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Original Research Article

# Extended Spectrum Beta-Lactamase Resistance among Klebsiella Pneumoniae in Various Clinical Samples at Tertiary Care Hospital

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**Abstract:**

**Background:** Rising antibiotic resistance presents a global healthcare crisis. Among Gram-negative bacteria, Extended Spectrum Beta-lactamases (ESBLs) are pivotal in undermining antimicrobial efficacy, with Klebsiella pneumoniae exemplifying multidrug resistance. This study investigates the concerning prevalence of ESBL resistance in Klebsiella pneumoniae across clinical samples within a tertiary care hospital. Notably, Klebsiella pneumoniae's intrinsic resistance mechanisms and nosocomial infection potential warrant increased attention. The study aimed to isolate, characterize, and detect ESBL production in Klebsiella pneumoniae, informing infection control and targeted treatment strategies.

**Methods:** This cross-sectional study, conducted at the Department of Microbiology, Government Medical College, Amritsar, over two years from July 2001 to May 2003, included 250 K. pneumoniae isolates from clinical specimens. Specimens were collected aseptically and processed before incubation on MacConkey agar and blood agar plates at 37°C. Microbiological identification confirmed K. pneumoniae strains. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion methods. Extended Spectrum Beta-lactamase (ESBL) production was detected through a combination of cephalosporins and beta-lactam inhibitors. Data were analyzed using SPSS version 20.0.

**Results:** Among 250 K. pneumoniae isolates, males (58.8%) slightly outnumbered females (41.2%), with the largest proportion in the 41-60-year age group (41.2%). Pus samples (66%) yielded the most Klebsiella species, followed by urine (24.8%) and sputum (9.2%). Hospitalized patients contributed 74% of the isolates, with outpatients at 26%. Antibiotic susceptibility revealed notable resistance to ceftazidime (90%) and cephalexin (79.6%), while cefotaxime (55.6%) displayed comparatively lower resistance. The sulbactam-cefoperazone combination showed a 66% resistance rate. Cefotaxime (36.4%) and cefoperazone (23.6%) demonstrated better efficacy. Furthermore, beta-lactamase positivity was associated with higher resistance, except for gentamicin. Beta-lactamase prevalence was notable in the 41-60-year age group, with 105 (71.42%) in males and 71 (68.93%) in females, primarily isolated from inpatients. A significant difference was observed between outpatients and inpatients.

**Conclusion:** The findings underscore the need for prudent antibiotic use, rigorous infection control measures, and continued surveillance to combat the growing challenge of antibiotic resistance and beta-lactamase production in K. pneumoniae.

**Keywords:** IPD, Pus, Klebsiella pneumoniae, ESBL producers, Gram-negative bacteria.



## Introduction

The emergence of antibiotic resistance in bacterial pathogens poses a formidable challenge to the global healthcare community, rendering once-effective antimicrobial agents increasingly ineffective [1]. Extended Spectrum Beta-lactamases (ESBLs) represent a critical mechanism of resistance among Gram-negative bacteria, particularly within the Enterobacteriaceae family. Klebsiella pneumoniae, a member of this family, has garnered significant attention due to its capacity to acquire and express ESBLs, rendering it a potent multidrug-resistant pathogen [2]. This study delves into the alarming prevalence of ESBL resistance in Klebsiella pneumoniae across various clinical samples within the confines of a tertiary care hospital, shedding light on the urgent need for continued surveillance and control measures [3]. Antibiotic resistance, driven by a combination of horizontal gene transfer, genetic

mutations, and selective pressures of antimicrobial use, threatens to undermine the foundations of modern medicine [4]. ESBLs, enzymes capable of hydrolyzing a wide range of beta-lactam antibiotics, have demonstrated a particularly worrisome ability to confer resistance to many clinically important drugs, including penicillins, cephalosporins, and monobactams [5]. The increasing prevalence of ESBL-producing strains, particularly in the clinical setting, has limited the therapeutic options available for treating infections caused by such strains, leading to higher mortality rates, prolonged hospital stays, and increased healthcare costs [6]. In the context of this escalating challenge, Klebsiella pneumoniae stands out as a formidable adversary [7]. This Gram-negative bacterium, known for its intrinsic resistance mechanisms, propensity to acquire plasmid-borne resistance genes, and survival in healthcare-associated environments, has become a notorious cause of nosocomial infections [8]. Its ability to colonize and infect various anatomical sites, including respiratory, urinary, and bloodstream infections, accentuates the urgency of monitoring and understanding the dynamics of ESBL resistance in this pathogen [9,10]. It is within this backdrop that this study aimed to isolate, identify, and characterize Klebsiella pneumoniae from clinical samples, conduct antimicrobial susceptibility testing using the Kirby Bauer disc diffusion method to inform treatment decisions, and detect the presence of Extended Spectrum Beta-lactamase (ESBL) production specifically in Klebsiellapneumoniae through the double disc potentiation technique, providing insights into Klebsiellapneumoniae -specific antibiotic resistance patterns and enabling targeted infection control and treatment strategies.

## Materials and Methods

### Study Design and Setting

This cross-sectional study was conducted under the department of Microbiology of Govt. Medical College, Amritsar. The study was carried out over a 2 years period from July 2001 to May 2003.The present study comprised of 250 isolates of Klebsiella from clinical specimens received in the Department of Microbiology.

### Sample Collection and Processing

Specimens were collected with all aseptic precautions before administering any antimicrobial therapy. The specimens were sputum, throat swabs, blood, urine, pus, vaginal and cervical swabs, body fluids as CSF, pleural, peritoneal, ascetic and synovial fluid. These were inoculated on the Mac Conkey agar, blood agar plates and incubated aerobically at 37°C for 18-24 hours.

### Microbiological Identification

After 24 hours of incubation, the plates were observed for bacterial growth. Colonies were examined with naked eye and magnifying lens, colony characters like size, shape, surface, edge, colour, opacity, consistency, emulsifiable and haemolysis on blood agar were noted. From these cultures, lactose fermenting colonies were selected. Morphology and staining characters of lactose fermenting colonies on MacConkey's agar was studied by Gram's staining method. Klebsiella species were identified as gram negative capsulated coccobacilli non motile, non-spring measuring 1-2µ x0.5 -0.8 µm . catalase and nitrate reduction test were positive, indole and MR test negative, VP and citrate test were positive. Urease test was positive and negative for PPA and gelatin liquefaction. On TSI medium, both acid and gas were produced without the production non sporing H2S. Indole was positive in K.pneumoniaesubsp. oxytoca. MR was positive in K.pneumoniaesubsp.aerogenes. Identified strains of K.pneumoniae species were tested for their antibiotic susceptibility and beta-lactamase production by NCCLS reference method, 2000.

### Antibiotic susceptibility Testing

The antibiotic sensitivity pattern of K. pneumoniae species, tested by Kirby Bauer's disc diffusion method. Plates of Mueller-Hinton agar was prepared and stored at 4 degrees Celsius. Before use, these plates were dried and inoculated within 15 minutes of the preparation of the test inoculums. Sterile, non-toxic cotton swabs were soaked in the inoculums and, with firm pressure, rotated several times on the inside wall of the tube to remove excess fluid.

Dried Mueller-Hinton agar plates were inoculated using the lawn culture technique. Antibiotic discs were applied over the plates, and these were incubated for 16-18 hours at 37°C. For precision and accuracy, a parallel set of control strains of K. pneumoniae (ATCC 25922) was set up. The zones of inhibition were measured using calipers or a transparent plastic ruler in millimeters across the disc. The diameter of the disc was included in this measurement. Results were interpreted according to the zone size in the Kirby–Bauer disc diffusion method.Three grades of sensitivity were recorded:Susceptible, Moderately susceptible, and Resistant.

### Extended Spectrum Beta-lactamase Detection

The clinical isolates were tested against third-generation cephalosporins, namely cefotaxime, ceftazidime, and cefoperazone, as well as a combination of third-generation cephalosporins with beta-lactam inhibitors (sulbactam + cefoperazone) using Magnex discs. The confirmation of ESBL production by a particular organism was based on a ≥ 3-5 mm increase in zone diameter when comparing the single disc to the combination disc. Interpretive criteria were defined for the 75µg/30µg (sulbactam/cefoperazone) susceptibility discs, following the US NCCLS disc method (National Committee for Clinical Laboratory Standards Performance Standards for Antimicrobial Disk

Susceptibility Tests, 2000). The categories were defined based on zone diameter measurements as follows: Susceptible (>20 mm), Intermediate (16-20 mm), Resistant (<16 mm).

**Data Analysis**

Data analysis was done using SPSS 20.0 version.

**Results**

Out of 250 isolates of K. pneumoniae, in terms of sex, the sample consisted of 147 (58.8%) males and 103 (41.2%) females, indicating a slight male predominance. Age distribution among the participants revealed a diverse representation of different age groups.

The age categories were as follows: 0-5 years, with 37 individuals (14.8%); 6-12 years, with 17 individuals (6.8%); 13-20 years, with 37 individuals (14.8%); 21-40 years, with 45 individuals (18%); 41-60 years, with 103 individuals (41.2%); and those over 60 years of age, with 12 individuals (4.8%).

This distribution emphasizes the heterogeneity of age groups within the study, with the highest proportion falling into the 41-60-year age category (Table 1).

**Table 1: Distribution of 250 isolates of K. pneumoniae in relation to age and sex of patients**

| Variables | Frequency | % |
|---|---|---|
| Sex | | |
| Male | 147 | 58.8 |
| Female | 103 | 41.2 |
| Age in Years | | |
| 0-5 | 37 | 14.8 |
| 6-12 | 17 | 6.8 |
| 13-20 | 37 | 14.8 |
| 21-40 | 45 | 18 |
| 41-60 | 103 | 41.2 |
| >60 | 12 | 4.8 |

The data show that the maximum number of Klebsiella species strains were isolated from pus (66%), followed by urine and sputum samples. Among the 250 strains of Klebsiella species, 185 (74%) were isolated from hospitalized patients, while the remaining 65 (26%) were from outpatient cases(Table 2).

**Table 2: Distribution of K. pneumoniae isolates among the clinical samples of the patients**

| Variable | Number | % |
|---|---|---|
| Sample | | |
| Pus | 165 | 66.0 |
| Urine | 62 | 24.8 |
| Sputum | 23 | 9.2 |
| OPD/IPD | | |
| Outpatients (OPD) | 65 | 26.0 |
| Inpatients (IPD) | 185 | 74.0 |

The antibiotic susceptibility of 250 strains of Klebsiella species was studied against the aforementioned seven antibiotics. The maximum resistance among third-generation cephalosporins was observed against ceftazidime (90%), followed by cephalexin (79.6%), cefoperazone (75.6%), and cefotaxime (55.6%). Piperacillin showed a resistance rate of 71.2%, while gentamicin exhibited a resistance rate of 68.8%. The sulbactam-cefoperazone combination demonstrated a resistance rate of 66%.Among the cephalosporins, cefotaxime was found to be the most effective drug, with a sensitivity rate of 36.4%, followed by cefoperazone at 23.6%. Gentamicin exhibited a sensitivity rate of 28%, while the sulbactam-cefoperazone combination showed a sensitivity rate of 32% (Table 3).

**Table 3: Antibiogram of 250 strains of K. pneumoniae**

| Drug | Disc content (µg/disc) | Susceptable | | Moderately susceptible | | Resistant | |
|---|---|---|---|---|---|---|---|
| | | Number | % | Number | % | Number | % |
| Piperacillin | 10 | 66 | 26.3 | 6 | 2.4 | 178 | 71.2 |
| Cephalexin | 30 | 49 | 19.6 | 13 | 5.2 | 189 | 79.6 |
| Cefotaxime | 30 | 91 | 36.4 | 10 | 4.0 | 139 | 55.6 |
| Ceftazidme | 30 | 23 | 9.2 | 2 | 0.8 | 225 | 90.0 |
| Cefoperazone | 30 | 59 | 23.6 | 2 | 0.8 | 189 | 75.6 |
| Sulbactam- cefoperazone | 75/30 | 80 | 32.0 | 5 | 2.0 | 165 | 66.0 |
| Gentamycin | 10 | 70 | 28.0 | 8 | 3.2 | 172 | 68.8 |

176 (70.4%) of the strains were positive for beta-lactamase production by NCCLS reference method (2000) and 74 (29.6%) showed negative results for beta-lactamase production (Figure1).

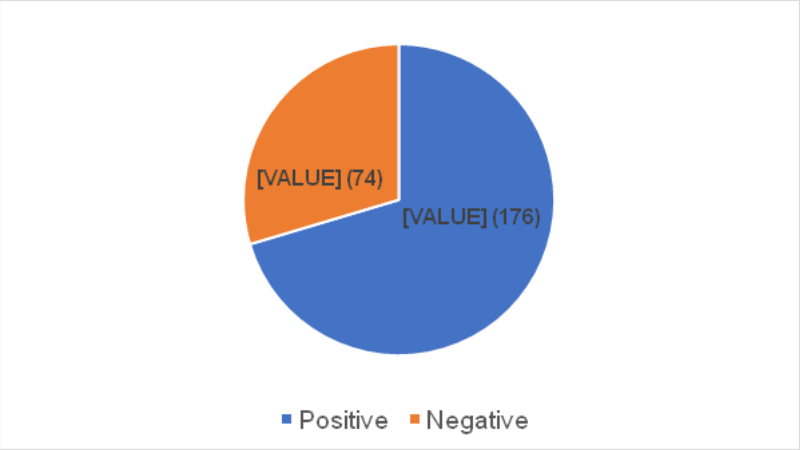


**Figure 1: Incidence of beta lactamase production in 250 isolates of K. Pneumonia**

Among the beta-lactamase positive strains, the highest sensitivity was observed towards cefoperazone (23.6%) and the sulbactam-cefoperazone combination. Gentamicin exhibited a sensitivity rate of 21%, while piperacillin had the lowest sensitivity at 4.4%.

Statistical analysis revealed a highly significant difference ($p < 0.001$) in antibiotic sensitivity rates between beta-lactamase producers and non-producers. However, this difference was not statistically significant ($p > 0.05$) in the case of gentamicin(Table 4).

**Table 4: K. pneumoniae isolates showing sensitivity pattern against beta-lactamase producers and nonproducers**

| Antibiotics | Beta- lactamase Positive Sensitivity (%) | Beta – lactamase Negative Sensitivity (%) |
|---|---|---|
| Piperacillin | 4.4 | 19.2 |
| Gentamycin | 21.0 | 29.6 |
| Cephalexin | 2.2 | 19.6 |
| Cefotaxime | 6.0 | 94.8 |
| Ceftazidime | 9.6 | 100.0 |
| Ceforperazone | 23.6 | 100.0 |
| Sulbactam-ceforperazone | 32.0 | 100.0 |

The highest number of beta-lactamase positive strains, i.e., 73 (29.2%), were observed in the 41-60 years age group. This was followed by 40, 30, 13, and 10 beta-lactamase positive strains among the 21-40, 0-5, 13-20, 6-12, and >60 years age groups, respectively. Among the beta-lactamase producing strains, 105 (71.42%) were found in males, while 71 (68.92%) were found in females. Statistical analysis indicated a nonsignificant difference ($P > 0.05$) in the occurrence of beta-lactamase producing strains between male and female patients.Out of the beta-lactamase producing strains, 148 (80%) were isolated from hospitalized patients, and 28 (43.07%) from outpatient cases. Statistical analysis revealed a highly significant difference ($p < 0.001$) in the occurrence of beta-lactamase producing strains between outpatients and inpatients (Table 5).

**Table 5: Age, Sex, and OPD/IPD based distribution of beta lactamase producing K. pneumoniae stains**

| Variables | Beta-lactamase positive strains | |
|---|---|---|
| | Number | % |
| Age group in years | | |
| 0-15 | 30 | 12.0 |
| 6-12 | 10 | 4.0 |
| 13-20 | 13 | 5.2 |
| 21-40 | 40 | 16.0 |
| 41-60 | 73 | 29.2 |
| >60 | 10 | 4.0 |
| Sex | | |
| Male | 105 | 71.42 |

| | | |
|---|---|---|
| Female | 71 | 68.93 |
| Group | | |
| Outpatient | 28 | 43.07 |
| Inpatient | 148 | 80.0 |

**Discussion**

The findings from this study provide valuable insights into the epidemiology and antibiotic resistance patterns of Klebsiella pneumoniae (K. pneumoniae) in a clinical setting. We observed a slight male predominance in the study population, with 58.8% of the participants being male and 41.2% female. This gender distribution, although not significantly skewed, is worth noting as it may have implications for the susceptibility and prevalence of K. pneumoniae infections among different sexes.Similarly, the study conducted by Deodurg et al., noted a similar pattern, with 56.66% male and 44.16% female patients [6].The observation of a male preponderance over females in our study aligns with findings reported by Chakraborty et al., where they observed 57% male and 43% female patients [5]. In contrast, Akila et al., study reported a different distribution, with Klebsiella pneumoniae observed in 38.76% of male patients and 61.24% of female patients [11].

The age distribution of the participants showcased a diverse representation across various age groups, emphasizing the heterogeneity of the study cohort. Among the age categories, the 41-60 years group stood out as the most represented, comprising 41.2% of the total sample. This observation may reflect the susceptibility of middle-aged individuals to K. pneumoniae infections or their higher likelihood of requiring medical care. The presence of K. pneumoniae infections in children and the elderly was also noteworthy, as it represented 14.8% in both the 0-5 years and 13-20 years age groups and 4.8% in those over 60 years. This broad age distribution underscores the pathogen's ability to affect individuals across the age spectrum. The similar age distribution was observed in the study by Raut et al., [12].

The clinical samples from which K. pneumoniae isolates were obtained revealed that pus samples yielded the highest number of strains, accounting for 66% of the isolates. This observation is consistent with K. pneumoniae's propensity to cause various types of infections, including wound and soft tissue infections, where pus samples are commonly collected. Urine and sputum samples also contributed to the isolate pool, representing 24.8% and 9.2%, respectively. The data indicate that K. pneumoniae can manifest in different anatomical sites, reflecting its versatility as a pathogen. The study by Faari et al., reported the isolation rates of Klebsiella pneumoniae from different clinical specimens as follows Swabs (55.7%), followed by Blood (17.1%), Urine (14.28%), and Sputum (12.85%) [13]. But the observation of our study was in contrast to Akila et al, study, where the distribution of Klebsiella species among various clinical samples was reported as follows Urine (52.15%), Sputum (29.67%), Pus (17.22%), and Blood (0.96%) [11]. In contrast, Ananthan et al., observed an even higher percentage, with 92.5% of blood samples containing ESBL-KP isolates [14]. Gupta et al. reported a higher prevalence of 69.2%, while, Sarojamma et al., found that 57.14% of blood samples contained ESBL-KP isolates [15,16]. Furthermore, the study showed that 74% of the isolates were from hospitalized patients, while 26% were from outpatient cases, and such similar finding was observed in the study by Chandramohan et al., [17]. This distinction is important as it highlights the nosocomial potential of K. pneumoniae, emphasizing the need for infection control measures within healthcare facilities.

Antibiotic susceptibility testing revealed the concerning issue of resistance among K. pneumoniae strains. The highest resistance was observed among third-generation cephalosporins, with ceftazidime, cephalexin, cefoperazone, and cefotaxime exhibiting resistance rates of 90%, 79.6%, 75.6%, and 55.6%, respectively. This trend underscores the alarming prevalence of extended-spectrum beta-lactamase (ESBL) resistance in these isolates, limiting treatment options with commonly used antibiotics. Piperacillin and gentamicin also showed substantial resistance at 71.2% and 68.8%, respectively. In contrast, cefotaxime was identified as the most effective drug, with a sensitivity rate of 36.4%, followed by cefoperazone at 23.6%. This data indicates that alternative antibiotics may be more effective in treating K. pneumoniae infections, suggesting the need for careful antibiotic selection. In study by Rahim et al., Imipenem exhibited an approximate susceptibility rate of 84.61%, making it one of the more effective treatment options. Amikacin followed closely with an approximately 76.92% susceptibility rate, suggesting its efficacy in managing Klebsiella pneumoniae infections. Gentamycin demonstrated a susceptibility rate of around 53.84%. Piperacillin/Tazobactam also exhibited a susceptibility rate of approximately 76.92%. In contrast, the effectiveness of Ciprofloxacin was comparatively lower, with a susceptibility rate of approximately 30.10%. Both Cefepime and Cefotaxime showed similar susceptibility rates of approximately 23.07% [18]. The similar antibiotic susesptible pattern was observed in the study by Menon et al., and Babypadmini et al., [19,20].

A significant proportion of the isolates, 70.4%, were found to be beta-lactamase positive, further complicating the antibiotic resistance landscape. The

prevalence of Klebsiella pneumoniae producing extended-spectrum beta-lactamases (ESBL) as reported by Rahim et al., (53.84%) and Chakraborty et al., (53%) aligns with the findings of our study [5,18].In India, the prevalence of ESBL-KP (Extended-Spectrum Beta-Lactamase-Producing Klebsiella pneumoniae) can vary significantly, with reported percentages ranging from 16% to 73% [21-24].These beta-lactamase producing strains exhibited variable sensitivity to different antibiotics, with cefoperazone and the sulbactam-cefoperazone combination showing the highest sensitivity rates at 23.6% and 32%, respectively. In contrast, piperacillin demonstrated the lowest sensitivity at 4.4%. Notably, statistical analysis revealed a highly significant difference ($p < 0.001$) in antibiotic sensitivity rates between beta-lactamase producers and non-producers, underscoring the challenge posed by beta-lactamase-mediated resistance.Correspondingly, Somily et al., presented findings concerning the antimicrobial resistance characteristics of ESBL-KP, indicating a greater susceptibility to amikacin (92.5%) and nitrofurantoin (67.43%) [25]. Conversely, Maina et al., revealed a substantial susceptibility rate of 99.4% to carbapenems among ESBL-KP isolates, but concurrently reported increased resistance to gentamicin, ceftazidime, and nitrofurantoin [26]. Goyal et al., have documented elevated resistance levels among ESBL (Extended-Spectrum Beta-Lactamase)-producing strains, with a higher prevalence of resistance to ciprofloxacin (93.8%), trimethoprim-sulfamethoxazole (79.1%), and gentamicin (66.7%). In contrast, these strains displayed a lower resistance rate to amikacin (14.7%) [27].

## Conclusion

In conclusion, the results of this study shed light on the demographic distribution, clinical sources, antibiotic resistance patterns, and beta-lactamase production among K. pneumoniae isolates.

The findings underscore the need for prudent antibiotic use, rigorous infection control measures, and continued surveillance to combat the growing challenge of antibiotic resistance and beta-lactamase production in K. pneumoniae. Understanding the epidemiology of K. pneumoniae infections and the factors contributing to resistance is critical for the development of effective strategies to manage and prevent these infections in both outpatient and inpatient settings.

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