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Risk factors, phenotypic and genotypic characterization of carbapenem resistant *Enterobacteriaceae* in Tanta University Hospitals, Egypt

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Abstract

Carbapenem resistant *Enterobacteriaceae* (CRE) is an increasing worldwide health problem with excess morbidity and mortality. The aim of this work is to study the risk factors, phenotypic and genotypic characteristics of CRE in our institute.

Seventy five isolates of *Enterobacteriaceae* from patients admitted to our institute with hospital acquired infections were included in this study. Identification and susceptibility testing were performed. The differentiation of the types of carbapenemases was achieved using inhibitor-based tests. The detection of *bla* genes was performed using multiplex PCR.

Out of 75 cases of *Enterobacteriaceae*, CRE was detected in 47 (62.7%). Cases of septicaemia and ventilator associated pneumonia with previous history of carbapenem intake, especially in neonatal ICU had higher risk for CRE. The overall detection rate for CRE by modified Hodge test (MHT) was 82.9%. Synergy combined disc tests were positive for both *Klebsiella pneumoniae* carbapenemase (KPC) (47%) and metallo-β-lactamases (MBLs) (19.1%). The most prevalent gene detected by multiplex PCR was bla_{KPC} (22; 46.8%), followed by bla_{VIM} (10; 21.3%), bla_{OXA-48} (6; 12.7%), bla_{KPC} + bla_{OXA-48} (5; 10.6%), bla_{KPC} + bla_{VIM} (3; 6.4%) while bla_{IMP} and bla_{NDM} genes were not detected.

Carbapenem resistance in Egypt is increasing, with limited treatment options. We hence suggest developing antimicrobial stewardship in Tanta University Hospital under governmental supervision, to prevent the unrestricted sale of non-prescribed anti microbial medicines.

Keywords: antimicrobial resistance, carbapenem resistance, carbapenemases, *Enterobacteriaceae*

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Introduction

Carbapenems are the only remaining therapy for the widespread incidence of extended-spectrum beta-lactamases (ESBLs) and AmpC enzymes producing *Enterobacteriaceae*. This has led to the emergence of carbapenem resistant strains, for which very few (if any) antibiotic options remain available.[1-3] Carbapenemases are representatives of ß-lactamase molecular classes A, B, and D. The most frequently reported carbapenem hydrolyzing enzymes are *Klebsiella pneumoniae* carbapenemase (KPC) (class A), New Delhi metallo-ß-lactamase (NDM), Verona Integron-encoded metallo-ß-lactamase (VIM), IMP–type carbapenemases (class B or metallo ß-lactamases (MBLs) and OXA-48-like enzymes (class D oxacillinases).[4,5] Carbapenemases are encoded by the genes bla_{NDM}, bla_{OXA}, bla_{VIM}, bla_{IMP}, and bla_{KPC}, which are present in plasmids and chromosomes.[6]

KPC enzymes are capable of hydrolyzing carbapenems, penicillins, cephalosporins and aztreonam, and are inhibited by clavulanic acid. The class B metalloenzymes (IMP, VIM etc.) have a broader substrate of hydrolysis including penicillins, cephalosporins, cephamycins, oxacephamycins and carbapenems, but not monobactams. Their activity is zinc dependant and is inhibited by the chelating agent ethylenediaminetetraacetic acid (EDTA).[1] OXA-48 is the most prominent enzyme of oxacillinases. OXA-48 is the most prominent oxacillinases enzyme. OXA-48-carrying isolates exhibit only low-level resistance to carbapenems, and remain susceptible to third-generation cephalosporins in the absence of concomitant ESBLs or plasmid-mediated AmpC enzymes, making their detection in diagnostic laboratories more difficult.[7]

While there have been several studies on carbapenem-resistance *Enterobacteriaceae* from various countries, Egyptian research in this area is limited. The present study was hence carried out to look for the risk factors, as well as the phenotypic and molecular epidemiology of *bla* genes responsible for carbapenem resistance in *Enterobacteriaceae* isolates seen during primary antibiotic susceptibility testing.

Patients, materials and methods

Patients

Patients who developed infections that met the criteria for hospital-acquired infection as defined by the Center for Disease Control and Prevention National Healthcare Safety Network (CDC/NHSN),[8,9] and who were referred by Tanta University Hospital to the microbiology laboratory of the Infection Control Unit of the Department of Medical Microbiology, Faculty of Medicine, Tanta University, during the period between September and November 2015, were included in this study.

The study was approved by the Institutional Ethical Committee, and written informed consent was obtained from the patients or their parents.

Tanta is the capital of Gharbia governorate, a town located in the middle of the Nile delta in the north of Egypt. Tanta University Hospital, one of the biggest and oldest hospitals in the region, is composed of five main buildings: the main hospital building, the ophthalmology and ENT hospital, the student hospital, the emergency hospital, and outpatient clinics with a total of 1,507 beds, with an occupancy rate of nearly 98% (77,938 in-patients). The hospital serves over half a million patients in its outpatient clinics (527,151 patients per year). In total, the hospital has 64 departments, divided as follows: 19 in the main hospital buildings; 3 in the ophthalmology hospital; 15 in the student hospital; 10 in the emergency hospital; and 17 in the outpatient clinics. There are 32 operating rooms, which serve the main hospital (18), the ophthalmology hospital (3), the student hospital (4), and the emergency hospital (7), performing a total of 14,450 operations per year. There are a total of eight ICUs with 185 beds spread across the main hospital (5 ICUs with 148 beds), the student hospital (1 ICU with 5 beds), and the emergency hospital (2 ICUs with 32 beds). There are also 25 renal dialysis bed units: 7 in the main hospital and 18 in the student hospital.

As in many low-income countries, the current infection control practices in the Tanta University Hospital (including hand hygiene, standard precautions, and transmission based precautions) face many obstacles both in terms of financial support and healthcare

workers' compliance, with no specific infection control measures in place regarding CRE. For many years, the use of third-generation cephalosporins and carbapenems as empirical treatment created a vicious circle of widespread multidrug-resistant organisms with very limited antibiotic treatment options.

Samples collection

Samples were collected from the patients with prior consent and with full aseptic precautions for wound swabs, endotracheal aspirates, blood and urine.

Identification and susceptibility testing

Identification of the bacterial strains was performed as per standard procedures, with verification by API20E (Biomereux, Inc., Hazelwood, MO). Susceptibility testing was performed using the Kirby–Bauer Method as per the Clinical and Laboratory Standards Institute.[8]

Susceptibility testing was done for the following antibiotics: ampicillin (10µg); amoxycillin clavulanate (30µg); cefuroxime (30µg); cefoxitin (30µg); cefepime (30µg); ceftriaxone (30µg); ceftazidime (30µg); cefotaxime (30µg); gentamicin (10µg); amikacin (30µg); tobramycin (10µg); ciprofloxacin (5µg); meropenem (10µg); and imipenem (10µg).

Isolates of Enterobacteriaceae which were resistant to either imipenem or meropenem or both, were selected for further phenotypic and molecular testing.

Detection of carbapenemases and their differentiation by inhibitor-based tests

The detection of carbapenemases by Modified Hodge Test (MHT) was performed using control strains of *E. coli* ATCC 25922,[10] while the detection of KPC (class A) ß-lactamases was performed using a combined disk test of meropenem with phenylboronic acid (CDT-MER+PBA). A zone diameter difference of >5 mm between a carbapenem disk with boronic acid and the carbapenem alone was considered positive for KPC production.[11] The detection of MBLs (class B) was performed using a combined disk test of imipenem with EDTA (CDT-IMP+EDTA), as described above. A zone diameter difference of >7 mm between imipenem disks and imipenem plus EDTA was interpreted as MBL-positive.[12]

All test interpretation was performed after incubation of the agar plates at 37°C for 18-24 hours.

PCR

DNA template preparation for the isolates was as follows

DNA extraction was performed using PrepMan Ultra Reagent Kit from Life Technologies (Applied BiosystemsTM distributor in Egypt, www.lifetechnologies.com).

Target amplification by multiplex PCR

Target amplification by multiplex PCR was performed to detect the five predominant carbapenemases (bla_{KPC}, bla_{NDM}, bla_{OXA-48}-like, bla_{VIM}, and bla_{IMP}) simultaneously using the protocol described by Doyle *et al.*[13]

The process was carried out using a 96-well thermal cycler instrument (Applied Biosystems at Life Technologies, Foster City, CA), with 300 ng of DNA added to a total volume of 50 µl: 25 µl of Taq PCR Master Mix Kit from QIAGEN. The concentrations of each of the forward and reverse primers used in a single PCR tube were as follows: 15 pmoles/ul for bla_{KPC}, bla_{IMP} and bla_{VIM}; 20 pmoles/ul for bla_{NDM}; and 25 pmoles/ul for bla_{OXA-48}. The PCR program began with an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of DNA denaturation at 95°C for 45 seconds, primer annealing at 60°C for 45 seconds, and primer extension at 72°C for 1 minute, followed by a final extension at 72°C for 8 minutes. The PCR products were analyzed by electrophoresis with 1.5% agarose gels in 0.5 x Tris-borate-EDTA (TBE) buffer. The PCR products were visualized in 2.5% agarose gel stained with ethidium bromide. The primer sequences and amplicon sizes are shown in Table I.

Statistical analysis

Data were analyzed using IBM SPSS version 20.0. Qualitative data were described using number and percentage. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Comparisons between different groups regarding categorical variables were carried out using Chi-square test and student T test. When more than 20% of the cells had an expected count of less than five, correction for chi-square was conducted

using Monte Carlo correction. The significance value was set at 0.05.

Results

In the present study, 62.7% (47/75) of isolated Enterobacteriaceae were carbapenem resistant, while 37.3% (28/75) were carbapenem sensitive.

Socio-demographics and isolate characteristics

The study included 75 patients – 41 males and 34 females – who were classified into two groups according to age. The first group ranged from 14-28 days, while the second group ranged from 38-79 years. No patients between 28 days and 38 years were enrolled in this study. The clinical diagnosis of these patients were wound infection (22 cases), urinary tract infection (UTI) (17 cases), septicemia (15 cases), pneumonia (11 cases) and ventilator associated pneumonia (VAP) (11 cases). The period of hospital admission of these patients ranged between 3-21 days (Table II).

When comparing patients in both CRE and CSE groups, we found significant increases in the following risk factors in the CRE group: neonates (14-28 days) (53.2%, p<0.001); prolonged period of hospital admission (3-21 days, p=0.001); patients admitted to neonatal ICU (53.2%, p<0.001); and previous treatment with antibiotics (p=0.003), with a statistically significant increase in the case of previous carbapenem (imipenem) use (p=0.007). According to the clinical diagnosis, all cases of ventilator-associated pneumonia (VAP) were CRE, while all cases of urinary tract infection (UTI) were CSE. Septicaemic patients also showed significant CRE (27.7%, p=0.032) (Table II).

CRE were more frequently found in *Enterobacter cloacae, Klebsiella pneumoniae* and *Klebsiella* spp. isolates, while CSE were more frequently found in *Escherichia coli* (53.5%) isolates, with statistical significance (Table III).

Prevalence and distribution of carbapenemase genes

The most prevalent carbapenemase genes detected by multiplex PCR in CRE were bla_{KPC} (46.8%), bla_{VIM} (21.3%), bla_{OXA-48} (12.7%), bla_{KPC} + bla_{OXA-48} (10.6%), and bla_{KPC} + bla_{VIM} (6.4%). Only a single case was PCR negative. The overall detection rate of CRE by MHT was 82.9%. However, the combined disk test used for the detection of KPC revealed 53.2% positive cases, while the combined disk test used for the detection of MBLs revealed 19% positive cases (Table IV).

Overall, bla_{KPC} was detected in *K. pneumoniae* (9/22 isolates) and *Klebsiella* spp. (7/22 isolates), while bla_{VIM} was detected in *Enterobacter* spp. (8/10 isolates). In the eight isolates that harboured dual mechanisms of carbapenem resistance, five had both bla_{KPC} + bla_{OXA-48}, mainly in *Enterobacter* spp. (n=2) and *Citrobacter* spp. (n=2), followed by *Klebsiella* spp. (n=1). Of the three isolates that had bla_{KPC} + bla_{VIM}, two were in *Enterobacter* spp. and one in *K. pneumoniae*, bla_{IMP}, while bla_{NDM} was not detected in any of the isolates (Figure 1).

Table I. Primers for the detection of *Enterobacteriaceae* that produce carbapenemases

Carbapenemase gene	Amplicon size (bp)	Primer sequence *a*
bla_{KPC}	900	5′-TGTCACTGTATCGCCGTC-′3 5′-CTCAGTGCTCTACAGAAAACC-′3
bla_{IMP}	587	5′-GAAGGCGTTTATGTTCATAC-′3 5′-GTACGTTTCAAGAGTGATGC-′3
bla_{VIM}	389	5′-GTTTGGTCGCATATCGCAAC-′3 5′-AATGCGCAGCACCAGGATAG-′3
bla_{NDM}	782	5′-GCAGCTTGTCGGCCATGCGGGC-′3 5′-GGTCGCGAAGCTGAGCACCGCAT-′3
$bla_{OXA-48-like}$	438	5′-GCGTGGTTAAGGATGAACAC-′3 5′-CATCAAGTTCAACCCAACCG-′3

The first and second primers for each gene are forward and reverse primers, respectively.[13]

Table II. Patient and specimen data of isolated carbapenem resistant and sensitive *Enterobacteriaceae*

	CRE (47)	CSE (28)	P
Sex			
Male	25(53.2%)	16(57.1%)	0.740
Female	22(46.8%)	12 (42.9 %)	
Age			
14-28 days	25(53.2%)	3(10.7%)	<0.001*
38-79 years	22(46.8%)	25(89.3%)	
Department			
NICU	25(53.2%)	3(10.7%)	<0.001*
EICU	11(23.4%)	3(10.7%)	0.172
General surgery	11(23.4%)	7(25.0%)	0.355
Urology wards	-------	15(53.6%)	<0.001*
Clinical Picture			
Septicaemia	13 (27.7%)	2(7.1%)	0.032*
VAP	10 (21.3%)	---------	0.011*
Pneumonia	9(19.1%)	2(7.1%)	0.193
Wound infection	15 (31.9%)	7(25.0%)	0.525
UTI	---------	17(60.7%)	<0.001*
Sample			
Blood	25(53.2%)	4 (14.3%)	0.001*
ETA	10(21.3%)	--------	0.011*
Wound swab	12(25.5%)	7 (25.0%)	0.959
Urine	------	17(60.7%)	<0.001*
Period of admission			
Min. - Max.	3D-21D	3D-7D	
Mean ± SD	6.47±3.64	4.03±1.13	
Median	5.0	4.0	p= 0.001*
Previous antibiotic Intake			
Negative	11(23.4%)	16(57.1%)	0.003*
Positive	36(76.6%)	12(42.9%)	
Aminoglycosides	4 (11.1%)	0 (0%)	0.2
3rd generation cephalosporins	11 (30.6%)	5 (41.7%)	0.4
Carbapenems	15 (41.6%)	0 (0%)	0.007*
Ampicillin/Clavulinic	5 (13.9%)	0 (0%)	0.17
Quinolones	1 (2.8%)	7(58.3%)	0.0001*

CRE: carbapenem resistant Enterobacteriaceae; CSE: carbapenem sensitive Enterobacteriaceae; NICU: neonatal intensive care unit; EICU: emergency ICU; VAP: ventilator associated pneumonia; UTI : urinary tract infection; ETA: endotraceal aspirate; Aminoglycosides: amikacin /gentamycin; 3rd generation cephalosporin: ceftriaxone; Carbapenem: imipenem; Quinolones: ciprofloxacin

**: Statistically significant at $p \leq 0.01$*

Table III. Distribution of isolated *Enterobacteriaceae* species

Species	CRE	CSE	P
K. pneumoniae	11(23.4%)	1(3.6%)	0.026*
Klebsiella spp.	11(23.4%)	8(28.6%)	0.619
Enterobacter spp.	20(42.6%)	1(3.6%)	<0.001*
Citrobacter spp.	4(8.5 %)	1(3.6%)	0.645
Serratia marcescens	1(2.1 %)	------	1.000
E. coli	------	15(53.5%)	<0.001*
Proteus spp.	------	2(7.1%)	0.136

CRE: carbapenem resistant Enterobacteriaceae; CSE: carbapenem sensitive Enterobacteriaceae;
**:Statistically significant at $p \leq 0.01$*

Correlation of the phenotype and genotype of carbapenem resistance

Our results showed that the MHT method is a good detector of KPC and OXA-48 producing organisms. Of 22 cases of KPC, 21 were detected by MHT with 95.6% sensitivity, while from 6 cases of OXA-48 producing isolates, 4 were detected by MHT with 75% sensitivity. Sensitivity for the detection of VIM was nearly 71%. Isolates carrying double genes were all detected by the MHT method. Regarding the CDT used for the detection of MBL, of 13 cases carrying VIM genes, nine were detected, with 76.4% sensitivity, while the sensitivity of the CDT used for detecting KPC was 85.7% (of 30 cases carrying KPC, 25 were detected) (Table IV).

Discussion

CRE are considered a major health problem worldwide and associated with increased mortality. The rapid detection of carbapenem resistance and appropriate treatment of such cases is therefore mandatory. The current situation in Egypt is not well studied. The goal of this study is therefore to determine the risk factors, as well as the phenotypic and genotypic characters of CRE. In the current study, using the disc diffusion method, 62.7% (47/75) of isolated *Enterobacteriaceae* were carbapenem resistant. This high detection rate was in line with that of a study carried out by Shanmugam *et al.* (93%).[14] However, lower incidences of antibiotic susceptibility were found in studies by Shahid *et al.* and Ramana *et al.* (1.8% and 36% respectively).[15,16] The high level of resistance in the current study can be attributed to the unrestricted use of antibiotics in our institute, which plays an important role in increasing carbapenem resistance.

In the present study, 53.2% of CRE isolates were from men, and 46.8 % were from women, with no statistically significant difference. This is supported by the findings of Qureshi *et al.* who showed that there is no relation between sex and carbapenem resistance.[17] Regarding age, CRE were more frequently isolated in infants (53.2%, 14-28 days), while 89.3% of CSE were isolated in adults aged between 38-89 years, with statistical significance. This concurred with results from studies by Tuona *et al.* and Ulu *et al.*, who considered young age a risk factor for CRE infection.[18,19] However, the current results disagreed with another study that found that CRE to be more frequently isolated in the elderly.[20]

About 53.2% of our cases harbouring CRE were admitted to the neonatal ICU, while CSE was more frequently isolated from the urology wards (53.6%), with statistical significance. These results concur with the results of another Egyptian study in which 30% of CRE were isolated from neonatal ICUs.[21] However, another study found that 56% of CRE cases were admitted to adult ICUs.[22] This variation may be due to differences in the antibiotic policies in each ICU. In contrast to our findings here, another study found that having the neurosurgical ICU unit as a place of admission is considered a risk factor for carbapenem resistance (an isolation rate of 76.9% compared with 43.5% in the rest of the hospital). The study postulated that these results were caused by a lack of infection control practices in the unit, and a preference for meropenem due to its ability to penetrate the blood-brain barrier.[19]

Table IV. Genotypic and phenotypic characters of carbapenem resistant *Enterobacteriaceae*

No =47	No. of isolates	(%)
Molecular Characterization		
bla $_{KPC}$	22	46.8
bla$_{VIM}$	10	21.2
bla$_{IMP}$	-	-
bla$_{NDM}$	-	-
bla$_{OXA-48}$	6	12.7
bla $_{KPC}$ + *bla*$_{oxa-48}$	5	10.6
bla$_{kpc}$ + *bla*$_{VIM}$	3	6.3
PCR-negative	1	2.1
Total carbapenemases	46	97.9%
Phenotypic carbapenemase producer		
MHT-positive	39	82.9%
MHT-negative	8	17.1%
Molecular Characterization	MHT Positive (39)	
bla $_{KPC}$ (22)	21	
bla$_{VIM}$ (10)	6	
bla$_{OXA-48}$ (6)	4	
bla $_{KPC}$ + *bla*$_{oxa-48}$ (5)	5	
bla$_{kpc}$ + *bla*$_{VIM}$ (3)	3	
PCR negative (1)	0	
Synergy combined disk test for the characterization of MBLs		
CDT-positive	9	19.1%
CDT-negative	38	80.9%
Synergy combined disk test for the characterization of KPC		
CDT-positive	25	53.2%
CDT-negative	22	46.8%

MHT: modified hodge test; MBL: metallo-beta-lactamase; CDT: combined disk test; KPC: Klebsiella pneumoniae carbapenemases

Regarding clinical presentation, all cases with VAP were CRE, and 27.7% of cases with CRE had septicaemia – a significantly higher rate in comparison with CSE isolates – while all cases with UTI were CSE. This may be due to the use of carbapenems as empirical therapy in our health facilities in cases of bacteremia and VAP, with its limited use for empirical treatment of UTI cases. Our findings are in accordance with an Indian study in which 25% of cases with blood stream infections were CRE.[22] A Canadian study, however, found three cases in which person-to-person transmission can occur with CRE. These three cases were also multidrug resistant. Two included meropenem resistance, while the third was initially susceptible to meropenem with some clinical improvement, only to relapse with fully resistant isolates.[23] A new strategy for antibiotic use could therefore be an efficient means to decrease the incidence of VAP, septicaemia, and other infections caused by carbapenem-resistant bacteria.

In our study, CRE were more frequently isolated from blood (53.2%), while 60.7% of samples carrying CSE isolates were from urine, which is statistically significant. Our findings are in accordance with the results of an Italian study, which found that CRE were most commonly isolated in the blood.[22] Conversely,

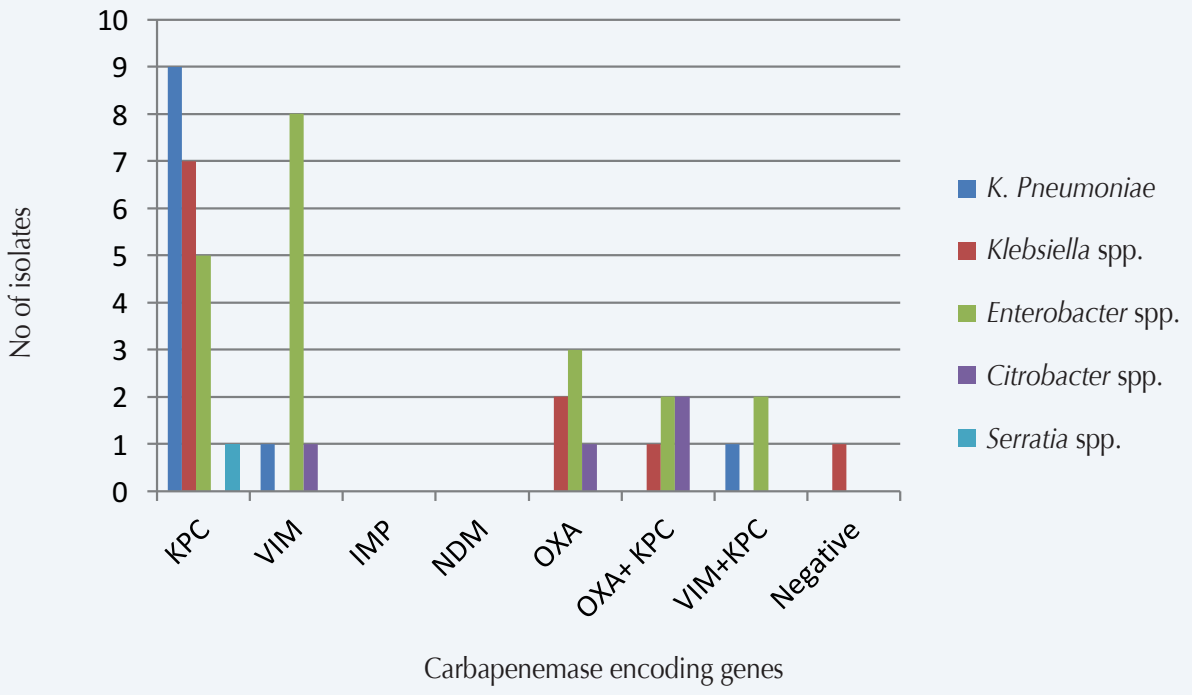

Figure 1. The distribution of carbapenemase encoding genes in the study isolates.

however, Shanmugam *et al.* documented that the majority of CRE isolates were from urine.[14]

In the current study, the duration of hospital admission in CRE cases was 6.47±3.64, which is significantly longer than for CSE cases. This is supported by the results of another study showing that a longer length of hospital stay was highly significant in terms of carbapenem resistance.[19] The study by Lin et al. showed the prevalence range of carbapenem resistance in long-term acute care hospitals to be 10%-54% versus 0%-29% in short stay hospitals.[20] However, our results disagree with the results of an Israeli study showing that the length of hospital stay before and after isolation did not differ between groups.[25]

One of the main predisposing factors for the dissemination of CRE is the abuse of antibiotics. The present study shows that 76.6% of cases harbouring CRE were previously exposed to antibiotic intake (mainly imipenem) prior to displaying culture and sensitivity results that indicated resistance. This was significantly higher than those for CSE isolates. This high prevalence may be due to the availability of non-prescribed antibiotics. This is supported by a study by Teo *et al.*, which found that 73% of cases receiving previous empirical antibiotics were infected with CRE.[26] A study by Savard and Perl also revealed that most patients with CRE received empirical carbapenem therapy.[27] Controversially, these results disagree with another study showing that previous exposure to antibiotics is not a risk factor for CRE infection.[28] We also found that previous exposure to ciprofloxacin was statistically significant for CSE isolates in comparison with CRE isolates. This was in agreement with a study by Tuona *et al.* showing that ciprofloxacin exposure during hospitalization was an independent risk factor for CRE infection.[18]

Regarding the type of isolated *Enterobacteriaceae,* there was significant isolation of CRE in *Enterobacter* spp., *K. pneumoniae* and *Klebsiella* spp. (42.6%, 23.4% and 23.4% respectively), while 53.6% of CSE isolates were *E. coli*. These results agree with other studies that found *Enterobacter* spp., *K. pneumoniae* and *Klebsiella* spp. to be the most common CRE isolates[29-31] This may be attributed to their ability to transfer multidrug resistance plasmids. A further another study showed that carbapenem resistance was seen mainly in *E. coli*.[32]

In the present study, the most prevalent genes were bla_{KPC} (22, 46.8%), while bla_{IMP} and bla_{NDM} genes were not detected. The total detection rate was 97.7%. This was in line with the results of a Mumbai study that found a high rate of carbapenamase production (more than 98%) by one or multiple genes.[33] The distribution of the studied genes were: bla_{NDM} alone 76.57%; bla_{OXA} alone 4.5%; double genes for carbapenamase production (bla_{OXA-48} + bla_{NDM} and bla_{NDM} + bla_{VIM}) 18.9%; with bla_{VIM}, bla_{IMP} and bla_{KPC} being undetected. Of the bla_{NDM} cases, more than 40% were *Klebsiella* spp. followed by *E. coli* (34.11%), *Enterobacter* spp. (21.17%), *Proteus* spp. (2.35%), *Citrobacter* spp. (1.17%), and *Morganella* spp. (1.17 %).

In contrast to results of the present study, carbapenamase producers were less than 30% in a study by Okoche *et al.*, with about 14% found to carry more than one encoding genes.[34] The most prevalent gene was blaVIM (10.7%), followed by bla_{OXA-48} (9.7%), bla_{IMP} (6.1%), bla_{KPC} (5.1%) and bla_{NDM-1} (2.6%). The highest number of these genes was found in *K. pneumoniae* (52.2%), followed by *E. coli* (28.4%), *Enterobacter* spp. (7.5%), *Serratia marcescens* (4.5%) and *Proteus mirabilis* (3.0%), while *Citrobacter freundii*, *Klebsiella oxytoca*, and *Pantoea agglomerans* were present in 1.5%. In another Moroccan study, results showed that carbapenamase production was less than 3%, and only two types of encoding genes were present: bla_{OXA-48} in 10 cases, and bla_{NDM-1} in 3.[35] A further Indian study showed that carbapenem resistance was less than 3% for MHT and PCR.[36]

We found also that 40.9 % (9/22) of the most commonly found gene (bla_{KPC}) were in *K. pneumoniae* isolates. This was consistent with the findings of other studies.[37,38]

Regarding the phenotypic methods used for the detection of carbapenem resistance, the present study revealed that the overall detection rate by MHT was 82.9%. This was in line with two other studies in which the overall detection rate of carbapenem resistance by MHT reached 96.46 % and 95%, respectively.[33,39] In another Indian study, results showed that MHT detected 80% of carbapenem resistant *K. pneumoniae isolates*.[40] Moreover, Amjad *et al.* reported that MHT reached 69%.[32] In contrast with these findings, a Ugandan study showed a relatively low MHT carbapenamase detection rate of 10.2%.[34] Similarly, a Moroccan study showed carbapenemase production by MHT to be 2.8%.[35] This may be explained by the restricted use of antibiotics in these countries compared to Egypt, where most drugs are available without prescription.

In this study, the synergy combined disc tests used for the characterization of MBLs and KPC revealed 19.1% and 47% of positive cases, respectively. Interestingly, comparative results vary by global location. Okoche et al. reported 11.2% positive cases detected by Boronic acid screen and 3.6% positive cases detected by EDTA test,[34] while Kazi *et al.* found positive MBLs tests reached 94.6 %, while no isolate was positive for KPC.[33]

Conclusion

Carbapenem resistance in Egypt is distressing, leading to an increased burden due to limited treatment options and increased mortality. We concluded that young age, previous antibiotic intake (especially carbapenems), long hospital admission period (particularly in neonatal ICUs), and bacteraemia are the most common risk factors for CRE infection. We attributed our high findings of carbapenam resistance to the following factors: (1) the empirical treatment by carbapenam antibiotics in Tanta University Hospital with the absence of antimicrobial stewardship; (2) the need for more effective infection control measures; and (3) the unrestricted over-the-counter sale of all types of antibiotics, even without prescription. We therefore suggest the development of antimicrobial stewardship in Tanta University Hospital, under governmental supervision, to prevent the unrestricted sale of non-prescribed antimicrobial medication.

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References

1. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-ß lactamases: the quiet before the storm? *Clin Microbiol Rev* 2005; **18:** 306–325. http://dx.doi.org/10.1128/CMR.18.2.306-325.2005
2. Poirel L, Pitout JD, Nordmann P. Carbapenemases: molecular diversity and clinical consequences. *Future Microbiol* 2007; **2:** 501–512. http://dx.doi.org/10.2217/17460913.2.5.501
3. Shahid M, Sobia F, Singh A, *et al*. Beta-lactams and beta-lactamase-inhibitors in current- or potential-clinical practice: a comprehensive update. *Crit Rev Microbiol* 2009; **35:** 81-108. http://dx.doi.org/10.1080/10408410902733979
4. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase producing *Enterobacteriaceae*. *Emerg Infect Dis* 2011; **17:** 1791–1798. http://dx.doi.org/10.3201/eid1710.110655
5. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V. Identification and screening of carbapenemase-producing *Enterobacteriaceae*. *Clin Microbiol Infect* 2012; **17:** 432–438. http://dx.doi.org/10.1111/j.1469-0691.2012.03815.x
6. Queenan AM, Bush K. Carbapenemases: the versatile ßlactamases. *Clin Microbiol Rev* 2007; **20:** 440–458. http://dx.doi.org/10.1128/CMR.00001-07
7. Cantón R, Akóva M, Carmeli Y, *et al*. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* 2012; **18:** 413–431. http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x.
8. Center of disease Control and prevention: CDC/NHSN Surveillance Definitions for Specific Types of Infections, 2016 http://www.cdc.gov/nhsn/PDFs/pscManual/2PSC_IdentifyingHAIs_NHSNcurrent.pdf [Accessed February 14, 2016]
9. Center of disease Control and prevention: Identifying Healthcare-associated Infections (HAI) for NHSN Surveillance, 2016 http://www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf [Accessed February 14, 2016]
10. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
11. Tsakris A, Themeli-Digalaki K, Poulou A, *et al*. Comparative evaluation of combined-disk tests using different boronic acid compounds for detection of *Klebsiella pneumoniae* carbapenemase-producing *Enterobacteriaceae* clinical isolates. *J Clin Microbiol* 2011; **49:** 2804–2809. http://dx.doi.org/10.1128/JCM.00666-11
12. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H. Evaluation of different laboratory tests for the detection of metallo-beta-lactamase production in *Enterobacteriaceae*. *J Antimicrob Chemother* 2008; **61:** 548–553. http://dx.doi.org/10.1093/jac/dkm535.
13. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout, JD. Laboratory detection of *Enterobacteriaceae* that produce carbapenemases. *J Clin Microbiol* 2012; **50:** 3877-3880. http://dx.doi.org/10.1128/JCM.02117-12.
14. Shanmugam P, Meenakshisundaram J, Jayaraman P. blaKPC gene detection in clinical isolates of carbapenem resistant *Enterobacteriaceae* in a tertiary care hospital. *J Clin Diagn Res* 2013; **7:** 2736-2738. http://dx.doi.org/10.7860/JCDR/2013/7759.3747
15. Shahid M, Umesh, Sobia F, *et al*. Molecular epidemiology of carbapenem resistant *Enterobacteriaceae* from a North Indian Tertiary Hospital. *N Z J Med Lab Sci 2012*; **66:** 5-7
16. Ramana KV, Rao R, Sharada ChV, Kareem MA, Reddy LR, Ratna MM. Modified Hodge test: A useful and the low cost phenotypic method for detection of carbapenemase producers in *Enterobacteriaceae* members. *J Nat Sc Biol Med* 2013; **4:** 346-348. http://dx.doi.org/10.4103/0976-9668.117009
17. Qureshi ZA, Paterson DL, Potoski BA, *et al*. Treatment outcome of bacteremia due to *KPC*- producing *Klebsiella pneumoniae*: superiority of combination anti-microbial regimens. *Antimicrob Agents Chemother* 2012; **56:** 2108–2113. http://dx.doi.org/10.1128/AAC.06268-11
18. Tuona FF, Rochac JL, Toledob P, *et al*. Risk factors for KPC-producing *Klebsiella pneumoniae* bacteremia. *Braz J Infect Dis* 2012; **16:** 416–419. http://dx.doi.org/10.1016/j.bjid.2012.08.006
19. Ulu AC, Kurtaran B, Inal AS, Kömür S, Kibar F, Çiçekdemir HY. Risk factors of carbapenem-resistant *Klebsiella pneumoniae* infection: A serious threat in ICUs. *Med Sci Monit* 2015; **21**: 219-224. http://dx.doi.org/10.12659/MSM.892516
20. Lin MY, Lyles-Banks RD, Lolans K, *et al*. The importance of long-term acute care hospitals in the regional epidemiology of *Klebsiella pneumoniae* carbapenemase-producing *Enterobacteriaceae*. *Clin Infect Dis* 2013; **57:** 1246-1252. http://dx.doi.org/10.1093/cid/cit500
21. Shawky SM, Abdallah A, Khouly M. Antimicrobial activity of colistin and tigecycline against carbapenem resistant *Klebsiella pneumoniae* clinical isolates in Alexandria, Egypt. *Int J Curr Microbiol App Sci* 2015; **4:** 731-742.
22. Sood S. Identification and differentiation of carbapenemases in *Klebsiella pneumoniae*: A phenotypic test evaluation study from Jaipur. *J Clin Diagn Res* 2014; **8:** DC01-3. http://dx.doi.org/10.7860/JCDR/2014/7027.4614.
23. Goldfarb D, Harvey SB, Jessamine K, Jessamine P, Toye B, Desjardins M. Detection of plasmid-mediated KPC-producing *Klebsiella pneumoniae* in Ottawa, Canada: evidence of intra-hospital transmission. *J Clin Microbiol* 2009; **47:** 1920–1922. http://dx.doi.org/10.1128/JCM.00098-09.
24. Tascini C, Tagliaferri E, Giani T, *et al*. Synergistic activity of colistin plus rifampin against colistin-resistant KPC-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2013; **57:** 3990–3993. http://dx.doi.org/10.1128/AAC.00179-13
25. Marchaim D, Navon-Venezia S, Schwaber MJ, Carmeli Y. Isolation of imipenem-resistant *Enterobacter* species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. *Antimicrob Agents Chemother* 2008; **52:** 1413–1418. http://dx.doi.org/10.1128/AAC.01103-07
26. Teo J, Cai Y, Tang S, *et al*. Risk factors, molecular epidemiology and outcomes of ertapenem-resistant, carbapenem-susceptible *Enterobacteriaceae:* a case-case-control study. *PLoS One* 2012; **7:** 1–8. http://dx.doi.org/10.1371/journal.pone.0034254
27. Savard P, Perl TM. A call for action: managing the emergence of multidrug-resistant *Enterobacteriaceae* in the acute care settings. *Curr Opin Infect Dis* 2012; **25:** 371–377. http://dx.doi.org/10.1097/QCO.0b013e3283558c17
28. Mouloudi E, Protonotariou E, Zagorianou A, *et al*. Bloodstream infections caused by metallo-beta-lactamase/*Klebsiella pneumoniae* carbapenemase producing *K. pneumoniae* among intensive care unit patients in Greece: risk factors for infection and impact of type of resistance on outcomes. *Infect Control Hosp Epidemiol* 2010; **31:** 1250–1256. http://dx.doi.org/10.1086/657135

29. Maalej SM, Meziou MR, Rhimi FM, Hammami A. Comparison of disc diffusion, Etest and agar dilution for susceptibility testing of colistin against Enterobacteriaceae. *Lett Appl Microbiol* 2011; **53:** 546–551. http://dx.doi.org/10.1111/j.1472-765X.2011.03145.x
30. Kiedrowski LM, Guerrero DM, Perez F, *et al*. Carbapenem-resistant *Enterobacter cloacae* isolates producing KPC-3, North Dakota, USA. *Emerg Infect Dis* 2014; **20:** 1583-1584. http://dx.doi.org/10.3201/eid2009.140344
31. Xu Y, Gu B, Huang M, *et al.* Epidemiology of carbapenem resistant *Enterobacteriaceae* (CRE) during 2000-2012 in Asia. *J Thorac Dis* 2015; **7:** 376-385. http://dx.doi.org/10.3978/j.issn.2072-1439.2014.12.33
32. Amjad A, Mirza IA, Abbasi, SA, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. *Iran J Microbiol* 2011; **3:** 189-193.
33. Kazi M, Drego L, Nikam C, *et al*. Molecular characterization of carbapenem-resistant *Enterobacteriaceae* at a tertiary care laboratory in Mumbai. *Eur J Clin Microbiol Infect Dis* 2015; **34:** 467-472. http://dx.doi.org/10.1007/s10096-014-2249-x
34. 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:** e0135745. http://dx.doi.org/10.1371/journal.pone.0135745
35. El Wartiti MA, Bahmani F, Elouennass M, Benouda A. Prevalence of carbapenemase-producing *Enterobacteriaceae* in a University Hospital in Rabat, Morocco: A 19-months prospective study. *Int Arab J Antimicrob Ag* 2012; **2:** 1–6. http://dx.doi.org/10.3823/718
36. Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1- and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the SENTRY antimicrobial surveillance program, 2006-2007. *Antimicrob Agents Chemother* 2011; **55:** 1274-1278. http://dx.doi.org/10.1128/AAC.01497-10
37. Robledo IE, Aquino EE, Va´zquez GJ. Detection of the *KPC* Gene in *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii* during a PCR-based nosocomial surveillance study in Puerto Rico. *Antimicrob Agents Chemother* 2011; **55:** 2968–2970. http://dx.doi.org/10.1128/AAC.01633-10
38. Pavelkovich A, Balode A, Edquist P, *et al*. Detection of carbapenemase-producing *Enterobacteriaceae* in the Baltic countries and St. Petersburg area. *BioMed Res Int* 2014; **2014:** 548960.
39. Birgy A, Bidet P, Genel N, *et al*. Phenotypic screening of carbapenemases and associated ß-lactamases in carbapenem-resistant *Enterobacteriaceae*. *J Clin Microbiol* 2012; **50:** 1295-1302. http://dx.doi.org/10.1128/JCM.06131-11
40. Bina M, Pournajaf A, Mirkalantari S, Talebi M, Irajian G. Detection of the *Klebsiella pneumoniae* carbapenemase (KPC) in *K. pneumoniae* isolated from the clinical samples by the phenotypic and genotypic methods. *Iran J Pathol* 2015; **10:** 199-205.