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Original Article

Prevalence of Carbapenemase Producing Klebsiella Pneumoniae Causing Urinary Tract Infection in Patients Visiting Kathmandu Medical College

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Abstract

Background

Urinary tract infections are among the most common bacterial infections worldwide, with *Klebsiella pneumoniae* being the leading cause. This study was done to ascertain the prevalence of Multi drug resistance, Extended Spectrum Beta Lactamase and carbapenemase production among urinary *Klebsiella pneumoniae* isolates.

Materials and Methods

A descriptive cross-sectional study was done in a Kathmandu Medical College, Kathmandu, Nepal from March-September, 2024 after obtaining ethical clearance. Total 89 urinary *Klebsiella pneumoniae*isolates were studied. Urine culture, isolation and identification of the isolates was done using standard microbiology techniques. Antibiotic susceptibility testing was performed by Kirby–Bauer disc diffusion method. Extended spectrum Beta lactamase producers were screened using ceftazidime and confirmed by ceftazidime (30µg) and ceftazidime plus clavulanic acid (30/10µg) disc. Carbapenemase producers were detected using modified carbapenem inactivation method. The data obtained were computed and analyzed using Statistical Package for Social Sciences 20.0 Version.

Results

Total 89(11.93%) *Klebsiella pneumoniae* were isolated from 743 urine sample. Among the isolates, 50 (56.17%) were multidrug resistant. Extended spectrum Beta lactamase detection by phenotypic confirmatory disc diffusion test identified 34 (38.20%) extended spectrum Beta lactamase producers. 21 (23.59%) were Meropenem resistant by Disc diffusion method while Carbapenemase production was confirmed in 6 (6.74%) isolates by Modified Carbapenem Inactivation method.

Conclusion

The present study concluded that multidrug resistance and Extended spectrum Beta lactamase production among uropathogenic*Klebsiella pneumoniae* is prevalent in our setting. The isolates producing carbapenemase were fewer but raises significant concern.

Keywords: *Carbapenem-Resistant Enterobacteriaceae, Drug Resistance, Klebsiella pneumoniae, Urinary Tract Infection*

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Introduction

Klebsiellapneumonaie (K. pneumoniae) is a rod-shaped gram negative, lactose fermenting capsulated bacteria, present in water and soil naturally and also considered a normal flora of mouth, skin, and intestines [1, 2]. It is associated with a variety of infections, such as urinary tract infection (UTI), pneumonia, intra-abdominal infection, bloodstream infection, meningitis and pyogenic liver abscess [3]. *K. pneumoniae* is also an "ESKAPE" pathogen, which is an abbreviation designated to bacteria for which resistance to current antibiotics poses a hazard. [4]

Reports of multidrug resistant (MDR) *K. pneumoniae* isolates are rising along with rapid emergence of *K. pneumoniae*carbapenemases (KPC) [5, 6]. KPCs are capable of hydrolyzing a broad spectrum beta lactams including penicillin, cephalosporin, carbapenem and monobactam [7]. Timely surveillance and instituting control measures for these Extended Spectrum Beta Lactamase (ESBL) and Carbepenemase producers is crucial as some of these enzymes are carried by plasmids and can be rapidly disseminated to other gram negative pathogens. [8]

This study was done to ascertain the prevalence of Multi drug resistance, Extended Spectrum Beta Lactamase and carbapenemase production among uropathogenic*Klebsiella pneumoniae* isolates in patients attending a tertiary care hospital.

Materials and Methods

A prospective, descriptive cross-sectional study was done in department of Microbiology at Kathmandu Medical College, Kathmandu, Nepal from March-September, 2024. Ethical clearance was obtained from the Institutional Review Committee of Kathmandu Medical College and Teaching Hospital (KMC-IRC No- 28022024/05).

Patients from all age group, sex, religion, ethnicity and locality indicated for urine culture and willing to participate in the study were included after obtaining informed consent. Patients from whom unlabeled or inadequate sample was collected or placed in a damaged/ broken container were excluded from the study.

Convenient sampling method was used and the sample size was calculated with a prevalence of 5% [9] as follows:

$n = z^2 \times p \times q / e^2$

where, z= 1.96; Prevalence of *K. pneumoniae* (p) = 5%[11];q = 100 – p = 95

e = allowable error= 5%; $n = (1.96)^2 \times 0.05 \times 0.95 / 0.0025 = 72.832$

n=73

Demographic data (age, gender etc.) was collected in a preset proforma from the patients. 743 Midstream clean catch urine sample were collected. Urine sample were inoculated in Cysteine Lactose Electrolyte Deficient (CLED) media using semi quantitative method and incubated at 37°C for 24 hours. Urine cultures with colony counts > 10^5 cfu/ml were considered significant. Identification was done by colony morphology, Gram staining and biochemical test. A total of 89 *K. pneumonaie*isolates were further studied. Antibiotic susceptibility test (AST) was performed for each isolates following the clinical and laboratory standard (CLSI) guidelines on Mueller Hinton Agar (MHA) by Kirby Bauer Disc diffusion method [10,11]. The antibiotics were used according to CLSI guidelines and for Quality control, *K. pneumoniae* (ATCC 13883) was used [10, 11].The isolates which showed resistance to any one or all of the Carbapenems , Cephalosporins or Cefoxitin were taken up for further phenotypic tests. Phenotypic screening test for ESBL production was done using Ceftazidime (30µg) and Cefotaxime (30µg) and Phenotypic confirmatory disc diffusion test (PCDDT) was done using Ceftazidime-clavulanic acid (30/10 µg) (CAC) [11]. For Carbapenemase detection, Modified Carbapenem Inactivation method (mCIM) was used as phenotypic confirmatory test as per CLSI guidelines [11].

The data obtained were computed and analyzed using Statistical Package for Social Sciences (SPSS) for Windows Version 20.0.

Results

Out of 743 mid-stream clean catch urine sample processed for culture and sensitivity, 89 (11.93%) *Klebsiella pneumoniae* were isolated. On screening test using Ceftazidime, 49 (55.05%) isolates were found to be ESBL producers. ESBL detection by PCDDT identified 34 (38.20%) ESBL producers. Out of 21 suspected carbapenemase producers, 6 (6.74%) isolates were confirmed using mCIM method.

Distribution of Samples among Patients

Isolation rate of *K. pneumoniae* was higher in females (61.8%) than that of males (38.2%). Among the departments, highest number were isolated from Medicine ward (37.07%). Age group 31-45 years had highest no. of *K. pneum-*

oniae isolates (30.3%). [Table 1]

Table 1: Comparison of demographic and clinical factors between ESBL and non-ESBL among UTI episodes

	Total (n=89)	ESBL n=34 (38.20%)	Non- ESBL n=55 (61.79%)
Age group			
<15 years	2 (2.2%)	0 (0%)	2 (3.63%)
16-30 years	20 (22.5%)	7 (20.58%)	13 (23.63%)
31-45 years	27 (30.3%)	12 (35.29%)	15 (27.27%)
46-60	14 (15.7%)	2 (5.9%)	12 (21.8%)
>60 years	26(29.2%)	13 (38.23%)	13 (23.63%)
Gender			
Male	34 (38.2%)	8 (23.5%)	26 (47.3%)
Female	55 (61.8%)	26 (76.5%)	29 (52.7%)
Departments			
Medicine ward	33 (37.07%)	9 (26.4%)	24 (43.63%)
ICU	17 (19.10%)	11 (64.71%)	6 (35.29%)
Gynecology ward	7 (7.86%)	4 (57.14%)	3 (42.86%)
Medicine OPD	18 (20.22%)	6 (17.64%)	12 (21.8%)
Others	14 (15.73%)	4 (28.57%)	10 (71.43%)

Distribution of ESBL positive *K. pneumoniae* isolates

The age group mostly infected with ESBL producing *K. pneumoniae*were >60 years (38.23%) followed by 31-45 years (35.29%). Isolation rate of ESBL producing *K. pneumoniae* was higher in females (76.5%) than that of males (23.5%). The distribution of ESBL and non-ESBL cases differed across the departments, with highest rate observed in ICU patients (64.71%). [Table 1]

Antibiotic susceptibility patterns in *Klebsiella pneumoniae* isolates

The highest sensitivity was exhibited towards Tigecycline (100%), followed by Meropenem (76.40%). The isolates were least sensitive towards Cefotaxime (44.94%). [Figure 1]
Among 89 isolates, 50 (56.17%) were MDR.

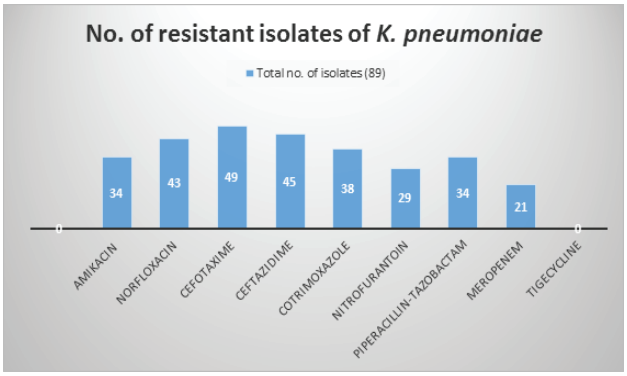

Figure 1: Antibiotic susceptibility patterns in *Klebsiella pneumoniae* isolated from urine samples

Phenotypic confirmation of carbapenemase production using mCIM method

21 (23.59%) were Meropenem resistant by Disc diffusion method. These isolates were further subjected to phenotypic tests for detection of Carbapenemases by Modified Carbapenem Inactivation method (mCIM) which confirmed 6 (6.74%) isolates to be MBL producers. [Figure 2]

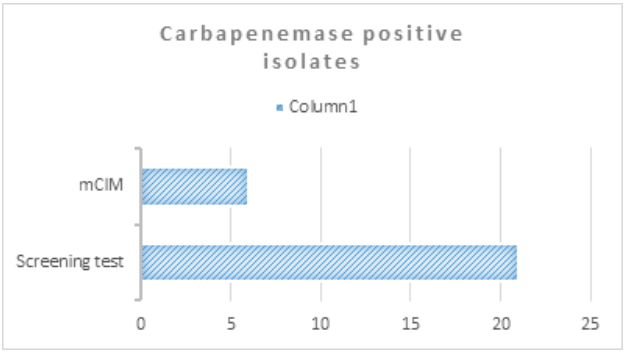

Figure 2: Carbapenemase producing isolates detected by Screening and mCIM method

Discussion

This study examined the ESKAPE pathogen *Klebsiella pneumoniae* isolates from suspected cases of UTI patients, with emphasis on their MDR pattern, ESBL and carbapenemase production since (CRKp) are considered a public health problem worldwide due to their rapid and efficient spread [12].Total 89 *Klebsiella pneumoniae* isolates (prevalence=11.93%) from 743 urine sample were studied. In our study, the highest percentage of UTI patients infected with *K. pneumoniae* belonged to 31-45 years age group 27 (30.33%) [Figure1], which is similar to study done in Nepal [13]. In contrast, another study from Nepal reported higher prevalence of UTI in older age groups [14]. In this study majority 55 (61.79%) patients were female, which correlates with findings of similar studies [15,16,17]. This could be attributed to biological factors, such as shorter urethra and proximity of urethra to anus.

ESBL detection by PCDDT identified 34 (38.20%) ESBL producers.Inotherstudies done in Nepal, ESBL producing *K. pneumoniae* were reported to be 8.3%, 18.4%, 25.8% [15, 16, 17]. Slightly higher prevalence of ESBL in our study may be due to the study site being a tertiary care center where complicated cases are referred. The prevalence of ESBL producing uropathogenic*K. pneumoniae* in present study has shown marked increase compared to similar study done in this hospital in 2012 (16.55%) [18]. This finding accords with the increasing global trend of ESBL

positive *K. pneumoniae* incidence and occurrence [19]. The majority of ESBL cases were in the >60 years age group (38.23%) and were predominantly female (76.47%).This can be attributed to higher rate of UTIs and its recurrence in old age and in females, leading to overuse of antibiotics. Similarly, highest ESBL producers were from ICU patients (64.71%), which can be attributed to ICUs having strong selection pressure for the organisms thus leading to high prevalence of MDR organisms.

In our study, the *K. pneumoniae* isolates exhibited lowest highest sensitivity towards Tigecycline (100%), followed by Meropenem (76.40%), and highest resistance was towards Cefotaxime (55.05%), followed by Ceftazidime (50.56%). This data is in range with similar studies done in Nepal [14,15,16,17]. Among the isolates, 56.1% were MDR. Similar data was reported by Pyakurel et al (56.8%) [20].

Carbepenem are often the last resort drugs for ESBL producing pathogens. However, the percentage of Carbepenem-resistant Enterobactericeae (CRE) has been on the rise. World Health Organization (WHO) has listed Carbepenem resistant *K. pneumoniae* in the critical priority list of pathogens; depicting urgency of need for new antibiotics against them [21]. In our study, 21/89 isolates were found to be Meropenem Resistant. This finding is of concern as the study done by Chander A and Shrestha CD in 2012 in our center showed 100% sensitivity of uropathogenic*K. pneumoniae* isolates towards Carbapenems [18].

Modified Carbapenem Inactivation method (mCIM) performed on the *K. pneumoniae* isolates confirmed 6 (6.74%) to be carbapenemase producers. This number is much lower compared to that given by other studies in Nepal 51.1%, 51.72% [20, 22].The difference between screening and mCIM may be attributed to non carbapenemase mechanism of resistance. Moreover the low rate of CRKp in our study could be attributed to carbepenems not being used primarily for treatment of UTIs.

Conclusion

Our study has concluded that multi drug resistant uropathogenic*K. pneumoniae* resistant to commonly prescribed antibiotics are highly prevalent in our settings. Moreover, high prevalence of ESBL producers and rising incidence of Carbapenem resistant uropathogenic*K. pneumoniae* observed in our study is a matter of concern.

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Conflict of interest: None

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