# Antibiogram of *Klebsiella pneumoniae* isolated from urine samples in a tertiary care hospital of Western Punjab

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**ABSTRACT**

**Aim:** *The present study was undertaken to determine the antibiotic susceptibility pattern of Klebsiella pneumoniae causing UTI in outdoor and indoor patients in a tertiary care hospital.*

***Material and Methods****: The samples were processed as per standard microbiological techniques. All isolates showing lactose fermenting, mucoid colonies on MacConkey agar and greyish, mucoid colonies on blood agar, gram negative coccobacilli on Gram staining were provisionally diagnosed as K. pneumoniae. The isolates were further subjected to various biochemical reactions for confirmation of K. pneumoniae. The antibiotic sensitivity testing was performed using the Kirby-Bauer disc diffusion method.*

***Results****: A total of 1854 urine samples were processed which yielded 92 K. pneumoniae isolates. Isolates were highly sensitive to colistin and moderately sensitive to imipenem, meropenem, cefepime, gentamicin and amikacin. High level of resistance was shown for piperacillin+tazobactum, cefixime, cotrimoxazole and ciprofloxacin. Very less sensitivity was recorded for norfloxacin and nitrofurantoin which are otherwise considered as key drugs for empirical treatment of UTI.*

***Conclusion****: This study highlights high level of resistance among K. pneumoniae isolates causing UTI. Therefore, the clinicians are left with very few options in the selection of optimal antimicrobials for empirical treatment of UTI.*

***Keywords****: Klebsiella pneumoniae, Urinary Tract Infections (UTI), Antibiotic susceptibility pattern.*

## Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections seen in clinical practice particularly in developing countries with a high rate of morbidity and financial costs.[1] The most common pathogenic organisms of UTI are *Escherichia coli, Staphylococcus saprophyticus, Staphylococcus aureus, Proteus* spp., *Klebsiella pneumoniae* , *Pseudomonas aeruginosa* and *Enterococcus* spp.[2] Some of the key factors predisposing to urinary tract infection have been attributed to poor personal hygiene and urinary tract abnormalities.[3-4]

*K. pneumoniae* is worldwide one of the most frequently isolated gram negative bacteria from clinical specimens of hospitalized patients.[5] It is the most relevant human pathogen within genus Klebsiella causing various infections in hospitals, long-term care facilities and communities worldwide, including lung, urinary tract, abdominal cavity, surgical sites and soft tissues infections and even bacteremia.[6] In the recent decades, appearance of plasmid mediated resistance to extended spectrum beta lactam drugs in hospital settings is a major cause for therapeutic failure leading to significant increase in morbidity and mortality.[7] Resistance to beta-lactams has been reported to be associated with ESBL production which hydrolyze oxyimino beta-lactams like cefotaxime, ceftriaxone, ceftazidime and monobactams.[8]

*K. pneumoniae* has developed resistance to most of the currently used antibiotics and even resistance to higher antibiotics like carbapenems is on the rise. Within the Enterobacteriaceae family, carbapenem resistant *Klebsiella pneumoniae* strains have recently been noted in many parts of the world. [9-10] In developing countries like India, UTI's are often managed empirically with wide range of antibacterial agents usually by family physicians even before the availability of laboratory report.[11] The antimicrobial resistance of urinary *K. pneumoniae* to commonly prescribed drugs vary significantly in different regions. Thus, it is important to know the local etiology and susceptibility patterns of uropathogens to optimize the rational empirical therapy This study was therefore undertaken to update our knowledge on recent trends in resistance patterns among *K. pneumoniae* isolates obtained from urine samples. This would guide the clinicians in a rational selection of antimicrobial agents and thus help in better patient management.

**Material and Methods**

The cross- sectional study was conducted at Adesh Institute of Medical Sciences and Research (AIMSR), Bathinda, Punjab for a period of six months from November 2019 to April 2020. The study protocol was approved by the Institutional Research Committee and Ethics Committee.

The urine samples which have been collected from both hospitalized inpatients and outdoor patients were received in Bacteriology Laboratory from various clinical departments. The samples were then processed as per standard microbiological techniques for bacteriological culture. Urine samples were inoculated using calibrated loop with holding capacity of 0.005 ml urine onto Blood agar and MacConkey agar medium (Hi-media, Mumbai, India). Inoculated culture plates were kept in the incubator at 37°C for 24 hours.[12--13]

The urine cultures of colony count $\geq 10^5$ CFU/ ml were considered as positive for UTI and cultures showing growth of more than two types of bacteria were considered contaminated as per Kass criteria. All isolates which formed lactose fermenting, rose pink, mucoid colonies on MacConkey agar and greyish, non-haemolytic, mucoid colonies on blood agar, and gram negative coccobacilli on Gram staining were provisionally diagnosed as *K. pneumoniae*. The isolates were further subjected to battery of various biochemical reactions (Sugar fermentation test, Indole test, MR test, VP test, Citrate utilisation test, Urease test, Nitrate reductase, catalase, oxidase test and TSI test) for confirmation of *K. pneumoniae*.[14-15]

The antibiotic sensitivity testing was performed using the Kirby-Bauer disc diffusion method. Two to three well isolated colonies were suspended in 5 ml of peptone water in a test tube and incubated for 2- 3 hrs at 37º C. The turbidity was matched to 0.5 McFarland standard. Using a sterile cotton swab, the broth culture was swabbed on the sterility checked Mueller-Hinton agar plate and the antibiotic discs were placed and plates were incubated at 37 ºC for 24 hours.[16]

The isolates were tested for following antibiotics: Gentamicin (10 µg), Amikacin (10 µg), Cefexime (30 µg), Cefepime (30 µg), Ciprofloxacin (5 µg) Norfloxacin (10 µg), Nitrofurantoin (300 µg), Imipenem (10 µg) Meropenem (10 µg), Piperacillin-Tazobactam (100/10 µg), Cotrimoxazole (25 µg), and colistin (10 µg) (Hi-media, Mumbai).

The diameter of the zone of inhibition was measured and results were interpreted as per the Clinical Laboratory Standards Institute (CLSI) guidelines.[17]

**Statistical Analysis:**

Statistical analysis of the data obtained was done by Descriptive statistics and calculating the percentages and ratios. Pie Charts are bar graphs were used for pictorial representation of the data.

**Results**

A total of 1,854 urine samples were received from outpatients and indoor patients of various clinical departments (Surgery, Medicine, Obstetrics and Gynaecology, Paediatrics, ICU, CCU) over a period of six months. Out of these, 974 (52.53%) samples were reported as culture positive for urinary tract infection and 880 (47.47%) were culture negative. Out of these 974 culture positive samples, 92 isolates were of *K. pneumoniae*. The prevalence of *K. pneumoniae* among total culture positive samples was found to be 9.4%. Among gram negative bacterial isolates, *E. coli* was the most commonly isolated organism followed by *K. pneumoniae*, *P. aeruginosa*, *Proteus* spp., *Acinetobacter* spp., *Citrobacter* spp. and *Enterobacter* spp. The prevalence of *K. pneumoniae* urinary infection was observed maximum from Surgery department (51.08%) followed by 18.47% from ICU, 14.13% from Medicine, 8.70% from CCU, 5.45% from OBG and lowest 2.17% from Paediatrics as shown in Table 1. Urinary tract infection was observed more in males than in females as shown in Figure 1. 60.8% (56) isolates were obtained from IPD (including ICU) and 39.2% (36) isolates were from OPD patients. Equal rate of UTI was found in age group of 41-60 years (29.34%) and 61-80 years (29.34%) followed by 21-40 years (26.08%), 0-20 years (8.70%), 81-100 years (6.54%), suggesting that urinary tract infection was more common in middle and elderly age group. (Figure 2)

**Table 1: *K. pneumoniae* isolates obtained from various departments**

| Department | No. of isolates | Percentage (%) |
|---|---|---|
| Surgery | 47 | 51.08% |
| ICU | 17 | 18.47% |
| Medicine | 13 | 14.13% |
| CCU | 08 | 8.70% |
| OBG | 05 | 5.45% |
| Paediatrics | 02 | 2.17% |
| **Total** | **92** | **100%** |

**Table 2: Antibiogram of *K. pneumoniae* isolates obtained in the study**

| Sr. No. | Antibiotics tested | No. of Sensitive isolates (% sensitivity) | No. of resistant isolates (% Resistance) |
|---|---|---|---|
| 1. | Gentamicin | 51 (55.5%) | 41 (44.5%) |
| 2. | Amikacin | 46 (50%) | 46 (50%) |
| 3. | Cefixime | 31 (33.6%) | 61 (66.4%) |
| 4. | Cefepime | 48 (52.2%) | 44(47.8%) |
| 5. | Ciprofloxacin | 28 (30.4%) | 64 (69.6%) |
| 6. | Norfloxacin | 38 (41.4%) | 54(58.6%) |
| 7. | Nitrofurantoin | 40 (43.4%) | 52 (56.6%) |
| 8. | Imipenem | 51 (55.5%) | 41 (44.5%) |
| 9. | Meropenem | 52 (56.6%) | 40 (43.4%) |
| 10. | Piperacillin+ Tazobactum | 27 (29.3%) | 65 (70.7%) |
| 11. | Cotrimoxazole | 32 (34.7%) | 60 (65.3%) |
| 12. | Colistin | 86 (93.4%) | 6 (6.6%) |

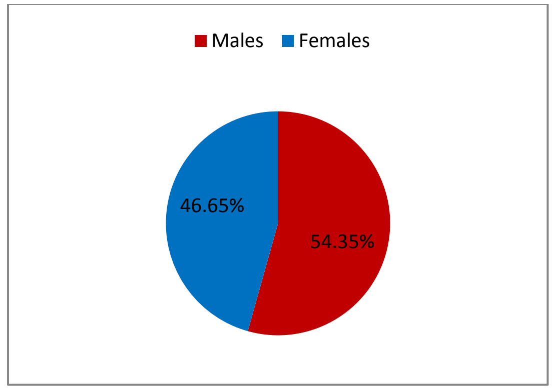


**Figure 1: Gender-wise distribution of patients with *K. pneumoniae* UTI**

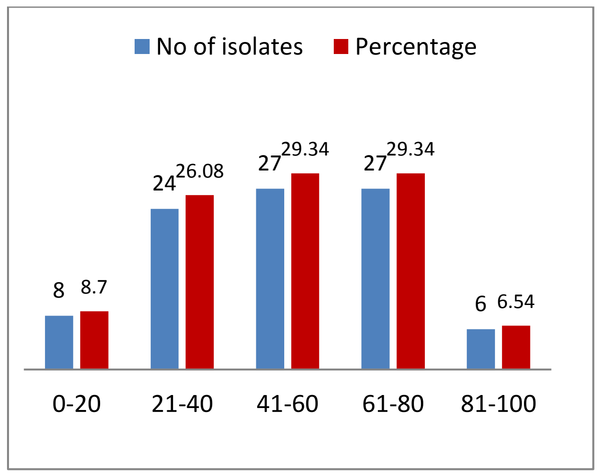


**Figure 2: Age- wise distribution of *K. pneumoniae* UTI**

*K. pneumoniae* isolates were highly sensitive to colistin (93.4%). They were equally sensitive to Imipenem (55.5%), Meropenem (56.6%), Cefepime (52.2%), Gentamicin (55.5%) and Amikacin (50%). High level of resistance was shown by *K. pneumoniae* for Piperacillin+Tazobactum (70.7%), Cefixime (66.4%), Cotrimoxazole (65.3%) and Ciprofloxacin (69.6%). Moreover, lesser sensitivity was recorded for Norfloxacin (41.4%) and Nitrofurantoin (43.4%) which are considered as key drugs for empirical treatment of UTI as shown in Table 2.

## Discussion

A total of 1854 urine samples were processed out of which, 974 (52.5%) yielded growth and 880 (47.5%) showed no growth on Blood agar and MacConkey agar. Mishra *et al* [18] and Khan *et al* [19] had also reported similar culture positivity of urine samples – 49.4% and

43.6% respectively. However, lesser culture positivity has been reported by Varghese *et al* [20] (31.8%); Dillirani and Suresh [21] (25.4%) and Sharanya *et al* [22] (12.09%). In this study, a total of 92 *K. pneumoniae* isolates were obtained from 1854 urine samples showing prevalence of 4.9%. In a study by Manikandan and Amsath[23] prevalence of *K. pneumoniae* has been reported as 3% among total urine samples which is similar to this study.

The prevalence of *K. pneumoniae* recorded among total urinary isolates was 9.4% (92/974). Manikandan and Amsath; Dillirani and Suresh had reported 11.07% and 13.8% prevalence among total isolates which similar is in line with this study. However, other studies have reported higher prevalence of *K. pneumoniae*: Bency *et al* [24] (15.8%);Verghese *et al* [20] (16.6%); Gill *et al* [25] (21.6%); Mishra *et al* [18]( 18.9%). This suggests that prevalence of particular organism varies from one place to another. In the present study, more isolates were obtained from males (54.35%) as compared to females (45.65%) which is contrary to study by Verghese *et al* [20] and Dillirani &Suresh [21] who have reported more rate of *K. pneumoniae* UTI in females than males. Sharanya *et al* [22] reported that 58.01% isolates were obtained from males and 41.03% from females. Gill *et al* [25 ]also reported similar results with more number of isolates from males (58%) than females (42%). In this study, *K. pneumoniae* UTI was seen maximum among the age group of 41 to 80 years. (58.6%).The findings are in concordance with studies of Bency *et al* [24] and Verghese *et al* [20] who have reported 57% and 60% *K. pneumoniae* isolates respectively from age group of 41-80 years.

Maximum sensitivity of *K. pneumonoiae* was recorded for colistin followed by imipenem, gentamicin and amikacin. In other studies, higher sensitivity has been reported for amikacin and imipenem as compared to this study. [19,21,23,24] whereas in another study, lesser sensitivity has been reported towards gentamicin (40%) and meropenem (36%) as compared to this study. [24]

Susceptibility towards cephalosporins was found lesser in this study which is similar to other studies. [19,20] Ciprofloxacin has been reported as lesser effective against *K. pneumoniae* which finds similarity with other studies.[21,24] In this study, *K. pneumoniae* isolates were found to have moderate susceptibility for nitrofurantoin (43.4%) and norfloxacin (41.4%). The results are in concordance with findings of Manikandan and Amsath but disconcordant with most of other studies [19,21,24] who have reported susceptibility towards nitrofurantoin as 81.6%, 64% and 76% respectively. Another study by Manjula *et al* [8] reported very high nitrofurantoin resistance (80%) in *K. pneumoniae* isolates. Susceptibility towards norfloxacin has been reported as 51.4% (Verghese *et al*); 100% (Bency *et al*); 5 0% (Manikandan and Amsath).

The study indicates that *K. pneumoniae* isolates have in general high rates of resistance to the commonly used antimicrobial agents. Resistance to various generations of cephalosporins and beta lactams is very high on account of the production of extended spectrum beta lactamases (ESBLs). Over the past two decades,there has been a wide use of extended broad-spectrum antimicrobial agents like carbapenems to meet the emerging challenge of treating UTIs due to gram-negative bacilli. However, these microbes have developed multiple antimicrobial resistance mechanisms including enhanced drug efflux, alterations of the drug target and the production of chromosomal and plasmid-mediated carbapenemases leading to treatment failure. In addition, the resistance to flouroquinolones and aminogycosides is due to mutations in the chromosomal genes encoding DNA gyrase of the bacteria and due to efflux of the drug.[26]

## Conclusion

Th study concludes that, the clinicians are left with very few options in the selection of optimal antimicrobials for empirical treatment of UTI due to the emergence of higher antimicrobial resistance among uropathogens. This study emphasizes the importance of current knowledge of the uropathogens and their susceptibility pattern for the initiation of appropriate empirical therapy and to minimize the evolving resistant strains. The susceptibility and resistance patterns as observed in the defined geographical area should be considered before starting empirical treatment for UTI. As susceptibility pattern is changing with the change in use of type of antibiotics, a regular monitoring of antibiotic resistance pattern is very helpful in ensuring proper therapy for patients with urinary tract infections. The treating physician should encourage accurate bacteriological diagnosis of each symptomatic patient as far as possible and refer to the records of local microbial isolation and their antibiogram in cases of emergency or in areas where the culture facility is not available.

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