7. RECOMMENDATIONS AND FUTURE DIRECTIONS

The resistance to antibiotics has now become an increasing problem worldwide. *P. aeruginosa* is an opportunistic nosocomial pathogen responsible for a wide range of infections that may present high rates of antimicrobial resistance. The present study provides a picture of moderate (34.48%) prevalence of multidrug resistant *P. aeruginosa* (MDRPA) strains in the hospital setting at Shimla, Himachal Pradesh. Such MDR isolates may lead to increased length of hospital stay and higher hospital costs which eventually enhances the morbidity and mortality rate. It is therefore necessary to utilize strategies that will minimize the effect of resistance on the susceptible patients and healthcare system. Effective treatment of infections caused by such MDRPA strains includes prevention, source control, using successful synergistic antibiotic combinations, including polymyxin B and E antibiotics that have adequate coverage. According to our study, a combination of piperacillin/tazobactam and levofloxacin are the antibiotics of choice for treating *P. aeruginosa* infections in this region of the country. Virulence factors play an important role in the pathogenicity of *P. aeruginosa* infections. We also evaluated the *in vitro* expression of some virulence factors (production of gelatinase, protease, hemolysin and lipase as well as biofilm formation) among multidrug resistant and sensitive isolates. However, all MDR and MDS isolates had no significant difference towards most of the virulence factors studied.

Phenotypically, MBLs and AmpC as a mechanism of resistance was found in 72.5% and 48.14% isolates respectively. The gene encoding New Delhi metallo β-lactamase enzyme i.e. *blaNDM*, was amplified in 37.93% strains of *P. aeruginosa*. An ampC β-lactamase derived from *P. aeruginosa* i.e. *blaPDC* was also found in 21.15% *P. aeruginosa* isolates. This might be the first study regarding the occurrence of *blaNDM* and *blaPDC* genes among *P. aeruginosa* strains in the state of Himachal Pradesh. The increase in the prevalence of the MBL and AmpC producing strains might be an indication of their spread between different genera or different species of the same genera through horizontal gene transfer. This study can be extended further to characterize other MBL genes (*(blaVIM, blaIMP, blaSPM, blaSIM, blaAIM, blaFIM)*) and AmpC genes (*(blaFOX, blaMOX, blaLAT, blaACC, blaMIR, blaDHA, blaBIL)*) to establish the full spectrum of genes inhabiting this region. The prevention of spread of such resistant strains and recognition of patients at risk is urgently needed in this region. We have to
focus on the basis of the evolution of such genes in addition to plan strict infection control strategy in hospitals.

This study can also be extended further to characterize MBL and AmpC encoding genes on the plasmid DNA, however, we only characterized MBL and AmpC genes present on the genomic DNA. This work can be further extended to know the incidence of MBL and AmpC producing \textit{P. aeruginosa} strains in large geographic region which might present better picture. The co-occurrence of both MBL and AmpC encoding genes was found in six isolates which is an alarming situation. The presence of multiple \( \beta \)-lactamases in a single isolate might contribute to the high degree of resistance which might lead to treatment failure and high mortality.

\( \beta \)-lactamases (Extended spectrum \( \beta \)-lactamases, AmpC \( \beta \)-lactamases and metallo-\( \beta \)-lactamases) have emerged as the most worrisome mechanism of resistance among the gram-negative bacteria, which pose a therapeutic challenge to the clinicians. The moderate prevalence such resistant strains in this region emphasize the need for an early detection of the \( \beta \)-lactamase producers in Microbiology laboratories by simple and inexpensive screening methods. Early detection of such isolates can help in providing an appropriate antimicrobial therapy and prevent the development and dissemination of these multidrug resistant strains. The emergence of \( \beta \)-lactamases as a mechanism of antimicrobial resistance necessitates the strict guidelines for limiting indiscriminate and irrational use of antibiotics. On the basis of local epidemiological data and international guidelines, every health care institution must build up its own antimicrobial stewardship program which should be based to optimize the antimicrobial use among the hospitalized patients, to improve the patient outcomes, to ensure a cost-effective therapy and to reduce the adverse consequences of the antimicrobial use.