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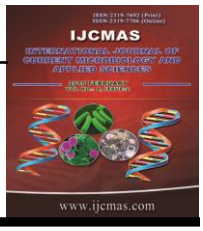

Original Research Article

An Insight into Antibiogram and β- Lactamase Production of *Pseudomonas aeruginosa* Isolated from River Water and Soil

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A B S T R A C T

Pseudomonas aeruginosa is a common nosocomial pathogen that causes infections associated with a high morbidity and mortality especially among immunocompromised patients. *P. aeruginosa* is a versatile ubiquitous bacillus that flourishes in a wide variety of ecological niches. Natural environment plays a major role in dissemination of drug resistant bacteria. A total of seventy five *P. aeruginosa* strains isolated from various natural environmental samples, were tested for antimicrobial susceptibility testing as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Beta- lactamase production was detected by standard procedures. Of the isolates, 70.7% showed resistance to cefepime, followed by ceftriaxone (40%), cefotaxime (30.7%), piperacillin/tazobatam (28%) and meropenem (2.7%). All the isolates were resistant to cefoxitin, of which 49% isolates revealed the presence of inducible AmpC β- lactamase and none of the isolates were plasmid mediated AmpC β-lactamase producers. Two isolates (2.7%) were MBL producers. Our study revealed presence of *P. aeruginosa* with high acquired antibiotic resistance in the environment. Better understanding of the factors responsible for the emergence of such pseudomonads in the environment may play a significant role in controlling spread of drug resistant *P. aeruginosa* in the community.

Keywords

P. aeruginosa, Antibiotic resistance, MBL, AmpC β- lactamase, River water

Introduction

Pseudomonas aeruginosa is an obligate aerobic Gram negative bacterium, with a remarkable capability to inhabit diverse niches (DebMandal *et al.*, 2011). *P. aeruginosa* is a ubiquitous bacillus and flourishes in a wide variety of ecological environment including different types of soil, rivers, wastewater, plant, animal and human (Goldberg, 2000). Its ability to survive on inert materials, live on minimal nutrition and tolerance to wide variety of physical conditions has contributed enormously to its ecological success and its role as an effective opportunistic pathogen (Gales *et al.*, 2001). *P. aeruginosa* can often lead to a broad spectrum of life threatening

diseases such as ventilator associated pneumonia, infections in cystic fibrosis, polymicrobial bacteremia, burns wound infection, urinary tract infection and ulcerative keratitis (Pier and Ramphal, 2005). This species is metabolically versatile, is intrinsically resistant to wide range of antimicrobials due to outer membrane permeability, extensive efflux pump system, and exhibits acquired resistance by β- lactamase production (Strateva and Yordanov, 2009). The multidrug resistant *P. aeruginosa* poses serious problems in the treatment of nosocomial infections (Suzuki *et al.*, 2013). However, there is a paucity of information on the distribution of *P. aeruginosa* in water and soil environment. Hence, the present study was designed to isolate *P. aeruginosa* from river water and agricultural soil, and to find out the resistance pattern and β-lactamase production.

Materials and Methods

Isolation and identification of *P.aeruginosa*

A total of 140 environmental samples were randomly collected from Payaswini river (n = 70) and agricultural soil (n = 70) in Sullia of South Canara district of Karnataka state. Samples were taken from locations considered to have a low risk of hospital effluent contamination to ensure the non-clinical origin of the environmental isolates. Samples were processed as follows: fifty ml of each river water sample was inoculated into 50 ml of double strength cetrimide broth and incubated at 37º C for 48 hours. Using a standard nichrome wire loop of 4mm diameter one loopful (0.01mL) of growth was plated on cetrimide agar and Mac Conkey's agar and incubated at 37º C for 24 hours. For soil samples, 20 gms of each soil sample collected from different areas to a depth of 30 cm were mixed with 20 ml of double strength cetrimide broth and incubated at 37º C for 48 hours. Further processing was similar to that done for water samples. The isolates from both water and soil were identified as *P. aeruginosa* by standard procedures (Govan, 2006).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of the isolates were performed on Mueller-Hinton agar (MHA) using commercial antibiotic discs (Hi-Media Laboratories Ltd, Mumbai) by the standard Kirby-Bauer disc diffusion method and interpreted as per CLSI recommendation (CLSI, 2011). The antibiotics used were: gentamicin (10µg), amikacin (30µg), tobramycin (10µg), ciprofloxacin (5µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), cefperazone/ sulbactam (75/30µg), piperacillin/ tazobactam (100/10µg), meropenem (10µg), imipenem (10µg), colistin (10µg), cefipime (30µg), cefoxitin (30µg), polymyxin B (300units). *P. aeruginosa* ATCC 27853 was used as control.

ESBL, AmpC β-lactamase & MBL detection

Isolates resistant to at least one of the third generation cephalosporins (ceftazidime, ceftriaxone and cefotaxime), cefoxitin and meropenem were tested for ESBL, AmpC β-lactamase and MBL production respectively. ESBL production was detected by CLSI phenotypic confirmatory test (Sreeshma *et al.*, 2013). Production of AmpC β-lactamases was detected by disc antagonism test and AmpC disc test as described earlier (Sreeshma *et al.*, 2013). MBL production was detected by disc potentiation test (Sreeshma *et al.*, 2013).

Result and Discussion

A total of 75 (54%) *P. aeruginosa* were isolated from 140 environmental samples. Out of which 49(65.3%) were from river water samples and 26(34.7%) were from soil (Table 1). Figure 1 presents the antimicrobial susceptibility pattern of the isolates to 15 antibiotics. All isolates were found to be susceptible to imipenem (100%), colistin (100%) and polymixin B (100%). Among the aminoglycosides tested, tobramycin (94%) was most active against *P. aeruginosa* followed by amikacin (88%) and gentamicin (78%). Ciprofloxacin showed 89% of susceptibility.

The resistance pattern of *P. aeruginosa* is showed in Figure 1. Isolates exhibited highest rate of resistance to cefepime (70%) followed by cefoperazone /sulbactam (56%), ceftriaxone (40%), cefotaxime (30%), piperacillin/ tazobactam (28%), ceftazidime (10%) and meropenem (2.7%).

From 75 cefoxitin resistant *P. aeruginosa*, 37 (49%) were found to be inducible AmpC β- lactamase producers by disc antagonism test. However, none of the isolates produced plasmid mediated AmpC β- lactamase. Thirty isolates were resistant to ceftriaxone, but no ESBL producing *P. aeruginosa* was detected. Among 75 isolates, only two were resistant to meropenem and both were confirmed as MBL producer by disc potentiation test. Both the MBL producers were isolated from river water. But no strains coproduced AmpC β-lactamase and MBL. The rate of AmpC β- lactamase and MBL production among the *P. aeruginosa* resistant isolates is summarised in (Table 2).

P. aeruginosa is a metabolically versatile bacterium and is responsible for a number of opportunistic and nosocomial infections. Soil and aquatic ecosystems, the largest and multifarious microbial habitats on earth, they could serve as storehouse of antibiotic resistant pathogens (Baquero *et al.,* 2008). Various agricultural and anthropogenic activities have led to the discharge of chemicals including antibiotic and heavy metals in the water and soil ecosystem. Aquatic ecosystem is a site where environmental bacteria interact with microbes originating from humans and animals, finally leading to exchange of genes through horizontal gene transfer. Thus facilitates the spread of drug resistance in bacteria ((Baquero *et al.,* 2008).

The isolation rate of environmental *P. aeruginosa* in the present study was 54%. On the contrary, a previous study reported higher isolation rate for *P. aeruginosa* (Ullah *et al.*, 2012). Our study indicated that occurrence of *P. aeruginosa* is common in river water (36%) than agricultural soil (19%), which was close to the observation by Pellett et al., (1983) who reported occurrence in water was 33.8%. A couple of other studies from South Africa (46.7%) and France (95.5%) reported high occurrence of *P. aeruginosa* in water (Igbinosa *et al.*, 2012; Slekovec *et al.*, 2012).

P. aeruginosa exhibited notable resistance to cephalosporins. Highest rate of resistance was against cefepime and cefaperazone/ sulbactam. Among the third generation cephalosporins, majority of the isolates were resistant to ceftriaxone followed by cefotaxime and ceftazidime. A previous study conducted in Assam reported higher antibiotic resistance for environmental isolates (Das *et al.,* 2014). Even though there was a high cephalosporin resistance, none of the isolates produced ESBL. The increase in cephalosporin resistance may be due to loss of certain outer membrane proteins, over expression of multidrug efflux pump, porin mutation and by hyper

production of AmpC β- lactamase (Strateva and Yordanov, 2009). All our isolates were resistant to cefoxitin and 49% of them were found to be inducible AmpC producer. This is a first known study reporting the production of inducible AmpC β- lactamase from environmental isolates. However, none of the isolates produced plasmid mediated AmpC β- lactamase.

A small percentage of *P. aeruginosa* showed resistance to meropenem. This was similar to the earlier reports (Suzuki *et al.*, 2013; Lutz and Lee, 2011). Carbapenem resistance in *P. aeruginosa* is mediated by various mechanisms such as outer membrane porin reduction, over expression of efflux pump or by production of metallo β-lactamases. In the current study, the meropenem resistant isolates were found to be MBL producers. Previous studies from different countries have observed higher percentage of MBL production (Das *et al.*, 2014; Shehabi *et al.*, 2011). However, in our study carbapenem resistance was completely absent in soil isolates. This observation was consistent with the previous study (Janam *et al.*, 2011). Furthermore, emergence of MBL producing *P. aeruginosa* in the environment is alarming.

Our findings showed that aminoglycosides were extremely effective against the isolates, which was in accordance with the previous study (Odjajare *et al.*, 2012). In addition, ciprofloxacin and piperacillin are the major antipseudomonal agents that were effective against the isolates.

Conclusion

Our study revealed that antibiotic resistant and beta lactamase producing *P. aeruginosa* are widely distributed in the river water than in soil. The emergence of drug resistant bacteria especially MBL producing bacteria in the environment poses a threat to public health. Therefore, there is a need for regular monitoring of various ecological niches to prevent the dissemination of these pathogens into the environment.

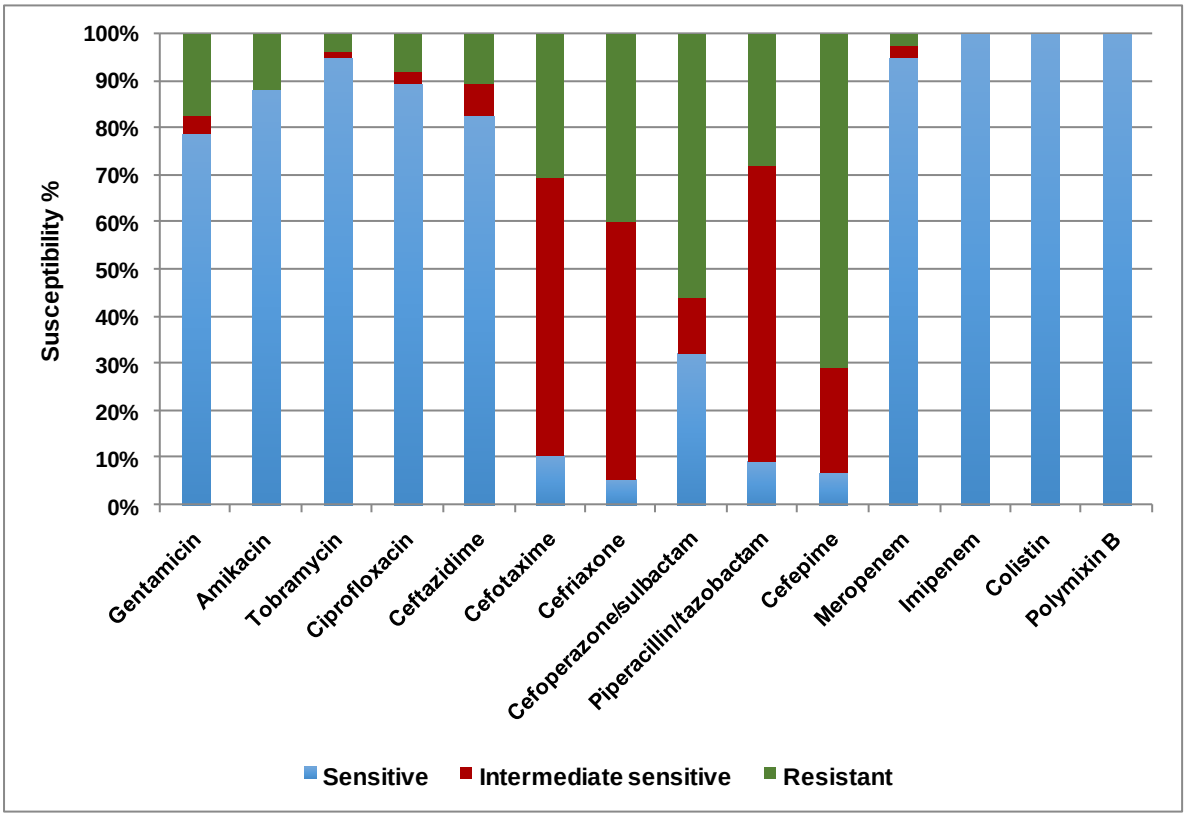
Table.1 Isolation rate of *P. aeruginosa* from environmental sample

Isolation source	No. of specimens	*P. aeruginosa* No (%)
River water	70	49(70%)
Agricultural soil	70	26(37%)
Total	140	75(54%)

Table.2 Rate of Inducible AmpC β-lactamase and MBL produced by *P. aeruginosa*

Enzymes produced	No. of isolates tested	No. of positive isolates (%)
Inducible AmpC β-lactamase	75	37 (49%)
MBL	75	2 (2.7%)

Fig.1 Antimicrobial susceptibility pattern of *P. aeruginosa*



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