Inducible Amp C Beta-lactamase Producing *Pseudomonas aeruginosa*: Predominant Resistance Mechanism and a Threat in a Tertiary Care Teaching Hospital

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Abstract

**Background:** Antibiotic resistance to multiple antibiotics among *P. aeruginosa* are on rise due to acquisition of various beta-lactamase enzymes. *P. aeruginosa* possessing such enzymes can cause major break down in therapy and are responsible for substantial clinical challenges.

**Objectives:** To know the antibiotic susceptibility pattern, common resistance mechanisms of *P. aeruginosa* and document baseline antibiotic resistance data to implement effective infection control program.

**Methods:** A total of 200 *P. aeruginosa* was isolated between January - June 2015 from various clinical samples of both hospitalized and outpatients. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method to ceftazidime, cefepime, cefpirome, piperacillin -tazobactam, ceferazone-sulbactam, ciprofloxacin, gentamicin, amikacin, imipenem, and piperacillin. ESBLs, inducible chromosomal and non-inducible plasmid Amp C beta-lactamase and metallo-beta-lactamase presence was investigated.

**Results:** Twenty five percent isolates were from the intensive care unit, 41.5% from in patients and 33.5% from outpatients. Inducible, non-inducible Amp C beta-lactamase, ESBLs and MBLs producers was 47.5%, 4%, 26% and 19% respectively. Highest resistance was recorded to ceftriaxone (44% - 56.5%). Least resistance to colistin (2%), imipenem (19.5%) and amikacin (21.5%). Resistance to piperacillin-tazobactam, ciprofloxacin, gentamicin and ceferazone-sulbactam was 24%, 31.5%, 32%, and 36.5% respectively. Multi-drug resistance were 37.5%. Overall resistance pattern were higher among ICU and inpatient than outpatient isolates.

**Conclusion:** Inducible Amp C beta-lactamases was found to be predominant resistance mechanism followed by ESBL and MBL among *P. aeruginosa*. Resistance was high to cephalosporins and among hospitalized patients. Usage of cephalosporins could be risk factor for inducible resistance. Colistin, imipenem and amikacin as first line with piperacillin-tazobactam as alternative may be preferred antibiotics in treating *P. aeruginosa* infections. Calls for screening, monitoring and infection control measures.

Introduction

*Pseudomonas aeruginosa* nosocomial infections have become a worldwide healthcare issue especially in intensive care units.¹ *P. aeruginosa* is notorious for its stubborness in the hospital settings, and multidrug resistance mechanisms are frequently seen in such hospital isolates.² There are two antibiotic resistance mechanisms among *P. aeruginosa*. One is by mutation in the intrinsic gene (intrinsic resistance) and the second is by acquiring antibiotic resistant genes from other bacteria. Further, acquired resistance in the form of over expression or by plasmid transfer of resistance genes, impart resistance to broad spectrum of antibiotics leading to increased frequency among clinical and environmental strains.³ Usually these resistance are mediated by enzymes like extended spectrum beta-lactamases (ESBLs), Amp C beta-lactamases and metallo-beta-lactamases (MBLs).⁴ Detection of such enzymes and timely report to the prescribing hands become crucial as the presence of these enzymes makes treatment of infection both hard and costly. Hence a preliminary attempt was undertaken to document their occurrence and to prepare a base line data for an effective infection control program.

**Material and Methods**

ESIC-MC and PGIMSR, Rajajinagar, Bengaluru is a tertiary care 500 bed teaching hospital. It is a closed system of health care delivery system where only patients insured under ESIC scheme represents the patient population. This study was an extension of a postgraduate dissertation work. The study period was from January – June 2015. Clinical specimens belonging to patients who stayed at least 3 days in hospital [ICU and in patients] and of all outpatients [irrespective of their previous hospitalization status] were included. Only one isolate per patient with clinical significance was considered. Institutional ethical clearance was obtained. *P. aeruginosa* were isolated and identified from various clinical samples (pus, urine, sputum, throat, vaginal and wound swab, body fluids like ascitic, cerebro spinal and pleural fluid) submitted to diagnostic microbiology by standard methods.⁵ Antibiotic susceptibility test was performed by Kirby- Bauer's
The data were analysed using Microsoft Excel.

Results

A total of 200 *P. aeruginosa* were isolated during the study period. Fifty (25%) were from the intensive care unit, 83 (41.5%) were from inpatients and 67 (33.5%) were from outpatients. Seventy-six (38%) isolates were from females and 124 (62%) isolates were from males. Isolation from pus, sputum, miscellaneous, urine and blood was 61 (30.5%), 49 (24.5%), 46 (23%), 35 (17.5%) and 4 (2%) respectively.

Isolates of *P. aeruginosa* revealed 47.5% of inducible and 4% of non-inducible plasmid mediated Amp C beta-lactamases. ESBLs and MBLs were 26% and 19% respectively (Table 1).
Detection of inducible and non inducible plasmid mediated Amp C beta-lactamases was high among ICU (60% and 4%) and inpatient isolates (49.3% and 4.8%) compared to outpatient isolates (32.8% and 2.9%). ESBL detection rate among ICU (28%), inpatients (21.6%) and outpatient isolates (29.8%) were nearly similar. Detection of MBL was high (32.8%) among outpatient isolates compared to inpatients (13.2%) and ICU isolates (10%). Two percent of ICU isolates were MBL positive by MHT whereas none could be found positive among inpatient and outpatient isolates by MHT (Table 1).

Highest resistance of 56.5% was noted to ceftazidime. Least resistance was recorded to colistin (2%), imipenem (19.5%) and amikacin (21.5%). Resistance to piperacillin-tazobactam, ciprofloxacin, gentamicin and ceferozone-sulbactam was 24%, 31.5%, 32%, and 36.5% respectively. Resistance to cefpirome and cefepime were 44% and 53% respectively. Multi-drug resistant was found to be 37.5%. Overall resistance pattern were higher among ICU and inpatient isolates than outpatient isolates (Table 2).

**Discussion**

ESBLs are enzymes that mediate resistance to third generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone) and are inhibited by beta-lactamase inhibitors (clavulanic acid, tazobactam, sulbactam), cefamycins (cefoxitin) and carbapenems (imipenem, meropenem). In the present study we observed 26% are ESBL producers which is high compared to studies from other parts of India revealing 19.4% - 21% as ESBL producers. Although ESBLs among *P. aeruginosa* is uncommon compared to other common Gram negative bacteria like *E. coli* and *K. pneumoniae*, the present increasing trend suggest possible horizontal gene transfer among *P. aeruginosa* and insists for better monitor and control measures.

Amp C beta-lactamases mediate resistance to cefamycins (cefoxitin), third and fourth generation cephalosporins, beta-lactamase inhibitors and are susceptible to carbapenems. *P. aeruginosa* produces both inducible chromosomal Amp C and non-inducible plasmid mediated AmpC. In the present study, 47.5% were inducible and 4% were non-inducible plasmid mediated Amp C beta-lactamase producers. In contrast, study from Varanasi reveals only 7% as inducible and 52.4% were non-inducible Amp C beta-lactamase. Besides, usage of cephalosporins in hospitals activates and hyper produces inducible Amp C enzymes. High cephalosporins resistance (44% - 56%) and inducible as a predominant resistance mechanism in the present study possibly indicates pressure of cephalosporins (personal interactions with clinician’s reveals frequent preference of cephalosporins) as an inducing antibiotic in the hospital environment. However, studies at one side show increase occurrence of Amp C beta-lactamase production in *P. aeruginosa* with use of inducers (like imipenem) and the other side without antibiotic inducers (mutational induction of Amp C gene). Further studies are needed in the present setting to understand the relation between use of inducing antibiotics and the occurrence of high inducible resistance among *P. aeruginosa*.

MBL is the most common carbapenem resistance mechanism in *P. aeruginosa*. Both beta-lactam and beta-lactamase inhibitors are ineffective against MBL producers. The present study records 19% isolates as MBL producers. However, studies from India ranges from 8% to 74.5% MBL producers among *P. aeruginosa*. Report from the global epidemiology of carbapenem-resistant *P. aeruginosa* documents that the geographical prevalence of MBL genes not only increasing steadily but also varies from country to country. Besides, the present study records 37.5% as multi-drug resistance seen in MBL producers. The appearance of MBL producing multidrug-resistant *P. aeruginosa* is a challenge in bringing the clinical infection under control and needs monitoring.

Overall resistance pattern in the present study were higher among ICU and inpatient isolates than outpatient isolates indicating a much antibiotic pressure in hospitalized patients. A study on trends of antibiotic resistance of *P. aeruginosa* from wound infections reveals a decreasing trend in resistance to ciprofloxacin, ceftazidime and carbapenems among in patients isolates due to reduced antibiotic usage.
whereas no change among outpatient isolates. Colonization pressure, cross transmission and exposure to multiple antibiotics have been identified to be important risk factors for the resistance acquisition of \textit{P. aeruginosa}. The present study reveals a higher resistance to cephalosporins (44\% to cefpirome, 53\% to ceftime and 56.5\% to ceftazidime). However, status of colonization, cross transmission between patients and the load of individual antibiotics used in the present study was not known. Although each resistance mechanism is related to a specific class of antibiotics, multiple mechanisms mediate variably resistance to each class of antibiotics and vary from country to country. Further studies also have to be carried out to look into other resistance mechanisms such as loss or reduced outer membrane protein channels and over production of active efflux pumps.

To conclude, inducible \textbf{Amp C beta-lactamase} was found to be predominant mechanism of resistance and a cephalosporins usage might be risk factor among \textit{P. aeruginosa}. Colistin, imipenem and amikacin as first line and piperacillin-tazobactam and imipenem and amikacin as first line and piperacillin-tazobactam producing \textit{Pseudomonas aeruginosa} in intensive care units. Australas Med J 2013; 6:686-93.

References


