Studies on multidrug resistant environmental isolates of *Klebsiella* spp. and their susceptibility towards metal nanoparticles

Key words: Antibiotic resistance, ESBLs, *Klebsiella*, Aquatic environment, Silver nanoparticles, Antibacterial activity.

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The present study was undertaken to determine the prevalence of Extended Spectrum β-lactamases (ESBLs) among *Klebsiella* isolates of anthropogenically influenced Delhi stretch of river Yamuna, India. An effort was made to unravel the molecular mechanisms contributing in β-lactamase mediated antibiotic resistance and co-resistance to other class of antibiotics. Furthermore, biosynthesis of silver nanoparticles using suitable bacterial culture supernatant and evaluation of their antibacterial activity against ESBL producing *Klebsiella* spp. was also studied.

A total of 263 non-duplicate bacterial isolates were obtained from 10 different sites of Delhi stretch of river Yamuna. Selective isolation and biochemical tests revealed 136 isolates as *Klebsiella* spp. Among them, 35 (25.73%) were found to be ESBL producer by phenotypic screening (Preliminary and PDCT). The 16S rRNA gene sequence analysis revealed 33 *Klebsiella* and the remaining two isolates, MK26 and NK8 as *E. coli* and *Enterobacter* sp. respectively. Of 33 *Klebsiella*, twenty five isolates were identified as *K. pneumoniae*, four *Klebsiella* sp., two *K. variicola*, one *K. oxytoca* and remaining one *K. quasipneumoniae*. Antibiotic susceptibility test of ESBL positive *Klebsiella* isolates showed highest resistance for β-lactam class of antibiotics viz. ampicillin (97.14%) followed by cefazolin (80%) and cefoxitin (65.71%) and co-resistance to non-β-lactam viz. trimethoprim, colistin and polymyxin B. Multidrug-resistance (MDR) defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, was observed in 91.42% of $ESBL^+$ *Klebsiella* isolates. Multiple antibiotic resistance (MAR) index values >0.2 among 85.71% of ESBL positive *Klebsiella* isolates indicating the high risk of contamination in the river. The MIC values of antibiotics against tested isolates varied from <4 μg/ml to >512 μg/ml. Highest MIC values were observed for the antibiotics ampicillin and trimethoprim against almost all test isolates. PCR and

sequencing analysis confirmed that *bla*TEM, *bla*SHV and *bla*CTX-M genes harbored by 57.57%, 54.54% and 45% of *Klebsiella* isolates. Mainly three variants of *bla*TEM viz. TEM-1, TEM-116 and TEM-206, eight of *bla*SHV viz. SHV-1, SHV-11, SHV-27, SHV-28, SHV-38, SHV-61, SHV-144 and SHV-148 and three of *bla*CTX-M viz. CTX-M-15, CTX-M-55 and CTX-M-188 were identified in among the isolates. ESBLs gene also presents in plasmid DNA of 17 *Klebsiella* isolates and can transfer to other bacteria through conjugation. In our best knowledge, this is the first report of environmental CTX-M-188, TEM-206, SHV-61 producing *Klebsiella pneumoniae* and first identification of TEM-116 in *Klebsiella quasipneumoniae* and *Klebsiella variicola* worldwide. Moreover, occurrence of ESBL genes *bla*TEM-206, *bla*SHV-27 and *bla*SHV-144 in any bacterial isolates is not reported from India so far.

Furthermore, metal resistant bacteria (AS1-AS11) were isolated from industrial effluents of Sahibabad Site-IV industrial area, U.P, India. Culture supernatant of all isolates separately screened for silver nanoparticles (AgNPs) synthesis. UV-vis spectrophotometric analysis showed maximum synthesis of AgNPs by one of the isolate AS3. 16S rRNA sequence analysis of isolate AS3 showed maximum homology with *Aeromonas dhakensis.* The maximum biosynthesis of AgNPs occurred when *Aeromonas dhakensis* AS3 culture supernatant mixed with silver nitrate solution (1 mM) at optimum reaction composition (1% v/v) and incubated at optimum temperature (40ºC) for 120 min under illumination. Brown color appearance of solution due to surface plasmon resonance (SPR) and absorption maxima centered at 405 nm was indicated formation of AgNPs. Fourier transform infrared spectroscopy (FTIR) spectrum analysis revealed the presence and association of supernatant biomolecules with AgNPs during synthesis. Atomic force microscopy (AFM), Field emission scanning electron microscopy (FE-SEM) and High resolution transmission electron microscopy (HR-TEM) showed spherical nanoparticles with an average size of 5 nm. X-ray diffraction (XRD) and Energy Dispersive X-ray (EDX) spectrum confirmed crystallinity and purity of AgNPs. Biosynthesized AgNPs showed promising synergistic and independent antibacterial activity against ESBLs producing *Klebsiella* isolates. The TEM images of treated *K. pneumoniae* cells with AgNPs indicated that it can act on bacterial cell wall, cell membrane and cell cytoplasm. This result suggests that biosynthesized AgNPs may be used as a next lot of candidate to combat ESBL producing multidrug resistant pathogens. This is the first report on extracellular biosynthesis of AgNPs using *Aeromonas dhakensis* AS3, an environmental isolate.