Summary & Conclusion

6. SUMMARY AND CONCLUSION

- A total of 100 isolates recovered from human clinical cases at Indira Gandhi Medical College (IGMC) Shimla (Himachal Pradesh) were collected and 87 isolates were confirmed as *P. aeruginosa* isolates on the basis of their morphology, cultural characteristics, microscopic examination of Gram’s stained preparations and standard biochemical tests in our laboratory.

- All the *P. aeruginosa* isolates were subjected to *in vitro* antibiotic cultural sensitivity assay using 12 antibiotics belonging to different antibiotic classes/groups. Out of 87 isolates examined, 30 (34.48%) isolates of *P. aeruginosa* were found as multidrug resistant (MDR) whereas 57 (65.52%) isolates were sensitive to multiple drugs (MDS).

- These MDR (n=30) and MDS (n=30) isolates were further screened *in vitro* for the production of different virulence traits i.e. gelatinase, protease, lipase, hemolysin and biofilm production. Statistically, there had no significant difference found among MDR and MDS isolates for the production of virulence factors studied.

- In addition to 87 *P. aeruginosa* isolates, 93 more isolates confirmed in our laboratory in another study were also included raising the number of isolates to 180.

- The isolates of *P. aeruginosa* (n=180) were characterized for the production of metallo β-lactamase (MBLs) and AmpC β-lactamase (AmpCs) enzymes.

- On the basis of resistance to carbapenems (meropenem/imipenem), 40/180 (22.22%) isolates were screened positive for MBL production. Of these, 29 (72.5%) isolates were confirmed as MBL producers in combined disc test (CDT) and MRP/MPR+EDTA E-strip test and further characterized for MBL encoding genes: *blaNDM* and *blaGIM*.

- For AmpC β-lactamase production, 108/180 (60%) isolates of *P. aeruginosa* were screened positive using standard disc diffusion breakpoint for cefoxitin. Of these, 52 (48.14%) were confirmed as AmpC producers on the basis of disc antagonism test (DAT) and Mix/Mix+E-strip test. The confirmed isolates were further characterized genotypically for AmpC genes: *blaPDC* and *blaCMY*. 
• The genomic DNA of 29 MBL and 52 AmpC positive *P. aeruginosa* isolates were used as templates for PCR amplification of MBL genes: *bla*<sub>NDM</sub>, *bla*<sub>GIM</sub> & AmpC-type genes (*bla*<sub>PDC</sub>, *bla*<sub>CMY</sub>) respectively.

• In case of MBL genes, the amplification was achieved in 11/29 (37.93%) isolates for *bla*<sub>NDM</sub> gene whereas no amplification was observed for *bla*<sub>GIM</sub> gene in any of the isolates tested.

• In case of AmpC-type genes, the amplification of *bla*<sub>PDC</sub> gene was achieved in 11/52 (21.15%) isolates while amplification was not observed for *bla*<sub>CMY</sub> gene in any of the isolates tested.

• The amplicons of *bla*<sub>NDM</sub> and *bla*<sub>PDC</sub> genes of three selected strains of *P. aeruginosa* were sequenced for their nucleotides and sequence homology to the published NCBI sequences was determined by BLAST n analysis.

• The present study revealed the occurrence of *bla*<sub>NDM-1</sub> and *bla*<sub>NDM-7</sub> gene variants among *P. aeruginosa* isolates in this region of country whereas the exact variant of *bla*<sub>PDC</sub> gene was not established as they showed homology with different *bla*<sub>PDC</sub> gene variants of standard *P. aeruginosa* strains of NCBI database.

• In the present study, we did observe the co-occurrence of MBL (*bla*<sub>NDM</sub>) and AmpC-type (*bla*<sub>PDC</sub>) β-lactamases in six isolates.

• The nucleotide sequences were submitted to the National Centre for Biotechnology Information (NCBI) through BankIt submission system and have been assigned accession numbers.