present study.

Prior to antibiotic era *Streptococcus pyogenes* was the common organism. In early 1950s, after the introduction of penicillin-G, *Staphylococcus aureus* became the common aetiological agent. But in last few decades *Pseudomonas aeruginosa* from patient's endogenous flora and / or environmental source is the most common cause of burn wound infection. The pre-emitant role of *P. aeruginosa* in hospital settings is due to resistance to common antibiotics, antiseptics and disinfectants used in hospital settings. It can survive and multiply even with minimal nutrients. [8]

Agnihotri N *et al* 2004, reported high culture positivity (96%) in the samples from patients of their burn unit. The most common isolate was *P.aeruginosa* (58.95%) followed by *S. aureus* (17.89%), Acinetobacter species (7.22%), Klebsiella species. [9]

Ganesamoni S *et al* 2011, noted that the predominant organisms colonizing the burn wound were *P.aeruginosa* (81.1%) followed by Acinetobacter species and MRSA. [10]

S. Shweta *et al* 2014 reported *P. aeruginosa* (47%), followed by *K. pneumoniae* (25.3%), *A. baumannii* (18.07%), *S. aureus* (7.2%) among burn wound infection. [11]

In our study *P. aeruginosa* showed highest resistance to cefepime (80%) followed by ceftazidime (63%), piperacillin (51%), ciprofloxacin (48.7%) and gentamycin (42.5%) (Table No.2). Among other gram negative bacilli, most were resistant to beta lactam antibiotics i.e. ampicillin (90%), followed by cefuroxime (80%) (Table No.3). Among staphylococci, methicillin resistance was 54% in *S. aureus* and 35% in CoNS. All methicillin resistant staphylococci were sensitive to vancomycin (Table no. 4).

Beta lactam antibiotics are the first line of treatment in burn wound infections. However most of the common organisms are resistant to these antibiotics. The mechanism of antibiotic resistance to beta lactams is production of beta lactamase enzymes such as ESBL and metallobatalactmases. If an isolate is ESBL producer, it is resistant to penicillins, cephalosporins and monobactams. [6]

In our study 37.16% of GNB were ESBL producers. Among these ESBL producers commonest organisms was *K. pneumoniae* (30.95%) followed by *E. coli* and *P. aeruginosa (23.80%)* (Table No.4). Mundhana S. et al (2015) at Solapur reported 27.21% of gram negative bacilli as ESBL producers, of which *K. pneumoniae* was predominant organism followed by *P. aeruginosa, E. coli* and proteus species. [12] Similar results were observed by Bandekar *et al* [13] and Anantkrishnan *et al* [14] in burn infections from India.

The treatment options for ESBL producers are limited and include carbapenems, aminoglycosides, betalactam - betalactamase inhibitor combinations. Carbapenems include imipenem, meropenem; newer drugs like ertapenem, faropenem are most effective and reliable as they are highly resistant to the hydrolytic activity of all ESBLs. Some cephalosporins like cefmetazole, cefotetan and latamoxef are also useful. Non betalactam antimicrobial agents like aminoglycosides and fluroquinolones may be beneficial, however,

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coresistance rates against these agents are frequent.[15]

Once multidrug resistant strains are established in hospital environment these can persist for months which further increases the overall burden. The development of resistance to particular antimicrobial agent is dependent on the use of that agent in that hospital setting. Overuse of any antimicrobial agent predisposes to development of resistance. The high incidence of beta lactamases production in burn wound infection in our study is alarming and needs urgent action. It is known that the antimicrobial susceptibility profile of burn unit microbial flora may not necessarily correlate with identical pathogens recovered from other units in the same hospital. Hence the general hospital antibiogram cannot be relied upon for guiding empirical antibiotic treatment in burn unit patients. [16] Ideally burn units should routinely determine and track the specific pattern of burn wound colonization and antimicrobial susceptibility profiles of organisms involved. Furthermore resistant strains should be screened for resistance mechanisms too.

Conclusion:

This it is very essential to screen all the isolates of burn wound infection for resistance pattern and the mechanism of resistance too. According to us easiest way would be surveillance of microbiology laboratory data that will facilitate the selection of appropriate empirical antimicrobial agent prior to availability of culture and sensitivity report. To reduce the morbidity and mortality in burn patients, strict infection control measures i.e. isolation of patient, use of gowns and gloves during patient care and hand washing before and after each patient visit, appropriate empirical antimicrobial therapy based on periodic survillence data and early detection of mechanism of resistance are key steps.

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