Summary:

Extended spectrum β-lactamase (ESBL) study in the drug resistant Gram negative clinical isolates

The emergence of multidrug resistant (MDR) bacteria is a worldwide health problem. Different mechanisms in the MDR Gram negative bacteria like enzyme deactivation, membrane permeability barriers and the efflux are involved to make antibiotics ineffective. This impairment of antibiotics is limiting the therapeutic options for the infections, once considered treatable. The *Klebsiella pneumoniae* is one of noscomial infection causing agent with high prevalence rate of β-lactamases. In the present study, we found 33% of the clinical isolates collected from GMCH 32, Chandigarh hospital, to be *K. pneumoniae* isolates. All isolates were ESBL positive and found co-resistance to other classes of antibiotics. Among the prevalence of ESBL enzymes, CTX-M15 was present in 100% isolates and NDM (carbapenamase) was recognized in 69% of isolates. In selected isolates ≥8 fold reduction in the MIC of monobactam, 3rd generation cephalosporin and carbapenem was observed. The expression analysis of these isolates for efflux pump genes shows upregulation in RND pumps (*kexD* and *acrB*) under antibiotic stress with exception of selected isolates showing greater upregulation in SMR (*kpnF*) and MATE (*kdeA*) family pumps. However, later were found with less fold increase in MIC as compared to isolates with RND pump upregulation. Regulation of different efflux pumps varies according to different antibiotic stress and reflects in the MIC of isolates. Further, simultaneous upregulation in

expression of efflux pumps suggests additive or synergistic effect in resistance. No mutational changes were observed for porins. The diverse resistance mechanisms, in clinical isolates are combined in the clinical setting and leads to therapeutic failures. Understanding of these factors is required to combat multidrug resistance.

Isolation of bioactive molecules from microbial sources

Besides understanding the resistance mechanisms in the pathogens, another aspect to combat the alarming situation of antimicrobial resistance is to explore the new entities to fight against them. The antibiotics arsenal for bacterial infection is scanty, and need to replenish. We explored the microbial diversity of around 4000 microbial culture, and extracts were screened for antimicrobial activity. Among the ten cultures which retained activity against multidrug resistant organism, the culture IMTB 1903 was preceded for the purification of active metabolites. IMTB 1903 belonged to genus *Streptomyces*, and was purified with three isomers of acetylated streptothricins (NP1, NP2 and NP3). The NP1, NP2 and NP3 were broad spectrum compounds exhibiting activity against various Gram positive and negative bacterial isolates with as good as MIC against sensitive and MDR bacterial strains for a species. The compound NP3, most active of them was inhibiting the protein synthesis process in the bacterial cells and posses less cytotoxicity activity comparable to the reported IC50 of standard streptothricins. The biosynthetic cluster for the streptothricin compound was analysed and bioinformatic analysis also indicates the high potential in genome of IMTB 1903 for the secondary metabolites.