Microbes and Infectious Diseases 2021; 2 (2): 295-307

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Phenotypic and genotypic characterization of metallo beta lactamases among Gram-negative uropathogens from a tertiary care hospital in Egypt: A cross sectional study

Doaa Alhussein Abo-Alella *[1], Mahmoud Mohamad Malek [2], Mai Mohamad Malek [1]

1- Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.
2- Urology Department, Faculty of Medicine, Zagazig university, Zagazig, Egypt.

ARTICLE INFO

Article history:
Received 12 January 2021
Received in revised form 14 February 2021
Accepted 18 February 2021

Keywords:
Gram-negative bacilli
Cabapenemases
NDM
VIM
IMP

ABSTRACT

Background: Gram negative bacilli (GNB) are the most common causes of urinary tract infections (UTIs). There is a worrying level of antimicrobial resistance emerging in UTIs pathogens. Metallo-β-lactamases (MBLs) are rapidly spreading β-lactamases with no available FDA approved inhibitor conferring resistance to most β-lactam antibiotics including carbapenems. We aimed to determine prevalence and antimicrobial resistance patterns of Gram-negative uropathogens in our tertiary care hospital, and to characterize MBLs production among them. **Methods:** A hundred and forty-three urine samples were collected from the Urology Department. Uropathogens were isolated on cystiene lactose electrolyte deficient agar and Mac-Conkey's agar. Gram negative bacilli were identified by conventional methods. Antimicrobial susceptibility was evaluated by disc diffusion method. Carbapenems resistant Gram-negative bacilli (CRGNB) were tested for MBLs production both phenotypically and genotypically. **Results:** Eighty-five GNB (75.9%) were isolated from 112 positive cultures. *Escherichia coli* (49.4%) was the most prevalent isolate. Most of GNB showed high resistance patterns to various antimicrobials. Twenty-nine GNB isolates were resistant to at least one carbapenem (28.2% for imipenem, 30.6% for meropenem). Twenty-three isolates (79.3%) were phenotypically positive for MBL activity by combined disc test (CDT). Twenty-two isolates (75.9%) were positive for one or more of MBL genes (*blaIMP*, *blaVIM* and/or *blaNDM*). **Conclusions:** Our study emphasized on the alarming rates of resistance to most of the common antimicrobials particularly carbapenems among GNB uropathogens, which represent a public health threat. CRGNB isolates in our hospital are phenotypically and genotypically associated with MBLs production so an effective strategy to combat these strains is mandatory.

Introduction

Urinary tract infections (UTIs) are the second most common bacterial infections arising in either community or hospital locales, particularly in developing countries [1]. Although UTIs are caused by different pathogens, yet Gram-negative bacilli (GNB) are the most common causes. The uropathogenic bacteria are mostly from the host bowel flora, which enters the bladder as an ascending infection through the urethra [2].

International guidelines for the treatment of UTIs recommend different antimicrobials, such

DOI: 10.21608/MID.2021.59593.1113
* *Corresponding author:* Doaa Alhussein Abo-alella
E-mail address: doaa.alhussein.1982@gmail.com

as β-lactams, fluoroquinolones, nitrofurantoin monohydrate or trimethoprim/sulfamethoxazole [3]. However, there is a worrying level of antimicrobial resistance emerging in UTI pathogens [4]. The resistance rates for these commonly used antibiotics are becoming too high to justify their empirical use for treatment of UTIs, which in turn raises the need to update the local antibiograms as these bacterial uropathogens are becoming a major health concern.

Metallo-beta-lactamases (MBLs) are special type of β-lactamases belonging to Amber class B. They cause resistance to all β-lactams, including carbapenems, except monobactams by means of their active-site zinc ions [5]. Metallo-beta-lactamases were thought to be medically irrelevant chromosomally encoded enzymes in nonpathogenic bacteria, for many decades [6,7]. This idea has been altered with the rising reports of clinically relevant acquired MBLs, especially Imipenemase enzyme (IMP), Verona Integron-encoded Metallo-β-lactamase (VIM) and New Delhi Metallo-β-lactamase (NDM) in GNB. The genes of these enzymes are chromosomally or plasmid encoded on transmissible genetic cassettes inserted into integrons and/or associated with composite transposons, which facilitates their intra- and inter-species horizontal dissemination among GNB [8]. Another problem with MBLs is the lack of FDA approved metallo-β-lactamase inhibitor (MBLI) available for clinical treatment of MBL-mediated resistant infections [9], which points to more concern needed for the occurrence of this class of β-lactamases in resistant bacteria.

Accordingly, the present study was designed to determine the prevalence and antimicrobial resistance patterns of Gram-negative uropathogens in our tertiary care hospital, and to characterize MBLs production both phenotypically and genotypically among them.

Methods

This cross sectional study was carried out in the period from September 2019 to August 2020 after obtaining ethical approval form Institutional Board Review IRB of Zagazig University Hospitals. The study was performed according to the Declaration of Helsinki. Written informed consent was acquired from the patients and their privacy rights were observed.

A total of 143 non-replicate urine samples were collected from patients admitted to the Urology Department in Zagazig University Hospitals. During the study period, patients admitted to the Urology department with a clinical manifestations of UTI were included. These manifestations were the presence of fever and/or any of the symptoms proposing UTI (eg increased frequency, burning micturition suprapubic pain and/or flank pain). Patients who received antimicrobials within 48hrs prior to urine sampling and those who refused to participate in the study were excluded. Besides, samples which grew more than one type of organism were considered as contaminated and hence, excluded from the study. The samples were collected as clean catch, mid-stream urine in sterile, wide mouth, screw capped containers. For catheterized patients, the catheter was clamped off above the outlet to allow collection of freshly voided urine through the catheter collection port or through puncture of the tubing with a needle [10]. Samples were transported to the Bacteriology Laboratory of Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University within one hour for processing. Uropathogens were isolated from samples by culture on cystiene lactose electrolyte deficient (CLED) agar and Mac-Conkey's (MAC) agar. The pathogen was initially identified by conventional methods including colonial morphology, Gram stained films and biochemical reactions. API 20E and API 20NE strips (bioMérieux, France) were used for further confirmation of some isolates.

All isolated Gram-negative uropathogens were tested for antimicrobial susceptibility by standardized Kirby-Bauer's disk diffusion technique as recommended by Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines [11]. The reference strain *Escherichia coli* ATCC® 25922 was used for quality control. The following antimicrobials (Oxoid, UK) were used; amikacin (30 µg), amoxicillin/clavulanic (20/10 µg), ampicillin-sulbactam (10/10 µg), cefepime (30 µg), cefoperazone (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), gentamicin (10 µg), imipenem (10 µg), levofloxacin (30 µg), meropenem (10 µg), nitrofurantoin (30 µg), pipracillin-Tazobactam (100/10 µg), trimethoprim-sulfamethoxazole(1.25/23.75µg).

Phenotypic screening for MBLs was performed by combined disc test (CDT) using imipenem (10 µg) and imipenem-EDTA (10 µg / 750 µg) discs. A 0.5 McFarland- turbidity suspension of each isolate to be tested was used to inoculate Muller- Hinton agar plate. Discs were

placed onto the inoculated medium, ensuring an adequate distance between the discs to permit the formation of clearly defined zones of inhibition. EDTA impregnated discs were used as negative controls. The procedure was repeated twice to ensure the reproducibility of results. Comparing the zone of inhibition of the imipenem disc to the zones of inhibition of the imipenem-EDTA discs, a zone difference 7 mm was recorded as demonstrating MBL activity [12].

Bacterial gnomic DNA was extracted from pure bacterial isolates by G-Spin^{TM} Total DNA Extraction Kit (iNtRON Biotechnology, Korea) according to the Manufacturer's instructions. DNA concentration and purity were determined by nanodrop 2000 (Thermofischer Scientific, USA) at 260 nm absorbance with a ratio between (1.7-1.8) for A_{260} /$A_{280.}$ The extracted DNA that were stored at -20^{o}C until used later. MBL genes (*blaIMP*, *blaVIM* and *blaNDM*) were detected by multiplex polymerase chain reaction (PCR). The primers sequences and product genes sizes were mentioned in **table (1)**. Polymerase chain reactions were performed in a final volume of 25µl of the amplification mixture containing 10ul of 2x PCR Master mix solution, 1.5µl (5-20pmol) of each primer, 2µ1 of template DNA and sterile distilled water to a total volume of 25µL. The gene segments were amplified using a DNA thermal cycler (Biometra, Germany). For amplification, PCR cycles were initial denaturation at 94°C for 10 minutes; 35 cycles of 94°C for 30 seconds, 52°C for 40 seconds, and 72°Cfor 50 seconds; and final extention step at 72°C for 5minutes as described by **Poirel et al.** [13]. Amplified PCR products were stored at -20°C until analyzed by electrophoresis on a 3% agarose gel after staining with ethidium bromide and visualized on a UV transilluminator (Cole-Parmer, USA).

Table 1. Primer sequences and product genes sizes.

Primer	Sequence (5′–3′)	Gene	Product size (bp)
IMP-F	GGAATAGAGTGGCTTAAYTC	*blaIMP*	232
IMP-R	TCGGTTTAAYAAAACAACCACC		
VIM-F	GATGGTGTTTGGTCGCATA	*blaVIM*	390
VIM-R	CGAATGCGCAGCACCAG		
NDM-F	GGTTTGGCGATCTGGTTTTC	*blaNDM*	621
NDM-R	CGGAATGGCTCATCACGATC		

Statistical analysis

The collected data were coded, entered, presented, and analyzed using Statistical Package for Social Science (SPSS) version 26. Qualitative data were represented as frequencies and percentages. For quantitative variables mean, standard deviation (SD), and (minimum-maximum) were computed. Chi square (X^2) test and Fisher's exact test were used to detect relation between different qualitative variables. The results were considered statistically significant and highly statistically significant when the significant probability (P value) was *<0.05** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), were calculated to measure the validity.

Results

The study included urine samples which were obtained from 143 patients; male patients (n=82, 57.3%) were more than females (n=61, 42.7%) with their ages range from 16 to 81 with the mean for age was 52.84±12.51.

On culture, only 112 samples (78.3%) revealed growth while 31 samples (21.7%) showed no growth. Regarding the positive culture results (n=112); GNB were the most commonly isolated organisms (n= 85, 75.9%) followed by candida (n=15, 13.4%) and Gram positive cocci (n=12, 10.7%). *Escherichia coli* (n=42, 49.4%) was the most commonly isolated organism among GNB, followed by *Klebsiella spp* (n=14, 16.5%),

Pseudomonas aeruginosa (n=13, 15.3%), *Proteus spp* and *Acinetobacter baumanii* (n=7, 8.2% for each), *Citrobacter freundii* and *Serratia marcescens* (n=1, 1.2% for each).

The antimicrobial resistance patterns of GNB isolates by disc diffusion method were shown in **table (2)**. Most of the isolates showed high resistance patterns with the least resistance to carbapenems (n=24, 28.2% for imipenem and n=26, 30.6% for meropenem) and aminoglycosides (n=33, 38.8% for amikacin and n=34, 40% for gentamicin). High resistance patterns were reported to various β-lactams (other than carbapenems) and β-lactam/ β-lactamase inhibitor combinations ranging from (88.2% up to 67.1%). In addition, resistance to trimethoprim/ sulfamethoxazole was (n=68, 80%) while resistance to nitrofurantoin was (n=58, 68.2%).

Table 3 presented the distribution of carbapenems resistance among GNB. Twenty-nine isolates (18/ 65 Enterobacteriaceae and 11/20 non-fermenter GNB) were resistant to imipenem and/or meropenem with non fermenter GNB being significantly more resistant than Enterobacteriaceae (78.6% vs 27.7% respectively, *P value=0.032*). Among Enterobacteriaceae, *Escherichia coli* (n=7, 16.7%) was significantly the least resistant to carbapenems compared to *Klebsiella spp* (n=6, 42.9%), *Proteus spp* (n=3, 42.9%), *Citrobacter freundii* (n=1, 100%) and *Serratia marcescens* (n=1, 100%) (P value =0.037). On the other side, no significant difference was detected among non-fermenter GNB where 6 *Pseudomonas aeruginosa* isolates representing 46.2% and 5 *Acinetobacter baumanii* isolates representing 71.4% were resistant to carbapenems (*P value =0.374*)

Figure 1 showed positive CDT for detection of MBL activity. The test isolate showed a zone diameter of >7 mm around the imipenem-EDTA disc compared to that of the imipenem disc alone.

Table 4 showed the results of both phenotypic and genotypic detection of MBLs among 29 carbapenems resistant Gram-negative bacilli (CRGNB). Twenty-three isolates (79.3%) were phenotypically positive for MBLs activity by CDT while 22 isolates (75.9%) were genotypically positive for MBLs genes by PCR. Evaluating the performance accuracy of CDT to detect MBL activity compared to PCR as a gold standard, CDT had a sensitivity of 90.9%, specificity of 57.1%, PPV of 86.9% and NPV of 66.6%.

Distribution of MBLs genes among CRGNB was shown in **table (5)**. Out of 22 isolates positive for MBL genes, *BlaNDM* (n=15, 68.2%) was the most prevalent gene detected either alone (n=7, 31.8%) or accompanied by *blaIMP* and/or *blaVIM* genes (n=8, 36.4%). *BlaVIM* (n= 13, 59.1%) was the next prevalent gene detected either alone (n=2, 9.1%) or accompanied by *blaNDM* and/or *blaIMP* genes (n= 11, 50%). The least prevalent gene was *BlaIMP* (n=9, 40.9%) detected either alone (n=1, 4.5%) or accompanied by *blaNDM* and/or *blaVIM* genes (n=8, 36.4%). Interestingly, 12 isolates representing 54.5% were positive for more than one MBL genes; 3 isolates representing 13.6% were positive for all three tested genes *BlaIMP+BlaVIM+ BlaNDM*, 4 isolates representing 18.2%were positive for *BlaIMP+BlaVIM* genes and similarly 4 isolates representing 18.2% were positive for *BlaVIM+BlaNDM* genes while only one isolate representing 4.5% was positive for *BlaIMP+BlaNDM* genes. **Figure 2** presented gel electrophoresis of some PCR product for MBL genes among GNB.

Table 2. Antimicrobial resistance pattern of Gram-negative bacilli isolates (n=85) by disc diffusion method.

Antimicrobial agents	**Total isolates (n=85)**	***Escherichia coli* (n=42)**	***Klebsiella spp* (n=14)**	***Proteus spp* (n=7)**	***Citrobacter freundii* (n=1)**	***Serratia marcescens* (n=1)**	***Pseudomonas aeruginosa* (n=13)**	***Acinetobacter baumannii* (n=7)**
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Amikacin	33 (38.8%)	13 (31%)	8 (57.1%)	Zero	Zero	1 (100%)	5 (38.5%)	6 (85.7%)
Amoxicillin/ Clavulanic acid	66 (77.6%)	32(76.2%)	12(85.7%)	3(42.9%)	1 (100%)	1 (100%)	11 (84.6%)	6 (85.7%)
Ampicillin/ Sulbactam	71 (83.5%)	39(92.9%)	12(85.7%)	2(28.6%)	1 (100%)	Zero	11 (84.6%)	6 (85.7%)
Aztreonam	66 (77.4%)	34 (81%)	13(92.9%)	5(71.4%)	1 (100%)	Zero	7 (53.8%)	6 (85.7%)
Cefepime	68 (80%)	35(83.3%)	13(92.9%)	6(85.7%)	1 (100%)	Zero	7 (53.8%)	6 (85.7%)
Cefoperazone	67 (78.8%)	32(76.2%)	12(85.7%)	4(57.1%)	1 (100%)	1 (100%)	10 (76.9%)	6 (85.7%)
Cefoxitin	75 (88.2%)	38(90.5%)	13(92.9%)	5(71.4%)	1 (100%)	1 (100%)	10 (76.9%)	7 (100%)
Ceftazidime	59 (69.4%)	26(61.9%)	11(78.6%)	5(71.4%)	1 (100%)	1 (100%)	9 (69.2%)	6(85.7%)
Ceftriaxone	66 (77.4%)	31(73.8%)	12(85.7%)	4(57.1%)	1 (100%)	1 (100%)	10 (76.9%)	6(85.7%)
Gentamicin	34 (40%)	14(33.3%)	7 (50%)	2(28.6%)	1 (100%)	1 (100%)	5 (38.5%)	4 (57.1%)
Imipenem	24 (28.2%)	7 (16.7%)	6 (42.9%)	3(42.9%)	Zero	Zero	4 (30.8%)	4 (57.1%)
Levofloxacin	42 (49.4)	18(42.9%)	10(71.4%)	3(42.9%)	Zero	Zero	6 (46.2%)	5(71.4%)
Meropenem	26(30.6%)	6(14.3%)	5 (35.7%)	2(28.6%)	1(100%)	1 (100%)	6 (46.2%)	5(71.4%)
Nitrofurantoin	58(68.2%)	26(61.9%)	13(92.9%)	2(28.6%)	Zero	Zero	13 (100%)	5 (71.4%)
Piperacillin/ Tazobactam	52(61.2%)	22(52.4%)	11(78.6%)	2(28.6%)	1(100%)	1 (100%)	10 (76.9%)	5 (71.4%)
Trimethoprim/Sulfamethoxazole	68(80%)	35(83.3%)	11(78.6%)	5(71.4%)	1(100%)	1 (100%)	10 (76.9%)	5 (71.4%)

Table 3. Distribution of carbapenems resistance (n=29) among Gram-negative isolates.

Gram-negative bacilli (n=85)	**Carbapenems resistant (n=29)**	***P value***	**Gram-negative bacilli (n=85)**	**Carbapenems resistant (n=29)**	***P value***
Enterobacteriaceae (n=65)	18 (27.7%)	***0.032*^***	***Escherichia coli* (n=42)**	7 (16.7%)	***0.037*^^***
			***Klebsiella spp* (n=14)**	6 (42.9%)	
			***Proteus spp* (n=7)**	3 (42.9%)	
			***Citrobacter freundii* (n=1)**	1 (100.0%)	
			***Serratia marcescens* (n=1)**	1 (100%)	
Non fermenter Gram negative bacilli (n=20)	11 (78.6%)		***Pseudomonas aeruginosa* (n=13)**	6 (46.2%)	*0.374^*
			***Acinetobacter baumannii* (n=7)**	5 (71.4%)	

Note: ^Fisher's exact test, ^^Chi square test, *Statistically significant ($p<0.05$)

Table 4. Phenotypic and genotypic detection of MBL activity among carbapenems resistance Gram-negative bacilli.

Carbapenems resistant Gram-negative bacilli (n=29)		**Phenotypic method (n=23) (79.3%)**	**Genotypic method (n=22) (75.9%)**
***Escherichia coli* (n=7)**		5 (71.4%)	4 (57.1%)
***Klebsiella spp* (n=6)**		6 (100.0%)	5 (83.3%)
***Proteus spp* (n=3)**		2 (67.7%)	2 (67.7%)
***Citrobacter freundii* (n=1)**		0 (0.0)	1 (100.0%)
***Serratia marcescens* (n=1)**		1 (100.0%)	1 (100.0%)
***Pseudomonas aeruginosa* (n=6)**		5 (83.3%)	6 (100.0%)
***Acinetobacter baumannii* (n=5)**		4 (80.0%)	3 (60.0%)
Phenotypic method		**Genotypic method**	
		Positive (n=22)	**Negative (n=7)**
Positive (n=23)		20	3
Negative (n =6)		2	4
Sensitivity	**Specificity**	**PPV**	**NPV**
90.9%	57.1%	86.9%	66.6%

PPV: Positive predictive value
NPV: Negative predictive value

Table 5. Distribution of MBLs genes among PCR positive Gram-negative bacilli.

PCR positive Gram-negative bacilli	***BlaIMP***	***BlaVIM***	***BlaNDM***	***BlaIMP+ BlaVIM***	***BlaIMP+BlaNDM***	***BlaVIM+ BlaNDM***	***BlaIMP+ BlaVIM+ BlaNDM***
***Escherichia coli* (n=4)**	Zero	zero	2	zero	1	1	zero
***Klebsiella spp* (n=5)**	Zero	zero	3	zero	Zero	1	1
***Proteus spp* (n=2)**	Zero	zero	1	zero	Zero	1	zero
***Citrobacter freundii* (n=1)**	Zero	zero	1	zero	Zero	Zero	zero
***Serratia marcescens* (n=1)**	1	zero	Zero	zero	Zero	Zero	zero
***Pseudomonas aeruginosa* (n=6)**	Zero	2	Zero	3	Zero	Zero	1
***Acinetobacter baumannii* (n=3)**	Zero	zero	Zero	1	Zero	1	1
Total (n= 22)	1	2	7	4	1	4	3
%	4.5%	9.1%	31.8%	18.2%	4.5%	18.2%	13.6%

Figure 1. Positive combined disc test for detection of MBL activity. The test isolate shows a zone diameter of >7 mm around the imipenem-EDTA disc compared to that of the imipenem disc alone.

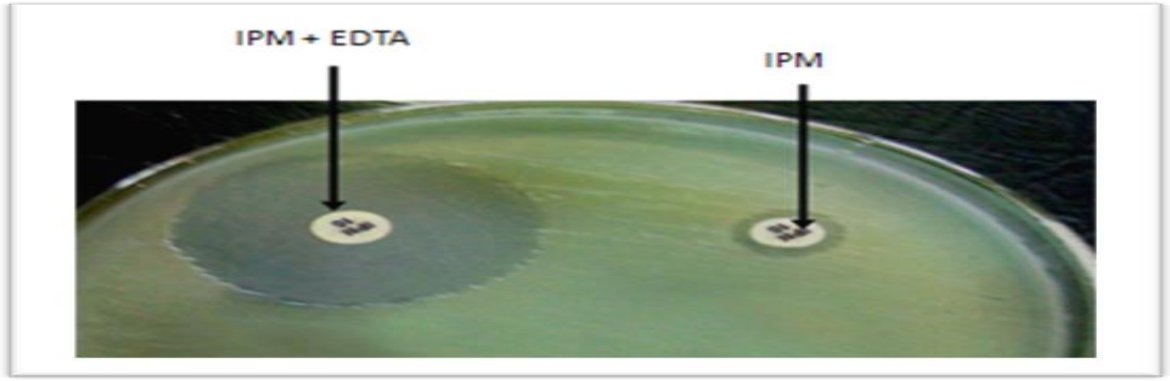
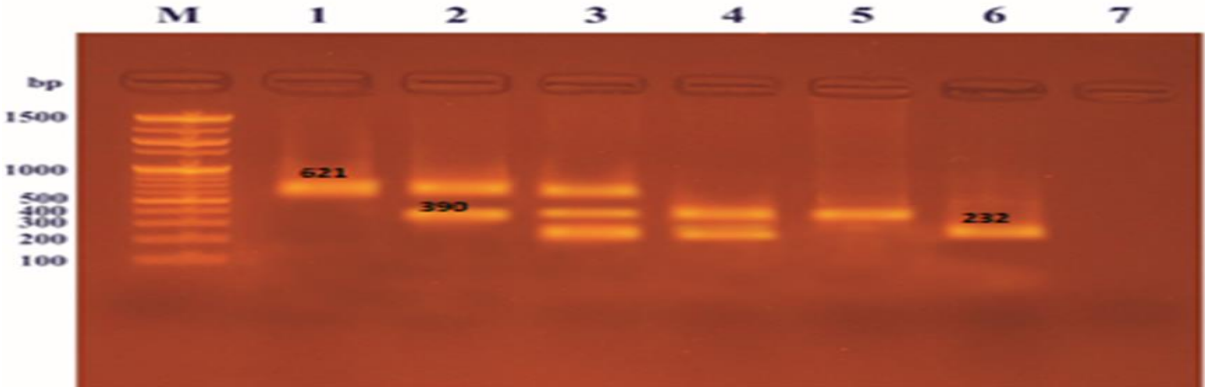


Figure 2. Gel electrophoresis of PCR product for MBL genes among Gram-negative bacilli.
M: DNA ladder 100-1500 bp. Lane 1: *blaNDM* positve *Escherichia coli*. Lane2: *blaVIM+ blaNDM* positive *Klebsiella spp*. Lane 3: *blaIMP+blaVIM+blaNDM* positive *Acinetobacter baumannii*. Lane 4: *blaIMP+blaNDM* positive *Escherichia coli*. Lane 5: *blaVIM* positive *Pseudomonas aeruginosa*. Lane 6: *blaIMP* positive *Serratia marcescens*. Lane 7: negative control. *blaNDM* at 621bp, *blaVIM* at 390bp and *blaIMP* at 232bp



Discussion

Urinary tract infection is one of the most prevalent bacterial infection affecting the population; it affects all age groups and both sexes worldwide. Several studies show geographic discrepancies in causative agents of UTIs and their resistance profiles to antimicrobials [1].

The therapy of UTIs is becoming more problematic as resistance rates for standard antimicrobials are rising. The rise of antimicrobial resistances and the existence of multi-drug resistance (MDR) pathogens in UTIs results in increasing rates of insufficient empirical therapy [4].

Carbapenems have been considered the final resort for treatment of infections caused by MDR pathogens. However, their effectiveness has been compromised due to the rising prevalence of CRGNB, which has turned into a worldwide problem. The production of carbapenemases including MBLs in GNB, associated with other resistance mechanisms, has limited down options of treatment for UTIs caused by these pathogens [14].

This study aimed to determine the prevalence and antimicrobial resistance profiles of Gram-negative uropathogens in our tertiary care hospital, and to characterize MBLS production among them both phenotypically and genotypically with special reference to IMP, VIM and NDM type MBLs which are the most prevalent types worldwide.

In our study, 85 GNB isolates were recovered from 143 urine samples from the Urology Department representing 75.9% of positive urine culture results (n=112), followed by *Candida* (13.4%) and Gram-positive cocci (10.7%). This finding was consistent with a previous study in Egypt by Ahmed et al [15] who reported that GNB were the most prevalent uropathogen accounting for 82%; however, they reported Gram-positive cocci to represent 13% and *Candida* spp to represent 5%. Regarding various types of isolated GNB, *Escherichia coli* was the most common isolate (49.4%), followed by *Klebsiella spp*

(16.5%) and *Pseudomonas aeruginosa* (15.3%). Similarly, **Ahmed et al.** [15] found that *Escherichia coli* was the most prevalent pathogen causing UTIs in their hospitals. However, they reported that the next uropathogen after *Escherichia coli*, causing UTI in their hospital was *Pseudomonas aeruginosa* (17%) followed by *Klebsiella spp*. (9%) *and Proteus spp* (1%). This variation in causative agents could be due to the diversity of bacterial ecology in different regions. We recovered only one isolate of *Citrobacter freundii* and one isolate of *Serratia marcescens (*representing 1.2% for each) and this supports the concept that these organisms are less pathogenic than other Enterobacteriaceae [16].

Regarding the antimicrobial resistance patterns of GNB isolates detected by disc diffusion method, the least reported resistant patterns were for carbapenems (28.2% for imipenem and 30.6% for meropenem) and aminoglycosides (38.8% for amikacin and 40% for gentamicin). These results are well matching with **Hasanin et al.** [17] who reported that the highest susceptibility of their isolates was to carbapenems followed by aminoglycosides. However, it still represents a high resistance pattern that can limit our treatment options.

In our study, high resistance patterns were reported to various β-lactams (other than carbapenems) and β-lactam/ β-lactamase inhibitor combinations ranging from (88.2% up to 67.1%). In addition, resistance to trimethoprim/ sulfamethoxazole was 80% while resistance to nitrofurantoin was 68.2%. Although, these classes of antimicrobial agents are recommended for treatment of UTIs by international guidelines [3], yet, their high resistance patterns reflect their inappropriate use in our locality and the need for newer and more effective treatment options.

Regarding the distribution of carbapenems resistance among GNB, we reported that non-fermenter GNB were significantly more resistant to carbapenems compared to Enterobacteriaceae (78.6% vs 27.7% respectively, *P value=0.032*). Similarly, **Kamel et al.** reported that non-fermenter GNB were more resistant to carbapenems compared to Enterobacteriaceae (85.71% vs 66.08%) [18]. *Escherichia coli* was significantly the least resistant to carbapenems among Enterobacteriaceae (P value =0.037) which matched the results of **Kotb et al.** [19].

Although 29 GNB isolates were phenotypically resistant to carbapenems by disc diffusion method, yet, not all these resistant isolates exhibited phenotypic and/or genotypic MBLs production. Carbapenems resistance could be mediated by a variety of enzymatic and non-enzymatic mechanisms. Enzymatic mechanisms include carbapenemases belonging to different Amber classes of beta lactamases. For example, KPC of class A, All MBLs of class B and OXA enzymes especially OXA-48 and OXA-23 of class D. Besides, AmpC of class C, although not a carbapenemase, but can act in context of permeability defects to mediate carbapenems resistance. On the other hand, non-enzymatic mechanisms include mutations or loss of expression of porin-encoding genes and overexpression of genes encoding efflux pumps. So, in addition to MBLs, all these mentioned mechanisms could have contributed to carbapenems resistance detected in our study [20].

A total of 29 carbapenems (imipenem and/or meropenem) resistant isolates (representing 34.1% of all GNB isolates) were screened for phenotypic MBL activity by CDT as described by **Yong et al.** [12]. Twenty-three isolates (79.3%) were phenotypically positive for MBL activity. After phenotypic screening, CRGNB isolates were tested by PCR for detection of MBL genes (*blaIMP*, *blaVIM* and *blaNDM*). Twenty-two isolates (75.9%) were positive for MBL genes. We noted some discrepancy between the results of the phenotypic screening and that of PCR, which is the gold standard, for detection of MBLs among different isolates of CRGNB. Combined disc test had a sensitivity of 90.9%, specificity of 57.1%, PPV of 86.9% and NPV of 66.6%. So, CDT represents a good screening test being simple, not time or money consuming with accepted sensitivity and specificity.

Some isolates showed phenotypic MBL activity with negative PCR results that could be simply explained by presence of other MBLs genes that we did not look for in our study eg *blaSPM*, *blaSIM*, *blaGIM* which were reported by a recent study in Egypt [21]. On the other hand, some isolates showed no phenotypic MBL activity although PCR results were positive that could be due to the environmental factors interfering with the phenotypic expression of the MBL genes and compromising the sensitivity of the phenotypic method compared to the molecular method [22].

Metallo-beta-lactamases are detected in GNB increasingly and with considerable clinical impact worldwide which represents a main threat to worldwide health care in both community and hospital locales [5]. The selective pressure exerted by the huge unjustified use of antimicrobials has largely contributed to their wide dissemination in the last 2 decades [23]. To date, several MBLs genes were

recognized worldwide such as the *blaVIM*, *blaIMP*, *blaGIM*, *blaSPM*, *blaDIM*, *blaSIM* and *blaNDM*. *BlaVIM*, *blaIMP* and *blaNDM* have emerged as the most prevalent in the clinical setting [23,5], of which more than 100 variants are collectively known at present [24]. So, our study was focused on these three genes. However other MBL genes have been reported in Egypt less commonly [21]. It is worth mention that although reports of MBL genes (IPM, VIM and NDM) among GNB have been published worldwide yet reports from Africa and Middle East countries [25-31] are relatively limited. This is not due to their absence but rather due to shortage of appropriate awareness, skillful staffs, resistance diagnosis, and surveillance systems [26].

Regarding our PCR results, *BlaNDM* (n=15, 68.2%) was the most prevalent gene detected, followed by *BlaVIM* (n= 13, 59.1%), while *BlaIMP* (n=9, 40.9%) was the least prevalent gene detected. In a previous study in Egypt, **Zafer et al.** [32] reported that *blaVIM* was the most prevalent MBL gene followed by *blaNDM* and *blaIMP*, however their study included *Pseudomonas aeruginosa* strains only, which could explain the inconsistency with our results.

Other studies in Egypt have reported the presence of MBL genes among GNB; **Kamel et al.** [18] reported the prevalence of *blaNDM* to be 27.58% while that of *blaVIM* to be 10.3% among 29 different carbapenemase positive isolates. **Soliman et al.** [33] found that 33.8% of GNB were positive for carbapenemase-encoding genes including 13 NDM-1, two NDM-5, three VIM-2 and four VIM-24. **Elkholy et al.** [34] found that the prevalence of *blaNDM-1* was 23.7% in *Klebsiella pneumoniae* isolates and 3.7% in *Escherichia coli* isolates.

Co-expression of MBL genes was obviously noted in our study. Twelve isolates representing 54.5% showed co-expression of more than one MBL gene. Coexistence of MBL genes have been previously reported in Egypt [21,35,36] and other countries [37-40] where misuse of antimicrobials is a common malpractice. The rising prevalence of coexisting MBL genes GNB could be attributed to the association of these genes with integrons and/or transposons that facilitates the movement of resistance genes between in plasmids. These highly mobile genetic tools allow transmission of the genes between different bacteria [8]. This remarkable coexistence of different MBL genes among GNB definitely contributed to the high-level resistance to carbapenems among GNB observed in our study and reported before in Egypt [19, 41]. **Lee et al.** reported that bacteria harboring plasmids encoding various carbapenemases may have better fitness and virulence [42] which could increase the spread of these strains so an effective strategy to combat these strains is mandatory.

Conclusion

In conclusion, our study emphasized on the alarming rates of resistance to most of the common antimicrobial classes particularly carbapenems among GN uropathogens, which represents a public health threat. We also reported that CRGNB isolates in our hospital are phenotypically and genotypically associated with MBLs production. These findings necessitate the continuous implementation of antimicrobial susceptibility testing to identify MDR strains and monitor the resistance trends, the combination of phenotypic and genotypic techniques as a reliable effective epidemiological tool to detect MBL activity among CRGNB, the adherence to infection control policies to confine the rapid spread of these pathogens and the application of an antimicrobial stewardship plan to rationalize the use of antimicrobials in our hospital particularly carbapenems

Declaration of interest and funding information

The authors report no conflicts of interest.

Contributors and authorship

All authors have made substantial contributions to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content. Finally, they have approved the version to be submitted.

References

1-**Tandogdu Z, Wagenlehner FM.** Global epidemiology of urinary tract infections. Curr Opin Infect Dis 2016;29(1):73-79.

2-**McLellan LK, Hunstad DA.** Urinary Tract Infection: Pathogenesis and Outlook. Trends Mol Med 2016;22(11):946-957.

3-**Naber KG, Bonkat G, Wagenlehner FME.** The EAU and AUA/CUA/SUFU Guidelines on Recurrent Urinary Tract Infections: What is the Difference? Eur Urol. 2020;78(5):645-646.

4-**Bischoff S, Walter T, Gerigk M, Ebert M, Vogelmann R.** Empiric antibiotic therapy in urinary tract infection in patients with risk factors

for antibiotic resistance in a German emergency department. BMC Infect Dis 2018;18(1):56.

5-**Mojica MF, Bonomo RA, Fast W.** B1-Metallo-β-Lactamases: Where Do We Stand? Curr Drug Targets 2016;17(9):1029-1050.

6-**Lim HM, Pène JJ, Shaw RW.** Cloning, nucleotide sequence, and expression of the Bacillus cereus 5/B/6 beta-lactamase II structural gene. J Bacteriol 1988;170(6):2873-2878.

7-**Walsh TR, Hall L, Assinder SJ, Nichols WW, Cartwright SJ, MacGowan AP, et al.** Sequence analysis of the L1 metallo-beta-lactamase from Xanthomonas maltophilia. Biochim Biophys Acta 1994;1218(2):199-201.

8-**Partridge SR, Kwong SM, Firth N, Jensen SO.** Mobile Genetic Elements Associated with Antimicrobial Resistance. Clin Microbiol Rev 2018;31(4): e00088-17.

9-**Bush K, Bradford PA.** β-Lactams and β-Lactamase Inhibitors: An Overview. Cold Spring Harb Perspect Med 2016;6(8): a025247.

10-**Albu S, Voidazan S, Bilca D, Badiu M, Truţă A, Ciorea M, et al.** Bacteriuria and asymptomatic infection in chronic patients with indwelling urinary catheter: The incidence of ESBL bacteria. Medicine (Baltimore) 2018;97(33):e11796.

11-**Clinical and Laboratory Standards Institute.** 2020. Performance standards for antimicrobial susceptibility testing, 30th ed CLSI supplement M100 Clinical and Laboratory Standards Institute.2020. Wayne, PA.

12-**Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y.** Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2002;40(10):3798-3801.

13-**Poirel L, Walsh TR, Cuvillier V, Nordmann P.** Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70(1):119-123.

14-**Martin A, Fahrbach K, Zhao Q, Lodise T.** Association between carbapenem resistance and mortality among adult, hospitalized patients with serious infections due to Enterobacteriaceae: Results of a Systematic Literature Review and Meta-analysis. Open Forum Infect Dis 2018;5(7): ofy150.

15-**Ahmed NH, Hussain T, Biswal I.** Comparison of etiological agents and resistance patterns of the pathogens causing community acquired and hospital acquired urinary tract infections. J Glob Infect Dis 2014;6(3):135-136.

16-**Pepperell C, Kus JV, Gardam MA, Humar A, Burrows LL.** Low-virulence Citrobacter species encode resistance to multiple antimicrobials. Antimicrob Agents Chemother 2002;46(11):3555-3560.

17-**Hasanin A, Eladawy A, Mohamed H, Salah Y, Lotfy A, Mostafa H, et al.** Prevalence of extensively drug-resistant Gram negative bacilli in surgical intensive care in Egypt. Pan Afr Med J 2014; 19:177.

18-**Kamel NA, El-Tayeb WN, El-Ansary MR, Mansour MT, Aboshanab KM.** Phenotypic screening and molecular characterization of carbapenemase-producing Gram-negative bacilli recovered from febrile neutropenic pediatric cancer patients in Egypt. PLoS One 2018;13(8): e0202119.

19-**Kotb S, Lyman M, Ismail G, Abd El Fattah M, Girgis SA, Etman A, et al.** Epidemiology of carbapenem-resistant Enterobacteriaceae in Egyptian intensive care units using National Healthcare-associated Infections Surveillance Data, 2011-2017. Antimicrob Resist Infect Control 2020;9(1):2.

20-**Nordmann P, Poirel L.** Epidemiology and Diagnostics of Carbapenem Resistance in Gram-

negative Bacteria. Clin Infect Dis 2019;69(7): S521-S528.

21-**Wasfi R, Rasslan F, Hassan SS, Ashour HM, Abd El-Rahman OA.** Co-Existence of carbapenemase-Encoding Genes in Acinetobacter baumannii from Cancer Patients. Infect Dis Ther 2020:1-5.

22-**Ugwu MC, Shariff M, Nnajide CM, Beri K, Okezie UM, Iroha IR, et al.** Phenotypic and Molecular Characterization of β-Lactamases among Enterobacterial Uropathogens in Southeastern Nigeria. Can J Infect Dis Med Microbiol 2020; 2020:5843904.

23-**Bush K, Bradford PA.** Epidemiology of β-Lactamase-Producing Pathogens. Clin Microbiol Rev 2020;33(2):e00047-19.

24-**U.S. National Library of Medicine.** Beta-Lactamase Data Resources [cited 6 Jan 2021]. https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources/

25-**Chairat S, Ben Yahia H, Rojo-Bezares B, Sáenz Y, Torres C, Ben Slama K.** High prevalence of imipenem-resistant and metallo-beta-lactamase-producing Pseudomonas aeruginosa in the Burns Hospital in Tunisia: detection of a novel class 1 integron. J Chemother 2019;31(3):120-126.

26-**Adam MA, Elhag WI.** Prevalence of metallo-β-lactamase acquired genes among carbapenems susceptible and resistant Gram-negative clinical isolates using multiplex PCR, Khartoum hospitals, Khartoum Sudan. BMC Infect Dis 2018;18(1):668.

27-**Pritsch M, Zeynudin A, Messerer M, Baumer S, Liegl G, Schubert S, et al.** First report on blaNDM-1-producing *Acinetobacter baumannii* in three clinical isolates from Ethiopia. BMC Infect Dis 2017;17(1):180.

28-**Singh-Moodley A, Perovic O.** Antimicrobial susceptibility testing in predicting the presence of carbapenemase genes in Enterobacteriaceae in South Africa. BMC Infect Dis. 2016;16(1):536.

29-**Okoche D, Asiimwe BB, Katabazi FA, Kato L, Najjuka CF**. Prevalence and characterization of carbapenem-resistant Enterobacteriaceae isolated from Mulago National Referral Hospital, Uganda. PLoS One 2015;10(8): e0135745.

30-**Aghamiri S, Amirmozafari N, Fallah Mehrabadi J, Fouladtan B, Samadi Kafil H.** Antibiotic Resistance Pattern and Evaluation of Metallo-Beta Lactamase Genes Including bla- IMP and bla- VIM Types in Pseudomonas aeruginosa Isolated from Patients in Tehran Hospitals. ISRN Microbiol 2014; 2014:941507.

31-**Jamal W, Rotimi VO, Albert MJ, Khodakhast F, Nordmann P, Poirel L.** High prevalence of VIM-4 and NDM-1 metallo-β-lactamase among carbapenem-resistant Enterobacteriaceae. J Med Microbiol 2013;62(Pt 8):1239-1244.

32-**Zafer MM, Al-Agamy MH, El-Mahallawy HA, Amin MA, Ashour MS.** Antimicrobial resistance pattern and their beta-lactamase encoding genes among Pseudomonas aeruginosa strains isolated from cancer patients. Biomed Res Int 2014; 2014:101635.

33-**Soliman AM, Zarad HO, Nariya H, Shimamoto T, Shimamoto T.** Genetic analysis of carbapenemase-producing Gram-negative bacteria isolated from a university teaching hospital in Egypt. Infect Genet Evol 2020; 77:104065.

34-**El-Kholy AA, Girgis SA, Shetta MAF, Abdel-Hamid DH, Elmanakhly AR.** Molecular characterization of multidrug-resistant Gram-negative pathogens in three tertiary hospitals in Cairo, Egypt. Eur J Clin Microbiol Infect Dis 2020;39(5):987-992.

35-**Abouelfetouh A, Torky AS, Aboulmagd E.** Phenotypic and genotypic characterization of carbapenem-resistant Acinetobacter baumannii isolates from Egypt. Antimicrob Resist Infect Control 2019; 8:185

36-**Gomaa FAM, Helal ZH, Khan MI.** High prevalence of blaNDM-1, blaVIM, qacE, and qacEΔ1 genes and their association with decreased susceptibility to antibiotics and common hospital biocides in clinical isolates of Acinetobacter baumannii. Microorganisms 2017;5(2):18.

37-**Ain NU, Iftikhar A, Bukhari SS, Abrar S, Hussain S, Haider MH, et al.** High frequency and molecular epidemiology of metallo-β-lactamase-producing gram-negative bacilli in a tertiary care hospital in Lahore, Pakistan. Antimicrob Resist Infect Control 2018; 7:128.

38-**Santimaleeworagun W, Samret W, Preechachuawong P, Kerdsin A, Jitwasinkul T.** Emergence of co-carbapenemase genes, bla(OXA23), bla(VIM) and bla(NDM) in carbapenem-resistant Acinetobacter baumannii clinical isolates. Southeast Asian J Trop Med Public Health 2016;47(5):1001-1007.

39-**Mohanam L, Menon T.** Coexistence of metallo-beta-lactamase-encoding genes in Pseudomonas aeruginosa. Indian J Med Res 2017;146(Supplement): S46-S52.

40-**Girija SA, Jayaseelan VP, Arumugam P.** Prevalence of VIM- and GIM-producing Acinetobacter baumannii from patients with severe urinary tract infection. Acta Microbiol Immunol Hung 2018;65(4):539-550.

41-**Khalifa HO, Soliman AM, Ahmed AM, Shimamoto T, Hara T, Ikeda M, et al.** High Carbapenem Resistance in Clinical Gram-Negative Pathogens Isolated in Egypt. Microb Drug Resist 2017;23(7):838-844.

42-**Lee H, Shin J, Chung YJ, Park M, Kang KJ, Baek JY, et al.** Co-introduction of plasmids harbouring the carbapenemase genes, blaNDM-1 and blaOXA-232, increases fitness and virulence of bacterial host. J Biomed Sci 2020;27(1):8.

Abo-Alella DA, Malek MM, Malek MM. Phenotypic and genotypic characterization of metallo beta lactamases among Gram-negative uropathogens from a tertiary care hospital in Egypt: A cross sectional study. Microbes Infect Dis 2021; 2 (2): 295-307.