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Surveillance of Antibiotic Resistant *Klebsiella pneumoniae* in Tertiary Care Hospital of Nepal

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ABSTRACT

Background

Klebsiella pneumoniae is a ubiquitous Gram-negative, non-motile, capsulated bacterium that resides in the mucosal surfaces of mammals and the environment such as soil, water, etc. Clinically, it is also associated with many infections and conditions that are aggravated by the acquisition of multidrug-resistant genes.

Methods

This descriptive study was conducted to find out the incidence of *Klebsiella pneumoniae* in different clinical samples and their antibiotic susceptibility pattern at B & B Hospital, Lalitpur from 12 June 2022 to 11 September 2022. The samples were cultured on MacConkey agar, and standard microbiological techniques were used to confirm the presence of *K. pneumoniae*. Antimicrobial susceptibility tests were performed using the Kirby-Bauer Disk Diffusion method.

Results

Of the 1967 different clinical samples, culture positivity was observed in 361 samples. Among these, total number of *Klebsiella pneumoniae* was 150 (41.55%) and from the urine samples exclusively, 63 (42%) *Klebsiella pneumoniae* were isolated. The most susceptible and resistant antibiotics were gentamicin and ciprofloxacin respectively. Among the isolates, 85 (56.66%) isolates were multi-drug resistant in which 69 (87.34%) isolates were resistant to carbapenem.

Conclusions

The diversified infections with a growing number of multi-drug resistant *Klebsiella pneumoniae* present a major public health concern. The limited availability of effective antibiotics emphasizes the prudent use of available antibiotics and the importance of regular national-wide surveillance of multi-drug resistance patterns.

Keywords: ciprofloxacin; gentamicin; *Klebsiella pneumoniae*; multi-drug resistant.

INTRODUCTION

Klebsiella pneumoniae is gram-negative capsulated bacterium which is widely distributed across various environment; from mammalian mucosal surface to soil and water. It is the most common opportunistic pathogen causing various severe life-threating infections.[1] Hence, prompt administration of effective antibiotics is crucial to arrest the spread of this bacterial infection. However, the emergence of multi-drug resistant (MDR) *K. pneumoniae* poses a formidable challenge in healthcare environment by considerably reducing the range of effective therapeutic options.[2] A comprehensive meta-analysis study conducted in Nepal revealed that the prevalence of *K. pneumoniae* infection was 16 %, accompanied by an alarming surge in MDR cases, reaching 64 %.[3] Therefore, regular monitoring of antibiotic use and resistance patterns is imperative to curb the spread of MDR *K. pneumoniae*. Hence, this study aimed to find out the different infections caused by *K. pneumoniae* and their antibiotic susceptibility patterns.

METHODS

A hospital-based trimester cross-sectional study was conducted at the Pathology Department, B &

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B Hospital, Lalitpur from June 12, 2022. The study group included patients enrolled in hospital seeking microbiological investigation. Ethical approval for this study was obtained from the Nobel Institutional Review Committee (reference number: 079/080/88). Clinical specimens (urine, wound swab, sputum, body fluids, pus, tissue, and tips) were collected in sterile, leak-proof, tight screw cap plastic vials following standard microbiological guidelines.[4] For isolation, each sample was cultured on MacConkey Agar at 37°C for 24 hours. Identification of the isolated bacteria was carried out through Gram staining, followed by battery of standard biochemical tests.[5] Antibiotic susceptibility testing was performed by following the Clinical and Laboratory Standard Institute (CLSI) guidelines using the disc diffusion method.[6] The commercially available antibiotic disks amikacin (30 µg), cefepime (30 µg), cefoperazone sulbactum (75 µg/30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cotrimoxazole (1.25 µg/23.75 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), ofloxacin (5 µg), piperacillin-tazobactam (100/10 µg), and tigecycline (15 µg) were used for antibiotic susceptibility test. The bacteria showing resistance to at least one antibiotic from three or more different classes were categorized as MDR.[7] The collected data were entered and analyzed using SPSS V23.

RESULTS

This study covers 1967 clinical specimens (urine, wound swab, sputum, body fluids, pus, tissue, and tips) from patients admitted and visiting the OPD of the hospital. Urine and tips were the most and least common clinical specimens, respectively (Figure 1).

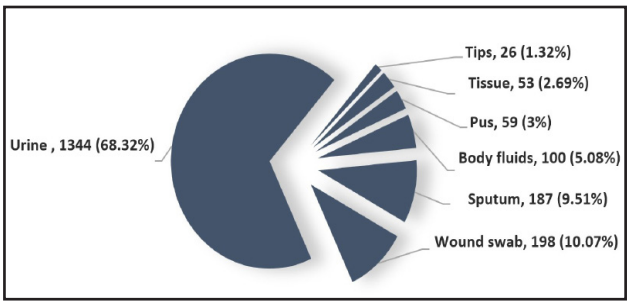

Figure 1. Distribution of total samples.

From 1967 clinical specimens, 361 clinical specimens showed microbial growth, of which 319 isolates were Gram-negative. The most frequently isolated organisms with their number were *Klebsiella pneumoniae* 150 (41.55 %) followed by *Escherichia coli* 101 (27.98 %), *Pseudomonas aeruginosa* 37 (10.25 %), *Klebsiella oxytoca* 27 (7.48 %), coagulase-negative staphylococci 26 (7.20 %), *Enterococcus* species 12 (3.32 %), *Staphylococcus aureus* 4 (1.11 %), *Acinetobacter* species 3 (0.83 %) and only one *Proteus vulgaris*. The distribution of *Klebsiella pneumoniae* isolates across different clinical samples showed that urine and tips had the highest and lowest occurrences respectively (Figure 2).

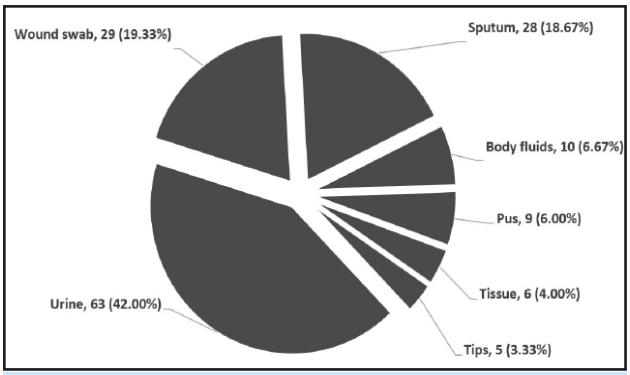

Figure 2. Distribution of *Klebsiella pneumoniae* in different samples.

From the age group 20-30 and below 10, highest and lowest numbers of *Klebsiella pneumoniae* were isolated respectively (Table 1).

Table 1. Age and sex wise distribution of *Klebsiella pneumoniae*.

Age group	Sex	
	Male (%)	Female (%)
0-10	1 (50)	1 (50)
10-20	8 (88.89)	1 (11.11)
20-30	31 (79.49)	8 (20.51)
30-40	15 (62.50)	9 (37.50)
40-50	7 (43.75)	9 (56.25)
50-60	10 (50)	10 (50)
60-70	9 (64.28)	5 (35.72)
70-80	15 (78.94)	4 (21.06)
80-90	2 (28.57)	5 (71.43)

Antibiotics amikacin, ceftazidime, ciprofloxacin, ceftriaxone, cotrimoxazole, and gentamicin were tested on all *Klebsiella pneumoniae* isolates from different clinical samples, and other antibiotics were tested on

the basis of different clinical samples and antimicrobial susceptibility test results based on above-mention antibiotics. Considering those only six antibiotics, the most susceptible and resistant antibiotic are gentamicin and ciprofloxacin, respectively. Overall, tigecycline was found to be the most effective antibiotic (Table 2). 85 (56.67%) isolates were confirmed as MDR, with carbapenem group of antibiotics being the most ineffective (Table 3).

Table 2. Antibiotic susceptibility pattern of *Klebsiella pneumoniae.*

Antibiotics used	Number of Isolates		
	Susceptible	Intermediate	Resistance
Amikacin	65 (43.33%)	18 (12.0%)	67 (44.67%)
Ceftazidime	65 (43.33%)	5 (3.33%)	80 (53.33%)
Ceftriaxone	65 (43.33%)	5 (3.33%)	80 (53.33%)
Ciprofloxacin	50 (33.33%)	9 (6%)	91 (60.67%)
Cotrimoxazole	67 (44.67%)	2 (1.33%)	81 (54.0%)
Gentamicin	71 (47.33%)	5 (3.33%)	74 (49.33%)
Norfloxacin	43 (68.25%)	-	20 (31.74%)
Cefepime	32 (31.06 %)	10 (9.7%)	61 (59.22%)
Ofloxacin	72 (50.70%)	8 (5.63%)	62 (43.66%)
Nitrofurantoin	24 (38.09%)	4 (6.35%)	35 (55.55%)
Chloramphenicol	45 (51.72%)	4 (4.60%)	38 (43.68%)
Cefoperazone-sulbactum	48 (46.6 %)	13 (12.62 %)	42 (40.77%)
Imipenem	10 (10.75%)	-	83 (89.25%)
Meropenem	10 (10.75%)	-	83 (89.24%)
Piperacillin-tazobactam	42 (40.77%)	12 (11.65%)	49 (47.57%)
Tigecycline	75 (72.81%)	-	28 (27.18%)

DISCUSSION

Klebsiella pneumoniae is one of the clinically significant opportunistic pathogen which typically infects patients with indwelling medical devices, especially urinary catheters and potential pathogen to cause nosocomial outbreaks. On average, one-third of Gram-negative infections belong to this bacterium.[1] With diversified infections, increased MDR rate, easy acquisition of drug resistance genes from other microbes and reduced effective antibiotics to curb the pace of MDR infections, this organism acquires much more public health concern. From 1967 total samples in our study, 361 (18.35%) samples showed significant growth, in which the number of Gram-negative isolates was 319. Among the

Table 3. Antibiotic susceptibility pattern of MDR *Klebsiella pneumoniae.*

Antibiotics used	Number of Isolates		
	Susceptible	Intermediate	Resistance
Amikacin	10 (11.76%)	10 (11.76%)	65 (76.47%)
Ceftazidime	14 (16.47%)	3 (3.53%)	68 (80%)
Ceftriaxone	14 (16.47%)	3 (3.53%)	68 (80%)
Ciprofloxacin	13 (15.30%)	2 (2.35%)	70 (82.35%)
Cotrimoxazole	17 (20%)	-	68 (80%)
Gentamicin	10 (11.76%)	10 (11.76%)	65 (76.47%)
Norfloxacin	16 (45.72%)	-	19 (54.28%)
Cefepime	19 (22.35%)	8(9.42%)	58 (68.23%)
Ofloxacin	27 (31.76%)	7 (8.24%)	51(60%)
Nitrofurantion	12 (34.28%)	1 (2.86%)	22 (62.85%)
Chloramphenicol	30 (44.12%)	4 (5.88%)	34 (40%)
Cefeperazone-sulbactam	32 (38.09)	10 (11.91%)	42 (50%)
Imipenem	10 (12.65%)	-	69 (87.35%)
Meropenem	10 (12.65%)	-	69 (87.35%)
Piperacillin-tazobactam	29 (34.94%)	8 (9.64%)	46 (55.42%)
Tigecycline	48 (85.71%)	-	8 (14.29%)

Gram-negative isolates, the most predominant was *Klebsiella pneumoniae* (150, 41.5%) followed by *Escherichia coli* (101, 28%), which is in congruence with a similar study conducted in Nepal.[8] However, a meta-analysis for the estimation of the prevalence of *Klebsiella pneumoniae* in four years from 2018 showed only 16%.[3] This difference may be attributed to different study designs, study population, study time, and placement of the study inside Kathmandu Valley etc. The number of isolates from males were roughly twice that of females (98 from males out of 1102 verses 52 from females out of 865). This finding correlates with the study conducted in others.[9, 10] Most of the bacteria were isolated from urine sample (42%) followed by wound swab (19.33%) and sputum (18.67%) etc which is similar to the study conducted in Sahid Gangalal National Heart Center, Nepal.[8] In our study, we evaluated six different antibiotics in all isolates, and other antibiotics were tested as second-line antibiotics or microbes isolated from specific clinical samples, such as the antibiotic nitrofurantoin on urinary isolates only. Considering those only six antibiotics, gentamicin was the drug of choice, with susceptibility rate of 44.37 %, while the least effective drug of choice was ciprofloxacin, with resistance

rate of 82.35%. The least effectivity of ciprofloxacin was similar to two different studies conducted in Bangladesh[11,12], and the increased resistance rate for ciprofloxacin is supported by 15 years' trend line study in China.[13] Overall, the most effective antibiotic is tigecycline with susceptibility rate of 72.81 %. We found that 85 (56.67%) isolates were MDR. A similar finding was estimated from Nepal and Indonesia which showed that prevalence of MDR *K. pneumoniae* was 64% and 54.5% respectively.[3, 9] In MDR *Klebsiella pneumoniae*, carbapenem groups of antibiotics, meropenem and imipenem were equally ineffective with resistance profile of 87.34% followed by ciprofloxacin 70 (82.35%), ceftazidime 68 (80%), ceftriaxone 68 (80%), and cotrimoxazole 68 (80%). Similar resistance pattern of ciprofloxacin (80%), and ceftazidime (87.8%) were also reported in study conducted in Bangladesh.[11]

CONCLUSIONS

The rising number of MDR *Klebsiella pneumoniae* has become a significant public health concern, causing treatment failure. Carbapenem group of drugs are considering last line drug for MDR bacteria but most of MDR isolates are resistant to carbapenem. This limits the therapeutic options for treating MDR *K. pneumoniae* and provides a shadowy picture of infection management. This shows the importance of establishing regular national wise surveillance of MDR strains to combat the various infections caused by MDR pathogens.

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