6.1 A concise report of the work done

In the present study, 239 clinical isolates of *K. pneumoniae* obtained from various clinical samples over a period of 2 years were characterised based on their morphological and biochemical features. Based on their antibiotic sensitivity profile, the isolates were assigned into 2 groups: non-ESBL producing and ESBL producing *K. pneumoniae* isolates. Antibiotic susceptibility, ESBL production, virulence factors such as hemolysin production, biofilm formation, production of capsule, hypermucoviscosity, MRHA, and PCR for detection of MBL genes was performed by standard procedures.

The study was to test the working hypothesis that ESBL-KP is more virulent than non-ESBL-KP strains, thus giving them the survival advantage and enhancing their ability to cause invasive infections. The results of the present study support the hypothesis as ESBL-KP were found to have more virulence factors than non-ESBL-KP isolates.

6.2 Salient findings of the study

This is one of the very few studies in the country that has studied the virulence potential of clinical isolates of *K. pneumoniae* in detail. Some of the highlights of the study are:

- This study included 239 clinical isolates of *K. pneumoniae*. During the study period the prevalence of *K. pneumoniae* infection in the hospital was found to be 15% and the isolation rate of this bacterium from various clinical samples ranged from 11.5% to 18.2%. Most of the isolates of *K. pneumoniae* were obtained from urinary tract samples (18.2%), followed by exudates (14.5%), respiratory samples (14.2%), and bloodstream samples (11.5%).

- Out of 239 *K. pneumoniae* isolates included in this study, 120 (50%) were from patients admitted to surgical and medical intensive care units. This was followed in decreasing order by isolates from surgical, medicine and orthopaedics wards.
The *K. pneumoniae* isolates (n=239) were grouped into two groups based on extended spectrum-β-lactamase production (ESBL). Group 1 included 166 (69.5%) clinical isolates of non-ESBL-KP that did not produce ESBL. Group 2 included 73 (30.5%) ESBL-KP that produced ESBL (ESBL-KP).

Antibiogram revealed that, 73 (30.5%) of them were ESBL producers showing *in vitro* resistance to third generation cephalosporins. Among *K. pneumoniae* strains, 29 (39.8%) isolates were from respiratory samples, followed by 19 (26.0%) from urinary tract, 13 (17.8%) from exudates and 12 (16.4%) from bloodstream infections.

Less than 40% of the isolates were sensitive to 3rd generation cephalosporins. *K. pneumoniae*, showed higher rates of sensitivity to amikacin (67.1%), followed by netilmicin. Higher rate of sensitivity is seen for piperacillin. Imipenem, the carbapenem antibiotic showed a greater sensitivity.

Long duration of hospitalisation, ICU admission, mechanical ventilation, bladder catheterisation, central line insertion, organ failure and parenteral nutrition were some of the clinical co-morbidities predisposing an individual to ESBL-KP infections.

ESBL production was seen in 73 (30.5%) of the *K. pneumoniae* isolates. Co-expression of more than one β-lactamase was seen in imipenem resistant ESBL-KP isolates.

PCR assay showed that, out of the 11 imipenem resistant ESBL-KP isolates, 4 (36.4%) were positive for *bla*<sub>NDM-1</sub> gene.

*K. pneumoniae* isolates showed polysaccharide capsular production (95%), followed by hypermucoviscosity (91.6%), biofilm formation (55.2%), MRHA (37.2%) and production of hemolysin (11.3%).

All ESBL-KP isolates (n=73) were positive for polysaccharide capsule and hypermucoviscosity followed by biofilm formation (89%), MRHA (53.4%) and hemolysin production (34.2%).
• This study showed occurrence of significant association between antibiotic resistance and production of multiple virulence factors. *K. pneumoniae* isolates from respiratory samples showed predominant expression of capsule and hypermucoviscosity. Expression of virulence factors such as MRHA and biofilm formation was more common among the *K. pneumoniae* isolated from the urinary tract samples.

• All the virulence factors studied were seen at higher rates among ESBL-KP isolates, in comparison to non-ESBL-KP. Production of hemolysin, hypermucoviscosity and biofilm formation rates were highly significant (*p*<0.001) among the ESBL-KP isolates.

• High prevalence of biofilm formation (89.0%) was seen among ESBL-KP isolates in comparison to non-ESBL-KP isolates.