Higher Rate of Extreme Drug-resistant *Klebsiella pneumoniae* Infections among Cardiac Patients

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ABSTRACT

Background: The accelerating rate of carbapenems resistance in *Klebseilla pneumoniae* isolates has put the treatment option worrisome. The effective strategy to ameliorate this alarming situation is possible through enhancing the combination therapy and appropriate laboratory diagnosis. Hence, the study was focused on identifying carbapenemase-producing *K. pneumoniae* and their antibiogram pattern.

Methods: A total of 944 clinical samples from patients attending Sahid Gangalal National Heart Center were processed from September 2019 to March 2020 to identify the possible bacterial pathogens following the standard microbiological procedures. *K. pneumonaie* isolates were further subjected to antibiotic susceptibility testing by the modified Kirby Bauer disc diffusion technique. Phenotypic confirmation of carbapenemase production was done by the modified carbapenemase inactivation method. The minimum inhibitory concentration of colistin was determined by the broth microdilution method.

Results: Of the total 944 samples, 15.47% (146) samples showed bacterial growth, among which 23.97% (35) were *K. pneumoniae*. Out of 35 *K. pneumoniae* isolates, 45.71% (16) were multidrug-resistant followed by 42.86% (15) extensively drug-resistant. Fourteen isolates of *K. pneumoniae* were carbapenemase producers among which 20% (7) were serine carbapenemase while 20% (7) showed metallo-β-lactamase production. All the carbapenemase-producing *K. pneumoniae* were susceptible to colistin with <0.125µg/ml. Carbapenemase activity showed statistically significant with multidrug resistance (p=<0.05).

Conclusions: An increasing resistance to the carbapenem drugs showed a great problem in the management of *K. pneumoniae* infections among immunocompromised patients especially cardiac patients however, colistin can be still an ultimate choice of drug for disease management.

Keywords: Broth microdilution; carbapenemase; colistin; extreme drug-resistant; *klebsiella pneumonia*.

INTRODUCTION

Klebsiella pneumoniae is an opportunistic pathogen to the susceptible host.[1] Moreover, the emergence of infection caused by multidrug-resistant (MDR) *K. pneumoniae* especially carbapenemase-producing strains, is becoming a therapeutic challenge in the recent two decades.[2,3] In Nepal, most of the previous study related to the prevalence of carbapenemase-producing *K. pneumoniae* in clinical isolates were mainly based on phenotypic characterization[4] but not by molecular method.[5] Pyakurel et al. and Pradhan et al. reported the highest prevalence of carbapenemase-producing *K. pneumoniae* with the rate of 51.1% and 51.8% in Nepal respectively.[6,7] Besides, the excessive use of antibiotics in animals and humans, antibiotics sold over-the-counter, increased international travel, poor hygiene, and release of non-metabolized antibiotics or their residues into the environment through manure and feces are major predisposing factors of antimicrobial resistance.[8] The infections by multidrug-resistant strains are huge problems among immunocompromised patients leading to a high mortality rate. Moreover, only few studies have been focused on the burden of the increasing rate of extreme drug-resistant (XDR) pathogens among such population. Therefore, this study was conducted to determine the rate of carbapenemase-producing *K. pneumoniae* and its association with MDR and XDR from different clinical samples among immunocompromised patients with cardiovascular

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diseases attending a tertiary care hospital in Kathmandu using mCIM and EDTA-mCIM methods.

METHODS

The study was a hospital-based prospective cross-sectional study. The study was conducted in the Microbiology Department of Sahid Gangalal National Heart Center, (SGNHC), Bansbari and Med Micro Research Laboratory (MMRL), Babarmahal, Kathmandu from September 2019 to March 2020. Sample collection and sample processing were carried out at the Microbiology laboratory of SGNHC. Confirmation of carbapenemase production and minimum inhibitory concentration of colistin were performed in MMRL. The study was approved by Nepal Health Research Council (NHRC) (Regd. No: 834/2019). Written consent was obtained from all the patients enrolled in the study.

All age groups of both sexes giving consent for the study were included in this study whereas those samples which were not properly labeled, improperly transported with visible signs of contamination and lacked patients' complete information were excluded.

A total of 944 non-duplicate clinical specimens including blood, sputum, urine, wound swab, pus, sputum, pericardial fluid, ET secretion tip and cardiovascular catheter tip collected from the inpatients of different wards (ICU, MICU, PSICU, general, medicine, surgery, and emergency) and outdoor patients (OPD) attending the hospital were processed for isolation and identification of possible bacterial pathogens.

The specimens were cultured simultaneously on Mac Conkey agar (MA), Blood agar (BA) and Michrom UTI agar by quadrant streaking and incubating at 37°C for 24 hours using a standard protocol.[9] The bacterial growth was identified based on standard Microbiological procedures. Only *K. pneumoniae* isolates obtained from the clinical specimens were further considered.

Antibiotic susceptibility testing (AST) was performed by using modified Kirby Bauer's disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines.[10] In this study, those isolates which acquired non-susceptibility to ≥3 classes of antibiotics of antimicrobial class and those isolates which showed susceptibility to only one or 2 classes of antibiotics of the antimicrobial class have been regarded as MDR and XDR respectively.[11]

K. pneumoniae isolates that were resistant to imipenem (10µg) and meropenem (10µg) with a zone of inhibition less than 20 mm were selected as screening positive.[12] The confirmation of carbapenemase production was done by the modified carbapenemase inactivation method (mCIM) and EDTA-carbapenemase inactivation method (eCIM) following CLSI guideline. Serine carbapenemase was confirmed when mCIM positive but eCIM negative. Likewise, Metallo-beta lactamase (MBL) was confirmed when both mCIM and eCIM were positive. eCIM is only valid if mCIM is positive.[13]

One-µl of test organisms were suspended in different tubes containing 2ml tryptic soy broth (TSB) and vortexed for 10 to 15 seconds. A meropenem disk was placed in each tube, and the tubes were incubated for 4 h ± 15 min with a 0.5 McFarland suspension of a carbapenem-susceptible strain (*Escherichia coli* ATCC 25922). The plates were incubated for 18 to 24 h at 35°C. The mCIM is considered as negative if the diameter of the zone of inhibition (ZOI) is ≥19 mm, or positive if ZOI is 6 to 15 mm or pinpoint colonies are present within a 16 to 18mm. An mCIM result is considered indeterminate if ZOI is 16 to 18 mm or if the ZOI is ≥19 mm with pinpoint colonies present within.[13]

One-µl of text organism was suspended in a 2-ml tube of TSB with a final concentration of 5 mM EDTA and vortexed for 10 to 15 seconds. A 10-µg meropenem disk was placed into the bacterial suspension. The tube was then incubated for 4 h ± 15 min at 35°C ± 2°C. Subsequently, the disk was applied on MHA plates with a 0.5 McFarland suspension of a carbapenem-susceptible strain (*Escherichia coli* ATCC 25922). The plates were incubated for 18 to 24 h at 35°C ± 2°C. The test isolate was considered as positive for MBL production when the size of ZOI increased by ≥5 mm compared to the ZOI observed for the mCIM. In contrast to the mCIM, pinpoint colonies within ZOI were ignored while reading eCIM results. *Klebsiella pneumoniae* ATCC BAA-1706 (negative for carbapenemase production), *K. pneumoniae* ATCC BAA-1705 (serine enzyme KPC positive), and *K. pneumoniae* ATCC BAA-2146 (MBL NDM enzyme positive) were used during the study as quality control strains.[13]

MIC for colistin was determined by broth microdilution according to the EUCAST.[14]

Data obtained were analyzed using SPSS version 21. Scatter plot diagrams were designed for MIC breakpoints from WHONET 2019. The p-value of <0.05 was considered statistically significant.

RESULTS

A total of 944 non-duplicative clinical samples processed during the study period contained 328 (34.75%) urine, 230 (24.36%) blood, 138 (14.62%) sputum, 111 (11.76%) wound/pus, endotracheal secretion tip 53 (5.61%), pericardial fluid 46 (4.87%), pleural fluids 14 (1.48%), 6 (0.64%) central venous catheter tips (CVPs), 6 (0.64%) mitral valve, 4 (0.42%) aortic valve and 8 (0.85%) others samples. Bacterial growth was observed only in 146 (15.47%) samples, while 798 (84.53%) were culture negative.

Out of 146 growth positive samples, *K. pneumoniae* was the predominant isolate with 35 (23.97%) followed by *E. coli* 33 (22.60%), *Acinetobacter* spp 19 (13.01%), *Serratia* spp 7 (4.79%), *Pseudomonas aeruginosa* 6 (4.11%), *Citrobacter freundii* 2 (1.37%) and *Proteus mirabilis* 2 (1.37%). Among Gram-positive bacteria, the most predominant one is *Staphylococcus aureus* accounting for 28 (19.18%), followed by coagulase-negative *Staphylococci* (CONS) 10 (6.85%) and *Streptococcus pneumoniae* 2 (1.37%) (Table 1).

Out of the total 35 isolates, a slightly higher rate of *K. pneumoniae* infection was observed in male patients; 19 (54.29%) as compared to female patients; 16 (45.71%) (p=0.162). A significantly higher rate of *K. pneumoniae* was isolated from the age group below 10 years among male patients (p=0.02). The age groups of 41-50, 51-60, 61-70 and >71-year patients showed an almost equal rate of isolation in males. The lowest number of bacteria was isolated from 21-30 years in both males and females (p>0.12) (Figure 1).

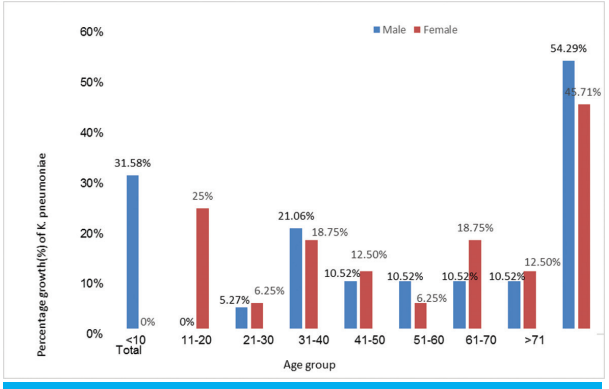

Figure 1. Distribution of *K. pneumoniae* according to patients' age and sex.

Out of 35 *K. pneumoniae* isolates, the highest number 18 (51.43%) were isolated from urine samples followed by sputum 7 (20%), pus/wound 4 (11.43%), CVP tip 3 (8.57%), blood 2 (5.71%) and pleural fluid 1 (2.86%).

None of *K. pneumoniae* isolates were resistant to colistin 0 (0%) followed by 4 (11.4%) to polymyxin B and 14 (40%) to meropenem. All these isolates were resistant to ampicillin (100%). Likewise, 91.43% of isolates were found to be resistant to ciprofloxacin followed by 82.86% to cefotaxime (Table 2).

Table 1. Distribution of organisms among growth positive samples.

Organisms /No. of samples (n)	Blood	Urine	Sputum	Pus	*Body fluids	**Others	Total no. (%)
K. pneumoniae	2	18	7	4	1	3	35 (23.97)
E. coli	0	31	1	0	0	1	33 (22.60)
S. aureus	7	10	1	6	1	3	28 (19.18)
Acinetobacter spp	5	2	3	3	0	6	19 (13.01)
*CONS***	3	1	2	3	0	1	10 (6.85)
Serratia spp	0	0	0	6	0	1	7 (4.79)
P. aeruginosa	2	1	2	1	0	0	6 (4.11)
P. mirabilis	0	2	0	0	0	0	2 (1.37)
C. freundii	0	1	0	1	0	0	2 (1.37)
S. pneumoniae	1	0	1	0	0	0	2 (1.37)
Enterobacter spp	0	0	0	1	0	0	1 (0.68)
Providencia spp	0	1	0	0	0	0	1 (0.68)
Total	**20**	**67**	**17**	**25**	**2**	**15**	**146**

**Body fluids: Pericardial fluid, Pleural fluid*
***Others: Endotracheal tube tips Mitral valve tissue, Aortic valve tissue, Foley's tip, CVP tip*
****CONS: Coagulase-negative Staphylococci.*

Table 2. Antibiotic susceptibility patterns of K. pneumoniae isolates from different clinical samples.

S. N.	Antibiotic categories	Antibiotics used	Number of *K. peumoniae* (%)	
			Sensitive	Resistance
A	Aminoglycosides	Amikacin	14 (40.0)	21 (60.0)
		Gentamicin	13 (37.14)	22 (63.86)
B	Antipseudomonal penicillin+ ß-lactamase inhibitors	Piperacillin / Tazobactam	12 (34.29)	23 (65.71)
C	Carbapenems	Meropenem	21 (60.0)	14 (40.0)
		Imipenem	18 (51.43)	17 (48.57)
D	Extended-spectrum cephalosporins 3rd and 4th generation cephalosporins	Ceftriaxone	10 (28.57)	25 (71.43)
		Ceftazidime	8 (22.86)	27 (77.14)
		Cefepime	7 (20.0)	28 (80.0)
		Cefotaxime	6 (17.14)	29 (82.86)
E	Fluoroquinolones	Ciprofloxacin	3 (8.57)	32 (91.43)
F	Folate pathway inhibitor	Cotrimoxazole	17 (48.57)	18 (51.43)
G	Penicillin	Ampicillin	0 (0.0)	35 (100.0)
H	Penicillin +ß-lactamase inhibitors	Amoxycillin / clavulanic acid	11 (31.43)	24 (68.57)
		Ampicillin / sulbactam	10 (28.57)	25 (71.43)
I	Polymyxin	Polymyxin B	31 (88.57)	4 (11.43)
		Colistin	100 (100.0)	0 (0.0)

Thirty-five *K. pneumoniae* isolates were classified into susceptible, MDR, and XDR strains following criteria revealed by Magiorakos et al (2012). Of 35 isolates, 4 were susceptible strains where they exhibited susceptibility to all the applied antibiotics except category G (Penicillin). Sixteen isolates were MDR and 15 isolates were XDR where they were resistant to all categories of applied antibiotics except polymyxin B. Of 15 XDR isolates, 4 are potential PDR that were intermediately resistant to polymyxin B (Figure 2).

Out of 35 *K. pneumoniae* isolates, 40% (14) were found to be carbapenemase producers. The majority of carbapenemase-producing *K. pneumoniae* were obtained from urine 7 (50%), sputum 4 (28.57%), CVC tips 2 (14.29%) and blood 1 (7.14%). Among 14 carbapenemases producing *K. pneumoniae* isolates, 7 (20%) were serine carbapenemase while 7 (20%) were metallo-ß-lactamase producers.

All non-MDR *K. pneumoniae* were non-carbapenemase producers. While, out of 15 isolates of XDR *K. pneumoniae*, 14 (93%) of them were carbapenemase producers. All isolates of carbapenemase-producing *K. pneumoniae* were found to be susceptible to colistin (Figure 3).

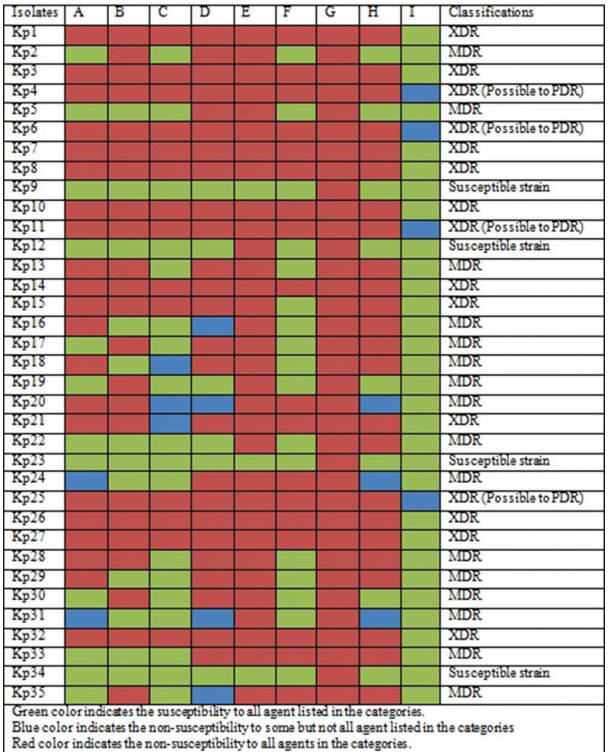

Figure 2. Categorization of *K. pneumoniae* into susceptible, MDR and XDR.

Note: A: Aminoglycosides; B: Antipseudomonal penicillin+ ß-lactamase inhibitors; C: Carbapenems; D: Extended spectrum cephalosporins 3rd and 4th generation cephalosporins; E: Fluoroquinolones; F: Folate pathway inhibitor; G: Penicillin; H Penicillin +ß-lactamase inhibitors; I: Polymyxin, MDR: Multidrug-resistant, XDR: Extreme drug-resistant.

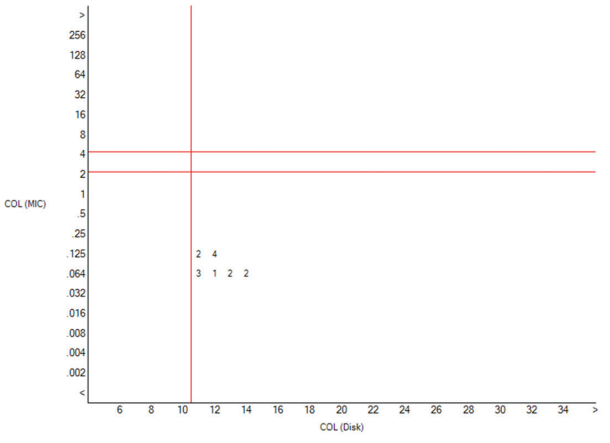

Figure 3. Scatterplot analysis of MIC and disk diffusion of colistin (output from WHONET after analysis).

DISCUSSION

The most striking finding of our study is the alarming prevalence of extreme drug-resistant organisms. In this study, we found 45.71% MDR isolates followed by 42.86% XDR while only 11.43% were susceptible ones. The highest prevalence of MDR and XDR were obtained from patients admitted to the critical care unit. They are more susceptible to get infections by multidrug-resistant pathogens from hospital environments as well as they have selective pressure due to overuse of antibiotics. This result indicated a slight increase in MDR and XDR than previous studies by Shrestha et al. who reported 32% MDR, and 5% XDR while Parajuli et al. found 64.9% MDR and 5% XDR.[15,16] The difference in MDR rates between the present study and the other studies could not be compared due to varying definitions of MDR used during categorizing isolates into MDR and XDR, types of study and sample size.

In this study, the carbapenemase enzyme among isolates was detected by mCIM and EDTA-mCIM. Among them, 40% of isolates were found to be carbapenemase producers, which is in harmony with the study performed by Shrestha et al from Nepal.[17] They reported 56.36% carbapenemase producers using the Modified Hodge test (not recommended nowadays). As very limited studies have been done in Nepal for the detection of carbapenemase producers, the exact clarification on the increasing or decreasing trend of carbapenemase producers in Nepal cannot be evaluated. A report from China CRE Network showed that 89% of clinical isolates (n=155) produce carbapenemase, with most isolates producing KPC (50%) or NDM (33.5%) particularly by *K. pneumoniae* and *E. coli*.[18] Though, the statistical association between carbapenemase-producing and resistance to meropenem antibiotics was found significant (<0.001).

Of the total 994 clinical samples analyzed, 15.47% (146) showed growth on different culture media. This finding is in harmony with the finding of Parajuli et al., Adhikari et al. and Shrestha et al. who reported similar bacterial growth of 19.68%, 17.79% and 16% respectively during their study.[16,19,15] Out of 146 isolated organisms, *K. pneumoniae* were predominant isolates with the number of 35 (23.97%) followed by *E. coli* 33 (23.60%) which is in concordance with the study conducted at B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal.[15] A high percentage of *K. pneumoniae* was isolated from the urine; 51%, followed by sputum (20%) and pus (11%). These findings identify *K. pneumoniae* as an important cause of hospital-acquired urinary tract infection. Similar to our study, Beirao et al and Singh et al have also reported a higher number of *K. pneumoniae* from urine samples.[20,21] Central venous catheter is frequently used in ICU settings however, their colonization with different types of the organism increases the hospital stay and mortality in these patients. It is usually done in an emergency procedure for patients with prolonged hospital stays, having critical conditions, especially those on ventilators and with multiple invasive devices. They are most likely to have a greater risk of infections. In a study, Sapkota and coworkers found the prevalence of *K. pneumoniae* in CVC tips was 3 % which is less in comparison to our study.[22] The highest percentage of isolation rate of *K. pneumoniae* in CVC tips in this study may be because of the particular sampling population. Cardiac patients are immunocompromised patients so they are more prone to be infected by normal flora of skin during catheter insertion. It has been stated that central venous catheterization longer than five to seven days was associated with a higher risk of a catheter-related infection.[23]

In this study, the highest frequency of *K. pneumoniae* was isolated from children below 10 age groups this may be due to children being more prone to the immune-compromised population. Most of the bacterial strains were found to be sensitive against colistin (100.0%), followed by polymyxin B (88.57%). However, the bacterial isolates exhibited 100% resistance towards ampicillin which is in harmony with the finding of Livermore and Woodford, Adhikari et al. and Beyene et al. [24,19,25] Aminoglycosides usually have better activity against clinically important Gram-negative bacteria, but in this study, we observed that the isolates were highly resistant to gentamycin (63%) and amikacin (60%). Khanal and coworkers reported a similar resistance rate of 69% and 54% to gentamycin and amikacin by *K. pneumoniae* respectively.[26] Carbapenems are an important class of ß- lactam antibiotics, however, around half of the isolates were resistant to imipenem; 48.57% followed by meropenem; 40%. Meropenem was found more effective than imipenem which differs from earlier studies.[15,27,28] This might be due to the reason that meropenem is more active against Gram-negative bacilli, while imipenem is more active against Gram-positive cocci. Consequently, the activity of carbapenem depends on the types and species of the organism as well as meropenem is newly introduced in comparison to imipenem.[29] The increasing trend of carbapenem resistance recorded in different studies of Nepal might be due to overuses and inappropriate uses of carbapenem antibiotics. Moreover, carbapenem antibiotics are more frequently used these days as an empirical option for the treatment of infections caused

by ESBL producers.[30]

Colistin is the drug of choice that shows the effectiveness for the treatment of infection caused by multidrug-resistant Gram-negative bacteria. However, the emergence of colistin-resistant among Gram-negative pathogens may lead to a serious infection making it extremely difficult to treat. In Nepal, there is an increasing trend of the use of colistin in poultry or animal farms for the growth promoter and food preservatives. Imprudent and uncontrolled use of colistin in animal farms leads to the infection to the people working on animal farms and related industries.[31] The dissemination of colistin-resistant isolates from animal or animal products to humans is a significant public health problem. In our study, determination of MIC values for colistin antibiotic for carbapenemase-producing *K. pneumoniae* were done using EUCAST-recommended guidelines whereas, all these isolates showed MIC values less than (≤ 2 mg/L) breakpoint. To the best of our knowledge, there is no literature of colistin-resistant *K. pneumoniae* from a human clinical specimen in Nepal to date but the prevalence of colistin resistance among clinical Gram-negative isolates was below 6%.[31]

CONCLUSIONS

An increasing resistance to the carbapenem drugs in the recent years showed a great problem in the management of *K. pneumoniae* infections among immunocompromised patients especially cardiac patients however, colistin can be still an ultimate choice of drug for disease management. In addition, it is very crucial to develop an effective protocol for proper diagnosis and effective antimicrobial therapy to minimize the burden of patients and the spread of such pathogens.

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