

Research Article

Extended Spectrum β -Lactamase Producing *Klebsiella pneumoniae* Infections

V. Sangamithra¹, S. Balamuruganvelu², B. Geethavani² and R. Sumathi^{3*}

¹Madha medical college, Chennai, Tamil Nadu, India.

²Department of microbiology, Sri lakshmi narayana institute of medical sciences, Pudhucherry, Tamil Nadu, India.

³Nandha College of Pharmacy, Erode-52, Tamil Nadu, India.

ABSTRACT

The increase in the incidence of extended-spectrum β -lactamase (ESBL)-producing bacteria has become a serious problem worldwide, but the distribution of ESBL-producing bacteria can vary according to geographical area or institution. The aim of this study was to analyze epidemiologic data on ESBL-producing bacteria and their genotypes in our hospital. The hospital microbiology laboratory databases were reviewed for ESBL-producing *Klebsiella pneumoniae* from 2004 to 2005. The ESBL-producers were also molecularly analyzed and included the CTX-M, TEM and SHV genes. In a 1-year study, there was 725 isolates of *K. pneumoniae*. Incidence of ESBL-producing *K. pneumoniae* increased. The most common genotype was TEM+SHV (30.0%) for *K. pneumoniae*. The increase of bacteria with multiple ESBL genes may be an emergent problem. Therefore, ESBL genotyping is needed for monitoring the important ESBLs that can lead to treatment failure and contribute to the appropriate use of antimicrobial agents and infection control.

Keywords: Extended-spectrum β -lactamase; *Klebsiella pneumoniae*.

INTRODUCTION

Infections caused by multidrug-resistant Gram negative bacilli that produce extended-spectrum β -lactamase (ESBL) enzymes have been reported with increasing frequency in intensive-care units and are associated with significant morbidity and mortality.¹⁻⁴ Because of resistance to numerous antimicrobial agents, treatment can be challenging. *K. pneumoniae* has emerged as an important cause of hospital-acquired infections, especially among patients in the neonatal intensive-care unit and mortality rates can be as high as 70%.^{5,6} Over the last two decades, the incidence of infections caused by multidrug-resistant *Klebsiella* strains has increased. Members of the family *Enterobacteriaceae* are frequently encountered in hospital acquired infection as they are more important in the spread of nonenteric 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. Gram negative challenges in the future include the potential for increasing rates of extended spectrum beta

lactamases (ESBL) and antibiotic resistance in *Acinetobacter*.⁷⁻⁹ ESBLs in *Klebsiella*, *E.coli*, *Proteus mirabilis* and AmpC resistance among the gram-negative organisms are of increasing concern. Nosocomial infections carry considerable clinical and economic burden.^{10,11} *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. It has been observed that there are many organisms that are able to produce extended spectrum.¹²⁻¹⁴ β -Lactamases (ESBL's), with *Klebsiella pneumoniae* being the most common species to produce ESBL's. Bacteria that are able to produce ESBL's are not susceptible to treatment with third generation cephalosporins, which poses a problem in effectively treating patients who are infected with an ESBL producing organism.^{15,16}

MATERIALS AND METHODS

A prospective study was undertaken from November 2004 to April 2005 in the Department of Microbiology, Sri Ramachandra

Medical College & Research Institute, a 1500-bed tertiary care centre. During this period all clinically significant, consecutive, nonrepetitive isolates of the genus *Klebsiella* from hospitalized 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, surgical wards and 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 [Proforma enclosed in Annexure I]. The samples were collected aseptically by standard techniques [Elmer W. Koneman *et al* 1997. Information on the isolated strains, including etiology and susceptibility to antibiotics, was also obtained.

Antimicrobial 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), Piperacillin (Pc-100 µg), Ciprofloxacin (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, Clavulanate-10µg), Amoxyclav (Amox-20µg, Clav-10µg) & Imipenem (I-10 µg) were obtained from Himedia. Antibiotic disc like Tzp (Piperacillin-100µg, Tazobactam-10µg) from BBL & 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. The plates were incubated at 37°C for 18 to 24 hours after which the zone of inhibition was measured with calipers and compared with control strains as per Clinical and Laboratory Standards Institute (CLSI) guidelines.¹⁷⁻²⁰ The reading of the test strain was taken only if the control strains showed satisfactory zone size in accordance with the CLSI (NCCLS) guidelines. [Annexure IV]

Preparation of agar plates

18ml of MHA was prepared, sterilized and cooled to 50°C. To this 2ml of the drug in required dilution was added to make up the volume to 20ml and poured in the glass petridish. To test the viability of the control organism and the test organism, a control plate without the antibiotic was included. The plates were allowed to set and the same were incubated overnight for quality checking. All the plates were inoculated the next day. After the plates with the media were set, they were allowed to dry for few minutes at 37°C before use.^{21,22}

Inoculation of the test organism

The test organism was inoculated into peptone water and incubated at 37°C until the turbidity matched 0.5 Macfarland standards. Then a platinum loop calibrated to deliver 0.001ml of the inocula was used to spot inoculates the culture. The control strains ATCC *E.coli* 25922 and ATCC *Klebsiella pneumoniae* 700603 were included in all the plates. Inoculated plates were left undisturbed until the spot of inoculums had dried. The plates were then inverted & incubated at 37°C for 16 to 18 hours.²³⁻²⁵

Interpretation

The concentration of the drug at which there was no visible growth of the organism at the spot of inoculation was taken as the MIC for that particular drug.

RESULTS

There was almost an equal distribution of the isolates among the genders, the males constituting 65(51%) and females 62(49%) of the total number (Figure 1). The male: female ratio was 1.04: 1.

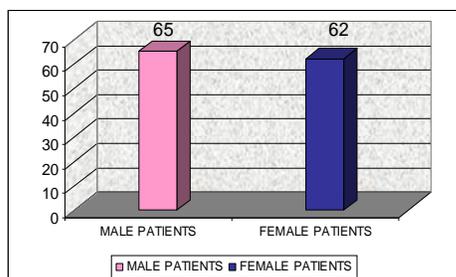


Fig. 1: Male-Female distribution

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, and 14(11%) of the patients were of the Pediatric age group which included 11(8.6%) neonates. Among the total (n=127), majority of the patients (54%) were between 31 to 60 years of age.

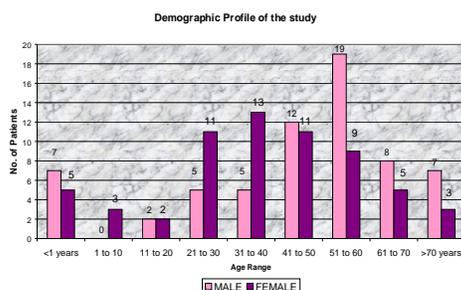


Fig. 2: Demographic Profile of study

The distribution of isolates in various samples is shown in **Figure 3**. Majority of the isolates were obtained from urine samples, followed by exudates, respiratory specimens and blood. The rate of isolation from urine samples was 46 % (n=58) followed by exudates which constituted 24 % (n=31) which included pus, wound swab, drain tips, and aural swab. Respiratory isolates accounted for 16 % (n=20) which include endotracheal secretions, bronchial wash, endotracheal tube tips and sputum and the isolation rate from blood accounted for 14 % (n=18).

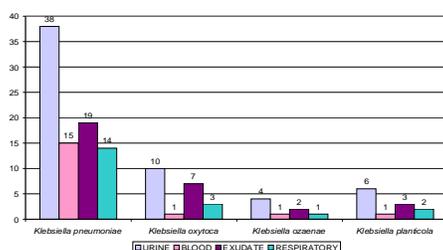


Fig. 3: Distribution of isolates in various clinical samples

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 urine isolates were predominantly recovered from non-ICU wards with one isolated from ICU. The organism was mostly isolated from male patients with advanced age, diabetes mellitus and underlying renal disease. The isolates obtained from exudates samples (n=3) 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 & 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 86(68%) followed by *Klebsiella oxytoca* 21(16%), *Klebsiella planticola* in 12(9%) and *Klebsiella pneumoniae* subspecies *ozaenae* in 8(6%).

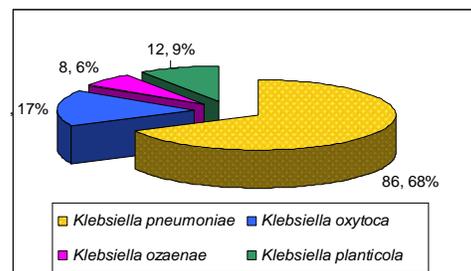


Fig. 4: *Klebsiella* species isolated

The species wise distribution of the isolate from various clinical samples and their correlation is given in **Table 1**.

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, produce alkali, and 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.

Figure 5 shows the distribution of *Klebsiella* in ICU and Non-ICU setting. The ICU included

medical, surgical, pediatric, neonatology, neurosurgical, cardiology and cardiothoracic ICU. Non-ICU wards were medical wards, surgical wards, and orthopedics, pediatrics and gynecology wards.

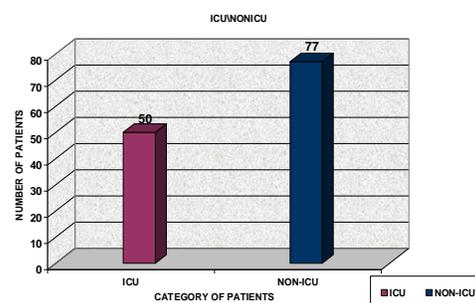


Fig. 5: ICU / NON-ICU distribution

Total number of isolates from ICU was 39% (n=50) and Non-ICU wards 61% (n=77) (p<0.05). In the ICU, majority of the isolates were from respiratory samples followed by blood, urine and exudate specimens. In the wards, *Klebsiella* was more commonly isolated from urine samples followed by exudates and blood samples.

Table 3: *Klebsiella* species isolated from various exudate samples

| Organism | Wound swab | Pus | Drain tip | Femoral line tip | Central line tip | Peritoneal swab | High vaginal swab | Aural swab | Total |
|---------------------|------------|-----|-----------|------------------|------------------|-----------------|-------------------|------------|-------|
| <i>K.pneumoniae</i> | 8 | 8 | 1 | 0 | 0 | 1 | 0 | 1 | 19 |
| <i>K.oxytoca</i> | 4 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 7 |
| <i>K.planticola</i> | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 |
| <i>K.ozaenae</i> | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| Total | 14 | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 31 |

Most of the exudate specimens had been from non-ICU wards (n=27) (87%). The most common isolate recovered from wound swab was *K.pneumoniae* (n=8) (57%) and the least being *K.planticola* (n=2) (14%). There were no *K. ozaenae* isolated from wound swab. Of the 11 pus samples, *K.pneumoniae* (n=8) (73%) was the predominant isolate followed by *K.oxytoca* (n=2) (18%). The least common was *K.ozaenae* (n=1) (9%). *K.planticola* was not found to be isolated from any of the pus samples. There was only one high vaginal swab (HVS) included in the study which grew *K.oxytoca*. There was only one femoral line tip and central line tip which grew *K.ozaenae* &

K.planticola respectively. *K.pneumoniae* was isolated from drain tip (n=1) & aural swab (n=1). ESBL producing *Klebsiella* species were recovered from 65% (20) exudate 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. The distribution of isolates among the respiratory samples is shown in the **Table 4** and **Figure 9A & 9B**. *K.pneumoniae* (n=14) (70%) was found to be the predominant isolate and the least common being *K.ozaenae* (n=1) (5%).

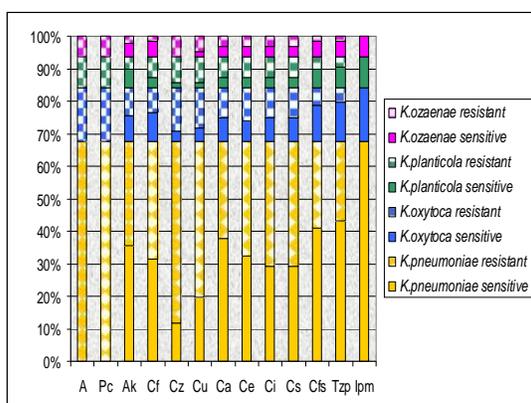
Table 4: Distribution of isolates among Respiratory samples

| Organism | E.T. tip | E.T.secretion | Bronchial wash | Sputum | Total |
|---------------------|----------|---------------|----------------|--------|-------|
| <i>K.pneumoniae</i> | 9 | 2 | 2 | 1 | 14 |
| <i>K.oxytoca</i> | 2 | 0 | 1 | 0 | 3 |
| <i>K.planticola</i> | 0 | 1 | 1 | 0 | 2 |
| <i>K.ozaenae</i> | 0 | 1 | 0 | 0 | 1 |
| Total | 11 | 4 | 4 | 1 | 20 |

K.pneumoniae (82%) (n=9) was the most common isolate grown followed by *K.oxytoca* (18%) (n=2). *K.ozaenae* and *K.planticola* were not recovered from any of the E.T.tips collected. *K.pneumoniae* was isolated from 50% (n=2) of E.T.secretion followed by one each of *K.ozaenae* and *K.planticola*. There was only one sputum sample collected which grew *K.pneumoniae*. Out of the 4 bronchial wash samples, 2 samples (50%) grew *K.pneumoniae* and one isolate of each *K.oxytoca* & *K.planticola* were also obtained.

Antibiotic Susceptibility Pattern

The antibiogram pattern of *Klebsiella* is depicted in **Figure 11**. The susceptibility exhibited by each isolate is shown in **Table 5**. The various classes of antibiotics tested are as follows, betalactam antibiotics such as Ampicillin, Piperacillin, Cephalosporins (Cefazolin, Cefuroxime, Ceftazidime, Cefotaxime, Ceftriaxone, and Cefaperazone), fluoroquinolones (Ciprofloxacin), Aminoglycosides (Amikacin), betalactam-betalactamase inhibitor combinations (Piperacillin-Tazobactam, Cefaperazone-Sulbactam) and Carbapenem (Imipenem).



In the present study, all the isolates (n=127) (100%) were found resistant to the betalactam antibiotics such as Ampicillin and Piperacillin. Of the total 127 strains, Amikacin resistance was found in 47% (n=60) isolates of which 48% (n=41) were found in *Klebsiella pneumoniae*, followed by 52% (n=11) *Klebsiella oxytoca*. Amikacin resistance was also found in *Klebsiella ozaenae* and *Klebsiella planticola* which accounted for 38%

(n=3) and 42% (n=5) respectively. However, Amikacin sensitivity was observed in 53% (n=67) *Klebsiella* isolates of which 52% (n=45) were in *Klebsiella pneumoniae*, 63% (n=5) in *Klebsiella ozaenae* and 58% (n=7) from *Klebsiella planticola*. There were only 48% (n=10) isolates of *Klebsiella oxytoca* found to be sensitive. Resistance to fluoroquinolones (Ciprofloxacin) was seen in 52% (n=66) of the isolates and this includes 53% (n=46) *Klebsiella pneumoniae*, 48% (n=10) *Klebsiella oxytoca*, 67% (n=8) *Klebsiella planticola* and 25% (n=2) isolates of *Klebsiella ozaenae*. Of the 127 strains, 48% (n=61) isolates were sensitive to Ciprofloxacin. Maximum sensitivity was observed in *Klebsiella pneumoniae* 47% (n=40) and least recorded in *Klebsiella planticola* 33% (n=4). In the present study, 30% isolates (n=38) were recorded resistant to all betalactam antibiotics which include Ampicillin, Piperacillin, and all cephalosporins. Among the isolates, 54% (n=68) (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 (n=72) (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% (n=48) and 57% (n=12) respectively. A high degree of resistance to Cefotaxime (p<0.01) was observed in *Klebsiella oxytoca* (n=13) (62%) next only to *Klebsiella planticola* (n=4)(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* (n=49) and *Klebsiella oxytoca* (n=12) followed by 50% in *Klebsiella ozaenae* (n=4). The susceptibility pattern of the betalactam-betalactamase inhibitor combinations in the study was found to be variable. Of the total 127 strains, 66% (n=84) and 62% (n=79) of the isolates were found to be sensitive to Piperacillin-Tazobactam (p=0.01) & Cefaperazone-Sulbactam (p<0.05) combinations. For *Klebsiella pneumoniae* and *Klebsiella oxytoca*,

the susceptibility to Piperacillin-Tazobactam was 64% (n=55) and 71% (n=15) respectively. But slightly lowered susceptibility rates were recorded with Cefoperazone- Sulbactam for both *Klebsiella pneumoniae* (n=52) (60%) and *Klebsiella oxytoca* (n=14) (67%). All the isolates were found to be sensitive to Carbapenems (n=127) (100%).

Of the 127 nonrepetitive isolates from the hospitalized inpatients, 74 isolates which showed a zone diameter of ≤ 22 mm for ceftazidime and / or ≤ 27 mm 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. The resistant organisms were isolated from blood (n=15), urine (n=26), exudates (n=20) and respiratory tract specimens (n=13). Of the 74 resistant isolates *Klebsiella pneumoniae* were 66% (n=49) and other *Klebsiella* species were 34% (n=25). The resistance pattern of the 74 isolates to different antibiotics by Kirby-Bauer disc diffusion techniques is shown in **Figure 12**. The antibiogram pattern of each resistant isolate is given in **Table 6**. All the resistant isolates exhibited different patterns of cross resistance to different classes of antibiotics.

DISCUSSION

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 127 isolates, the isolation rate of *Klebsiella pneumoniae* was 68% followed by *K.oxytoca* 17%, *K.planticola* 9% and finally *K.ozaenae* 6%. In our study, the rate of isolation of various *Klebsiella* species from the intensive care units includes 70% (35) *K.pneumoniae*, 16% (8) *K.oxytoca*, 10% (5) of *K.planticola* and only 4% (2) isolates of *K.ozaenae*. In our study *K.pneumoniae* was isolated at the rate of 68% from various clinical samples that included urine 44%, exudates 22%, and blood 17%, and respiratory tract specimen's 16%. Among the *Klebsiella pneumoniae* isolates 41% (35) were from ICU and 59% (51) were from Non-ICU wards.

Out of the 44% *K.pneumoniae* isolated from urine in our study, 16% (n=6) were from ICU and 84% (n=32) were from Non-ICU wards. The presence of indwelling catheter was observed in 48% (n=41) of the patients in both ICU and Non-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. The organism was isolated in significant numbers from postnatal women and in women who underwent catheterization during surgical procedures. In male patients the risk factors were age, underlying illness like diabetes, CRF, urogenital tract abnormality and BPH with indwelling catheter. 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. Total *K.pneumoniae*, 17% (n=15) was isolated from blood samples, 60% (n=9) were from newborn infants and 67% (n=6) of them were preterm. Of the 6 preterm infants, inborn error of metabolism was found in one patient, respiratory distress in 3 patients. The predisposing risk factors in these patients were the presence of vascular access, intubation, ICU stay, and antibiotic therapy.

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