DOI: 10.7860/JCDR/2016/18016.7612

Original Article

Microbiology Section

ESBL and MBL in Cefepime Resistant *Pseudomonas aeruginosa*: An Update from a Rural Area in Northern India

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ABSTRACT

Introduction: Cefepime, a fourth generation cephalosporin, is widely used for the empirical treatment of serious infections in critically ill hospitalized patients. *Pseudomonas aeruginosa (P. aeruginosa),* one of the commonest bacteria causing nosocomial infections has a propensity to develop antibiotic resistance quite promptly.

Aim: We undertook this study to assess the efficacy of cefepime against current clinical isolates of *P. aeruginosa* and to study existence of different beta-lactamase enzymes among cefepime resistant *P. aeruginosa* isolates.

Materials and Methods: Total of 618 isolates of *P. aeruginosa* recovered consecutively from various clinical samples of a tertiary care hospital were analysed. Their Antimicrobial sensitivity profile against piperacilin (100µg), piperacillin/tazobactam (100µg/10µg), ceftazidime (30µg), cefoperazone (75µg), cefepime (30µg), ciprofloxacin (5µg), gentamycin (10µg), amikacin (30µg) and imipenem (10µg) (Himedia) was tested by Kirby-Bauer disc diffusion method (Clinical and Laboratory Standards Institute guidelines). We further looked for ESBL, MBL and ESBL + MBL co producers among the cefepime resistant isolates by two different methods (combined double disc synergy test, imipenem-EDTA combined disc test and vitek2).

Results: Among 618 consecutive clinical isolates of *P. aeruginosa*, we observed resistance to cefepime in 457 (74%) isolates. We observed resistance to ciprofloxacin (n=506, 82%) in maximum number of isolates followed by that to Gentamycin (n=475, 77%), amikacin (n=366, 60%), and cefoperazone (n=350, 56.6%). Among all our cefepime resistant *P. aeruginosa* isolates only 27(6%) were ESBL producers, 18(4%) MBL producers and 2(0.4%) were ESBL+ MBL co-producers. All the ESBL and MBL isolates were also tested by VITEK 2 advanced expert system (bioMırieux Vitek Systems Inc, Hazelwood, MO, France) which revealed a 100% concordance with the phenotypic method tested.

Conclusion: This paper highlights the need to reconsider prescribing empirical antibiotics for *Pseudomonas* infections in this region and formulate a strong antibiotic policy to curb the menace of spread of multidrug resistant strains.

Keywords: Beta lactamases, Co-producers, Drug resistance, Fourth generation cephalosporin

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa), one of the common causes of nosocomial infections accounts for almost 10% of all hospital acquired infections [1,2]. These infections are difficult to eradicate and are often life threatening because of widespread occurrence of antibiotic resistance. Unfortunately, wrong choice of drugs has led to an increase in mortality rates and hence appropriate selection of antibiotics for these infections is the need of the hour [3,4]. In sync with the occurrence of widespread antibiotic resistance, recent reports of the emergence of Multidrug Resistance (MDR) in this pathogen has further limited therapeutic options [5,6]. Cefepime, a fourth-generation zwitteronic cephalosporin, is one of the few antibiotics reported to have a consistent activity against *P. aeruginosa* infections. However, reports on resistance to cefepime among these organisms are rising [7-9]. This is being reported more often from hospital settings resulting in a significant increase in attributable mortality among in-patients [10]. Since cefepime is one of the latest antibiotics introduced in clinical use, rapid emergence of widespread resistance to it could indicate a potentially disturbing trend and necessitate changes in current antibiotic prescription practices.

In *P. aeruginosa*, resistance to antibiotics may be due to outer membrane impermeability, target site modification and multidrug efflux pumps. Acquired resistance is due to the production of beta lactamase enzymes like extended spectrum beta lactamase (ESBL), metallo β-lactamases (MBL) and AmpC β-lactamases [11,12]. ESBLs are beta-lactamases that hydrolyze penicillins, cephalosporins and aztreonam and MBLs hydrolyze carbapenems and other beta-lactams. Various authors have reported growing occurrence of co-expression of these beta-lactamases in clinical isolates, underscoring the need for their early detection so that appropriate policy on curtailing empirical prescription of antibiotics is established [13].

AIM

We conducted a prospective study to analyze the susceptibility pattern of *P. aeruginosa* to cefepime and also determine the frequency and coexistence of ESBL and MBL-producing cefepime resistant *P. aeruginosa* strains at our tertiary care predominantly rural catering centre, in Northern India.

MATERIALS AND METHODS

This study was performed over a period of 2 years, from January 2010 till December 2012 in a 750 bedded, predominantly rural-serving, tertiary care teaching hospital in northern India. An approval was obtained from the institutional ethics committee of the institution. All positive *P. aeruginosa* culture isolates recovered from clinical samples, received during the period of study were included. In a previous study [8], the antimicrobial sensitivity of all the isolates were tested against piperacilin (100µg), piperacillin/tazobactam (100µg/10µg), ceftazidime (30µg), cefoperazone(75µg), cefepime (30µg), ciprofloxacin (5µg), gentamycin (10µg), amikacin (30µg) and imepenem (10µg) (Himedia) by Kirby-Bauer disc diffusion method on Mueller-Hinton agar following the recommendations of the Clinical and Laboratory Standards Institute [14].

We further carried the study forward and screened the cefepime resistant *P. aeruginosa* isolates for production of beta lactamase enzymes viz. ESBL and MBL. A double disc synergy test was used

as a screening tool to look for ESBL production among the strains. A 30µg disc of ceftazidime alone, and another in combination with 10µg clavulanic acid were placed at a distance of 20mm apart on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37°C. The strains showing at least 5mm differentiation between the inhibition zone around ceftazidime discs alone in comparison with the inhibition zone around ceftazidime+clavulanic acid were flagged as ESBL producing strains.

For detection of Metallo β-lactamases producing strains a 10µg disc of imipenem alone and another in combination with EDTA were placed at a distance of 20mm apart on a plate of Muller Hinton agar inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37°C. The strains which showed a greater than 7mm distinction between the inhibition zone around imipenem discs alone and the inhibition zone around imipenem+EDTA discs were considered as MBL-producing.

RESULTS

Among all the antibiotics tested for anti- pseudomonal efficacy in 618 isolates of *P. aeruginosa* recovered in our study, we observed resistance to ciprofloxacin (n=506, 82%) in maximum number of isolates followed by that to Gentamycin (n=475, 77%), cefepime (n=457, 74%), amikacin (n=366, 60%), and cefoperazone (n=350, 56.6%) [Table/Fig-1]. Further, among the 457 cefepime resistant isolates 27(6%) were ESBL positive and in 18(4%) strains MBL was detected [Table/Fig-2]. The co-existence of ESBL and MBL was reported in 2(0.4%) isolates. These two ESBL and MBL producers were resistant to almost all classes of antibiotics tested. The phenotypic results showed concordant results with VITEK 2 advanced expert system (bioMırieux Vitek Systems Inc, Hazelwood, MO, France).

Antibiotics	No. of resistant isolates	Percentage of Resistant isolates
Ciprofloxacin	506	82%
Gentamycin	475	77%
Cefepime	457	74%
Amikacin	366	60%
Cefoperazone	350	56.6%
Ceftazidime	247	40%
Imipenem	172	28%
Piperacillin	67	10.8%
Piperacillin/tazobactam	44	7.1%

[Table/Fig-1]: Invitro resistance pattern of *P.aeruginosa* isolates.

Total No. of Cefepime resistant *P.aeruginosa* isolates	ESBL producers	MBL producers	ESBL + MBL producers
457	27(6%)	18(4%)	2(0.4%)

[Table/Fig-2]: ESBL, MBL and co producers among cefepime resistant *P.aeruginosa* strains.
ESBL-Extended Spectrum Beta- Lactamases, MBL-Metallobeta-Lactamases.

DISCUSSION

Cefepime is one of the few antibiotics reported to have a consistent activity against *P. aeruginosa* infections. Nevertheless, resistance to cefepime among *P. aeruginosa* is rising which is complicating the clinical management of patients infected with such isolates [8,15,16]. We found an alarming level (74%) of resistance to cefepime among the *P. aeruginosa* isolates recovered from patients of our hospital. These results were comparable to the findings of studies done by Jazani et al., and Satti et al., in which resistance rate of cefepime among the *P. aeruginosa* were notable(75.4% and 71% respectively) [17,18].

Extended-Spectrum β-Lactamases (ESBLs) and Metallo-Betalactamases (MBL) have emerged as an important cause of resistance in Gram-negative bacteria. Acquired resistance by the production ESBL and MBL enzymes is a common reported mechanism in *P. aeruginosa* also [19]. In our previous study we reported resistance to commonly prescribed antibiotics in an alarmingly higher proportion of *P. aeruginosa* isolates recovered from this region [8]. In continuation to that we explored the frequency of ESBL and MBL production among cefepime resistant *P. aeruginosa* isolates and found that among the 457 cefepime resistant *P. aeruginosa* isolates, only 27 (6%) were ESBL producers which was much lower than the percentage of ESBL producing isolates of *P. aeruginosa* as reported by Aggarwal et al., (20.27%) and Goel et al., (42.31 %) [20,21]. The prevalence of MBL producers among our cefepime non susceptible *P. aeruginosa* isolates was also substantially lower as compared to the findings of other authors. Among, the 457 cefepime resistant *P. aeruginosa* isolates 18(4%), were MBL producers. In India, a prevalence of MBL in *P. aeruginosa* isolates ranging from 7.5% to 71% has been reported [22]. We found coexisting ESBL + MBL enzymes in 2(0.4%)of our isolates which is in contrast with the study by Chaudhary et al., in which 14.36% of the isolates co-produced ESBL and MBL enzymes [13]. This again emphasizes the need for screening for these enzymes before prescribing cefepime in *P. aeruginosa* infections. If remain undetected, these inducible enzymes can lead to treatment failures and increased mortality in critically ill patients.

CONCLUSION

Our study clearly demonstrates that although frequency of ESBL and MBL mediated resistance among the *P. aeruginosa* isolates recovered from the study group is quite low, the percentage of cefepime resistant *P. aeruginosa* was substantially notifiable. The possibility of some other resistance mechanism imparting resistance to cefepime needs to be explored. We plan to look for that possibility in our future projects. Nevertheless, bearing in mind the high rate of cefepime resistance in *P. aeruginosa* isolates clinicians need to reconsider prescribing cefepime empirically for *Pseudomonas* infections in this region.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Nov 30, 2015**
Date of Peer Review: **Dec 29, 2015**
Date of Acceptance: **Feb 11, 2016**
Date of Publishing: **Apr 01, 2016**