Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producing *Pseudomonas aeruginosa* Isolates from Burns Patients

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* is a leading cause of nosocomial infections. It shows intrinsic and acquired resistance to many structurally related antibiotics including beta lactam antibiotics.There are few studies on beta lactamase producing *Pseudomonas aeruginosa* in hospitalized strains. The aim of this study was to know the prevalence of ESBL producing *Pseudomonas aeruginosa* isolates from burn wound infections and to study the susceptibility of these ESBL producing *P.aeruginosa* to various other antimicrobial agents.

Material and Methods: A total of one hundred and eight pus samples were collected from 108 randomly selected patients admitted in burns unit of a tertiary care hospital between January 2012 to June 2013.The organisms were processed and identified using standard bacteriological conventional culture methods. ESBL producing *Pseudomonas aeruginosa* isolates were detected using "Double Disc Synergy test" (DDST) and "Phenotypic Confirmatory Disc Diffusion Test" (PCDDT).

Results: Among the 108 organisms isolated, the predominating isolate was *Pseudomonas aeruginosa* 78 (72.22%), out of these 19 isolates were sensitive to 3rd generation cephalosporins (3rd GCs) and 59 were resistant. ESBL production was detected in 22 (37.28%) isolates, 19(86.36%) were positive by DDST and all 22(100%) were positive by PCDDT.

Conclusions: 37.28% strains of *Pseudomonas aeruginosa* produced ESBLs. Phenotypic Confirmatory Disc Diffusion Test was most sensitive in the screening of ESBLs than Double Disc Synergy test.

Keywords: *Pseudomonas aeruginosa*, 3rd generation cephalosporins, Extended spectrum beta lactamases, Burn wound infection.

INTRODUCTION

Pseudomonas aeruginosa is a leading cause of nosocomial infection and can cause fatal illness in a variety of patients.[1] The infections caused by these bacteria are commonly ventilator associated pneumoniae, burn wound infections and urinary tract infections.[2] ESBLS(extended spectrum beta lactamases) represent a major group of β-lactamases currently being identified world wide in large numbers, and are now found in significant percentage of *Escherichia coli* and *Klebsiella pneumonia* strains. They have also been found in *Pseudomonas aeruginosa* and other *Enterobacteriaceae* strains like *Enterobacter, Citrobacter, Proteus, Serratiamarsescens* etc.[3,4]

Development of generalised β lactam resistance is mainly because of inappropriate use of third generation cephalosporins.[5] Development of resistance to beta lactam group of antibiotics is mainly due to beta lactamase enzyme production which is either plasmid or chromosomally mediated.There is limitation of therapeutic options because of increased incidence of ESBL producing strains among clinical isolates. *Pseudomonas aeruginosa* shows intrinsic and acquired resistance to many structurally related antibiotics including beta lactam antibiotics and previous exposure to antibiotics often leads to multidrug resistant *Pseudomonas aerusinosa* strains.[6,7] Therefore it is important to isolate and identify the resistant strains so that appropriate antibiotic therapy can be given.[7] The lack of sensitivity and specificity in traditional susceptibility tests to detect ESBLs has led to the search for an accurate, less cumbersome and cost effective test to detect the presence of ESBL in clinical isolates.

ESBL production in gram negative bacteria particularly in *Escherichia coli* and *Klebsiella* species has been studied by various workers in the past but limited data on beta lactamase producing *Pseudomonas aeruginosa* in hospitalized strains prompted us to undertake this study.

The aim of this study was to determine the prevalence of ESBL producing hospital strains of *Pseudomonas aeruginosa* and also to study the susceptibility of these ESBL producing *P.aeruginosa* to various other antimicrobial agents.

MATERIAL AND METHODS

A total of one hundred and eight pus samples were collected from 108 randomly selected patients admitted in burns unit of a tertiary care hospital between January 2012 to June 2013, after clearance from ethical committee. The organisms were processed and identified using standard bacteriological conventional culture methods. *P aeruginosa* isolates that were obtained as a pure and predominant growth from the clinical specimens were only considered for the present study. The organisms were identified based on the colony morphology and biochemical reactions. The sensitivity of the isolates to the third-generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone, 30 µg each) was determined by disc diffusion method using *P. aeruginosa* ATCC 27853 as control strain. Commercially available (Hi-Media) 6-mm antibiotic discs were used on Mueller Hinton agar (MHA). Results were interpreted according to the CLSI guidelines,

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which suggest a diameter of inhibition zone ≥22 mm for ceftazidime, ≥27 mm for cefotaxime and ≥25 mm for ceftriaxone as susceptible. ESBL producing *Pseudomonas aeruginosa* isolates were detected using "Double Disc Synergy test" (DDST) and "Phenotypic Confirmatory Disc Diffusion Test" (PCDDT).

Double Disc Diffusion Test (Double disk approximation method or Double disk synergy test): 0.5 McFarland standardized inoculum was swabbed onto Mueller-Hinton agar plate. An amoxycillin with clavulanic acid disc was placed in the center of the plate and one ceftriaxone disc and ceftazidime disc were placed at a distance of 20 mm (Center to center) from the amoxycillin with clavulanic acid disk. The plates were incubated overnight at 37° C and results were read. Enhancement of zone of inhibition of the cephalosporin disc towards clavulanic acid containing disc was inferred as synergy and the strain considered as ESBL producer. This increase occurs because the clavulanic acid present in the amoxyclav disc inactivates the ESBL produced by the test organism.

Phenotypic Confirmatory Disc Diffusion Test (PCDDT): 0.5 McFarland standardized inoculum was swabbed onto Mueller –Hinton agar plate. Ceftazidime disc containing 30 mcg and Ceftazidime with clavulanic acid disc 20+10 mcg were placed at a distance of 30 mm from each other. Plates were incubated overnight at 37°C and results were read. Increase in zone diameter >5 mm with ceftazidime with clavulanic acid was inferred as positive test and the organism considered ESBL producer.

STATISTICAL ANALYSIS

Results are based on the descriptive statistics.

RESULTS

Of the 108 patients 50 (46.29%) were males and 58 (53.70%) were females. Female to Male ratio was 1.16:1. Out of the 108 samples studied 84 (77.77%) showed burn wound colonization, 54(50%) showing monomicrobial and 30 (27.77%) showing polymicrobial growth. 24 samples yielded no growth. Among the 108 organisms isolated, the predominating isolate was *Pseudomonas aeruginosa* (72.22%), followed by *Klebsiella pneumoniae* (8.33%), *Staphylococcus aureus* (8.33%) and the least isolate was *Enterococcus* species comprising about 0.92% of the total isolates (Fig-1). Out of 78 isolates of *Pseudomonas aeruginosa* tested for their antibiogram, 19 isolates have shown sensitivity to 3rd generation cephalosporins (3rd GCs) and 59 have shown resistance, the percentage being 24.35% and 75.65% respectively. Among the 59 isolates of *Pseudomonas aeruginosa* resistant to 3 GCs, ESBL production was detected in 22 (37.28%) isolates and 37 (62.72%) were Non-ESBL producers, as per DDST and PCDDT (Table1). Out of the 22 ESBL producing isolates of *Pseudomonas aeroginosa* ESBL production was detected using CAZ and CAC in 22(100%) and ESBL production was detected using CTR and CIS in 20(90.90%). Among the total (22) number of ESBL producing *Pseudomonas aeruginosa* isolates 19(86.36%) were positive by DDST and all 22(100%) were positive by PCDDT.(Table2)

DISCUSSION

The main cause of death in burns patients is shock which is prevented by the use of effective fluid resuscitation regimens. The outcome of burns patients also depends on proper wound healing for which infection is the predominant determinant. Other improvements in care that protect organ function and prevent complications have further accentuated infection as the most frequent cause of burn patient morbidity and mortality. As there is a great variability of both local and systemic clinical manifestations of invasive burn wound infection, great emphasis is being put on proper identification of the burn wound microbial flora for appropriate treatment. Moreover, microbial surveillance has a very important role in any kind of nosocomial infection including burn wound infection, more so in today's era of drug resistance.

In the present study an attempt was made to know the pattern

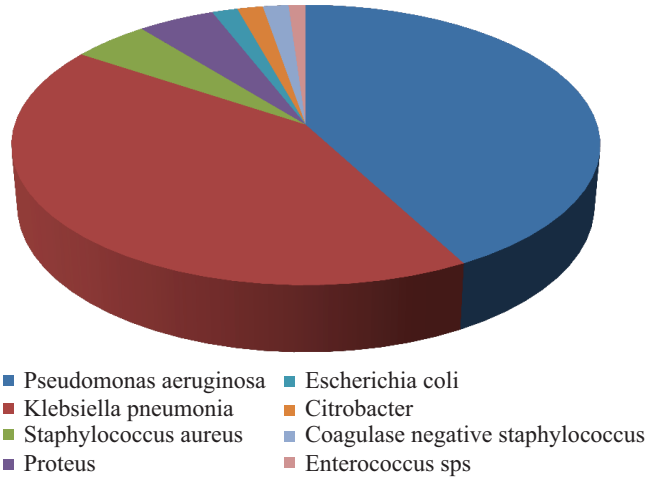

Figure-1: Showing distribution of various organisms isolated from burn wound surfaces.

Total No. of isolates resistant to 3GC	ESBL producers		Non-ESBL producers	
	No.	Percentage	No.	Percentage
59	22	37.28%	37	62.72%

3GC- 3rd generation cephalosporins

Table-1: Distribution of *Pseudomonas aeruginosaisolates* based on ESBL production

Total No. of ESBLs isolated	Zone enhancement by DDST		Zone enhancement by PCDDT	
	No.	Percentage	No.	Percentage
22	19	86.36%	22	100%

DDST- Double Disc Synergy test, PCDDT - Phenotypic Confirmatory Disc Diffusion Test

Table-2: Comparison of DDST and PCDDT in detecting ESBL production.

of burn wound bacterial colonization and the antimicrobial sensitivity profile of *Pseudomonas aeruginosa* isolates and screening for Extended spectrum beta lactamase producers. In the present study it is noted that 77.77% samples showed burn wound colonization with bacteria. B.S.Nagoba et al.[8] Agnihotri et al.[9] and Singh et al.[10] have reported 100%, 96% and 87.5% colonization respectively. This high culture positivity rate may be attributed to the selection of cases with deep and major burn wounds.

It is also noted in the present study that monomicrobial growth (50%) was more common than polymicrobial growth (27.77%), as in various other studies such as done by Anuradha Rajput et al.[11]

B.S.Nagoba et al.[8] however have reported polymicrobial growth from 61.5% of samples and single isolate from only 38.5% of samples which is contrasting with the present study. In the present study, the most common isolate was *Pseudomonas aeruginosa* (72.22%), followed by *Staphylococcus aureus (8.33%)* and *Klebsiella pneumoniae* (8.33%), *Proteus* (2.77%). And the least isolate was *Enterococcus* spp comprising about 0.92% of the total isolates.

The incidence of Gram positive isolates to Gram negative isolates were 11.1% to 88.9% respectively, where the ratio of these was 1:8. The predominance of *Pseudomonas aeruginosa* and other Gram negative isolates were the most common agents which has also been reported by other workers such as O.Oncul et al,[12] Anuradha Rajput et al,[11] Manjula Mehta et al.[13] This suggests that *Pseudomonas aeruginosa* is the classical pathogen in burn wound infections. This is because *Pseudomonas aeruginosa* thrives on moist wound surfaces and is highly pathogenic in thermally injured immunosuppressed patients. These bacteria usually gain access to burn patients through cross-contamination of burn wounds. The second most common isolate in this study was *Staphylococcus aureus* as in other studies from economically developing countries such as O.Oncul et al,[12] Rastegar Lari et al.[14] This contrasts however with some other studies, especially from economically developed countries, which reports *Staphylococcus aureus* as the predominant organism in burn infection as stated by Ozumba et al.[15]

During the first week of admission the predominant isolate was *S.aureus* (89%) of which most of them were monomicrobials whereas Gram negative isolates comprised only (11%). The predominance of Gram-positive bacteria in the early phase switches to Gram-negative species 4-10 days after injury. In the polymicrobial isolates of first week *Klebsiella pneumoniae* was the major isolate among Gram negatives. During the second week of admission, Gram-negatives (65%) predominated significantly over the Gram-positives (35%) of which most of them were polymicrobial isolates, and *Pseudomonas aeruginosa* was the predominating organism. Statistically, there exists a negative correlation between Gram positives and Gram negatives as time increases. In the present study it was found that more than 80% of the *P.aeruginosa* isolates were sensitive to Meropenem, Piperacillin/Tazobactam, Imipenem/Cilastin. More than 50% sensitivity was shown to Amikacin, Ciprofloxacin, Aztreonam and Colistin. Less than 40% sensitivity was shown to Gentamycin, Ceftazidime, Ceftriaxone and Cefotaxim. In a study done by Sharma and Taneja et al,[16] ninety per cent of *P. aeruginosa* were resistant to amikacin and ceftazidime, 45 per cent to ciprofloxacin and 25 per cent to piperacillin.

ESBL DETECTION

Out of 78 total isolates of *Pseudomonas aeruginosa,* 59 were found to be resistant to third generation cephalosporins (3GCs).ESBL detection was performed by Double Disc Synergy Test (DDST) using Ceftazidime, Amoxyclav and Ceftriaxone discs and Phenotypic Confirmatory Disc diffusion disc (PCDDT) using Ceftazidime and Ceftazidime-Clavulunic acid, Ceftrioxone and Ceftrioxone-Sulbactam discs. Among the 59 resistant isolates, ESBL production was detected in 22 (37.28%) isolates and 37 (62.72%) were Non-ESBL producers. There is geographic variation in ESBL producing strains of *Pseudomonas aeruginosa.* In our study 37.28% strains were ESBL producers, which is similar to a south Indian study done by Senthamarai.S, Sivasankari S et al[17] among 144 strains of *P.aeruginosa*, 51 (35.4%) showed ESBL production in the combined disc diffusion test, where in a study done by Abiola Olukeri Okesola and Anthony Alaba Oni from Nigeria,[18] out of the 90 isolates of *P.aeruginosa*, 20 (22.2%)isolates were found to be positive for ESBL production while 70(77.8%) were negative and in another study done by Varun Goel et al[19] showed higher incidence of 42.31% ESBL producers. However our study is in contrast to studies conducted by others who depicted low rates, 3.7% Woodford et al.,[20] 4.2% Lim et al.,[21] respectively of ESBL production in *P. aeruginosa*. In our study among the total (22) number of ESBL producing *Pseudomonas aeruginosa* isolates, 19(86.36%) were positive with DDST and all 22(100%) were positive by PCDDT, which roughly correlates with thestudy done by Xiaofei Jiang et al[22], among the total of 34 isolates that were considered ESBL producers, 20 strains were positive with PCDDT and 10 were positive with DDST.

CONCLUSIONS

Pseudomonas aeruginosa was found to be commonest cause of burn wound infections. Isolates of *P.aeruginosa* were resistant to many routine antibiotics like Ceftazidime, Gentamycin and Meropenem.

Phenotypic Confirmatory Disc Diffusion Test was most sensitive in the screening of Extended Spectrum beta lactamases than Double Disc Synergy test. Screening of burn wound infections for ESBLs will guide in therapeutic option of antibiotic and to institute the appropriate antimicrobial agent to the patient and to prevent the spread of ESBL positive organisms. Use of the simple ESBL screening test like PCDDT will be crucial step in large scale monitoring of these emerging resistant determinants. As the therapeutic options are very less, strict infection control measures and proper antibiotic regimens are the measures which will help in reducing the high morbidity and mortality in burn wound cases.

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