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ORIGINAL ARTICLE

Detection and Characterization of Nosocomial Carbapenem-Resistant Gram Negative Bacilli from Assuit University Hospitals

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Key words:

Carbapenem resistance, Nosocomial, Tigecycline and Colistin

ABSTRACT

Background: *Carbapenem resistant gram negative bacilli (CRGNB) pose significant risks. They are difficult to detect, they have a role in unnoticed spread within hospitals and they are able to participate in horizontal gene transfer with other pathogens in hospitals.* ***Objectives:*** *This study was conducted to determine the frequency of carbapenem resistance among nosocomial GNB infections, to compare different phenotypic methods to PCR for accurate detection of CRGNB isolates, to define the risk factors for carbapenem resistance in GNB infections and to define possible treatment alternatives for CRGNB.* ***Methodology:*** *Three hundred fifty five specimens were collected from 134 nosocomially infected patients admitted to different hospital wards at Assuit University Hospitals. Phenotypic identification of CRGNB isolates were performed by antimicrobial susceptibility testing. Further confirmation and biotyping were done by API20E and API20NE kits. Phenotypic detection of carbapenemases was done by determination of MIC by Imipenem-E test and Meropenem-E test, CHROM agar KPC™ medium, modified Hodge test (MHT), combined disc method (CD), double disc synergy test (DDST).The isolated CRGNB were further identified genetically using the conventional polymerase reaction (PCR).* ***Results:*** *out of total 745 isolates, GNB represented 47.9%. Ninety seven isolates of GNB (27.17%) were resistant to carbapenems. All CRGNB isolates were tested by PCR for detection of bla genes (bla kpc, bla IMP-1, bla VIM-1 and bla oxa 48). 32.84% isolates were positive to bla KPC gene only, 17.92% were positive to bla IMP-1 gene only, 16.42% were positive to bla VIM-1 gene only, 13.43% were positive to both bla IMP-1 and bla VIM-1 genes, 7.46% were positive to bla OXA-48 gene only, 7.46% were positive to bla KPC and bla IMP-1 / bla VIM-1 and 4.47% strains were positive to bla KPC, bla IMP-1 / bla VIM-1 and bla OXA-48. Comparing the different phenotypic methods used for detection of CRGNB to PCR, E-test had 100% sensitivity and 93.33% specificity, DDST had 100% sensitivity and 96.67% specificity, CD test had 100% sensitivity and 23.33% specificity, CHROM agar KPC medium had 88.06% sensitivity and 73.33% specificity and MHT had 98.5% sensitivity and 60% specificity. In this study, both tigecycline and colistin were the most effective antimicrobial agents against carbapenem resistant GNB strains.* ***Conclusions:*** *resistance to carbapenems raises a significant alarm signal to put the possibility of carbapenem resistant pathogens as an important cause of worsening the patient condition especially in ICUs. DDST can be used as a convenient screening method in microbiology laboratories but genetic confirmation by PCR and analysis of carbapenemases producers is mandatory for positive isolates.*

INTRODUCTION

The occurrence of carbapenem resistant gram negative bacilli (CRGNB) isolates in a hospital environment poses not only a therapeutic problem, but also a serious concern for infection control management with the global increase in the occurrence and types of different carbapenemases, early detection is crucial. Because of being difficult to detect, such strains pose significant risks, particularly due to their role in unnoticed spread within hospitals and their ability to participate in horizontal gene transfer with other pathogens in the hospital [1].

Carbapenem-resistant Enterobacteriaceae (CRE) were relatively uncommon before 2000. Carbapenem

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resistance is complex; it can occur in different Enterobacteriaceae and be mediated by several mechanisms. It was first identified from a *Klebsiella* isolate in 2001 and has now spread widely throughout the United States and around the world [2].

Carbapenem-resistant Enterobacteriaceae can spread in health-care settings and cause infections with mortality rates of 40% to 50%. Carbapenem resistance develops as a result of the production of carbapenem-hydrolyzing enzymes. These enzymes are usually encoded by genes carried on plasmids which can rapidly spread among related bacterial genera [3].

Although carbapenem resistant enterobacteriaceae (CRE) appear to have been uncommon before 2000, however, over the last decade CRE have been reported more commonly. In the Meropenem Yearly Susceptibility Test Information Collection Program, meropenem resistance among clinical isolates of *klebsiella pneumoniae* increased significantly from 0.6% in 2004, 5.6% in 2008 to 12% in 2012. Among isolates reported to the NHSN in 2012, carbapenem resistance was reported in up to 4% of *E. coli* and 12% of *klebsiella pneumoniae* isolates that were associated with certain device-related infections [4].

Independent risk factors for CRE infection include, but aren't limited to, use of beta-lactam antibiotics and the use of mechanical ventilation. Patients who have been diagnosed with diabetes have also been shown to be an elevated risk for acquiring CRE [5].

Those at the highest risk are patients receiving an organ or stem cell implantation, use of mechanical ventilation, or have to have an extended stay in the hospital along with exposure to antimicrobials. It was shown that the prevalence of CRE was proportional to the lengths of stays of the patients in hospitals [6].

The aim of this study was to determine the frequency of infection with carbapenem resistance among nosocomial GNB infections in different wards/ICUs at Assuit University Hospital, to compare between different phenotypic methods and PCR as a reference test for carbapenemase production among clinical CRGNB isolates, to determine the antimicrobial sensitivity profile of these isolates and in-vitro detection of the best recommended antimicrobial therapy for CRGNB isolates by Kirby-Bauer method, and to identify different risk factors associated with occurrence of nosocomial CRGNB infections.

METHODOLOGY

1. Study Population:

This study was conducted on 355 specimens of 134 patients admitted to the ICUs at Assuit University Hospital, who developed nosocomial infections. Specimens were collected according to the site of infection: sputum samples (n=71), endotracheal tube (n=87), throat swabs (n=21), surgical site infection swabs (n=56), blood (n=57), and urine samples (n=63).

Isolation and identification of GNB isolates:

All specimens were cultured on blood agar and incubated at 37˚C for 24 hours. Suspected colonies of GNB were identified with: Gram stain, culture on MacConkey's agar, Herellea agar and EMB agar.

- ***Biochemical reactions:*** as oxidase , triple sugar iron, Simmon's citrate agar, Christensen's urea, motility, catalase, motility indole ornithine (MIO) tests were done.
- ***Phenotypic identification of CRGNB isolates:*** were performed by antimicrobial susceptibility testing which was done by Kirby-Bauer disc diffusion method according to CLSI [7].
- ***Further confirmation and biotyping of CRGNB isolates:*** were done by API20E and API20NE kit (BioMerieux, Marcy L Etoile. France).
- ***Phenotypic detection of carbapenemases in CRGNB isolates:*** was done by: determination of MIC of different CRGNB isolates by Imipenem (IPM)-E test and Meropenem (MP)-E test, CHROM agar KPC™ medium, modified Hodge test (MHT), combined disc method (using EDTA as inhibitor), double disc synergy test (DDST) (using EDTA as inhibitor).
- ***PCR:***
  The isolated CRGNB (identified by disc diffusion method) were further identified genetically using the conventional polymerase reaction (PCR).

Table 1: Sequence of primers used in PCR amplification of *bla* genes

Primers	*Nucleotide sequence (5'-3')*	*Size(bp)*
bla IMP-1 [8]	**F** (-GTT CCT AAA CAT GGT TTG GTG GT-)	587
	R (-TTT AAC CCT TTA ACC GCC TGC TCT-)	
bla VIM-1 [8]	**F** (-TCT ACA TGA CCG CGT CTG TC-)	747
	R (-TGT GCT TTG ACA ACG TTC GC-)	
bla KPC [9]	**F** (-ATG TCA CTG TAT CGC CGT CT-)	925
	R (-TTT TCA GAG CCT TAC TGC CC-)	
bla OXA-48 [10]	**F** (-TTG GTG GCA TCG ATT ATC GG-)	390
	R (-GAG CAC TTC TTT TGT GAT GGC-)	

Data entry and data analysis were done using Statistical Package for Social Science (SPSS Inc., Chicago, version 16). P-value was considered significant when $p<0.05$.

RESULTS

The present work is a hospital based observational study, conducted on 134 nosocomially infected patients admitted to different hospital wards and ICUs at Assuit University Hospitals.

Three hundred fifty five (355) clinical specimens were screened for CRGNB.

Seven hundred and forty five pathogens (745) were isolated. Of these 47.92% GNB isolates were identified, *Staphylococci* spp. represented 32.62% of the total isolates, and *Candida* spp. represented 19.46% of the isolates (Table 2).

The distribution of GNB isolates among different samples is shown in table 3. The higher percentage of GNB isolates were *Klebsiella spp.* (31.1%), followed by *pseudomonas spp.* (29.4%) then *E.coli* (25.2%).

Table 2: Distribution of pathogens isolated from 355 samples collected from nosocomially infected patients

Samples collected	*Total No. of pathogens*	Gram negative bacilli	Staphylococci spp.	Candida spp.
		No (% from total no of pathogens)	No (% from total no of pathogens)	No (% from total no of pathogens)
Endotracheal aspirates (n=87)	213	105 (49.29%)	61 (28.64%)	47 (22.07%)
Sputum samples (n=71)	159	75 (47.17%)	57 (35.85%)	27 (16.98%)
Blood culture (n=57)	154	61 (39.61%)	77 (50%)	16 (10.39%)
Wound swabs (n=56)	87	57 (65.52%)	21 (24.14%)	9 (10.34%)
Urine samples(n=63)	92	36 (39.13%)	13 (14.13%)	43 (46.74%)
Throat swabs (n=21)	40	23 (57.5%)	14 (35%)	3 (7.5%)
Total (n=355)	**745**	**357 (47.92%)**	**243 (32.62%)**	**145 (19.46%)**

Table 3: Distribution of different types of GNB among different samples

Samples collected	*No. of GNB isolates*	*Enterobacteriaceae (n=223)*				*Non Enterobacteriaceae (n=134)*		
		E.coli	*Klebsiella spp.*	*Enterobacter spp.*	*Proteus spp.*	*Pseudomonas spp.*	*Acinetobacter spp.*	*Stenotrophomonas spp.*
Urine samples (n=63)	36	15	8	-	2	9	1	1
Sputum samples (n=71)	75	18	29	1	2	23	-	2
Endotracheal aspirates (n=87)	105	16	35	2	6	33	9	4
Blood culture (n=57)	61	20	17	-	5	15	2	2
Throat swabs (n=21)	23	12	5	-	-	6	-	-
Wound swabs (n=57)	57	9	17	3	1	19	8	-
Total (% from total no. of GNB isolates)	**357**	**90 (25.2%)**	**111 (31.1%)**	**6 (1.9%)**	**16 (4.4%)**	**105 (29.4%)**	**20 (5.6%)**	**9 (2.4%)**

Antimicrobial Resistance Rates of GNB isolates:

The resistance rates of GNB were found as 27.17% for both meropenem and imipenem, 84.31% (n=301) for ampicillin, 81.79% for amoxicillin-clavulanic acid, 78.43% for piperacillin, 73.39% for aztreonam, 72.83% for cefaclor, 77% for cefoperazone, 73.39% for both ceftriaxone and cefazolin, 48.74% for cefipeme, 53.22% for ciprofloxacin, 49.86% for levofloxacin, 51.82% for lomefloxacin, 45.1% for netlimicin, 47.62% for both amikacin and tobramycin, 76.75% for tetracycline, 15.97% for tigecycline, 76.75% for polymyxin b and 62.18% for chloramphenicol.

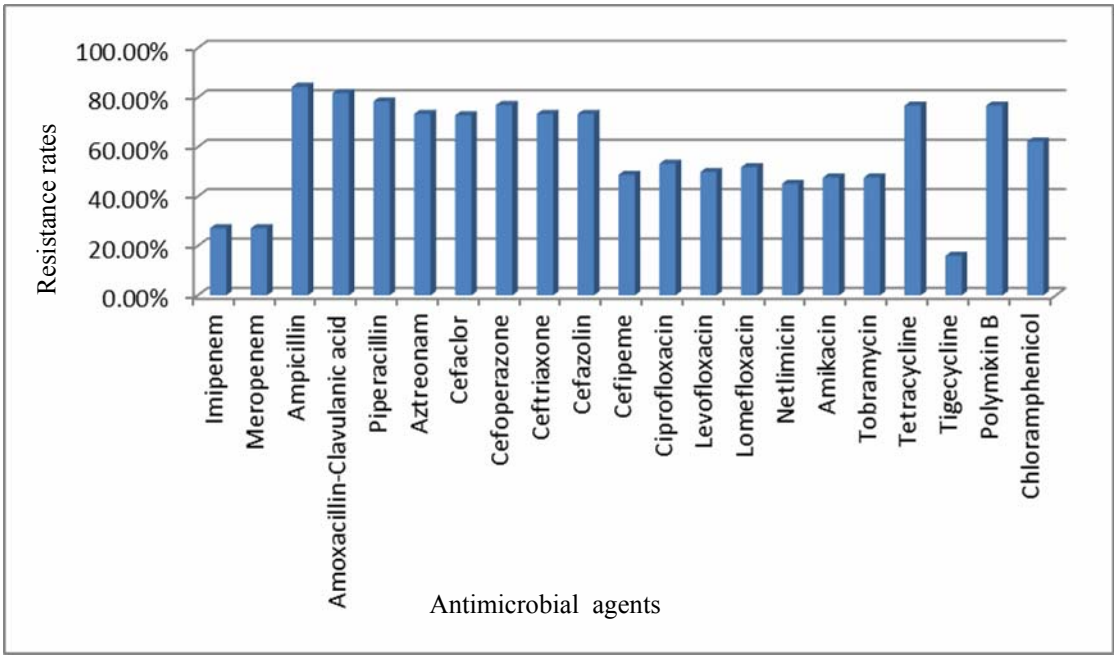

Fig. 1: Resistance rates of 357 GNB isolates to various antibiotics.

In the present study, 56.91% of GNB isolates were multidrug-resistant (MDR) and exhibited resistance to three or more of the representatives of antimicrobial classes mentioned previously, and 43.09% GNB isolates were non-multidrug resistant (non-MDR).

27.17% isolates of GNB were found to be resistant to carbapenems both imipenem and meropenem. Of these 34.02% isolates were *Klebsiella* spp., 23.71% were *Pseudomonas* spp., 18.56% were *E.coli*, 8.25% were *Proteus* spp., 6.19% were *Acinetobacter* spp., 5.15% were *Stenotrophomonas* spp., 4.12% were *Enterobacter* spp.. All strains found as imipenem and meropenem resistant were MDR.

Identification of CRGNB by API:

The 97 conventionally identified CRGNB isolates by disc diffusion method were further tested using the API 20E and API 20NE strips for confirmation and further identification to the species level.

The API 20E Index system identified 33 *Klebsiella* spp. isolates as *KLebsiella pneumoniae* with two different analytic profile index numbers, 18 *E.coli* spp. isolates as *E.coli* 1, eight *Proteus* spp. isolates (six isolates as *Proteus vulgaris* and two isolates as *Proteus mirabilis*), four *Enterobacter* spp. isolates (two as *Enterobacter cloacae* and two *as Enterobacter aerogenes*).

The API 20NE Index system identified 20 isolates of *Pseudomonas* spp. as *Pseudomonas aeruginosa* with two different analytic profile index numbers and three strains as *Pseudomonas luteola*, all six isolates of *Acinetobacter* spp. as *Acinetobacter baumanii* with three different codes and all five isolates of *Stenotrophomonas* spp. as *Stenotrophomonas maltophilia* with two different codes.

Identification of CRGNB by different phenotypic methods compared to conventional disc diffusion method:

Comparing the different phenotypic methods used for detection of CRGNB to the conventional disc diffusion method, it was noticed that CRGNB was identified in 71.13% of isolates by IPM E-test, 69.07% by MP E-test, 85.9% by CHROMagar KPCTM medium, 80.41% by Modified Hodge Test (MHT), 100% by Combined test (CD) test and 70.1% by Double Disc Diffusion Test (DDST) test.

Detection of Carbapenem Resistance Genes by PCR:

During the study period, a total of 97 CRGNB isolates were identified by disc diffusion method. All isolates were tested by PCR for detection of *bla* genes (*bla KPC* , *bla IMP-1*, *bla VIM-1* and *bla OXA48*).

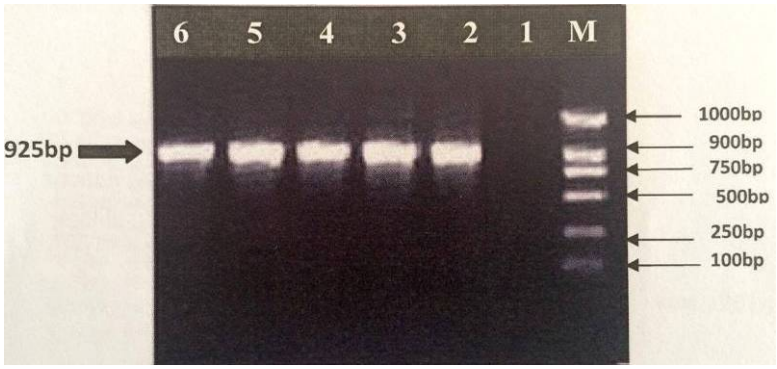

Fig. 2: Gel electrophoresis of the PCR-amplified products for detection of *bla* $_{KPC}$ gene. **Lane M** is DNA marker **(100-1000 bp)**; **lane 1** is a negative control and **Lanes 2, 3, 4, 5& 6** are positive for *bla* $_{KPC}$ gene (925 bp).

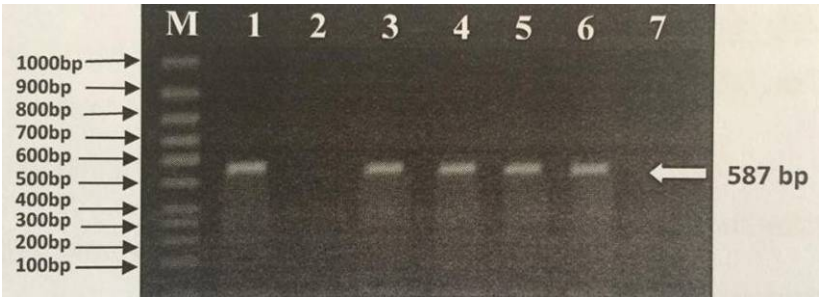

Fig. 3: Gel electrophoresis of the PCR-amplified products for detection of *bla* $_{IMP-1}$ gene. **Lane M** is DNA marker **(100-1000 bp)**; **Lanes 1, 3, 4, 5& 6** are positive for *bla* $_{IMP-1}$ gene (587 bp); **Lane 2** is negative for *bla* $_{IMP-1}$ gene and **Lane 7** is a negative control.

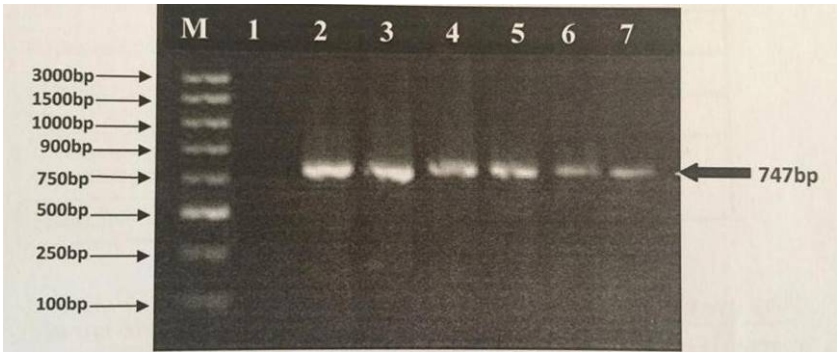

Fig. 4: Gel electrophoresis of the PCR-amplified products for detection of *bla* $_{VIM-1}$ gene. **Lane M** is DNA marker **(100-3000 bp)**; **Lanes 2, 3, 4, 5, 6& 7** are positive for *bla* $_{VIM-1}$ gene (747 bp); **Lane 1** is a negative control.

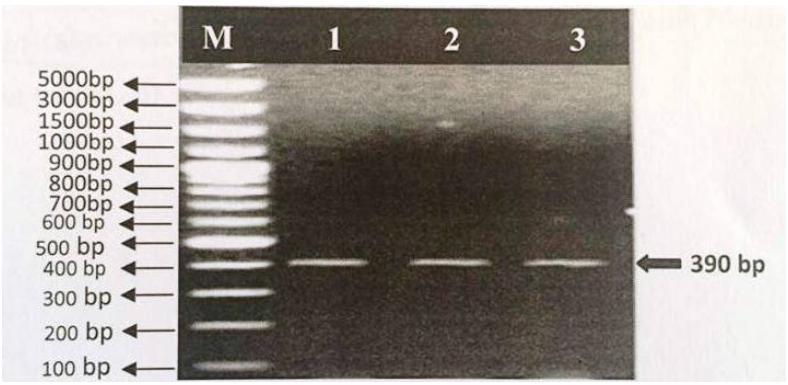

Fig. 5: Gel electrophoresis of the PCR-amplified products for detection of *bla* $_{OXA-48}$ gene. **Lane M** is DNA marker **(100-5000 bp)**; **Lanes 1, 2& 3** are positive for *bla* $_{OXA-48}$ gene (390 bp).

Out of 97 tested isolates, 69.07% were found to harbor one or more of the carbapenemases genes. These included 75.76% of *Klebsiella* spp., 82.61% of *Pseudomonas* spp., 72.22% of *E.coli*, 66.67% of *Acinetobacter* spp., 37.5% of *Proteus* spp., 50% of *Enterobacter* spp. and 20% of *Stenotophomonas* spp.

In the present study, 32.84% isolates were positive to *bla KPC* gene only, 17.92% were positive to *bla IMP-1* gene only, 16.42% were positive to *bla VIM-1* gene only, 13.43% were positive to both *bla* $^{IMP-1}$ and *bla VIM-1* genes, 7.46% were positive to *bla OXA-48* gene only, 7.46% were positive to *bla KPC* and *bla IMP-1 / bla VIM-1* and 4.47% strains were positive to *bla KPC, bla IMP-1 / bla VIM-1* and *bla OXA-48*.

Sensitivity and specificity of different phenotypic methods compared to PCR:

Double disc synergy test and IPM E-test showed the best sensitivity (100%) and specificity (96.7% and 93.3%, respectively).

Table 4: Comparison between different phenotypic tests and PCR as a reference test for detection of carbapenemases in 97 CRGNB isolates

Phenotypic test	*sensitivity*	*specificity*	*PPV*	*NPV*
IPM E-test	100%	93.33%	97.1%	100%
CHROMagar KPCTM medium	93.2%	36.8%	82.09%	63.63%
Modified Hodge Test	98.5%	60%	84.6%	94.74%
Combined disc test	100%	23.33%	74.44%	100%
Double disc synergy test	100%	96.67%	98.5%	100%

Antimicrobial sensitivity of CRGNB:

The antimicrobial sensitivity of 97 carbapenem-resistant GNB isolates had shown that both tigecycline and colistin were the most effective antimicrobial agents against CRGNB strains; both demonstrated good sensitivity (85.57% and 80.41% respectively). Ertapenem sensitivity among these isolates reached 52.58%. Among aminoglycoside class, tobramycin was more effective than amikacin against the isolated strains, where the susceptibility to tobramycin was 34.02% and to amikacin was 26.8%. Concerning fluoroquinolones; only levofloxacin was more effective than ciprofloxacin (95% resistance rate), where the susceptibility rate to levofloxacin was 20.62%. Similar to levofloxacin, 20.62% of isolates were susceptible to piperacillin-tazobactam comination. Only the above-mentioned antimicrobial agents were effective to CRGNB strains, where about more than 97% of these strains exhibited high resistance rate to other antimicrobial agents.

Risk factors for Carbapenem Resistance:

A total of 134 patients were included in this study. Patients positive for CRGNB (n=65) were compared with patients negative for CRGNB to determine factors associated with carbapenem resistance. Patients aged above 40 years are at a higher risk for acquiring CRGNB infection than other age groups (p value=0.045, OR=3.22). Concerning gender, male sex is considered risk factor (p value=0.03, OR=6.28). Prior antibiotic treatment (p value=0.000, OR=43.20), the presence of a urinary catheter (p value=0.000, OR= 41.07), mechanical ventilation (p value=0.000, OR=25.88) and central line in ICU (p value=0.000, OR= 5.65) were the most prevalent risk factors associated with the occurrence of nosocomial CRGNB infection. P value was not significant for immune-suppression and diabetes mellitus variables. Parentral nutrition and surgery had OR<1.

DISCUSSION

In the present study, 745 pathogens were isolated from 355 clinical samples collected from 134 nosocomially infected patients. The highest numbers of samples collected were 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%) (table 2). The highest numbers of endotracheal samples may be due to the largest numbers of patients admitted at the ICUs with association of mechanical ventilation. This is in accordance with one Senegalese study conducted in an ICU; the proportion of ventilated patients affected by ventilator-associated pneumonia was 50% WHO[11]. Also Jombo *et al.*[12] supported our finding about the prevalence of urinary tract infection which was about 12.3% in Nigeria.

In the present study, GNB represented 47.9% of total isolates (357/745) and 56.91% of GNB isolates were MDR. These results are discordant with Jayaprada *et al.* [13] who reported that 1.3% isolates of GNB were resistant to all β-lactams, carbapenem, fluroquinolones and aminoglycosides. This difference may be attributed to the misuse of antibiotics in our locality.

The carbapenem resistance rate among GNB in our study is 27.17% (97/357). This resistance rate is in

accordance with Wattal *et al.* [14] 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. Misuse and abuse of antibiotics in Egypt has adversely contributed to the rate of antibiotic resistance in the study and might also be the cause of the emergence of carbapenemase enzymes.

Phenotypic detection of carbapenemases using E-test was done. E-test results showed that it had 100% sensitivity and 93.33% specificity compared to PCR as a reference test. This is in agreement with Timothy *et al.* (2002) [15] who found that E-test had a100% sensitivity for detection of MBLs in GNB.

In the current study, we report 93.2% sensitivity and 36.8% specificity of CHROMagar KPC medium for detection of CRGNB compared to PCR. These results are in agreement with those of Gilad *et al.*[16], who observed a high sensitivity but low specificity yields (93.8% and 62.7% respectively). However our results are different from some investigators. It was reported by Samra *et al.*[17] that the sensitivity and specificity relative to PCR were 100% and 98.4% respectively, for CHROMagar KPC for detection of carbapenemase. It should be kept in mind that this media can't be used in screening of *Proteus* spp., *Acinetobacter* spp. and *Stenotrophomonas* spp. as it doesn't support their growth which limits its use in screening of CRGNB without initial isolation and identification.

In this study, phenotypic detection of carbapenemases using modified Hodge test was done. Our results revealed that it had 98.5% sensitivity and 60% specificity which are largely in keeping with previous reports. According to McGettigan *et al.*[18] the MHT is reported to have high sensitivity (94.5%) and 69% specificity and to be suitable for confirming carbapenemase production.

Combined disc test, another phenotypic test for detection of carbapenemases showed that it was highly sensitive (100%) but weakly specific (23.33%) at detecting CRGNB. It was reported by Franklin *et al.*[19] that CD test as a phenotypic detection system was highly sensitive (100%) and specific (98%) at detecting carbapenem-resistant MBL-carrying isolates across a wide range of GNB from clinically important specimens.

In our study, the sensitivity of DDST in detection of MBL was 100% with 96.67% specificity. These findings are in accordance with those of Yan *et al.*[20] who reported that DDST had a high sensitivity and specificity (100% each). A similar result was observed by Franklin *et al.*[19] who reported that the use of DDST for phenotypic detection of MBL in GNB had a sensitivity of 79% and a specificity of 98%. To the contrary, a lower sensitivity of DDST (33.3%) was reported by Lee *et al.* [21] in detection of MBL in GNB. This difference may be attributed to different patient population.

In the present study, the antimicrobial susceptibility patterns (by disc diffusion method) of the isolated CRGNB strains showed high levels of resistance (97-100%) to most commercially available antimicrobial agents. Similar resistance rates were detected by Kucukates and Kocazeybek [22] who reported that Gram negative enteric bacilli were uniformly resistant to β-lactam antibiotics as well as β-lactam β-lactamase inhibitors. Resistance to ciprofloxacin and ceftriaxone ranged from 50-100% and 25-83.3% respectively. The increased prevalence of resistant organisms in our ICUs probably reflects lack of proper antibiotic policy resulting in prolonged and indiscriminate use of antimicrobial agent. These high rates of resistance reflect a threat limiting the treatment options in our hospitals.

In this study, both tigecycline and colistin were the most effective antimicrobial agents against CRGNB isolates; both demonstrated good sensitivity (85.57% and 80.41%, respectively). It was found by Koomanachai *et al.*[23] that 63 (80.8%) of patients included in their study had a favorable clinical response and 94.9% had a microbiological response to colistin. Our results are also supported by a review done by Hirsch and Tam[24], who gathered data from 15 publications on the treatment of 55 patients with *Klebsiella pneumoniae* carbapenemases-related infections and a favorable outcome was achieved in 75% of patients treated with tigecycline.

As dictated by the susceptibility patterns, it appears that medicine is returning to drugs which have been phased out. Colistin (Polymixin E), the cationic peptide with the potential nephrotoxic effect was successful in treating critical cases with MDR-GNB infections. So, until other new efficient agents arise colistin is advocated as the empirical drug of choice in the setting of MDR-GNB infections.

This study shows that prior antibiotic treatment, mechanical ventilation, presence of a urinary catheter and central line in ICU have been shown to be playing an important role in acquiring nosocomial CRGNB infection. P values were not significant for immunosuppression and diabetes mellitus. These results were similar to those reported by Ortega *et al.*, Surasarang *et al.* and Vasudevan *et al.*[25, 26 & 27]. However they are different from those of Choi *et al.*(2008) [28], who reported that the most common risk factors were solid cancer (35.7%), followed by biliary disease (22.7%), hypertension (22.4%), neurologic disease (18.9%), diabetes mellitus (18.3%), liver cirrhosis (6%), multiple trauma (3.4%) and COPD (1.8%). This difference in the rate may be due to difference in study population as our study was done in Upper Egypt, difference in life style, socioeconomic pattern of patients and difference in facilities of infection control measures.

The present study concluded that:

- 27.17% of GNB isolates were resistant to carbapenems which raises a significant alarm signal to put the possibility of carbapenem resistant pathogens as an important cause of worsening the patient condition especially in ICUs.
- This study validates simple and highly sensitive phenotypic methods for the detection of carbapenemases and MBL production. DDST is superior to CDT and MHT in detection of carbapenemases and it is equally effective to E-test for MBL detection in Gram negative bacilli. Genetic confirmation by PCR and analysis of carbapenemases producers is mandatory for positive isolates screened by phenotypic tests.
- Treatment of CRGNB infections is made problematic by high rate of GNB resistance to cephalosporins, all penicillins, and to monobactems as aztreonam. This makes the use of tigecycline or colistin is the last resort for treatment of infections caused by highly resistant GNB in critically ill patients.

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