



Evaluating the performance of different detection methods of Carbapenemase producing Gram-negative bacilli isolated from surgical site infections

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Received: 2 March, 2021; Accepted: 14 April, 2021; Published online: 16 April, 2021



Abstract

Surgical site infections (SSIs) are still the most prevalent infections in health care facilities. The magnitude of the problem increased with the development of health care associated infections caused by Gram negative bacilli (GNB), which are resistant to Carbapenem antibiotics. This study aimed to assess the performance of various detection methods of carbapenemase-producing GNB; isolated from healthcare associated SSIs at different surgical units, Mansoura University Hospitals, Al- Dakahliya Governorate, Egypt. A total of 186 wound specimens were collected from patients showing symptoms and signs of SSIs; used for isolation of bacteria and then identification of these bacterial isolates according to colony morphology; microscopic examination and biochemical reactions. About 173 specimens were positive for bacterial pathogens; out of them 83 were GNB isolates. The most commonly isolated bacteria were; *Klebsiella* spp. 31 (37.3%), followed by *Escherichia coli* 22 (26.5%), *Pseudomonas aeruginosa* 17 (20.5%), *Proteus* spp. 10 (12.0%) and *Enterobacter* spp. 3 (3.6%). The antibacterial sensitivity testing of the total 178 bacterial isolates was assessed using the disc diffusion assay. Bacterial pathogens that were carbapenemase producers were tested using phenotypic, rapid colorimetric (Carba NP test) and genotypic methods. Among these isolated bacteria 31 (83.8%), 26 (70.3%) and 28 (75.7%) were carbapenem resistant; confirmed by MHT, Carba NP test and multiplex Polymerase chain reaction (PCR), respectively. Continuous screening of the bacterial antimicrobial susceptibility at local level and rational use of the antibacterial agents; is essential to decrease the emergence and spread of resistant bacterial pathogens.

Keywords: Surgical site infections, Carbapenemase, Metallo beta-lactamase (MBL), Multiplex PCR, Carba NP test

1. Introduction

Health Care-Associated Infections (HAIs) still occupy a large portion of the medical health concern (Tariq *et al.*, 2017). Despite the remarkable progress in the science of surgical and wound management

systems, SSIs lead to notable rates of morbidity and mortality worldwide ([Singh et al., 2014](#)).

The post-operative SSIs are classified by the Centers for Disease Control and Prevention (CDC) and National Nosocomial Infections Surveillance System (NNIS) 30 days post-surgery into three different types; superficial incision that involves only the skin and subcutaneous tissue, deep incision in which infection penetrates into deep soft tissue such as fascia and muscle layer, and organ/space infections that involves any organ or space other than the incision site ([Berríos-Torres et al., 2017](#)). Several studies reported that the highest incident rate of SSIs was recorded in superficial incision; followed by deep wound, while the less frequent rate of infection was observed in wounds that involve an organ or space ([Onyekwelu et al., 2017](#)).

The National Academy of Sciences and the National Research Council Cooperative Research in 1964 accepted another classification of the post-surgical wound infections; according to level of microbiological contamination of the surgical site at the time of surgery into; clean, clean-contaminated, and contaminated with dirty wounds ([Malpiedi et al., 2013](#)).

Several risk factors increase the rate of SSIs including; diabetes mellitus, malnutrition, high body mass index, anemia, use of immune-suppressive drugs and corticosteroids, antibiotics use, smoking, infection control measures and type of wound ([Labib et al., 2012](#)). Moreover, [Ansari et al., \(2015\)](#) reported that long duration of surgery; type of surgical technique, poor post-operative glycemic control, prolonged post-operative stay, presence of drains, and presence of infection at distant sites, hypoxia and hypothermia, are other reasons for increasing the rate of SSIs.

Postoperative wound infections may be of exogenous or endogenous sources ([Shriyan et al., 2010](#)). Several studies revealed that the most common bacteria causing SSIs include; *Staphylococcus aureus* (*S. aureus*), followed by *Klebsiella pneumonia* (*K.*

pneumonia), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), Coagulase- Negative Staphylococci (CNS), *Enterobacter* spp., *Proteus mirabilis*, *Enterococcus* spp. and *Streptococcus* spp. ([Dessie et al., 2016](#)).

The emergence of antibiotic resistant bacteria such as extended spectrum beta lactamases (ESBLs), in addition to the prevalence of different carbapenemases among bacteria that cause HAIs, represent a big problem to the health care facilities; affecting proper antibiotics used in treatment of surgical patients ([Shaikh et al., 2015](#)).

Ambler classification divides the β -lactamase enzymes into four classes (A, B, C and D) based on their amino acid sequences. The most frequent carbapenemase-producing Gram negative bacilli (CR-GNB) belongs to the Ambler class A (*bla*_{KPC}), class B (*bla*_{VIM}, *IMP*, and *NDM*), and class D (*bla*_{OXA-48}) like ([Albiger et al., 2015](#)). In *Acinetobacter* spp., the most common carbapenemases include; OXA-23, OXA-24, and OXA-58-like enzymes ([Noël et al., 2017](#)). However, in *Pseudomonas* spp., class B metallo β -lactamases (MBL) mainly; *bla*_{VIM} and *IMP* are the most common ([Tamma and Simner, 2018](#)). Several methods for detecting CR-GNB have been evolved. These methods include; phenotypic, rapid colorimetric and molecular methods ([Al-Zahrani, 2018](#)).

The objective of this study was to evaluate the different detection methods of carbapenemase-producing GNB; isolated from healthcare associated SSIs at different surgical units, Mansoura University Hospitals, Al- Dakahliya Governorate, Egypt.

2. Patients and methods

2.1. Study design

This study is a cross-sectional study conducted in Mansoura University Hospitals during the period from April, 2019 to March, 2020. About 186 patients that had symptoms and signs of SSIs; fever, pain, tenderness and discharge from the surgical site were enrolled in this study. Meanwhile, patients that had

any surgical operations outside of Mansoura University Hospitals were not included in this study. Written consents from the patient's and/or their relatives were obtained. Demographic data (age, sex and occupation), clinical data (i.e. ICU admission devices, co-morbid disease including; diabetes, obesity, malignancy, hepatic and renal impairment, chronic lung disease, pre-operative antibiotic intake, use of immunosuppressive drugs, were collected from the patients) and surgical history (including; type of operation; emergent or elective, duration of the procedure, type of the surgical wound, prosthesis insertion and length of post-operative stay), were collected from each patient.

2.2. Samples collection

The patient's edge of the wound was pressed to discharge ooze, whereas pus samples were aspirated using sterile syringes. Wound swabs were collected by rolling the sterile cotton swabs over 1 cm area of the wound for 5 sec, and then the swabs were placed in Stuart's transport medium (Oxoid, UK). Samples from drainage tube after operations were also collected. All samples were transported to Microbiology Diagnostics and Infection Control Unit (MDICU), Medical Microbiology and Immunology department, Faculty of Medicine, Mansoura University, within 2 h of collection; if there was any delays in transportation to the laboratory, the samples were kept at 4 °C in the refrigerator.

2.3. Samples processing

2.3.1. Microscopic examination

Gram stained films of the different wound samples were carried out and examined to detect the presence of any pus cells or bacteria.

2.3.2. Cultivation

Samples were cultivated on Nutrient agar, Blood agar and MacConkey agar Petri plates (Oxoid, UK) using the streaking method, according to [Kallstrom,](#)

[\(2014\)](#). Plates were incubated at 37°C for 24-48 h, and were examined daily for bacterial growth.

2.4. Identification of the bacterial isolates

Gram negative bacteria (GNB) were identified according to colony morphology; microscopic examination and biochemical reactions including; Oxidase test using Oxidase detection strips (Oxoid, UK), Kligler iron agar (KIA) test, Lysine iron agar (LIA), Motility, Ornithine production, Indole (MIO), Citrate utilization test (Oxoid, UK)), in reference to [Mahon et al., \(2014\)](#). Reference strains including; *E. coli* (ATCC 25922), *K. pneumonia* (ATCC 33495), *P. vulgaris* (ATCC 15315), *E. aerogenes* (ATCC 13048) and *P. aeruginosa* (ATCC 27853), were obtained from NAMRU-3 Institute (Naval Medical Research Unit Three), Cairo, Egypt.

2.5. Antibiotic sensitivity assay

Bacterial growth were tested for carbapenemase production; according to the clinical and laboratory standards institute guidelines ([CLSI. 2017](#)), using the disc diffusion method. Muller Hinton (MH) agar plates were seeded individually on their surfaces with 0.1 ml (1×10^6 cells/ml) of the test bacterium, which was spread using a sterile glass spreader. After dryness, the test antibiotic discs were placed individually on the seeded agar surface. Plates were incubated at 37°C for 24 h. After incubation, the diameters of inhibition zones were measured using a calibrated ruler. The commercially prepared antibiotics discs used include; Ampicillin (AM) (10 µg), Amoxicillin/Clavulanic acid (AMC) (20/10 µg), Cefotaxime (CTX) (30 µg), Ceftazidime (CAZ) (30 µg), Ceftriaxone (CRO) (30 µg), Azteronam (ATM) (30 µg), Gentamicin (CN) (10 µg), Amikacin (AK) (30 µg), piperacilline/Tazobzctam (TPZ) (100/10 µg), Ciprofloxacin (CIP) (5 µg), Imipenem (IMP) (10 µg) and Meropenem (MEM) (10 µg) (Oxoid, UK). Bacterial isolates that showed an inhibition zone diameter \leq 19 mm for Imipenem and Meropenem (10 µg each) were considered as carbapenemase producers.

2.6. Phenotypic detection of carbapenemase producers

Carbapenem resistant GNB were examined for carbapenemase production using the Modified Hodge Test (MHT), in reference to [Tamma and Simner, \(2018\)](#). In this assay, 0.5 McFarland dilution of the reference strain *E. coli* (ATCC 25922) was prepared in 5 ml of sterile saline. A 1:10 dilution was made by adding 0.5 ml of the 0.5 McFarland suspensions to 4.5 ml of saline. This 1:10 dilution of *E. coli* was streaked individually on the surface of Muller Hinton (MH) agar plates, and allowed to dry for 3-5 min. A carbapenem susceptibility disks (10 µg) were placed individually in the center of the test areas. The tested bacterium was streaked in a straight line from the edge of the disk to the edge of the plate, and this step was repeated with the reference strain in another direction. Plates were incubated at 35± 2°C for 16-24 h. After incubation, plates were examined for a clover leaf like indentation of the test bacterium and the reference strain of *E. coli* (ATCC 25922) within the zone of inhibition of the carbapenem disc.

The Metallo-β-lactamases (MBL) were identified by Imipenem EDTA double disc synergy test ([Joji et al., 2019](#)). A 0.5 M EDTA solution was prepared by dissolving 186.1 g of Disodium EDTA in 1000 ml of dist. water, the pH was adjusted to 8.0 using NaOH, and then the mixture was sterilized at 121°C for 15 min. using an autoclave. A Muller Hinton agar plate was seeded at its surface by the tested bacterial strain, and then an Imipenem disc (10 µg) was placed on the surface of the plate. A sterile blank filter paper disc was placed at a distance of 10 mm from the antibiotic disc, and then 10 µl of the 0.5 M EDTA solution was added to this blank disc. The plate was incubated at 37°C for 24 h. If the zone of inhibition in Imipenem + EDTA disc is (≥ 7 mm) more than the Imipenem disc alone, this indicates presence of MBL.

2.7. Rapid detection of carbapenemase production by Carbapenemase-Nordmann-Poirel (Carba NP) assay

A rapid chromogenic biochemical method named Nordmann/Dortet/Poirel (Carba NP) test was used to detect for carbapenemase production ([Nordmann et al., 2012](#)). This test depends on *in vitro* hydrolysis of the Imipenem antibiotic (Sigma-Aldrich, Germany) that reduces the pH of the medium, and thus causes a change in the color of phenol red indicator (Oxford) from red to yellow. Tazobactam antibiotic (Sigma-Aldrich, Germany) used as a control (that inhibits carbapenemase activity), was added in another tube to confirm the obtained results.

2.8. Genotypic detection of carbapenemase genes using Multiplex PCR

The presence of *bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM} and *bla*_{OXA-48} carbapenemase genes were detected in Imipenem and meropenem resistant isolates using the multiplex PCR, according to the method conducted by [Monteiro et al., \(2012\)](#).

2.8.1. Genomic DNA extraction

DNA was extracted using High Pure PCR Template Purification Kit (Roche, Germany), according to manufacturer's instructions.

2.8.2. DNA amplification by multiplex PCR

Five pairs of primers (Bioline, UK) presented in Table (1) were used to detect the presence of *bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM} and *bla*_{OXA-48} carbapenemase genes ([Monteiro et al., 2012](#)). Amplification of DNA was carried out according to the manufacturer's instructions.

2.8.3. Multiplex PCR technique

DNA amplification was carried out through a 50 µl reaction mix. containing 10 mM Tris-HCL (pH 8.3), 50 mM KCL, 1.5 mM MgCl₂, 0.1% Triton X-100, 200 mM of each of the deoxynucleoside triphosphate, 1U of *Thermus aquaticus* DNA polymerase, 5 µl of template DNA, and 0.4 mM of each primer. The amplification conditions include: initial denaturation at 94°C for 5 min.; 30 cycles of 94°C for 25 s, 52°C for 40 s and 72°C for 50 s; with a

Table 1. Multiplex PCR primers used in detection of the carbapenemase genes

Primer	Primer Sequence 5'-3'	Amplicon size (bp)	Reference
bla_{KPC} type	KPC-F TCGCTAAACTCGAACAGG	785	Monteiro <i>et al.</i>, (2009)
	KPC-R		
	T TACTGCCCCGTTGACGCCCAATCC		
bla_{NDM-1}	NDM-F TTGGCCTTGCTGTCCTTG	82	Monteiro <i>et al.</i>, (2012)
	NDM-R		
	ACACCAGTGACAATATCACCG		
bla_{IMP} type	IMP-F GAGTGGCTTAATTCTCRATC	120	Mendes <i>et al.</i>, (2007)
	IMP-R AACTAYCCAATAYRTAAC		
bla_{VIM} type	VIM-F	382	Mendes <i>et al.</i>, (2007)
	GTTTGGTCGCATATCGCAAC		
	VIM-R		
bla_{OXA-48}	AATGCGCAGCACCAGGATAG	177	Monteiro <i>et al.</i>, (2012)
	OXA-48 F TGTTTTTGGTGGCATCGAT		
	OXA-48 R GTAAMRATGCTTGGTTTCGC		

final elongation step at 72°C for 5 min. For multiplex PCR assay of the carbapenemase genes, the annealing temperature was optimal at 55°C for amplification of bla_{VIM}, bla_{IMP} and bla_{KPC} genes, 45°C for bla_{NDM} gene and optimal at 57°C for amplification of bla_{OXA-48} genes. Amplicons were visualized after running at 100 V for 1 h on a 1.2 % agarose gel containing Ethidium bromide (Sigma, USA). A 100 bp DNA ladder (Promega, Madison, USA) was used as a size marker ([Monteiro *et al.*, 2012](#)).

2.9. Statistical analysis

Data were statistically analyzed using Statistical Package of Social Science (SPSS) software version 25 (SPSS Inc., Chicago, IL, USA). Categorical data were described as numbers and percentages. Comparison was performed by Qi-square and P was considered significant if P was < 0.05.

3. Results

3.1. Patients characteristics

This study was conducted on 186 patient; 117 (62.9 %) were males while 69 (37.1 %) were females. Patient's ages ranged from 20- 70 years. The mean age of the studied population was 49.2± 13 years (Table 2). Risk factors of SSIs among the studied population included 43.5 % diabetics; however, 40.3 % of them were obese. About 100 % of the patients had received post-operative antibiotics. Emergent surgical procedures were 54.8 % (Tables 3 and 4).

3.2. Isolation and identification of the bacterial isolates

A total 178 bacterial isolates were recovered from 173 positive specimen's growths. Out of these, 83 (46.6 %) isolates were GNB (Fig. 1). They were identified according to the colony morphology;

microscopic characteristics and biochemical assays as; *Klebsiella* spp. 31 (37.3 %), *E. coli* 22 (26.5 %), *P. aeruginosa* 17 (20.5 %), *Proteus* spp. 10 (12.0%) and

Enterobacter spp. 3 (3.6%), as demonstrated in Fig. (2 and 3).

Table 2. Demographic criteria of the studied patient's groups

	Age group (years)	Frequency	Percentage%	Mean ± SD	P value
N=186	20-30	19	10.2	49.2± 13	0.010
	30-40	36	19.4		
	40-50	58	31.2		
	50-60	43	23.1		
	60-70	30	16.1		
	Gender	Male	117		
	Female	69	37.1		

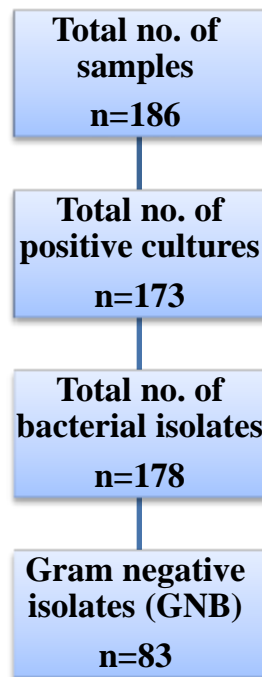
Where; SD: Standard deviation

Table 3. Clinical criteria of the studied patient's groups

N=186		Frequency	Percentage (%)
Diabetes	Yes	81	43.5
	No	105	56.5
Liver disease	Yes	23	12.4
	No	163	87.6
Renal and other co-morbid diseases	Yes	15	8.1
	No	171	92.0
Obesity	Yes	75	40.3
	No	111	59.7
Immunodeficiency	Yes	17	9.1
	No	169	90.9
ICU admission	Yes	34	18.3
	No	152	81.7

Table 4. Operative characteristics of the studied patient's groups

		Frequency	Percentage (%)
Pre-operative antibiotics	Yes	11	5.9
	No	175	94.1
Post-operative antibiotics	Yes	186	100
	No	0	0
Procedure type	Emergent	102	54.8
	Elective	84	45.2
Class of wound	Clean	29	15.6
	Clean-contaminated	73	39.2
	Contaminated	47	25.3
	Dirty	37	19.9
Post-operative period	Less than 7days	116	62.4
	7-14 day	53	28.5
	More than 14 day	17	9.1

**Fig. 1:** Flow chart of the studied patient's groups

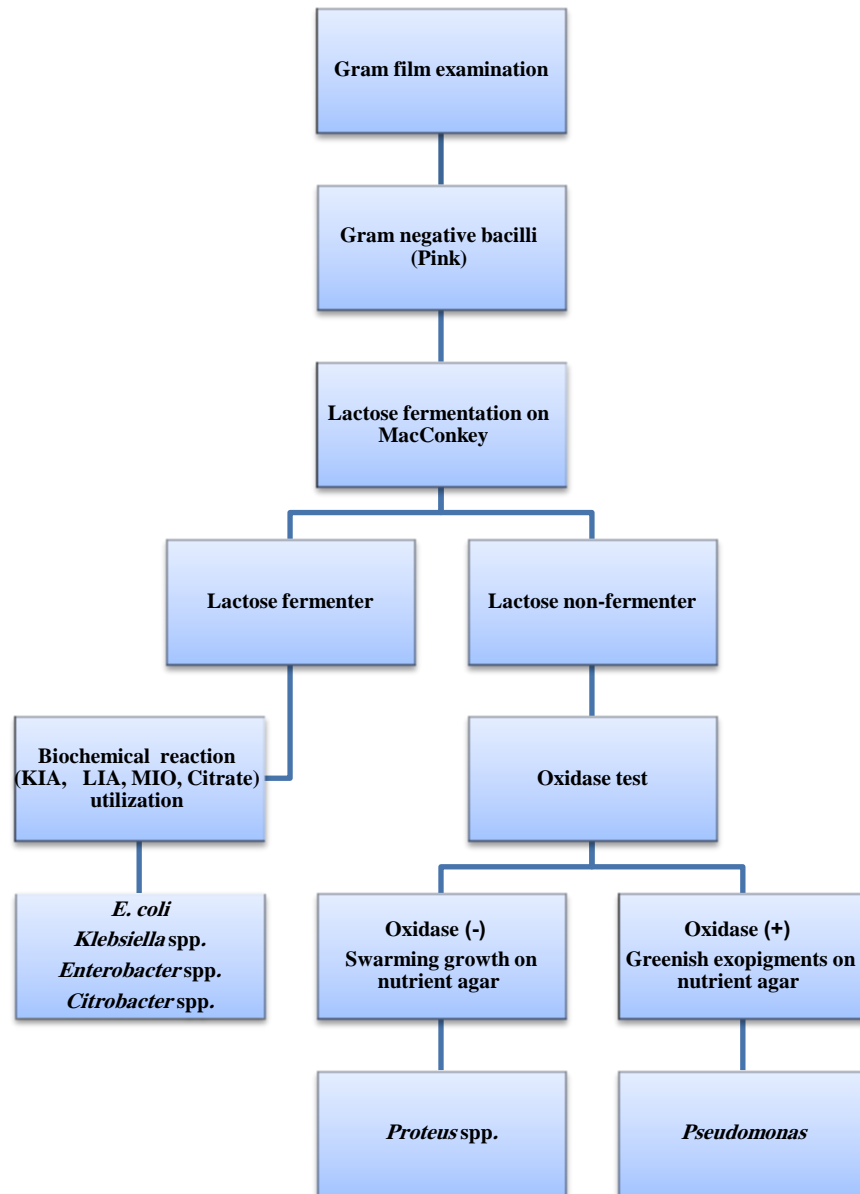


Fig. 2: Flowchart of the identification process of the Gram negative bacterial (GNB) isolates, according to their colony morphology, microscopical examinations and biochemical assays

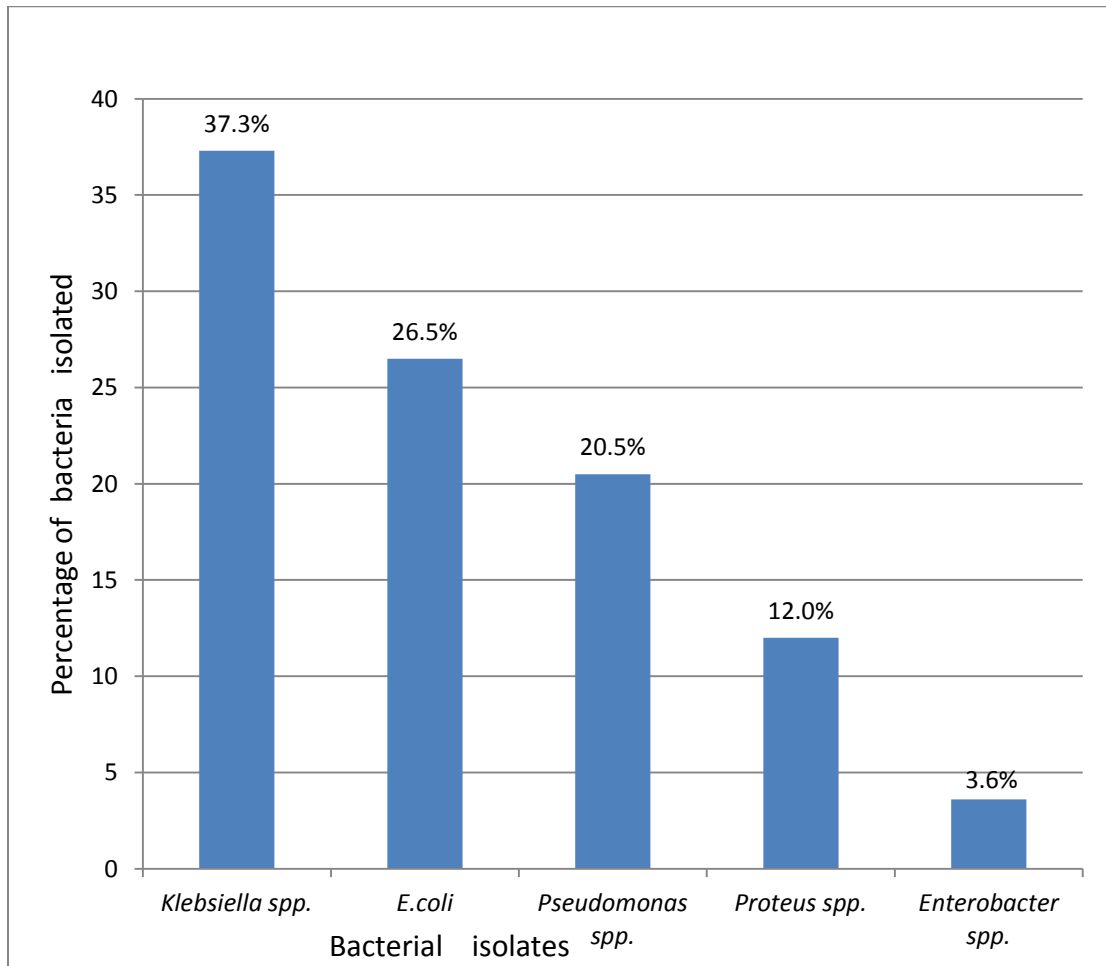


Fig. 3: Prevalence of the Gram negative bacterial (GNB) isolates recovered from the studied patient's specimens

3.3. Antibiotic susceptibility pattern of the recovered GNB

The antibiotic sensitivity patterns are displayed in Tables (5 and 6). As shown in Table (5), the highest resistance is recorded against ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), gentamicin (10 µg) and ciprofloxacin (50 µg) are; 100, 78.3, 77.1, 77.1, 77.1, 91.6 and 78.3 %,

respectively. Resistance to azteronam (30 µg); amikacin (30 µg), piperacilline/Tazobzctam (100/10 µg) are; 45.8, 69.9 and 33.7 %, respectively. About 44.6 % of the isolates are carbapenem resistant using the disc diffusion method, where; *P. aeruginosa* expressed resistance of (58.8 %), *K. pneumonia* (45.2%), *E. coli* (40.9 %); *Enterobacter* spp. (33.3%) and of *Proteus* spp. (30.0 %), are carbapenem resistant, as demonstrated in Table (6).

Table 5. Antibiotic resistance pattern of carbapenem resistant GNB, expressed through the disc diffusion method (n=83)

Antibiotic discs	Resistance to Carbapenem	
	No.	%
Ampicillin (10 µg)	83	100
Amoxicillin/clavulanic acid (20/10 µg)	65	78.3
Cefotaxime (30 µg)	64	77.1
Ceftriaxone (30 µg)	64	77.1
Ceftazidime (30 µg)	64	77.1
Azteronam (30 µg)	38	45.8
Amikacin (30 µg)	58	69.9
Gentamicin (10 µg)	76	91.6
Ciprofloxacin (5 µg)	65	78.3
piperacilline/Tazobzctam (100/10 µg)	28	33.7
Imipenem (10 µg)	37	44.6
Meropenem (10 µg)	37	44.6

Table 6: Antibiotic resistance pattern of the isolated GNB using the disc diffusion method

Antibiotic discs	Frequency of efficacy (%)									
	<i>Klebsiella</i> spp. n=31		<i>E. coli</i> n=22		<i>P. aeruginosa</i> n=17		<i>Proteus</i> spp. n=10		<i>Enterobacter</i> spp. n=3	
	R	%	R	%	R	%	R	%	R	%
Ampicillin (10 µg)	31	100	22	100	17	100	10	100	3	100
Amoxicillin/clavulanic acid (20/10 µg)	23	74.2	20	90.9	13	76.5	7	70.0	2	66.7
Cefotaxime (30 µg)	25	80.6	11	50.0	16	94.1	8	80.0	3	100
Ceftriaxone (30 µg)	25	80.6	12	54.5	16	94.1	8	80.0	3	100
Ceftazidime (30 µg)	27	87.1	11	50.0	15	88.2	9	90.0	2	66.7
Azteronam (30 µg)	13	41.9	8	36.4	8	47.1	8	80.0	1	33.3
Amikacin (30 µg)	21	67.7	14	63.6	14	82.4	7	70.0	2	66.7
Gentamicin (10 µg)	29	93.5	19	86.4	16	94.1	9	90.0	3	100
Ciprofloxacin (5 µg)	24	77.4	17	77.3	13	76.5	9	90.0	2	66.7
pipracilline/Tazobzctam (100/10 µg)	9	29.0	7	31.8	9	52.9	3	30.0	0	0
Imipenem (10 µg)	14	45.2	9	40.9	10	58.8	3	30.0	1	33.3
Meropenem (10 µg)	14	45.2	9	40.9	10	58.8	3	30.0	1	33.3

3.4. Phenotypic characterization of the carbapenemase producers

Carbapenemases are detected in 31 (83.8%) out of 37 carbapenem resistant GNB isolates using MHT, compared to the reference strains (100 %) (Fig. 4). Meanwhile, among the *P. aeruginosa* isolates (17), 9 (52.9%) isolates are found to be positive for carbapenemase production, *Klebsiella* spp. 13 (41.9%), *E. coli* 7 (31.8%), *Proteus* spp. 2 (20.0%), whereas none of *Enterobacter* spp. are recorded to be positive for carbapenemase production (Table 7).

3.5. Detection of Metallo-β-lactamases (MBL) production

Using the Imipenem EDTA double disc synergy assay, out of a total of 37 carbapenem resistant GNB isolates, 28 (75.7%) are recorded to be positive for MBL. This enzyme is detected in 11 (35.5%) of *Klebsiella* spp., 9 (52.9%) of *P. aeruginosa*, 7 (31.8%) of *E. coli* and 1 (10.0%) of *Proteus* spp., through the Imipenem-EDTA disc. On the other hand, no *Enterobacter* spp. are recorded to be positive for MBL, using the same assay (Table 8).

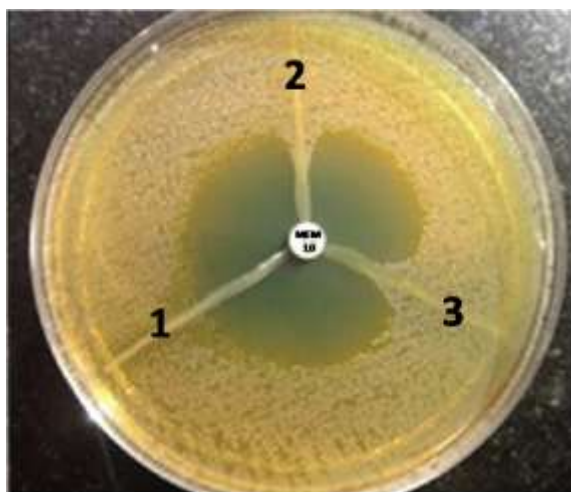


Fig. 4. Detection of carbapenemases production by MHT. Positive result leaf-like indentation of the *E. coli* ATCC 25922 growing along the test organism growth streak was seen in (2 and 3), while negative result was seen in (1).

Table 7. Detection of carbapenemases production by carbapenem-resistant GNB isolates using the Modified Hodge test (MHT)

Carbapenem resistant GNB (n=37)	Modified Hodge test (MHT)	
	No.	%
<i>P. areuginosa</i> (n=17)	9	52.9
<i>Klebsiella spp.</i> (n=31)	13	41.9
<i>E.coli</i> (n=22)	7	31.8
<i>Proteus spp.</i> (n=10)	2	20.0
<i>Enterobacter spp.</i> (n=6)	0	0
Total positive	31	83.8
Total negative	6	16.2

Table 8. Detection of Metallo- β -lactamases (MBL) production by carbapenem-resistant GNB isolates, using the Imipenem EDTA double disc synergy assay

Carbapenem resistant GNB (n=37)	Imipenem EDTA double disc synergy assay	
	No.	%
<i>P. aeruginosa</i> (n=17)	9	52.9
<i>Klebsiella</i> spp. (n=31)	11	35.5
<i>E. coli</i> (n=22)	7	31.8
<i>Proteus</i> spp. (n=10)	1	10.0
<i>Enterobacter</i> spp. (n=6)	0	0
Total positive	28	75.7
Total negative	9	24.3

3.6. Conformation of carbapenemase production ability using Carba NP assay

Results of the Carba NP assay are interpreted as shown in Table (9). Among the 9 *P. aeruginosa* isolates that are recorded by MHT to be positive for carbapenemase production, 7 are proven to be carbapenemase producers using Carba NP test, while 2 isolates are recorded as non-carbapenemase producers. Among the 11 carbapenemase producers *Klebsiella* spp. isolates, 10 are confirmed to be carbapenemase producer by Carba NP test, while 1 isolate is recorded as non- carbapenemase producer. All the 7 *E. coli* and 2 *Proteus* spp. isolates previously recorded as positive carbapenemase producers by MHT, are confirmed also as

carbapenemase producer by the Carba NP assay (Table 10).

3.7. Detection of the presence of carbapenemase genes using Multiplex PCR

In the present study, all carbapenem resistant GNB were tested for presence of carbapenemase genes such as *bla*_{KPC}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM} and *bla*_{OXA-48}, using the multiplex PCR. About 28 (75.7 %) out of the 37 carbapenem resistant GNB isolates that were proved to be carbapenemases producers by phenotypic methods, are recorded to be positive for carbapenemase genes using the multiplex PCR (Table 11). Agarose gel electrophoresis analysis of the multiplex PCR amplification products showed *bla*_{NDM-1} of 82 bp fragments, *bla*_{IMP} of 120 bp

fragments, bla_{VIM} of 382 bp fragments, bla_{OXA-48} of 177 bp fragments and bla_{KPC} of 785 bp fragments. The agarose gel of multiplex PCR amplification products of carbapenemase genes is demonstrated in Fig. (5). Correlation among results of the different

methods used in this study is shown in Table (12). It is observed that there is a good agreement between the 3 methods used for detection of carbapenemase production, where the recorded sensitivity is 83.8 %, whereas the specificity is 75.0 %.

Table 9. Interpretation of results of the Carba NP assay

Tube A	Tube B	Tube C	Tube D	Interpretation of result
(Bacteria+ diluted Phenol red+ 0.1mM zinc sulfate) Control	(Bacteria+ diluted Phenol red+0.1mM zinc sulfate Imipenem)	(Bacteria+ diluted Phenol red+0.1mM zinc sulfate Imipenem +tazobactam)	(Bacteria+ diluted Phenol red+ Imipenem+0.003M EDTA)	
Red	Red	Red	Red	Non-carbapenemase producer Class A
Red	Yellow/Orange	Red	Yellow/Orange	carbapenemase (KPC) Class B
Red	Yellow/Orange	Yellow/Orange	Red	carbapenemase (MBL) Class D
Red	Yellow/Orange	Yellow/Orange	Yellow/Orange	carbapenemase (OXA)
Yellow/Orange	Yellow/Orange	Yellow/Orange	Yellow/Orange	Non-interpretable result

Table 10. Detection of carbapenemases production by carbapenem-resistant GNB isolates using Carba NP test

Carbapenem resistant GNB (n=37)	No.	%
<i>P. aeruginosa</i> (n=17)	7	41.2
<i>Klebsiella</i> spp. (n=31)	10	32.3
<i>E. coli</i> (n=22)	7	31.8
<i>Proteus</i> spp. (n=10)	2	20
Total	26	70.3

Table 11. Detection of the presence of carbapenemase genes among the carbapenem-resistant GNB isolates using the Multiplex PCR

Genes	No.	%
bla _{KPC}	6	21.4
bla _{VIM}	1	3.6
bla _{IMP}	5	17.9
bla _{NDM}	9	32.1
bla _{OXA-48}	7	25.0



Fig. 5: Agarose gel electrophoresis analysis of multiplex PCR amplification products of carbapenemase genes Where; M: is 100 bp DNA marker, and numbers from 1-18 are isolate lanes

Table 12. Correlation among results of the phenotypic tests with the Carba NP assay and the multiplex PCR

Carbapenemases production by MHT		Carbapenemases production by Carba NP test		Carbapenemases production by multiplex PCR		Sensitivity	Specificity	P. value
No.	%	No.	%	No.	%			
31	83.8	26	70.3	28	75.7	83.8	75.0	< 0.001
Total (n=37)		CI= 91.2		Accuracy= 81.6				

Where; CI: Confidence interval

4. Discussion

Postoperative SSIs remain the most common types of post-operative nosocomial infections especially in developing countries where resources are still limited ([Allegranzi et al., 2011](#)), considering SSIs as major sources of morbidity and mortality for patients undergoing surgical procedures especially with increasing the prevalence of carbapenem resistant strains, as reported in the recent study conducted by [Metwally and Aamir, \(2020\)](#). A previous study of [Kalayu et al., \(2019\)](#) highlighted

that it is essential for every health care unit to screen for nosocomial pathogens and assess their antibiotic susceptibilities, to allow the availability of data required for implementation of antimicrobial policies and infection control measures in the different health care units. In this study, the most prevalent isolated GNB was *Klebsiella* spp. (37.3%) followed by *E. coli* (26.5%). Most of *Klebsiella* spp. isolates were carbapenem resistant (45.2 %) by disc diffusion method, in agreement with [Metwally and Elnagar, \(2019\)](#). This may be attributed to increasing the use of carbapenem drugs during treatments of

SSIs. However, [Ibrahim et al., \(2017\)](#) reported that the most common bacterial pathogens causing post-operative SSIs were *P. aeruginosa* and *E. coli*. This difference in results may be attributed to the different surgical operations, and the engagements of different infection preventions and control measures. Currently, the antibiotic susceptibility pattern of carbapenem resistant GNB revealed high resistance to; ampicillin, amoxicillin/ clavulanic acid, cefotaxime, ceftriaxone and ceftazidime. These results are in agreement with [Zahran et al., \(2017\)](#); [Kalayu et al., \(2019\)](#), who stated that 100 % of the carbapenem resistant bacteria were resistant to ampicillin, amoxicillin/ clavulanic acid and ceftriaxone. The recorded percentages of resistance to gentamicin, amikacin and ciprofloxacin were 91.6, 69.9 and 78.3 %, respectively. A less resistance pattern was previously recorded by [Baran and Aksu, \(2016\)](#), who reported resistance level to aminoglycosides of 63.8 %. According to [Hammam et al., \(2020\)](#), these results necessitate the helpful use of alternatives such as tigecycline or colistin; however, their use will increase the risk of emergence of new strains resistant to both antibiotics.

Carbapenem resistant GNB isolates were tested for carbapenemase production using the MHT assay. Among the 37 carbapenem resistant isolates, 31 (83.8 %) isolates were positive and 6 (16.2 %) isolates were negative. Higher results were recorded by [Zhou et al., \(2018\)](#), as they detected 96.2 % of positive carbapenem resistant GNB isolates using the assay. Through Imipenem EDTA double disc synergy assay, MBL was detected in 28 (75.7 %) of carbapenem resistant GNB isolates. In another previous study conducted by [Eser et al., \(2014\)](#), they revealed MHT positivity in only 1 out of 7 isolates (14.2 %), and this assay showed that MHT might present false negative results, especially in the presence of ESBL or AmpC gene.

Using Carba NP test, GNB isolates that are resistant to carbapenem were detected to be 26 (70.3 %). On contrary, higher result was recorded by

[Creighton, \(2020\)](#), as they detected 97.7 % of carbapenem resistant GNB of Enterobacteriaceae using the Carba NP assay.

Generally, bla_{OXA-48} and bla_{NDM}-positive are considered as the most common carbapenemase genes in the Middle-Eastern countries ([Shibl et al., 2013](#)). In this study, among 37 carbapenemase-producing isolates; 9 (32.1 %) were bla_{NDM}, and 7(25.0 %) were bla_{OXA-48}. In contrast to our results, the recent study [Al-Zahrani and Alasiri, \(2018\)](#) had detected bla_{OXA-48} in 81.5 % of carbapenem resistant GNB isolates.

In the current study, comparing MHT assay with Carba NP test and multiplex PCR for detection of carbapenemase production; revealed good agreements among the 3 detection methods, recording sensitivity of 83.8 % and specificity of 75.0 %. In another study by [Mancini et al., \(2017\)](#), the detected sensitivity and specificity were 75.0 % and 99.1 %, respectively. In countries with high prevalence rate of carbapenem resistance; the Carba NP assay may be helpful for rapid epidemiological and screening purposes, which allow early treatment of patients and decrease the antimicrobial resistance.

Conclusion

Health care associated infections especially SSIs continue to expand, and with the high rate of carbapenemase resistant isolates; the need for continuous assessment of carbapenemase is essential. Detection of carbapenemase genes with PCR is expensive and time consuming. Thus, the phenotypic methods and Carba NP test could be hopeful alternatives, due to their lower costs and less efforts. Bacterial culture and antibiotic sensitivity testing's should be carried out before beginning of the antibiotic therapy of SSIs. The antibiotics use policy should be settled; to decrease the unnecessary use of antibiotics and reduce the emergence of carbapenemase producing bacterial pathogens.

Conflict of interest

There is no conflict of interests.

Funding source

No funding was received for this work.

Ethical approval

This study was approved by Mansoura University Institutional Review Board 1222.R1.R2. The patient's consents and statement of protection of the patient's privacy are provided.

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