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ORIGINAL ARTICLE

Detection of bla OXA-23-like and bla OXA-40- like genes in Carbapenem Resistance *Acinetobacter* Species Isolated from Ain Shams University Hospitals

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Abbreviations
ETA: endotracheal tube aspirate
CVP: cardiovascular pressure catheter (CVP catheter tips)
CKD: chronic kidney diseases.

ABSTRACT

Background: *The emergence and rapid spread of the important nosocomial drug-resistant Acinetobacter spp., in particular, A. baumannii, are of great concern worldwide.* ***Objectives:*** *This study aimed to analysis of the antibiotics susceptibility profile in Acinetobacter spp., and detection of genes encoding bla OXA-23-like and blaOXA-40-like genes as a source of Carbapenem resistance in MDR Acinetobacter spp.* ***Methodology:*** *A total of 40 Acinetobacter spp. were collected from Ain Shams University Hospitals in the period from January to October 2016 to determine the distribution of blaOXA-23-like and blaOXA-40-like genes in Carbapenem-resistant Acinetobacter spp. All isolated species were cultured, subjected to biochemical testing, and antimicrobial susceptibility testing. The distribution of blaOXA-58-like and blaOXA-40-like genes was investigated in the MDR Acinetobacter spp. by multiplex polymerase chain reaction (PCR).* ***Results:*** *As regards the resistance pattern 26 (65%) of Acinetobacter spp. were resistant to ampicillin/sulbactam, 23 (57.5%) were resistant to imipenem, 20 (50%) were resistant to levofloxacin, 15 (37.5) were resistant to ceftazidime, 13 (32.5%) were resistant to cefipime and 10 (25%) were resistant to gentamycin. Thirteen (13/40, 32.5%) of Acinetobacter species isolates were MDR. eight isolates (8, 61.5%) from MDR Acinetobacter species were positive for bla OXA-23 like genes while all isolates were negative for bla OXA-40 like genes (0/13, 0%) .* ***Conclusions:*** *The prevalence of Carbapenem-resistant Acinetobacter spp. was high in ASUHs. The distribution of blaOXA-23-like and blaOXA-40-like among Carbapenem-resistant Acinetobacter species was low and there was no association between Carbapenem-resistant isolates and distribution of these genes. This confirms that Acinetobacter has different mechanisms for MDR other than the blaOXA carriage.*

INTRODUCTION

Acinetobacter is a formidable challenge to managing critically ill patients. This pathogen ability to rapidly develop antimicrobial resistance to all currently available antimicrobial agents is concerning because increasing data support attributable mortality to these bacteria when associated with hospitalized patients with comorbidities and severe illness[1].

Acinetobacter is often causing outbreaks in intensive care units (ICU). The role of dual therapy is currently unclear and might be associated with increased toxicities without proven synergy or ability to prevent the development of resistance. Infection control and antibiotic control measures might have the greatest impact on these bacteria. The problem is complicated by increasing rates of resistance to broad-spectrum antibiotics including carbapenems which have been the drugs of choice in severe infections due to this organism. Continued efforts are needed to develop new antimicrobial agents against this pathogen and assess the ideal currently available agents [2,3].

The mechanisms that are responsible for conferring resistance on *Acinetobacter* spp., including the production of β-lactamases, changes in penicillin-binding proteins that prevent activities of β-lactam drugs, alterations of porin proteins that result in decreased permeability to antibiotics, and the activity of efflux pumps that decreases the concentration of antibiotics within the bacteria [4].

B-Lactamases involved in acquired resistance are of molecular classes A, B, and D. Class D carbapenemases are increasingly reported in *Acinetobacter baumannii* [5]. Class D beta-lactamases are also referred to as OXA-type enzymes, because of their preferential ability to hydrolyze oxacillin. OXA enzymes (encoded by bla OXA genes) can be sub classified into eight distinct subgroups, of which OXA-23-like, OXA-24-like, and OXA-51-like have been identified in *Acinetobacter* spp.[1].

bla OXA-23 and -51 are the most common and frequently occur simultaneously within a single isolate. While OXA-51 is an intrinsic enzyme, OXA-23 is an acquired enzyme and frequently leads to A. baumannii outbreaks in intensive care units around the world [6].

This study aims to analysis of the antibiotics susceptibility profile in *Acinetobacter* spp. and detection of genes encoding blaOXA-23-like and blaOXA-40-like genes as a source of Carbapenem resistance in MDR *Acinetobacter* spp. isolated from patients in intensive care unit in ASUHs, and correlates between infection with acinetobacter spp. and risk factors in ICU.

METHODOLOGY

This study was conducted on 175 patients attended Intensive Care Units (ICUs) of Ain Shams University Hospitals in the period from January to October 2016. An informed consent was taken from the authorized person in ICUs and from the patients or from their relatives. Full details about the study, its benefit were explained to them. We collected data about the duration of hospital stay, using invasive procedures (mechanical ventilation, urinary catheter, and intra vascular devices), and prior antibiotics intake.

Out of the 175 specimens; fifty seven respiratory specimens (sputum and endotracheal tube aspirate ETA), 58 urine specimens (from non catheterized patients; a mid-stream urine (MSU) and from catheterized patients; the sample was taken from the catheter tube by a sterile syringe), 32 pus specimens (superficial skin swabs were collected from open wound or open abscesses using a sterile swab), 18 blood specimens (samples were taken by venipuncture under complete aseptic condition) and 10 CVP tips. All samples were collected in sterile containers to be examined bacteriologically.

The following variables were analyzed: duration of stay in the ICU, mechanical ventilation, endotracheal intubation, instrumentation at various sites such as Central venous catheters, the presence of underlying diseases or conditions, including chronic obstructive pulmonary disease (COPD), diabetes mellitus, hypertension, chronic kidney disease (CKD), chronic liver disease, malignancy.

Bacterial identification:

Identification of the isolated organisms was done according to Cheesebrough[7]. Based on colonial morphology, microscopic examination of Gram stained films and biological activity of the isolated organisms**.** Preliminary identification of Gram negative bacilli was done by using triple sugar iron agar medium (TSI) inoculation, Lysine Iron Agar (LIA) medium inoculation' Motility Indole Ornithine (MIO) agar medium inoculation' citrate utilization test, urease production test and detection of cytochrome oxidase enzyme using Oxidase strips (All media and reagents were supplied from Oxoid, UK).

Antimicrobial susceptibility testing:

The antibiogram of the obtained isolates was done by disc diffusion method using overnight cultures at a 0.5 McFarland standard on Muller Hinton agar plates (bioM´erieux, France). After overnight incubation the results were interpreted according to the CLSI (Clinical and Laboratory standard institute 2016). Multidrug resistance isolates were defined as non-susceptible to one or more agent in three or more antimicrobial categories including the following: β-lactam/β-lactamase inhibitor combination (ampicillin/sulbactam), extended spectrum cephalosporins (ceftazidime and cefepime), carbapenems (imipenem), quinolones (levofloxacin) and aminoglycosides (gentamicin) (Becton Dickinson Microbiology Systems).

From the 175 specimens 40 *Acinetobacter* spp. were isolated, 13 (32.5%) were identified as MDR *Acinetobacter* strains which were all resistant to carbapenems. The carbapenem-resistant genes of this MDR *Acinetobacter* spp. were subsequently investigated by multiplex polymerase chain reaction (PCR) assay, to detect blaOXA-23-like and blaOXA-40-like genes.

PCR amplification of blaOXA alleles:

For preparation of genomic DNAs, a fresh bacterial colony was suspended in100 µl of sterile distilled water and boiled for 10min followed by centrifugation for 1 min. 2 ml supernatant was used to amplify the genes encoding Carbapenemases a multiplex -PCR assay was run using the primers specific for the *blaOXA-23 like* (501 bp): 5′- GAT CGG ATT GGA GAA CCA GA -3′and 5′-ATT TCT GAC CGC ATT TCC AT-3′ and *blaOXA-40-like* (246 bp): 5´-GGTTAG TTG GCC CCC TTA AA and 5´-AGT TGACGC AAA AGG GGA TT)[8].

Amplification was performed in a final volume of 50 µl containing reaction buffer 1X 2 mM MgCl2, 2 mMdNTP, 500nM primers, 1.6 U Taq polymerase and 10 - 100 ng of DNA templates. The thermo-cycler was programmed at 94°C for 5 min followed by 30 cycles of 25 s at 94°C, 40 s at 53°C, 50s at 72°C, and a final cycle of 6 min at 72°C. The PCR products were separated by agarose gel electrophoresis.

Statistical analysis:

The data was coded and entered using the statistical package SPSS version 15. The data was summarized using number and percentage for qualitative values. Statistical differences between independent groups were tested using Chi Square test for the qualitative variables.

RESULTS

Acinetobacter species (40 /175) were isolated from different sites of infection, sputum and endotracheal tube aspirate ETA 17 (42.5%), pus 11 (26.5%), urine 6 (15%) blood 4 (10%) and cardiovascular pressure catheter (CVP) tips 2 (5%) collected from Ain Shams University Hospitals in the period from January to October 2016.

Risk factors associated with these infections caused by *Acinetobacter* species were analyzed; the most

common risk factor associated with *Acinetobacter* species infection was instrumentation (central venous catheters at various sites) followed by endotracheal intubation then mechanical ventilation. Out of the 40 patients infected with *Acinetobacter* species 9 were diabetic, 7 had chronic kidney diseases (CKD), 5 had chronic liver disease and 3 had malignancy (Table 1).

Table 1: Risk factors associated with infection by *Acinetobacter spp*.

Risk factor	*Number (%)*
Instrumentation	40 (100%)
Intubation	22 (55%)
Mechanical ventilation	17 (42.5%)
Diabetes	9 (22.5%)
CKD	7 (17.5%)
Chronic liver diseases	5 (12.5%)
Malignancy	3 (7.5%)

We found that, there was a significant difference regarding the duration of hospital stay and the infection with *Acinetobacter* spp. detected; 33 (82.5%) of patients had a prolonged duration of hospital stay (5 days or more). There is increase association between prolonged stay in ICU more than 5 days and infection with MDR *Acinetobacter* spp. and also a poor outcome.

As regards the resistance pattern 26 (65%) of *Acinetobacter* spp. isolates were resistant to ampicillin/sulbactam, 23 (57.5%) were resistant to imipenem, 20 (50%) were resistant to levofloxacin, 15 (37.5) were resistant to ceftazidime, 13 (32.5%) were resistant to cefipime and 10 (25%) were resistant to gentamycin. Thirteen (13/ 40, 32.5%) of Acinetobacter species isolates were MDR (Table 2).

Table 2: Pattern of antimicrobial susceptibility in *Acinetobacter spp*. isolates (N=40).

Antibiotics	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Ampicillin/sulbactam	9	22.5%	5	12.5%	26	65%
Imipinem	12	30%	4	10%	23	57.5%
Levofloxacin	12	30%	8	20%	20	50%
Ciftazidim	16	40%	9	22.5%	15	37.5%
Cifipim	15	37.5%	12	30%	13	32.5%
Gentamycin	19	47.5%	11	27.5%	10	25%

We found that, there was a significant difference regarding the duration of hospital stay and the infection with *Acinetobacter* spp. There was a significant association between longer duration of hospital stay with invasive procedures (mechanical ventilation, urinary catheter, and intra vascular devices) as 35 (87.5%) patients were with one or more of these devices, also with prior antibiotics intake.

As regards the detection of bla OXA genes in the MDR Acinetobacter isolates (13, 32.5%), these MDR *Acinetobacter* strains were subsequently investigated by multiplex-PCR assay. Four isolates (4/13, 30.8%) from MDR Acinetobacter species were positive for bla OXA-23 like genes while all isolates were negative for bla OXA-40 like genes (0/ 13. 0%) (Fig. 1).

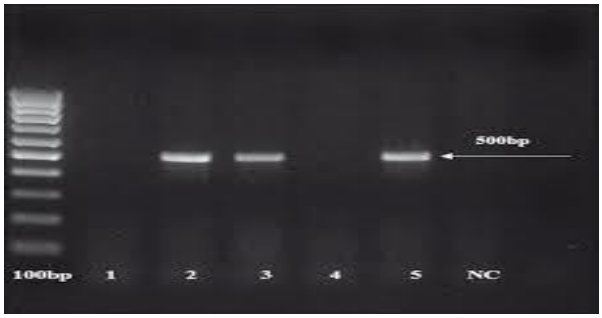

Fig. 1: ***Detection of bla OXA-23-like and bla OXA-40- like genes*** *by multiplex-PCR. 100 bp DNA ladder, lane 2, 3,5Acinetobacter spp. containing blaOXA23-like gene, while lane 1,4 Acinetobacter species lacking any OXA genes; NC Negative control.*

DISCUSSION

Acinetobacter spp. had emerged as one of the most troublesome pathogens due to its ability to acquire genetic determinants for antibiotic resistance. Carbapenem, aminoglycosides and quinolone antibiotics have been used efficiently in the treatment of infection with Acinetobacter spp., However, multidrug-resistant isolates resistant to these antibiotics have been increasingly reported [9].

Carbapenem resistance among *Acinetobacter* spp. can be mediated by two groups of β-lactamases such as: carbapenem- hydrolyzing oxacillinases and molecular class B metallo-β-lactamases. However, the most widespread β-lactamases are carbapenem- hydrolyzing oxacillinases belonging to molecular class D [10].

In the present study, sputum was the most common sample from which Acinetobacte spp. were isolated, sputum 17 (42.5%), pus 11 (26.5%), urine 6 (15%) blood 4 (10%) and CVP tips 2 (5%). These results go with that of Feizabadi et al, and Elhady et al.[1,11], who reported that the respiratory samples and wound swabs were the most common sites of isolation of *Acinetobacter spp.*

In our study, there was a significant association between risk factors as central venous catheters at various sites and endotracheal intubation then mechanical ventilation with MDR *Acinetobacter* spp. also there is a significant association between longer duration of hospital stay and infection with MDR

Acinetobacter spp., similarly the study of Ji et al. and Elhady et al.[11,12], who reported a significant difference regarding the duration of hospital stay and the infection with *Acinetobacter* spp, and there was also a significant association with invasive procedures.

As regards the resistance pattern of Acinetobacter spp. in this study, 65% of Acinetobacter spp. isolates were resistant to ampicillin/sulbactam, 57.5% were resistant to imipenem, half of isolates 50% were resistant to levofloxacin, 37.5 were resistant to ceftazidime, 32.5% were resistant to cefipime and 25% were resistant to gentamycin. 32.5% of Acinetobacter species isolates were MDR. These results are similar that of Yang et al.[4] who reported that, among the tested β- lactam antimicrobial agents, as high as 75% of *A.baumannii* isolates were resistant to ampicillin/sulbactam. Approximately half of the isolates were resistant to imipenem (55%) and meropenem (49%). Less than half of the *A. baumannii* isolates were resistant to other β-lactam antimicrobial agents, including cefepime (40%), ceftazidime (27%) and cefoperazone (17%). Also in Egypt Elhady et al.[11], who reported that as high as 74% of *Acinetobacter* spp. isolates were resistant to ampicillin/sulbactam and 62% to levofloxacin. Approximately half of the isolates were resistant to imipenem (56%) and meropenem (48%). *Acinetobacter* spp. was resistant to other β-lactam antimicrobial agents, including cefepime (39%), ceftazidime (28%), and cefoperazone (13%). Out of fifty *Acinetobacter* spp. isolates 38% were resistant to gentamycin.

Comparing our result of this study with other studies Pannika et al.[13], found that all isolates with the exception of one were resistant to extended-spectrum Cephalosporins and imipenem and meropenem. Yoon et al.[14], reported that *A. baumannii* isolates showed resistance or intermediate susceptibility to ampicillin/ sulbactam, ceftazidime, cefotaxime, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin. However, Kock et al.[15], demonstrated that, the overall percentage of resistance to the tested antibiotics was amikacin (5%), cefepime (62%), ceftazidime (45%), ciprofloxacin (65%), colistin (0%), gentamicin (58%), imipenem (59%), meropenem(63%) and piperacillin-tazobactam (60%).

In our study there was a statistically significant association between both the duration of hospital stay and the use of invasive procedures as urinary catheter, intravascular devices or mechanical ventilator and the infection with Acinetobacter, 35 (87.5%) patients were with one or more of these devices. Similarly Ji et al.[12], reported a significant association between longer duration of hospital stay with invasive procedures. Afaf et al. [16], reported that, there was no statistically significant difference appeared regarding the application of urinary catheter, application of intra vascular devices, and mechanical ventilation. Also Anke et al.[17], stated that, mechanical ventilation, urinary catheter use, prior antibiotic therapy and surgery don't have any significance in acquiring infections. These differences in the results could be explained by the difference in hospital environment of this study and the other studies, also difference in patient's risk factors predisposing to infection and difference in number of samples.

In the present study among the all MDR *Acinetobacter* spp. isolates, blaOXA-40- like gene was not detected among any isolates, while blaOXA-23-like gene has been detected in 4 isolates (30.8 %). Irfan et al.[18], reported that blaOXA-40- like and blaOxA-58-like were absent in all isolates (0/50), and Tahiry et al.[19], found that all 53 (Carbapenem resistant Acinetobacter spp.) isolates showed the presence of blaOXA-23 and blaOXA-51 but none had blaOXA-24- like, blaOXA-58.Also Elhady et al.[11], found that all carbapenem resistant *Acinetobacter* spp. isolates, blaOXA-40- like gene was not detected among any isolates, while blaOXA-58-like gene has been detected in 3 isolates. One of the limitations of this study was the small number of samples. So, more researches are necessary to monitor the spread of antibiotic-resistance genes that are associated with *Acinetobacter* spp. in clinical settings.

CONCLUSIONS

The prevalence of Carbapenem-resistant *Acinetobacter* spp. was high in ASUHs so continuous studies of their resistance mechanisms in the hospital are important, and an intervention is urgently needed to prevent further dissemination of these antibiotic resistance genes. The distribution of blaOXA-23-like and blaOXA-40-like among Carbapenem-resistant *Acinetobacter* species was low and there was no association between Carbapenem-resistant isolates and distribution of these genes. This confirms that *Acinetobacter* has different mechanisms for MDR other than the blaOXA carriage.

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