1. INTRODUCTION

The widespread use of antimicrobial agents in the treatment of infections in the tropics has led to serious problems of antimicrobial resistance. The emergence and spread of antibiotic resistance in bacteria of medical importance imposes serious constraint on the option available for treatment of many infections (Nikaido, 1998; Li & Lim, 2000) mentioned that in the last 10 years, the extensive spread of multiple antibiotic-resistant *Klebsiella pneumoniae* strains, especially the extended-spectrum beta lactamase producing strains (ESBLs), has become a major threat to the ever-increasing number of immunocompromised patients; the ESBLs are usually plasmid mediated (R plasmid) offer resistance to antibiotics and are transmissible from one cell to another by direct cell contact (conjugation).

The genus *Klebsiella* belongs to the tribe *Klebsiellae*, a member of the family *Enterobacteriaceae*. The organisms are named after Edwin Klebs, a 19th century German microbiologist. *Klebsiella* are non-motile, rod-shaped, Gram negative bacteria with prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on Gram’s stain and provides resistance against many host defense mechanisms.

*Klebsiella* species is ubiquitous in nature. *Klebsiellae* probably have two common habitats, one being the environment, where they are found in surface water, sewage, and soil and on plants (Podschum & Ullman 1998), and the other being the mucosal surfaces of mammals such as humans, horses, or swine, which they colonize. Seven species of the *Klebsiella* genus, with demonstrated similarities in DNA homology are known. These are (1) *Klebsiella pneumoniae*, (2) *Klebsiella ozaenae*, (3) *Klebsiella terrigena*, (4) *Klebsiella*
rhinoscleromatis, (5) *Klebsiella oxytoca*, (6) *Klebsiella planticola*, and (7) *Klebsiella ornithinolytica*.

*Klebsiella pneumoniae* is clinically the most important species and in recent years have become important pathogens in nosocomial infections. It is closely related to *K. oxytoca* from which it is distinguished by being indole-negative and by its ability to grow on both melezitose and 3-hydroxybutyrate (Podschun & Ullman 1998). Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infections. In particular, accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections. The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks. Hospital outbreaks of multidrug-resistant *Klebsiella* spp., especially those in neonatal wards, are often caused by new types of strains, the so-called extended-spectrum-β-lactamase (ESBL) producers.

The incidence of ESBL-producing strains among clinical *Klebsiella* isolates has been steadily increasing over the past years. The resulting limitations on the therapeutic options demand new measures for the management of *Klebsiella* hospital infections. While the different typing methods are useful epidemiological tools for infection control, recent findings about *Klebsiella* virulence factors have provided new insights into the pathogenic strategies of these bacteria. *Klebsiella* pathogenicity factors such as capsules or lipopolysaccharides are presently considered to be promising candidates for vaccination efforts that may serve as immunological infection control measures.
ESBLs are enzymes produced by Gram negative bacilli that mediate resistance to extended spectrum cephalosporins and aztreonam. Most ESBLs are plasmid encoded enzymes derived from classical TEM and SHV type β-lactamases. ESBL types at institution and countries are intensely varied. SHV type ESBL, predominates in Western Europe, while TEM type ESBLs are the most frequently isolated in United States, South America, Eastern Europe and more recently, Spain, Kenya, India and Korea have a predominance of the CTX M type. Most outbreaks are limited to areas where high risk patients are cared for, but several epidemics have been noted among neonates, elderly patients and even outpatients (French et al., 1996). This is due to the bacterial antibiotic resistance properties.

*Klebsiella* species may contain resistance plasmid (R-plasmids) which confer resistance to such antibiotics as ampicillin and carbenicillin. To make matter worse, the R-plasmids can be transferred to other enteric bacteria not necessarily of the same species (Ze-Qing et al., 2005). *Klebsiella pneumoniae* tends to affect people with underlying diseases, such as alcoholism, diabetes and chronic lung disease. Mortality in *K. pneumoniae* is fairly high due to the underlying disease that tends to be present in affected persons. With the extensive use of third and fourth generation cephalosporins as an important component of empirical therapy in intensive care units (ICUs) and high risk wards, resistance to these drugs has become a major problem all over the world (Bradley et al., 2001, Srujuna et al., 2004). Resistance has developed in bacteria by possessing ESBLs capable of hydrolyzing these new cephalosporins. β-lactamase mediated resistance may be overcome by combining β-lactam antibiotics with β-lactamase inhibitors which bind irreversibly to the β-lactamases and render them inactive thus, sparing the β-lactam antibiotics.
Researchers have found that bacteria can exchange among themselves packets of genetic material that make them resistant to several antibiotics such as *K. pneumoniae* have developed strains that are resistant to several antibiotics at the same time and these strains are known as ESBL producing *K. pneumoniae* (Paterson *et al.*, 2004).

During the last decade, the effort to combat multi drug resistant (MDR) microorganisms have been coming up. A large number of hospitals have implemented more rigorous infection control measures while; drug companies have developed novel antimicrobial agents to combat these bacteria, resulting in several new compounds with novel mechanisms of action (Schito, 2006). Paralleling the developments in gram-positive bacteria, infections caused by MDR Gram-negative bacilli have become a growing problem (Talbot *et al.*, 2006). Moreover, there are now a growing number of reports on cases of infections caused by Gram-negative organisms for which no adequate therapeutic option exist. This return to the preantibiotic era has become a reality in many parts of the world. (Christian *et al.*, 2008).

These types of infections not only affect the developing countries, but also represent a major medical and economic problem in developed countries. The worldwide emergence of multidrug resistant bacterial strains is a growing concern because of their variation at strain level and increasing intrinsic antibiotic resistance.

Therefore the study of the distribution and determination of diseases frequently in human population is a necessary step for the improvement of health care system and the prevention of illness. The measurement of disease frequently relates to the quantification of disease occurrence in human populations involves the distribution of health status in terms of age, sex, race, geography, etc. (Mac Mahon and Pugh, 1970). The identification of factors,
those are responsible for the increase or decrease of disease risks in order to obtain the knowledge necessary for primary prevention, the prediction of disease trends to facilitate the adaptation of the health services to future needs and to identify research priorities and also to control the spread of contagious diseases by targeted vaccination programs.

The first aim of epidemiological study is to target the biomedical prospective i.e, the etiology of diseases and disease process. This includes the description of the disease spectrum, disease entities to know about the various outcomes that may be caused by particular pathogens.

The methods used to describe the distribution of diseases may be considered as a prerequisite to identify the determination of human health and diseases. This is based on two fundamental assumptions (1) The occurrence of diseases in populations is not a purely random process; (2) It is determined by causal and preventive factors (Hennekens and Buring, 1987). These factors have to be searched for systematically in populations defined by place, time, or other different ecological models. These factors explain interrelationship of host, agent and environment, which constitute the epidemiological triangle as shown in Fig:1. Changes in any of these three factors will influence the balance among them and thereby represents increase or decrease in the disease frequency (Mausner and Bahn, 1974). The discipline of epidemiology, together with the applied fields of economics, management sciences, and the social sciences, provide the essential quantitative and analytical methods, principles of logical inquiry, and rules for evidence for diagnosing, measuring and projecting the health needs of community and populations, determining health goals, objectives and priorities; allocating and managing health care resources. Assessing intervention strategies
and evaluating the impact of health services. (White and Henderson, 1976). Epidemiology pursues three major targets: (1) to describe the spectrum of diseases and their determinations, (2) to identify the causal factors of diseases and (3) to apply this knowledge for prevention and public health practice.

![Epidemiological triangle](image)

**Fig-1: The Epidemiological triangle.**

Epidemiologic typing systems can be used for outbreak investigations, to confirm and delineate the patterns of transmission of one or more epidemic clone(s), to test hypotheses about the sources and vehicles of transmission of these clones and to monitor the reservoirs of epidemic organisms. Typing also contributes to epidemiologic surveillance and evaluation of control measures, by documenting the prevalence over time and circulation of epidemic clones in infected populations. These distinctive characters, called epidemiological markers are scored by typing systems which are designed to optimize discrimination between epidemiologically related and unrelated isolates of the pathogen of interest (Maslow and Mulligan 1996). Although pathogenic microorganisms constitute a small proportion of the microbial species these are characterized by high genetic diversity. Understanding genetic diversity of pathogens may have far reaching implications for public health intervention.
strategies such as tracking the global spread of pathogens, understanding emergence of new and drug resistant microbes and rational development of diagnostics, therapeutics and vaccines.

The epidemiology of *Klebsiella pneumonia* infection usually studied by the analysis of phenotypic markers, including biotype, phage type, serotype, bacteriocin typing and antimicrobial susceptibility (Rennie R P., et al 1974, Pieroni, p., et al, 1994, Orskov, I., et al 1984, Bauernfeind, A. 1984). In recent years epidemiology has been enriched tremendously with tools from molecular biology. It has branched into a complex field called Molecular Epidemiology.

From the epidemiologic point of view, it is necessary to determine the clonality of the bacterial isolates. Particularly it is important in endemic and epidemic nosocomial out break to improve the management of such outbreaks. In recent approaches molecular epidemiology typing methods utilizes nucleic acids. This provides higher discriminatory power than phenotypic parameters (Nouvellon, M., 1994). These methods facilitate the elucidation of transmission routes, genetic variability and phylogenetic distances between strains (Arlet, G et al 1994, Hartstein, A. I., et al 1993).

Although conventional methods for epidemiological methods are widely used, their theoretical and inherent limitations led to the development of molecular methods. Typing is important for eradication of environmental sources as well as prevention of cross infections and monitoring of antimicrobial therapy efficacy (Poh, C. L., et al., 1993). And also typing procedures are the integration of epidemiology, taxonomy and evolutionary genetics.
Multidrug-resistant Enterobacteriaceae are emerging as a serious infectious disease challenge. These strains can accumulate many antibiotic resistance genes though horizontal transfer of genetic elements, those for \( \beta \)-lactamases being a particular concern. Some \( \beta \)-lactamases are active on a broad spectrum of \( \beta \)-lactams including the last-resort carbapenems.

Carbapenems are one of few antimicrobials that have been effective against multidrug-resistant bacteria, but their utility is threatened by the emergence of carbapenem-resistant Enterobacteriaceae (CRE). Klebsiella pneumoniae is the most common CRE species in the United States, typically encountered as a hospital acquired infection with high morbidity and mortality, and resistant to nearly all available antibiotics. (Gupta et al; 2011, Centers for Disease Control and Prevention; 2013).

One of the most important mechanisms of antibiotic resistance in Klebsiella spp. is the production of AmpC \( \beta \)-lactamases (Class C \( \beta \)-lactamases) which are an important group of enzymes that are broadly distributed in the world; it is the second most common \( \beta \)-lactamase group (Bush et al., 1995). The inducible chromosomal AmpC genes were detected on plasmids of Klebsiella spp., E. coli, or Salmonella spp. In E. coli AmpC is poorly expressed, while in Klebsiellae and Salmonella species the AmpC gene is missing from the chromosome and found on the plasmids (Philippon et al., 2002). Recently, more than 100 different AmpC enzymes were commonly isolated from extended-spectrum cephalosporin-resistant Gram-negative bacteria (Jacoby and Bush, 2009).

Many clinical laboratories have problems detecting extended-spectrum beta-lactamases (ESBLs) and plasmid-mediated AmpC beta-lactamases. Confusion exists about the importance of these resistance mechanisms, optimal test methods, and appropriate
reporting conventions. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures.

The above introduction reveals the importance of the study of molecular epidemiology of bacterial infectious diseases and its applications towards the *Klebsiella pneumoniae* infections. The progress in molecular epidemiological investigations on bacterial infections, especially with respect to *Klebsiella pneumoniae* in Indian scenario is very less and that of Hyderabad Karnataka including Gulbarga region is almost negligible.

During present work, an attempt has been made to isolate and characterize the *Klebsiella pneumoniae* from various clinical specimens collected from health care centres of Gulbarga region and also to explain epidemiological distribution of multidrug resistant *Klebsiella pneumoniae* by comparing and evaluating few phenotypic and genotypic methods.

**The objectives**

- Screening and isolation of the *Klebsiella pneumoniae* isolates from clinical samples.
- Morphological, cultural characterization and antimicrobial activity of *K. pneumoniae* isolates.
- Study of extended spectrum beta-lactamases and biofilm formation of *Klebsiella pneumoniae* isolates.
- Molecular characterization of *K. pneumoniae* isolates
  - SDS-PAGE analysis of whole cell protein
  - Isolation of Genomic DNA
  - Isolation of Plasmid DNA
  - PCR amplification of imipenem and AmpC gene.