5. DISCUSSION

Epidemiological practice and the results of epidemiological analysis make a significant contribution to emerging population-based health management frameworks. Population-based health management encompasses the ability to: Assess the health states and health needs of a target population, implement and evaluate interventions that are designed to improve the health of that population efficiently and effectively provide care for members of that population in a way that is consistent with the community's cultural, policy and health resource values.

Biomedical sciences, clinical sciences and public health sciences with epidemiology as their core discipline, contributing in improvement of health care system, by investigating the determinants of health-related states or events in specified populations. In particular the main objective of epidemiology is to promote, protect and restore the health, by understanding the requirements, identifying the risk conditions. The worldwide increase of infectious diseases explains the need of systematic surveillance and prevention by means of epidemiological investigation. On this basis of public health risk can be determined and interventions in the spread of disease can be designed and their efficacy can be assessed. Presently epidemiological investigations supplemented with the expanding knowledge of different molecular biological techniques and branched as molecular epidemiology.

Klebsiella spp are ubiquitous in nature. Klebsiellae probably have two common habitats, one being the environment, where they are found on surface water, sewage and soil and on plants, and the other being the mucosal surfaces of mammals such as humans, horses
or swine, which they colonize. In this respect, the genus *Klebsiella* is like *Enterobacter* which are common in humans but not in the environment. (Brown and Seidler; 1973).

Members of the genus *Klebsiella* are increasingly important opportunistic pathogens associated with severe hospital–acquired infections such as septicaemia, pneumonia, respiratory tract infection and urinary tract infections. An estimated 8% of all nosocomial bacterial infections in the United States and in Europe are caused by *Klebsiellae*, mainly by *Klebsiella pneumoniae* and *Klebsiella oxytoca* (Podschun and Ullman, 1998). Nosocomial bacterial pneumonia is frequently polymicrobial, with Gram-negative bacilli predominating worldwide (American Thoracic Society, 1996). Delay in antimicrobial treatment can lead to adverse outcomes hence, the choice of empirical therapy is vital. Many effective antimicrobial agents are available but the treatment of nosocomial pneumonia still remains challenging (Ugur et al., 2004).

The epidemiology of *Klebsiella pneumoniae* infections are usually studied by the analysis of phenotypic markers including serotype, phage type, biochemical characterization (Rennie et al; 1974, Pieroni et al, 1994, Orskov et al; 1984, Bauernfeind, 1984) and molecular typing methods (Nouvellon; 1994).

The present investigation was an effort to assess the need of systematic surveillance, as the value of applying various precautions to *Klebsiella pneumoniae* carriers by isolating and characterizing the epidemiologically important, multi drug resistant *Klebsiella pneumoniae* in various clinical isolates obtained from the health care centers of north eastern region (Gulbarga region) of Karnataka, India.
In the present study, a total of 384 *Klebsiella pneumoniae* strains were isolated from 1064 samples of different locations, economic groups and age groups. The overall incidence of *Klebsiella pneumoniae* colonization observed to be 36.09%. This is comparatively higher than the earlier reported incidence of 27.74% from this region (Vinod Kumar and Neelagund; 2004). Though the highest isolation rate of *Klebsiella pneumoniae* was observed in sputum (31.25%), urine (22.91%), blood (14.58%), pus (14.06%) and stool (6.77%), cervical swab (6.51%), ear swab (1.82%), CSF (1.04%), peritoneal fluid (1.04%) accounted comparatively less in our studies. On the contrary higher incidence of *Klebsiella pneumoniae* in urine samples then in other clinical samples is in agreement with the several reports that *Klebsiella spp* are nosocomial pathogens commonly encountered in urinary tract infection (UTI), septicemia or respiratory tract infections (Podschun; 2000; Barman et al, 2008 and Bercion et al; 2009).

The colonization by *Klebsiella pneumoniae* on an average showed no significant difference between males and females but it was slightly high in males than the females in some locations. The incidence of *Klebsiella pneumoniae* colonization is high in individuals of zone D and zone E, when compared with other zones but economic background of the individuals has very little effect on the *Klebsiella pneumoniae* colonization as it was observed in zone A, low rate of incidence was noted with MIG (22.95%) but age group had a significant effect on *Klebsiella pneumoniae* colonization especially in 26-50 age group colonization was high followed by 50> years of age groups in zone B, C and E, whereas in zone D *Klebsiella pneumoniae* colonization was significant in age group of 26-50 years followed by 0-5 years.
In the hospital setting, the local antibiotic policy is a major determinant of the colonization pattern. Further more wide spread use of antimicrobial therapy has often been held responsible for the occurence of multiple resistant *Klebsiella* in hospitals.

This may be because of weak immune system as observed the high frequency of infection especially in immunocompromised hosts because of bruns, respiratory diseases like cystic fibrosis and cancer chemotherapy and diabetic patients etc. One of the reports states that in 50-60 years age group, the most prevalent organism was *Klebsiella pneumoniae* closely followed by *E-coli* (Shah et al 2002). Studies on age dependent variation of bacterial isolations revealed that neonates are particularly vulnerable to infections because of weak immune barrier (Das et al; 2008).

Antibiogram typing was done for all the strains isolated in our study. Antibiogram typing results of *Klebsiella pneumoniae* against five groups of antibiotics represents the high degree of resistance with sulphonamides (64.58%) followed by β-lactams (57.60%) then fluoroquinoloes (46.95%), aminoglycosides (43.48%) and chloramphenicol (16.92%), when considered individual antibiotics the incidence of resistance was high in isolated strains to amoxicillin / clavulanic acid (83.59%), cefpodoxime (75.26%), ceftazidime (72.13%), cefuroxime (69.27%), aztreonam (67.96%), cefotaxime (65.36%), co-trimaxozole (64.58%), ceftriaxone (63.54%). But with norfloxacin (51.56%), gentamycin (48.69%), nalidixic acid (47.39%), neomycin (45.05%), ciprofloxacin (41.92%), amikacin (36.71%) the incidence of resistance was moderate while with meropenem (27.86%), pipericillin (25.26%), imipenem (25.78%) and chloramphenicol (16.92%) it was recorded relatively lower.
Comparatively the incidence of resistances observed in present study was higher than earlier reports, however available data indicates that the prevalence of resistance among *Klebsiella pneumoniae* isolates varies between countries and difference can be attributed to the variation of resistance pattern to antimicrobials based on extent of exposure to various antibiotics and their difference in prescription patterns and or the quality of infection control practice. So our results moderately correlating with the earlier reported results across the world.

The analysis of the data for multiple antibiotics resistance showed minimum percentage of resistance among the isolates was 1.30% with all 18 antibiotics tested and more was 13.80% against 13 antibiotics.

On contrary the overall resistances offered by the isolates against different antibiotics are as follows: 95% resistance offered to tetracycline, 94% to ampicillin, 92% to azactam, 88% to ceftriaxone, 85% to ciprofloxacin and augmentin, 78% to cefotaxime, 63% to ceftazidime, 60% to gentamicin and 49% to tobramicin. Only 9% isolates showed resistance to imipenem (Shafaq Aiyaz Hassan *et al.*, 2011).

However in our investigation amoxicillin / clavulanic acid showed high resistance and moderate resistance was with cefpodoxime, ceftazidime and low resistance was shown by imipenem, piperacillin followed by chloramphenicol from our observations we suggest these antibiotics for more effective treatment of *Klebsiella pneumoniae* infection.

Although carbapenems are widely regarded as the drug of choice for treatment of infection caused by ESBL producing organisms, production of β-lactamase capable of
hydrolyzing carbapenems has recently been reported across the world including the Indian sub-continent. Few studies from India reported imipenem resistance (12%) in *Klebsiella pneumoniae* (Baby Padmini and Appalaraju, 2004; Gupta et al; 2006 and Akram et al; 2007) similarly our studies showed 27.86% of meropenem and 25.78% imipenem resistant strain which is higher when compared to previous reporters.

Carbapenem resistance also arises from the production of large quantities of chromosomal and plasmid mediated cephalosporinases combined with decreased drug permeability through the outer membrane (Poirel et al; 2004). It has been suggested that such low level resistance to imipenem may arise from AmpC hyper production and loss of porin (Stapleton, 1999; Odeh et al; 2002 and Poirel et al; 2004).

In our study, the comparison of the distribution of antibiotic resistance pattern in *Klebsiella pneumoniae* isolated from various clinical specimens of different zones indicated 17 diversified patterns (Table-10a & 10b). The analysis of the data for multiple antibiotics resistances showed minimum percentage of resistance among the isolates was 1.30% with 18 antibiotics and more was 13.80% against 13 antibiotics (Table-11).

Over all high resistance was observed with sulfonamides (64.58%) then β-lactams (57.60%) followed by fluoroquinolnes (46.95%), aminoglycosides (43.48%) and less with chloramphenicol (16.92%).

In zone D and zone E incidence of resistance was high (38.83%) and (38.61%) followed by zone C (36.66%), zone B (35.66%) and zone A (34.88%). In zone A aganist incidence of resistance to sulphonamides and β-lactams is high when compared with
fluroquinolones and aminoglycosides followed by chloramphenicol, this was analogous with all other zones, but lower rate of resistance was observed with fluroquinolones, aminoglycosides and chloramphenicol in all zones and shows less usage of this group of antibiotics when compared with other groups and also explain the efficacy of this group to cure the *Klebsiella pneumoniae* infections.

Since it was difficult to analyze all the isolated strains by advance methods involved in the present study (protein profiling, molecular detection of AmpC and imipenem gene amplification, plasmid profiling, phylogenetic tree construction), only few multidrug resistant and epidemiologically important *K. pneumoniae* strains were used and reliable outcome were represented.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that completely suppressed bacterial growth after 18 hr incubation at 37°C (Andrews 2001). NCCLS approved the standard methods for dilution in antimicrobial susceptibility tests published in 2000 and recommended that MICs of >2µg/ml for cefpodoxime, ceftazidime, aztreonam, cefotaxime, and ceftriaxone should be regarded as possibly indicating ESBL production. It further suggested that for all ESBL producing strains the test result should be reported as resistant for all pencillins, cephalosporins and aztreonam. In this content all our representative isolates showed MIC > 6µg/ml for ESBL screening agents and therefore likely to posses an acquired resistance mechanism (Bell et al., 2007). On contrary ESBL producing organism may go undetected because they are associated with MIC as low as 0.5µg/ml for ceftazidime, cefotaxime, ceftriaxone and aztreonam (Datta et al., 2004). These enzymes do not always increase the MIC to a high enough level to be
considered resistant by the NCCLS interpretation guideline (Mackenzie et al., 2005). Therefore great caution is needed in interpreting the MICs cut off for ESBL harbouring isolates.

In the present study, the differences in the MIC’s obtained for the same antibiotic against various isolates carrying identical ESBL-type can be attributed to additional resistance mechanisms, such as enzyme hyper production, plasmid copy variation, production of other β-lactamases and changes in permeability (Bradford, 2001 & Sturenburg & Mack, 2003).

The new multi-resistant *Klebsiella spp* produce outbreaks similar to those of the 1970’s and they can pass aminoglycoside and cephalosporin resistances to other species (Brun *et al.*, 1987; Naumovski *et al.*, 1992; Sirot *et al.*, 1991). Nevertheless, most strains produce β-lactamases that remain susceptible to inhibition by clavulanic acid and other commercially available β-lactamase inhibitors. Thus, although these strains are resistant to cephalosporin they are usually susceptible to amoxicillin-clavulanic acid and other β-lactam – β-lactamase inhibitor combinations (French *et al.*, 1996). On the contrary, our studies shows resistant strains which is new pattern of resistance shown in this region. The prevalence of ESBL producing organisms have been reported from worldwide. The first ESBL producing organism was detected in Europe in the year 1983 by Knothe *et al*.

*K. pneumonias* with ESBLs production were more prevalent in Latin America (45%), followed by the Western pacific region (24%), Europe (22%), the United State (7.6%) and Canada (4.9%). In Middle East, Saudi Arabia (12.2%), Lebanon (20%), Egypt (37.5%), Israel (79%), and Turkey (50%), Jordan (80%) and Pakistan (36%) (Jain *et al.*, 2003).
The frequency of ESBL producing *Klebsiella pneumoniae* is different in countries ranging from 5% in Japan to maximum of 86% from India (Lewis *et al.*, 1999; Jain *et al.*, 2003). Interestingly, these ESBL producing *Klebsiella pneumoniae* were isolated from ICU that to from tertiary care hospitals. The prevalence of ESBL producing *Klebsiella pneumoniae* from community is very low as compared to clinical isolates (Gupta, 2007; NNIS Report, 2002).

The report on ESBL producing *Klebsiella pneumoniae* was reported from Nagpur in the year 1997 by Hansotia *et al.*, Subsequently, report from various parts of India showed increased ESBL producing *Klebsiella pneumoniae* from clinical samples as well as from community isolates. The rate of prevalence of ESBL producing *Klebsiella pneumoniae* ranging from 70% to 80% in the clinical isolates and 40-50% in community isolates. The prevalence rate was reported to be 22.7% from neonatal septicaemia (Vinod kumar and Neelgund, 2006).

Resistance to fluoroquinolones, co-trimoxazole and trimethoprim is frequently observed among ESBL producers. Thus, the presence of an ESBL is a good marker of the MDR phenotype. The carbapenems i.e, imipenem, meropenem and ertapenem are considered the drugs of choice for the treatment of infections caused by extended spectrum cephalosporin resistant *E. coli* and *Klebsiella pneumoniae*; however, carbapenem resistance is emerging in certain geographical areas (Leavitt *et al.*, 2007; Paterson *et al.*, 2006; Naavon *et al.*, 2006). The tetracycline derivative showed promising in vitro activity against many of these MDR organisms (Giakkoupi *et al.*, 2003) but the clinical experience with this agent is still limited
and low-grade resistance in members of the family *Enterbacteriaceae* has been reported and attributed to efflux pump mechanisms (Kang *et al.*, 2004; Ruzin *et al.*, 2005).

The frequency of ESBL producing isolates from specimens of patients who attended the community health centers was lower than what was reported in India (Gupta and Datta, 2007) but higher than 1.48% reported from Brazil (Mathur *et al.*, 2002). The high rates of ESBL was seen among the isolates of patients treated in the community is not because of over the counter sales of antibiotics practiced in India but, mostly because of poorly directed therapy among our general practitioners in which there is indiscriminate practice of antibiotics prescription for patients with various infections in the community (Gupta and Datta, 2007). Although, the ESBL producing isolates from the intensive care hospital of the Streit *et al.*, 2004, study showed *E. coli* 2.1% and 8.2% *Klebsiella pneumoniae* respectively, yet the rates are still lower than 11.2% in *E. coli* and 16.2% *Klebsiella pneumoniae* reported in USA (Streit *et al.*, 2004) and 80% in *Klebsiella pneumoniae* isolates reported in India (Mathur *et al.*, 2002). The single most reason for this high rate of ESBL production among these *Klebsiella pneumoniae* and *E. coli* isolates in this hospital is the extensive inappropriate use of cephalosporins in the country as reported by Pinto *et al.*, 2004. This selective pressure created by the use of this third generation cephalosporins has also been described as one of the most important factors elsewhere (Thomson *et al.*, 1999; Philippon *et al.*, 1994).

Stephan *et al.*, (2009) showed that the high prevalence might be a possible consequence of inappropriate use of these antibiotics without proper diagnosis. A heavy use of antibiotics has been reported to be a risk factor for acquisition of ESBL producing organisms (Asensio *et al.*, 2000). Carbapenem class antibiotic are considered as choice of
chemotherapy now a days (Caio et al., 2004), whereas increase in prevalence and geographic spread of carbapenem resistant strains has increased worldwide (Fritsche et al., 2007) as also found in our results.

The spread of MDR microorganisms is associated to the selective pressure caused by the over use of the broad spectrum cephalosporins. The ESBL production is also related to cross-resistant to other classes of drugs, leading to therapeutic failure (Daniella et al., 2008; Thouraya et al., 2003). Usually ESBL production frequently is accompanied by multiresistance to antibiotics.

Ugur et al., 2004 showed Klebsiella isolates were more susceptible to third-generation cephalosporins (42.6% vs. 81.6% for ceftazidime; 65.8% vs. 85.7% for cefotaxime), aztreonam (44.0% vs. 65.6%) and ticarcillin / clavulanate (37.0% vs. 60.3%). Klebsiella spp were 81.6% susceptible to ceftazidime in their study; these rates are 96.6% in North America (NCCLS 1998), 86.7% in china, 80.5% in Korea (Hoban et al., 2000), 69.4% in Latin America (Xu et al., 1999) and 51.9% in India (The Korean antimicrobial resistance study group et al., 1999).

The present study demonstrated that the phenotypic confirmatory disk diffusion test (PCDDT) was the most sensitive in detecting ESBL than double disk synergy test (DDST). DDST detected 32.3% whereas PCDDT detected 78.21% of ESBL producers. Presence of ESBLs can be masked by the expression of AmpC β-lactamase, which can be generated by chromosomal of plasmid genes. Duttaroy et al from Gujarat, India in 2005 reported 58% prevalence of ESBL producing Klebsiella pneumoniae, isolated from different clinical specimens using DDST. Padmini et al from Coimbatore, India in 2004 reported 40%
prevalence of ESBL producing *Klebsiella pneumoniae*, in urinary isolates. The higher prevalence 78.21% in the present investigation is due to the use of more than one confirmation test compared to single test applied by various reporters.

Despite the discovery of ESBLs and AmpC β-lactamases at least a decade ago, there remains a low level of awareness of their importance and many clinical laboratories have problems in detecting ESBLs and AmpC β-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 some times to therapeutic failures. Our screening demonstrates the co-existence phenotype of both ESBL and AmpC in thirteen isolates out of thirty nine which gives 33.33%. AmpC production was reported to be 3.3% and 7% by Ratna et al (2003) and Rodrigues et al (2004). Similarly 6.7% prevalence was found in a study conducted in Kolkata by Suranjana et al; 2005 which was comparably very much lower than our findings. On the contrary AmpC production was found to be as high as 47.3%, 43% and 36% by Hemalatha et al (2007), Manchanda et al (2006) and Singhal et al (2005) respectively. In a study in Korea by Lee et al (2003) 12% of the isolates were shown to be AmpC produces. This could be because plasmid mediated AmpC enzyme have also been shown to disseminate among *Enterobacteriaceae*, sometimes in combination with ESBLs.

In contrast, all the AmpC harbouring organisms were found only in clinical specimens from admitted patients. It has been reported that at present in India AmpC harboring isolates are largely restricted to the hospitalized patients only (Manchanda et al; 2003). The AmpC disk test was an easier, reliable and rapid method of detection of isolates that harbour AmpC
\(\beta\)-lactamases. This suggests that AmpC disk test can be used for routine screening of the AmpC enzyme in the clinical laboratory.

The standard assay for minimal inhibitory concentration and biofilm formation of MDR *Klebsiella pneumoniae* originated from clinical isolates, testing the antibiotic susceptibility of bacteria is minimum inhibitory concentration (MIC), which tests the sensitivity of bacteria in their planktonic phase. The MIC is of limited value in determining the true antibiotic susceptibility of bacteria in their biofilm phase. The minimum inhibitory concentration (MIC) assay, on the other hand, allows direct determination of the bacteria in their biofilm phase of development (Ceri et al., 1997, Ceri et al., 2007). Biofilms are defined as microbially derived sessile communities characterized by the cells that are irreversibly attached to a substratum or to each other. They are embedded in a matrix of extracellular polymeric substances (EPS) they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription (Donlan and Costerton 2002). Within a biofilm, bacteria communicate with each other by production of chemotactic particles or pheromones, a phenomenon called quorum sensing (Thomas and Day 2007).

The establishment of biofilms by *K. pneumoniae* on the tissues of susceptible hosts is believed to protect the pathogen against host defense mechanisms, facilitate bacterial communication leading to expression of virulence determinants (Stewart and Costerton 2001). Biofilm resistance to antibiotics necessitates new measures for the management of the infections produced by the responsible biofilms. High antimicrobial concentrations are required to inactivate organisms growing in a biofilm, as antibiotic resistance can increase 1000 fold. According to publication by the National Institute of Health, more than 80% of all
infections involves in biofilm formation (Reid 1999). Biofilms are associated with many medical conditions including indwelling medical devices, dental plaque, upper respiratory tract infections, peritonitis, and urogenital infections (Donlan 2001). Both Gram-positive and Gram-negative bacteria have the capability to form biofilms (CLSI 2008).

Detailed studies of the isolates lead to the conclusion that the penetration of the biofilms through its matrix and rapid efflux of the antibiotic that still manages to penetrate and found that *Klebsiella* biofilm had impaired the resistance.

The present study for MIC demonstrated that 72 isolates were cefotaxime antibiotic resistant among them 3 isolates were resistant to 8-16µg /ml, 3 isolates were resistant to 32 µg /ml, 6 isolates to 64 µg /ml and 60 isolates showed resistance to 128 µg /ml concentration. For cefuroxime antibiotic, 69 isolates were resistant out them 62 isolates showed resistance to 128 µg /ml, 1isolate showed to 64 µg /ml, 2 isolates showed to 32 µg /ml and 4 isolates showed to 8-16µg /ml. Gentamycin antibiotic, 11 isolates ranged concentration of 8-16µg /ml, 9 isolates 32 µg /ml, 6 isolates to 64 µg /ml and 26 isolates showed resistant to 128 µg /ml compared to 52 resistant isolates. Among the 45 isolates of *K. pneumoniae* resistant to ciprofloxacin, 6 isolates were resistant to 8-16 µg /ml, 10 isolates were resistant to 32 µg /ml, 4 and 25 isolates showed to 64 and 128 µg /ml respectively. Imipenem antibiotic 4 isolates has showed resistant to 128 µg /ml, 3 isolates showed to 64 µg /ml and 1 and 4 isolates showed to 32 and 8-16 µg /ml respectively of total 20 MDR isolates.

The increase in genetic complexity of resistance mechanism in *K. pneumoniae* organism complicates identification of the mechanism of resistance to particular antibiotics. The ability to adhere to materials and to form biofilm is an important feature in the
pathogenesis of *Klebsiella* associated CAI due to the colonization of the polymeric surface by forming multi-layered cell clusters, embebbed in extra cellular material. In this work, a modification of a rapid technique was set up to detect the strains able to form biofilm isolated from clinical sources (Toole and Kolter; 1998). The detection of the strains with a high capability to form biofilm is remarkable, basically by the potential modification of the therapy applied to patients and also to avoid the remotion of the implanted device.

The MIC of all nine strains to ceftazidime was greater than 32 µg/ml, and the MIC value with the ceftazidime-clavulanate combination was 0.5 µg /ml in four strains and 1.0 µg/mL in five strains. A reduction of more than 32-fold in the MIC of ceftazidime in the presence of clavulanate was seen in all the epidemic strains, confirming ESBL production. (Navaratnam et al., 2000). In our study cefotaxime antibiotic 3 isolates were resistant 8-16µg /ml, 3 isolates were resistant to 32 µg /ml 6 isolates to 64 µg /ml 60 isolates to 128 µg /ml concentration. For cefuroxime antibiotic concentration among the 68 isolates were resistant out of them 62 isolates showed 128 µg /ml, 1 isolate showed to 64 µg /ml, 2 isolates showed to 32 µg /ml and 4 isolates showed to 8-16µg /ml.

Biofilm formation in test tube method among 40 isolates at incubation for 24 hrs and 48 hrs along with glucose and antibiotic 10 (25%) showed high exhibiting strong formation 28 (70%) in case of 1% glucose and with imipenem antibiotic 08 (20%) was observed. The high biofilm formation was observed with 1% glucose incubation after 48 hrs among 24 (60%) with glucose 12 (30%) of imipenem antibiotic 08 (20%) as showed high biofilm formation and with glucose 14 (35%) respectively incubation of 48 hrs. In microtitre plate method 11 (27.5%) isolates with addition of glucose, 21 (52.5%), 08 (20%) of containing imipenem antibiotic showed strong biofilm formation at 24 hrs and in contrast less formation
was observed in glucose 09 (22.5%) as of normal 11 (27.5), with addition of imipenem antibiotic along with glucose more biofilm formation was of 09 (22.5%) after incubation of 48 hrs as compared study of Jeff et al., 2000 showed more formation than of our isolates. The microtitre plate method is more sensitive and accurate technique in enhancing and observing the biofilm formation as compared to test tube method in elucidating with antibiotic and glucose as *K. pneumoniae* as become multidrug resistant to different antibiotic.

In past klebocin typing has been used in epidemiological investigation of *K. pneumonia* isolates (Chugh et al; 1980). Recently, molecular methods including whole cell protein analysis (WCPA) by SDS-PAGE, ribotyping for different multi drug resistant *Klebsiella* isolates. The discriminatory power of WCPA, have been reported to be better than bacteriocin typing including the klebocin typing (Grundman et al., 1995). Though ribotyping appear to be reliable methods for distinguishing *K. pneumonia* strains they are expensive, time consuming and required skilled staff. In contrast, SDS-PAGE appears to be relatively economic and does not require skilled staff. A bacterial strain, growing in standardized conditions always produces the same set of proteins. SDS-PAGE is currently one of the most commonly used techniques for the characterization and analysis of proteins and it has been used as taxonomic tool for identification of various bacterial species. (Jeyaseelan et al., 1987).

In the present study the whole cell protein profiles corresponds a distinct banding pattern. The SDS-PAGE of whole cell extracts of isolates showed thirteen distinct protein bands, with molecular mass ranging from 18 to 130kd, were visualized after the gels were stained with coomassie brilliant blue (CBB).
Polymerase chain reaction technique has been used to amplify gene encoding the AmpC β-lactamase. The results clarify that isolates carry AmpC gene. AmpC enzyme was first reported in 1988 as plasmid mediated enzyme (Bauernfeid et al., 1989). Plasmid mediated AmpC have arisen through the transfer of chromosomal genes for the inducible AmpC β-lactamase into plasmids; this transfer has resulted in plasmid-mediated AmpC β-lactamases in isolates of *K. pneumoniae*, *E.coli* and *Enterobacter aerogenes* as reported by Bauernfeid *et al.*, (1989). The same study also pointed out that plasmid-mediated AmpCs differ from chromosomal AmpCs in being uninducible. AmpC-encoded β-lactamases are now predominant in many strains of *Klebsiella* spp., *E.coli*, *Proteus mirabilis* and *Salmonella* spp. (Koh *et al.*, 2007). Plasmid-mediated AmpC have been described from diverse geographic areas including the United states, United kingdom and Asia (Hernandez *et al.*, 2000). Lee *et al.*, (2005) pointed out that the reduced susceptibility to cefoxitin in *Enterobacteriaceae* may be an indicator to AmpC activity, but cefoxitin resistance may also be mediated by alterations to the outer membrane permeability. Organisms over expressing AmpC β-lactamases a major clinical concern because these are usually resistant to all β-lactam drugs except for cefepime, cefpirome and carbapenemes, but in contrast to ESBLs, AmpC β-lactamases hydrolyze cephamycins and are not inhibited by β-lactamase inhibitors (Girlich *et al.*, 2000). Robberts *et al.*, (2008) reported that *K. pneumoniae* harbours only plasmid mediated AmpC, the same study revealed that 33.33% of *K. pneumoniae* isolates were positive for AmpC β-lactamase, from those 5 isolates were selected for amplification of AmpC gene using PCR technique.

Phylogenetic tree was constructed with significantly aligned sequence of 16S rDNA and deposited in NCBI GenBank under accession numbers KM377643, KM377644, KM377645 and KM377647 in BLAST search.