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Emerging New Delhi metallo-β-lactamase-1-type-producing Gram-negative bacteria isolated from Cairo University Pediatric Hospital, Cairo, Egypt

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ABSTRACT

New Delhi metallo-β-lactamase (NDM) compromises the efficacy of almost all β-lactam antibiotics, including carbapenems. This study aimed to screen for the $bla_{\text{NDM-1-type}}$ gene and NDM-1-type carbapenemase production among Gram-negative bacteria in Cairo University Pediatric Hospital (Cairo, Egypt). Among 382 Gram-negative clinical isolates collected over the period October 2013 to May 2014, 100 clinical isolates showing reduced carbapenem (imipenem and meropenem) susceptibility were included in this study. Initial phenotypic screening for NDM enzyme production was performed by Etest for metallo-β-lactamases (EMBL). Genotypic detection of the $bla_{\text{NDM-1-type}}$ gene was done by *Taq*Man real-time PCR. Metallo-β-lactamase production was detected in 23% of the isolates by EMBL, whereas 24% of the isolates were found to be positive for the $bla_{\text{NDM-1-type}}$ gene by real-time PCR. The EMBL sensitivity was 79.2%, specificity was 94.7%, positive predictive value was 82.6%, negative predictive value was 93.5% and overall accuracy was 91.0%. Seventeen (70.8%) of $bla_{\text{NDM-1-type}}$-positive cases were hospital-acquired in origin, whilst 7 cases (29.2%) were community-acquired. Eleven isolates (45.8%) harbouring $bla_{\text{NDM-1-type}}$ were found in critical care units. In conclusion, the high prevalence of $bla_{\text{NDM-1-type}}$ carbapenemase gene among Gram-negative bacteria, with its great potential for spread in intensive care units, warrants the attention of a nationwide surveillance programme to contain its spread.

1. Introduction

Carbapenemase-producing multidrug-resistant (MDR) Gram-negative bacteria are a threat to public health not only due to the rapidly increasing rate of resistance but also due to the limited antibiotics active against them [1].

Enzymatic resistance to carbapenems in Gram-negative bacteria is mainly due to two types of β-lactamases. The first group of enzymes uses serine as the active site amino acid to inactivate carbapenems, and the main inhibitors of the serine β-lactamases are tazobactam, clavulanic acid and sulbactam. The second group of enzymes uses a zinc ion, hence their name, the metallo-β-lactamases (MBLs). The ion chelator ethylene diamine tetra-acetic acid (EDTA) is a common inhibitor of MBLs [2,3].

One of the emerging MBLs is the New Delhi metallo-β-lactamase (NDM) enzyme, which was first identified in Sweden in a patient who had travelled from India; the NDM-1-type enzyme has been detected in Pakistan, the UK, the USA, Kenya, Japan, Canada, Belgium, The Netherlands, Taiwan, Singapore, the Sultanate of Oman, Australia and Egypt [4,5]. The widespread nature of NDM may be ascribed to the location of the causative gene in a mobile genetic element near a pathogenicity island. The $bla_{\text{NDM-1-type}}$ gene is carried on plasmids and other mobile genetic elements, facilitating its rapid spread between bacterial strains and species. Thus, it is essential to study the epidemiology of bacteria harbouring NDM-1-type to avoid its further spread and to establish effective control measures [6–11].

The aim of this study was to screen for the $bla_{\text{NDM-1-type}}$ gene and NDM-1 carbapenemase production among Gram-negative bacteria in Cairo University Pediatric Hospital (Cairo, Egypt) to highlight its prevalence and the mandatory role of strict control measures to curb this outbreak that threatens public health.

2. Materials and methods

This was a cross-sectional study including 382 consecutive Gram-negative clinical isolates collected over the period October 2013 to May 2014 from Cairo University Pediatric Hospital.

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2.1. Selection of test isolates

A total of 382 Gram-negative bacterial isolates were screened for reduced susceptibility to carbapenems (imipenem and meropenem) by the disk diffusion method. All isolates were identified by API® 20E and API® 20NE (bioMérieux, Marcy-l'Étoile, France).

Antimicrobial susceptibility testing was performed by the modified Kirby–Bauer disk diffusion method using Muller–Hinton agar (Oxoid Ltd., Basingstoke, UK) with aerobic incubation at 35 °C for 16–18 h according to Clinical and Laboratory Standard Institute (CLSI) guidelines [12]. The following antimicrobial disks were obtained from Oxoid Ltd.: ampicillin (10 μg); amoxicillin/clavulanic acid (10 μg); ceftazidime (30 μg); cefotaxime (30 μg); ceftriaxone (30 μg); cefepime (30 μg); cefoxitin (30 μg); imipenem (10 μg); meropenem (10 μg); aztreonam (30 μg); trimethoprim/sulfamethoxazole (1.25/23.75 μg); cefoperazone (75 μg); nitrofurantoin (300 μg); piperacillin/tazobactam (100/10 μg); gentamicin (10 μg); amikacin (30 μg); ciprofloxacin (5 μg); and polymyxin B (300 U).

Definitions of multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) organisms were according to Magiorakos et al. [13].

Of the 382 consecutive Gram-negative clinical isolates collected over the study period, only 100 isolates showed reduced susceptibility to imipenem and meropenem by Etest minimum inhibitory concentrations (MICs) (AB bioMérieux, Solna, Sweden) and were included in this study [12].

2.2. Phenotypic screening for metallo-β-lactamase (MBL) production

All 100 selected isolates were screened for MBL production by the imipenem/EDTA synergy Etest (AB bioMérieux). Interpretation of results according to the manufacturer's recommendations was as follows: the result was considered positive for MBL when the MIC ratio for the imipenem/imipenem + inhibitor (IP/IPI) was ≥8 or there was a reduction in the MIC of imipenem of ≥3 dilutions in the presence of EDTA. Also, a rounded 'phantom' inhibition zone between IP/IPI or deformation of the ellipse was considered positive for MBL regardless of the IP/IPI ratio according to the manufacturer's recommendations.

Stocks of all isolates were stored in tryptic soy broth (Sigma–Aldrich, St Louis, MO) with 20% glycerol at −80 °C for subsequent real-time PCR analysis.

2.3. bla$_{NDM-1-type}$ gene detection by TaqMan real-time PCR

Automated DNA extraction was performed using a MagNA Pure DNA Isolation and Purification Kit I (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendations.

The LightCycler® *Taq*Man probe and primer sequences derived were from the GenBank sequence **AB571289**: primer sequence F, 5′-GCT GGC GGT GGT GAC TC-3′; R, 5′-GGC AAG CTG GTT CGA CAA C-3′; and *Taq*Man probe sequence FAM-TGG CAT AAG TCG CAA TCC CCG C—BBQ [14].

The final 20 μL PCR mixture contained 5 μL of extracted DNA and 15 μL of PCR buffer composed of 2 μL each of the forward and reverse primers and 1 μL of the NDM-1 TM Probe, 7.4 μL of LightCycler® 480 DNA Master Mix (containing FastStart™ *Taq* DNA Polymerase, reaction buffer, dNTP mix and 10 mM $MgCl_2$), 2.9 μL of nuclease-free water, 0.4 μL of LightCycler® uracil *N*-glycosylase and 1.3 μL of activator [$Mn(OAc)_2$] to achieve maximum amplification specificity and efficiency.

The real-time PCR assay was performed using a LightCycler® 480 instrument programmed according to the following cycling conditions: denaturation for 10 min at 95 °C, followed by 45 cycles each of 10 s at 95 °C, 1 min at 58 °C and 1 s at 72 °C.

2.4. Bacterial control strains

DNA was extracted from strains of *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumannii* 19609 and *Pseudomonas aeruginosa* ATCC 27853 (Microbiologics Inc., St Cloud, MN) and was included in the testing panel as negative control strains.

Klebsiella pneumoniae ATCC-BAA-2146 was included as a positive control [14].

3. Results

The patient age ranged from 7 days to 72 months, with a median of 5.5 months. Of 100 Gram-negative clinical isolates showing reduced susceptibility to carbapenems, 55% were identified to be of hospital-acquired origin, whilst 45% were community-acquired. Moreover, 16% of the isolates were MDR, 78% were XDR and 6% were PDR.

The distribution of different bacterial isolates in relation to sample types is shown in Table 1. The results of imipenem and meropenem susceptibility testing by the disk diffusion method were found to be in agreement with their MICs.

MBL production was detected in 23/100 isolates (23%) by Etest MBL IP/IPI strip (EMBL), whereas 24/100 (24%) were positive for the *bla*$_{NDM-1-type}$ gene by real-time PCR. Four isolates initially tested positive by the EMBL and negative by real-time PCR. Summarised data for the 24 *bla*$_{NDM-1-type}$-positive cases are given in Table 2.

4. Discussion

Use of β-lactam antibiotics is preferred by paediatricians owing to their limited side effects. The NDM carbapenemase is one of the emerging enzymatic mechanisms of resistance compromising the efficacy of almost all classes of β-lactams [14]. The microbiology laboratory plays an important role in highlighting the prevalence of various types of antibiotic resistance by using rapid and sensitive assays such as real-time PCR, in addition to testing using

Table 1
Distribution of different types of bacteria in relation to different sample types.

Sample type	*Klebsiella pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Enterobacter cloacae*
Blood culture	17	7	7	2	3
Sputum	26	0	6	5	0
Urine	5	13	2	0	0
Drain	1	0	0	0	0
Peritoneal fluid	0	3	0	0	0
Pleural fluid	2	0	0	0	0
Pus	0	1	0	0	0
Total	51	24	15	7	3

Table 2
Summary of data for the 24 cases positive for the $bla_{\text{NDM-1-type}}$ gene by real-time PCR.

No.	Section	Sample type	Organism	Imipenem		Meropenem		EMBL	
				MIC (μg/mL)	Interpretation	MIC (μg/mL)	Interpretation	MIC ratio of IP/IPI	Interpretation
1	ICU	Blood culture	*Klebsiella pneumoniae*	12	R	16	R	17.7	P
2	Neonatology	Blood culture	*K. pneumoniae*	24	R	12	R	8	P
3	ICU	Blood culture	*K. pneumoniae*	32	R	24	R	Phantom[a]	P
4	Outpatient	Urine	*Escherichia coli*	16	R	16	R	35.5	P
5	ICU	Drain	*K. pneumoniae*	16	R	24	R	Phantom	P
6	ICU	Blood culture	*K. pneumoniae*	6	R	32	R	Phantom	P
7	Outpatient	Urine	*E. coli*	6	R	2	I	Phantom	P
8	ICU	Sputum	*K. pneumoniae*	32	R	16	R	Phantom	P
9	ICU	Sputum	*K. pneumoniae*	6	R	32	R	2	N
10	Neonatology	Sputum	*K. pneumoniae*	32	R	16	R	12	P
11	ICU	Blood culture	*Acinetobacter baumannii*	24	R	24	R	Phantom	P
12	ICU	Blood culture	*K. pneumoniae*	4	R	6	R	8	P
13	Outpatient	Urine	*K. pneumoniae*	8	R	16	R	12	P
14	ICU	Sputum	*K. pneumoniae*	24	R	16	R	Phantom	P
15	Neonatology	Sputum	*Pseudomonas aeruginosa*	24	R	16	R	Phantom	P
16	Neonatology	Sputum	*K. pneumoniae*	8	R	12	R	0.7	N
17	Neonatology	Pus	*E. coli*	32	R	24	R	21.4	P
18	ICU	Pleural fluid	*K. pneumoniae*	8	R	32	R	Phantom	P
19	ICU	Blood culture	*A. baumannii*	48	R	24	R	32.1	P
20	Neonatology	Blood culture	*P. aeruginosa*	8	R	24	R	6.6	N
21	Ward	Pleural fluid	*K. pneumoniae*	32	R	24	R	4	N
22	Neonatology	Sputum	*K. pneumoniae*	6	R	8	R	Phantom	P
23	Neonatology	Blood culture	*P. aeruginosa*	32	R	8	R	17.7	P
24	Neonatology	Blood culture	*E. coli*	4	R	24	R	2	N

MIC, minimum inhibitory concentration; EMBL, Etest for metallo-β-lactamase; IP/IPI, imipenem MIC/imipenem MIC in presence of the inhibitor ethylene diamine tetra-acetic acid (EDTA); ICU, intensive care unit; R, resistant; P, positive; N, negative.
[a] Rounded 'phantom' inhibition zone between IP/IPI.

other affordable alternative techniques for routine surveillance programmes, such as EMBL.

In this study, the high percentage of XDR (91.7%) and PDR (8.3%) phenotypes found among the 24 isolates harbouring the $bla_{\text{NDM-1-type}}$ gene leaves very limited options for treatment. The existence of the $bla_{\text{NDM-1-type}}$ gene near a pathogenicity island gives the gene the genetic basis of the MDR phenotype, beside other causes [6].

Many studies have shown that intensive care units (ICUs) are deemed to be the epicentre and the main source of development and amplification of antimicrobial resistance [15], a finding that was also observed among the 24 NDM-1-type-positive cases in the current study; 11 cases (45.8%) were isolated from ICUs, whilst 9 cases (37.5%) were isolated from the neonatology unit.

Of 24 samples positive for the $bla_{\text{NDM-1-type}}$ gene, the most commonly identified organisms were *K. pneumoniae* (15; 62.5%), followed by *E. coli* (4; 16.7%), *P. aeruginosa* (3; 12.5%) and *A. baumannii* (2; 8.3%). This is similar to a study by Rahman et al. in India in 2014, in which the distribution of the 44 $bla_{\text{NDM-1-type}}$-positive isolates was as follows: *K. pneumoniae*, 20 (45%); *E. coli*, 14 (32%); *Citrobacter* spp., 4 (9%); *Providencia* spp., 4 (9%); and *Enterobacter* spp., 2 (5%) [16].

In 2012, Halimi et al. recommended the use of EMBL to identify NDM-1-producers in comparison with genotypic results. They found also that the Etest MBL IP/IPI showed a sensitivity of 98% and a specificity of 100% [17]. Yet, being a phenotypic test, it also has a lengthy turnaround time, with reportable results not available for up to 24 h, hence the test is more useful as a confirmatory test rather than as a screening tool, Moreover, several isolates harbouring $bla_{\text{NDM-1-type}}$ genes exhibit low MICs to carbapenems that may fail to be interpreted using the EMBL strip.

On the other hand, the PCR assay offers the advantage of faster genotyping and a shorter turnaround time (4 h for PCR compared with >24 h for EMBL), with less subjectivity in interpreting the results [18].

In this study, four isolates initially tested positive by the EMBL and negative by real-time PCR, including two *E. coli*, one *K. pneumoniae* and one *P. aeruginosa*. In such cases, MBL production could be ascribed to other MBLs [18]. The reduced carbapenem susceptibilities detected in 76 organisms negative for $bla_{\text{NDM-1-type}}$ by PCR could be explained by production of other carbapenemases or loss of a porin channel combined with the presence of an AmpC β-lactamase [19].

We recommend the use of other phenotypic tests, such as the KPC and MBL ConfirM Kits of Rosco as well as the Carba NP test with a short turnaround time ca. 2 h. Also, using meropenem/EDTA Etest could improve the results because it includes a wider range of dilutions in screening for carbapenemases.

This study highlights the high prevalence of the $bla_{\text{NDM-1-type}}$ carbapenemase gene among Gram-negative bacteria with its great potential for spread in ICUs. A nationwide surveillance programme for detection of antibiotic resistance is mandatory, with appropriate implementation of infection control measures in healthcare settings.

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Competing interests

None declared.

Ethical approval

Not required.

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