CHAPTER 1: INTRODUCTION

1.1 Introduction to *Klebsiella pneumoniae*

*Klebsiella* are ubiquitous rod shaped bacteria that commonly inhabit the environment including soil, water and plants. Some strains of *Klebsiella* are considered a part of normal flora of human gastrointestinal tract and nasopharynx. The genus *Klebsiella*, family *Enterobacteriaceae*, was named by V. Trevisan (1885), in honor of the German bacteriologist, Theodor Albrecht Edwin Klebs (1834-1913). These bacteria are categorized microbiologically as facultative anaerobic gram negative bacilli (GNB) of the family *Enterobacteriaceae*. These bacteria are generally non-motile and encapsulated with a prominent polysaccharide-based capsule. They can grow well on routine laboratory culture media and have no special growth requirements. Among these, *Klebsiella pneumoniae* (*K. pneumoniae*) is the most important species affecting man and is responsible for some of the serious debilitating and life-threatening infections such as pneumonia, septicemia, urinary tract, nosocomial and opportunistic infections. For the first time, German microbiologist and pathologist Carl Friedlander (1882) described *K. pneumoniae* isolated from respiratory samples of patients who had suffered from pneumonia.

1.1.1 Physiology and morphology of *Klebsiella pneumoniae*

*K. pneumoniae* is a physiologically versatile bacterium, living in ecological environment and commonly found in soil and water. Uptake of atmospheric nitrogen gas and its reduction to ammonia and amino acids, by metabolic pathways of nitrogen fixation is observed in *K. pneumoniae*. *K. pneumoniae* makes use of nitrogen fixation only in anaerobic conditions, as damage can occur to part of the nitrogenase enzyme in the presence of oxygen. Prominent polysaccharide capsule, facilitates the bacterium to protect itself from phagocytosis making it more virulent and pathogenic. In the hospital environment, this bacterium is isolated from sites such as polluted medical equipment, sink drains and handles, bar soaps and cleaning equipment leading to contamination. It is a highly adaptable organism with a large genome spanning over 5 million base pairs, encoding 5300 genes. It comprises of a relatively invariable core genome, that encodes metabolic and resistance factors. This highly
variable genome harbors the genes encoding virulence factors, antibiotic resistance and specific catabolic pathways, that allow its persistence in diverse environments.\textsuperscript{9} Typical biochemical characteristics of this bacterium are negative oxidase test, positive citrate utilisation test, reduction of nitrate, lysine decarboxylation, positive urea hydrolysis test and fermentation of lactose. \textit{K. pneumoniae} isolates can produce several colony morphotypes. Large, smooth, mucoid colony morphotype is commonly isolated from clinical samples. Small colony morphotype with rough and convex appearance is commonly obtained from environmental sources.\textsuperscript{10,11} The mucoid hypermucoviscous morphotype is often obtained from urinary tract and respiratory tract secretions.\textsuperscript{12}

1.1.2 Infections caused by \textit{Klebsiella pneumoniae}

\textit{K. pneumoniae} is the most common bacterium found in a wide range of nosocomial and community acquired infections.\textsuperscript{13} This bacterium causes nosocomial infections such as pneumonia, urinary tract infections, bacteremia, wound infections, intra abdominal infections and neonatal septicemia.\textsuperscript{14-16} Other than being a nosocomial pathogen, \textit{K. pneumoniae} has been associated with community-acquired infections such as invasive and systemic infections including liver abscess, meningitis, endophthalmitis and septic arthritis in diabetics and immunocompromised individuals.\textsuperscript{17-20} One of the reasons for its success as nosocomial pathogen is its high rate of intrinsic resistance to antimicrobials, including antibiotics and disinfectants. Pathogenic stains of \textit{K. pneumoniae} are also associated with chronic lung infections in individuals with cystic fibrosis.\textsuperscript{21} Occasionally, \textit{K. pneumoniae} can colonize human body sites such as the nasal mucosa, throat, skin and intestine. Higher rates of colonization are reported following hospitalization, treatment with broad-spectrum antibiotics, disruption of the physical barriers (skin or mucous membrane), presence of indwelling invasive devices, and/or when there is an underlying dysfunction of the immune defense mechanisms. Therefore, \textit{K. pneumoniae} is mostly a nosocomial pathogen associated with respiratory tract infections including ventilator-associated pneumonia (VAP), urinary tract infections (UTIs), burn wound infections, soft tissue infections, bacteraemia, bone and joint infections and a variety of systemic infections, particularly in immunosuppressed patients. The community-acquired infections caused by pathogenic \textit{K. pneumoniae} are: urinary tract infections, acute lower
respiratory tract infections, bacteremia, liver abscess, meningitis and endophthalmitis. The mucoid hypervirulent or hypermucoviscous phenotype of \textit{K. pneumoniae} is significantly associated with purulent infections in the liver causing liver abscess.

1.1.3 Virulence factors of \textit{K. pneumoniae}

\textit{K. pneumoniae} possesses an arsenal of both cell-associated and secreted virulence factors. Some of the important cell associated virulence factors that enable its survival in diverse environmental conditions and help in establishing infections are capsular polysaccharide, fimbrial adhesins, biofilm and lipopolysaccharide. The prominent extracellular or secreted virulence factors are hemolysins, proteases, cytotoxin, siderophores, exotoxins, etc. Production of several virulence factors is coordinated by a cell density regulating mechanism termed as quorum sensing (QS). \textit{K. pneumoniae} possesses a polysaccharide capsule which provides resistance against phagocytic defense mechanism of the host. Fimbrial adhesins allow the bacteria to adhere to host cell surfaces, to facilitate bacterial colonization and biofilm formation. Two prominent bacterial fimbriae, the mannose sensitive type 1 and the mannose resistant type 3 fimbrial adhesins, confer adherence of \textit{K. pneumoniae} to the host cells. Type 1 adhesins bind to mannose-containing trisaccharides of the host glycoproteins mainly on epithelial cells of the urogenital, respiratory, and intestinal tracts. Tannin treated erythrocytes are only agglutinated by Type 3 adhesins. Quorum sensing is a cell to cell communication system involving signaling molecules called autoinducers and is responsible for controlling biofilm formation.\textsuperscript{22} \textit{K. pneumoniae} secretes siderophores, the iron chelating molecules that are critical for bacterial growth, multiplication and for greater virulence. The phenolate-type enterobactin and the hydroxamate-type aerobactin are the two most important iron chelating siderophores secreted by \textit{K. pneumoniae}.

1.1.4 Antibiotic resistance in \textit{K. pneumoniae}

Hospital acquired infections of \textit{K. pneumoniae} often show greater association with antibiotic resistance. Mechanisms of antibiotic resistance seen in this bacterium can be broadly categorized as intrinsic, acquired or adaptive, with an overlap between categories. Intrinsic resistance is due to the failure of the antibiotic to accumulate in the cell, whereas acquired and adaptive resistance results from the changes in the
antibiotic target sites or enzymatic inactivation of the drug. *K. pneumoniae* is intrinsically resistant to several antibiotics due to its poor outer membrane permeability and active efflux of antibiotics. This along with the restricted uptake through its outer membrane with secondary resistance mediated by the β-lactamases makes it a difficult pathogen to treat. Chromosomal mutations within its genome can lead to changes in the regulation of resistance genes. It can also acquire resistance genes from other bacteria via plasmids, transposons, and bacteriophages. Acquired resistance genes predominantly confer resistance to β-lactams and aminoglycosides whereas, chromosomal mutation often leads to fluoroquinolone resistance. Multidrug resistant *K. pneumoniae* (MDR-KP) and extended-spectrum β-lactamase (ESBL) producing *K. pneumoniae* are emerging rapidly as a challenge in clinical practice. An isolate of *K. pneumoniae* is defined as MDR-KP when it shows *in vitro* resistance to at least 3 classes of antibiotics such as cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. MDR is a pervasive clinical problem often associated with significant morbidity, mortality and increased economic burden, which stems from the inappropriate empiric therapy. For a while now, carbapenems were considered as the drug of choice for treatment of MDR infections. However, in the recent past carbapenem resistance has become widely prevalent and is often mediated by metallo-β-lactamase (MBL) enzymes that hydrolyze carbapenems. These enzymes are encoded by genes like imipenemase (*bla*IM), Verona imipenemase (*bla*VIM) and New Delhi metallo-beta-lactamase-1 (*bla*NDM-1), carried either on the bacterial chromosome or in a plasmid. Therefore, there is a need to reconsider therapeutic strategies for the effective and early appropriate empiric treatment of infections caused by MDR isolates. Inappropriate initial empiric therapy is a major contributor to therapeutic failure which in turn translates into high mortality rates.

### 1.1.5 Correlation between antibiotic resistance and virulence of *Klebsiella pneumoniae*

As there is higher incidence of MDR, along with ESBL production among *K. pneumoniae* strains, there is a need to study virulence potential in these clinical isolates. The conventional antibiotic chemotherapeutic knowledge from past indicates steady increase in antibiotic resistance as a result of, either overuse or misuse of antibiotics. This was the dogma in infectious diseases for long. The regulation of
virulence and antibiotic resistance genes is a very complex mechanism which was thought to occur as separate events. However, it is now more evident that the genetic regulation of both is intertwined and connected. Studies have suggested that, the expression of antibiotic resistance genes could be influenced by regulation of virulence genes. At this point of time, there is no published data from our country comparing the virulence determinants of non-extended-spectrum β-lactamase producing *K. pneumoniae* (non-ESBL-KP) and extended-spectrum β-lactamase producing *K. pneumoniae* (ESBL-KP) isolates. Therefore, this study was planned to compare the virulence potential of non-ESBL-KP with that of ESBL-KP isolates, so that it can serve as a guide for future studies on newer therapeutic strategies tackling both, antibiotic resistance and virulence in *K. pneumoniae* simultaneously.

### 1.2 Aim and objectives

#### 1.2.1 Aim of the study

To detect the virulence factors and antibiogram pattern among clinical isolates of *K. pneumoniae* in a tertiary care hospital.

#### 1.2.2 Objectives of the study

1. To isolate and identify *K. pneumoniae* from various clinical samples.

2. To study antibiogram pattern of these isolates.

3. To phenotypically detect ESBL production in cephalosporin resistant *K. pneumoniae* isolates.

4. To detect virulence factors such as capsule, hypermucoviscosity, mannose sensitive and mannose resistant pili, biofilm formation and hemolysin in these isolates.

5. To compare production of virulence factors among non-ESBL producing and ESBL producing *K. pneumoniae* isolates.

6. To identify presence of *bla*<sub>NDM-1</sub> gene among imipenem resistant ESBL producing *K. pneumoniae* isolates.
1.3 Social relevance

*K. pneumoniae* is a significant cause of hospital-acquired infections because of its ability to survive in low nutrient environments, inherent resistance to antibiotics, and its ability to form biofilms, which makes it a difficult pathogen to eradicate. The persistence of this bacterium in the environment can be related to its ability to form biofilms that also increase its resistance to antibiotics and disinfectants. The large genome of this bacterium contributes to its adaptability and metabolic versatility. Currently, carbapenems are the antibiotics of choice for treatment of severe *K. pneumoniae* infections. However, in the recent past, resistance to this class of antibiotics is increasing worldwide and very often carbapenem resistance mediated by enzymes such as metallo-β-lactamases (MBL) are plasmid mediated and have a potential for rapid dissemination. Therefore, early and rapid detection of carbapenemase production is necessary to initiate effective antibiotic treatment, and also to put in place infection control measures to prevent their dissemination in hospital settings. With the rapid spread of ESBL, MBL and carbapenemase producing *K. pneumoniae* and with no newer antibiotics in the pipeline, we are left with a very limited treatment options. These options include the highly toxic reserve antibiotics such as colistin and polymyxin B which need to be used judiciously in patients with other underlying co-morbidities like compromised renal function. Thus, there is a pressing need to know the local antibiotic sensitivity pattern of *K. pneumoniae* isolates which can help identify the appropriate agents for initial empiric antibiotic therapy. Studying the virulence factors in the context of antibiotic resistance will also help in designing newer therapeutic strategies to tackle MDR-KP. Therefore, this research proposal was designed to study the local antibiogram pattern of *K. pneumoniae* which will help in designing the in-house hospital antibiotic policy. It will also co-relate the virulence factors produced by ESBL-KP and non-ESBL-KP isolates which will help in identifying the pathogenic potential of multidrug-resistant strains. This study will also pave the way for future research on newer modalities of treatment for highly virulent MDR-KP.