Extended Spectrum Beta Lactamase and AMP C Beta Lactamase Production among Gram Negative Isolates Obtained from Burn Infections

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ABSTRACT

Introduction: Extended spectrum β -lactamases (ESBLs) and AmpC beta lactamses continue to be a major problem in health care settings. Due to the scarcity of information regarding the antibiotic susceptibility patterns particularly from burn infections, the current study was carried out to assist the clinicians to prescribe appropriate antibiotics against gram-negative clinical isolates.

Material and methods: In the current study, a total of 210 samples were collected from burn patients. A total of 134 bacterial isolates were obtained during study period of one year, out of 134 bacterial isolates 114 gram negative isolates were screened for ESBL and MBL production. In the AST testing, the isolates that exhibited reduced zone of inhibition to one or more of the antibiotics such as cefotaxime (≤27mm), ceftriaxone (≤25mm), ceftazidime (≤22 mm), cefpodoxime (≤17mm) and aztreonam (≤27mm) were considered as potential ESBL producers and the ESBL production was confirmed using phenotypic screening test (double disk synergy test) and phenotypic confirmatory test (combined disk test). However, isolates showing resistance or decreased sensitivity to cefoxitin, cefotaxime, ceftriaxone, ceftazidime, cefpodoxime or aztreonam and sensitive to cefepime were considered as a screen positive AmpC producer and subjected to AmpC disk tests.

Results: The current study showed that 33.3% and 14% of ESBL and AmpC producers were detected respectively in our hospital.

Conclusion: Routine screening for ESBL and AmpC production should be done as theses are simple, easy to perform and interpret requiring less expertise.

Keywords: Extended Spectrum Beta Lactamases, AmpC Beta Lactamases, Gram Negative Isolates, Antibiotic Susceptibility Testing

INTRODUCTION

In burn patients thermal destruction of the skin barrier and concomitant depressions of local and systemic host cellular and humoral immune responses are contributing factors for infectious complications. Although burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized by gram positive bacteria, gram negative bacteria and yeasts, derived from host's normal gastrointestinal and upper respiratory tract flora and/or from the hospital environment or that are transferred via a health care worker's hands.[1] The pattern of infection differs from hospital to hospital; the varied bacterial flora of infected wound may change considerably during the healing period. In the past 65 years, antibiotics have been critical in the fight against infectious disease caused by the bacteria and other microbes. Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the Twentieth Century. Despite the advances in patient care and the use of a large number of antimicrobial agents, infections which complicate the clinical course of patients who had sustained severe thermal injures continue to be a major unsolved problem.[2] Multidrug resistant bacteria have been frequently reported as the cause of nosocomial outbreaks of infection in burn units or as colonizers of wounds of burn patients. Incidence of ESBL, AmpC and MBL producing strains among clinical isolates has been steadily increasing over last few years.[3] Antimicrobial susceptibility pattern varies from region to region, 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. It is necessary to study the different mechanisms of beta lactmases among gram negative bacilli, so that early detection of such strains will help for appropriate treatment and to prevent the spread of these isolates in hospital.[4] The present study was undertaken to detect different mechanisms of resistance of isolates from burn wound infection

MATERIAL AND METHODS

The study was conducted in a tertiary care hospital among all the patients having signs of burn infections. During the study period, a total 210 samples were collected from different sites and were immediately transported to the department of Microbiology. Immediately after receipt, specimens were subjected to culture and antibiotic susceptibility testing.

Bacterial strains and Antibiotic susceptibility testing

All the pus samples were cultured on blood agar (Hi media, Mumbai, India) and MacConkey agar (Hi media, Mumbai, India), and incubated at 37^{0}C for 18 to 24 h. After incubation, the bacterial isolates were identified by standard laboratory protocols[5]

The antibiotic susceptibility testing of gram negative bacilli was performed on Muller Hinton agar (Hi media, Mumbai, India) by modified Kirby Bauer disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI)[6] using Ampicillin (10µg), Amoxycillin/ clavulanic acid (20µg +10µg), piperacillin/tazobactum (100+10µg), piperacillin

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(100µg), cinoxacin (100µg), carbencillin (100 µg), ceftriaxone (30 µg), cefipime (30µg), ceftazidime (30 µg), cefoxitin(10 µg), cefpodoxime (30µg), cefotaxime(30µg), ceftizoxime (30µg), aztreonam (30µg), imipenem (10µg), gentamicin (10µg), amikacin(30 µg), tobramycin(10 µg), tetracycline (30µg), co trimoxazole(1.25/23.75µg), ciprofloxacin (5µg). The inoculated AST plates were incubated at 37°C for 16-18 h and the results were interpreted as per CLSI guidelines.[6]

Screening test for ESBL and Amp C beta lactamases

Isolates that exhibited reduced zone of inhibition to one or more of the antibiotics such as cefotaxime (≤27mm), ceftriaxone (≤25mm), ceftazidime (≤22 mm), cefpodoxime (≤17mm) and aztreonam (≤27mm) were considered as potential ESBL producers.[7] However, isolates showing resistance or decreased sensitivity to cefoxitin, cefotaxime, ceftriaxone, ceftazidime, cefpodoxime or aztreonam and sensitive to cefepime were considered as a screen positive AmpC producer and subjected to AmpC disk test.[8]

Phenotypic confirmatory test for ESBL and Amp C beta lactamases (Combined disk test)

This test was carried as per CLSI recommendations, briefly; ceftazidime (30 µg) versus ceftazidime/ clavulanic acid (30 µg/10 µg), (Himedia, Mumbai, India), used as a phenotypic confirmatory test wherein a greater than or equal to 5 mm increase in the zone diameter for the antimicrobial agent tested in combination with B-lactamase inhibitor versus its zone when tested alone indicates ESBL production.[6] AmpC disk test was carried out as recommended by Black et al, briefly; a sterile disk (6 mm) moistened with sterile saline (20 µl) and inoculated with several colonies of test organism was placed beside a cefoxitin disk (almost touching) on the MHA plate lawned with a culture of E. coli ATCC 25922 and incubated overnight at 35°C. A positive test appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk and a negative test had an undistorted zone.[8]

Quality control

Every new batch of culture media was incubated at 37^{0}C for overnight to ensure the sterility. E. coli ATCC 25922 and P. aeruginosa ATCC 27853 were used as quality control strains for antimicrobial susceptibility testing. However, a non-ESBL producing organism E.coli ATCC 25922 and an ESBL-producing organism K. pneumoniae ATCC 700603 was used while testing ESBL screening and phenotypic confirmatory tests.

STATISTICAL ANALYSIS

Descriptive statistics like mean and percentage were used for interpretation with the help of Microsoft office 2007.

RESULTS

Table 1 demonstrates the distribution of all gram negative bacilli isolates obtained from burn infecions. Of the 210 non repetitive specimens processed, a total of 134 bacterial isolates were obtained. Out of the 134 non-repetitive isolates, 20(14.9%) isolates were Gram positive. After exclusion of these Gram positive organisms, a total of 114(85%) Gram negative isolates were subjected to further analysis. Of the 114 GNB isolates, 51(44.7%) showed resistance or decreased sensitivity to any one of these antibiotics such as cefotaxime (≤27mm), ceftriaxone (≤25mm), ceftazidime (≤22 mm), cefpodoxime (≤17mm), aztreonam(≤27mm) and confirmed using combined disk test (Phenotypic confirmatory test). Of these 51 isolates, 38(33.3%) isolates were confirmed for ESBL production using combined disk test. Of these, 12 isolates were E. coli, K. pneumoniae (n=10), P. aeruginosa (n=12), P. mirabilis (n=4), respectivley. Of the 114 Gram negative isolates, 39(34.2%) isolates showed resistance or decreased sensitivity to cefoxitin, cefotaxime, ceftriaxone, ceftazidime, cefpodoxime, aztreonam and sensitive for cefipime. Of theses, 16(14%) isolates were confirmed for AmpC beta lactamase production using AmpC disk test method. Out of the 16 AmpC beta lactamasesse confirmed cases, 4 and 3 isolates were Klebsiella and E. coli spps, respectively.

Gram negative isolates obtained	
P. aerugonosa	38(33.3)
E. coli	19(16.6)
K. pneumoniae	17(14.9)
Proteus spp	10(8.7)
Klebsiella Oxytoca	10(8.7)
Morganlella morganii	07(6.1)
Citrobacter freundii	06(5.2)
Acinetobacter spp	4(3.5)
Enterobacter aerogenes	3(2.6)
Total	114

Table-1: Distribution of all gram negative bacilli isolates obtained from burn infections

DISCUSSION

The ability to produce β-lactamases enzymes is the major cause of resistance of bacteria to β-lactam antibiotics. Numerous β-lactamases are encoded either by chromosomal genes or transferable genes located on plasmids or transposons.[9] Based on amino acid and nucleotide sequence studies, four distinct classes of β-lactamases have been defined. Class A (Extended

Microorganism	Screening test (Positive)		Confirmatory test (Positive)	
	ESBL	AmpC	ESBL	AmpC
P. aeruginosa (n=38)	15 (29.4)	4 (10.2)	8 (21)	2 (13.3)
E.coli(n=19)	13 (25.4)	16 (41.2)	12 (31.5)	3 (20)
K. pneumoniae (n=17)	12 (23.5)	12 (30.7)	10 (26.3)	4 (26.6)
Proteus mirabilis (n=10)	5 (9.8)	3 (7.6)	4 (10.5)	3 (20)
Klebsiella Oxytoca (n=10)	3 (5.8)	3 (7.6)	3 (7.6)	2 (13.3)
Citrobacter spp (n=6)	3(5.8)	-	2 (5.2)	-
Acinetobacter spp (n=8)	-	2 (5.1)	-	2 (13.3)
Total = 114	51 (44.7)	39 (34.2)	38 (33.3)	16 (14)

Table-2: Detection of ESBL and Amp C beta lactamases among Gram negative isolates as indicated by various detection methods

spectrum β-lactamases) class B (Metallo β-lactamases), class C (AmpC β-lactamases) and Class D (Cloxacillin hydrolysing β-lactamases).[10,11] Despite the discovery of ESBLs and AmpC β-lactamases at least a decade ago, there remains a low level of awareness of their importance and many clinical laboratories have problems in detecting ESBLs and AmpC β-lactamases. Confusion exists about the importance of these resistance mechanisms, optimal test methods, and appropriate reporting conventions. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures.[12]
The most common pathogen isolated from burn wounds in our study was *P. aeruginosa* (33.3%), followed by *E.coli* (16.6%), *K.pneumoniae* (14.9%) Proteus spp and Klebsiella oxytoca (8.7%) each. However in study of Varsha Gupta et al, *P. aeruginosa* (34.4%) was found to be most common pathogen followed by *Acinetobacter spp* and *K. pneumoniae* (22.2%) each, *E. coli* and *S. aureus* (8.8%).[13]
In the present study 33.3% isolates were ESBL producers the results were similar to studies by Shukla et al (30.18%) and Taneja et al (36.5%).[14,15] In our study 14% isolates produced inducible Amp C beta lactamases, similar to studies of Ruturaj M. Kolhapure (10.3%) and Rodrigues et al (7%), but study of Sinha P et al showed 24% prevalence of AmpC.[16-18] Our study showed maximum ESBL production in E.coli (31.5%), followed by Klebsiella pneumonia(26.3%) and Pseudomonas aeruginosa (21%) which was similar with the studies conducted by Varsha Gupta et al and Singh et al.[13,19] AmpC β-lactamases are clinically important cephalosporinases encoded on chromosomes of many of the Enterobacteriaceae and a few other organisms[8], where they mediate resistance to cephalothin, cefazolin, cefoxitin, most of the penicillins and β-lactamase inhibitor.[20] In many bacteria Amp C enzymes are inducible and can be expressed at high levels by mutation. Over expression confers resistance to broad spectrum cephalosporins.[21] In our study the maximum AmpC production was seen in K.pneumoniae(26.6%) followed by E.coli(20%) and Acinetobacter spp.(13.3%) which differ from the study conducted by Singhal et al where AmpC β-lactamases was seen mainly in Acinetobacter spp. (28.57%) followed by E. coli (6.97%) and Klebsiella spp. (6.18%).[20] The AmpC disk test was an easier, reliable and rapid method of detection of isolates that harbour AmpC β-lactamases. This suggests that AmpC disk test can be used for routine screening of the AmpC enzyme in the clinical laboratory. The ESBL and AmpC co production was detected in 7.1% of the isolates in the present study, which was in concordance with the studies done by Loveena et al (6.5%) and Kolhapure RM et al (9.7%).[16,22]

CONCLUSION

Drug resistance to antimicrobial agents is a serious threat in burn infection. The present study highlights the high prevalence of various β-lactamases among the multi drug resistant gram negative isolates in burn infection. It also reflects narrow future of the treatment options available for these notorious pathogens. The high incidence of β-lactamases production due to multiple mechanisms in burn infection is alarming and urgent action needs to be taken from both the therapeutic and infection control perspective. Early detection of these β-lactamase producing isolates in a diagnostic laboratory could help to avoid treatment failure, as often the isolates producing this enzyme show a susceptible phenotype in routine susceptibility testing.

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