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Hospital Acquired Infections by Carbapenem Resistant Enterobacteriacea

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ABSTRACT

Background: *The emergence and spread of carbapenem resistant Enterobacteriaceae (CRE) infections have become an increasing concern for healthcare services worldwide. Community and hospital-acquired infections caused by these bacteria have been associated with significant morbidity and mortality with limited treatment options. Rapid and accurate detection of carbapenem resistance in these bacteria is important for infection control.* ***Objectives:*** *To detect the prevalence of carbapenem resistant Enterobacteriaceae (CRE) species and determine their antimicrobial susceptibility profile using the Vitek 2 system and detect the presence of carbapenemases genes using Multiplex PCR.* ***Methodology:*** *Various clinical samples were collected from 469 patients admitted in Sohag university hospitals in the period between April 2016 and August 2017, CRE isolates were identified by conventional methods and antimicrobial susceptibility testing using disc diffusion method and also performed by Vitek 2 automated system, Multiplex PCR was used for detection of carbapenemases genes as blaKPC, blaVIM, blaIMP, blaNDM-1 and blaOXA-48.* ***Results:*** *The prevalence of carbapenem resistant Enterobacteriaceae (CRE) species was 19.9%, Klebsiella pneumoniae was the most common species (51.4%), Escherichia Coli (28.6%), Enterobacter aerogenes(8.6%), Acinetobacter baumannii (5.7%), Vitek 2 system identified CRE isolates with 82.7% sensitivity, 98.6 % specificity and 90.6% diagnostic accuracy, 25.7 % of CRE strains were isolated from the internal ICU and 20 % from chest department and mostly isolated from urine(40%) and from endotracheal tubes swabs(28.6%), 77.1% of CRE isolates contained carbapenemases genes, 62.1 % were blaKPC positive, 20.7 % were blaVIM-positive, 3.4 % were blaNDM-positive, 13.8 % were blaOXA-48-positive and none was blaIMP-positive.* ***Conclusion:*** *Conventional methods supported by Vitek 2 system is a valuable method for identification of CRE species, the detected carbapenemases genes in this study indicates that carbapenem resistance is spreading in Egypt and support the use of molecular methods for the rapid detection of CRE for successful implementation of infection control measures, we recommend routine testing to determine carbapenem resistance in Enterobacteriaceae in health facilities in Egypt.*

Keywords: Carbapenem, Enterobacteriacea, Vitek 2, Multiplex PCR

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INTRODUCTION

Enterobacteriaceae are common human pathogens and colonizers of the intestinal tract which can cause a broad range of diseases including urinary tract infections, pneumonia, bloodstream infections, intraabdominal, skin and soft tissue infections in both community and hospital settings (1).

The increase in antibiotic resistance in the Enterobacteriaceae family has become a major threat to public health. Especially in recent years, Enterobacteriaceae species have been identified as important nosocomial pathogens that can lead to severe morbidity and mortality, particularly in intensive care units (ICU), internal medicine and surgical units (2).

Dissemination of infections caused by extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases producing Enterobacteriaceae has compromised susceptibility to cephalosporins in many areas of the world and has led to increased use of carbapenems which are stable antibiotics against these enzymes. However widespread use of carbapenems resulted in the emergence of carbapenem-resistant Enterobacteriaceae (CRE) (3,4). Mainly two different mechanisms are responsible for carbapenem resistance in Enterobacteriaceae: (i) hyper production of ESBL or AmpC enzymes combined with porin loss or upregulated efflux pump and/ or (ii) production of carbapenem-hydrolyzing carbapenemases (5).

Carbapenem-resistant Enterobacteriaceae (CRE) have been reported worldwide as a consequence largely of acquisition of carbapenemase genes (2). The first carbapenemase producer in Enterobacteriaceae (NmcA) was identified in 1993 (6). These enzymes have primarily been described in *Klebsiella spp*. and *Serratia marcescens*, and throughout different countries (7,8)

While resistance of chromosomal-mediated (intrinsic) carbapenemases is limited, some plasmid-mediated (extrinsic) carbapenemases have emerged in recent years. Plasmid-mediated carbapenemases can hydrolyse β-lactam antibiotics in addition to carbapenems. These are the members of Ambler class A, B and D β-lactamases. The SME, NMC, IMI, KPC, and GES enzymes comprise class A carbapenemases. Moreover, IPM, VIM, GIM, SPM, SIM, and NDM-1 enzymes comprise class B, and OXA enzymes that hydrolyse oxacillins comprise class D (9).

Several molecular methods including plasmid profile analysis, restriction endonuclease analysis (REA) of chromosomal DNA, restriction fragment length polymorphism (RFLPs), real time and multiplex PCR, ribotyping, pulsed-field gel electrophoresis (PFGE) and DNA sequencing are used to detect the presence of carbapenemases genes in Carbapenem-resistant Enterobacteriaceae .(10,11).

Although the recent emergence and dissemination of carbapenem resistance in Enterobacteriaceae is frequently reported worldwide but a limited number of published data about resistant species are available (12,13).

The aim of this study was to determine the prevalence and characteristics of carbapenem resistant Enterobacteriacea isolated from patients admitted in different departments of Sohag university hospitals and determine their antimicrobial susceptibility profile using phenotypic methods and to detect the presence of carbapenemases (bla) genes using a molecular method

METHODOLOGY

Study Design:

This was a cross-sectional laboratory based study conducted in the infection control unit laboratory of Sohag university hospitals and included 469 patients admitted to different departments and ICUs who acquired nosocomial infections in the period between April 2016 and August 2017. All patients, diagnosed on the basis of clinical presentation, were subjected to the following: detailed history of associated risk factors, thorough clinical examination and laboratory investigations, institutional ethical board approval and informed consent were taken from all the patients.

Phenotypic identification of Carbapenem Resistant Enterobacteriacea(CRE) :

Enterobacteriaceae isolates from different clinical samples (urine, pus, sputum, blood and endotracheal tubes swabs, etc) were identified using conventional methods as gram staining, culture on MacConkey's, EMB and MIO agars, and biochemical reactions.

Phenotypic identification of CRE isolates were performed by antimicrobial susceptibility testing using Kirby-Bauer disc diffusion method according to CLSI 2013 M100-S23 breakpoint values (14); Susceptibilities were determined to imipenem(IPM)(10µg), meropenem(MEM)(10µg), ertapenem(ERT)(10µg), ceftriaxone(30µg), cefotaxime(30µg), ceftazidime(30µg), cefoxitin(30µg), cefepime(30µg), ciprofloxacin(5µg), levofloxacin(5µg), ampicillin(10µg), piperacillin(100µg), amoxicillin/ clavulanic acid (20/10ug), piperacillin/tazobactam(100/10µg), aztreonam(30µg), amikacin(30µg), gentamicin(10µg), tobramycin(10µg) and trimethoprim sulphamethoxazole (1.25/23.75µg) (Oxoid, UK), isolates were considered as CRE if they were found resistant or intermediate susceptible to one or more of the carbapenems (IPM, MEM and ERT).

Vitek 2 system identification of CRE:

Identification of the CRE isolates was also performed by Vitek 2 automated system, pure subcultures of Enterobacteriaceae isolates were suspended in sterile saline and measured by the DensiChek turbidity meter (bioMérieux) to obtain 0.5 McFarland turbidity, then inoculated to the colorimetric ID-GN cards, the Vitek 2 compact instrument automatically filled, sealed, and incubated the cards, results were compared to the database of the unknown organism. Final identifications listed as "excellent," "very good," "good," "acceptable" or "low discrimination" was considered correct, antibiotic susceptibility tests were done using Vitek 2 AST-GN cards performed according to manufacturer's protocol. (bioMérieux, Marcy l'Etoile, France)

Multiplex PCR amplification of Carbapenemases genes:

Multiplex PCR was used for detection of carbapenemases (bla) genes as *bla*KPC gene, metallo-β-lactamase genes as *bla*VIM, *bla*IMP and *bla*NDM-1, oxacillinase genes as *bla*OXA-48, DNA extraction for all carbapenem resistant Enterobacteriacea(CRE) isolates was performed using boiling method to obtain bacterial DNA, primers sequences used for detection of carbapenemases genes are presented in Table (1).

Table 1: Primers sequences used in PCR detection of Carbapenemases genes

Primers	*Primer sequences*	*Amplicon size*
bla KPC	F 5'-CAT TCA AGG GCT TTC TTG CT-3'	521bp
	R 5'-ACG ACG GCA TAG TCA TTT GC-3'	
bla IMP	F 5'-TTG ACA CTC CAT TTA CDG-3'	139bp
	R 5'-GAT YGA GAA TTA AGC CAC YC-3'	
bla VIM	F 5'-GAT GGT GTT TGG TCG CAT A-3'	390 bp
	R 5'- CGA ATG CGC AGC ACC AG-3'	
bla NDM-1	F 5'-GGT TTG GCG ATC TGG TTT TC-3'	339 bp
	R 5'-CGG AAT GGC TCA TCA CGA TC-3'	
bla OXA-48	F 5'-GCT TGA TCG CCC TCG ATT-3	281 bp
	R 5'-GAT TTG CTC CGT GGC CGA AA-3'	

Amplification was carried out in T-Gradient thermal cycler (Biometra, Germany) using 50 µL reaction volume containing 5uL of template DNA (50ng/ uL) added to a 45uL mixture containing 200 uM of dNTP mixtures (Roche, Switzerland), 0.4uM of each primer, 2.5U Taq DNA polymerase (Invitrogen, Germany), and appropriate PCR buffer (0.2uM MgCl2, 2.5uM KCL, 0.5uL 10% Tween 20, 1uL of Gelatin, and 3.8uL of pure water). For *bla*VIM, *bla* KPC, *bla* NDM, and *bla* OXA-48, the programme was denaturation at 94°C for 45 seconds, annealing at 52°C for 1 minute, and elongation at 72 °C for 1 minute. For *bla* IMP the same programme was used except that the annealing temperature was adjusted to 45°C for 1 minute, a total of 40 cycles were performed. This was followed by a final extension at 72° C for 10 minutes. PCR amplicons were resolved by electrophoresis on a 1.5 % agarose gel stained with ethidium bromide by comparing with 100 base-pairs standard DNA ladder and visualized by gel documentation system (15)

Statistical Analysis

Data were analyzed using computer program SPSS (Statistical Package for the Social Science (SPSS) version, data were expressed as number and percent, quantitative data were analyzed using student Mann-Whitney test. Qualitative data was compared using Chi square test; P value was considered significant if less than 0.05.

RESULTS

A total of 469 patients were included in the study, with mean age 51.4 y (range 14–83y), 294 (62.7 %) were males and 175 (37.3 %) were females.

590 pathogens were identified, these consisted of 176 (29.8%) Enterobacteriaceae, 96 (16.4%) non enterobacteriaceae gram –ve bacilli, 211(35.8%) gram +ve cocci and 107(18.1%) fungal pathogens.

Enterobacteriaceae isolates:

From the 176 Enterobacteriaceae isolates identified, *Klebsiella pneumoniae* was the most common isolate 68(38.6%), followed by *Escherichia Coli* 54(30.7%), *Enterobacter aerogenes* 24(13.6%), *Proteus mirabilis* 13(7.4%), *Acinetobacter baumannii* 11(6.3%), *Enterobacter cloacae complex* 3(1.7%), *Klebsiella oxytoca* 2(1.1%), *Citrobacter spp* 1(0.6%) (Table 2)

The cumulative antibiogram of Enterobacteriaceae isolates were as follow; resistance rate to imipenem(21.8%), meropenem(20.1%), ertapenem(19.9%), ceftriaxone(47%), cefotaxime(50.7%), ceftazidime(63.9%), cefoxitin(50%), cefepime(42.8%), ciprofloxacin(35.8%), levofloxacin(29.9%), ampicillin(93.8%), piperacillin(80.1%), amoxicillin/clavulanic acid (83%), piperacillin/tazobactam (71%), aztreonam(19.9%), amikacin(35.9%),gentamicin(63%), tobramycin(76.7%) and trimethoprim-sulphamethoxazole (87.1%).

CRE isolates:

35(19.9%) CRE isolates were identified from the 176 Enterobacteriaceae isolates by the disc diffusion method, the most common CRE species were *Klebsiella pneumoniae* 18(51.4%), *Escherichia Coli* 10(28.6%), *Enterobacter aerogenes* 3(8.6%), *Acinetobacter baumannii* 2(5.7%), *Proteus mirabilis* 1(2.8%) and *Klebsiella oxytoca* 1 (2.8%) (Table 2), also Vitek 2 system identified correctly 29 CRE isolates with 82.7% sensitivity, 98.6 % specificity and 90.6% diagnostic accuracy, there was no statistically significant differences between percentages of different species of carbapenem resistant and carbapenem sensitive Enterobacteriacae(P value=0.06) (Table 2,3)

Table 2: Comparison between Carbapenem Resistant and Carbapenem Sensitive Enterobacteriacae species:

Enterobacteriacae species:	*Total*	*Carbapenem resistant Enterobacteriacae*	*Carbapenem sensitive Enterobacteriacae*	P-value
Klebsiella pneumoniae	68(38.6%)	18 (51.4%)	50 (35.7%)	
E. Coli	54(30.7%)	10 (28.6%)	44 (31.2%)	
Enterobacter aerogenes	24(13.6%)	3 (8.6%)	21 (14.9%)	
Acinetobacter baumannii	11(6.3%)	2 (5.7%)	9 (6.3%)	0.06
Enterobacter cloacae	3(1.7%)	0 (0%)	3 (2.1%)	
Proteus mirabilis	13(7.4%)	1 (2.8%)	12 (8.5%)	
Klebsiella oxytoca	2(1.1%)	1 (2.8%)	1 (0.7%)	
Citrobacter	1(0.6%)	0 (0%)	1 (0.7%)	
Total	176(100%)	35(19.9%)	141(80.1%)	

Table 3: Differential diagnostic values of manual versus Vitek 2 system in detecting Carbapenem Resistantance in Enterobacteriacae

Antibiotic sensitivity test		*Vitek 2 system AST-GN (Carbapenem) Resistant*	*Sensitive*	*Total*
Disc diffusion method (Carbapenem)	*Resistant*	29	6	35
	Sensitive	2	139	141
	Total	31	145	176
Sensitivity	*Specificity*	*PPV*	*NPV*	*Accuracy*
82.7%	98.6 %	93.6%	95.9 %	90.6%

25.7 % of CRE strains were isolated from the internal ICU, 20 % from chest, 17.1 % from surgery, 17.1 % from internal medicine, 11.4 % from neuropsychiatry, 8.6% from plastic surgery departments. As regard the clinical samples, most of the 35 CRE isolates were collected from urine (14 isolates, 40%), from endotracheal tube (10 isolates, 28.6%), from sputum (6 isolates, 17.1 %), and from pus (3 isolates,8.6%), there was no statistically significant differences regarding percentages of carbapenem resistant and carbapenem sensitive enterobacteriacae between different departments and clinical samples with non significant P values (0.19 and 0.08) simultaneously (Table 4, 5)

Table 4: Comparison between Carbapenem Resistant and Carbapenem Sensitive Enterobacteriacae according to Departments

Departments	*Total 176*	*Carbapenem resistance 35(19.9%)*	*Carbapenem sensitive 141(80.1%)*	*P- value*
ICUs	58	9(25.7%)	49 (34.7%)	
Chest	42	7 (20%)	35 (24.8%)	
Internal medicine	31	6 (17.1%)	25 (17.7%)	0.19
Neuro psychiatry	23	4 (11.4%)	19 (13.8%)	
General surgery	17	6 (17.1%)	11 (7.8%)	
Plastic surgery	5	3 (8.6%)	2 (1.4%)	

Table 5: Comparison between Carbapenem Resistant and Carbapenem Sensitive Enterobacteriacae according to Clinical samples

Clinical samples	*Total 176*	*Carbapenem resistance 35(19.9%)*	*Carbapenem sensitive 141(80.1%)*	*P- value*
Urine	**84**	**14 (40%)**	**70 (49.6%)**	
Pus	**22**	**3 (8.6%)**	**19 (13.8%)**	
Sputum	**28**	**6(17.1%)**	**22(15.6%)**	**0.08**
Blood	**5**	**0 (0 %)**	**5 (3.5%)**	
Urinary catheter	**7**	**2 (5.7%)**	**5 (3.5%)**	
Endotracheal tube	**30**	**10(28.6%)**	**20(14.2%)**	

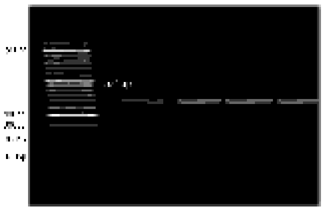
The use of external devices as intravenous cannula, urinary catheter and drainer was the most common risk factor of CRE infections, it was positive in 85.7% patients with significant P value (P value=0.02), previous antibiotic treatment, with augmentin and third generation cephalosporins were the commonest antibiotics, was positive in 40% of CRE patients (p value=0.08), diabetes mellitus was positive in 34%, renal disease was positive in 26% of CRE patients with non significant P values(0.06 and 0.4) simultaneously, there were no statistically significant differences between patients with CRE and patients without CRE as regard age, sex, liver diseases, duration of hospital stay and duration of antibiotics intake (p values > 0.05)

Carbapenemases genes identified by multiplex PCR:

27(77.1%) isolates from a total of 35 CRE isolates contained at least one of the carbapenemases (bla) encoding genes. Of these, 18 (62.1 %) were *bla*KPC positive, 6(20.7 %) were *bla*VIM-positive, 1 (3.4 %) were *bla*NDM-1-positive, 4 (13.8 %) were *bla*OXA-48-positive and 0 (0 %) was *bla*IMP-positive, 2 (7.4 %) of CRE isolates contained both of *bla*KPC and *bla*VIM genes, most of the carbapenemase encoding CRE isolates were *Klebsiella pneumoniae* 18/18(100 %), *Escherichia Coli* 8/10(80 %), *Enterobacter aerogenes* 2/3(66.7%), *Acinetobacter baumannii* 1/2(50 %), in *Klebsiella pneumoniae* and *Escherichia Coli*, the predominating *bla* gene was *bla*KPC gene (66.7% & 50 %) simultaneously. (Table 6) (Figures 1-5)

Table 6: Distribution of Carbapenemase (bla) genes in Carbapenem Resistant Enterobacteriacae pecies

CRE species	*blaKPC*	*blaVIM*	*blaNDM*	*blaOXA48*	*blaIMP*	*Total*
Klebsiella pneumoniae(n=18)	**12(66.7%)**	**2(11.1%)**	**1(5.6%)**	**3(16.7%)**	**0 (0%)**	**18**
E. Coli (n=10)	**4(50%)**	**3(37.5%)**	**0 (0%)**	**1(12.5%)**	**0 (0%)**	**(100%)**
Enterobacter aerogenes(n=3)	**1(50%)**	**1(50%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**8 (80%)**
Acinetobacter baumannii (n=2)	**1(100%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**2 (66.7%)**
Proteus mirabilis(n=1)	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**1 (50%)**
Klebsiella oxytoca(n=1)	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**
						0 (0%)
Total	18(62.1 %)	6(20.7 %)	1 (3.4 %)	4 (13.8 %)	0 (0%)	29 (100%)



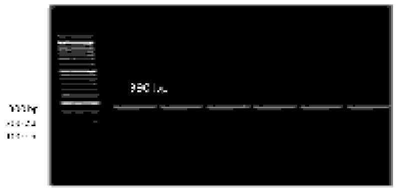


Figure 1: Agarose gel electrophoresis of amplified bla KPC gene (521 bp).
Figure 2: Agarose gel electrophoresis of amplified bla VIM gene (390 bp).

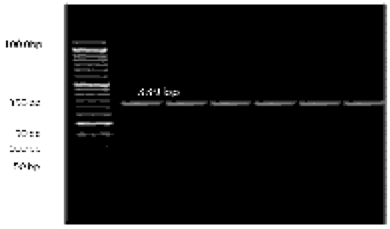


Figure 3: Agarosel gel electrophoresis of blaNDM-1 gene (339 bp)
Figure 4: Agarosel gel electrophoresis of blaOXA-48 gene (281 bp)

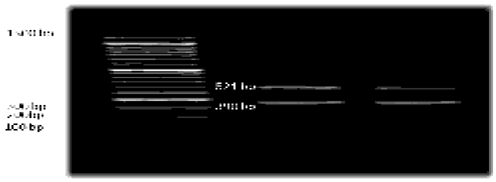
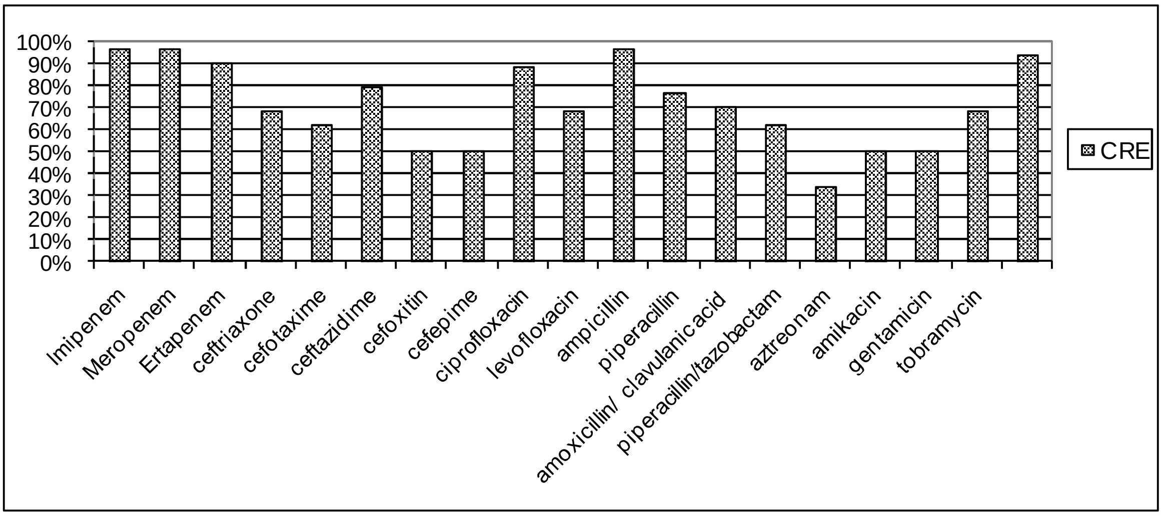


Figure 5: Agarosel gel electrophoresis of multiplex blaVIM and blaKPC genes (390 bp & 521 bp)

Cumulative Antibiogram of CRE:

Figure 6 presents the cumulative antibiogram of the 35 CRE isolates studied. There was no statistically significant difference in the resistance rates of CRE to imipenem, meropenem or ertapenem(97%, 97% and 91%); as regard cephalosporins, resistance rate of CRE were 69% to ceftriaxone, 63% to cefotaxime, 80% to ceftazidime and 51% to cefoxitin and cefepime.

31/35 isolates (89%) were resistant to ciprofloxacin and 24/35 isolates (69%) to levofloxacin As regards penicillins, resistance rates of CRE were 97% to ampicillin, 77% to piperacillin, 71% to amoxicillin/clavulanic acid and 63% to piperacillin/tazobactam, 33/35 of isolates (94%) were resistant to trimethoprim-sulfamethoxazole, no significant difference was noted in resistance rates to amikacin (51%) compared to gentamicin (51%) or tobramycin (69%). (Figure 6)



DISCUSSION

Carbapenem resistance among Enterobacteriaceae, in particular *Klebsiella pneumoniae* and *Escherichia coli*, is an emerging problem worldwide; production of carbapenemases is the most common mechanism of this resistance, rapid identification of carbapenemase producing Enterobacteriaceae strains is crucial for preventing hospital infections and outbreaks (16)

In this study, 590 pathogens from 469 patients were identified, 176 (29.8%) Enterobacteriaceae isolates were identified; *Klebsiella pneumoniae* was the most common species (38.6%), followed by *Escherichia Coli* (30.7%), *Enterobacter aerogenes* (13.6%), these results were in agreement with the results reported by *Elraghya etal.* who found that *E. coli* (48.4%) and *K. pneumoniae* (18.3%) were the two most common Enterobacteriaceae species, followed by *Enterobacter spp.* (13.3%) (17), this also correlates with the findings of *Shaaban et al*. and *AbdEl-Mongy and Reyad* (18,19)

The rate of CRE among Enterobacteriaceae species in our hospital during this study was found as 19.9 %., the most common CRE species were *Klebsiella pneumoniae* (51.4%), *Escherichia Coli* (28.6%), *Enterobacter aerogenes* (8.6%), *Acinetobacter baumannii* (5.7%), *Proteus mirabilis* (2.8%) and *Klebsiella oxytoca* (2.8%)

These results correlate with the study performed by *El-Rehewy* and his colleagues who found that carbapenem resistance rate among gram negative bacilli in nosocomially infected patients from Assuit university hospitals is 27.17%(20), and in other two different Egyptian studies performed by *Elraghya etal.* in Menoufia university hospitals and by *El-Kazzaz and Abou El-khier*. in Mansoura university hospitals who found that the rates of CRE were 45% and 47% simultaneously (17,21), also in accordance with *Wattal et al.* who reported high prevalence of resistance to carbapenems ranging from 13 to 51% in *E.coli* and *Klebsiella spp*. from ICUs and wards from tertiary care hospital in Delhi (22)

In controversy to our results, a low rate of carbapenem resistance among Enterobacteriaceae (2.82 %) was found in a Turkish study perfomed by *Irmak and Neriman*(23) , also in the United States prevalence of CRE was found to be between 1.4 and 4.2 % (24), similar low CRE isolation rates have been reported; 1.2 % in Lebanon, 4.05 % in Malaysia.(25,26)

In our study, there was no statistically significant difference in the resistance rates of CRE isolates to imipenem, meropenem or ertapenem (97%, 97% and 91%) and this in agreement with the study peformed by *Sahin* and his colleagues on 43 CRE strains, 100% were ertapenem-resistant, 95.3 % were meropenem-resistant and 83.7 % were imipenem-resistant. (16)

The antibiogram of the CRE isolates of our study showed variable degrees of resistance to different antibiotics, as regard cephalosporins, resistance rate of CRE were 69% to ceftriaxone, 63% to cefotaxime, 80% to ceftazidime and 51% to cefoxitin and cefepime, 89% were resistant to ciprofloxacin and 69% to levofloxacin, similar resistance rates were detected by *Kucukates and Kocazeybek* (27) who reported that resistance of gram negative enteric bacilli to ciprofloxacin and ceftriaxone ranged from 50-100% and 25-83.3% respectively.

25.7 % of CRE strains were isolated from the internal ICU, 20 % from chest, 17.1 % from surgery; these results are in agreement with results obtained by *Irmak and Neriman* that showed that the majority of CRE strains were isolated from ICUs (27%) (23)

as regard the clinical samples, most of the CRE isolates were collected from urine (40%), from sputum (17.1 %), from pus (8.6%) and from endotracheal tubes swabs (28.6%), these results correlate with the study performed by *El-Rehewy etal* who found that the highest numbers of isolates were collected from the endotracheal aspirate (24.5%), followed by sputum samples (20%), urine samples (17.75%), blood samples (16.06%), wound swabs (15.77%), and then throat swabs (5.92%) (20)

Certain risk factors were found to be related to the acquirement of CRE infections, the use of external devices was positive in 85.7%, previous antibiotic treatment especially augmentin and third generation cephalosporins in 40%, diabetes mellitus in 34%, renal disease was positive in 26% of CRE infected patients, two previous studies reported that staying in the ICU, surgical procedures, using catheter, length of hospitalization and using of cephalosporins and aminoglycosides are risk factors for carbapenem-resistant *K. pneumoniae* infections (28,29)

In the current study, 77.1% of CRE isolates contained at least one of the carbapenemases genes identified by multiplex PCR, 62.1 % were *bla*KPC positive, 20.7 % were *bla*VIM-positive, 3.4 % were *bla*NDM-positive, 13.8 % were *bla*OXA-48-positive and non was *bla*IMP-positive, in *Klebsiella pneumoniae* and *Escherichia Coli*, the predominating *bla* gene was *bla*KPC gene (66.7% & 50 %) simultaneously.

As regard *bla*KPC gene, a study performed in Menoufia university hospitals on multidrug-resistant enterobacteriaceae nosocomial uropathogens showed that 24.07% of carbapenem resistant isolates were positive for *bla*KPC using real time PCR(17), and also in agreement with a study by *Girgis et al.* in Ain Shams university hospitals; they reported that 21% of isolates were *bla*KPC gene positive using PCR. (30)

Two previous studies evaluated carbapenem resistance in ESBL-producing carbapenem-resistant *K. pneumoniae* strains, of the 14 strains examined, the OXA-1 gene was detected in all, the OXA-48 gene in two, and the NDM-1 gene in two (31,32), a multi-central surveillance study performed at a Turkish university hospital showed that more than 96% of *K. pneumoniae* isolates harbored *bla*OXA-48 gene (33), *bla*NDM-1 gene in a previous study was observed in 6.5% of the resistant *K.pneumoniae* isolates recovered from infection sites and rectal swabs (34).

In controversy to our results, some studies reported that *bla*KPC, *bla*IMP, and *bla*VIM genes were not determined from any of the *K.pneumoniae* isolates, two recent studies performed on carbapenem-resistant *K. pneumoniae* clinical isolates from different hospitals of Turkey, no positive results for *bla*KPC, *bla*IMP, and *bla*VIM genes were detected (16), also, an Egyptian study performed by *Hassan etal* on 30 *Acinetobacter baumanii* carbapenem resistant clinical isolates reported that none of the isolates was positive for *bla*NDM-1 gene using real time PCR. (36)

CONCLUSION

The detected carbapenemases genes as *bla*KPC, *bla*VIM, *bla*NDM-1 and *bla*OXA-48 in this study indicate that carbapenem resistance is spreading in our locality and Egypt and support the use of molecular methods for the rapid detection of CRE, successful implementation of infection control measures is a must to reduce the problem of bacterial resistance, and to prevent its spread, we recommend routine testing to determine carbapenem resistance in Enterobacteriaceae isolates in our hospital and other health facilities in Egypt, in addition, antibiotics such as colistin, tigecycline and fosfomycin should be tested to provide alterative treatment to CRE.

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