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Research Article

CO-EXISTENCE OF BETA LACTAMASES (ESBL AND MBL) IN *Pseudomonas aeruginosa* ISOLATES FROM PUS SAMPLES

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Abstract- Background: Resistance to broad spectrum beta lactamase mainly those mediated by ESBL's and MBL's enzymes is an increasing problem worldwide. Detection of their prevalence and co-existence is essential so as to formulate an effective antibiotic policy and hospital infection control measures. Thus, this present study was undertaken to determine the prevalence and co-existence of ESBL & MBL in *P. aeruginosa* isolates from pus samples. **Material and methods:** A total of 1100 pus samples were screened, of which 90 isolates of *P. aeruginosa* were isolated and subjected for ESBL and MBL phenotypic tests. Double disc synergy test and Imipenem (IMP) - EDTA combined disc test were used for their detection respectively. **Results**: Out of the 90 *P. aeruginosa* isolated from pus samples, 55(61%) were Cefazidime sensitive and 35(38.8%) were resistant. Of the 35 *P. aeruginosa* resistant to ceftazidime, DDST detected 16(45.7%).65(72.2%) were Imipenem sensitive and 25(27.7%) were resistant. Of the 25 *P. aeruginosa* resistant to Imipenem, IMP-EDTA CDT detected 16(64%) of ESBL producers.12(13.3%) showed Resistance to both Imipenem and Ceftazidime and only 6 of the 12 showed Co-existence of ESBL and MBL accounting for 50% of them and 6.6% of the total 90 *P. aeruginosa* isolates. **Conclusion:** There is a need for screening tests for detection of not only individual occurrence of different beta lactamases but also their co-existence in the same organism to be made a mandatory routine in all microbiology laboratories.

Key words- *Pseudomonas aeruginosa*, Beta lactamases, ESBL, MBL

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Introduction

Multidrug-resistant *P.aeruginosa* is a major pathogen encountered in pyogenic infections. An alarming increase in the incidence of antibiotic resistance in them has become a serious concern. The commonest cause of bacterial resistance known in them are production of β- lactamases [1]. A variety of ß-lactamases like ESBLs, AmpC ß-lactamases, metallo-ß- lactamases and oxacillinases have emerged as the most worrisome mechanism of resistance among the gram negative bacteria, mainly *P.aeruginosa*, which has posed a therapeutic challenge to the health care settings. Other modalities which might contribute to beta-lactam resistance are antibiotic target site alterations, efflux pumps and finally porin channel deletion. Because of their efficacy, broad spectrum of action and low toxicity β-lactam antibiotics are the most frequently prescribed worldwide. A number of mutated forms of β-lactamases such as the ESBLs, AmpC β-lactamases and metallo-β-lactamases have emerged as a therapeutic challenge to the health care settings due to the indiscriminate use of beta-lactam antibiotics [2]. ESBLs are Class A β- lactamases with mutant, plasmid mediated enzymes, that hydrolyze all cephalosporins, penicillins and monobactams but not cephamycins or carbapenems. They are also known to be inhibited in-vitro by clavulanate [3]. Metallo β- lactamases are carbapenamases, which require zinc divalent cation, as cofactor for enzyme activity and are able to hydrolyze all β-lactams except monobactam and known to be inhibited by chelating divalent cations like ethylenediamine tetraacetic acid (EDTA). The gene responsible for MBL production may be chromosomal or plasmid mediated and hence pose a threat of spread of resistance by gene transfer among the Gram negative bacilli, commonly known to occur in *P. auruginosa* [4].

The treatment options in these ESBL and MBL producing *P. aeruginosa* are limited due to their capability to hydrolyze a wide range of β-lactam antibiotics, mainly the extended-spectrum penicillins as well as the third and fourth generation cephalosporins, including carbapenams [5,6]. Due to the lack of guidelines from CLSI Clinical Laboratory Standards Institute and standardized phenotypic tests, the detection of ESBL and MBL mediated resistance in *P. aeruginosa* have posed a problem. A large number of phenotypic methods have been proposed by different researchers for their detection but the coexistence of different classes of β-lactamases in a single bacterial isolate is a real challenge for both diagnosis and treatment. When ESBL and MBL co-exist they mask each other causing misreporting of phenotypic test results and further failure in clinical treatment of patients [7]. Thus this present study was designed to investigate the prevalence of the β-lactamase producing *Pseudomonas aeruginosa* strains individually and also as a co-existence (ESBL and MBL) which will help to formulae empirical therapy policy.

Material and methods

The study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College, KLE Academy of Higher Education and Research, DR. Prabhakar Kore Charitable Hospital & MRC, Belagum. A total of 1100 pus samples were received at the Department of Microbiology, over a period of eleven months, of which 90 isolates of *P. aeruginosa* were isolated. All the isolates were identified as *P. aeruginosa* by standard conventional methods [8]. The antimicrobial susceptibility testing of the isolates was performed on Mueller-Hinton agar (MHA) using commercially available antibiotic discs (Hi-Media Laboratories Ltd, Mumbai) -

by standard Kirby-Bauer disc diffusion method and results were interpreted as per CLSI recommendation [9]. The antibiotics tested were gentamicin (10µg), cefperazone /sulbactam (75/30µg), amikacin (30µg), meropenem (10µg), colistin (10µg), tobramycin (10µg), cefotaxime (30µg), ciprofloxacin (5µg), ceftazidime (30µg), cefpodoxime (10µg), ceftriaxone (30µg), piperacillin/tazobactam (100/10µg), imipenem (10µg), cefipime (30µg), cefoxitin (30µg). *P. aeruginosa* ATCC 27853 was used as control.

ESBL detection

Isolate that showed resistant to at least one of the third generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone, cefpodoxime) were tested for ESBL production by Double Disc Synergy Test.30mcg disc of each third generation cephalosporin antibiotics: Cefotaxime, Ceftriazone and Ceftazidime, are placed on MHA plate lawn cultured with standard inoculum (0.5 McFarland) of the test organism at distance of 15mm center to center from Augmentin disc (Amoxicillin/Clavulanic acid- 20mcg/10mcg), followed by overnight incubation at 37°C. Increase in the inhibition zone of any one of the three third generation antibiotic disc towards augment disc is considered as an ESBL producer [10].

MBL detection

Isolates resistant to meropenem or imipenem were screened for MBL production. All isolates positive by screen test were subjected to Imipenem (IMP) - EDTA combined disc test.

A 0.5M solution of EDTA was prepared by dissolving 186.1gm of disodium EDTA 2H2O in 1000mL of distilled water and pH was adjusted to 8.0 by using NaOH. The mixture was sterilized by autoclaving. Lawn culture of standard inoculum (0.5 McFarland) of the test organism was made on MHA. Two 10mcg Imipenem discs are placed on the plate. To one of the Imipenem discs, 10µl (750mcg) of 0.5M EDTA solution is added, followed by incubation at 37°C overnight. If increase in inhibition zone with IMP and EDTA disc was >7mm than IMP disc alone, it is considered as MBL producer [11].

Results

Total pus samples processed 1100.

Out of the 90 *P. aeruginosa* isolated from pus samples, 55(61%) were Cefazidime sensitive and 35(38.8%) were resistant, as shown in [Table-1] of the 35 *P.aeruginosa* resistant to ceftazidime, DDST detected 16(45.7%).

Table-1 *Showing the Ceftazidime susceptibility pattern*

Total number of *Pseudomonas aeruginosa* isolates from pus sample	Ceftazidime resistant	Ceftazidime sensitive
90	35(38.8%)	55(61.1%)

Out of the 90 *P.aeruginosa* isolated from pus samples, 65 (72.2%) were Imipenem sensitive and 25(27.7%) were resistant as depicted in [Table-2]

Of the 25 *P. aeruginosa* resistant to Imipenem, IMP-EDTA CDT detected 16(64%) of ESBL producers.

Table-2 *Showing the Imipenem susceptibility pattern.*

Total number of *Pseudomonas aeruginosa* isolates from pus sample	Imipenem resistant	Imipenem sensitive
90	25(27.7%)	65 (72.2%)

Of the 90 *P. aeruginosa* isolates, 12(13.3%) showed Resistance to both Imipenem and Ceftazidime and only 6 of the 12 showed Co-existence of ESBL and MBL accounting for 50% of them and 6.6% of the total 90 *P. aeruginosa* isolates as in [Table-3]

Table-3 *Showing the resistance pattern to both Imipenem and Ceftazidime.*

Total number of *Pseudomonas aeruginosa* isolates from pus sample	Resistant to both Imipenem and Ceftazidime	Co-existence of ESBL & MBL
90	12(13.3%)	6 (50%)

Discussion

P. aeruginosa is one of the most commonly encountered causative agent of opportunistic nosocomial pathogen. Intrinsic and acquired antibiotic resistance, in *P. aeruginosa* has led to an increased incidence of multidrug resistance causing infectiveness of antimicrobial agents [1], In our study, *P. aeruginosa* showed 100% sensitivity to colistin which is similar to the study findings done by Somily, *et al*; but is in contrast to the Varaiya, *et al*; study findings which showed low colistin susceptibility of 57.5% [13,14]. Difference in Study Environmental conditions could be one of the reasons causing this colistin susceptibility disparity [15]. Hence Polymyxin B and colistin remains the first line of drug choice for the treatment of multidrug resistant *P. aeruginosa* [16]. 72.2% sensitivity was seen for Imipenem in *P. aeruginosa* which was in concordance with the findings of other studies done by Aggarwal, *et al*; Rudresh, *et al*; Rawat, *et al*; and Dutta, *et al* [17-20].

In our study 38.8% of the isolates showed ceftazidime resistance which is almost near to the resistance pattern seen in a study done at Turkey by Gencer, *et al*; showing 22% ceftazidime resistance [21].

Co-existence of ESBL and MBL was seen in for 50% of the *P.aeruginosa* isolates showing resistance to both Ceftazidime and Imipenem which accounts to 6.6% of the total 90 *P. aeruginosa* isolates. This findings of ours is akin to that done by Oberoi, *et al*; who reported coexistence of ESBL and MBL in 8.79% of the isolates [22]. But the findings of the study done by Umadevi, *et al*; showed only 2(4%) and 0% in a study by Picao, *et al*; for co-existence of ESBL and MBL, which was in contrast to our study [23,24]. Differences in the antibiotic usage pattern which in turn might have led to the mutation in the β- lactamase producing gene could be the probable cause for this difference seen in the co-existence of ESBL and MBL. Normal flora of individuals vary from one area to another due to the differences in cultural, nutritional and ethnic practices and this could also be the contributing cause for variation in the co-existence of ESBL and MBL. Use of different types of phenotypic methods for the detection of ESBL and MBL by different researchers due to the lack of CLSI guidelines could be another possible reason [25]. As a result of inappropriate use of extended spectrum cephalosporins, variation in prevalence of various β-lactamases in seen within the same hospital. [26] The increased incidence and prevalence of the MBL and the ESBL producing isolates is an indication of more and more isolates acquiring the resistance mechanisms, thus causing antimicrobial treatment to be ineffective.

Due to this treatment failure caused by production of multiple β- lactamases by *P. aeruginosa*, its occurrence has to be looks up as a serious issue and needs urgent actions to be taken to prevent and control the spread of such resistant strains.

Conclusion

Considering the magnitude of the issue, need to understand the requirement/importance and to carry out screening tests for detection of not only individual occurrence of different beta lactamases but also their co-existence in the same organism has to be made a mandatory routine in all microbiology laboratories.

The limitation of our study was molecular analysis and characterization of β-lactamases were not done due to financial constrains.

Application of research

It is necessary to implement screening tests for detection of ESBL and MBL as a routine microbiological laboratory investigation.

Authors Contribution: All author equally contributed.

Abbreviations

ESBL- Extended spectrum-ß- lactamase
MBL- Metallo-ß- lactamase
DDST- Double Disc Synergy Test
EDTA- Ethylenediamine tetraacetic acid

Conflict of interest:

There are no conflicts of interest.

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