**Antimicrobial Susceptibility Pattern and *Distribution of blaOXA-58-like and blaOXA-40-like genes among* Carbapenem resistant *Acinetobacter spp. Isolates* inAin Shams University Hospitals**

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**Abstract**: Carbapenem-resistant *Acinetobacter*spp.has emerged globally. Theobjective of this study was to analyze the prevalence of antibiotics resistance and the distribution of blaOXA-58-like and blaOXA-40-like genes in Carbapenem resistant *Acinetobacter* spp. isolates. *Subjects and methods:*A total of 50 independent clinical *Acinetobacter* spp. isolates were collected from Central laboratories of Ain Shams University Hospitals (ASUHs) during the period from February to October 2014 to determine the distribution of blaOXA-58-like and blaOXA-40-like genes in Carbapenem-resistant *Acinetobacter* spp.All isolates were cultured, subjected to biochemical testing, and antimicrobial susceptibility testing. The distribution of blaOXA-58-like and blaOXA-40-like genes were investigated in the Carbapenem-resistant *Acinetobacter* spp. isolates by multiplex polymerase chain reaction (PCR) techniques.*Results:* Resistance pattern of clinical isolates were 74% to ampicillin /sulbactam, 62% to levofloxacin, 56% to imipenem, 48% to meropenem, 39% to cefepime, 38% to gentamicin, 28% to ceftazidime, and 13% cefoperazone. Overall, 38% (19/50) of the isolates were characterized as Carbapenem-resistant. The study of distribution of carbapenemase blaOXA-58-like and blaOXA-40-like genes in the Carbapenem-resistant *Acinetobacter* spp. isolates, revealed that all Carbapenem-resistant *Acinetobacter* spp. (n=19) tested were negative for blaOXA-40-like, on the contrary, alleles encoding OXA-58-like enzymes (blaOXA58-like) were detected in three isolates (3/19). It could be concluded thatthe prevalence of Carbapenem-resistant *Acinetobacter* spp. was high in Ain ShamsUniversity hospital. And the distribution ofblaOXA-58-like and blaOXA-40-like among Carbapenem-resistant*Acinetobacter* species was low and there was no association between antibiotic resistance and the presence of these genes inCarbapenem-resistant*Acinetobacter* spp.

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**Keywords:** *Acinetobacter,*Carbapenem-resistant*, Antimicrobial susceptibility testing, multiplex PCR, bla OXA genes.*

1. **Introduction**

*Acinetobacter* spp. is a group of non fermentative, non motile, oxidase negative Gram-negative bacilli. The emergence and rapid spread of the important nosocomial drug-resistant *Acinetobacter* spp., in particular, *A. baumannii*, are of great concern worldwide [1].

*Acinetobacter* spp. is an important cause of nosocomial infections, such as pneumonia, urinary tract infections, wound infections and septicemia particularly in intensive care settings affecting mainly the severely immuno-compromised, and is typically selected by prior antimicrobial therapy [2].

*A. baumannii* is considered a serious pathogen being characterized by multidrug resistance (MDR); long-term survival on inanimate surfaces such as computer keyboards, pillows, curtains and other dry surfaces; and propensity for epidemic spread [3]. This longevity is thought to contribute to the clonal spread of isolates, facility of person-to-person transmission and environmental contamination. For the control of a hospital outbreak, strict adherence to infection control measures and sometimes even the closure of wards are required [4].

Hospital strains of *Acinetobacter* spp. are usually multidrug resistant. The problem is complicated by increasing rates of resistance to broad-spectrum antibiotics including carbapenems. Carbapenems have been the drug of choice for the treatment of *Acinetobacter* spp., however the number of isolates showing resistance to these antibiotics has increased [5].

Several mechanisms are responsible for conferring the resistance to β-lactam 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 [1].

However resistance to these antibiotics has emerged due to the production of carbapenem-hydrolyzing β-lactamases among these pathogens. Two classes of molecular carbapenemases classes B, D have been identified [5], but those belonging to molecular class D OXA enzymes have emerged globally as the main mechanism responsible for carbapenems resistance [6].

Four families of OXA carbapenemases (OXA-23-like, OXA-40-like, OXA-51-like, and OXA-58-like) are limited to isolates of *Acinetobacter* spp. The rapid detection of strains that produce these beta-lactamases in clinical bacteriology laboratories allows appropriate therapy to be implemented promptly in order to reduce patient morbidity and mortality [7].

Due to the global spread of *Acinetobacter* spp. and its importance as one of the most common nosocomial infection nowadays, in this study, we reported an analysis of the antibiotics susceptibility profile in *Acinetobacter* spp. Isolates. Additionally, the distribution of genes encoding blaOXA-58-like and blaOXA-40-like genes as a source of Carbapenem resistance in MDR *Acinetobacter* spp. isolated from patients in ASUHs.

**2. Material and methods:**

Thisstudy was conducted on A total of 50 independent *Acinetobacter* spp. clinical isolates were isolated from different sites of suspected nosocomial infection including sputum 27(54%), pus 11 (22%), urine 7(14%), blood 3(6%) and CVP tips 2(4%) collected from Central laboratories of ASUHs from February to October 2014.We collect data about the duration of hospital stay, using invasive procedures (mechanical ventilation, urinary catheter, and intra vascular devices), and prior antibiotics intake.

**Bacterial identification:**

All isolates were first cultured on appropriate agar plates to check for purity and identificationand then incubated aerobically at 37°C for 24 hrs. Biochemical identification of the isolated organisms based on colonial morphology, microscopic examination of Gram stained films and biological activity of the isolated organisms according to *Collee, et al* [8].

**Antimicrobial susceptibility testing:**

Antimicrobial susceptibility testing of the obtained isolates was performed using the standard Kirby-Bauer disc diffusion method for antibiotics, and the results were interpreted according to the CLSI guidelines (2013) [9].Antibiotics susceptibility of the isolates towards β-lactam antibiotics (meropenem, imipenem, ampicillin/sulbactam, ceftazidime, cefepime, and cefoperazone), gentamicin, and levofloxacin. (Becton Dickinson Microbiology Systems) was performed on Mueller- Hinton agar (bioM´erieux, France), using overnight cultures at a 0.5 McFarland standard followed by incubation at 35°C for 16 to 18 h.

Multidrug resistance was defined as resistance to three or more representatives of the following classes of antibiotics: quinolones (levofloxacin), extended-spectrum cephalosporins (ceftazidime and cefepime), β-lactam/β-lactamase inhibitor combination (ampicillin/sulbactam), aminoglycosides (gentamicin), and carbapenems (imipenem and meropenem). Among all of the clinical isolates, 19 out of 50 (37%) were identified as MDR *Acinetobacter* strains which were all resistant to carbapenems.

The carbapenem-resistant alleles of this MDR *Acinetobacter* spp. were subsequently investigated by multiplex polymerase chain reaction (PCR) assay, to detect blaOXA-58-like and blaOXA-40-like genes.

**PCR amplification *ofblaOXA alleles:***

DNA was extracted from the strains by boiling one to three colonies in 100 ul of sterile water for 10 min followed by centrifugation for 1 min. 14,000rpm (10). To amplify the genes encoding Carbapenemases a multiplex -PCR assay was run using the primers specific for the *blaOXA-40-lik*e (246 bp: 5´-GGT TAG TTG GCC CCC TTA AA and 5´-AGT TGA CGC AAA AGG GGA TT), and OXA-58-like (599 bp: 5´-AAGTAT TGG GGC TTG TGC TG and 5´-CCC CTCTGCGCTCTACATAC)[10]. Amplification was performed in a final volume of 50 µl containing reaction buffer 1X 2 mM MgCl2, 2 mMdNTP, 500 nM primers, 1.6 U Taq polymerase (Metabion, Martinsried, Germany), and 10 - 100 ng of DNA templates. The thermo-cycler (Eppendorf, Hamburg, Germany) 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. DNA from a clinical isolate of P. aeruginosa was used as a negative control in the amplification study as previously described [10,11].

###### Statistical Methods:

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

**3. Results:**

Biochemical and conventional methods enabled the identification of 50 *Acinetobacter* spp. isolates were collected from different sites of infection: including sputum 27(54%), pus 11 (22%), urine 7(14%), blood 3(6%) and CVP tip 2(4%) collected from Central laboratories of ASUHs during the period from February to October 2014.

As regards to the resistance pattern of *Acinetobacter* spp. isolates, among the tested β-lactam antimicrobial agents, as high as 74% of *Acinetobacter* spp. isolates was resistant to ampicillin/sulbactam and 62% to levofloxacin. Approximately half of the isolates were resistant to imipenem (56%) and meropenem (48%). Less than half of the *Acinetobacter* spp. were 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. Overall, 38% (19/50) of the isolates were characterized as MDR strains Table 1.

***Table 1:*** *Pattern of antimicrobial susceptibility in Acinetobacter spp. isolates (N = 50).*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Sensitivity*** | ***Sensitive*** | | ***Intermediate*** | | ***Resistant*** | |
| ***Name of antibiotic disc*** | ***No. of isolates*** | ***%*** | ***No. of isolates*** | ***%*** | ***No. of isolates*** | ***%*** |
| *ampicillin/sulbactam* | *4* | *8%* | *9* | *18%* | *37* | *74%* |
| *Levofloxacin* | *12* | *24%* | *7* | *14%* | *31* | *62%* |
| *Imipenem* | *15* | *30%* | *7* | *14%* | *28* | *56%* |
| *Meropenem* | *17* | *34%* | *9* | *18%* | *24* | *48%* |
| *Cefepime* | *19* | *38%* | *11* | *23%* | *20* | *39%* |
| *Gentamycin* | *19* | *38%* | *12* | *24%* | *19* | *38%* |
| *Ceftazidime* | *26* | *52%* | *10* | *20%* | *14* | *28%* |
| *Cefoperazone* | *31* | *62%* | *13* | *25%* | *6* | *13%* |

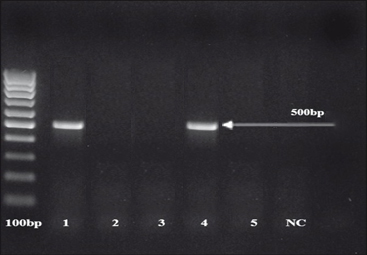
We found that, there was a significant difference regarding the duration of hospital stay and the infection with *Acinetobacter*spp detected (table 2).

There wasasignificant association between longer duration of hospital stay with invasive procedures (*mechanical ventilation, urinary catheter, and* intra vascular devices*) as all infected patients were with one* or more of these devices, also with prior antibiotics, environmental contamination, understaffing and poor adherence of staff to hand hygiene as observed.

Among the carbapenem-resistant *Acinetobacter* spp. isolates (n=19), carbapenem-resistant alleles of these MDR *Acinetobacter* strains were subsequently investigated by multiplex-PCR assay. All MDR *Acinetobacter* spp. (n=19) isolated, tested were negative for blaOXA-40-like, on the contrary, alleles encoding OXA-58-like enzymes (blaOXA58-like) were detected in three isolates (3/19) (15.8%). (Figure1).

***Table 2:*** relationship between the duration of hospital stay and the infection with *Acinetobacter*spp (N = 50).

|  |  |  |
| --- | --- | --- |
| **Risk factors** | ***Acinetobacter* spp.** | ***P Value*** |
| **Duration of Hospital stay(days)**  **Mean ± SD** | 17.340±12.857 | 0.02459\* |



***Figure 1;* Detection of genes encoding OXA carbapenemases by multiplex –PCR.**M, 100 bp DNA ladder; Lane 2, 3 ,5*Acinetobacter* spp. Lacking any OXA genes; 1,4*Acinetobacter* spp. Containing *blaOXA58-like* gene; NC Negative control (Pseudomonas aeruginosaDNA, field strain).

**4. Discussion:**

The rapid emergence and global dissemination of *Acinetobacter* spp. as a major nosocomial pathogen is remarkable and demonstrates its successful adaptation to the 21st century hospital environment. Invariably, one of the most alarming characteristics of this gram-negative pathogen is its ability to develop resistance to all available antibiotics including carbapenems which are drugs of choice in the treatment of severe infections [12].

Carbapenem resistance among *Acinetobacter* spp. can be mediated by two groups of β-lactamases such as: carbapenem- hydrolyzing oxacillinases as well as molecular class B metallo-β-lactamases. However, the most widespread β-lactamases are carbapenem- hydrolyzing oxacillinases belonging to molecular class D (CHDLs) [13].

In the present study, sputum was the most common sample from which *Acinetobacter* spp. were isolated 27(54%) followed by pus 11 (22%), urine 7(14%), blood 3(6%) and CVP tip 2(4%). Similarly Anke et al. [14], Feizabadi et al., [15] and Afaf et al., [16] reported that the respiratory samples and wound swabs were the most common sites of isolation of *Acinetobacter* spp.

As regards to the resistance pattern of *Acinetobacter* spp. isolates, among the tested β-lactam antimicrobial agents, we found 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%). Less than half of the *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. Overall, 38% (19/50) of the isolates were characterized as MDR strains. Similarly, Yang et al., [1] 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%).

Comparing to another study in Egypt by Afaf et al., [16] who detected that the resistance pattern of isolates 100% were resistant to ceftazidime, 83% to levofloxacin, 80% to amikacin, 67% to ampicillin/sulbactam, 57% meropenem while only 23% to colistin. On the other hand Pannika et al., [17] found that all isolates with the exception of one were resistant to extended-spectrum Cephalosporins and imipenem and meropenem. Savov et al., [18] reported that more than 90% of *A. baumannii* strains were resistant to ciprofloxacin and amikacin and that 75% were resistant to meropenem. Yoon et al., [19] reported that *A. baumannii* isolates showed resistance or intermediate susceptibility to ampicillin/sulbactam (SAM), ceftazidime, cefotaxime, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin.

However, Kocket al., [20] 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%). These differences in the results could be explained by the difference in working environments and number of isolates examined. Further population-based prevalence studies are required to observe the true resistance pattern of isolates.

In our study, there was a significant difference regarding the duration of hospital stay and the infection with *Acinetobacter*spp detected, and there was a significant association between longer duration of hospital stay with invasive procedures (mechanical ventilation, urinary catheter, and intra vascular devices), also with prior antibiotics, environmental contamination, understaffing and poor adherence of staff to hand hygiene. Similarly Ji et al., [21] reported a significant association between longer duration of hospital stay with invasive procedures, prior antibiotics, environmental contamination, and poor hand hygiene.

Afaf et al., [16] reported that, there was a statistically significant difference regarding the duration of hospital stay and the infection with *Acinetobacter*, but no statistically significant difference appeared regarding the application of urinary catheter, application of intra vascular devices, and mechanical ventilation. On the other hand, Jang et al., [22] has done a multivariate analysis which identified mechanical ventilation, prior infection, antimicrobial therapy, prior colonization, and colonization pressure as independent risk factors for bacteraemia. While Anke et al., [14] 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 (isolates).

In the present study among the 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 (15.8%). Similarly Bamford et al., [23] reported that the prevalence of the OXA- 58 gene in clinical isolates of *A. baumannii* was found to be 3% (3/97) and no OXA-24 genes were detected in any of the clinical isolates of *A.baumannii*.

Another studies conducted by Feizabadi et al.,[15] where 15% of clinical isolates of *A. baumannii* tested positive for OXA-58 genes, and Mendes et al., [24] who found that, 12% of *A. baumannii* isolates were found to be OXA-58 positive. However, Kocket al., [20] showed that 3% (3/97) of the *A. baumannii* isolates were positive for OXA-58, and none of the isolates was positive for OXA-24.

WhileIrfan et al., [25] reported that blaOXA-40-like and blaOxA-58-like were absent in all isolates (0/50), and Tahiry et al., [26] 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 Yang et al., [1] found that, alleles encoding blaOXA58-like were not detected in their study.

One of the limitations of this study was the small number of samples (isolates). So continuous research and surveillance is necessary to monitor the prevalence and spread of antibiotic-resistance genes that are associated with *Acinetobacter* spp*.* in clinical settings. Future research should include confirmation of the distribution of the OXA-40 and OXA-58 like genes, and determination of its relatedness with Carbapenem-resistant *Acinetobacter* spp.

**5. Conclusions**

The prevalence of Carbapenem-resistant *Acinetobacter* spp. was high in ASUHs so continuous surveillance and elucidation of their resistance mechanisms in the hospital are important, and an intervention policy is urgently needed to prevent further dissemination of these antibiotic resistance genes. The distribution of blaOXA-58-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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