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Original article

Prevalence of New Delhi metallo-beta lactamase gene among *Klebsiella* species isolates: An Egyptian study

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ABSTRACT

Background: Emergence of carbapenemase producing microorganisms, specifically the New Delhi metallo-β-lactamase (NDM) and its different groups, in different areas all over the world, raised a global health concern. New Delhi metallo-beta-lactamase destructs carbapenems, that are considered one of the last lines of treatment for infections caused by resistant strains. **Aim:** Our study aimed for detection of the NDM gene among the carbapenem resistant *Klebsiella* spp. isolated from a variety of clinical samples and detection of the associated risk factors for acquiring such infection. **Methods**: Two hundred carbapenem-resistant *Klebsiella* isolates were enrolled and subjected to microbiological identification and antibiotic sensitivity testing. Isolates were gathered from 560 diverse clinical specimens collected from various medical departments in Ain Shams University Hospital. *Klebsiella* spp. isolates were exposed to Imipenem-EDTA combined disk method for the identification of metallo-β-lactamase (MBL) production, at that point real time polymerase chain reaction (RT-PCR) was done for the detection of the New Delhi metallo-β-lactamase *(bla_{NDM-1})* gene among MBL phenotypic producing organisms. **Results:** Using Imipenem-EDTA, 82 *Klebsiella* isolates were detected to be MBL producers. The PCR showed that the blaNDM-1 gene is carried by 80 of the 82 MBL positive isolates (97.6%). There was no statistical significance regarding the risk factors and the gene acquisition. **Conclusion:** Real time-PCR used for detection of MBL is more sensitive than the phenotypic detection methods. There was no specific risk factor identified for acquiring of bla_{NDM-1} gene in this study . Increasing in the incidence of MBL prompt the need to evolve preventive measures to reduce their spread.

Introduction

Antibiotics were mainly invented to fight microorganisms causing infections, but the development of new resistant genes like the New Delhi-metallo-beta-lactamases (NDM) diminished the capacity of beta lactam antibiotics to treat such diseases. This might be due to the development of new patterns in gene mutations [1], which might lead to delay in the invention of new anti-microbials for treatment and subsequently the infections with these organisms will turn into a disaster worldwide [2].

Among the *Enterobacteriaceae* family, the production of carbapenemases has become an essential factor for the wide range of β-lactam opposition. Carbapenemases are subdivided into 3 molecular classes A, B and D [3]. Species which are positive for NDM genes, are dangerous as they tend to cause unnoticed asymptomatic carriers among general community, in addition to the deficiency of

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a successful antimicrobials against NDM-1-positive superbugs [4]. The NDM is an adaptable molecular class B β-lactamase that was first recuperated from *Klebsiella pneumoniae (K.pneumoniae)* and *Escherichia coli*, detached in Sweden in 2008, from an Indian patient who moved one day ago from a hospital in New Delhi [5].

Since the discovery of NDM it has become a source of concern. It has been recognized around the world in the UK, India, Pakistan [6], the Sultanate of Oman, Morocco, Algeria, Iraq, Egypt, Taiwan, America, France, Kenya, Italy, Japan, the Netherlands, Norway, Morocco, Lebanon and Emirates [7]. Experiments showed an expansion in the recurrence of blaNDM among *K. pneumoniae* species that were isolated from clinical specimens since the year 2009 till now [8]. New Delh metallo-beta-lactamases-1 has been distinguished in both chromosomes and plasmids. Plasmids conveying the bla NDM-1 gene additionally convey various different genes conferring resistance giving protection from a lot more classes of antibiotics apart from beta-lactam. In this manner, NDM-carrying strains are impervious to all classes of antibiotics accessible [9]. Only aztreonam, tigecycline, colistin and fosfomycin can be effective but these antimicrobial also have limitations.. However, colistin has uncertain efficacy in lung infections, and its utilization has consistently been hampered by the event of renal impairment, and less significantly its neurological side effects [10].

Thus, reliable detection and active surveillance are crucial to prevent the dissemination of such resistance [11]. A group of non-molecular-based tests like modified Hodge test (MHT) and inhibition studies by ethylene diamine tetraacetic acid (EDTA) have been proposed for detecting the carbapenemase action. Anyway, no currently accessible tests is known to have 100% explicitness or affectability [12].

A variety of culturing modalities have been used for screening of carbapenem resistant strains like the commercially available chromogenic agar media with variability in the sensitivity and specificity ranging from 75.9–81.3% [13]. Though, the molecular techniques remained the golden test for the detection of carbapenemases. The vast majority of them depend on polymerase chain reaction (PCR), and might be trailed by sequencing for exact identification of carbapenemase variations (e.g. *VIM*-type, *KPC*-type, *NDM*-type, and *OXA-48*-type). The real-time PCR (RT-PCR) technique done directly on the isolated colonies can give results within 4–6 hours or less, with excellent sensitivity and specificity [14]. New Delhi metallo beta lactamase producing organisms also carries other resistance enzymes like ESBL and Amp C β-lactamases [15].

Our study aimed for detection of the NDM gene among the carbapenem resistant *Klebsiella* spp. isolated from a variety of clinical samples and detection of the associated risk factors for acquiring such infection.

Samples and Methods

The type of our study is a cross sectional analytic study, that was conducted at the Microbiology Laboratory, Clinical Pathology department, Ain Shams University Hospital during the period from January 2019 and July 2019. The study was approved by the Research ethics Committee, Ain Shams University, Faculty of Medicine (FWA 00017585). The study was conducted on 200 carbapenem-resistant Klebsiella species collected from different clinical samples (560 specimens) handled to the microbiology laboratory (from various hospital departments) for culturing and antimicrobial susceptibility testing. Following microbiology standard operating procedures, isolates were classified based on their culture characteristics, Gram stained film morphology, and various biochemical reaction tests. Antimicrobial sensitivity testing was done for all the isolates to carbapenems (imipenem and/or meropenem) according to the Clinical and Laboratory Standards Institute recommendations, using imipenem (10 ug) and/or meropenem (10 ug) disk diffusion [16].

Information were gathered in a retrospective manner from patients who were infected with the carbapenem-resistant organisms regarding their age, sex, antibiotic intake, previous hospitalization and the presence of medical device. **All *Klebsiella* isolates were subjected to the following**:

1- Detection of metallo-β-lactamase production

Using the imipenem-EDTA (IMP-EDTA) disk method where the suspension was spread onto the Mueller-Hinton agar plate. The plate was dried for 3-5 minutes. Then two imipenem (10μg) disks were added on the plate, and 10ul of 0.5M EDTA were added to one of them to adjust the concentration needed. Plates were incubated overnight at 36± 1°C under aerobic conditions. The inhibition zone

around the IMP disk was compared to that around IMP-EDTA disk. An extension in the diameter of the inhibition zone ≥7mm around IMP-EDTA disk to that of IMP alone reveals the presence of MBL(a positive test result) [17].

2- Real time polymerase chain reaction (RT-PCR) for the detection of (*bla$_{NDM}$*) gene

Positive MBL producing isolates only were exposed to RT-PCR. The primers for PCR detection of *bla$_{NDM}$* were outlined agreeing to GenBank (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA) [5] as follows: NDM forward primer: 5'- GAC CGC CCA GAT CCT CAA -3' and NDM reverse primer: 5'- CGC GAC CGG CAG GTT -3'. Reaction components were added QuantiTect® SYBR ®Green qPCR Master Mix (2X)12.5 µl , Forward Primer 0.75 µl , Reverse Primer 0.75 µl, Template DNA 5 µl, Template DNA 5 µl , RNase-free water with a total volume of 25 µl. After mixing the reaction mix, it was loaded into the PCR cycler (Rotor Gene Q). Thermal denaturation was used to create a melting curve, which involved heating the products for 1 minute at 95°C, 30 seconds at 55°C, and 30 seconds at 95°C. Per amplicon has a distinct peak at its melting temperature (Tm: temperature at which 50 percent of the DNA is single stranded). When Tm is reached, there is a sudden decrease in fluorescence (due to separation of DNA strands and the discharge of the color). The presence of other peaks indicates a DNA contaminant or a primer dimer [18] **(Figure 1)**.

Statistics

The data gathered was double-checked, coded, organized and presented to a PC utilizing a measurable bundle for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Information was displayed and sufficient analysis was conducted in accordance with the type of data collected for each parameter.

Descriptive statistics

- Quantitative normally distributed data are presented by the mean (measure of the central tendency) and SD (measure of the variation).
- Categorical data is expressed as a number or a percentage.

Analytical statistics

When the predicted count is less than 5 in more than 20% of cells, the Fisher exact test was used to search for a relationship between two subjective variables.

Results

Out of the 560 specimen collected, 200 *klebsiella* species were isolated Imipenem/Imipenem-EDTA double disk synergy test was positive in 82 (41%) of 200 *Klebsiella* isolates denoting MBL-production. Polymerase chain reaction showed that the blaNDM-1 gene is carried by 80 of the 82 MBL positive isolates (97.6%), while the remaining isolates were non-MBL producers **(Figure 2)**.

The MBL producing *Klebsiella* isolates were collected from different infections, 58.8% were isolated from wound infections, 29.1% from respiratory tract infections, 7.3% from urinary tract infections, and 4.8% from bloodstream infections. However, there was no statistically significant relation between infection type and isolation of MBL-producing *Klebsiella* species ($p>0.05$).

There was no statistically meaningful difference between the various hospital departments and the isolation of MBL producing *Klebsiella* species ($p>0.05$). 36.6% of the MBL producing *Klebsiella* species were isolated from medical departments, 31.7% from the surgical departments and 31.7% from the different hospital ICUs.

A correlation was done between isolation of MBL among cases with history of previous hospital admission and the presence of any medical device or intake of antibiotics, but no statistical significance was observed as shown in **table (1)**.

Figure 1. Positive isolates for *bla$_{NDM-1}$*.

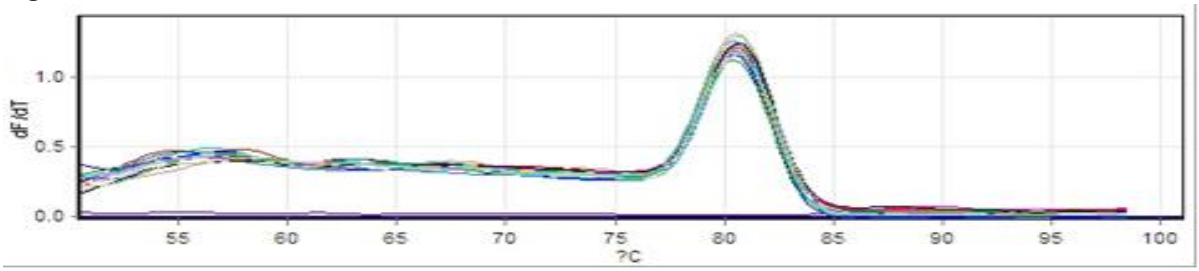

Figure 2. Percentage of MBL producing isolates among the *Klebsiella*.

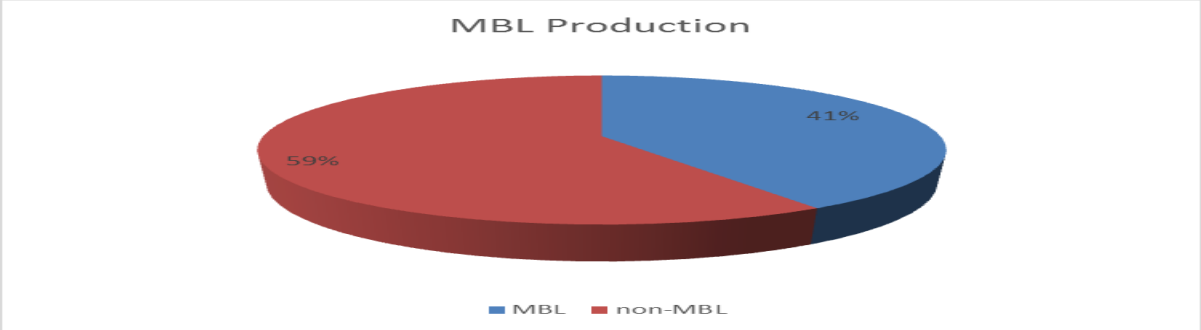

Table 1. Correlation between risk factors and acquisition of MBL among *Klebsiella* species.

Risk Factors		MBL		Chi square	
		Positive N (%)	Negative N (%)	*P value*	Sig.
Previous hospitalization	Yes	20 (24.4)	38(32.2)		
	No	62(75.6)	80(67.8)	*0.397*	NS
Presence of medical device	Device	30(36.6)	54(45.8)		
	No device	52(63.4)	64(54.2)	*0.360*	NS
Antibiotic intake	Yes	70(85.4)	104(88.1)		
	No	12(14.6)	14(11.9)	*0.685*	NS

Discussion

Metallo-beta-lactamase producers formed 41% of the overall isolates in our sample, as determined by using one phenotypic and one genotypic approach. The combined disk strategy of imipenem-EDTA (IMP-EDTA) was used to classify MBL, while RT-PCR was used to assess the existence of the bla NDM-1 gene. During the last decade, a remarkable increase in carbapenemase production was observed among isolates of *Enterobacteriaceae*, *Pseudomonas spp*, and *Acinetobacter baumannii* [19]. Since the discovery of NDM, it became a source of serious concern because NDM-positive isolates are broadly resistant to all beta-lactam antibiotics and the rapid spread of *bla* $_{NDM}$ among the hospital adapted clones, can lead to horizontal gene transfer and environmental persistence [20].

New Delhi metallo-β-lactamase variants are most widely expressed among the *Enterobacteriaceae*, according to recent research. The most frequently implicated species is *K. pneumoniae*, which accounts for slightly more than half of all positive isolates, followed by *Escherichia coli* and the *Enterobacter cloacae* complex. *Pseudomonas* spp. and *Acinetobacter baumannii* isolates that are NDM-positive are also becoming more common [21].

The frequency of MBL producing isolates, in our study was (41%) which was slightly lower than that reported in studies by **Mariappan et al.** [22], who reported the MBL to be (58.6%) and **Shenoy et al. [**15], who reported the MBL to be (93.24%) in India**. Ugwu et al.** [23], found that the most common ESBL gene was blaTEM, and nine out of ten ESBL-positive *K. pneumoniae* strains were both phenotypically and PCR-positive**.**

The frequency of bla_{NDM-1} carrying isolates, in our study (97.6%) was similar to that reported in the studies of **Abaza et al.** who tested 30 carbapenamases producing isolates of *Pseudomonas aeruginosa (P. aeruginosa)* for MBL genes and all were positive [24]. **El-Ghazzawy et al.**, a study conducted in Egypt, registered a similar frequency (96.4%) [25]. But **Raro et al.** found out only nine isolates (11.2%) out of 80 isolates collected carrying blaNDM$_{-1}$ [26]. While **Devkota et al.** reported 30% frequency of bla_{NDM-1} carrying isolates in a study done in Nepal [27]. **El-Kazzaz and Abou El-Khier** conducted a study in 2015 in El-Mansoura, Egypt,

and found that although neither NDM nor IMP genes were detected, 60 percent of MBL had VIM (Verona Integron-encoded Metallo—lactamase) genes [28].

In the current study, comparison between the different hospital departments regarding the isolation of MBL producing strains showed there was no statistically significant difference between the strains (*p>0.05*). Similar results reported by **Lavagnoli et al.** who observed no association between the acquisition of carbapenemase-producing Enterobacteriaceae (CPE) and the admission at different hospital departments in a Brazilian study [29]. However, **Masaeli and associates**, who collected 423 clinical isolates reported that admission to the ICU was a major risk factor for the acquisition of MBL by using univariate analysis [30]. Discordant results might be due to the smaller number of CPE examined in our study and the statistical method used.

Despite the fact that the majority of MBL-producing isolates (85.4%) in our sample were recovered from patients taking antibiotics, though no statistically significant association was found between antibiotic intake and the isolation of MBL producing organisms (*P> 0.05*). However, **Masaeli et al.** reported by univariate analysis a significant association between antibiotic intake and the recovery of MBL [30]. These discrepancies maybe due to difference in the numbers of the studied groups and differences in the types of antibiotics taken by the patients.

Conclusion

Polymerase chain reaction detected the blaNDM-1 gene in 97.6% of MBL producing strains, suggesting that NDM carbapenemase is responsible for 40% of carbapenemases in our hospital. The detection of carbapenemase genes in *Enterobacteriaceae* starts with a screening step and is followed by a PCR-based test to detect carbapenemase genes. While traditional susceptibility testing may easily identify carbapenem-resistant bacteria, there is an urgent need for quick and responsive detection of resistant genes in carbapenem-resistant *Enterobacteriaceae* from clinical samples. Our findings highlight the importance of using both phenotypic and genotypic MBL detection methods in routine practice to detect carbapenem resistance early and avoid the spread of this resistant pathogen. More research is required to look into the clinical and epidemiologic factors linked to MDR isolates, as well as acquisition trends, high-risk exposures, and comorbidities linked to MDR infection in Egypt. To control transmission and provide adequate empiric therapy to individuals with bacterial infections in settings with limited health care services, cost-effective management and surveillance strategies are needed to improve clinical outcomes.

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Authors contributions

Names	Credit Role
Marwa A. El-Ashry	Conceptualization
Marwa A. El-Ashry, Nadia M. ElSheshtawy	Formal analysis
Marwa A. El-Ashry	Investigation
Marwa A. El-Ashry, Nadia M Sheshtawy	Methodology
Marwa A. El-Ashry, Nadia M. ElSheshtawy	Supervision
Nadia M. ElSheshtawy	Software and Statistics
Marwa A. El-Ashry, Nadia M. ElSheshtawy	Writing - original draft
Marwa A. El-Ashry, Nadia M. ElSheshtawy	Reviewing & editing

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