High Prevalence of New Delhi Metallo-beta-Lactamase (NDM) producing Klebsiella pneumoniae in Al Najaf province

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Abstract

Background: Carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) particularly New Delhi metallo-B-lactamase (NDM) is a serious public health concern globally. The aim of the study to determine the molecular epidemiology of *bla*$_{NDM}$-producing clinically isolated *K. pneumoniae* **Methods:** about 121 *K. pneumoniae* clinical isolates were collection from Private Laboratories and Public Health Laboratory in Najaf province during the period of study, determine the antibiotic susceptibility of *K. pneumoniae* isolates by using Kirby-Bauer disk diffusion method to 25 antimicrobial agents according to the CLSI (2021). Molecular detection for MBL genes by multiplex PCR **Results:** Antibiotic susceptibility test revealed that from 121 *K. pneumoniae* clinical isolates CR-KP isolates had represented in 17(14%). All isolates of CR-KP were extensively drug-resistant (XDR). Frequency of CR-KP isolates among 23 XDR *K. pneumoniae* isolates were 17 (73.9%). The PCR data of MBL genes revealed that the frequency of MBL genes among XDR *K. pneumoniae* as following *bla*NDM, and *bla*IMP were 73.9% and 8.7% respectively **Conclusion:** Among the XDR *K. pneumoniae* isolates, *bla*$_{NDM}$ was the most prevalent carbapenemase gene. The extensivlely resistant lineage of NDM-producing *K. pneumoniae* is prevalent in the clinical setting.

Keywords: *Klebsiella pneumoniae*; Carbapenem; Carbapenem-resistant; New Delhi Metallo-beta-Lactamase (NDM)

Introduction

K. pneumoniae is the most clinically relevant *Klebsiella* species, with significant virulence and antibiotic resistance. *K. pneumoniae* infections are the most common healthcare-associated infection worldwide (1). Carbapenems are regarded to be effective agents for the treatment of severe infections caused by ESBLs producing. *K. pneumoniae* isolates (2). The incidence of carbapenem-resistant *K. pneumoniae* detected at alarming rates worldwide in Europe, South America, Africa, North America, and Asia including Iraq (3,4). *K. pneumoniae* isolates resistant to Carbapenem are spreading all over the world, which poses a therapeutic dilemma since fewer medicines are effective in treating them. This, in turn, has sped up the development of XDR and PDR Gram-negative bacteria (5). The infections with *K. pneumoniae* resistant to carbapenem, the treatment options become restricted, where used colistin as the last-resort antimicrobial (6). Mechanisms are accountable for the creation of carbapenem resistant *K. pneumoniae*, firstly mechanism suggests the procuration of the carbapenemase genes, which express enzymes that degrade carbapenem, while secondly needs reducing in antimicrobial intake by lack of porin expression correlated with overexpression of β-lactamases with a limited affinity to carbapenem antibiotics (7). As of now, β-lactamases are categorized according to Ambler's categorization of their molecular structures. The Ambler classification divides β-lactamases into four classes A, B, C, and D based on conserved features in the core sequences that make up the proteins. Class B beta-lactamase is MBL that has the metal Zn2+ at the enzyme active center, as opposed to a serine residue in classes A, and D(8). With the exception of monobactams like aztreonam, MBLs have a wide substrate range and can catalyze the hydrolysis of almost all β-lactam antibiotics (9). Due to their metalloenzyme nature, MBLs are resistant to the currently known beta-lactamase inhibitors but may be inhibited by metal ion chelators such as ethylene diamine tetra acetic acid (EDTA) (10). The MBL gene has been localized to chromosomes, plasmids, integrons, transposons, and other genetic elements. There are several different integrons that include gene cassettes encoding the most prevalent MBL enzymes (IMP, VIM, NDM, GIM, and SIM). In the presence of plasmids or transposons, these integrons transfer with relative ease (10). The *bla*NDM gene was identified in Indian patient who had previously been treated in New Delhi hospitals in 2008 in Sweden (11). Worldwide attention is now focused on NDM-1 positive *Enterobacteriaceae*, as this gene can be acquired from the environment by bacteria and can be distributed from India into another country during travel. *bla*NDM-gene was mostly identified in important strains of *K. pneumoniae* and *E. coli*, however, also identified in *P. aeruginosa* and *A. baumannii* isolates (12,13,14). In this group, at least 21 variants were described and identified (15). Unlike several other genes for carbapenemase, *bla*NDM-1 is easy to spread within *Enterobacteriaceae* and unrelated species because the plasmids encoding this gene have a broad range of hosts (16). Currently, β-lactamases of the NDM type are regarded as the most important in terms of clinical and

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epidemiological significance. Microbiologists, epidemiologists, and physicians have been more concerned about antibiotic resistance since the discovery of different carbapenemases (17).

Materials and Methods

This a cross-sectional analysis was performed at the Private Laboratories and Public Health Laboratory in Najaf province during six months from September to February 2022. The study population consisted of 121 *K. pneumoniae* clinical isolates isolated from patients clinically suspected by the physician to have infections, and the samples immediately transported to the microbiology laboratory, all *K. pneumoniae* isolates collected in this study had been confirmed and identified depending on Morphological characteristics, Microscopically examination (gram-stain), and Biochemical tests according to the standard method described by MacFaddin's instructions(18). *K. pneumoniae* isolates were also identified by the VITEK-2 compact system.

Antimicrobial Sensitivity Testing

Antimicrobial sensitivity testing of *K. pneumoniae* isolates was performed by using disk diffusion methods (Kirby-Baure method), The resistance profile to 25 antibiotics disks including: piperacillin(100µg), ticarcillin(75µg), piperacillin-tazobactam(10µg), ticarcillin-clavulanic acid(75µg), cefoxitin(30µg) ceftazidime(30µg), cefotaxime(30µg), ceftriaxone(30µg), aztreonam(30µg), cefepime(30µg), meropenem(10µg), imipenem(10µg), gentamicin(10µg), amikacin(30µg), tobramycin(10µg), netilmicin (30µg), ciprofloxacin(5µg) , levofloxacin(30µg), oflaxacin(5µg), lomefloxacin(10µg), moxofloxacin(5µg), chloramphenicol(30µg),trimethoprim(5µg),
Sulfonamides (300µg), colistin(25µg). All susceptibility results were interpreted according to the standard values performed by CLSI 2022(19). Following antibiotic susceptibility testing, the multiple antimicrobial resistance profiles of tested *K. pneumoniae* isolates were determined based on the standardized international terminology proposed by CDC center and the European Centre for Disease Prevention and Control (ECDC) definitions (20) and according to the CLSI 2022(19) recommendations using the Kirby-Bauer method. They were categorized into groups according to their classification as MDR, XDR or PDR.

Molecular detection of NDM gene

DNA was extracted from the isolates by using the protocol kit of the manufacturing company (Favorgen,Taiwan), XDR *K. pneumoniae* isolates were screening for detection the presence MBLs genes by multiplex PCR, the primers sequence was published in previous article by Ellington *et al.* (21). Amplicons were separated by agarose gel electrophoresis in 1.5 % (w/v) agarose gel, stained with ethidium bromide. The positive results were detection when the DNA band base pairs of sample equal to the target product size. The PCR were prepared in total volume 50µl PCR mixture including 25 µl Promega Master mix, 1.5µl forward primer (10µM), 1.5µl reverse primer (10µM), 2µl DNA template (10-250 ng), and 8µl nuclease free water. PCR conditions had performed in T3000 thermocycler (Biometra).

Results

Of all the 121 *K. pneumoniae* clinical isolates following antimicrobial susceptibility tested, present study classified 23 (19%) isolates as XDR, bacteria that have developed resistance to at least one agent across all classes of antimicrobials, but still responding to just one or two classes of antimicrobials. antibiotic susceptibility testing indicated that 17 (73.9%) of the 23 XDR isolates showed resistance to at least one carbapenem and categorized as carbapenem resistant isolates.
Molecular Detection and Distribution of MBLs genes: XDR *K. pneumoniae* isolates were examined by multiplex PCR for the occurrence gene determinants encoding MBL genes (*bla*IMP, *bla*VIM, and *bla*NDM). Overall, these XDR isolates had a high prevalence rate of carbapenemase genes, PCR analysis showed the presence of genes conferring resistance to carbapenems in 18 (78.3%) isolates. Whereas, only 5 (21.7%) isolates were not determined to have carbapenemase-related resistance genes, 3 of them were resistant to both meropenem, imipenem. The results of MBL genes detected among these isolates are defined in Table (1). The results appeared that *bla*NDM, and *bla*IMP were the only detected MBL genes. The agarose gel electrophoresis of the PCR-amplified products for interest genes are shown in Figure (1). In total, 17 (73.9%) XDR isolates were found to be MBL producers. Among the three tested genes encoding for MBL, bla_{IMP}, it was detected in two (8.7%) isolates: KP8 and KP9, while bla_{NDM}, being the most predominant gene, was detected in 17 (73.9%) isolates: KP1, KP2, KP3, KP4, KP5, KP6, KP7, KP8, KP9, KP10, KP11, KP15, KP16, KP17, KP19, KP20, and KP23. The bla_{VIM}, MBL gene was not detected in any isolates in this study. combined of *bla*NDM, and bla_{IMP} appear in (2 isolate, 8.7%).

Table (1): Detailed results of the carbapenemase genes carrying XDR *K. pneumoniae* isolates (n= 23)

Type of *bla* gene	No. of isolates (%)	Isolate symbol	Susceptibility to:	
			Imipenem	Meropenem
bla_{NDM}	15(26.1)	KP1, KP2, KP5, KP6, KP4	R	R
		KP10, KP15, KP16, KP17	R	R
		KP11	I	R
		KP3	S	R
		KP7, KP19, KP20	S	S
		KP23	S	I
bla_{NDM}+ bla_{IMP}	2 (8.7)	KP8, KP9	R	R
bla_{KPC}	1 (4.3)	KP21	R	R
No gene	5(21.7)	KP12	R	R
		KP13	S	S
		KP14	I	S
		KP18, KP22	R	R

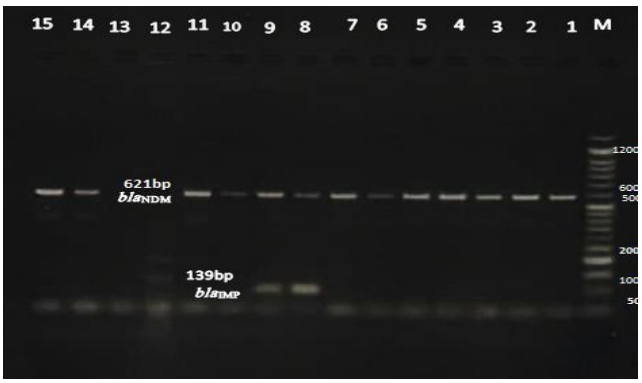

Figure (1): Agarose gel with ethidium bromide - stained of multi-plex PCR amplified product from extract DNA of K. pneumoniae isolates with blaNDM, blaIMP blaVIM genes primeres. The electrophoresis performed at 80 volt for 90 minutes. Lane (M) is DNA molecular size marker (1500 -bp ladder). 1,2,3,4,5,6,7,8,9,10,11,14, and15 show positive results with blaNDM gene (621 bp) , 8 and 9 show positive results with blaIMP gene (139).

Discussion

Extensively drug resistant isolates, particularly those resistant to carbapenems, have become a major concern of health institutions. Unfortunately, during the last decade, *K. pneumoniae* with carbapenemase production emerged noticeably. (22). The primary goal of this research was to conduct an epidemiological analysis of XDR *K. pneumoniae* in Najaf province by determining its prevalence and the kinds of carbapenemases it produces.

MBLs have a wide range of substrates and can hydrolyze practically all beta-lactam antibiotics outside monobactams (9). According to the results presented here, the most frequently detected carbapenemases in study population was NDM, 73.9% of the XDR isolates were bla_{NDM} positive. The observation of the NDM carbapenemase in majority of XDR isolates during the study period is alarming. This may suggest a more serious endemic of this gene among *K. pneumoniae* isolates in Najaf province or even in Iraqi, and is worthy of further epidemiological studies. The rise of NDM has become a significant public health problem that provides a challenge for treatment of severe illnesses around the globe. In 2008, a Swedish patient hospitalized in New Delhi, India with a *K. pneumoniae* UTI was the first report to bla_{NDM} harboring *K. pneumoniae* (11). Several studies found that NDM-mediated carbapenem resistance was widespread in Indian subcontinent and majority of the patients that have acquired the NDM-producing isolates have been either travelled or in contact with India, Bangladesh or Pakistan (23). Annually, many peoples come to Najaf from numerous countries including Indian subcontinent and in the latest years, many Iraqi patients were travel "medical tourism" to India and other countries for treatment purpose, which may help in the acquisition of this gene. However, in Iraq, NDM producing *P. aeruginosa* was first identified in two hospitalized patients in Najaf (14). However, the extent to which *Enterobacteriaceae* in Najaf hospitals have developed resistance to carbapenems as a result of NDM is yet unclear. Recently, Al-Hasnawi (4) from Najaf reported the first detection of bla_{NDM} in 4 (18.2%) of *K. pneumoniae* isolates. One general concept from this study may be the endemic outbreak of NDM producing *K. pneumoniae* isolates in Najaf laboratories which can be a serious concern. The high rate (73.9%) of NDM producing *K. pneumoniae* isolates found in this study is alarming threat and more than the rates reported in, Saudi Arabia, 14% (24), China , 22.8% (25) and Turkey 38.9% (26). Since the bla_{NDM} gene is located on a plasmid that can be easily transferred from one isolate to another, so bla_{NDM} harboring *K. pneumoniae* is has a particularly important (27). Thus, the detection of this enzyme in Iraqi hospitals attracted much attention and publicity.

Conclusion

Among the carbapenems resistant isolates, bla_{NDM} was the most prevalent carbapenemase gene. The extensivlely resistant lineage of NDM-producing *K. pneumoniae* is prevalent in the clinical setting.

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