

ABSTRACT

Background: Development of antibiotic resistance is one of the major causes of treatment failure in bacterial infections like *Pseudomonas aeruginosa* which is one of the leading opportunistic pathogen. Increasing resistance to carbapenems has further worsened the scenario. The major mechanism of resistance to carbapenems includes production of metallo beta lactamases, loss of porin channels, outer membrane protein defect and over expression of antibiotic efflux pumps. Efflux pumps of the RND family are prominent in clinically significant MDR strains of *P.aeruginosa* of which, *Mex* efflux pumps contribute significantly. Evidencing their expression in clinical isolates would help in rationalizing antibiotic selection.

Objectives: The present study was carried out to detect the presence of efflux pump-mediated drug resistance in clinical isolates of *Pseudomonas aeruginosa* using phenotypic and genotypic methods.

Methods: Clinical isolates ($n = 50$) of MDR *Pseudomonas aeruginosa* were collected from various samples, and their efflux-mediated drug resistance was detected by three simple phenotypic assays - ethidium bromide (EB)-agar cartwheel method efflux pump inhibitor (EPI)-based assay, employing phenylalanine-arginine β -naphthylamide as inhibitor, and synergy test using Carbonyl Cyanide 3-Chloro phenyl hydrazone (CCCP). Molecular characterization was done using PCR, to look for the presence of *mexE* gene which is a constituent of mexEF- OprN efflux system.

Results: out of the fifty strains of *Pseudomonas aeruginosa*, the EB-agar cartwheel method of screening revealed efflux activity in 40% ($n=20$) of the strains. The efflux activity was revealed at a minimum concentration of EB at 1 mg/l up to a concentration of 2.5 mg/l. The confirmatory EPI-based micro plate assay and the synergy test showed efflux activity only in 32% ($n=16$) strains. These positive strains ($n = 16$) for efflux were subjected to PCR for the presence of *mexE* gene and found that MexEF-OprN efflux system was predominant type.

Conclusion: This study reports on the emergence of efflux pump-based multidrug-resistance in *Pseudomonas aeruginosa*. Our results showed that 32% of drug resistance in MDR strains of *P.aeruginosa* is attributable to efflux-related mechanisms, thereby emphasizing the need for inclusion of efflux-related tests in the diagnostic regimen for MDR clinical bacteria.

Key words: *Efflux pump, Pseudomonas, Carbapenems, MexEF-OprN*