Original Article

Mechanisms of beta lactamase production in GNBs from burn wound infection

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Abstract

Infection is one of the common causes of morbidity and mortality among hospitalized burn patients. Infection by the multidrug resistant bacteria reduces the therapeutic options for the effective treatment. The increasing incidence of beta-lactamase production due to multiple mechanisms in isolates from burn infection is alarming and poses a significant challenge if remains undetected. The present study was undertaken to evaluate the different mechanisms of resistance in burn wound isolates. A total 181 swabs were collected from patients admitted in burn unit from Jan 2013-Dec 2013. Among 134 culture positive swabs 157 isolates were studied for identification and antimicrobial susceptibility testing. All Gram negative bacilli were subjected to detection of Extended Spectrum of Beta Lactamase (ESBL) by predictor disc approximation method. All *P. aeruginosa* isolates were screened for Metallobetalactamse (MBL) production by Imipenem – EDTA disc diffusion method. *K. pneumoniae* was the commonest ESBL producing organism. Among 31 beta lacatmase producing *P. aeruginosa* strains, 21 were MBL producing, 6 were AmpC and 4 were plain ESBL producers. Among 32 ESBL producing Gram negative bacilli (other than *P. aeruginosa*) 22 isolates were ESBL+Derepressed mutants and 10 were plain ESBL producers.

Keywords: beta lactamase.

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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] Among the wide array of antimicrobial agents, betalactams are most widely used agent accounting for 50% of all systemic antimicrobial agents in use. Gram negative bacteria produce a much greater variety of beta lactmaseses than gram positive bacteria and are secreted intracellularly.[2] Over last 20 years many new beta lactam antibiotics have been developed that were specifically designed to be resistant to hydrolytic action of beta lactamases. However, with each new class that has been used to treat patients, new beta lactamases emerged that caused resistance to that class of drug.[3] The selective pressure of the use and overuse of new antibiotics for the treatment of patients are the contributing factors for the emergence of new variants of beta lactamases.[4] 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.[5] As the 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. The present study was undertaken to detect different mechanisms of resistance of isolates from burn wound infection.

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MATERIAL AND METHODS

A total of 181 swabs of all age groups and both sexes admitted to burn care unit during Jan 2013 to December 2013 were collected under aseptic precautions and processed immediately. Swabs were cultured on blood and Mac Conkeys agar and incubated aerobically. The bacteria were identified by standard biochemical tests and antimicrobial susceptibility was done by Kirby-Bauer disc diffusion method according to CLSI guideline 2014.[6,7] All gram negative bacilli were subjected to detection of different mechanisms of ESBL by Predictor disc approximation method. The results for plain ESBL producers, ESBL plus derepressed mutants, derepressed mutants, AmpC, multiple mechanisms were noted according to crieteria by Dr. Rodrigues C. 8 All *P. aerginosa* isolates were tested for MBL production by disc potentiation test (Imipenem – EDTA combined disc test).[9]

RESULTS

In present study out of 113 gram negative bacilli, 42 (37.16%) isolates were ESBL producers.

Table 1: Mechanisms of beta-lactamase production in gram negative bacilli (Except *P.aeruginosa*)

Organism	Total beta lactamase producers	Plain ESBL producers	ESBL+ Derepressed mutants	Derepressed mutants	Amp C
K. pneumoniae (20) (20)(20)	13	5	8	-	-
*E.coli (*16)	10	2	8	-	-
Proteus spp(n=11)	4	1	3	-	-
Citrobacter spp(n=7)	2	1	1	-	-
Nonfermenter (n=3)	1	1	0	-	-
Acinetobacter (n=2)	2	0	2	-	-
Enterobacter spp.(4)	0	0	0	-	-
K.oxytoca (n=3)	0	0	0	-	-
TOTAL (66)	**32 (48.48%)**	**10 (15.15%)**	**22 (33.33%)**	**-**	

*Percentages are for gram negative bacilli except *P.aeruginosa*.

Table 2: Mechanisms of beta-lactamase production in *P. aeruginosa*

Organism	Totalbeta-lactamase producers	ESBL	AmpC	MBL
Pseudomonas aeruginosa(47)	31 (65.94%)	4* (8.51 %)	6* (12.76 %)	21* (44.68 %)

*percentages are from total number of *P.aeruginosa* isolates

DISCUSSION

The most common isolate in our study was *P. aeruginosa* followed by *S. aureus, K. pneumoniae* and Coagulase Negative Staphylococci (CoNS). 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 metallobetalactmases. ESBLs are either chromosomally mediated or plasmid mediated. Initially these were commonly found in Klebsiella spp. and *E.coli*, but now these enzymes are produced by all members of *Enterobacteriaceae* and a few other gram negative bacilli. These enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but not cephamycins and imipenem.[10] AmpC beta-lactamases are well defined enzymes with broad substrate specificity. These enzymes are chromosomal as well as plasmid mediated. In many bacteria. AmpC enzymes are inducible and can be expressed at high levels by mutation. Over-expression confers resistance to broad spectrum cephalosporins.10 Metallobetalactamase (MBL) is group of carbapenem hydrolyzing beta-lactamase. The MBLs are inhibited in vitro by CuCl3, FeCl3, EDTA and thiol compounds like 2-mercaptopropionic acid but not by beta-lactamase inhibitors like clavulanic acid and / or sulbactum.10 If an isolate is ESBL producer, then it should be reported as resistant to Penicillins, Cephalosporins and Monobactams. AmpC producer isolates should be reported as resistant to Penicillins, Cephalosporins including Cephamycins and Monobactam. If an isolate is MBL Producer, then it should be reported as resistant to Penicillins, Cephalosporins, Carbapenems. Note-This is irrespective of in vitro susceptibility.[11] In the present study, out of total 66 gram negative bacilli other than *P. aeruginosa,* 32 (48.48%) were betalactamase producers among which 22 (33.33%) were ESBL with derepressed mutants while remaining 10 (15.15%) were plain ESBL producers. None of the strain was found to be AmpC producer. This is in accordance with Rodrigues C *et al* 2004 8, and Nitin Bandekar *et al* 201110 in which ESBL

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production was the most common mechanism followed by AmpC production. Majority of ESBL producers and derepressed mutants were *Klebsiella pnemoniae* and followed by *E.coli, Proteus mirabilis*. *P. aeruginosa* may be intrinsically resistant or have acquired resistant to antibiotics due to permeability barrier of the cell surface, multidrug efflux pumps and production of beta lactamases. Table no.2 Shows different mechanisms of beta-lactamase production in *P. aeruginosa*. Out of 47 *P. aeruginosa* isolates 31 isolates were producing beta-lactamase, out of which, 21 (44.68%) were MBL producers, 6 (12.76%) were AmpC producers, and 4 (8.51%) were ESBL producers. There are many reports with different percentages for beta-lactamases production in *P. aeruginosa.* In the present study, production of MBL (44.68%) found to be most common beta-lactamase in *P. aeruginosa* which is comparable with studies by Goel V *et al* 201312 (53.85%) and Saderi *et al* 200813 (53.2%). In contrast to present study, Kumar V *et al* 2012 4, Altun S et 201314, Shahid M *et al* have reported AmpC production is the most common mechanism of resistance in *P. aeruginosa.* The plain ESBL production in present study is 8.51% which is in accordance with, and Varsha Gupta *et al* 2015 (16.1%), Altun S *et al* 201314 (14%). These observations suggest that the betalactmases which are generally widespread in members of *Enterobacteriaceae* are also increasingly found in *P.aeruginosa.* All the gram negative bacilli in our study were sensitive to imipenem. Carbapenems are most effective and reliable as they are highly resistant to hydrolytic activity of all ESBL enzymes due to the trans-6-hydroxy ethyl group. For infections due MBL producing bacteria Polymixins may be the alternative therapeutic option. In vitro studies reveal that tigecycline and colistin are the antimicrobial agents with consistent activity against MBL producing strains. 11 These beta lactamase producing strains especially MBL producing *P. aeruginosa* in burn patients remain as a therapeutic challenge for surgeons and these strains may cause nosocomial outbreaks. Our study emphasizes that it is absolutely necessary that all gram negative bacilli should be screened for mechanisms of antimicrobial resistance. Detection of these strains will help to avoid therapeutic failures. Aggressive infection control measures can be applied to prevent the further nosocomial outbreaks. The routine screening for mechanisms of antimicrobial resistance detection will be helpful to formulate the antibiotic policy in burn unit that will further reduce the morbidity and mortality in burn patients.

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