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Original Research Article

Molecular detection and antimicrobial resistance pattern of carbapenem-resistant *Klebsiella pneumoniae* isolated from patients with lower respiratory tract infections

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ABSTRACT

Background: Recently worldwide, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infection has attracted a lot of attention because of the scarcity of effective drugs and the severity of the illness as well as the high death rate. This study was conducted to assess the antimicrobial susceptibility profiles and the molecular identification of CRKP isolates that were obtained from patients with lower respiratory tract infection (LRTI) at a tertiary care hospital in Sylhet, Bangladesh.
Methods: This cross-sectional study was conducted in the Department of Microbiology at Sylhet MAG Osmani Medical College, Sylhet, from July 2021 to June 2022. A total of 153 sputum samples were collected from adult patients (≥18 years) clinically diagnosed with LRTIs. Data were analyzed using SPSS version 26.
Result: Among 153 sputum samples from LRTI patients, 66.0% showed bacterial growth, with Klebsiella pneumoniae accounting for 20.9% of isolates. The mean age was 52.43±17.12 years with male predominance (69.3%). Of the Klebsiella pneumoniae isolates, 25.0% were carbapenem-resistant (CRKP), among which 75.0% produced carbapenemase and 50.0% harbored the KPC gene. Overall isolates showed high resistance to cephalosporins (up to 81.2%) and moderate sensitivity to carbapenems (around 72–75%) and amikacin (56.2%). CRKP isolates demonstrated marked multidrug resistance, with high resistance to most antibiotics, including carbapenems (75.0%).
Conclusion: In conclusion, one in every fourth KP isolate was CRKP, which highlighted the need for careful prescription of carbapenems for the therapy of infectious diseases with ESBL-producing KP. Among different genes conferring resistance to carbapenems, only the blaKPC gene was investigated in this study.

Keywords: Antimicrobial resistance, Carbapenem resistance, *Klebsiella pneumoniae*, Respiratory tract infections

INTRODUCTION

Klebsiella pneumoniae is a rod-shaped, gram-negative bacterium that is frequently the cause of serious community-acquired and hospital-acquired infections.[1] Besides, it is a proved one among the risk factors independently associated with death in severe community-acquired pneumonia.[2] Because of KP infections, the

therapy has become even more complicated, to the extent that via the evolution of multidrug-resistant (MDR) strains the majority of KP isolates were found to be resistant to ceftriaxone, ceftazidime, gentamicin, amikacin, and meropenem.[3,4] Three very important authorities, the World health organization, the centers for disease control and prevention (CDC), and public health England, have most seriously labelled MDR KP as one of the most urgent threats to human health.[5] The acquisition of antibiotic resistance in KP occurs mainly through genetic elements such as those that code carbapenemases, which results in the development of CRKP.

These CRKP strains not only cause major nosocomial infections but are also resistant to almost all antibiotics currently available.6 Resistance to drugs resulting from carbapenemase production has become a serious issue in KP species, especially with enzymes such as *Klebsiella pneumoniae* carbapenemase, which are capable of hydrolysing a broad spectrum of beta-lactam antibiotics including penicillin, cephalosporins, and carbapenems. There are several phenotypic and genotypic approaches that can be used to identify carbapenemases; however, the modified hodge test is quite popular, mainly because it is very easy to perform. blaKPC is a gene coding for carbapenemase and it is one of the most common and widespread worldwide.

Particularly with KP it is contributing to severe forms of infections such as pneumonia, bloodstream infections and urinary tract infections. Usually, PCR for the blaKPC gene is performed to confirm the genetic basis of carbapenem resistance and therefore help in targeted antimicrobial therapy and infection control measures.[7,8] Since antimicrobial resistance among carbapenemase-producing KP has been increasing, the current study was carried out to evaluate the antimicrobial susceptibility patterns and identify CRKP among patients with lower respiratory tract infections in a tertiary care hospital in Sylhet, Bangladesh for the purpose of making selection of antimicrobials more effective and limiting the spread of these infections.

Klebsiella pneumoniae has shown great capacity to survive in hospital environments particularly in intensive care units where invasive procedures, long hospital stays and broad use of antibiotics provide the perfect conditions for transmission.[9] Colonization of respiratory and gastrointestinal tracts acts as the first step in infection, and the role of cross-transmission through hands of healthcare workers or medical equipment that are contaminated cannot be overlooked in the outbreaks.[10,11] Besides, biofilm production makes the bacteria not only more resistant, but also able to survive longer in the environment, especially on surfaces of endotracheal tubes and indwelling devices, which largely results in LRTIs. Recently, the discovery of the hypervirulent strains of *Klebsiella pneumoniae*, producing capsular material in abundance and having virulence factors such as rmpA and siderophore systems, has raised concerns, as these bacteria can lead to very serious infections even in healthy individuals.[12] Alarmingly, there have been reports of convergence between hypervirulence and carbapenem resistance, leading to exceedingly pathogenic and hard-to-treat infections.[13] Diagnostically speaking, identifying the bacteria and determining their susceptibility should be done very fast so as to get on with the treatment in time and thus, there is an increased use of novel methods such as automated systems and molecular diagnostics together with the traditional techniques.[14] Infection control measures, such as rigorous hand hygiene, antimicrobial stewardship programs, and continuous monitoring of resistance patterns, are crucial for successfully controlling the spread of these organisms in healthcare facilities.[15]

METHODS

This cross-sectional study was performed at the Department of Microbiology, Sylhet MAG Osmani Medical College Sylhet during July, 2021 to June, 2022. For our study, we took 153 sputum samples from adult patients (18-87 years) clinically diagnosed with LRTIs who visited the inpatient and outpatient departments of Medicine of Sylhet MAG Osmani Medical College Hospital. Patients undergoing immunosuppressive therapy and those with diagnosed TB were not included in the study. Sputum samples were obtained in sterile, wide-necked, leak-proof containers and were sent to the microbiology lab without delay for immediate processing. Initially, every sample was subjected to macroscopic and microscopic observations, then cultured on both Blood agar and MacConkey's agar media for incubation at 37°C over 24-48 hours.

The identification of presumptive *Klebsiella pneumoniae* isolates was done by Gram staining technique and standard biochemical tests including indole, catalase, urease, citrate, oxidase and triple sugar iron (TSI) tests. Antimicrobial susceptibility testing was done with Kirby Bauer disc diffusion method on Mueller-Hinton agar in line with the Clinical and Laboratory Standards Institute guidelines (CLSI), 2021. At the molecular level, polymerase chain reaction (PCR) was conducted to identify carbapenemase genes using specific primer sequences (Forward: ATGTCACTGTATCGCCGTCT; Reverse: TTTTCAGAGCCTTACTGCCC). The amplified products were separated and visualized on 1.5% agarose gel stained with ethidium bromide under a gel documentation system using a 100 bp DNA ladder. The Ethical Review Committee of Sylhet MAG Osmani Medical College gave us permission to start the research. Then, we used the software SPSS version 26 to analyze the data, and with the help of descriptive statistics, we presented the findings.

RESULTS

A total of 153 sputum samples were analyzed, with a mean age of 52.43±17.12 years and a male predominance (69.3%; male-to-female ratio 2.3:1). Culture positivity was observed in 66.0% of samples, predominantly showing

single bacterial growth (56.8%), while mixed growth was noted in 9.2% of cases. Among all isolates, *Klebsiella pneumoniae* accounted for 20.9% (n=32). Of these, 25.0% (n=8) were identified as CRKP, while 75.0% (n=24) were carbapenem-sensitive. Phenotypic detection using the Modified Hodge Test revealed carbapenemase production in 75.0% (n=6) of CRKP isolates. Furthermore, genotypic analysis demonstrated the presence of the KPC gene in 50.0% (n=4) of CRKP isolates, confirming the molecular basis of resistance in a subset of cases (Table 1). KP isolates were mostly resistant to cefotaxime (81.2%), ceftazidime (75.0%), amoxicillin-clavulanic acid (65.6%), cefepime (65.6%), cefoxitin (62.5%), azithromycin (56.2%) and ciprofloxacin (43.8%); while sensitive to meropenem (75.0%), imipenem (71.9%), piperacillin-tazobactam (65.6%) and amikacin (56.2%) (Table 2). High levels of resistance were observed against multiple antibiotics, particularly amoxicillin–clavulanic acid and ciprofloxacin (87.5% each), as well as cefoxitin, meropenem, imipenem, and azithromycin (75.0% each). Moderate resistance (50.0%) was noted for ceftazidime, cefotaxime, and cefepime. Sensitivity was relatively higher for piperacillin-tazobactam, ceftazidime, cefotaxime, and amikacin (50.0% each), while carbapenems (meropenem and imipenem) retained limited effectiveness, with only 25.0% sensitivity. Intermediate susceptibility was minimal across most antibiotics (Table 3).

Table 1: Demographic characteristics, culture findings, and distribution of carbapenem resistance and kpc gene among *Klebsiella pneumoniae* isolates (n=153).

Variables	Findings
Age range (in years)	18–87
Mean age (in years)	52.43±17.12
Gender distribution	Male: 69.3%
Male: Female ratio	2.3:1
Culture positivity	101 (66.0%)
Single growth	87 (56.8%)
Mixed growth	14 (9.2%)
***Klebsiella pneumoniae* isolates (KP)**	32 (20.9%)
Carbapenem-sensitive KP	24 (75.0%)
Carbapenem-resistant KP (CRKP)	8 (25.0%)
Carbapenemase production (MHT) among CRKP (n=8)	6 (75.0%)
KPC gene detected by PCR among CRKP (n=8)	4 (50.0%)

Table 2: Antimicrobial susceptibility patterns of *Klebsiella pneumoniae* isolates (n=32).

Antimicrobials	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Piperacillin–tazobactam	21 (65.6)	6 (18.8)	5 (15.6)
Ceftazidime	7 (21.9)	1 (3.1)	24 (75.0)
Cefotaxime	6 (18.8)	0 (0.0)	26 (81.2)
Cefoxitin	12 (37.5)	0 (0.0)	20 (62.5)
Amoxicillin–clavulanic acid	8 (25.0)	3 (9.4)	21 (65.6)
Meropenem	24 (75.0)	1 (3.1)	7 (21.9)
Imipenem	23 (71.9)	2 (6.2)	7 (21.9)
Amikacin	18 (56.2)	7 (21.9)	7 (21.9)
Azithromycin	14 (43.8)	0 (0.0)	18 (56.2)
Cefepime	5 (15.6)	6 (18.8)	21 (65.6)
Ciprofloxacin	8 (25.0)	10 (31.8)	14 (43.8)

Table 3: Antimicrobial susceptibility patterns of carbapenem-resistant *Klebsiella pneumoniae* in LRTI (n=8).

Antimicrobials	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Piperacillin–tazobactam	4 (50.0)	1 (12.5)	3 (37.5)
Ceftazidime	4 (50.0)	0 (0.0)	4 (50.0)
Cefotaxime	4 (50.0)	0 (0.0)	4 (50.0)
Cefoxitin	2 (25.0)	0 (0.0)	6 (75.0)
Amoxicillin–clavulanic acid	1 (12.5)	0 (0.0)	7 (87.5)
Meropenem	2 (25.0)	0 (0.0)	6 (75.0)
Imipenem	2 (25.0)	0 (0.0)	6 (75.0)
Amikacin	4 (50.0)	1 (12.5)	3 (37.5)
Azithromycin	2 (25.0)	0 (0.0)	6 (75.0)
Cefepime	2 (25.0)	2 (25.0)	4 (50.0)
Ciprofloxacin	1 (12.5)	0 (0.0)	7 (87.5)

DISCUSSION

The present study provides important insights into the epidemiology, resistance profile, and molecular characteristics of *Klebsiella pneumoniae* in LRTIs. In this study, the mean age of patients was 52.43±17.12 years with a male predominance (69.3%, male-to-female ratio 2.3:1). Similar demographic patterns have been reported by Sharma et al who observed a mean age of 50.6 years with 66% male predominance among LRTI patients, and Kumar et al., who also documented higher infection rates among males (approximately 70%).[16,17] This male predominance may be attributed to higher exposure to risk factors such as smoking and occupational hazards.

Regarding culture findings, 66.0% of sputum samples in the current study showed bacterial growth, with *Klebsiella pneumoniae* accounting for 20.9% of isolates. In contrast, Tanni et al reported a higher isolation rate of *Klebsiella pneumoniae* (28.4%) among respiratory samples in Bangladesh, while Joseph et al documented a lower prevalence of 15.2%.[4,18] Furthermore, CRKP constituted 25.0% of KP isolates in this study. This is comparable to findings by Tzouvelekis et al who reported CRKP prevalence rates ranging from 20–30% in hospital settings.[19] Carbapenemase production was detected in 75.0% of CRKP isolates using the Modified Hodge Test, whereas Yong et al reported carbapenemase positivity in 68% of CRKP isolates.[20] Additionally, the blaKPC gene was detected in 50.0% of CRKP isolates in this study, while Nordmann et al found KPC gene prevalence in approximately 40–60% of carbapenem-resistant isolates globally.[21] The antimicrobial susceptibility profile of Klebsiella pneumoniae isolates revealed high resistance to third-generation cephalosporins, with cefotaxime (81.2%) and ceftazidime (75.0%) showing poor sensitivity.

Similarly, Paterson and Bonomo reported resistance rates exceeding 70% for third-generation cephalosporins among ESBL-producing *Klebsiella pneumoniae*.[22] In contrast, carbapenems such as meropenem (75.0% sensitivity) and imipenem (71.9% sensitivity) retained relatively good activity in this study. Comparable findings were reported by Das et al who observed carbapenem sensitivity rates of around 70% among *Klebsiella pneumoniae* isolates.[23] Amikacin also demonstrated moderate effectiveness (56.2% sensitivity), which aligns with findings by Sader et al who reported aminoglycoside sensitivity rates of approximately 50–60%.[24]

Among CRKP isolates, the resistance profile was markedly higher. In this study, resistance to amoxicillin-clavulanic acid and ciprofloxacin reached 87.5%, while resistance to carbapenems (meropenem and imipenem) was 75.0%. Gupta et al reported similar findings, with CRKP isolates showing over 80% resistance to fluoroquinolones and beta-lactam antibiotics.[25] Notably, piperacillin-tazobactam and amikacin retained moderate sensitivity (50.0%), which is comparable to the findings of Falagas et al who reported partial effectiveness of these agents against CRKP.[26] Additionally, cephalosporins such as cefepime showed 50.0% resistance in this study, whereas Lee et al. documented even higher resistance rates (>70%) among CRKP isolates.[27]

Limitations

The study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

CONCLUSION

In conclusion, one in every fourth KP isolate was CRKP, which highlighted the need for careful prescription of carbapenems for the therapy of infectious diseases with ESBL-producing KP. Among different genes conferring resistance to carbapenems, only the blaKPC gene was investigated in this study. In order to prevent the spread of resistant strains, other resistance mechanisms, including efflux pump over-expression, porin loss, and other beta-lactamase genes, need to be studied in the future.

Recommendations

It is recommended that routine antimicrobial susceptibility testing and early screening for carbapenemase-producing *Klebsiella pneumoniae* should be implemented in all tertiary care settings to guide appropriate therapy. Strict adherence to infection prevention and control measures, along with antimicrobial stewardship programs, is essential to limit the spread of multidrug-resistant and carbapenem-resistant strains. The use of higher antibiotics should be reserved and guided by culture sensitivity reports to avoid further resistance development.

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