

Low Prevalence of Carbapenem-Resistant *Klebsiella pneumoniae* in Zabol, Southeast of Iran

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Abstract

Introduction: *Klebsiella pneumoniae* is a well-known pathogen which causes different kinds of infections including pneumoniae, urinary tract infection, and bloodstream infection. Therefore, the present study aimed to investigate the incidence of carbapenem-resistant *K. pneumoniae* (CRKP) in Zabol, which is located in Sistan and Baluchestan province, southeast of Iran.

Methods: A total of 70 clinical specimens of *K. pneumoniae* were collected from patients who referred to Amirmomenin hospital affiliated with Zabol University of Medical Sciences during (December) 2017-2018. Then, resistance to nine different antibiotics was evaluated based on the purpose of the study. Finally, polymerase chain reaction (PCR) amplification was performed using specific primers for detecting *bla*AIM, *bla*VIM, *bla*NDM-1, and *bla*SPM genes.

Results: The highest sensitivities of the isolates were related to ertapenem (n=68, 97.1%), meropenem (n=67, 95.7%), followed by gentamicin (n=65, 92.8%) and amikacin (n=65, 92.8%). In addition, 3 isolates were imipenem-resistant (4.3%), which were metallo-beta-lactamase positive as well. Eventually, based on the results of PCR, two isolates were found to be *bla*NDM positive.

Conclusion: In general, the results of this study revealed that the prevalence of CRKP was low in the region under investigation. Therefore, continued monitoring of antibiotic resistance profile is required for hindering the emergence and spread of drug-resistant bacteria.

Keywords: *Klebsiella pneumoniae*, *bla*NDM, Metallo-beta-lactamase, Antibiotic resistance

Introduction

Enterobacteriaceae are well-known gram-negative bacteria, many of which are present as the normal flora of the gut microbiota in humans and animals while the other bacteria are isolated from the soil or water. *Klebsiella pneumoniae* is regarded as one of the most important members of the family Enterobacteriaceae and is responsible for different kinds of infections like pneumoniae, urinary tract, bloodstream, and liver abscesses.^{1,2} In addition, carbapenems such as meropenem, imipenem, ertapenem, and doripenem are the members of beta-lactam antimicrobials with substantial efficacy in treating penicillin and cephalosporin-resistant infections.³

Several mechanisms such as porin loss, the over-expression of efflux pumps, and hydrolyzing enzymes are identified in carbapenem-resistant (CR) isolates.^{4,5} One of the most essential mechanisms of CR is to produce metallo-beta-lactamase (MBL) enzymes including imipenemase,

Verona integron-encoded MBL, New Delhi MBL, German imipenemase, São Paulo MBL, Adelaide imipenemase, and Seoul imipenemase.^{4,5}

Carbapenem-resistant *K. pneumoniae* (CRKP) infection has been considered a major cause of mortality and morbidity worldwide, especially in developing countries including Iran.^{5,6} However, limited information is available regarding the prevalence of CRKP in Zabol. Therefore, the current study sought to evaluate the prevalence of CRKP in this region in Sistan and Baluchestan province, located in the southeast of Iran.

Materials and Methods

Specimen Collection and Bacterial Identification

The present study was conducted using the clinical specimens of *K. pneumoniae* obtained from patients referring to Amirmomenin hospital affiliated to Zabol University of Medical Sciences,

Zabol, Iran during (December) 2017-2018. Presumptive identification was performed by conventional biochemical tests including lactose fermentation, Gram stain, catalase, and oxidase. Furthermore, *K. pneumoniae* species-specific polymerase chain reaction (PCR) was performed by primers amplifying internal transcribed spacer region based on previously described conditions.^{6,7}

Antimicrobial Susceptibility Testing

Antibiotic resistance was assessed using ertapenem (10 µg), meropenem (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), amikacin (30 µg), ceftazidime (30 µg), and ceftazidime (30 µg) antibiotics which were purchased from the MSAT Company (Merseyside, UK). Then, the resistance was ascertained by Kirby-Bauer's disk diffusion method and Clinical and Laboratory Standards Institute guidelines.⁸ Further, the minimum inhibitory concentration of imipenem (Sigma-Aldrich, USA, within the range of 64-0.5 µg/mL) was determined using microdilution method.⁸ Moreover, *Pseudomonas aeruginosa* ATCC 27853 was used as quality control. Finally, imipenem-resistant isolates were subjected to phenotypical detection of MBL enzymes by imipenem-EDTA combined disk test as described earlier⁹ and the known clinical isolate of MBL-producing *P. aeruginosa* was utilized as quality control.

PCR

DNA Extraction

The boiling method was employed for DNA extraction.¹⁰ Briefly, 2 fresh colonies of *K. pneumoniae* were completely dissolved in 400 µL sterile distilled water. Then, the obtained suspension was heated at 100°C for 10 minutes. Eventually, the suspension was centrifuged at 13000 g for 10 minutes in order to separate DNA and the supernatant was used for the target amplification.

PCR Method

PCR amplification was performed using specific primers (Table 1) in order to detect *bla*AIM, *bla*VIM, *bla*NDM-1, and *bla*SPM genes.¹¹ Ampliqon (Ampliqon, Danish) ready to use master mix was employed for PCR amplification based on the condition described earlier.¹¹ Finally, the separated PCR products were visualized by staining with

Table 1. The Primers Used in This Study

Gene	Sequence (5-3)	Amplicon	Ref.
<i>bla</i> NDM	F-GGTTTGCGATCTGGTTTTTC R-CGGAATGGCTCATCAGGATC	621	11
<i>bla</i> VIM	F-GATGGTGTGGTCCGATA R-CGAATGCCGAGACCAG	390	11
<i>bla</i> AIM	F-CTGAAGGTGTACGGAACAC R-GTTCGGCCACCTCGAATTG	322	11
<i>bla</i> SPM	F-AAAATCTGGGTACGCAAACG R-ACATTATCCGCTGGAACAGG	271	11
ITS	F- ATTTGAAGAGTTGCAACCGAT R-CCG AAGATGTTCACTTCTGATT	260	7

Sybr safe (Thermo Fisher Scientific Inc., USA).

Results

Seventy isolates of *K. pneumoniae* were collected from sputum (5 cases, 7.1%), urine (58 cases, 83%), blood (3 cases, 4.2%), and wound (4 cases, 5.7%). Among these, 40 (57.1%) isolates belonged to males and 30 (42.9%) of them were related to females. The results of *K. pneumoniae* species-specific PCR are illustrated in Figure 1. As shown, the isolates are mostly susceptible to ertapenem (n=68, 97.1%), imipenem (n=67, 95.7%), meropenem (n=67, 95.7%), followed by gentamicin (n=65, 92.8%), amikacin (n=65, 92.8%), ceftazidime (n=60, 85.7%), ciprofloxacin (n=58, 82.8%), ceftazidime (n=57, 81.4%), and ceftazidime (n=57, 81.4%) antibiotics. Totally, 3 (4.3%) isolates were imipenem-resistant with a MIC of 16 µg/mL. Additionally, all imipenem-resistant isolates were MBL positive by imipenem-EDTA combined disk test. Based on the results of PCR displayed in Figure 2, two isolates were *bla*NDM positive while other resistant genes were not detected.

Discussion

The current study investigated the antibiotic resistance profile of 70 *K. pneumoniae* isolated from clinical specimens of patients who referred to Amiralmomenin hospital of Zabol as the only referral hospital in the region. The findings revealed that the majority of isolates (80%) were susceptible to the tested antibiotics, namely, ceftazidime, carbapenems, ciprofloxacin, gentamicin, ceftazidime, amikacin, and ceftazidime.

Different antibiotics such as aminoglycosides and beta-lactamases are commonly used to treat the infection caused by *K. pneumoniae* while the prevalence of antibiotic resistance has rapidly increased in recent years threatening global public health, especially in developing countries.^{12,13} In fact, treating drug-resistant infections has become more challenging owing to the evolution of several antibiotic-resistance mechanisms resulted from

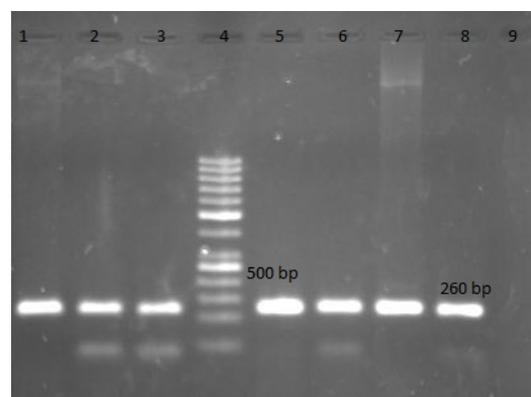


Figure 1. Species-Specific PCR (ITS-Region) Electrophoresis for *K. pneumoniae*; Line 1: Positive control (260 bp); Line 4: DNA 100 bp ladder; Line 9: Negative control; Lines 2, 3, 5, 6, 7, and 8: Positive *K. pneumoniae* isolates.

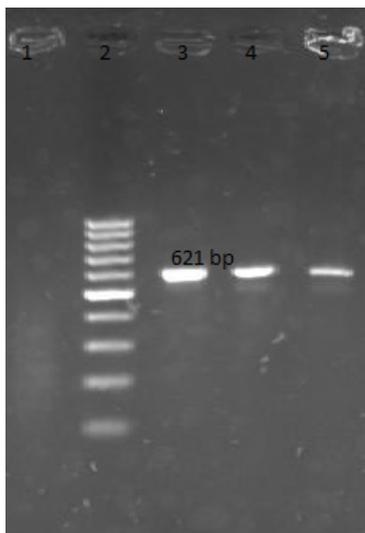


Figure 2. *bla*NDM (621 bp) Electrophoresis. Line 1: Negative control; Line 2: DNA 100 bp ladder; Line 3: Positive control (*E. coli bla*NDM positive); Lines 4 and 5: Positive clinical isolates

widespread use and misuse of antibiotics.^{12,13}

In addition, based on the results of the present study, the isolates were mainly found to be susceptible to ertapenem, imipenem, meropenem, gentamicin, and amikacin. Overall, 3 isolates were imipenem-resistant. Further, the prevalence of antimicrobial resistance isolates varied between and within different countries. For instance, Fallah et al, in their study which was conducted in Tehran, reported that 59%, 36%, and 55.5% of the investigated *K. pneumoniae* isolates were resistant against ceftriaxone, ceftazidime, and ciprofloxacin, respectively.¹³ In addition, the findings of a comprehensive meta-analysis showed that the resistance rate of *K. pneumoniae* against different antibiotics in different provinces of Iran was at an alarming level.¹⁴ For example, based on a report, resistance to amikacin ranged from 6.1% in Qom province to 47% in Isfahan province. Furthermore, resistance to ciprofloxacin and gentamicin in different provinces of Iran differed greatly, ranging from 12% to 53% and 6% to 59%, respectively.¹⁴

Internationally, resistance to different classes of antibiotics such as beta-lactam and aminoglycoside in the majority of European countries such as Denmark, Finland, Sweden, Germany, and Norway are much lower compared to Iran.¹⁴ These differences may be attributed to the low quality of personal hygiene, the indiscriminate use of antibiotics, infection control policies, and the presence of some risk factors such as previous hospitalization and long-term staying at ICU.^{14,15}

Since carbapenems are relatively resistant to hydrolysis by most β -lactamases, they are currently used to treat serious infections caused by multi-drug resistance bacteria, showing resistance against cephalosporins, penicillins, and β -lactam/ β -lactamase inhibitor combinations.¹⁶ CR isolates of *K. pneumoniae* are known to be associated with

high mortality and morbidity rates due to limited available therapeutic alternatives, indicating the importance of detection strategies.¹⁴⁻¹⁸

Based on the results of the current study, 3 (4.3%) *K. pneumoniae* isolates were CRKP among which two of them were *bla*NDM positive. Moreover, a comprehensive meta-analysis conducted to estimate the prevalence of CRKP in different provinces of Iran indicated that 11% of the isolated *K. pneumoniae* were CR, ranging from 0.004 in Tehran to 58% in Isfahan provinces.¹⁴ Conversely, the reported prevalence of CRKP in Romania and Greece with respective frequencies of 24% and 61% were much higher than that of Iran, which contradicts the results of the present study.¹⁵ On the other hand, no CRKP isolates were detected in reports from Sweden, Finland, Estonia, and Iceland.¹⁵

These contradictory results may be related to the low quality of personal hygiene,¹⁴⁻¹⁸ variable sample sizes, the indiscriminate use of antibiotics, poor infection control policies, and the presence of some risk factors like previous hospitalization and long-term staying at ICU.¹⁴⁻¹⁸ The results of the current study further revealed that 4.3% of isolates were MBL enzymes positive using imipenem-EDTA combined disk test. Among them, two isolates were *bla*NDM positive while the other investigated resistance genes were not detected. Several independent studies reported *bla*NDM-harboring *K. pneumoniae* from different provinces of Iran.^{18,19}

The mechanisms of resistance to carbapenems are multi-factorial including the overexpression of efflux pumps, the production of extended spectrum beta-lactamases (ESBL), and mutations in porins or Penicillin-binding Proteins. Producing ESBL is regarded as the most important mechanism employed by the bacteria to destroy the structure of beta-lactam antibiotics. Based on their structural properties, ESBLs are classified into A, B, C, and D groups. Class B beta-lactamases (i.e., *bla*VIM, *bla*IMP, *bla*NDM, and *bla*SPM), which include MBLs, are among the most essential mechanisms.¹⁴⁻¹⁹

The findings of different independent studies demonstrated that plasmid-expressed *bla*NDM-1 gene encoding the NDM-1 metallo- β -lactamase is spreading worldwide. For example, different outbreaks of *bla*NDM producing isolates are reported from patients referring to hospitals in India, Pakistan, Bangladesh, Poland, the US, Canada, and Spain.²⁰ Rapid and accurate detection of MBL genes is of paramount importance since these genes are normally located at transposable genetic elements such as plasmids and transposons facilitating the spread of resistance genes between different isolates. In addition, these resistance genes are accompanied by other resistance genes such as beta-lactam hydrolyzing enzymes, aminoglycoside modifying enzymes, and 16SrRNA methylase genes (i.e., *armA*, *rmtA*, *rmtB*, and *rmtC*), conferring resistance to all β -lactams and aminoglycosides.¹⁸⁻²⁰

Conclusion

In general, the incidence of CRKP was not at a high level in Zabol region. Accordingly, the detection of *bla*NDM-producing *K. pneumoniae* highlights the necessity of continued monitoring of antibiotic resistance profile in the hospitals. More importantly, failure in accurate and timely detection of MBL genes may result in the emergence of multi-drug resistant bacteria.

Ethical Approval

The study was approved by the Ethics Committee of the Zabol University of Medical Sciences (Zbmu.1.REC.139.228).

Conflicts of Interest

The authors declare that they have no competing interests.

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