CHAPTER 1: INTRODUCTION

1.1. General introduction and habitat diversity

The *Klebsiella* species are gram-negative, rod shaped, non-motile, lactose fermenting bacteria that are found in the environment and also present as the normal flora in the gut of humans. Genus *Klebsiella*, which belongs to the tribe Klebsiellae, is classified under *Enterobacteriaceae* family. The clinical relevance of the genus *Klebsiella* led to it being divided into *Klebsiella pneumoniae* (*K. pneumonia*) and *Klebsiella oxytoca* (*K. oxytoca*). *K. pneumoniae* is further classified into three species based on diseases caused: *K. pneumoniae*, *K. ozaenae*, and *K. rhinoscleromatis*. *K. pneumoniae* is the most common species isolated from the hospitalized patients and hence often considered a nosocomial pathogen. *K. pneumoniae* was first isolated by Friedlander, (1883), from fatal cases of pneumonia.¹

1.2. *Klebsiella* species- clinical importance

*Klebsiella* species causes urinary tract infection (UTI), pneumonia, septicemia, soft tissue infection, meningitis, and bacteremia.² *K. pneumoniae* is the most significant pathogen within the genus of *Klebsiella*, being responsible for 75-86% *Klebsiella* related infections; however, *K. oxytoca* is the second most common *Klebsiella* species, which is responsible for 13-25% of infections.³

*Klebsiella* species are responsible for community acquired and nosocomial infections, which are responsible for Friedlander's pneumonia - a community acquired pulmonary infection, which affects the chronic alcoholic patients. However, other community acquired infections with *K. pneumoniae* are skin and soft tissue infections, including diabetic foot infections, ulcerative keratitis, and otitis externa.⁴

Moreover, few severe clinical diseases are emerging in Asia. In which, community-acquired pyogenic liver abscess (PLA) is common, which is mainly caused by emerging hypervirulent trait, i.e., hypermucoviscous.⁵ Majority of PLA cases are generally associated with K1 serotype and have been detected mainly in Taiwan.⁶ *K. pneumoniae* also causes endophthalmitis, which may be a secondary complication of PLA, and is identified in Asian countries as well. It is
strongly associated with the presence of primary liver abscesses and an underlying diabetic condition.\textsuperscript{7} \textit{K. pneumoniae} is also responsible for community-acquired bacterial meningitis in adults, without having any other body infection.\textsuperscript{8} In view of nosocomial infections, \textit{Klebsiellae} are considered as important pathogenic agents, which are responsible for infections such as UTI, pneumonia, bloodstream infections, surgical site, soft tissues, wound infections and also cause septicemia.\textsuperscript{2} In the hospital settings, the rate of colonization by \textit{Klebsiella} species is directly proportion at the duration of the hospitalization, and it is the second most frequent cause of catheter associated UTI, followed by pneumonia. The urinary tract is one of the most common sites of \textit{Klebsiella} infection, particularly in females; 20-30\% of women have recurrent UTI like cystitis, urethritis, and pyelonephritis.\textsuperscript{1}

1.2.1. Identification of \textit{Klebsiella} species

The genus \textit{Klebsiella} is divided into several species, i.e., \textit{K. pneumoniae}, \textit{K. oxytoca}, \textit{K. planticola}, \textit{K. ornitholytica}, and \textit{K. terrigena}. \textit{K. pneumoniae} is further subdivided into subspecies \textit{pneumoniae}, subspecies \textit{ozaenae}, and subspecies \textit{rhinoscleromatis}. Identification of \textit{Klebsiella} species is based on the biochemical reaction.\textsuperscript{9} Among the genus \textit{Klebsiella} most common clinically isolated pathogen is \textit{K. pneumoniae}, which ferments all sugars (glucose, lactose, sucrose, mannitol) with the production of acid and abundant gas. It is indole and methyl red negative, voges-proskauer and citrate positive, as shown in Table 1.1.
### Table 1.1 Biochemical reactions of Klebsiella species

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<td></td>
<td></td>
<td>subsp. p</td>
<td>subsp. o</td>
<td>subsp. r</td>
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<tr>
<td>1</td>
<td>Indole</td>
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<td>2</td>
<td>Methyl red test</td>
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<td>3</td>
<td>Voges proskauer</td>
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<td>+</td>
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<td>4</td>
<td>Simmons citrate utilization</td>
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<td>5</td>
<td>Lysine decarboxylase</td>
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<td>±</td>
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<td>6</td>
<td>Ornithine decarboxylase</td>
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<td>7</td>
<td>Urease</td>
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<td>9</td>
<td>Pectate degradation</td>
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<td>10</td>
<td>Gas from glucose</td>
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<td>±</td>
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<td>11</td>
<td>Utilization of malonate</td>
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**Abbreviations used:** subsp. p - subspecies pneumoniae, subsp. o- subspecies ozaenae, subsp. r- subspecies rhinoscleromatis, K. O - K. oxytoca, K. P - K. planticola, K. T- K. terrigena, K .Or - K. ornitholytica, TSI- Triple sugar iron). ‘+’ – positive; ‘-’ – negative; ‘±’- variable

### 1.3. Pathogenicity of Klebsiella

The pathogenicity of *Klebsiella* species is because of its ability to elaborate a wide array of virulence factors, such as capsule, fimbriae (pili), lipopolysaccharides, and siderophores.\(^\text{10}\), \(^\text{11}\) These help the bacteria to adhere and invade their host by damaging the host’s immune responses and forming a barrier to antibiotics.

Another strain of *Klebsiella* species has emerged in Asia with the virulence factor, hypermucoviscous, which is associated with *magA* and *rmpA* genes. The *magA* gene is denoted as a mucoviscosity associated gene, which plays an important role in serious infection of *Klebsiella* like bacteremia, septicemia, and pneumonia, as well as lung and liver abscesses. The chromosomal *magA* gene has hyperviscous phenotype, and is characterized by forming a mucoviscous string of $>5$ mm diameter while passing the loop through a colony. It is also
causing increased levels of resistance to phagocytosis. The *rmpA* (regulator of the mucoid phenotype) gene is responsible for regulating the synthesis of extracellular polysaccharide capsule.\textsuperscript{12}

Fimbriae are non flageller, filamentous projection on the bacterial surface. It is the first step in the infectious process, when the microorganism comes close to host mucosal surfaces and maintains this proximity by attaching to the host cell (adherence). The adhesive properties in the *Enterobacteriaceae* are generally mediated by different types of pili or fimbriae. Fimbrial adhesins are bacterial proteins which are able to recognize molecular receptors and facilitate adherence to specific tissue surfaces of the host. *K. pneumoniae* produces two major fimbrial adhesions, i.e., type 1 and type 3 fimbriae.\textsuperscript{13} Type 1 fimbriae have mannose-sensitive hemagglutinins, while type 3 fimbriae have mannose-resistant hemagglutinins.\textsuperscript{14} Type 1 fimbriae (type 1 common pili) are the most common adhesive organelle in *Enterobacteriaceae*. Type 1 fimbriae and its adhesive subunit FimH, play an important role in UTI caused by *K. pneumonia*. Type 1 fimbriae adhesion protein is of pilus type, is located on the fimbrial shaft, and is capable of binding to mannose containing trisaccharides of the host glycoproteins to cause UTI.\textsuperscript{15, 16} Antibacterial agents with high intrinsic activity have to be used against *K. pneumoniae* infection. Such agents includes third-generation cephalosporins (e.g., cefotaxime, ceftriaxone), carbapenems (e.g., imipenem, meropenem), aminoglycosides (e.g., gentamicin, amikacin), and quinolones.\textsuperscript{17}

Biofilm formation by *Klebsiella* is a major problem in hospital settings. *K. pneumoniae* is commonly found in hospital infections where it adheres to implant material or human cell surfaces and adapts to a biofilm environment.\textsuperscript{18} The biofilm formed on implanted devices such as catheters, and pacemakers allow the bacteria to get direct access to the bloodstream, which leads to a spread throughout the body and, results in life threatening systemic infections. Additionally, biofilm provides protection from environmental stress and allows horizontal transfer of genes coding for drug resistance. Hence, biofilm forming bacterial strains are highly resistant to antimicrobial agents and host immune factors and they are a challenge to clinical management because they are often difficult to treat and may require device removal.\textsuperscript{19, 20} Majority of the infections caused by this organism, particularly those infections associated with indwelling medical devices, are due to biofilm formation. The biofilm mode of growth is one of the reasons
for increased resistance to antimicrobial therapy with an increased risk of treatment failure. Most of the time, microorganisms are resistant to the commonly used antibacterial agents because of biofilm formation. Hence, knowledge on biofilm formation in bacterial infections may provide awareness on the importance of initiation of treatment in the early stage of infection as a life saving measure.\textsuperscript{21}

1.4. Antimicrobial resistance

Increasing antimicrobial drug resistance, including drug resistant\textit{Klebsiella} species, is seen worldwide. This may be due to the direct effect of individual antimicrobial exposure, or due to the indirect effect of transmission of multidrug resistant (MDR) organisms from other patients as a result of the higher colonization process. Therefore, this increased antibiotic resistance trend severely limits the choice of effective antimicrobial agents.\textsuperscript{22}

Multidrug resistance in gram negative bacteria, particularly in \textit{Enterobacteriaceae}, such as \textit{E. coli} and \textit{K. pneumoniae} have become one of the biggest issues worldwide. Infections with these bacteria lead to prolonged hospital admissions and higher mortality rates.\textsuperscript{23} The emergence of MDR \textit{Klebsiella} isolates during therapy is reported. The frequency of isolation of fluoroquinolone resistance among \textit{Klebsiella} isolates has increased from 65\% to 94\% over a decade.\textsuperscript{24, 25} The intrinsic resistance of the species is due to the low permeability of the outer membrane, constitutive expression of $\beta$-lactamase, and the mechanism of efflux pumps. It also possesses remarkable ability to acquire mechanisms for development of resistance to multiple groups of antimicrobial agents, including $\beta$-lactams, aminoglycosides, and fluoroquinolones.

\textit{Klebsiella} represents a phenomenon of bacterial resistance. Mechanisms such as, production of plasmid mediated $\beta$-lactamases by different molecular classes, over-expression of active efflux systems, synthesis of aminoglycoside modifying enzymes (phosphoryltransferases, acetyltransferases, and adenylyl transferases), structural alterations of topoisomerase II and IV determining quinolone resistance and derepression of chromosomal mutation are documented. Therefore, these mechanisms are often present simultaneously, thereby conferring MDR phenotypes.\textsuperscript{26}
Drug resistant *K. pneumoniae* is one of the important nosocomial and community acquired bacterial infections, which is emerging worldwide and has led to increased mortality and morbidity in humans.\(^{27}\)

### 1.4.1. Mechanism of fluoroquinolone resistance in *Klebsiella*

The resistance against quinolone group of drugs, widely used for treatment of *Klebsiella* species, is mainly acquired by intra or inter species exchange of transferable plasmid encoded antibiotic resistant genes *qnrA, qnrB* and *qnrS*.\(^{28}\) Quinolone resistance mechanisms are associated with PMQR genes, i.e., target protection proteins encoded by *qnr* genes, specific efflux pumps encoded by *qepA*, and a multidrug efflux pump encoded by *oqxA, oqxB* and an altered aminoglycoside acetyltransferase encoded by *aac(6')Ib-cr*. Quinolone resistance is mainly caused by mutations in the chromosomal genes for DNA gyrase and DNA topoisomerase IV.\(^{29}\) The *qnr* gene is found to encode a 218 amino acid protein, and the purified *qnr* protein is shown to protect DNA gyrase activity *in vitro*.\(^{30}\) Another plasmid mediated drug resistance mechanism, i.e., *aac(6')Ib-cr* has emerged, which is responsible for enzymatic modification of some fluoroquinolone drugs. The aminoglycoside acetyltransferase variant, *aac(6')Ib-cr*, is capable of acetylating and subsequently reducing the activity of norfloxacin and ciprofloxacin against the bacteria which then develop resistance to these drugs.\(^{31}\) The *qepA* is a fluoroquinolone (FQ) specific efflux pump protein. It is a new plasmid-mediated gene responsible for reduced fluoroquinolone susceptibility. *qepA* encodes for an efflux pump belonging to the major facilitator subfamily that decreases susceptibility to hydrophilic fluoroquinolones, especially ciprofloxacin and norfloxacin.\(^{32}\)

### 1.5. Social relevance of the study

Infections caused by *Klebsiella* species are on the rise and difficult to treat. Antibiotic resistance due to diverse mechanisms in these bacteria has complicated the problem. The present study has enabled us to understand the virulence mechanisms in *Klebsiella* isolates from our hospital and also helped in knowing the differences in such factors among the community acquired and the hospital acquired strains. The present study has highlighted the presence/absence of an emerging
variant of *Klebsiella* species which is a dangerous pathogen known as hypervirulent *K. pneumoniae*.

Currently, the drugs of choice for infections caused by resistant isolates of *Klebsiella* species have been carbapenems and β-lactams – β-lactamase inhibitor (BL-BLI) combination. With increasing reports of resistance to these drugs, the alternatives for treatment of such infections have to be looked at. This study may help to understand the potential of quinolone group of drugs in treating such infections and using them as an alternate therapy for drug resistant *Klebsiella* species.

This study may help in developing strategies to prevent the spread of resistance among *Klebsiella* species and also manage drug resistant *Klebsiella* infection, which in turn will reduce complications and mortalities due to such infections. Hence this study was taken up to investigate the following aim and objectives.

The increasing incidence of drug resistance in *Klebsiella* leads to an emerging need for complete understanding of all the possible mechanisms of drug resistance, in order to predict novel drug targets, so that novel therapeutic strategy can be developed to prevent significant morbidity and mortality. Therefore, this *in vitro* study on drug resistance mechanism is the base for understanding the approaches in treating PMQR resistant *Klebsiella* isolates.

This prospective study, will provide data regarding the common virulence factors responsible for *Klebsiella* infection along with the genes which are mainly responsible for drug resistance of *Klebsiella*. Hence it maybe possible to treat the patients in a better and proper way in hospital setup; and with an effective drug regimen, the spreading of drug resistance maybe controlled in the vicinity of the hospitals.
1.6. **Aim of the study**

This study aims to detect virulence genes and quinolone drug resistance mechanisms in extended spectrum beta lactamase producing *Klebsiella* species from the clinical isolates.

1.7. **Objectives of the study**

1. To isolate and identify *Klebsiella* species from clinical samples.
2. To determine the antimicrobial susceptibility pattern of *Klebsiella* isolates by Kirby Bauer disc diffusion method and confirm ESBL production by double disc diffusion test.
3. To detect the MIC of fluoroquinolone drugs by E-strip method.
4. To phenotypically determine the frequency of hypermucovirulent *Klebsiella* species and the presence / absence of type 1 and type 3 fimbriae by hemagglutination assay.
5. To detect the biofilm production among *Klebsiella* species by microtitre plate assay.