Molecular characterization of colistin- and carbapenem- resistance mechanisms in *Pseudomonas aeruginosa* isolates

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Introduction

P. aeruginosa causes severe nosocomial infections in cystic fibrosis and immunocompromised patients. The spread of carbapenem resistant *P. aeruginosa* and infection caused by it is a burgeoning problem. These patients were saved by antibiotics like colistin. However, patients are succumbing due to such infections inspite of colistin therapy. This is alarming. This prompted us to look into the problem of colistin resistant *P. aeruginosa* (CRPA) and also probable resistance mechanisms involved.

Methods

Nineteen CRPA isolates were collected from microbiology department, JIPMER, Puducherry. E-Strips were used to identify the MIC level of colistin and Polymyxin B resistance in *P. aeruginosa*. Quantitative real time PCR (qRT-PCR) was optimized to evaluate the expression levels of *pmrA* and *phoP* genes. The *rpsL* gene was used as reference gene. The differential gene expression of *pmrA* and *phoP* between CRPA and CSPA (Colistin sensitive *P. aeruginosa*) isolates were analyzed with student's t-test, using the normal significance level $P \leq 0.05$. The *pmrB* and *phoQ* genes of CRPA isolates were amplified and sequenced. The amino acid variations were evaluated using online expasy tool. In addition, PCR assay was carried out to detect the presence of MCR-1 mediated colistin resistance. Multiplex PCR was optimized to evaluate the presence of carbapenemases genes (blaNDM-*1, blaIMP, blaVIM, blaOXA-48* and *blaKPC*). Reverse transcriptase PCR (RT-PCR) was optimized to detect *OprD* deficiency and efflux pump activity was determined by evaluating the expression levels of *mexA* and *mexC* genes using qRT-PCR.

Results

Of the total 19 CRPA isolates, most of strains were collected from wound swab (n=15) followed by blood (n=2), pus (n=1) and urine (n=1). Nineteen CRPA isolates were found to be resistant to different antimicrobial agents including colistin, polymyxin B, meropenem, imipenem, ciprofloxacin, gentamycin, amikacin, ceftazidime and piperacillin-tazobactam. The study isolates were confirmed as *P. aeruginosa* by 16SrRNA gene sequencing. Overall, 11 and 9 isolates showed enhanced expression of *pmrA* and *phoP* genes in CRPA isolates, respectively. Nucleotide variations were found in 14 sites of *pmrB* gene and 7 sites of *phoQ* gene. Amino acid alterations were observed in 12 sites of *PmrB* gene and 3 sites of *phoQ* gene. The amino acid substitutions in colistin resistant isolates (MIC, 8µg/ml) were

PmrB; Lys333Asn, Val378D, Gly393Asp, His345Leu, Trp352Arg, Asn363Ser, Ala364Thr, Gly436Val, Gly439Val, Ser411Arg, Val389Glu, Phe405Leu and Val417Asp; and ***PhoQ***: Phe197Leu, Leu324Arg Leu29Arg. No MCR-1 gene was detected in this study. In addition, *blaVIM* was observed in six CRPA strains and one isolate possess *blaIMP*. However, none of the CRPA isolates were found to be harboring with *OprD* deficiency or activated efflux pump activity.

Conclusion

Co-occurrence of colistin- and carbapenem- resistance mechanisms in same bacteria remains a challenge for clinicians to consider therapeutic options against such infections. While our studies highlighted the mechanisms of colistin- and carbapenem resistance in CRPA isolates, further studies are required to trace the source and the origin of colistin resistance in this specific environment.