Original Research Article

Prevalence of Extended Spectrum Beta-Lactamase Producing Klebsiella Species in an Intensive Care Unit

V. Sangamithra*, Shamsadh, Kalyani and Mallika

Department of Microbiology, SRM Medical College and RI, Chennai, India

*Corresponding author

**ABSTRACT**

Klebsiella is well known to the clinicians as a cause of community acquired bacterial pneumonia which has a high fatality rate if untreated. Klebsiella is among the five gram negative pathogens most commonly encountered in hospital acquired infections. As opportunistic pathogens, Klebsiella species primarily attack immunocompromised individuals. Reports from India show the occurrence of ESBL producers in Klebsiella species to range from 6.6 to 53% but the exact magnitude of the problem is not known. ICUs are often the epicentre of ESBL production in hospitals. Patients with ESBL producing organisms are often seriously ill patients with prolong hospital stays and in whom invasive medical devices are present for a prolonged duration. Heavy antibiotic use is also a risk factor since ESBL production frequently is accompanied by multiresistance to antibiotics, therapeutic options become limited. So far, however, ESBL producing Klebsiella strains have been susceptible to carbapenems and are the drugs of choice in the treatment. In this respect, emergence of imipenem â€“resistant ESBL producing Klebsiella strains will have a serious impact on remaining therapeutic options. To date, two diagnostic tests have been most commonly used to detect such isolates- double disc synergy test and Etest strip.

**Keywords**

ESBL, Nosocomial infections, Gram negative pathogen, Multidrug resistance.

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**Introduction**

Members of the family Enterobacteriaceae are frequently encountered in hospital acquired infection as they are more important in the spread of non enteric infection in hospital. This is due to the antibiotic resistance, transmissibility, and virulence of the organism, which interact among the patients with similar medical problems who undergo similar procedure and receive similar antibiotics. Nosocomial infections carry considerable clinical and economic burden. Klebsiella species are ubiquitous in nature. They probably have two common habitats, one being the environment, where they are found in 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 (Ullmann et al., 1998). Klebsiella is well known to most clinicians as a cause of community acquired bacterial pneumonia occurring particularly in chronic alcoholics (Aggarwal et al., 2003).

In hospitals, colonization rates increase with the duration of stay and the hospital personnel can carry the organism. The high rate of colonization in patients is associated with the use of antibiotics (Patrick Grimont et al., 2005).
As opportunistic pathogens, *Klebsiella* species primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying diseases like diabetes mellitus or chronic obstructive pulmonary disease (COPD) (Podschun *et al.*, 1998).

The bowel is the major site of colonization with infection of the urinary tract, respiratory tract, and wounds. In addition to prior antibiotic use, risk factors for infection and colonization include the presence of an indwelling catheter, prolonged use of invasive medical devices, feeding tube, or central venous catheter; poor health status; severe illness, including major surgery and treatment in an intensive care unit (ICU) or nursing home, inadequate infection control practices. Acquisition of these species has become a major problem in most hospitals because of resistance to multiple antibiotics and potential transfer of plasmids to other organisms (Obiamive *et al.*, 2002). Morbidity and mortality rates are comparable to those for other gram-negative organism causing sepsis and septic shock. In neonatal units, outbreaks caused by ESBL producing strains result in more serious problem and may be associated with increased mortality (Obiamive Umeh *et al.*, 2002). Among *Klebsiella* species, *Klebsiella pneumoniae* can cause primary community acquired pneumonia as well as nosocomial pneumonia. The typical case is a middle or elderly male with underlying problems such as alcoholism, COPD, or diabetes mellitus. Necrosis and abscess formation is more likely with *Klebsiella pneumoniae* infections than with any other bacterial pneumonia. In addition to pneumonia, *Klebsiella* can cause urinary tract and wound infections, bacteremia, and meningitis.

*Klebsiella pneumoniae* rank 7th as a cause of nosocomial UTI, blood stream, cardiovascular, and ear, nose and throat infections (Sharon Abbott *et al.*, 2003). They rank 4th as a cause of hospital acquired pneumonia (Sharon Abbott *et al.*, 2003).

In contrast, infections due to *Klebsiella pneumoniae* subspecies *ozaenae* and *Klebsiella pneumoniae* subspecies *rhinoscleromatis* are restricted to certain body sites and in most cases affect only the nose (Ingo Stock *et al.*, 2000, Sharon Abbott *et al.*, 2003) causing atrophic rhinitis and rhinoscleroma respectively. Both are chronic diseases of the upper respiratory tract; occurring most frequently in tropical areas of the world; transmission is thought to be from person to person.

In pediatric wards, nosocomial *Klebsiella* infections are especially troublesome particularly in premature infants and ICUs. *Klebsiella* species are often the pathogens involved in neonatal sepsis in both early manifestation and late manifestation infections (Podschun and Ullmann, 1998). *Klebsiella oxytoca* in particular has been implicated in neonatal bacteremia, especially among premature infants and in neonatal ICUs. It is among the top 4 pathogens that cause infection in patients in neonatal intensive care units. It is the second most frequent cause of gram-negative neonatal bacteremia (Obiamive Umeh *et al.*, 2002).

Almost universally, the members of this genus are resistant to the early beta lactam antimicrobials such as penicillin, ampicillin, and amoxicillin. They are usually susceptible to the cephalosporins which are the drugs of choice. However in recent years *Klebsiella* species resistant to cephalosporin are emerging rapidly. This resistance is due the presence of a group of enzymes called extended spectrum beta lactamases. The emergence of extended spectrum beta lactamases is an increasing problem worldwide and is due to indiscriminate use of
the 3rd generation cephalosporins. They were unknown before the introduction of these antibiotics in the early 1980s.

ESBL producing organisms are now a problem in the hospitalized patients worldwide. The ESBL phenomenon began in Western Europe, most likely because extended spectrum beta lactam antibiotics were first used there clinically; however it did not take long before ESBL had been detected in the United States and Asia (Obiamive Umeh et al., 2002).

ESBLs are enzymes that have the ability to inactivate beta lactam antibiotics containing oxyiminogroup (3rd generation cephalosporins and Aztreonam). Hence ESBLs are capable of hydrolyzing broad spectrum cephalosporins, penicillins, and monobactams, but are inactive against Cephamycins and Carbapenems.

The ESBL producing bacteria are typically associated with Multidrug resistance because genes for other mechanisms of resistance often reside on the same plasmid as the ESBL genes. Thus some ESBL producing strains also show resistance to Quinolones, Aminoglycosides and Trimethoprim sulfamethoxazole.

Infections with ESBL producing bacteria can result in avoidable failure of treatment with resultant increase in the cost of patient care with prolong hospital stay. ESBL producing organisms also exhibit cross resistance to various other classes of antibiotics in common use resulting in limitation of therapeutic options.

The concern for the accurate detection of ESBLs is twofold. First, there is an increasing prevalence of ESBLs worldwide. Second, many strains producing ESBLs demonstrate an inoculum effect, in that the Minimum inhibitory concentrations of extended spectrum cephalosporins rise as the inoculum increases (Patricia Bradford, 2001) Extended spectrum beta lactamases producing Klebsiella pneumoniae was first reported in 1983 from Germany. Since then the usage of 3rd generation cephalosporin in the treatment of multidrug resistant Klebsiella pneumoniae infections has been limited as resistant strains have been reported from other parts of the world and recently from South India also (Jerestin Hansotia et al., 1997). Production of these enzymes is either chromosomally mediated or plasmid mediated. Point aminoacid substitution of the classical plasmid mediated betalactamases like TEM-1, TEM-2 and SHV-1 increases the spectrum of activity from earlier generation betalactams to 3rd generation cephalosporins and monobactams.

The chromosomally mediated betalactamases production is mainly through the expression of AmpC gene which is either constitutive or inducible (Rodrigues et al., 2004). Klebsiella are a part of normal life and live inside almost every individual. As opportunistic pathogens, they take advantage of weakened host defenses to colonize and elicit a variety of disease states. Many hospital-acquired infections occur because of the invasive treatments that are often needed in hospitalized patients leading to an increase in the susceptibility to infection. Due to extensive spread of antibiotic resistance, especially extended spectrum betalactamase producing strains, there has been renewed interest in Klebsiella infections.

The main aim of this study includes, to isolate and speciate the Klebsiella isolates from various clinical specimens from patients admitted in ICU. To determine the antibiotic susceptibility pattern of Klebsiella species by disc diffusion method. And to detect the presence of extended spectrum betalactamases (ESBL) by double disc synergy test and Inhibitor Potentiated disc diffusion test.
Materials and Methods

A prospective study was undertaken from November 2004 to April 2005 in the Department of Microbiology, Sri Ramachandra Medical College and Research Institute, a 1500-bedded tertiary care centre. During this period all clinically significant, consecutive, non-repetitive isolates of the genus *Klebsiella* from ICU patients were included in the study. The isolates were collected from various specimens like blood, urine, pus, wound swab, sputum, bronchial wash, endotracheal secretions and body fluids from patients admitted in medical and surgical intensive care units (medical, surgical, cardiothoracic, cardiology, neurosurgery and burns units).

A detailed clinical history was taken and recorded from the patients whose culture grew *Klebsiella* from any of the above clinical specimens. The proforma included the patient’s age, sex, date of admission, admitted ward, brief clinical history, diagnosis, presence of any risk factors (DM, intake of steroid or immunosuppressant, HIV, HBV), presence of associated illness and antibiotic therapy. The samples were collected aseptically by standard techniques (Elmer Koneman *et al.*, 1997).

Methodology

Specimen processing

A direct smear for assessment of the cellularity and presence of bacteria was carried out in all cases. The media for the study were procured from Himedia (Mumbai). The media and the biochemicals were prepared by following standard procedures (Collee *et al.*, 1996) (Annexure II). Each batch of media and biochemicals were tested with suitable controls and was utilized only if it was satisfactory. The primary isolation of the specimen was done on 5% sheep blood agar, MacConkey agar and incubated overnight at 37°C. The isolates produced large grey colonies with a mucoid consistency on blood agar and lactose fermenting large pink coloured mucoid colonies on MacConkey agar. The isolates were subjected to Gram stain which showed capsulated gram-negative short straight rods uniformly stained with parallel sides and rounded ends. A preliminary biochemical reaction which includes catalase test, oxidase tests, test for indole production, triple sugar iron (TSI) reaction, urease test, citrate utilization and mannitol motility test were performed. The Oxidative-Fermentative test for glucose was put for each isolate to show the ability of the organism to breakdown carbohydrates both aerobically and anaerobically.

Biochemical reactions

Once presumptively identified as belonging to the family *Enterobacteriaceae* and genus *Klebsiella* the organism was subjected to further identification up to species level based on Bergey’s Manual. The isolates were also subjected to tests for specific breakdown products formed from fermentation of glucose, Methyl Red and Voges-Proskauer (MR/VP).

Citrate utilization test and tests for enzymes which included Urease and nitrate reduction test was performed. Finally aminoacid decarboxylation reactions were performed for amino acids lysine and ornithine, colour change was observed at the end of each day. A consequent change in colour to violet or reddish-violet was observed and considered a positive result. Based on these tests the isolates were identified as *Klebsiella pneumoniae* subspecies *pneumoniae*, *Klebsiella oxytoca*, *Klebsiella planticola* and *Klebsiella pneumoniae* subspecies *ozaenae*.
Antibiotic susceptibility testing

Antibiotic susceptibility testing was done on Muller Hinton agar plates by Kirby Bauer disc diffusion method. ATCC, *E.coli* 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as control strain and was included for each batch of antibiogram of the test strain.

The antibiotic discs namely Ampicillin (A-10μg), Pipericillin (Pc-100μg), Ciprofloxacain (Cf-5 μg), Amikacin (Ak-30 μg), Cefazolin (Cz-30 μg), Cefuroxime (Cu-30 μg), Ceftazidime (Ca-30μg), Cefotaxime (Ce-30μg), Ceftriaxone (Ci-30μg), Cefaperazone (Cs-75 μg), Cefoxitin (Cfx-30 μg), Cefepime (Cpm-30 μg), Ceftazidime-clavulanate (Ca-30μg,Cr;Clavulanate-10μg), Amoxyclav Amoxicillin-20μg,Clav-10μg) and Imipenem (I-10 μg) were obtained from Himedia. Antibiotic disc like Tzp (Pipericillin-100μg, Tazobactum-10μg) from BBL and Cefaperazone-Sulbactam (Cs-75μg, Sulbactam-30μg) from Pfizer were also included to study the antibiotic susceptibility pattern of these isolates.

Commercially available antibiotic disc were checked for quality using standard strains and then used for the test. For doing antibiogram, 4 -5 well demarcated colonies from the culture were inoculated into nutrient broth and incubated at 37° C till the density of the suspension to be inoculated matched the opacity standard of 0.5 McFarland (barium sulphate suspension) turbidity. A lawn culture of the test organism was made on MHA plate with a sterile cotton swab soaked in the broth, after removing the excess broth by pressing against the sides of the test tube. A disc of Amoxyclav (20μg Amoxicillin/10μg Clavulanic acid) was placed in the center of the lawn culture, on the three sides of this disc at a distance of 30mm from the edge of the above disc; discs containing Ceftazidime, Cefotaxime and Ceftriaxone were placed. Plates were then incubated at 37°C for 18 to 24 hours.

The isolates interpreted as ESBL if the inhibition zone around one or more cephalosporin disc was extended on the side nearest to the Amoxyclav disc or clear extension of the edge of the inhibition zone of any of the antibiotic towards the disc containing clavulanic acid was interpreted as an indication of ESBL production.
Inhibitor potentiated disc diffusion technique

The test organism was grown at 37°C on a nutrient agar plate incubated overnight. Isolated colonies of the organism were inoculated into peptone water and incubated at 37°C and the turbidity adjusted to 0.5 Macfarland standards.

A lawn culture of the test organism was made on the MHA plates with a sterile cotton swab soaked in the broth, after removing the excess broth by pressing against the sides of the test tube and the following discs were placed,

Ceftazidime (30µg) / Ceftazidime-clavulanate (30µg + 10µg) (Himedia)
Cefaperazone (30µg) / Cefaperazone-sulbactam (30µg + 75µg) (Himedia)
Piperacillin (10µg) / Piperacillin-tazobactam (10µg + 100µg) (BBL, USA)

After placing these discs, the plates were incubated at 37°C for 18 to 24 hours.

Zone diameter of the antibiotic (alone) and antibiotic with the inhibitor combination were compared. If the difference in zone size was ≥ 5mm it was indicative of ESBL production.

Results and Discussion

The present study was carried out from November 2004 to April 2005 in the Department of Microbiology, Sri Ramachandra Medical College and Research Institute which is a tertiary care centre. A total of 50 non-repetitive isolates of *Klebsiella* obtained from patients admitted in the ICU for more than 48 hours were included in the study. The samples for study were collected from patients with underlying cardiac or renal diseases, malignancy, diabetes with complications, road traffic accidents etc. They subsequently acquired infection with *Klebsiella* at varying periods after a minimum of 48 hours of hospitalization.

There was almost an equal distribution of the isolates among the genders, the males constituting 29(58%) and females 21(42%) of the total number (Figure 1). The male: female ratio was 1.04: 1.

The demographic profile of the study subjects is shown in figure 2. The age distribution shows that infection with *Klebsiella* was common in middle and older age group. Among the total (n=50), majority of the patients (54%) were between 31 to 60 years of age.

The distribution of isolates in various samples is shown in figure 3. Majority of the isolates were obtained from respiratory specimens, blood followed by urine and exudates samples. The rate of isolation of Respiratory isolates accounted for 40%(n=34) which include endotracheal secretions, bronchial wash, endotracheal tube tips and sputum and the isolation rate from blood accounted for 36%(n=18) from urine samples was 14% (n=7) followed by exudates which constituted 10%(n=5) which included pus, wound swab, drain tips.

The respiratory isolates were recovered from bronchial wash and endotracheal tube secretion from patients who were on ventilatory support. The outcome of these patients was fatal which is attributed to the underlying illness. The risk factors in these patients were stay in ICU, intubation and exposure to multiple antibiotics.

The organism isolated from urine was mostly isolated from male patients with advanced age, diabetes mellitus and underlying renal disease.

The isolates obtained from exudates samples were from elderly diabetic male patients with severe underlying illness such as epidural hemorrhage, chronic kidney disease and road traffic accident. There were surgical
Interventions in all these patients. Risk factors in these patients were presence of indwelling devices, recent surgery and use of multiple antibiotics.

The isolates were identified based on Bergey’s manual of determinative bacteriology Annexure III. Gram stain showed gram-negative bacilli which were capsulated, uniformly stained with parallel sides and rounded ends. They produced the characteristic large grey mucoid colonies on blood agar and lactose fermenting large pink mucoid colonies on MacConkey agar. The isolates had gas production with acid slant and acid butt in TSI medium, fermented glucose both aerobically and anaerobically and were nonmotile with mannitol fermented.

The various isolates of the genus Klebsiella obtained in the present study are depicted in figure 4. Out of the total Klebsiella isolates, Klebsiella pneumoniae subspecies pneumoniae was the commonest isolate 34(68%) followed by Klebsiella oxytoca 9(17%), Klebsiella planticola in 4(9%) and Klebsiella pneumoniae subspecies ozaenae in 3(6%). The species wise distribution of the isolate from various clinical samples and their correlation is given in table 1 and figure 5

In the present study, all Klebsiella pneumoniae subspecies pneumoniae were found to produce gas and ferment all the carbohydrates tested. None of the isolates were able to produce indole and acid during fermentation of glucose in MR tests. All the isolates were able to produce acetoin, utilize citrate, reduce nitrate and decarboxylate the amino acid lysine.

All the Klebsiella oxytoca in the study were able to produce indole, ferment all the carbohydrates with gas production, reduce nitrates, and produce alkali and acetoin. Most of them decarboxylated the amino acid lysine and did not produce acid from glucose in MR test.

Most strains of Klebsiella planticola did not produce indole and none of the isolates were able to ferment dulcitol. All the isolates were found to ferment glucose in MR tests, produce acetoin, utilize citrate, reduce nitrate, and form alkali and decarboxylate lysine.

None of the isolates of Klebsiella pneumoniae subspecies ozaenae was able to produce indole, acetoin in VP test and ferment the carbohydrates sucrose and dulcitol. All the isolates were able to reduce nitrates, decarboxylate lysine and utilize citrate. However, none of the strains produced alkali by the urease test.

Total number of isolates from MICU was 80% (n=40) and SICU wards 20% (n=10) (p<0.05) (Figure 6). In the ICU, majority of the isolates were from respiratory samples followed by blood, urine and exudate specimens. The immune status of each patient was assessed depending on the following conditions like diabetes mellitus, malignancy, intake of steroids, HIV and HBsAg. The breakup of the immunocompromised states is shown in figure and 7.

One of the above immunocompromised factor was found in 39 %( n=19). About 12 %( n=6) of patients had more than one immunocompromised factor. Of the total, 49 % (n=24) (p<0.05) patients were immunocompetent. Of the 39 % (n=19) immunocompromised patients, 43% (n=8) had diabetes, malignancy was recorded in 2% (n=1), long term steroid use / immunosuppressant drugs was seen in 13% (n=3)(p<0.01) of the patients, and 6% (n=1) patients were on dialysis.

The predisposing risk factors (p<0.01) like any surgical procedures, vascular line access,
ventilators, urinary catheters, presence of any drain tubes etc were analyzed and given in table 2.

About 57% patients (n=29) had more than one intervention (p <0.001). Only 15% (n=19) had no intervention. History of previous or recent surgical procedures was recorded in 53% of patients. Prior antibiotic therapy was considered to be the most important risk factor for the acquisition of Klebsiella infection among hospitalized patients. Majority of the patients 31% had at least one antibiotic followed by 30% patients with three antibiotics. Only one patient (0.7%) had no antibiotic therapy. However, in 37% patients there was usage of 3 to 5 antibiotics. This might have been due to the frequent changeover of different antibiotic classes.

In the present study, a total of 18 blood samples of which K. pneumoniae was 14 and K. oxytoca collected were 7. Figure 8 shows the distribution of isolates in blood samples.

In the present study, a total of 20 respiratory tract specimens which includes E.T. tip (n=11), E.T.secretion (n=4), sputum (n=1) and bronchial wash (n=4) were collected. K. pneumoniae was found to be isolated in all the respiratory samples (Table 3).

**Antibiotic susceptibility pattern**

The susceptibility exhibited by each isolate is shown in table 4. The various classes of antibiotics tested are as follows, beta-lactam antibiotics such as Ampicillin, Piperacillin, Cephalosporins (Cefazolin, Cefuroxime, Cefazidime, Cefotaxime, Ceftriaxone, and Cefaperazone), fluoroquinolones (Ciprofloxacin), Aminoglycosides (Amikacin), beta-lactam-beta-lactamase inhibitor combinations (Piperacillin-Tazobactam, Cefaperazone-Sulbactam) and Carbapenem (Imipenem). In the present study, all the isolates (n=50) (100%) were found resistant to the beta-lactam antibiotics such as Ampicillin and Piperacillin. Of the total 50 strains, Amikacin resistance was found in 47% isolates of which 48% were found in Klebsiella pneumoniae, followed by 52% (Klebsiella oxytoca. Amikacin resistance was also found in Klebsiella ozaenae and Klebsiella planticola which accounted for 38% and 42% respectively. However, Amikacin sensitivity was observed in 53% Klebsiella isolates of which 52% were in Klebsiella pneumoniae, 63% in Klebsiella ozaenae and 58% from Klebsiella planticola. There were only 48% isolates of Klebsiella oxytoca found to be sensitive.

Resistance to fluoroquinolones (Ciprofloxacin) was seen in 52% of the isolates and this includes 53% Klebsiella pneumoniae, 48% Klebsiella oxytoca, 67% Klebsiella planticola and 25% isolates of Klebsiella ozaenae. Of the 50 strains, 48% isolates were sensitive to Ciprofloxacin. Maximum sensitivity was observed in Klebsiella pneumoniae 47% and least recorded in Klebsiella planticola 33%.

In the present study, 30% isolates were recorded resistant to all beta-lactam antibiotics which include Ampicillin, Piperacillin, and all cephalosporins. Among the isolates, 54% (p<0.01) strains were found resistant to all the third generation cephalosporins. Maximum resistance to all the 3rd generation cephalosporins was observed in Klebsiella planticola.

Among the 3rd generation cephalosporins, ceftazidime was found to be the most resistant antibiotic (p<0.03) varying from 67% (n=8) in case of Klebsiella planticola to 50% (n=4) in Klebsiella ozaenae. Ceftazidime resistance in Klebsiella pneumoniae and Klebsiella oxytoca were 56% and 57% respectively. A high degree of resistance to Cefotaxime (p<0.01) was observed in Klebsiella oxytoca (62%) next only to Klebsiella planticola (67%).
Resistance to Ceftriaxone and Cefaperazone (p<0.05) was almost equal in the various species of *Klebsiella* which includes 57% in both *Klebsiella pneumoniae* and *Klebsiella oxytoca* followed by 50% in *Klebsiella ozaenae*.

The susceptibility pattern of the betalactambetalactamase inhibitor combinations in the study was found to be variable. Of the total 50 strains, 66% and 62% of the isolates were found to be sensitive to Piperacillin-Tazobactam (p=0.01) and Cefaperazone-Sulbactam (p<0.05) combinations. For *Klebsiella pneumoniae* and *Klebsiella oxytoca*, the susceptibility to Piperacillin-Tazobactam was 64% and 71% respectively. But slightly lowered susceptibility rates were recorded with Cefaperazone-Sulbactam for both *Klebsiella pneumoniae* (60%) and *Klebsiella oxytoca* (67%). All the isolates were found to be sensitive to Carbapenems (100%).

Of the 50 nonrepetitive isolates from the hospitalized inpatients, strains which showed a zone diameter of ≤ 22mm for ceftazidime and ≤ 27mm for cefotaxime or found resistant to any one of the third generation cephalosporin on routine antibiotic susceptibility testing by Kirby-Bauer disc diffusion technique were subjected for identification of ESBL.

Of the 50 resistant isolates *Klebsiella pneumoniae* were 68% (n=34) and other *Klebsiella* species were 32% (n=16). The resistance pattern of the 50 isolates to different antibiotics by Kirby-Bauer disc diffusion techniques is shown in figure 9. All the resistant isolates exhibited different patterns of cross resistance to different classes of antibiotics.

Screening for ESBL based on Ceftazidime was performed by agar dilution technique. All the 50 strains on screening with double disc synergy test 21% (n=21) of the isolates showed either enhancement of the zone size in the presence of Clavulanic acid or clear extension of the edge of the inhibition zone of any of the third generation cephalosporin towards the Clavulanic acid disc (Figure 10).

Inhibitor potentiated disc diffusion test was done using three drug/inhibitor combination. An increase in zone size by ≥5mm with Ceftazidime/Clavulanic acid, Piperacillin/Piperacillin-Tazobactam and Cefaperazone/Cefaperazone-Sulbactam was observed in 84% (n=42), 100% (n=50) and 94% (n=47) (p<0.05) of the isolates respectively (Table 5).

*Klebsiella pneumoniae* is the species most frequently isolated in clinical laboratories. In the present study also, *K. pneumoniae* was the most common species isolated from clinical samples. Among the total 50 isolates, the isolation rate of *Klebsiella* species from the intensive care units includes *Klebsiella pneumoniae* was 68% followed by *K. oxytoca* 17%, *K. planticola* 9% and finally *K. ozaenae* 6%. In a study done by Arora et al., 2003 and Subha et al., 2003, *K. pneumoniae* was isolated at the rate of 84% and 83% from various clinical samples followed by *K. oxytoca* in 16% and 17% in their study. David Livermore and Yuan, 1996 reported 74% *K. pneumoniae* and 26% *K. oxytoca* followed by only 2 isolates (0.2%) of *K. ozaenae*. However Priya Datta et al., 2004 isolated only 35.7% of *K. pneumoniae* followed by 4% *K. oxytoca* from clinical samples.

David Livermore and Gioia Babini, 2000 reported 70.6% *K. pneumoniae*, 26.6% *K. oxytoca* and only (2.8%) one isolate of *K. ozaenae* recovered from patients admitted in ICUs of 21 hospitals.

In our study *K. pneumoniae* was isolated at the rate of 68% from various clinical samples that included urine, exudates and blood, and
respiratory tract specimens. Among the *Klebsiella pneumoniae* isolates 41% (35) were from SICU and 59% (51) were from MICUs.

Subha *et al.*, (2003) reported 83% *K. pneumoniae* at the rate of 21% in blood, 58% in urine and 4% in respiratory tract specimens. In a study done by the same author in 2001 the rate of isolation of *K. pneumoniae* (84%) from various clinical samples was found to be 22% (17) blood, 57% (43) urine, 18.4% (14) from stool, and 2% (2) from throat swab.

Supriya Tankhiwale *et al.*, 2005 and Robert Lewis *et al.*, 1978 who reported 37% and 38% of *K. pneumoniae* from urine samples. However, Subha *et al.*, 2001 had a slight increase (56%) in the isolation rate of *K. pneumoniae* from urine.

Out of the 44% *Klebsiella species* isolated in our study, 16% were from SICU and 84% were from MICU wards. The presence of indwelling catheter was observed in 48% of the patients in both ICU setting. Age, presence of urinary catheters, BPH, CRF, surgical procedures, urogenital abnormality, prolonged hospital stay and exposure to antibiotics were found to be the associated risk factors for isolation from urinary tract.

Among the 16% of *Klebsiella pneumoniae* isolated from respiratory tract samples, a high rate of isolation from endotracheal tube tips was noted 82%. The rate of isolation from endotracheal secretion, bronchial wash and sputum were 14%, 14% and 7% respectively. In our study, 15% of the *Klebsiella* isolates were obtained from ventilated patients. Hans-Jurgen Woske *et al.*, (2001) recovered *Klebsiella* species from 9% of ventilated patients similar to the present study.

Of the 16% (n=14) *Klebsiella pneumoniae* respiratory isolates, 93% (n=13) of them were obtained from patients on ventilatory support. The outcome in some of these patients was fatal which is attributed to the underlying illness. The risk factors observed in these patients were prolonged stay in ICU, intubation, exposure to multiple antibiotics and immunocompromised states like diabetes, steroid therapy.

In our study, 17% of *K. pneumoniae* was recovered from blood samples which were correlating well with the study done in North India by Manjula Mehta *et al.*, 2005 who reported 15% of *K. pneumoniae* from blood samples. However, there was a slight variation in the isolation rate of Subha *et al.*, 2001 who reported 22% of *K. pneumoniae* from blood. Thukral *et al.*, (2005) isolated *K. pneumoniae* at the rate of 31% from wound swabs. In our study 41% of *K. pneumoniae* was isolated from various patients admitted in the ICU wards whereas only one isolate of *K. pneumoniae* was recovered from ICU in the study done by Thukral *et al.*, 2005.

In our study *K. pneumoniae* was isolated from 18.6% of surgical wound infections however there was a slight increase in the incidence (26.8%) of *K. pneumoniae* reported by Karyakarte *et al.*, (1999) from surgical wounds.

*K. oxytoca* accounts for 17% of the clinical samples included in the present study. Subha *et al.*, 2001 and Priya Dutta *et al.*, (2004) isolated the organism from 15% and 4% of the total samples respectively.

Among the total, *Klebsiella oxytoca* was isolated from one blood sample from an ICU patient with demyelinating syndrome. The mortality rate associated with *K. oxytoca* in this study was 10% which is similar (9%) to the mortality rate reported by Rong-Dih Lin *et al.*, (1997). In our study the risk factors associated with *K. oxytoca* infection includes catheterization (52%) intubation (33%).
steroid medication (10%), diabetic (52%) and 67% patients who had undergone surgery recently. The use of more than 2 antibiotics was recorded in 43% of the patients and presence of i.v. line found in 76.1% of the patients.

*K. ozaenae* was isolated from urine3 (100%) specimens. Katherine Murray *et al.*, (1981) isolated 40% of *K. ozaenae* from various clinical samples which included exudates, urine and blood. *K. ozaenae* associated bacteremia has been reported only in one case in our study.

In this study, *K. placticola* was isolated from only 9% of the total clinical specimens which include 100% (4) urine sample. Podschun *et al.*, (1998) and Brian Mee *et al.*, (1997) have isolated only 8.7% and 9% of *K. placticola* from clinical specimens. Freney *et al.*, (1986) has reported 18% to be *K. placticola* isolated from 14 tracheal aspirates, 3 urine, 2 sputum, 2 throat swabs, 1 CSF, 1 nasal swab, and 1 venous catheter.

In the present study, 57% of *K. pneumoniae* was found to be resistant to any one of the 3rd generation cephalosporins. However, Hansotia *et al.*, (1997) isolated 26% of *K. pneumoniae* that were found resistant to 3rd generation cephalosporins. The prevalence of ESBL producing *Klebsiella* species was found to be 58% in our study. Mathur *et al.*, (2002) reported a higher proportion of ESBL positive *Klebsiella* species (80%). Sumeeta Khurana *et al.*, (2002) reported 38.5% of *Klebsiella* species to produce ESBL. Das *et al.*, (2004) reported ESBL in *Klebsiella* species to be 76.34%

In a study in south India done by Subha *et al.*, 2002 ESBL mediated resistance was 25.8% and in another study by Hansotia *et al.*, (1997) a very low prevalence of 6% was reported.

Emily Hyle *et al.*, (2005) observed ESBL in *K. pneumoniae* to be 50.7% and 5.8% in *K. oxytoca*. Priya Dutta *et al.*, (2004) reported 35.7% *K. pneumoniae* and 16.6% of *K. oxytoca* as producing ESBL. In the present study *K. pneumoniae* harbouring ESBL were 66% and *K. oxytoca* with ESBL were 17.5%.

In our study ESBL producing *K. pneumoniae* was found in 66% of the isolates. Huseyin Tash *et al.*, (2005) reported the prevalence of ESBL producers among *K. pneumoniae* to be 57.1%.

In the ICUs, ESBL producing *K. pneumoniae* isolates were more frequently detected in blood (46%) and respiratory tract samples (37%) similar to the study done by Carmen Pena *et al.*, (1998). In MICUs, isolates were detected in urine (56%) and exudate samples (40%) similar to the study of Carmen Pena *et al.*, 1998. In the present study ESBL producing *K. pneumoniae* was isolated from blood (n=14) respiratory tract specimen (n=20). In a study from North India, performed by Shukla *et al.*, (2004) similar rate of isolation was recorded in blood isolates (24%) in contrast to variable values obtained for urine (20.5%) and pus (36.1%). This variation might be due to the selection of the study group involved.

Carmen Pena *et al.*, 1998 reported 35% ESBL producing *K. pneumoniae* isolates. The author also found the isolates to be more frequently recovered from blood (40%) and respiratory samples (26%) in an ICU setting, whereas the non-ICU wards showed greater incidence of ESBL producing *K. pneumoniae* in urine samples (55%) followed by surgical wound samples (34%).

In the present study, ESBL positive *K. pneumoniae* found to be 68%. Presence of renal failure, obstructive uropathy which included stricture urethra and benign prostatic hypertrophy were found to be the risk factors
responsible for the acquisition of ESBL producing *K. pneumoniae* infection. *K. pneumoniae* strains are mostly isolated from urine but unexpectedly, the percentage of *K. pneumoniae* strains resistant to extended spectrum cephalosporins and Aztreonam was lower for UTI than for other infections, especially bacteraemia and respiratory tract infections. The ESBL positivity in urinary isolates of *K. pneumoniae* in Latin American hospitals, USA and Canada was reported as 37.7%, 6.4%, and 6.2% respectively.

In our study, the isolation rate of ESBL producing *K. pneumoniae* from blood was 24% whereas David Paterson et al., 2004 isolated 30.8% *K. pneumoniae* with ESBL. In a report by Lautenbach et al., (2001), only 9.1% of the patients had blood stream infections with ESBL producing *K. pneumoniae*.

In our study, the overall mortality rate due to *K. pneumoniae* was 8.1% (*n=7*), of which 5 isolates of *K. pneumoniae* were found to have ESBL. In a report of 216 patients with *K. pneumoniae* bacteraemia, Paterson et al., (2004) indicated that mortality rate was 46% for 32 patients with bacteraemia caused by ESBL producing *K. pneumoniae* strains and 34% for patients with bacteraemia caused by ESBL nonproducing *K. pneumoniae* strains.

Reduction in the use of betalactam antibiotics containing an oxyiminogroup and infection control measures may reduce the spread of ESBL producing organisms within a hospital (David Paterson et al., 2004). Among the ESBL isolates of our study, the resistance among aminoglycosides and quinolones was found to be 73% and 78%. Resistance to 3rd generation cephalosporins was found in 97% isolates for ceftazidime, 95% strains to cefotaxime and 99% isolates for ceftriaxone and cefaperazone. There were 62% and 32% of the isolates found sensitive to cefoxitin and cefepime.

As observed by Hernandez et al., (2005), the present study also showed the concurrence of ciprofloxacin resistance with ESBL production, particularly in isolates of *K. pneumoniae*. Cross resistance to other antibiotic classes was common, in our study 73% and 78% of strains were resistant to amikacin and ciprofloxacin compared to 41% and 100% resistance observed by Janis Weiner et al., (1999) for aminoglycosides and quinolones.

Subha et al., (2002) from Chennai had reported 75% of the isolates to be resistant to amikacin which is comparable to the present study where there were 73% of the isolates of *K. pneumoniae* harbouring ESBLs found resistant to amikacin.

In concurrence with the present study, Jerestin Hansotia et al., 1997 observed variable sensitivity to the non-betalactam like Amikacin (64%) and Ciprofloxacin (35%).

Mathur et al., (2002) reported 63% of the isolates resistant to amikacin and 81% to ciprofloxacin similar to the present study.

In our study Amikacin and Ciprofloxacin resistance was seen in 74% isolates compared to 61% and 31% reported by Livermore and Gioia Babini (2000).

In the present study ESBL mediated resistance against cephalosporins was found in 51% of *K. pneumoniae* isolates which is much less than the study done by Shukla et al., (2004) where 72% strains of *K. pneumoniae* were involved.

In the present study, all isolates were susceptible to Imipenem and resistant to ampicillin, piperacillin, aztreonam, cefazolin and cefuroxime. Synergy to clavulanate was found in 43% of the isolates and 57% had no synergy.
In our study only 43% of the isolates were detected as ESBLs by the DDST compared to 27.3% isolates detected by DDST by Shukla et al., (2004). In our study 42 strains were positive by DDST. 8 strains which were DDST negative could have had the AmpC profile, porin deficiency or other factors operating. Of these 42 strains, 27 were cefoxitin resistant, thus reflecting the possibility of chromosomal AmpC (Martinez-Martinez et al., 1996). The sensitivity of DDST of the present study was only 43% similar to Priya Datta et al., (2004). Vercauterens et al., (1997) observed variable degrees of 79% and 93% sensitivity to the DDST.

**Table.1 Correlation of clinical samples and species of Klebsiella**

| samples     | Klebsiella pneumoniae | Klebsiella oxytoca | Klebsiella ozaenae | Klebsiella planticola |
|-------------|-----------------------|--------------------|--------------------|-----------------------|
|             |                       |                    |                    |                       |
| Respiratory | 20                    | 0                  | 0                  | 0                     |
| Blood       | 14                    | 7                  | 0                  | 0                     |
| Urine       | 0                     | 1                  | 3                  | 4                     |
| Exudate     | 0                     | 1                  | 0                  | 0                     |

**Table.2 Predisposing risk factors**

| Type of Interventions      | Numbers (n=127) | Percentage |
|-----------------------------|-----------------|------------|
| Line access                 | 42              | 84%        |
| Urinary catheter            | 25              | 50%        |
| Ventilator                  | 18              | 35%        |
| Others (dialysis, wound drain) | 8             | 17%        |
| More than one interventions  | 29              | 57%        |

**Table.3 Distribution of isolates among respiratory samples**

| Organism    | E.T. tip | E.T. secretion | Bronchial wash | Sputum | Total |
|-------------|----------|----------------|----------------|--------|-------|
| *K. pneumoniae* | 9        | 6              | 4              | 1      | 14    |
Table 4 Antibiotic Susceptibility Pattern of *Klebsiella* species

| Antibiotic               | *Klebsiella pneumoniae (n=34)* |  |  |  | *Klebsiella planticola (n=4)* |  |  |  | *Klebsiella oxytoca (n=9)* |  |  |  | *Klebsiella ozaenae (n=3)* |  |  |
|--------------------------|--------------------------------|---|---|---|--------------------------------|---|---|---|--------------------------------|---|---|---|--------------------------------|---|---|
|                          | S                              | R | S  | R  | S                              | R | S  | R  | S                              | R | S  | R  |
| Ampicillin               | 0(0%)                          | 34(100%) | 0(0%) | 4(100%) | 0(0%)                          | 9(100%) | 0(0%) | 3(100%) | 0(0%)                          | 3(100%) | 0(0%) | 3(100%) |
| Piperacillin             | 0(0%)                          | 34(100%) | 0(0%) | 4(100%) | 0(0%)                          | 9(100%) | 0(0%) | 3(100%) | 0(0%)                          | 3(100%) | 0(0%) | 3(100%) |
| Amikacin                 | 18(52%)                        | 16(48%) | 3(67%) | 1(33%) | 4(48%)                          | 5(52%) | 5(63%) | 3(38%) | 18(52%)                        | 16(48%) | 3(67%) | 1(33%) |
| Ciprofloxacin            | 16(47%)                        | 18(53%) | 1(33%) | 3(67%) | 5(52%)                          | 4(48%) | 6(75%) | 2(25%) | 16(47%)                        | 18(53%) | 1(33%) | 3(67%) |
| Cefazolin                | 6(17%)                         | 28(83%) | 1(33%) | 1(67%) | 1(19%)                          | 8(81%) | 0(0%) | 8(100%) | 6(17%)                         | 28(83%) | 1(33%) | 1(67%) |
| Cefuroxime               | 10(29%)                        | 24(71%) | 1(33%) | 1(67%) | 2(24%)                          | 7(76%) | 0(0%) | 3(100%) | 10(29%)                        | 24(71%) | 1(33%) | 1(67%) |
| Ceftazidime              | 19(56%)                        | 15(44%) | 1(33%) | 3(67%) | 3(43%)                          | 6(57%) | 1(50%) | 1(50%) | 19(56%)                        | 15(44%) | 1(33%) | 3(67%) |
| Cefotaxime               | 16(48%)                        | 18(52%) | 1(33%) | 3(67%) | 3(38%)                          | 6(62%) | 1(50%) | 1(50%) | 16(48%)                        | 18(52%) | 1(33%) | 3(67%) |
| Ceftriaxone              | 15(43%)                        | 19(57%) | 1(33%) | 3(67%) | 3(43%)                          | 6(57%) | 1(50%) | 1(50%) | 15(43%)                        | 19(57%) | 1(33%) | 3(67%) |
| Cefaperazone             | 15(43%)                        | 19(57%) | 1(33%) | 3(67%) | 3(43%)                          | 6(57%) | 1(50%) | 1(50%) | 15(43%)                        | 19(57%) | 1(33%) | 3(67%) |
| Cefaperazone-Subbactam   | 20(60%)                        | 14(40%) | 3(67%) | 1(33%) | 6(67%)                          | 3(33%) | 3(100%) | 0(0%) | 20(60%)                        | 14(40%) | 3(67%) | 1(33%) |
| Piperacillin-Tazobactam  | 22(64%)                        | 12(36%) | 3(67%) | 1(33%) | 6(71%)                          | 3(29%) | 3(100%) | 0(0%) | 22(64%)                        | 12(36%) | 3(67%) | 1(33%) |
| Imipenem                 | 34(100%)                       | 0(0%)  | 4(100%) | 0(0%)  | 9(100%)                         | 0(0%)  | 3(100%) | 0(0%) | 34(100%)                       | 0(0%)  | 4(100%) | 0(0%) |
| Aztreonam                | 0(0%)                          | 34(100%) | 0(0%)  | 4(100%) | 0(0%)                          | 9(100%) | 0(0%)  | 3(100%) | 0(0%)                          | 34(100%) | 0(0%)  | 4(100%) |

Table 5 Inhibitor Potentiated Disc Diffusion Test

| Isolate       | CaC >5mm | CaC <5mm | Tzp >5mm | Tzp <5mm | Cfs >5mm | Cfs <5mm |
|---------------|----------|----------|----------|----------|----------|----------|
| *K. pneumoniae* | 28       | 6        | 34       | 0        | 31       | 3        |
| *Klebsiella oxytoca* | 7       | 2        | 9        | 0        | 9        | 0        |
| *Klebsiella ozaenae* | 3       | 0        | 3        | 0        | 3        | 0        |
| *Klebsiella planticola* | 4      | 0        | 4        | 0        | 4        | 0        |
**Fig. 1** Male-Female distribution

![Male-Female distribution chart](image)

**Fig. 2** Demographic profile of study

![Demographic profile chart](image)
Fig. 3 Distribution of isolates in various clinical samples

![Bar chart showing distribution of isolates in various clinical samples.]

Fig. 4 **Klebsiella** species isolated

![Pie chart showing percentage of different Klebsiella species isolated.]

Fig. 5 Correlation of clinical samples and species of **Klebsiella**

![Bar chart showing correlation of clinical samples and species of Klebsiella.]

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Fig. 6 MICU /SICU distribution of Klebsiella

Fig. 7 Immune status of the patient
Fig. 8 Distribution of isolates in blood samples

![Graph showing distribution of isolates in blood samples]

Fig. 9 Antibiotic Susceptibility pattern of ESBL isolates

![Antibiotic susceptibility pattern graph]

Fig. 10 *Klebsiella* isolates positive for DDST

![Graph showing *Klebsiella* isolates positive for DDST]
The other confirmatory test used in this study was the IPDD test. CLSI states that this test can be performed with either ceftazidime/clavulanic acid or cefotaxime/clavulanic acid, but screening with both increases the sensitivity (NCCLS, 2002). In our study, 92% of the isolates were identified by IPDD test similar to 100% sensitivity observed by Ho et al., (1998).

In our study, 92% strains of the total 74 ESBL producers could be identified by IPDD test similar to Priya Datta et al., (2004). However, use of piperacillin/tazobactam was able to pick up more strains compared to ceftazidime/clavulanic acid and cefaperazone/sulbactam. Tazobactam is a more potent inhibitor of both plasmid and chromosomal mediated betalactamases.

In the present study, only 43% of Klebsiella species were susceptible to piperacillin-tazobactam combination compared to 35% and 70% susceptibility noted by Jill Rebuck et al., (2000) and Livermore et al., (1996). Likewise in our study also there were 57% of isolates found resistant to piperacillin-tazobactam and 65% to cefaperazone-sulbactam.

Of the total ESBL producing Klebsiella isolates 68% were resistant to Cefepime and all the isolates 100% resistant to Aztreonam. Nearly 68% of the ESBL producing K. pneumoniae were resistant to cefepime in our study but Cheol-In Kang et al., (2004) reported only 4.6% of the isolates to be resistant. Barroso et al., (2000) reported resistance to aztreonam in all the 138 isolates of his study.

In accordance with this study, Imipenem and cefepime were sensitive in 100% and 33% of the strains. Aksaray et al., (2000) found 98.6% and 70% of these strains to be sensitive to Imipenem and cefepime.

None of the Klebsiella isolates were found to be resistant to Imipenem which demonstrates the highest degree of sensitivity similar to Bradley Jett et al., (1995).

In conclusion,

- Nosocomial Klebsiella infections continue to be a heavy burden on the economy and on the life expectancy of patients worldwide.
- K. pneumonieae is the most frequently isolated organism from clinical specimens and found to be associated with drug resistance.
- Apart from K. pneumoniae and K. oxytoaca, K.planticola in particular, has been isolated with increasing frequency from human infectious clinical samples.

In our study so far, ESBL producing Klebsiella strains have been susceptible to carbapenems.

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