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Detection of Colistin Resistance in Uropathogenic Carbapenem Resistant *Klebsiella pneumoniae*

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Abstract

Background: Colistin resistance among carbapenem-resistant *Klebsiella pneumoniae* (CR*Kp*) strains at an accelerated rate poses a serious global threat which limits therapeutic options. **Objective:** In this study, 203 laboratory-archived uropathogenic *K. pneumoniae* isolates were screened for carbapenem and colistin resistance. **Methods:** Carbapenam resistance screening was performed using combined disc test. Colistin resistance was determined for CR*Kp* isolates, according to clinical and laboratory standards institute- European Committee on Antimicrobial Susceptibility Testing guidelines. Polymerase Chain Reaction for carbapenamase genes (bla_{NDM-1}, bla_{KPC-2} and $bla_{OXA-1)}$ and plasmid-mediated colistin resistance genes ($mcr\text{-}_{1\text{-}2}$) were performed for the resistant isolates. **Results:** About 30% of the CR*Kp* isolates were resistant to colistin; 57.1% and 35.7% of the isolates carried bla_{NDM-1} and bla_{OXA-1} gene, respectively. None of the isolates showed positive for bla_{KPC-2} and $mcr_{(1-2)}$ genes. **Conclusion:** Colistin resistance in the absence of a plasmid borne transfer mechanism was observed among our CR*Kp* strains. Escalating colistin resistance is a crucial obstacle in treatment of *K. pneumoniae* infections and repurposing their usage is important to combat and control the resistance.

Keywords: Carbapenem resistance, Colistin resistance, *Klebsiella pneumoniae,* Urinary tract infection

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Introduction

For decades, antibiotic resistance has been a global public health risk that has been overlooked. The emergence of resistance in carbapenems, widely recognized as successful broad-spectrum antibiotics against multidrug resistant (MDR) *Klebsiella pneumoniae,* has generated huge burden worldwide and persistence of CR*Kp* infections is a serious health concern.[1] Production of carbapenamases, which is encoded by the genes KPC, NDM, and OXA, is one of the most important mechanisms of resistance in carbapenem resistant *K. pneumoniae* (CR*Kp*).[1] Colistin is one of the last resort therapeutic options available to treat against CR*Kp* infections. The clinical potential of colistin has been significantly compromised by plasmid-borne colistin resistance genes (*mcr)*.[2] Furthermore, ongoing colistin resistance among CR*Kp* is another unanswered concern that is limiting treatment options for *K. pneumoniae* infections.[2] The root to rise of carbapenem and colistin resistance is multi-factorial. Major factor in developing resistance is a lack of good antibiotic stewardship which leads to misuse of antimicrobials, inappropriate empiric therapy, and leading to overuse of antimicrobials in food and animal industry.[3,4]

Recent report of colistin-resistant CR*Kp* isolates in other countries are alarming.[5] There is limited report on colistin resistance among CR*Kp* in our region. Hence, we conducted this study to determine colistin resistance among the CR*Kp* isolates to understand the underlying resistance mechanisms.

Materials and Methods

We conducted a cross-sectional descriptive study on 203 archived uropathogenic *K. pneumoniae* isolates, which were collected from December 2017 to January 2019. The study isolates were revived on MacConkey agar and confirmation of *K. pneumoniae* isolates was done by standard biochemical tests. CR*Kp* screening was performed for all isolates on Muller Hinton agar using combined Disc Test (CDT), with imipenem (10 ug) and EDTA (0.5 M). Isolates with a 7 mm increase in zone diameter in imipenem-EDTA compared to imipenem were classified as CR*Kp* as per Clinical and laboratory standards institute (CLSI) guidelines.[6] To screen for colistin resistance, CR*Kp* isolates were inoculated on EMB agar plate supplemented with 3.5 g/ml colistin using the three-phase streaking method.[7] Appropriate positive and negative control strains were included in the study. Colistin resistant isolates were subjected to MIC assay by broth micro dilution method using cation adjusted Muller Hinton broth. Results were interpreted, MIC >2 mg/L was considered as colistin resistance, according to CLSI and European Committee on Antimicrobial Susceptibility Testing joint working group on colistin.[8] DNA extraction was performed using boiling lysis technique. Simplex Polymerase Chain Reaction (PCR) was performed to detect carbapenamase genes (bla_{NDM-1}, bla_{KPC-2} and bla_{OXA-1}) and plasmid mediated colistin resistant genes ($mcr\text{-}_1$ and $mcr\text{-}_2$) for the colistin resistant CR*Kp* isolates. The PCR primers and cycle conditions were followed as per previous literature.[9,10] The amplified PCR products were resolved in 1.5%

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agarose gel electrophoresis containing 0.5 mg/ml ethidium bromide with 100 bp ladder (Gene Direx) and visualized in gel documentation system (Carestream Gel Logic 212 Pro, USA).

Results

Among the 203 *K. pneumoniae* isolates, 54.8% (111/203) isolates were resistant to carbapenem, of which 62.2 % (69/111) isolates were resistant to imipenem and 95. 5% (106/111) isolates were resistant to meropenem. Colistin resistance was observed in 13% (9/69) and 13.2 % (14/106) of the imipenem and meropenem resistant *K. pneumoniae* isolates, respectively. Among the 14 colistin resistant CR*Kp* isolates, 57.1% (8/14) and 35.7% (5/14) possessed bla_{NDM-1} gene and bla_{OXA-1} gene, respectively. Three isolates (21.4 %) (3/14) found to possess both bla_{NDM-1}, and bla_{OXA-1} gene. None of the isolates possessed bla_{KPC-2} gene and the $mcr_{(1-2)}$ genes in our study.

Discussion

Antibiotic resistance is increasingly a major health concern, resulting in treatment failure to human infections. UTIs caused by MDR *K. pneumoniae* isolates decreases the efficacy of many antimicrobial medications which limits the therapeutic options and challenges the right treatment. Colistin is prescribed as the last line drug to treat aggressive infections caused by drug-resistant Gram-negative bacteria including CR*Kp* isolates.[2] However, due to inappropriate use, Gram-negative bacteria have developed resistance to carbapenem and colistin antibiotics.[3,4] Several reports indicate a rapid surge of plasmid-mediated colistin resistance among the clinical isolates of *K. pnuemoniae*.[2,4,5,7] This study focussed on the MDR *K. pneumoniae* isolates from UTI. Rate of carbapenem resistance was high in our study, this is in agreement with a previous study conducted in North India.[11] In the present study, meropenem resistance was higher (52.2%) than imipenem resistance (34%) among the *K. pneumoniae*, which is similar to the findings of another study in our region.[12] Majority of colistin resistant CR*Kp* possessed bla_{NDM-1} (57.1%) followed by bla_{OXA-1} (35.7%) and none of the isolates possessed bla_{kpc-2} gene. The absence of bla_{kpc-2} in our isolates indicates that the transposon carrying the gene has mutated and further plasmid DNA sequence analysis will be required to determine the changes in the resistant gene. In our study, 13% of CR*Kp* isolates were colistin resistant, with MICs ranging from 4g/ml to 512g/ml and none of the colistin resistant *K. pneumoniae* isolates had plasmid-mediated $mcr_{(1-2)}$ genes. This is in accordance to a study conducted in North India, were 18.5% of colistin resistance were observed in uropathogenic *K. pneumoniae* and none of the isolates possessed *mcr* genes.[13] Colistin resistance in *K. pneumoniae* could be related to the existence of chromosomally mediated gene - *mgrB* in the absence of *mcr* genes. Previous studies have reported that mutations in the *mgrB* gene are the most common cause of colistin resistance in *K. pneumoniae*.[14-16]

Conclusion

The colistin resistance among the CR*Kp* isolates pose a serious threat to patient care around the world. In this study, bla_{NDM-1} was the predominant carbapenamase gene among the colistin resistant CR*Kp* isolates. Colistin resistance in CR*Kp* isolates emphasises the significance of antibiotic judiciousness, and the need for ongoing surveillance of carbapenem and colistin resistance in common Gram-negative bacteria to avoid plasmid mediated resistance.

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