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Original Article

Incidence of hypermucoviscous *Klebsiella pneumoniae* and phenotypic detection of their virulence factors along with classical strains among patients visiting tertiary care hospital

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Abstract

Introduction: A new strain of *Klebsiella pneumoniae* named hypermucoviscous *K. pneumoniae* emerges with a distinctive feature to classical strains. Infections due to hypermucoviscous strains have increased with significant mortality and morbidity. This study aimed to determine the prevalence of hypermucoviscous *K. pneumoniae* and compare their virulence with the classical strains phenotypically.

Methodology: One hundred-five clinical isolates of *K. pneumoniae* isolates proceeded for the study. A modified string test evaluated the hypermucoviscosity. The determination of antibiotic susceptibility was done using the Kirby-Bauer disk diffusion method. A phenotypic combination disk test was used to detect β-lactamases (ESBL, MBL, and KPC). Serum resistance was determined by the viable count method, and biofilm production by the microtiter plate method.

Results: The modified string test detected 27.6% (29/105) of isolates as hypermucoviscous and 72.4% (76/105) as classical *K. pneumoniae*. Most *K. pneumoniae* were resistant to ceftazidime (80%) and cefotaxime (78%), and 46.7% were resistant to both imipenem and meropenem. A combination disk test identified 53.3% of ESBL, 28.6% of MBL, and 17.2% of KPC producers. Furthermore, 24.8% of *K. pneumoniae* were biofilm producers, and 39% were found to be serum resistant.

Conclusions: In comparison, classical strains were more likely to develop ESBL, MBL, KPC, and biofilms while hypermucoviscous strains have higher serum resistance. The present study revealed that hypermucoviscous *K. pneumoniae* strains are prevalent and can be associated with metastatic invasive infections. Therefore, appropriate treatment strategies and timely diagnosis of these strains to limit their infection are crucial.

Key words: *Klebsiella pneumoniae*; hypermucoviscosity; multi-drug resistance; biofilm; serum resistance.

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Introduction

Klebsiella pneumoniae was first isolated in 1882 by Carl Friedlander and was initially known as Friedlander's bacterium [1]. It is a gram-negative, non-motile, encapsulated bacterium of the family *Enterobacteriaceae* that resides in the environment [2,3]. *K. pneumoniae* quickly colonizes human mucosal surfaces, including the gastrointestinal (GI) tract and oropharynx, from where their colonization begins [2-4]. They are responsible for many community-onset and nosocomial infections. Typically, classical *K. pneumoniae* (cKP) strains cause severe diseases like pneumonia, bacteremia, or meningitis, including in immunocompromised people, such as diabetes or malignancies [5-7]. However, since the 1980s, a new strain of *K. pneumoniae* has been causing severe infections in healthy individuals. These strains are hypervirulent due to their ability to infect both immunocompetent and immunocompromised populations. In addition, they have an increased tendency for infections to be invasive and can establish metastatic diseases [8-10]. Although hypervirulent *K. pneumoniae* (hvKp) can cause infection in healthy individuals, most of them have been found to be associated with a secondary infection and now are considered an important nosocomial pathogen [11,12]. The capsule surrounding the surface of *K. pneumoniae* serves as the main virulence factor associated with the viscous phenotype. Most of the hypermucoviscous *K. pneumoniae* (hmvKP) isolates were capsular serotype K1 and K2. Hypermucoviscosity in *K. pneumoniae* have been mainly found due to mucoviscosity-associated gene A (*magA*) and regulator of mucoid phenotype A (*rmpA*) [13]. Capsules and siderophores are significant virulence factors that play an important role in hypermucoviscosity phenotype infections [14].

Over the past few decades, using different resistance mechanisms, *K. pneumoniae* has been increasingly resistant to most antibiotics. Consequently, it is challenging to treat common infections like urinary tract infections and severe diseases like bacteremia and pneumonia, which become life-threatening [15,16]. Majorly two types of resistance mechanisms have been commonly seen in *K. pneumoniae*. First is the expression of extended-spectrum β-lactamases (ESBLs) [17], which render bacteria resistant to penicillin, cephalosporin, and monobactam. In contrast, the second common resistance mechanism is the expression of carbapenemase by *K. pneumoniae*, which makes bacteria resistant to almost all available β-lactam antibiotics, including carbapenem drugs [18]. The increasing incidence of ESBL and carbapenemase-producing *K. pneumoniae* is a global concern. However, this scenario will be exaggerated when they develop biofilm production, causing increased resistance to multiple antimicrobial agents [17]. The relatively high number of infections caused by cKp is compounded by multidrug resistance, most notably carbapenem resistance [19]. Treatment options are often limited, and mortality for individuals with infections caused by carbapenem-resistant *K. pneumoniae* is relatively high (~42%) [20]. Previously, hypervirulent *K. pneumoniae* were commonly susceptible to a variety of antibiotics, including cephalosporins and carbapenems; however, multi-drug resistant hypervirulent (MDR-hv) strains have recently emerged, most of them were found to be because of horizontal transfer of resistant plasmid. Based on how they acquire the multidrug resistance, these MDR-hvKp is classified into two types; type I MDR-hvKp and type ii MDR-hvKp [21].

The prevalence of hypermucoviscous *K. pneumoniae* in Asian regions like Taiwan (80%), China (45.7%), and South Korea (78%) is high. Different studies have now reported the emergence of MDR hvKp-associated infections over the past few years, and to the best of our knowledge, no study has been documented in Nepal on infection with hypermucoviscous *K. pneumoniae* so far. Thus, we aimed to investigate the prevalence of hypermucoviscous strains and study their virulence factors phenotypically along with classical strains *K. pneumoniae* among the patients who visited our hospital.

Methodology

This laboratory-based cross-sectional study was done in the microbiology department of Manmohan Memorial Teaching Hospital, Swoyambhu, Kathmandu, Nepal. The study included all the microbiological samples received in the microbiology department for culture.

Bacterial isolation and identification

All clinical samples collected in the microbiology department were cultured on appropriate culture media. Urine samples are inoculated on Cystine-Lactose-Electrolyte- Deficient (CLED) agar (HiMedia, India), Sputum samples on Chocolate agar, Blood agar, and Mac-Conkey agar (HiMedia, India), Wound swabs on Blood and Mac-Conkey agar, Blood samples were first inoculated on Brain Heart Infusion broth. They then sub-cultured on Blood and Mac-Conkey agar. All the *K. pneumoniae* isolates were identified by standard microbiological tests.

Antimicrobial susceptibility testing

We tested the antibiotic susceptibility of all the *K. pneumoniae* isolates to 16 commonly used antibiotics that covered almost all available antibiotics using the Kirby- Bauer disk diffusion method according to CLSI guidelines [22]. The antibiotic disks used were gentamicin (10 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), ceftazidime (30 µg), tetracycline (30 µg), nitrofurantoin (300 µg), tobramycin (10 µg), tigecycline (15 µg), chloramphenicol (30 µg), levofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), polymyxin B (300 units), colistin sulphate (10 µg), piperacillin/tazobactam (100/10 µg), co-trimoxazole (25 µg). The dilution of bacterial isolates was prepared equivalent to 0.5 McFarland standards and then lawn culture on Muller Hinton Agar (MHA) plate surface using a sterile cotton swab. The result was classified as sensitive, intermediate, and resistant according to inhibition zone interpretation.

Detection of MDR isolates

All the *K. pneumoniae* isolates were non-susceptibility to at least one agent in three or more antimicrobial categories defined as multidrug resistance (MDR) isolates [23].

Phenotypic detection of ESBL-producing isolates

The initial screening was performed by using cefotaxime (CTX) (30µg) and ceftazidime (CAZ), where ZOI $\leq$ 27 mm for CTX and $\leq$ 22 mm for CAZ were considered as a potential ESBL producer as recommended by CLSI. The combination disc test (CDT) was performed to confirm Extended-Spectrum Beta-Lactamase (ESBL). First, a standard suspension of isolates equivalent to 0.5 McFarland was swabbed on the surface of the Muller Hinton Agar (MHA) plate. Then, the ceftazidime (CAZ), ceftazidime/clavulanic acid (CAC), cefotaxime (CTX), and cefotaxime/clavulanic acid (CEC) discs (HiMedia, India) placed on the surface of MHA plates and incubated aerobically overnight at 37 °C. The test is positive if the difference between CAZ, CTX, and CAC, CEC inhibitory zone is ≥ 5 mm, respectively [24].

Detection of Metallo beta-lactamase (MBL) and Klebsiella pneumoniae carbapenemase (KPC)

Isolates resistant to Imipenem (IPM), or Meropenem (MPR) are considered potential MBL producers as recommended by CLSI. The MBLs production was confirmed by the EDTA-combination disc test method. First, two Meropenem (10 µg) discs are placed on the MHA surface 20 mm from the center. One with 10 µL (0.1M EDTA, containing 292 µg) of EDTA and another meropenem alone. The agar plate was then incubated aerobically overnight at 37 °C. The positive test indicated an increase in the inhibition zone of the meropenem disc containing EDTA with ≥ 5 mm than that of meropenem alone [25].

Similarly, for detecting *Klebsiella pneumoniae* carbapenemase (KPC), two meropenem (MRP) discs were placed 20 mm away from the center, one with 20µL of 400µg/mL phenylboronic acid (PBA) and another meropenem alone. The increase in the zone of inhibition of meropenem containing phenylboronic acid by ≥ 5mm than that of meropenem alone is considered a positive test [25].

Detection of virulence factors

Detection of hypermucoviscosity

Hypermucoviscosity was evaluated by the modified string test, where a standard bacteriological loop was used to stretch a muco-viscous string from the bacterial colony to determine the mucoid phenotype. The string test was modified to exclude the discrepancy between the laboratory staff. The formation of a viscous string > 10mm is regarded as a positive confirmation of the hypermucoviscous (Hmv) phenotype [26].

Detection of biofilm

The isolates were subjected to biofilm detection by microtiter or tissue culture plate. Organisms isolated from fresh agar plates were inoculated in 2 mL of Luria Bertani broth (HiMedia, India) with 2% glucose and incubated at 37 °C for 24 hours. The culture was then diluted at a ratio of 1:100 with a fresh medium. Each well of sterile 96-well polystyrene tissue culture plates was inoculated with 200µL of the diluted culture of different strains isolated from the various samples and incubated at 37 °C for 24 hours. After incubation, the contents of each well were removed by gentle tapping and washed with 0.2 mL of phosphate buffer saline (pH 7.2) three times, removing free-floating bacteria. Biofilm formed by bacteria adherent to the wells was fixed by keeping it at 60 °C for 1 hour and was stained by crystal violet (2%). The excess stain was removed using deionized water by rinsing it three times and decolorizing it with 30% acetic acid. The stained adherent biofilm's optical density (OD) was obtained using a micro-ELISA auto reader at 570 nm.

Un-inoculated wells containing broth were considered a negative control. The experiment was performed in triplicate two times. First, the average optical density (OD) values of each test strain and negative control were calculated. The final OD values of a test strain were expressed as the average OD value of the strain reduced by the OD cut-off value (ODI) of the negative control. According to Stepanovic *et al.* ODI, the interpretation of biofilm production is defined as three standard deviations (SDs) above the mean OD of the negative control [3,27,28].

Serum bactericidal activity

An inoculum of 25µL (adjusted to 10^6 CFU/mL) prepared from the mid-log phase was diluted by 0.9% saline and added to 75 µL of pooled human sera contained in a tube. Viable counts (VC) were checked at 1, 2, and 3 hours of incubation at 37 °C. Each strain was assessed at least three times, and the mean results were expressed as percent inoculums. Results expressed as percentage inoculation and responses regarding viable counts were graded from 1 to 6. Serum sensitive at grades 1 to 2, intermediate at grades 3 to 4, and serum resistant at grades 5 and 6 [29].

Statistical Analysis

Data analysis was performed using IBM SPSS version 23.0 (IBM Corp., USA). Descriptive statistics were conducted, such as frequency and percentage. The comparison of antibiotic resistance and virulence factors between hypermucoviscous and classical strains was assessed by Bivariate and Multivariate analysis using the chi-square test and binary logistic regression. The odds ratio with its 95% CI was used to declare statistically significant at $p < 0.05$ in binary logistic regression.

Results

Isolation and identification of Klebsiella pneumoniae

A total of one hundred-five non-repetitive pure and microbiologically identified as *K. pneumoniae* were isolated during the study period from various clinical samples collected in the department of microbiology. The majority of *K. pneumoniae* isolates were isolated from urine (40%), followed by sputum (30.5%), wound swabs (16.2%), and blood (9.5%), respectively. Hypermucoviscosity was seen in 29 (27.6%) *K. pneumoniae* (hmvKP) isolates and 76 (72.4%) were classical *K. pneumoniae* (KP), which was determined by a modified string test (Figure 1). In this study, 52% of *K. pneumoniae* were isolated from females, while 48% were male patients. The mean age of patients infected with *K. pneumoniae* was 45.52 ± 19.46 (Ranging from 10 to 86 years).

Antibiotic susceptibility of isolated Klebsiella pneumoniae

67.6% of the *K. pneumoniae* isolates were multidrug-resistant (MDR). The *K. pneumoniae* showed the highest resistance to tested β-lactam antibiotics such as ceftazidime (80%), cefotaxime (78%), imipenem (46.7%) and meropenem (46.7%). Similarly, 66.7% of isolates were resistant to nitrofurantoin, and 62.9% were resistant to piperacillin-tazobactam. Polymyxin B and colistin showed the highest inhibitory activity against the *K. pneumoniae* isolates (Table 1).

Detection of beta-lactamases

Among the 105 *K. pneumoniae* isolates, 53.3% of *K. pneumoniae* isolates were phenotypically confirmed positive for ESBL production. Moreover, among the ESBL producers, 21.9% were hypermucoviscous strains, and 31.4% were classical strains (Table 2). Likewise, 28.6% of *K. pneumoniae* were MBL producers, 12.4% were hypermucoviscous, and 16.2% were classical strains. Similarly, 17.2% of *K. pneumoniae* were KPC producers, of which 8.6% were hypermucoviscous strains, and 8.6% were classical strains (Table 2). The logistic regression analysis demonstrated a negative association or less likely to be associated with hypermucoviscosity for strains producing ESBLs [OR: 0.200; 95% CI (0.073-0.548)], MBLs [OR: 0.355; 95% CI (0.143-0.880)], and KPCs [OR: 0.299; 95% CI (0.104-0.853)].

Figure 1. String Test Positive (≥ 10mm).

Table 1. Antibiotics resistance pattern of *Klebsiella pneumoniae.*

Antibiotics	Resistant isolates	
	No.	%
Gentamycin	47	44.8
Ciprofloxacin	58	55.2
Cefotaxime	82	78.1
Ceftazidime	84	80.0
Tetracycline	38	36.2
Nitrofurantoin	70	66.7
Tobramycin	46	43.8
Tigecycline	31	29.5
Chloramphenicol	36	34.3
Levofloxacin	47	44.8
Imipenem	49	46.7
Meropenem	49	46.7
Polymyxin B	0	-
Colistin	0	-
Piperacillin/Tazobactam	66	62.9
Cotrimoxazole	57	54.3

Virulence factors detection

The quantitative microtiter plate method assessed biofilm formations of the *K. pneumoniae* isolates. Biofilm production is categorized as biofilm producers and non-producers. 24.7% (26/105) of *K. pneumoniae* were found to be biofilm producers. Among the total biofilm producers, 11.4% (12/105) were hypermucoviscous strains, and 13.3% (14/105) were classical strains (Table 3). Similarly, 39% (41/105) of the tested *K. pneumoniae* were serum resistant, and 61% (64/105) were serum sensitive. Among the serum-

Table 2. β-lactamases among the *K. pneumoniae* isolates.

Beta-lactamase	Total (%)	hmvKP (%)	cKP (%)	COR [95% CI]	*p*
ESBL-producer	53.3	21.9	31.4	0.200 [0.072-0.548	**0.002***
MBL-Producer	28.6	12.4	16.2	0.335 [0.143-0.880]	**0.025***
KPC-producer	17.2	8.6	8.6	0.299 [0.104-0.853]	**0.024***

bold * = Significance at $p < 0.05$; hmvKP: hypermucoviscous *K. pneumoniae*; cKP: classical *K. pneumoniae*; COR: crude odds ratio; CI: confidence interval.

Table 3. Biofilm and serum bactericidal activity.

Tests	Total (%)	hmvKp (%)	cKP (%)	COR [95% CI]	*p*
Biofilm					
Biofilm Producers	24.8	11.5	13.3	0.320 [0.125-0.818]	**0.017***
Non-BiofilmProducers	75.2	16.2	59.0		
Serum BactericidalActivity					
Serum Sensitive (Grade 1-4)	61.0	11.5	49.5	3.069 [1.269-7.42]	**0.013***
Serum Resistant (Grade 5-6)	39.0	16.2	22.8		

bold* = Significance at $p < 0.05$; hmvKP: hypermucoviscous *K. pneumoniae*; cKP: classical *K. pneumoniae*; COR: crude odds ratio; CI: confidence interval.

resistant isolate of *K. pneumoniae*, 16.2% (17/105) were hypermucoviscous types, while 22.8% (24/105) were classical types (Table 3). The statistical analysis indicated that *K. pneumoniae* strains forming biofilms exhibited a negative association [OR: 0.320; 95% CI (0.125-0.880)] with hypermucoviscosity. In contrast, strains resistant to serum demonstrated a positive association [OR: 3.069; 95% CI (1.259-7.42)], suggesting that they were 3.069 times more likely to be associated with hypermucoviscosity.

Discussion

K. pneumoniae is a common pathogen associated with community and healthcare-associated infections, including respiratory tract infections, urinary tract infections, wounds, and bloodstream infections [30]. Klebsiella's pathogenicity is due to its virulence factors and ability to acquire multiple antibiotic resistances [31,32]. *K. pneumoniae* is the most common bacteria after *E. coli* among the *Enterobacteriaceae* family, causing invasive infections. In addition, those infections by *K. pneumoniae* have been associated with comorbidities such as cancer and diabetes [33].

A total of 105 *K. pneumoniae* isolates were collected from different clinical samples, and hypermucoviscosity was determined by a modified string test. Hypermucoviscous *K. pneumoniae*, namely hmvKP, is more invasive and associated with severe infections that can be monitored in routine practice by a modified string test [31,34,35]. In our study, a modified string test identified 29 (27.6%) hmvKP, which is lower than that reported in Taiwan (80%), China (45.7%), and South Korea (78%) [36]. However, this indicates that the hmvKP is present in our hospital settings, as they can cause invasive infections, they are a real threat to human health.

K. pneumoniae isolates, like other *Enterobacteriaceae*, are increasingly resistant to multiple antimicrobial agents, including aminoglycosides, quinolones, and third generation cephalosporins [37]. Beta-lactams are the antibiotic of choice for *K. pneumoniae* infections, but bacteria develop resistance to these beta-lactams through the production of β-lactamase which hydrolyzes oxyimino-cephalosporins leads to acquired resistance and significantly impacts treatment efficacy [38]. Broad-spectrum antibiotics such as carbapenems and fluoroquinolones are typically used to treat infections caused by extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae*; however, carbapenem-resistant *K. pneumoniae* (CR-Kp) are significantly increasing that resulting in an escalated global risk of disease [39]. In our present study, the highest number of *K. pneumoniae* resistant to ceftazidime (80%), followed by cefotaxime (78%), nitrofurantoin (66.7%), piperacillin-tazobactam (62.8%), ciprofloxacin (55.2%), cotrimoxazole (54.2%), imipenem (46.7%) and meropenem (46.7%). In a similar study carried out by Parajuli *et al.* in 2017, all *K. pneumoniae* were resistant to cotrimoxazole and cefotaxime, 86.4% to ciprofloxacin, 81.0% to piperacillin-tazobactam, 48.6% of isolates resistant to both imipenem and meropenem [40]. In another study by Gharrah *et al.* in 2017, 49% of isolates were resistant to cefotaxime, and 40% were resistant to ceftazidime [31]. Similarly, in the study by Li *et al.* from China, out of 88 *K. pneumoniae* isolates highest resistance was observed in chloramphenicol (42%), followed by tetracycline (39.7%), cefotaxime (37.5%), ciprofloxacin (25.0%), ceftazidime (23.8%) and piperacillin-tazobactam (22.7%) [41]. In developing countries, where the health care delivery system is inferior, prior use of antibiotics like third-generation cephalosporins and carbapenems without susceptibility testing might be the reason for increasing resistance towards the option drugs.

Multi-drug resistance (MDR) is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [23]. In the study by Nepal *et al.* in 2017, 59% of *K. pneumoniae* were multidrug-resistant [42]. However, in our study, 67.6% (71/105) of *K. pneumoniae* were multidrug-resistant. Over the last decades, the prevalence of MDR *K. pneumoniae* in clinical settings has increased progressively; a meta-analysis study have found 55 % pool prevalence of MDR *K. pneumoniae* in the southeast Asia [43]. The number of multidrug-resistant *K. pneumoniae* isolates in Nepal is also growing yearly. A systematic study by Odari *et al.* from Nepal in 2022 have reported 66%

MDR *K. pneumoniae* [44]. This shows that antimicrobial resistance is becoming a matter of concern in developing countries, which might be due to cheap access to antimicrobials and a lack of antibiotic use recommendations. Therefore, the government should implement a proper strategy to benefit antibiotic use, and antibiotics such as carbapenems should only be used after susceptibility testing.

Beta-lactams are the choice of drug in the infections of *K. pneumoniae*, but bacterial resistance to beta-lactam antibiotics is increasing globally, leading to morbidity and mortality [45]. Extended-spectrum beta-lactamase (ESBL) production is substantially growing and is now recognized as a worldwide problem [46]. The proportion of *K. pneumoniae*-producing ESBL varied among the various countries, 12% in the United States, 33% in Europe, 52% in Latin America, and 28% in Western Pacific [47]. In the recent study by Gharrah *et al*., 50% of *K. pneumoniae* isolates were ESBL producers [31], which is similar to our study. Carbapenems are the preferred antibiotics for treating ESBL *K. pneumoniae* infections. However, carbapenem resistance in *K. pneumoniae* is attributed by the production of carbapenemase like Metallo beta-lactamase (such as IMP, VIM, and NDM) and the output of *K. pneumoniae* carbapenemase (KPC), causing the severe clinical problems and treatment complexity [48-50]. In our study, 46.7% (49/105) of *K. pneumoniae* were resistant to carbapenem drugs (imipenem and meropenem) detected by the disk diffusion method. However, by the combination disk test (CDT), 28.7% (30/105) of *K. pneumoniae* were MBL producers, and 17.1% (18/105) were KPC producers. Our study revealed that the rate of carbapenem resistance is increasing in comparison to the study performed by Nepal *et al.* (12.8% MBL producer) [42] and Parajuli *et al.* (10.8% KPC producer) [40]. This increase in the rate of carbapenem resistance might be due to the shared use of carbapenems during the initial treatment course without their susceptibility testing. The high clinical and economic burden caused by these carbapenem resistant *K. pneumoniae* are the major concern. The annual economic burden of multidrug resistant bacteria amounted to more than US$ 45 billion worldwide [51]. Therefore, appropriate strategies and resources should be adopted to control carbapenem resistant *K. pneumoniae* could be helpful for reducing the clinical as well as economic burdens to the patients [52].

In addition to the production of beta-lactamases, biofilm formation, and serum resistance contribute to these isolates becoming more virulent [48,50,53]. Microbial biofilm formation and development have been reported to be a significant pathogenic factor of *K. pneumoniae* infections, as biofilm protects bacteria from exposure to antimicrobials [54]. Our study reported 24.7% of *K. pneumonia*e as a biofilm producer, which is comparatively less than Vuotto *et al.* (49.2%) [54]. Similarly, serum resistance in *K. pneumoniae* has been an essential pathogenic factor for their disease establishment [55]. 39% of *K. pneumoniae* in our study were serum-resistant, like the study of Gharrah *et al.* (40%) [31]. In our present study, it was found that classical strains have a higher rate of ESBL, MBL, KPC, and biofilm production than hypermucoviscous strains. But the serum resistance was associated with the hypermucoviscous strains more than that of classical strains supporting the statement that hypermucoviscous strains can be associated with metastatic invasive infections.

Although this study was conducted in a single hospital center and the overall prevalence of hypermucoviscous strains couldn’t be made only based on our data. However, our study has documented the presence of hypermucoviscous strains in our hospital and increasing MDR-hmvKP could be an alarming sign. We evaluated the hypermucoviscosity of *K. pneumoniae* by the string test, but we couldn’t perform the molecular tests like gene detecting *megA* and *rmpA* to confirm our phenotypic string test results. In the study by Shoja *et al.*, 5.5% (22/400) of *K. pneumoniae* were found to be string test positive and 64% (14/22) of string test positive isolates were also positive for *rmpA* gene [56]. Nevertheless, the string test is not considered as a gold standard for identifying the hypermucoviscous strains, however majority isolates can be determined by the string test. In the developing country like Nepal where in routine clinical laboratory molecular methods like *rmpA* or megA gene detection would not be possible, string tests could be a tool to identify these hypermucoviscous strains.

Conclusions

This study showed that hypermucoviscous *K. pneumoniae* is prevalent in our hospital and they can be differentiated from the classical strains by a string test. Phenotypic comparisons of beta lactamases, biofilms, and serum bactericidal activity revealed that classical strains were more likely to produce beta lactamases (ESBLs, MBLs and KPCs) and biofilms while hypermucoviscous strains were found to be more resistant to serum bactericidal activity. This has indicated that the hypermucoviscous strains are more likely to be associated with metastatic invasive infections, and classical strains are more likely to develop antibiotic resistance. Therefore, an appropriate and timely diagnosis with correct treatment strategies is crucial to reduce their dissemination. Furthermore, our current investigation highlights the necessity for comprehensive research on hypermucoviscous *K. pneumoniae* in Nepal.

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Consent
Written informed consent was obtained from the patients to publish this research article.

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No conflict of interests is declared.

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