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PHENOTYPIC AND GENOTYPIC DETECTION OF CARBAPENEM RESISTANT ACINETOBACTER BAUMANNII IN SURGICAL AND INTENSIVE CARE UNITS IN AL AZHAR UNIVERSITY HOSPITAL – NEW DAMIETTA

By

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ABSTRACT

Background: Acinetobacter baumannii (A. baumannii) has emerged as a healthcare-associated pathogen worldwide. Several epidemiological studies have reported the occurrence of multi-drug resistant A. baumannii infections in different regions of the world. The spread of carbapenem-resistant A. baumannii is of a global concern.

Objectives: This work was carried out to detect carbapenem-resistant A. baumannii in Surgical Departments and intensive care units (ICUs), Al-Azhar University hospital, New Damietta. It was also aimed to determine the occurrence of bla OXA -51-like and bla OXA-23 like genes among the isolated strains.

Patients and methods: The current study was conducted on 500 patients attending the Surgical Departments and ICUs during the period between May 2016 and September 2018. The choice of samples differs according to the clinical manifestations.

The isolates were obtained from various clinical specimens, i.e. sputum, endotracheal aspirate (ETA), pus, urine and blood samples. All clinical specimens were collected under complete aseptic conditions. The clinical specimens were traced to the species level using API 20NE system followed by an assesment of the different phenotypic assays for detection of carbapenemase production using multiplex polymerase chain reaction (PCR).

Results: Post-operative infections were detected in 217 (43.4%) out of 500 of patients. A. baumannii was considered the third common isolated Gram-negative organisms (27, 12.3 %). A. baumannii isolates were predominant in ICUs (14, 51.9%). Carbapenemase production was detected in A. baumannii isolates using the modified Carbapenem Inactivation Method (mCIM), the modi?ed Hodge test (MHT) and the Carba NP test. Using multiplex PCR analysis, most isolates (12; 44.4%) carried bla OXA-51-like gene, followed by ten (37%) isolates that carried both bla OXA-51-like and bla OXA-23-like genes. Only one (3.7%) isolate carried bla OXA-23-like gene.

Conclusion: The current study suggested that A. baumannii is one of the most commonly detected isolates in our hospital. The mCIM is the most useful phenotypic method for detection of carbapenemase production. Detection of carbapenem resistance genes is alarming a serious healthcare problem in our hospital.

Key words: A. baumannii, bla OXA -51-like genes, Gram-negative bacteria, herellea agar,LAM agar, mCIM, Carba NP, modi?ed Hodge test, carbapenemases, healthcare-associated pathogen and PCR.

INTRODUCTION

Hospital acquired infections (HAIs) are considered as one of the most common complications of healthcare. According to a study by the Centers for Disease Control and Prevention *(CDC, 2018)*, at any given time, nearly one in every 25 hospitalized patients in the US has an HAI. Nosocomial infections accounts for 7% in developed and 10% in developing countries *(Khan et al., 2017)*.

A. baumannii is one of the most important pathogens in HAIs especially in ICUs *(Ghajavand et al., 2015)*. A. baumannii is primarily a health care-associated pathogen and many reports indicated, it as the cause of outbreaks and nosocomial infections including septicemia, bacteremia, ventilator - associated pneumonia (VAP), wound sepsis, endocarditis, meningitis, and urinary tract infections *(Vashist et al., 2011)*.

A. baumannii was identified as Gram-negative coccobacilli, non-motile, non-lactose fermenting, pale yellow colonies with entire margins of 1 to 1.5 mm in diameter after 24 hours on MacConkey medium as primary medium for isolation *(Tille, 2017)*.

The WHO declared that A. baumannii is one of the most serious ESKAPE organisms (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter species) that effectively escape the effects of antibacterial drugs *(Boucher et al., 2009)*. A number of A. baumannii resistance mechanisms are known, including enzymatic degradation of drugs, target modifications, multidrug efflux pumps, and permeability defects *(Gordon and Wareham, 2010*; *Kim et al., 2012*; *Lin and Lan, 2014)*.

Carbapenems have been used as the last-line drugs for the treatment of A. baumannii infections until 1991 when the first Carbapenem-resistant A. baumannii (CRAB) was recognized *(Kim et al., 2012)*. CRAB had been reported worldwide and become a significant health problem due to the limited options for antibiotic treatment *(Alsan & Klompas, 2010*; *Asadollahi et al., 2012* and *Abbott et al., 2013)*. In multi-resistant strains of A. baumannii, the main mechanism is being production of carbapenemases; enzymes belonging to Ambler classes B, A and D *(Bush and Jacoby, 2010)*.

The present study was conducted to detect carbapenem resistant A. baumannii through the occurrence of bla OXA-51-like, bla OXA-23 – like genes among CRAB in Surgical Departments and ICUs in Al Azhar University Hospital - New Damietta. It was also aimed to detect, screen and assess the biotyping and antimicrobial susceptibility patterns of different clinical isolates.

PATIENTS AND METHODS

This study was conducted on 500 patients attending ICUs and the Surgical Departments, Al Azhar University Hospital, New Damietta between May 2016 and September 2018. The ethical research and review committee of the hospital approved the study protocol, and informed consents were obtained from the patients or his relatives.

Plan of the study :

A. Collection of clinical specimens, i.e. sputum, endotracheal aspirate (ETA), pus, urine and blood samples from patients of any age at the ICUs and Surgical Departments. It also included the health care workers (HCWs) and environmental samples.

B. Isolation in pure culture with complete identi?cation of all isolates by conventional microbiological methods, antibiotic susceptibility testing using Kirby-Bauer Disc Diffusion Method (CLSI, 2018).

C. Phenotypic and genotypic detection of A. baumannii.

Inclusion criteria:

Random sampling of A. baumannii that fulfill the definition of multidrug resistance.

Different terminology like multidrug resistant (MDR), extensively drug resistant (XDR) and Pan drug resistant (PDR) have been used with various definitions to describe the degree of antimicrobial resistance for Acinetobacter spp. MDR Acinetobacter spp. can refer to being resistant to a minimum of three classes of antimicrobial drugs e.g. all penicillins and cephalosporins fluoroquinolones, and aminoglycosides *(Jung and Park, 2015)*. MDR Acinetobacter spp. are defined as the isolate resistant to at least three classes of antimicrobial agents; all penicillins and cephalosporins (including inhibitor combinations), fluroquinolones, and aminoglycosides. XDR Acinetobacter spp. are the Acinetobacter spp. isolates that are resistant to the three classes of antimicrobials described above MDR and are also resistant to carbapenems. PDR Acinetobacter spp. are the XDR Acinetobacter spp. that are resistant to polymyxins and tigecycline *(Manchanda et al., 2010)*.

Exclusion criteria:

Patients who had a HAI during their stay in another hospital from which they had been transferred or patients not admitted to hospital.

Subjects:

A. Patients:

Patients admitted to the selected different surgical wards and ICUs, during the study period were followed- up prospectively for occurrence of one or more HAIs. Data collected from each patient included age, sex, length of hospital stay, use of invasive medical devices, receipt of antibiotics, the measure of obesity was body mass index (BMI), which is a formula of weight in kilograms divided by height in meters squared (BMI =kg/m2)(Obese; Greater than 30) and problem necessitating admission.

B. Health care worker and environmental Assessment:

HCWs bacteriological swabs were collected from hand and nose. Environmental bacteriological swabs were also collected from surfaces, walls, furniture, beds, floor and trolleys of ICU and different surgical wards.

In the present study, MacConkey medium was used as an initial step for isolation of A. baumannii with further growing on Herellea agar medium and Leeds Acinetobacter agar medium. These were followed by identification and biotyping using catalase test, oxidase test

followed by further confirmation by API 20 NE system to the species level. Phenotypic detection of carbapenemase production *(CLSI, 2013)*, Modified Hodge test (MHT), rapid diagnosis using CarbAcineto NP test and Modified Carbapenem Inactivation Methods (mCIM)/ EDTA-modified Carbapenem Inactivation Method (eCIM) were done *(CLSI, 2018)*.

Multiplex polymerase-chain reaction (PCR was used for detecting A. baumannii harboring bla OXA-51-like and bla OXA-23-like genes *(Zavascki et al., 2010)*.

Statistical Analysis:

It was done using Excel Program & Statistical Package of Social Science (SPSS) software version 25 (SPSS Inc., Chicago, IL, USA). Continuous variables were analyzed as mean values ± standard deviation (SD) or median (range) as appropriate. Percentages were calculated for categorical data. For categorical variables, differences were analyzed with X^2 (chi square) test and Fisher's exact test when appropriate. Differences among continuous variables with normal distribution were analyzed by Student's T-test; for continuous variables without normal distribution, we used non-parametric tests and differences were analyzed by the Mann –Whitney U-test. Friedman test was done to assess difference among repeated measures regarding different study parameters in each study group. P value of ≤ 0.05 was considered statistically significant. PCR results were considered the gold standard.

RESULTS

The study population included 293 (58.6%) males and 207 (41.4%) females. Age distribution ranged from 14 - 85 years with a mean age of 38.8±15.9 years. Most of the total population was subjected to elective surgery (407, 81.4 %). Forty-five (9%) out of five hundred patients were obese, while 34(6.8%) had history of smoking, 26(5.2%) were a diabetic ones and 14 (2.8%) had a history of malignancies, other patients had a history of liver and kidney diseases (16, 3.2% and 13, 2.6%; respectively) **(Table 1)**.

Table (1): Demographic and clinical characteristics of patients

		Frequency	Percent %
Age (yrs)			
Mean ±SD	**38.9±15.9**		
Range	**(14-85)**		
	10-20	**53**	**10.6 %**
	21-30	**141**	**28.2 %**
	31-40	**95**	**19.0 %**
	41-50	**78**	**15.6 %**
	51-60	**78**	**15.6 %**
	60+	**55**	**11.0 %**
Gender	**Male**	**293**	**58.6**
	Female	**207**	**41.4**
ICU and Surgical departments	**ICU**	**168**	**33.6**
	General surgery	**172**	**34.4**
	Orthopedic surgery	**75**	**15**
	Urological surgery	**61**	**12.2**
	Gynecologic and obstetric surgery	**20**	**4**
	Neurosurgery surgery	**3**	**0.6**
	E.N.T surgery	**1**	**0.2**
Types of operative procedure	**Emergency**	**93**	**18.6**
	Elective	**407**	**81.4**
Average length of stay **in hospitals (ALOS) (Days)**	**3 – 7 days**	**283**	**56.6**
	7 – 10 days	**151**	**30.2**
	More than 10 days	**66**	**13.2**
Underlying risk factors	**Obesity**	**45**	**9**
	Smoking	**34**	**6.8**
	Diabetes	**26**	**5.2**
	Liver disease	**16**	**3.2**
	kidney disease	**13**	**2.6**
	malignancies	**14**	**2.8**
	shocked	**14**	**2.8**
	Cardiovascular disease	**6**	**1.2**

Postoperative infections were detected in 217 (43.4%) of patients with no significant difference between the infected and non-infected groups. One hundred and thirty one male patients (26.2%) had postoperative infections, while 162(32.4%) of them had no such infections. Eighty six (17.2%) of the female patients developed post-operative infections with no significant difference between the infected and non-infected groups ($p = 0.482$). Surgical site infections (SSIs) were developed in 76 (35%), urinary tract infection (UTI) in 55 (25.3 %), blood stream infection (BSI) in 34 (15.7 %), ventilator associated pneumonia (VAP) in 29 (13.4%) and hospital acquired pneumonia (HAP) in 23 (10.6%) of patients **(Table 2)**.

Table (2): Prevalence and distribution of A. baumannii isolates according to each ward and different clinical specimens

Parameters / Wards	Frequency of A.baumannii	Percentage	Specimen	Frequency of A.baumannii	Percentage
ICU	14	51.9	Endotracheal T	10	37
			Blood	1	3.7
			Wound swab	1	3.7
			Urine	2	7.4
General surgery	11	40.7	Blood	7	25.9
			Wound swab	1	3.7
			Urine	1	3.7
			Sputum	2	
Urology surgery	1	3.7	Urine	1	3.7
Orthopedic surgery	1	3.7	Sputum	1	3.7

In the current study, Pseudomonas spp. was the most frequently isolated pathogen among the Gram-negative organisms (34, 28.6%). A. baumannii was considered as the third most common Gram-negative organism isolated (27, 22.7%). Coagulase negative Staphylococcus (CONs) (33, 32.7%) were the most frequently isolated pathogens among Gram-positive organisms followed by S. aureus (26, 25.7%). Antibiogram analysis of the A. baumannii isolates showed- -a high resistance pattern. All A. baumannii isolates showed 100% resistance to Ampicillin/sulbactam, Amoxicillin / clavulanic acid, Amoxicillin, Oxacillin, Ceftazidim, Ofloxacin, Gentamicin, Erythromycin, Clindamycin and Nitrofurantoin. High profile of resistance to Cefotaxim, Ceftriaxon, Ciprofloxacillin, Rifampicin, Norfloxacin, Cefoperazone / sulbactam (92.6%); each. Imipenem, Tobramycin and Chloramphenicol (88, 9%); each and Tazobactam (85.2%). Meropenem, Amikacin, Levofloxacin and Sulfamethoxazole/trimethoprim (81,5); each. Vancomycin (77.5%), Cefepime (74.1) and Aztironam (70.4%) were observed. Only two isolates (7.4%) were resistant to Colistin and 25 isolates (92.6%) were susceptible to this antibiotic. Eighteen (66.7%) A. baumannii isolates showed multidrug resistant (MDR) pattern, also 7, (25.9%) extensively drug resistant (XDR) strains were isolated with no pan drug resistant (PDR) strains **(Table 3)**.

Table (3): Evaluation of antimicrobial resistance pattern among A. baumannii isolates.

Resistance grades	N, (%)
Not MDR	2, (7.4%)
MDR	18, (66.7%)
XDR	7, (25.9%)
PDR	0, (0%)
Total isolates	27(100%)

Multidrug-resistant (MDR), Extensively Drug-resistant (XDR), Pan Drug-resistant (PDR)

The 25 Imipenem-resistant strains out of 27 A. baumannii (92.6%) which were evaluated phenotypically using Modified Hodge test (MHT), Carba NP and mCIM tests for detection of carbapenemase producing isolate. Carbapenemase production was detected in the A. baumannii isolates using the mCIM, MHT and the Carba NP test (p value = 0.000). The Sensitivity of the mCIM method was (92%), the Specificity (100%) .The MHT had sensitivity and specificity that was (76% and 100%) respectively. Out of 25 (92.6%) Imipenem resistant isolates were analyzed with MHT with meropenem 10 µg (MEM) for carbapenemase production; 19 (70.4 %) isolates were found to be true positive with MHT (MEM) and six (22.2 %) isolates were false positive results. Carba NP test showed positive result in 22 (81.5%). However, three (11.1 %) of isolates were false positive, Sensitivity with (70.04%; 95.83%) 95% Conf. Ints, Specificity with (34.24%: 100.00%) 95% Conf. Ints. The current study suggests that the mCIM assay is the most useful phenotypic method for detection of carbapenemase production **(Figure 1)**.

Figure (1): Positive mCIM and eCIM, Interpretation: (carbapenemase and metallo-β-lactamase detected). A mCIM positive result (zone diameter 5 mm) on right side and an eCIM positive result (zone diameter = 13 mm with pinpoint colonies throughout the zone of inhibition). A ≥ 5 mm increase in zone diameter for eCIM vs zone diameter for mCIM (13 mm − 5 mm = 8 mm) demonstrates the inhibition of the metallo-β-lactamase in the presence of EDTA. NOTE: The pinpoint colonies throughout the zone of inhibition was ignored when measuring the zone for the eCIM test, A narrow ring of growth around the meropenem disk results from carry-over of the tested organism in the trypticase soy broth and should be ignored.

Using multiplex PCR analysis, most isolates (12; 44.4%) carried was bla OXA-51-like gene, followed by ten (37%) isolates that carried both bla OXA-51-like and bla OXA-23-like genes. Only one (3.7%) isolate carried blaOXA-23-like gene **(Figure 2)**.

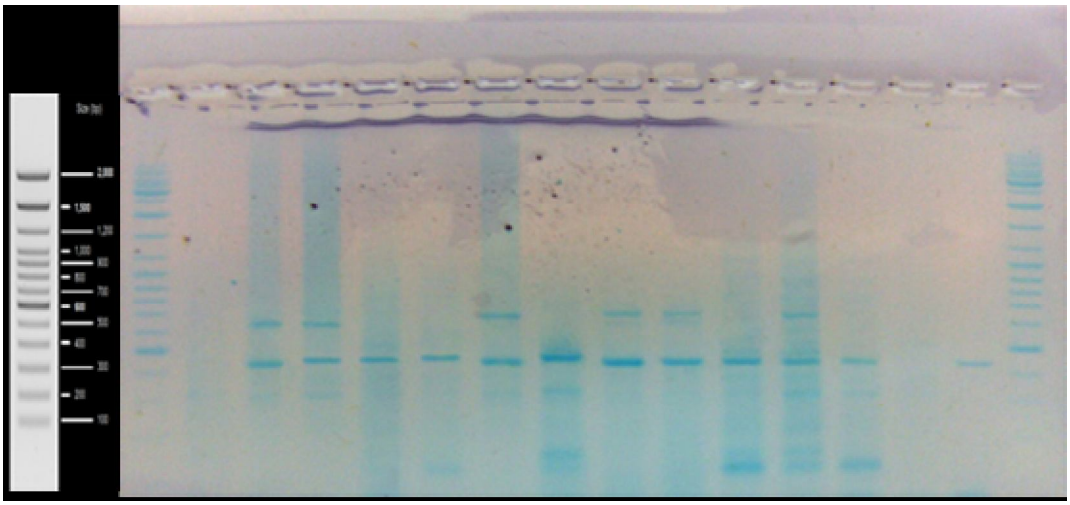

Figure (2): Gel electrophoresis of the multiplex PCR amplified products of blaOxa51-like and blaOxa 23-like genes of A. baumannii isolates. PCR amplified products of bla Oxa51-like gene with 353 bp amplification fragment, PCR amplified products of bla Oxa23-like gene with 501 bp amplification fragment, This figure demonstrates M: 100-2000 bp DNA Ladder, NC = negative control; lines 1-11& 13: isolates carrying bla OXA-51-like genes; line 1, 2, 5, 7, 8, 10; isolates carrying bla OXA-23-like genes; line 12: isolate negative for both bla OXA-51-like and bla OXA-23-like genes.

Overall, a total of 50 swabs were taken from different surfaces of the hospital environment. Positive cultures were only detected in 30 (60%), of the 50 selected sites. P. aeruginosa was the most common organism (9, 30%), followed by Escherichia coli (7, 23.3%), CONs (5, 16.7%), K. pneumoniae (4, 13.3%), Enterococcus spp., Serratia spp, Acinetobacter spp (lwoffii and baumannii) and Aspergillus Niger (1.3.3 %; each). The affected major wards were ICUs and General Surgery (8;26.7% and 9;30%); respectively . Among fifty health care workers (HCWs), 150 swabs (100 hand and 50 Nose) were investigated. Positive cultures were detected in 15 (15 %) and 3 (6%); respectively. The HCWs samples were observed that, a total of (18) microorganisms were isolated, CONs was the most common organism (9, 50%), followed by S. aureus (3, 16.7%), followed by P. aeruginosa (5, 27.8%) and E. coli (1, 5.5%). Among the antibiogram for the isolated strains from the Hospital environment, Gram-positive strains were sensitive to Amikacin, Colistin, Amoxicillin clavulanic acid, Ceftriaxone, Cefotaxim, Vancomycin (6;85.71%) followed by Ciprofloxacin and Oxacillin (5; 71.43%). Intermediate susceptibility were mainly for Ceftazidim and Ampicillin/Sulbactam (2; 28.57%). The most resistant strains had resistance to Amoxicillin (6; 85.71%). Gram-negative strains were sensitive to Colistin (23;100%), followed by Cefoperazone sulbactam, Meropenem and Aztreonam (21; 91%). Intermediate susceptibility were mainly for Amoxicillin clavulanic

acid (8; 35%). The most resistant strains had resistance to Amoxicillin (22; 96%), followed by Trimethoprim-sulfamethoxazole and Oxacillin (16; 70% and 12;52%) respectively.

Among the antibiogram for the isolated strains from the HCWs, the most sensitive antibiotics were Amikacin, Colistin, Meropenem , Imipenem, Aztreonam, Cefotaxim, Cefoperazone-sulbactam, Levofloxacin and Chloramphenicol for Gram- positive and Gram- negative strains (12;100% and 6; 100%); respectively. The most resistant strains had resistance to Amoxicillin (12;100% and 6; 100%); respectively, followed by Trimethoprim-sulfamethoxazole (12;100% and 5; 83.3%); respectively, followed by Oxacillin (6; 50% and 2; 33.3%) respectively.

In the present study, the only one strain of A. baumannii was isolated from ICUs (monitor alarm button). The isolated A. baumannii was investigated using previous mentioned different phenotypic tests. It was identi?ed to be positive by MHT only, while all further tests used were shown negative results. Using multiplex PCR analysis, A. baumannii isolated pathogen was found to carry the bla OXA-51-like gene only.

DISCUSSION

Acinetobacter baumannii has emerged as a healthcare-associated pathogen worldwide. The spread of carbapenem-resistant A. baumannii is of global concern. The study population included 293 (58.6%) males and 207 (41.4%) females. Age distribution ranged from 14 - 85 years with a mean age of 38.8±15.9 years. The current results was agreed with a study published by *Mawalla et al. (2011)* who found that, the mean age was 38 years with standard deviation of 22.12 years, There were 116 (46.4%) males and 134 (53.6%) females, SSI rate between male and female was 58% and 40% respectively. Similarly, the study of *Pal et al. (2017)* reported a higher rate of A. baumannii infection among male as compared to female patients. Many studies noted that, the incidence of post-operative infection was more common in males than in female's patients *(Insan et al., 2013)*. In addition, the study is coordinated with a study done by *Rebic et al. (2018)* who found that, A baumannii infections were more common in males (54.20%) as compared with females (45.80%) with a mean age of the study population at 42.5±23.22 years. The age of male and female patients it was 42.9±22.3 and 36.3±22.6 years, respectively, but it was in disagreement with the ratio of isolates in comparison with the gender; 50.80 % isolates were from females, and 49.18 % were from male. This could be explained by more frequent hospital admission of male patients compared with females, also multiple risk factors in male such as cigarette smoking, extended hospital stay, prolonged antibiotic usage and high exposure to external environment than do female patients. In contrast, *Khairy et al. (2011)* found that the rate of SSI was higher in females than in males. This could be explained by more frequent hospital admission of females patients compared with male.

The results of this current study were similar to the results of *Fouad et al. (2013)*, who reported that A. baumannii strains were isolated most frequently from respiratory tract and wound infections by 45% (24/53) and 42 % (22/53); respectively, followed by urinary tract infection (UTI) (11% ,6/53), and lastly blood stream infection (BSI) (2%,1/53). *Sahu et al. (2016)* showed that lower respiratory tract infections (LRTIs) after cardiac surgery accounted the most of the infections (44.2%) followed by SSI (11.6% (BSI (7.5%), UTI (6.9%) and infections from combined sources (29.8%) and Acinetobacter, Klebsiella, Escherichia coli and Staphylococcus were the most frequent pathogens isolated in those patients with LRTI, BSI, UTI, and SSI; respectively.

In the present study, the most commonly risk factors were diabetes mellitus (18.5%), obesity (14.8%), shocking and cardiovascular disease (11.1% each), followed by malignancy and liver disease (7.4% each), and finally Smoking (3.7%) with no significant difference between development of MDR and XDR within A. baumannii infections and the underlying risk factors. Prolonged length of hospital stay mostly in period more than 10 days showed statistically no significant to development of MDR and XDR within A. baumannii infections (12, 44.4%). Our results agreed with study carried by *Rebic et al. (2018)* who found that, the major risk factors associated with Acinetobacter infection were post-surgical (48%), followed by diabetes mellitus (11%), I.V. catheterization (25%), extended hospital stay (21%) and mechanical ventilation (92%).

The present data were coordinated with many reports, as the study done by *Mirnejad et al. (2018)* who found that, the frequencies of MDR and XDR isolates were 70 and 19%; respectively, No PDR isolates were identified. *Rebic et al. (2018)* showed that, out of which 78.4 % were MDR. of these MDR isolates, 17.24% were PDR, most of the positive isolates, 68.92 %, were from the general surgery (48, 65%) and ICUs (20, 27%) ($p<0.01$). In a study carried by El - *Bassuony et al. (2016)*, A. baumannii showed the highest resistance to imipenem (81%), MDR were (63.64%) and XDR were (18.18%), there is no PDR strains. *Čiginskienė et al. (2019)* found that, the proportions of MDR, XDR and potentially pandrug-resistant (pPDR) A. baumannii were 13.3, 68.3, and 18.3% respectively.

The current study agreed with many of reports. In Egypt, *El- Masry and El-Masry (2018)* reported that 14 out of 22 (63.6%) A. baumannii isolates were carbapenem resistant as detected by the antibiotic susceptibility test. A. baumannii is resistant to sulfamethoxazole-trimethoprim, amoxicillin / clavulanic acid, ciprofloxacin, piperacillin, tazobactam and ceftazidime (100, 90.9, 90.9, 90.9, 81.8 and 81.8%; respectively); Imipenem resistance was 14/22(63.6%); Colistin showed the highest activity against A. baumannii isolates; the resistance rate was 4.5%. Resistance rates of carbapenems was observed to be high in previous studies in Egypt. In *Al-Agamy et al. (2014)* for example the resistance rate to imipenem was high (70%) among A. baumannii isolates. A. baumannii isolates were all resistant to amoxicillin–clavulanate, aztreonam, cefepime,

ceftazidime, and cefotaxime. The resistance rates to ciprofloxacin, imipenem, and amikacin were 85% (34/40), 70% (28/40), and 45% (18/40); respectively. Colistin showed the highest activity against A. baumannii isolates; the resistance rate was 5% (2/40). *EL - Bassuony et al. (2016)* noted that all A. baumannii isolates showed 100% resistance to Nitrofurantoin, 3rd, and 4th generation cephalosporens. A significantly high prevalence of resistance to ampicillin/ sulbactam (75%), Ciprofloxacillin (79.5%), imipenem (81.81%), meropenem (88.63%) and ciprofloxacin were observed. All isolates was sensitive to Colistin. On the contrary, 56.4 and 78.2% of Acinetobacter isolates were susceptible to Tigecycline and Colistin, respectively. Moreover, the resistance rates of Acinetobacter isolates for Imipenem and Meropenem were 67.9 and 64.1%, respectively *(Baker et al., 2017)*.

In the current study, the mCIM is the most useful phenotypic method to be a significant for detection of carbapenemases.The present study is in coordination with *CLSI (2017)* reports in which, the mCIM had > 99% sensitivity and > 99% specificity for detection of carbapenemase production. A study done by *Pierce et al. (2017)* showed that, the sensitivity of the mCIM was 99% (95% confidence interval [CI], 93 to 100) and the specificity was 100% (95% CI, 82 to 100), in the second stage of this study, the range of sensitivities observed across nine laboratories was 93% to 100%, with a mean of 97%; the range of specificities was 97% to 100%, with a mean of 99% compared to the genotype. *Yamada et al. (2016)* study reported that, the sensitivity of mCIM using meropenem was 100% and the speci?city was 100%. MHT has been widely used as the preferred phenotypic method for detection of MBL, it is not recommended in latest version of CLSI, due to its low sensitivity. CarbaNP method is recommended instead *(CLSI, 2018)*. *Kuchibiro et al. (2018)* with compared some phenotypic methods and MHT and showed an acceptable specificity (100%), but the sensitivity was very low (50%).

The mCIM is simple, inexpensive, less subjective, reproducible and most sensitive method *(Aktas et al., 2017*; *Datta et al., 2017*; *Pierce et al., 2017* and *Pragasa et al., 2017)*. In a limited setup laboratory, early phenotypic detection of carbapenem-resistant Enterobacteriaceae (CRE) is of great importance as this will guide clinicians and help to control the spread of carbapenemase producer by contact precautions of the patient. In a microbiology laboratory where molecular methods are unavailable, mCIM method will play important role for easy and early detection of CRE *(Pawar et al., 2018)*.

The current study is in agreement with many studies, which revealed that, A. baumannii harboring bla OXA-51-like gene that has been identified as a marker for species identification. A. baumannii harboring blaOXA-51-like gene has been identified as a marker for species identification *(Cicek et al., 2014*; *El-Abd et al., 2015*; *Lin et al., 2016*; *Ghaith et al., 2017*; *Joshi et al., 2017* and *Pal et al., 2017)*. Similarly, *Hou and Yang (2015)* reported that, the main resistant genes in Acinetobacter baumannii were bla OXA-51 and blaOXA-23, and the main multidrug-resistant genes were found in

ICU. An intrinsic bla OXA-51-like gene detected in all isolates in this study supports the use of this gene as a surrogate marker of A. baumannii identification. Also, the current results are agreed with the studies carried out by *Amr and Abdel Razek (2016)* at Zagazig University Hospitals, Egypt, using the multiplex PCR; there results showed that all A. baumannii isolates were positive for blaOXA-51-like gene while 69.7% of carbapenems resistant isolates were positive for blaOXA-23like gene, while 10 (30.3%) were not carrying this gene. *Ahmed et al. (2015)* reported that, 131 out of 150 isolates (87.3%) were resistant to imipenem. A study was found that 115/150 (76.7%) isolates were bla OXA-23-like positive and 150/150 (100%) were bla OXA51-like positive .

High prevalence of positive bla OXA-23-like gene among carbapenem-resistant A. baumannii isolates were observed by many studies in Egypt, *El- Masry and El-Masry (2018)* found that, 14 A. baumannii isolates screened for carbapenemase production by MHT, carbapenemase activity was detected among 10 (71.4%) of carbapenem-resistant A. baumannii isolates. Molecular detection of carbapenem resistant genes showed that bla OXA-23 was the common detected gene 6/14 (42.8%). *El-Abd et al. (2015)* showed that, bla OXA-23 carbapenemase was detected in 48 (85.6%) of the 56 carbapenem-resistant isolates. According to the results of multiplex PCR, *Shoja et al. (2016)* noted that, All isolates (100%) carried bla OXA-23-like gene, bla OXA-23-like and bla OXA-24-like genes which were detected in 85.6% and 6.2% of carbapenem resistant isolates, respectively. The acquired oxacillinase (OXA) genes, notably bla OXA-23-like were prevalent in the A. baumannii isolates *(Lowe et al., 2018)*. Different ratios of bla OXA-23-like gene were reported in Egypt as 50% *(Al-Agamy et al., 2014)*, 52.9% *(Al-Hassan et al.,2013)*, 100% by *Fouad et al. (2013)*, and others also reported all over the world as those reported by *Cherkaoui et al. (2015)* (51.8%), *El-Abd et al. (2015)* (85.7%), *Vali et al. (2015)* (85%) and *Rolain et al. (2016)* (100%). The presence of numerous resistance genes led to a high level of resistance to most of the antibiotics *(Al maghrabi et al., 2018* and *Mosavat et al., 2018)*.

The results of environment and HCWs was agreement with *Gelaw et al. (2011)* study, who found that, seventy-two swab specimens were collected from the dominant hand and nostrils of health professionals at Gondar University Teaching Hospital. Seventy-seven bacterial pathogens of nosocomial importance were isolated, 36 (46.8%) and 41(53.2%) were from nostril and hands, respectively. Seventy-two of the isolates were Gram positive, while five (6.5%) were Gram negatives. CoNs was the predominant isolate 44 (57.1%) followed by S. aureus (28; 36.4%). Inanimate surfaces and equipment contamination play a major role in cross-transmission of pathogens in ICUs. Bacteria, including MDR organisms, may survive for a long time to environmental physical and chemical agents and have been isolated from different surfaces and equipment of the patient zone and of the healthcare area *(Russotto et al., 2015)*. Contamination of the ICU environments and carriers with important bacterial pathogens that are the main risk factors for HAIs *(Tajeddin et*

al., 2016). The contamination rate of A. baumannii for environments as well as healthcare workers is higher than other MDR organisms *(Morgan et al., 2012*; *Escudero et al., 2017* and *Thom et al., 2017)*.

Once contaminated, an environment can be a CRAB reservoir for a long period of time since the pathogen can survive for more than several months under dry conditions *(Chemaly et al., 2014)*. Therefore, avoiding contamination is far more important with CRAB than other MDR organisms. In addition, the results of the present study was agreed with *Aljanaby and Aljanaby (2018)* who showed that, A. baumannii isolates were resistant to the most used antimicrobials in high percentages. The resistance of bacteria due to the presence of bla OXA - 51-like as well as the acquired gene can be illustrated for the presence of Insertion sequence (IS) elements that play an important role in the mobilization and expression of OXA-type β-lactamases and in the acquisition of resistance by A. baumannii *(Correa et al., 2018)*. This explains why these bacteria have firmly established themselves as MDR nosocomial pathogens whose infections no longer respond to treatment by commonly used antibiotics *(Odewale et al., 2016)*.

Most of the Gram negative organisms were highly sensitive to Amikacin and Colistin followed by Meropenem and Cefoperazone / sulbactam. Gram positive organisms were highly sensitive to Vancomycin and Colistin. High resistant for both was Amoxicillin and Trimethoprim-sulfamethoxazole. These results are coordinated with the study of *Vijayanarayana et al. (2014)* who found that, the sensitivity pattern of Gram negative organisms such as K. pneumonia, Acinetobacter species and E. coli was comparatively identical showing high sensitivity towards colistin P. aeruginosa was highly sensitive to only Colistin. Whereas Gram positive organisms, Methicillin-resistant Staphylococcus aureus (MRSA) and Methicillin-sensitive Staphylococcus aureus (MSSA) were highly sensitive to Linezolid, Cotrimoxazole, Tetracycline and Vancomycin and highly resistant to Cotrimoxazole. In contrast, *Zahran et al. (2017)* reported that, Enterobacteriaceae isolates showed 100% resistance to Ceftazidime, Cefepime, Cefotaxime, Ceftriaxone, Cefoxitin, Aztreonam, and Chloramphenicol. Resistance to Carbapenems was 88.2% to Imipenem and 82.4% to Ertapenem and Meropenem. In the current study, the only one A. baumannii strain was isolated from ICU (monitor alarm button). The isolated A. baumannii was investigated by different phenotypic tests, it was found to be positive by MHT only, and all further tests were shown negative results. Using multiplex PCR analysis, A. baumannii isolated pathogen was identi?ed to be carried the bla OXA-51-like gene only. The present study was in agreement with a study carried out by *Nowak et al. 2012* who reported that seven isolates of carbapenem resistant A. baumannii only had bla OXA-51-like. Carbapenem resistance in these isolates may be associated with other mechanisms such as: modification of penicillin binding proteins, loss of porins and decreased permeability or over expression of efflux pump *(Zarrilli et al., 2009* and *Kulah et*

al., 2010). The relationship between harboring bla OXA-51-like genes and resistance to carbapenems still need more investigation.

CONCLUSION

The current study suggests that A. baumannii is one of the most commonly detected isolate in our hospitals. The mCIM assay is the most useful phenotypic method for detection of carbapenemase production. Detection of Carbapenem resistance genes is alarming as a serious healthcare problem in our hospitals.

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الكشف المظهرى و الجزيئى للراكدة البومانية المقاومة للكاربابينيم بوحدات الجراحة والعناية المركزة بمستشفى جامعة الأزهر - دمياط الجديدة

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خلفيـــة البحـــث: بـــرزت الراكـــدة البومانيـــة كأحـــد العوامـــل المســـببة للأمـــراض المرتبطـــة بالرعايـــة الصـــحية فـــي جميـــع أنحـــاء العـــالم. كمـــا أظهـــرت العديـــد مـــن الدراســـات الوبائيـــة عـــن حـــدوث عـــدوى الراكـــدة البومانيـــة متعـــددة المقاومـــة للمضـــادات الحيويـــة فـــي منـــاطق مختلفـــة مـــن العـــالم. ولعـــل انتشـــار عـــدوى هـــذا الميكـــروب المقـــاوم للكاربـــابينيم ليعـــد مصدر قلق عالمي.

الهـــدف مـــن البحـــث : الكشـــف عـــن الراكـــدة البومانيـــة المقاومـــة للكاربـــابينيم بأقســـام الجراحـــة ووحـــدات العنايـــة المركـــزة بمستشـــفى الأزهـــر الجـــامعي (دميـــاط الجديـــدة) و تحديد مدى حملها لبعض الجينات المسببة لمقاومة الكاربابينيم.

المرضـــى وطـــرق البحـــث : أجريـــت الدراســـة الحاليـــة علـــى 500 مـــريض بأقســـام الجراحـــة ووحـــدات العنايـــة المركـــزة بمستشـــفى الأزهـــر الجـــامعي (دميـــاط الجديـــدة) فـــي الفتـــرة مـــا بـــين مـــايو 2016 وســـبتمبر 2018. وقـــد تـــم اختيـــار عينـــات الدراســـة المختلفـــة حســـب الاعـــراض الســـريريه.وقد شـــملت العينـــات بـــاختلاف أنـــواع العـــزلات علـــى عينـــات مثـــل البصـــاق والشـــفط للســـوائل داخـــل الحنجـــره والقـــيح والبـــول وعينـــات الـــدم. وقـــد تـــم تتبـــع جميـــع العينـــات إلـــى مســـتوى الأنـــواع وذلـــك لتقيـــيم الاشـــكال المظهريـــة المختلفـــة ايضـــا" والكشـــف عـــن إنتـــاج الكاربابينيمـــاز باســـتخدام تفاعـــل البـــوليميريز المتسلسل.

النتـــائج: تـــم الكشـــف عـــن عـــدوى مـــا بعـــد العمليـــات الجراحيـــة فـــي 217 (43.4 ٪) حالـــة مـــن أصـــل 500 مـــن المرضـــى. وقـــد كانـــت الراكـــدة البومانيـــة ثالـــث أكثـــر الكائنـــات الحيـــة الدقيقـــة ســـلبية الجـــرام المعزولـــة (27، 12.3%). وقـــد ظهـــرت عـــزلات الراكـــدة البومانيـــة بشـــكل ملحـــوظ ، وانتشـــار كبيـــر بوحـــدات العنايـــة المركـــزة (14، 51.9 ٪).

وقد تم الكشف عن إنتاج الكاربابينيماز في عزلات الراكده البومانيه باستخدام طريقة تعطيل الكاربابينيم المعدلة، واختبار هودج المعدل و كاربا ان بى. و بإستخدام تفاعل البوليميريز المتسلسل، وقد حملت 12 عزلة (44.4 ٪) الجين الذي يشبه bla OXA-51 ، يليه عشر عزلات (37 ٪) حملت كلا من الجينات التي تشبه bla OXA-51 و bla OXA-23 يحمل واحد فقط (3.7 ٪) عزل الجين الذى يشبه bla OXA-23.

الاستنتاج: تشير الدراسة الحالية إلى أن الراكدة البومانية تعد ثالث أكثر العزلات سالبة الجرام المكتشفة شيوعًا في مستشفي جامعة الأزهر بدمياط الجديدة. وقد وجد أن الفحص (mCIM) تعطيل الكاربابينيم المعدلة هو الأسلوب المظهري الأكثر فائدة للكشف عن إنتاج انزيمات الكربابينيماز، وأن وجود مثل هذه العزلات الحاملة لجينات مقاومة للكاربابينيم إذ يمثل مشكلة صحية خطيرة بالمستشفيات.