Abstract

The increasing number of hospital and community acquired infections by *Klebsiella pneumoniae* (*Kp*), especially those associated with high resistance rates, including last-resort antibiotics led to the declaration of *Kp* as an 'urgent threat' and 'priority pathogen' by CDC and WHO. *Kp* ranks 2nd after *E. coli* in urinary tract infections (UTIs) and bacteraemia. Capsular polysaccharides (CPS), exopolysaccharides (EPS) and biofilm are the major virulence factors involved in pathogenesis of *Klebsiella* spp. Despite *Kp* being extensively studied in literature, antibiotic resistance and virulence factors of clinical isolates of *Klebsiella* from UTIs are less studied compared to liver abscess conditions. Further, the mechanism of strong biofilm formation in *Kp* is underexplored. Hence, in the present study, we have studied the clinical isolates *Klebsiella* spp. from UTIs and investigated antibiotic resistance and virulence factors.

Clinical isolates (n=56) of Gram-negative bacteria from patients suffering from UTIs were collected from pathology labs of Gujarat, India. n=29 isolates were identified as *Klebsiella* spp. after biochemical tests and molecular identification. These isolates were proceeded further for study of antibiotic resistance and virulence factors.

To study antibiotic resistance in the isolates, antimicrobial susceptibility testing was performed using disk-diffusion and MIC. The isolates were categorized in MDR, XDR, and PDR categories based on their resistance pattern. Phenotypic assays were performed to investigate the presence of ESBL and MBL enzymes. qRT-PCR was performed to study the expression of important carbapenemase and ESBLs genes. Whole genome sequencing was performed using Illumina NGS for n=8 to investigate the genomic features of PDR, XDR, MDR and susceptible isolates. Oxford nanopore sequencing was performed for high-resolution analysis of PDR isolate DJ. Global analysis of CG147 (n=217) and ST231 (n=95) genomes deposited from worldwide including our isolates DJ, M2 and M6 was performed. From the collected isolates, n=21, n=6, n=1 and n=1 were categorized as MDR, XDR, PDR and susceptible, respectively. Based on the results of antimicrobial susceptibility testing and phenotypic assays, five isolates, 1 PDR (DJ), 3 XDR (M2, M6, M17B) and 1 susceptible (M25) were selected for WGS by Illumina NGS. Statistically significant difference in the

relative expression of *bla*NDM and *bla*OXA-48-like (carbapenemase genes) was observed between PDR/XDR and susceptible isolates. During analysis of whole genome of selected isolates, significant difference in number of resistance genes and plasmids were observed between XDR and susceptible isolates. During Oxford nanopore sequencing, two copies of each *bla*NDM-5 and *bla*OXA-181 as well as three copies of *bla*CTX-M-15 were found to be present on chromosome and plasmid. Importantly, one copy of blaNDM-5 was found to be located on the chromosome due to insertion of ISEcp1/IS26. In the genome of DJ, 1057 bp insertion of IS5 transposase was found in *mgrB*, which was responsible for colistin resistance. Further, a premature stop codon caused by a A580T substitution was found in *ramR*, which was responsible for tigecycline resistance in DJ. Various other resistance genes and plasmids and association between them were detected based on the global analysis.

For investigation of virulence factors, CPS and EPS were extracted from the collected isolates and quantification of CPS and EPS was done from all n=29 isolates. String test was performed for detection of string phenotype of all isolates. Study of *rmpA* gene was done using PCR and qRT-PCR. Phagocytosis assay was performed to analyse virulence of few selected isolates. Analysis and visualization of phagocytosis was done using flow-cytometry and CLSM. Regarding study of biofilms, to identify factors contributing to strong biofilm formation, isolates were categorized in strong, moderate and weak biofilm producers; strong and weak biofilm formation on various catheters and in different media conditions was studied. Quantification of matrix components (eDNA, protein, EPS, and bacterial cells), CLSM, FEG-SEM and flow-cytometry analysis were performed to compare strong and weak biofilm matrix. Isolates produced wide range of CPS and EPS. No correlation between CPS and EPS production by isolates was found. String positive phenotype is not associated to *rmpA* or higher amount of CPS production. .... amount of CPS, EPS or overexpression of *rmpA* do not affect the ability of an isolate to resist phagocytosis. Isolate with string positive phenotype (M20) was phagocytosed the least compared to other isolates. Hence, string phenotype may play a role in virulence. Regarding study of biofilm, our results suggest increased biofilm formation on latex catheters compared to silicone and silicone-coated latex

catheters. Higher amounts of eDNA, protein, EPS, and dead cells were observed in the strong biofilm of *Kp*. High adhesion capacity and cell death seem to play a major role in formation of strong *Kp* biofilms. The enhanced eDNA, EPS, and protein in the biofilm matrix appear as a consequence of increased cell death.