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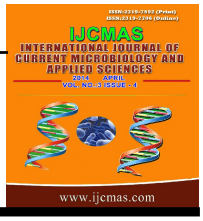


**Original Research Article**

# Emergence of Carbapenem-Resistant *Acinetobacter baumannii* in the Intensive Care Unit in Sohag University Hospital, Egypt

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**Keywords**

*Carbapenem resistant, Acinetobacter baumannii, ICU, Nosocomial infection, MBLs.*

**A B S T R A C T**

Carbapenem resistant *Acinetobacter baumannii* (*A. baumannii*) has emerged as a serious challenge causing nosocomial infection worldwide. We collected 24 *Acinetobacter* isolates from various clinical samples from patients in the intensive care unit, Sohag University Hospital during the period from August 2013 to February 2014. Identification of the *Acinetobacter* species was done by using API 20 E strips as a biochemical identification system. PCR was done to detect *blaOXA-51* like gene which is unique to *A. baumannii*. The isolates were tested for antibiotic sensitivity by the disc diffusion method. Imipenem resistant isolates were further tested for metallo-β-lactamase (MBLs) production by the imipenem/EDTA combined disk test (CDT). PCR was done for the detection of VIM-1 and IMP-1 genes coding for MBLs production. Using API 20E strips; 21 (87.5%) isolates were identified as *A. baumanii* and 3 (12.5%) isolates were identified as *A.heamolyticus. A. baumannii blaOXA-51* like gene was detected in all the 21(87.5%) isolates. Of them; 15 (71.4 %) isolates were imipenem resistant. MBLs were produced by 13 (86.7%) isolates of the 15 imipenem resistant *A. baumannii* isolates. Both MBLs genes VIM-1 and IMP-1 were completely absent in all isolates. This is the first investigation in our Hospital that has identified carbapenem-resistant *A. baumannii* in our clinical samples. Colistin was very effective against such isolates. Laboratories should routinely screen for MBLs production among *A. baumannii* isolates as their prompt detection is necessary to prevent further dissemination.

## Introduction

At present, one of the most serious issues in medicine is increasing resistance of bacterial pathogens to antimicrobial agents. This fact is associated with higher mortality and morbidity rates, prolonged hospital stays and increased treatment-related costs *(Senkyrikova et al., 2013).* Such negative trends have been observed in *Acinetobacter baumannii* which is responsible for a variety of nosocomial infections, including bacteraemia, urinary tract infections, diabetic ulcers, pneumonia, especially in mechanically ventilated patients and intravenous devices infections; with mortality rates range from 19% to 54 % (*Eveillard et al., 2010*).

*Acinetobacter baumannii* was sensitive to most antibiotics in the 1970s, but now it is resistant to virtually all antibacterial drugs *(Howard et al., 2012).* Multidrug resistant (MDR) *Acinetobacter spp*. is defined as the isolate resistant to at least three classes of antimicrobial agents; all penicillins and cephalosporins (including inhibitor combinations), fluroquinolones, and aminoglycosides. Extreme-drug-resistant (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. Finally, pandrug-resistant (PDR) *Acinetobacter spp.* are the XDR *Acinetobacter spp.* that are resistant to polymyxins and tigecycline (*Manchanda et al., 2010).* In multiresistant strains of *Acinetobacter baumannii*, the drugs of choice are carbapenems. Unfortunately, the development of resistance did not spare even this group of antimicrobial drugs, with the main mechanism being production of carbapenemases; enzymes belonging to Ambler classes B, A and D *(Ambler, 1980; Bush and Jacoby, 2010)*. MBLs-positive *A. baumannii* strains are a serious therapeutic problem due to co-resistance to numerous antibiotic groups and difficulties in treating the infections caused by them (*Szejbach et al., 2013).* There is a huge risk of these "superbugs" extending into the community and threatening public health. *Acinetobacter baumannii* is responsible for approximately 2–10% of all Gram-negative infections in intensive care units (ICUs) and significantly increased mortality of infected patients *(Tan et al., 2013).* Emergence of metallo –β-lactamases (MBL) producing multidrug resistant (MDR) *A.baumannii* is a matter of concern in an intensive care unit (ICU). MBL genes from such organisms can spread rapidly to other gram negative bacilli, making them resistant to other antibiotics *(Bose et al., 2013).* The aims of this study were to isolate *A.baumannii* from various clinical samples from patients in the ICU and to find out the in vitro antimicrobial activity against these isolates. Imipenem resistant *A.baumannii* isolates were further tested for MBLs production and PCR was done for the detection of VIM-1 and IMP-1 genes coding for carbapenemase production among these isolates.

## Materials and Methods

This study was carried out in the Department of Medical Microbiology & Immunology, Faculty of Medicine, Sohag University during the period from August 2013 to February 2014. Clinical samples were collected from ICU patients in Sohag University Hospital. Specimens, such as, blood, endotracheal aspiration, urine, pus, and wound swabs were sent to the microbiology laboratory and were processed as per conventional methods. Blood was collected in blood culture bottles containing Brain heart infusion broth. Subcultures were done on blood agar and MacConkey's agar and incubated aerobically at 37□ C for 24 hours. Isolates were identified as *Acinetobacter* by conventional bacteriological tests; as colony morphology, Gram staining, Oxidase and catalase tests. Differentiation of *Acinetobacter* species by using API 20 E strips *(BioMerieux, France)* as a biochemical identification system. Patients were eligible for participation in the study if they had signs and symptoms of nosocomial surgical site infection, nosocomial urinary tract infection, infected tracheostomy, or bacteraemia.
At presentation:

Full history was obtained from all patients including:

- Duration of hospitalization.
- Presence of associated risk factors as; diabetes mellitus, previous antibiotic therapy shortly before the occurrence of infection.
- Presence of external associated medical devices as; urinary catheters, mechanical ventilation or peripheral I.V. catheters.

In cases of hospital acquired surgical site infection or bed sores: Sterile cotton swabs were used for sampling of pus and exudates from infected wounds.

In cases of catheter-associated urinary tract infection: the catheter collection port was disinfected and 5-10 ml urine was aspirated. Diagnosis of UTI was defined as the presence of > 100.000 CFU / 1 ml fresh uncentrifuged urine (presence of > 100 colonies by using 0.001ml caliberated nichrome loop).

**Microbiological analysis**

All samples were subjected in parallel to the following methods of detection:-
Samples were inoculated on MacConkey's agar medium *(Oxoid Limited, Basingstoke, England)* for primary isolation of *Acinetobacter* isolates.

**Microscopic examination**

Gram staining examination of *Acinetobacter* colonies showing gram negative coccobacilli arranged singly, in pairs or in short chains.

**Biochemical reactions**

Colonies were identified as *Acinetobacter* by being catalase positive, and oxidase-negative**.**

**Differentiation of *Acinetobacter* species:**
by using API 20E strips *(BioMerieux, France)* as a biochemical identification system (Figure 1).

**Molecular detection of blaOXA-51-like gene:**

All isolates were subjected to PCR to detect *bla*OXA-51-like gene which is unique to *A. baumannii* species *(Karmostaj et al., 2013).* DNA was extracted from the isolates by the use of QIAamp DNA Kits according to the manufacturer's instructions. PCR assay was run using the primer *bla*OXA-51-like (OXA-51-F 5'-TAA TGC TTT GAT CGG CCT TG-3' and OXA-51-R 5'-TGG ATT GCA CTT CAT CTT GG-3'). PCR were carried out in 25 µl reaction volumes containing 12.5 µl PCR master mix, 4.5 µl PCR grade water, 2 µl of each primer and 4 µl of the extracted DNA sample was added. In each set of experiments, a negative control was included. The negative control was prepared by replacing the DNA template with PCR grade water. Amplification of the sample according to the following directions (Amplification cycle profile), by using a Biometra thermal cycler *(T Gradient software PCR system version 4 - Biometra Whatman company, Goettingen, Germany*). The PCR amplification cycling 95°C for 4 min, and then 34 cycles at 95°C for 45 s, 52°C for 45 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min *(Karmostaj et al., 2013).*

**Antimicrobial Susceptibility Testing**

The susceptibility testing of *Acinetobacter baumannii* isolates to different antibiotics was carried out by the disc diffusion method (Modified Kirby- Bauer method) according to the Clinical Laboratory Standards Institute (CLSI) guidelines *(CLSI, 2011).* The antibiotics included

were grouped as follows: β-lactams (ceftazidime (30μg), and aztreonam (30μg)); carbapenems (imipenem (10μg), meropenem (10μg)); beta-lactam/inhibitor combination (ampicillin–sulbactam (20/10 μg)); quinolones (ciprofloxacin (5μg)); aminoglycosides (amikacin (30μg), gentamicin (10 μg)); polymixin (colistin (10μg)), and Extended-spectrum β-lactams (Piperacillin (100 μg)). *(Oxoid Ltd., Basingstoke UK*).

**Phenotypic Detection of MBL - Producing Isolates**

The isolates were confirmed as MBLs producers by imipenem/EDTA combined disk test (CDT). To make 0.5 M Ethylenediamine tetra acetic acid (EDTA) solution was prepared by dissolving18.6 g of disodium EDTA.$2H_2O$ was dissolved in 100 mL of distilled water, and the pH was adjusted to 8 by sodium hydroxide (NaOH). Then the mixture was sterilized in the autoclave to prepare a sterile EDTA solution.

To prepare EDTA-containing imipenem disks (930 μg EDTA); 5 μL of EDTA solution was added to imipenem disks (10 μg imipenem). These disks were dried immediately in a 37°C incubator and stored at 4°C in airtight vials until use. For each isolate, 1 imipenem disk and 1 EDTA-containing imipenem disk were placed on a suitable distance on the surface of Mueller-Hinton agar plates inoculated with a bacterial suspension equivalent to 0.5 McFarland Standard. After 24 hours of incubation at 37°C, an increase of ≥7 mm in the zone diameter of EDTA-containing imipenem disk compared to imipenem disk was considered to be a positive test for the MBLs producer imipenem resistant strains *(Saderi et al., 2010)* Figure (2a&b).

**Molecular detection of MBLs genes (*bla IMP-1* and *bla VIM-1* genes) by PCR**

MBLs genes were detected by Polymerase Chain Reaction (PCR) for detection of *bla IMP-1* and *bla VIM-1 genes* coding for Imipenem resistance in our *Acinetobacter baumannii* isolates.

**Sample treatment & DNA extraction**

(according to the manufacturer's instructions). Bacterial DNA was extracted by the use of QIAamp DNA Kits (QIAGEN GmbH, Lot No 127155347).

**Primers**

Oligonucleotide primer sequences used *(synthesized by metabion international AG, Germany)* were as follows: The 2 oligonucleotide primers $A_1$ and $A_2$ resulting in the amplification of a 587-bp PCR fragments for detection of *bla IMP-1* gene; Primer $A_1$ *(blaIMP-1-F1)*: 5′-ACC GCA GCA GAG TCT TTG CC -3′. Primer $A_2$ *(blaIMP-1-R1)*: 5′- ACA ACC AGT TTT GCC TTACC -3′ (*Jeon et al., 2005*). The 2 oligonucleotide primers $B_1$ and $B_2$ resulting in the amplification of an 801 -bp PCR fragments for detection of *blaVIM-1* gene; Primer $B_1$ *(blaVIM-1-F1)*: 5′- ATGTTAAAAGTTATTAGTAGT -3′. Primer $B_2$ *(blaVIM-1-R1)*: 5′- CTACTCGGCGACTGAGCGAT -3′ for amplification of *bla VIM*-1 gene (*Fiett et al., 2006*).

**PCR**

In a sterile thermal cycler 0.5ml tube, 25μl PCR reaction mix containing 12.5μl PCR master mix, 4.5 μl PCR grade water, 2 μl of each primer and 4 μl of the extracted DNA sample was added. In each set of experiments, a negative control was

included. The negative control was prepared by replacing the DNA template with PCR grade water. Amplification of the sample according to the following directions (Amplification cycle profile), by using a Biometra thermal cycler *(T Gradient software PCR system version 4 - Biometra Whatman company, Goettingen, Germany*). The PCR amplification cycling profile of *bla IMP-1* gene was 5 min of denaturation at 95°C (1 cycle), followed by 35 cycles of amplification; each of heat denaturation at 95 °C for 60 s, primer annealing at 55 °C for 60 s, and DNA extension at 72 °C for 90 s then one cycle for final extension at 72°C for 5 minutes. The PCR amplification cycling profile of *blaVIM-1* gene was 5 min of denaturation at 95°C (1 cycle), followed by 35 cycles of amplification; each of heat denaturation at 95 °C for 60 s, primer annealing at 55 °C for 60 s, and DNA extension at 72 °C for 60 s then one cycle for final extension at 72°C for 5 minutes *(Azimi et al., 2013)*. The amplified DNA was electrophoresed using 1.5% agarose gel electrophoresis *(Electrophoresis power supply-Biometra Whatman company, Goettingen, Germany)*, stained with ethidium bromide, and visualized under UV transillumination and photographed.

### Statistical Analysis

Qualitative (categorical) data were represented by the number and percent (%).The Chi-square test (X2 value) was used to assess the association between categorical data. *P values* < 0.05 were considered significant. *P values* < 0.01 and *P values* < 0.001 were considered highly significant.

## Results and Discussion

A total of 24 *Acinetobacter* isolates were collected from patients in the ICU of Sohag University Hospital during the period from August 2013 to February 2014 as detected by conventional bacteriological tests.

### Species distribution of *Acinetobacter* isolates

Using API 20E strips *(BioMerieux, France)* as a biochemical identification system for identification of the species of the isolated *Acinetobacter*; 3 (12.5% ) isolates were *Acinetobacter heamolyticus* and 21(87.5%) isolates were *Acinetobacter baumanii* which were also be confirmed by using PCR to detect *bla*OXA-51-like gene which is unique to *A. baumannii* species. *Acinetobacter baumannii blaOXA-51* like gene was detected in the 21(87.5%) isolates which were detected by the API 20E strips as *Acinetobacter baumannii*. (Figure 3)

### Sources of the *A. baumannii* isolates

The isolates were obtained from different clinical samples including; 7(33.3%) Endotracheal secretion, 6 (28.6%) urine, 4 (19%) blood, and 4 (19%) pus (Table.1).

### Susceptibility to antibiotics using disc diffusion method:

Using disc diffusion method to test the susceptibility of *A. baumannii* isolates to different antibiotics, according to the Clinical Laboratory Standards Institute's (CLSI) guidelines *(CLSI, 2011)* we found that; the highest sensitivity of *Acinetobacter baumanii* was for Colistin (100% of isolates). Followed by Ciprofloxacin (42.9% of isolates); then Aztereonam (38% of isolates). The lowest sensitivity of *A. baumannii* was for Ampicillin/Sulbactam (14.3%), followed by Imipenem, Meropenem, Piperacillin and Gentamycin (28.6% of isolates); then

Ceftazidime and Amikacin (33.3% of isolates). 13 (61.9%) *A. baumannii* isolates were MDR with resistance to Ampicillin/Sulbactam, Piperacillin, Gentamicin and Amikacin, Ceftazidim, and Aztreonam, and 12 (57.1%) isolates were XDR with resistance to Imipenem and Meropenem in addition. (Table 2)

**Phenotypic Detection of MBL-Producing *Acinetobacter baumanii* Isolates:**

High level of Imipinem resistance was detected by disc diffusion method in 15 (71.4%) of 21 isolates of *A. baumannii*. Of the 15 Imipenem resistant *A. baumannii*; 13 isolates (86.7%) were metallo β lactamase producers (increase of ≥7 mm in zone diameter of EDTA-containing imipenem disk compared to imipenem disk).Two isolates (13.3%) were MBL negative.

**Types of nosocomial infections caused by Imipenem resistant *Acinetobacter baumanii*:**

There were 21 cases of health care-associated infections caused by *A. baumannii*, of these cases there were15 cases caused by Imipenem resistant *A. baumannii*; 4 (26.7%) cases of infected tracheostomy, 4 (26.7%) cases of nosocomial urinary tract infection (UTI), 3 (20%) cases of septicaemia, 3 (20%) surgical site infection and 1(6.7%) case of infected bed sores in ICU patients. (Table 3)

**Risk factors associated with Imipenem resistant *Acinetobacter baumanii* infection**

In our study, we found that imipenem resistant *A. baumannii* infections were more among patients with history of prior exposure to broad spectrum antibiotics shortly before the occurrence of imipenem resistant *A. baumannii* infection with a highly statistically significant difference compared to patients with Imipenem sensitive *A. baumannii* infections *(P = 0.004)*. We found also; patients who have external devices were more liable to develop imipenem resistant *A. baumannii* infections with a statistically significant effect as a risk factor *(P = 0.03)*. There was also a highly statistically significant association between prolonged hospital stay for 10 days or more and infection with imipenem resistant *A. baumannii (P = 0.002)*.Imipenem resistant *A. baumannii* infections were more among patients aged over 35 years; however the difference was not statistically significant when compared with the distribution among Imipenem sensitive *A. baumannii* infected patients *(P = 0. 2)*. (Table 4)

**Genotyping of Imipenem resistant *Acinetobacter baumanii* isolates using PCR**

Among the 15 IPM-resistant *A. baumannii* isolates detected, PCR screening for the presence of *bla IMP-1* and *bla VIM-1 genes* coding for MBLs production; revealed that none of the genes detected in any of the studied isolates.

Carbapenem-resistant *A. baumannii* has increasingly become a serious therapeutic problem worldwide *(Gordon and Wareham, 2010)*. In the current study a total of 24 *Acinetobacter* isolates were collected from patients in the ICU of Sohag University Hospital during the period from August 2013 to February 2014. In our study identification of the *Acinetobacter* species was done by using API 20 E strips as a biochemical

identification system. Since the *bla*oxa-51 – like gene is consistently found and is also unique to *A. baumannii* species, its detection could provide a simple method of identifying *A. baumannii* which would be more reliable than biochemical identification *(Karmostaj et al.,2013)*. So; all isolates were subjected to PCR to detect *blaOXA-51* like gene. Using API 20E strips; 21 (87.5%) isolates were identified as *Acinetobacter baumanii* and 3 (12.5%) isolates were identified as *Acinetobacter heamolyticus. Acinetobacter baumannii blaOXA-51* like gene was detected in all these 21(87.5%) isolates. Our isolates were collected from ICUs, but in other studies *A. baumannii* was isolated from different wards of hospitals. In our study high rates of resistance of *A. baumannii* to imipenem and meropenem (71.4%) and Ceftazidime (66.7%) were observed. These results agreed with those of the study conducted in Tehran Hospitals, as high rates of resistance of *A. baumannii* to imipenem (52.5%), meropenem (52.5%), and cefotaxime (92.5%) were observed (*Taherikalani et al., 2009)*. Another point in the current study was that, 13 (61.9%) isolates were MDR with resistance to Ampicillin/Sulbactam, Piperacillin, Gentamicin and Amikacin, Ceftazidim, and Aztreonam, and 12 (57.1%) isolates were XDR with resistance to Imipenem and Meropenem in addition. It has been considered by *Poirel and Nordmann (2006)*; that resistance against carbapenems is, in itself, sufficient to define an *A. baumannii* as highly resistant. These high resistance rates of the isolated *A. baumannii* from our clinical samples can be explained by the over consumption of antibiotics in our hospital, which is usually empirical and without documented proof of infection. As expected, the majority of isolates were multiply resistant to antimicrobials. So far, 100% of the isolates in the current study were susceptible to colistin. Others also observed that; colistin with rifampin and/or tigecycline were useful against carbapenem resistant strains (*Noyal et al., 2011)*. Susceptibility to colistin was reported as 91.2–100% in various studies *(Dizbay et al.,2008 and Taherikalani et al.,2009 )*, and it seems to be a good option in the treatment of MDR *A. baumannii*, but adverse reactions, such as, renal toxicity has limited use of this agent. While many underlying mechanisms may account for carbapenem resistance, the possession of MBL genes is of particular concern because they are able to hydrolyze most beta-lactams, including imipenem and meropenem, drugs considered of reserve for the treatment of Gram-negative pathogens especially in *A. baumannii* multidrug-resistant strains. Therefore, the reliable detection of the MBL-producing strains is essential for the optimal treatment of infected patients and to control the nosocomial spread of resistance. Several phenotypic methods are available for the detection of MBL-producing bacteria. All of these methods are based on the ability of metal chelators, such as EDTA and thiol-based compounds, to inhibit the activity of MBLs. A much simpler test has been approved by the CLSI *(CLSI, 2006)*; the combined disk test using EDTA with imipenem is simple to perform and interpret and can be easily introduced into the workflow of a clinical laboratory *(Saderi et al., 2010)*. By using imipenem/EDTA combined disk test (CDT) a high percentage of Imipenem resistant *A. baumannii* isolates (86.7%) were metallo-β-lactamase producers. A majority of our isolates also showed a resistance to other important groups of antibiotics including third generation

**Figure.1** API 20E strip with a panel that was identified as *Acinetobacter baumannii*

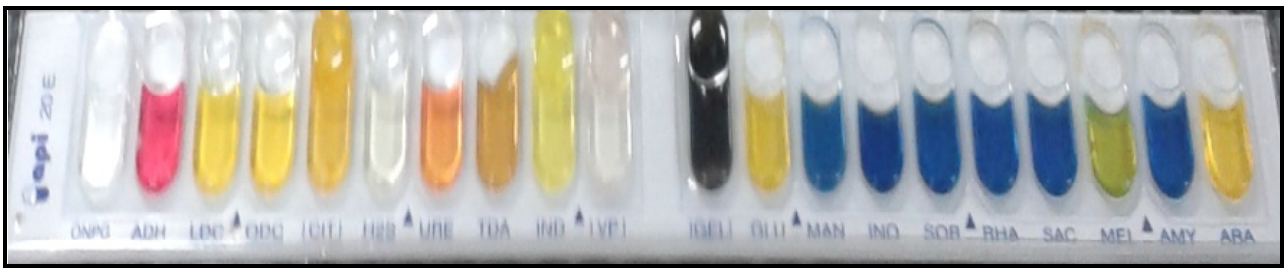
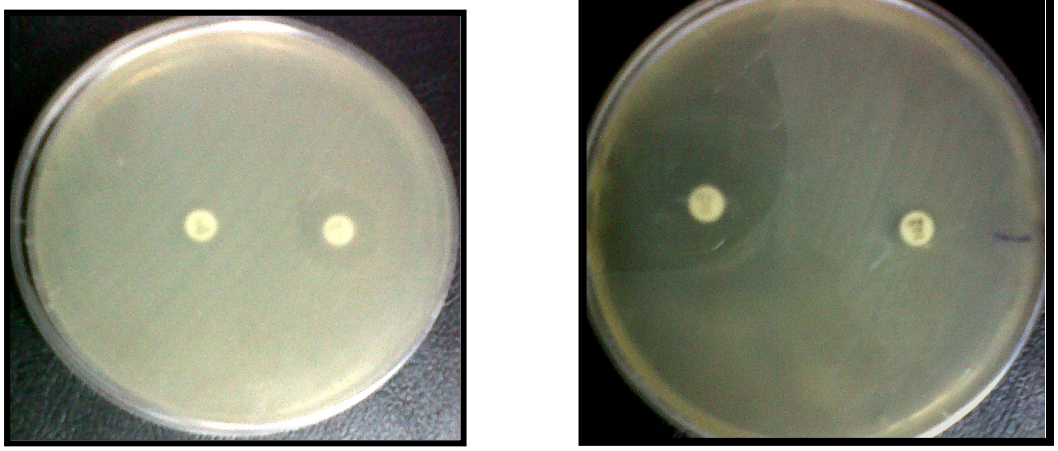



**Figure.2a&b** Combined disk test showing enhanced inhibition zone of ≥7 mm around IPM + EDTA disc indicating MBL producers



a) b)

**Figure.3** *bla*OXA-51-like gene was detected by PCR; M: M.W. marker 100 bp. Lane1: negative control. Lanes 2-7: *bla*OXA-51-like gene -353 bp positive samples

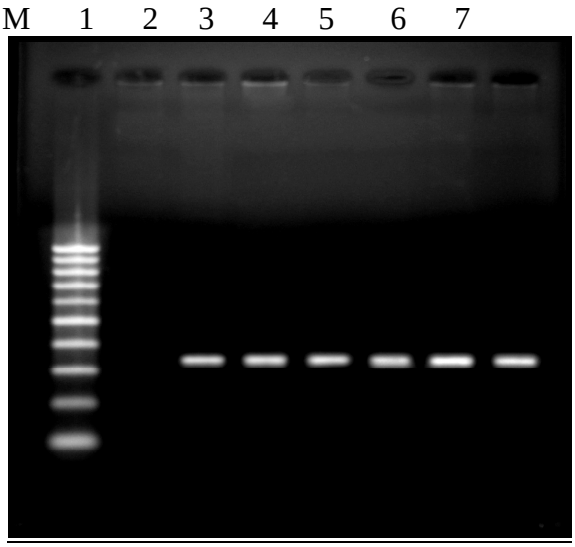


**Table.1** Sources of the *A. baumannii* isolates

| Samples | Total | Percentage % |
|---|---|---|
| Endotracheal secretion | 7 | 33.3 |
| Urine | 6 | 28.7 |
| Blood | 4 | 19 |
| Pus | 4 | 19 |
| Total | 21 | 100 |

**Table.2** Susceptibility pattern of *Acinetobacter baumanii* isolates to different antibiotics

| Antibiotic | Antibacterial class | Sensitive | Resistant |
|---|---|---|---|
| Ampicillin/Sulbactam | β-lactam/inhibitor combination | 3(14.3%) | 18(85.7%) |
| Imipenem | Carbapenemes | 6 (28.6%) | 15(71.4%) |
| Meropenem | Carbapenemes | 6 (28.6%) | 15(71.4%) |
| Gentamycin | Amino glycosides | 6 (28.6%) | 15(71.4%) |
| Piperacillin | Extended-spectrum β-lactams | 6 (28.6%) | 15(71.4%) |
| Ceftazidime | Third generation Cephalosporin | 7 (33.3%) | 14(66.7%) |
| Amikacin | Amino glycosides | 7 (33.3%) | 14(66.7%) |
| Aztereonam | β-lactams | 8 (38%) | 13 (62%) |
| Ciprofloxacin | Fluoroquinolone | 9 (42.9%) | 12(57.1%) |
| Colistin | polymixin | 21(100%) | 0 (0%) |

**Table.3** Different types of health care-associated infections caused by Imipenem resistant *Acinetobacter baumanii*

| Site of infection | Carbapenem Sensitive *Acinetobacter baumanii* | Carbapenem resistant *Acinetobacter baumanii* | Total |
|---|---|---|---|
| **Infected tracheostomy** | 3(50%) | 4(26.7%) | 7 (33.3%) |
| **UTI** | 2(33.3%) | 4(26.7%) | 6 (28.6%) |
| **Sepsis** | 1(16.7%) | 3(20%) | 4(19%) |
| **SSI** | 0 | 3(20%) | 3(14.3%) |
| **Infected bed sores** | 0 | 1(6.7%) | 1 (4.8%) |
| **Total** | 6 (28.6%) | 15(71.4%) | 21(100%) |

**Table.4** Risk factors associated with imipenem resistant *Acinetobacter baumanii* infection

| Risk Factor | | Imipenem resistant *Acinetobacter baumanii* n=15 | | Imipenem sensitive *Acinetobacter baumanii* n=6 | | *P* value |
|---|---|---|---|---|---|---|
| | | Number | Percentage % | Number | Percentage % | |
| Age | >35 | 6 | 66.7 | 3 | 33.3 | 0.2 |
| | ≤35 | 9 | 75 | 3 | 25 | |
| External device | Yes | 6 | 60 | 4 | 40 | 0.03* |
| | No | 5 | 45.5 | 6 | 54.5 | |
| Previous antibiotic use | Yes | 8 | 53.3 | 7 | 46.7 | 0.004** |
| | No | 2 | 33.3 | 4 | 66.7 | |
| Prolonged hospital stay > 10 days | Yes | 8 | 57 | 6 | 43 | 0.002** |
| | No | 3 | 42.9 | 4 | 57.1 | |

***Significant *p*<0.05 **highly significant *p*<0.01, <0.001**

cephalosporins, aminoglycosides and quinolones which is a characteristic of metallo-β-lactamase producing isolates. The same results were observed by *Mohanty et al. (2013)* who reported a higher prevalence of resistance in MBL-positive *A. baumannii* isolates as compared to the MBL-negative ones for all antibiotics except colistin. Since MBLs genes are carried on plasmids, this may explain the higher prevalence of co-resistance to other antibiotics found in MBL-positive isolates *(Mohanty et al., 2013)*. The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern with regard to the future of antimicrobial chemotherapy. The rapid detection of MBL-positive gram-negative bacilli is necessary to aid infection control and to prevent their dissemination. A PCR method was simple the results of PCR molecular tests for to use in detecting MBL-producing isolates initially, but it became more difficult with the increased number of types of MBLs *(Young et al., 2002).* The prevalence of carbapenem resistance mediated by acquired MBL including imipenem (IPM) and Verona integron-encoded metallo-β-lactamase (VIM), are increasing from different parts of the world *(Chin et al.,2011).* In the present study we tried to identify the presence of the genes coding for these two common carbapenem hydrolyzing enzymes*; blaIMP-1*and *blaVIM-1* using PCR among our clinical isolates of *A. baumannii*. But none of them was detected in our clinical isolates. Our results agreed with those of *Eser et al. (2009)* which were done on 124 strains of *A. baumannii* in Turkey in 2009, 80% imipenem- resistant strains were detected as CDT positive; however detection of *blaVIM-1* and *blaIMP-1* were

negative. Of much concern were the results observed in the study was done by *Azimi et al. (2013)* PCR assay was done for 93 *A. baumannii*; 85% of them were resistant to imipenem. 34% of them have a positive CDT. The *VIM-1*, and *IMP-1* genes were not detected in PCR molecular method, however in 74% of strains with positive results in combination disc, were positive for the OXA-23 gene after PCR test. This study showed that the blaOXA-23 resistance determinant may become an emerging therapeutic problem. There are a lot of studies highlighting presence of multiple genes encoding carbapenem hydrolyzing enzymes in *A. baumannii* including the novel MBL *blaNDM-1 (Decousser et al., 2013)*. We examined only 2 MBL genes and some of the MBL-producing isolates may have MBL genes other than the examined ones. Various risk factors for infection with multidrug-resistant *Acinetobacter* species were found and summarized as; Prolonged length of hospital stay, Exposure to an intensive care unit (ICU), Receipt of mechanical ventilation, Exposure to antimicrobial agents esp., carbapenems, and Invasive procedures. All these risk factors also previously reported by *Manchanda et al. (2010).* Many case control studies have revealed that prior exposure to antimicrobial therapy has been the most common risk factor identified in multivariate analysis. The second most common risk factor identified in case-control studies is mechanical ventilation. Other risk factors include a stay in an ICU, length of ICU and hospital stay, severity of the illness, recent surgery, and invasive procedures *(Katsaragakis et al., 2008).* Emergence of MBLs producing *A. baumannii* in our clinical isolates is alarming and reflects excessive use of carbapenem. Therefore, early detection and prompt application of infection control measures to prevent further spread of MBLs to other gram negative bacteria. Additionally, it is important to follow antibiotic restriction policies to avoid excessive use of carbapenem and other broad spectrum antibiotics. A limitation of the current study was the lack of genotypic confirmation for the presence of carbapenem-hydrolyzing genes. There is need for genetic analysis of MBLs enzymes.

Carbapenem-resistant *A.baumannii* is emerging as an important pathogen in our ICU. Antibiotic selective pressure is an important cause of emergence of MDR *A.baumannii* infection in our health care settings. The high prevalence of antibiotic resistance observed in the current study is significant, as only few therapeutic options would be available for treatment. New antibiotics are needed to treat MDR *A. baumannii*. The Present study provided valuable information about the effect of colistin that can be used in our health care settings. A strict antibiotic policy should be implemented in our health care facility. Infection control practices and antibiotic resistance surveillance should be carried out regularly to decrease the spread and impact of *A. baumannii*. Detection and molecular characterization of MBL-producing *A. baumannii* strains is recommended for the purposes of infection prevention and control.

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