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Prevalence and Phenotypic Detection of AmpC Betalactamase, Extended Spectrum Beta-Lactamases, and Metallo Betalactamase Producing *Pseudomonas aeruginosa* at a Tertiary Hospital, South India

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Abstract

Background: *The reducing susceptibility of broad-spectrum betalactam antibiotics, mediated by AmpC betalactamase, extended-spectrum betalactamases (ESBL), and metallo betalactamase (MBL) enzymes, is a rapidly growing problem globally.*

Aim of the study: *This study was conducted to phenotypically detect the predominance and incidence of AmpC beta-lactamase, ESBL, and MBL-producing Pseudomonas aeruginosa from different clinical specimens.*

Materials and Methods: *Over the course of a year, a prospective cross-sectional observational investigation had been performed in a tertiary hospital in Nalgonda. AmpC betalactamase, ESBL, and MBL enzymes were detected phenotypically in 46 nonrepetitive isolates of Pseudomonas aeruginosa. An antagonistic disc test was used to identify the enzyme AmpC betalactamase phenotypically. Based on CLSI guidelines, the double disk synergistic test and combined disk diffusion approach were employed to phenotypically detect ESBL. The Imipenem and Imipenem plus EDTA disk synergistic/potentiation test identified MBL.*

Results: *Twenty segregates (43.4%) in total tested positive for AmpC beta-lactamase. Six strains (13%) of them produced inducible AmpC. Co-production of AmpC with MBL and ESBL was described in 15% and 40% of the details, respectively.*

Conclusion: *The research highlights the significant incidence of multidrug-resistant (MDR) Pseudomonas aeruginosa producing beta-lactamases with different constituents. Therefore, appropriate antimicrobial strategies and actions to restrict the erratic application of cephalosporins and carbapenems are necessary to prevent the spread of these various pathogens that produce beta-lactamases.*

Keywords

AmpC beta-lactamase, Extended spectrum beta-lactamase, Metallo beta-lactamase, Pseudomonas aeruginosa.

INTRODUCTION

As an opportunistic Gram-negative bacterium, *Pseudomonas aeruginosa* can cause a variety of illnesses that are difficult to treat because of the bacteria's resistance to several antibiotics. *Pseudomonas aeruginosa* has intrinsic resistance and aquired resistance to numerous anti-microbials. In expansion, it can moreover happen due to the nonsensical utilization of antibiotics.[1] The most prevalent resistance mechanism in Gram-negative bacteria against this class of anti-microbials is the hydrolysis of beta-lactam anti-microbials by beta-lactamase. *Pseudomonas aeruginosa* diseases are difficult to treat because most isolates show varying degrees of natural resistance. Furthermore, “the production of plasmid-interrupted AmpC beta (β)-lactamase, extended-spectrum betalactamases (ESBL), and metallo betalactamase (MBL) enzymes provides details about the acquired resistance.[2] In *Pseudomonas aeruginosa* counting, a few factors may provide resistance to β-lactam anti-microbial: genetic changes that result in consistent overexpression of chromosome-mediated AmpC cephalosporinases, preservation of transferable β-lactamase properties, excessive synthesis of efflux frameworks, and reduced porosity. Ambler course A ESBLs and course B MBLs are two of the different β-lactamases that are described as rapidly developing proteins in clinical segregates of *P. aeruginosa*. Penicillins, cephalosporins, and aztreonam can all be hydrolyzed by ESBLs (but not cephamycins or carbapenems).[3] A β-lactamase inhibitor, like clavulanic acid, inhibits these enzymes. Of the more than two hundred distinct ESBLs identified in Gram-negative bacteria, 32 are

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found in *Pseudomonas aeruginosa* and are linked to class D (OXA sort) and course A (SHV, CTX-M, BEL PER, VEB, GES, and TEM) β-lactamases. Cephamycins (like cefoxitin as well as cefotetan), oxyimino-cephalosporins (like ceftazidime, cefotaxime, & ceftriaxone), and monobactams (like aztreonam) are all hydrolyzed by AmpC β-lactamases. Early" detection is crucial due to the rising prevalence and types of these different β-lactamase chemicals.[4] This will allow for the implementation of appropriate antimicrobial treatment and disease control plans, among other benefits like reduction in hospital stay and strategic approach towards hospital's premises sterilisation. *P. aeruginosa* conveys a range of resistance components challenging conventional anti-microbial treatments. Inborn resistance, a foundational properties encoded in its genome, consist variables like lack of external layer penetrability, Mex-type efflux pumps, as well as AmpC β-lactamase.[5] These components collectively build up a basal resistance level shown in overall strains. Obtained resistance incorporates changes affecting anti-microbial targets and the securing of altering proteins or resistance plasmids. This versatility empowers the bacterium to advance quickly, undermining the adequacy of customary anti-microbials. Intriguingly, *P. aeruginosa* shows versatile resistance components, reacting powerfully to natural boosts. This highlight permits the bacterium to alter its anti-microbial resistance profile, assisting in complicating treatment techniques. The interaction of these resistance components contributes to the improvement of MDR strains and poses a genuine danger to open well-being. Resistance is taken after by advancement from bacteria. Contamination due to *P. aeruginosa* is troublesome to treat, because of the expanded inborn resistance of the microscopic organisms and the capacity to be less susceptible to various antibiotics. The utilization of anti-microbials is right now very wide with lack of information about the utilization of appropriate anti-microbials, so that it can cause resistance. In common, the instruments of anti-microbial resistance to *Pseudomonas aeruginosa* can be partitioned naturally and acquired. Inborn alludes to a resistance instrument that is hereditarily coded and obtained alludes to resistance through the procurement of extra components or is a result of a transformation occasion under selective weight. The synthesis of amplified range beta-lactamases proteins is a crucial part of *Pseudomonas aeruginosa* resistance to beta-lactam anti-microbials. Penicillin, cephalosporin, and monobactam anti-microbials can all be hydrolyzed by ESBL, a substance that bacteria deliver and which can lead to resistance to all of these anti-microbials.[6]

AIM AND OBJECTIVES

This consideration was attempted to distinguish the predominance "of ESBL, AmpC beta-lactamase, and metallo-beta-lactamase creating *Pseudomonas aeruginosa* from different clinical specimens.

MATERIALS AND METHODS

In a tertiary" hospital in Nalgonda, a 12-month observational, prospective cross-sectional study was carried out. Phenotypical tests were performed on 46 nonrepetitive isolates of *Pseudomonas aeruginosa* to detect the enzymes MBL, ESBL, and AmpC betalactamase. An antagonistic disc test was employed to phenotypically detect the enzyme AmpC betalactamase. By employing the double disk synergistic test as well as the "combined disk diffusion approach based on (Clinical and Laboratory Standards Institute) CLSI guidelines, the ESBL was detected phenotypically. Imipenem and Imipenem plus EDTA disk synergistic/potentiation test" revealed the presence of MBL.

Antimicrobial susceptibility testing

As per the guidelines provided by the CLSI, the strains were subjected to antibiotic susceptibility testing through the Kirby-Baeur disc diffusion technique. The following antibiotic discs were positioned 24 mm apart on an agar plate that had been lawn cultured with a subculture of isolates of *Pseudomonas aeruginosa*. Amikacin (30µg), Gentamicin (10µg), Piperacillin (100µg), Co-trimoxazole (25µg), Cefuroxime (30µg), Ciprofloxacin (5µg), Ceftizoxime (30µg), Ampicillin (30µg), and Piperacillin/tazobactam (100µg/10µg) (Hi-Media)

Phenotypic detection by Double disc synergistic test for ESBL exhibition

The classic double disc collaboration test, which uses ceftazidime (30 µg) as well as cefotaxime discs (30µg) with or without clavulanic acid (10µg) as directed by the CLSI, was used to identify ESBL generation. After an overnight incubation on Muller Hinton agar, an increment of ≥ 5 mm in the hindrance zones was identified as ESBL production, as compared to cephalosporin alone or in combination with clavulanic acid.

Phenotypic detection of MBL exhibition

The Imipenem-EDTA disk test was used to identify the generation of metallo beta-lactamases. Employing fitting sums of 10 µl of 0.5M EDTA arrangement, one of the two 10µg imipenem disks that were on the plate was filled with imipenem at the indicated concentration (750µg)/Imipenem-EDTA (IE) disk. After overnight incubation at 37˚C, the hindrance zones of imipenem and imipenem-EDTA disks were compared. Ipenem disk alone was regarded as the MBL maker when the hindrance zone increment with imipenem and EDTA disk was larger than 7mm.

Phenotypic screening/detection of AmpC β-lactamase exhibition

The Cefoxitin disk diffusion test was used to detect resistance and screen for the production of AmpC β-lactamase. After achieving a zone breadth of <18mm (screen positive), the segregates were moved forward and put through corroborative testing.

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The disk antagonistic test was employed to identify the presence of inducible AmpC β-lactamase in each *Pseudomonas aeruginosa* isolate. Over a Mueller Hinton agar (Hi-Media) plate, a test isolate was lawn cultured with turbidity similar to 0.5 McFarland guidelines. Twenty millimeters separated the Hi-Media disks containing cefoxitin (30µg) and cefotaxime (30µg). The screening of AmpC β-lactamase revealed positive results for separations exhibiting blunting of the cefotaxime zone of inhibition next to the cefoxitin disk.

RESULTS

Twenty segregates (43.4%) in total tested positive for AmpC beta-lactamase. Six strains (13%) of them produced inducible AmpC. AmpC's co-production with ESBL & MBL was described in 3/20(15)% & 8/20(40)%, accordingly. Strains of *Pseudomonas aeruginosa* produced with 100% AmpC β-lactamase enzymes showed reduced susceptibility to the majority of commonly used antibiotics in hospitals. Employing the Kirby-Bauer disc diffusion approach, the *Pseudomonas* isolates showed maximum susceptibility (79.1%) to imipenem, moderate susceptibility (50.5%) to piperacillin/tazobactam, followed by amikacin (45.5 percent) and co-trimoxazole (42.5 percent), & poor susceptibility patterns (the remaining drugs).

Table 1. Total number of AmpC β-lactamase producers

Total Number of isolates	Cefoxitin disk test	Disc antagonism test (inducible AmpC producers)
46	20	6

Table 2. Distribution of β-Lactamase Genes Among *Pseudomonas aeruginosa*

AmpC	AmpC +ESBL	AmpC +MBL
20	3	8

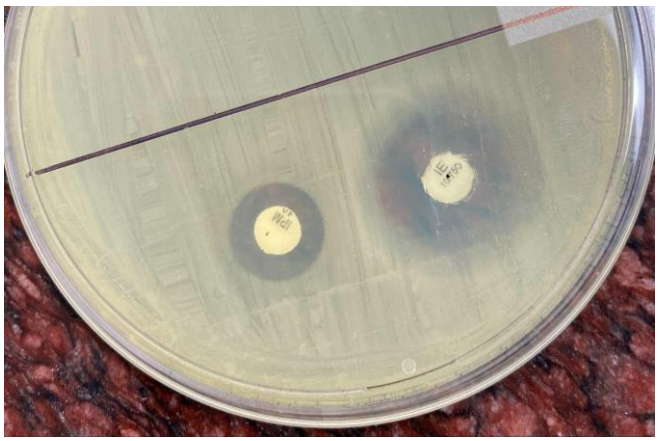
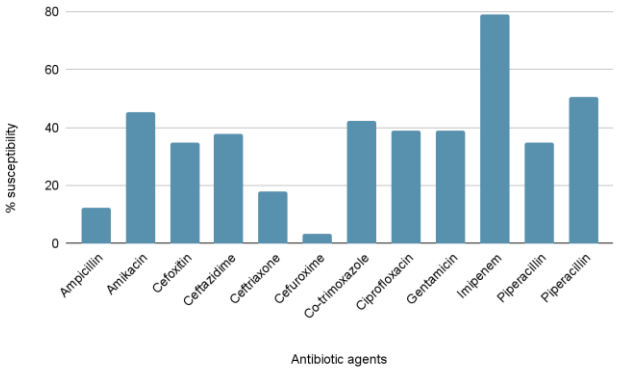


Figure 1. Demonstration of MBL through Imipenem-EDTA disk test



Graph 1. Antibiotic susceptibility pattern of *P. aeruginosa*

DISCUSSION

Pseudomonas aeruginosa is an opportunistic pathogenic bacterium that primarily affects patients with underlying medical conditions as well as those with a remarkably high threshold for resistance.[6,7,8] Trouble in treating *Pseudomonas aeruginosa* bacterial contamination also occurs due to bacterial resistance to beta-lactam anti-microbials.[7,8] There have been a few studies done to address the issue of antimicrobial resistance. If left undiagnosed, several *Pseudomonas aeruginosa* strains that produce β-lactamases are a major source of restorative disappointment and present significant clinical challenges.[9] There is no strategy for AmpC β-lactamase detection in the current CLSI rules. The original purpose of the AmpC disc test was to differentiate between AmpC β-lactamases mediated by plasmids.[3] In any case, Black et al. described how to use the AmpC disc test to identify chromosomally interceded inducible AmpC β-lactamases in a variety of microbes, that includes *Pseudomonas aeruginosa*.[13,14] Among the *Pseudomonas aeruginosa* confines under consideration, AmpC generation (43.47%) was higher using the Cefoxitin disk test (20/46) and antagonistic disc test (6/46). According to our analysis, *Pseudomonas aeruginosa* has a high rate of ESBL (15%), in contrast to a previous study that found 3.3% of ESBL generation [1]. Among AmpC-producing isolates, our results indicated a high percentage (40%) of MBL-producing *Pseudomonas aeruginosa*; however, earlier research in this nation suggested a low (7.5%) to direct (20.8%) predominance of MBLs [10,11]. With an apparent defenselessness of 89.1%, imipenem was the most effective drug, according to our study, which is in line with other studies. [1,7,12].

CONCLUSION

The current investigation highlights the significant frequency of MDR *Pseudomonas aeruginosa* that generates β-lactamase enzymes through various mechanisms. To address the current issue, epidemiological studies must be carried out in hospital settings to identify the source of infection. Early identification of these isolates that produce β-lactamase can help prevent treatment failure in the

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microbiology lab. To prevent this pathogen that produces multiple β-lactamases from spreading and eliminating all other options for treating Gram-negative nosocomial infections, strict antimicrobial guidelines and strategies are necessary. These guidelines and strategies should limit the careless application of cephalosporins as well as carbapenems in hospital settings.

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