Molecular Characterization and Antibiotic Susceptibility Pattern of Acinetobacter Baumannii Isolated in Intensive Care Unit Patients in Al-Hassa, Kingdom of Saudi Arabia

Abstract

Background: Acinetobacter baumannii is an emerging nosocomial multidrug resistance pathogen with the rapid spread of clones being reported in health-care settings and hospitals worldwide. Carbapenem resistance in this bacterium has been attributed to D OXA β-lactamases with OXA-51-like β-lactamase, being present in all A. baumannii isolate. The present study looks into the antibiotics susceptibility and molecular characterization of clinical A. baumannii isolates from Intensive Care Unit (ICU) samples in Al-Hofuf, South-eastern region of Saudi Arabia.

Materials and Methods: Eleven strains of ICU A. baumannii isolates were used for the investigation. Bacteria isolation was by basic microbiological techniques. Organisms identification and antibiotic susceptibility testing was by the BioMerieux VITEK 2 compact automated system (BioMerieux, Marcy l’Etoile France), according to the manufacturers guidelines. Confirmation of A. baumannii was by the presence of the OX-51 gene, also, carbapenemase encoding resistant genesbla<sub>OXA-23</sub> and bla<sub>OXA-40</sub> and bla<sub>OXA-51</sub> were analyzed using multiplex PCR. The Student’s t test was used to analyze the obtained data for between group comparisons with statistically significance level set at P < 0.05.

Results: Eight of the isolates were confirmed to be A. baumannii. Five of which were resistant to the carbapenems against which they had been tested. One isolate was resistant to tigecycline, whereas three tested intermediate to the drug. OXA-23 was detected in isolates 1, 4, 5, 6, and 7.

Conclusion: It can, therefore, be concluded that the probable predominate carbapenems resistant genes in ICU isolates from the present investigation, are those associated with OXA-23.

Keywords: Acinetobacter baumannii, Intensive Care Unit, multidrug resistance, OXA-23, OXA-51, extensive drug resistance

Introduction

The increase in difficult to treat multi-drug resistant bacteria has created an unprecedented problem in the 21<sup>st</sup> century. Acinetobacter baumannii has been listed among the most difficult antimicrobial resistant Gram-negative bacilli. A. baumannii an opportunistic pathogen has evolved to be an important pathogen associated with nosocomial infections worldwide and is known to have developed strains that are multidrug resistance (MDR), extensive drug resistance (XDR) and Pandrug resistance. Researchers have carried out extensive investigations into various aspects of this bacteria super bug in which they had looked into the various genes responsible for carbapenemase resistance. A. baumannii is postulated to have emerged as a nosocomial pathogen with the rapid spread of clones being reported in hospitals and health-care settings. Outbreaks of MDR A. baumannii in critical care units have also been reported in various regions of the world. Furthermore, colonization with this bacterium in immunocompromised patients have been reported by researchers with diabetes being shown to be a significant risk factor for A. baumannii nosocomial infections. Other risk factors which have been associated with patient colonization by A. baumannii are exposure to ICUs as well as prolonged hospital stays. Patients at risk could be through exposure to mechanical ventilation, recent surgery, invasive procedures as well as items used in the care of patients.

The carbapenems had been the drug of choice in treatment due to their effective antibacterial activity as well as having low

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toxicity. However, A. baumannii has emerged resistant to this drug of choice resulting in a major global health concern.\cite{18,19} Resistance to the carbapenem manifested in plasmid-encoded β-lactamase (OXA-23, OXA-40, and OXA-58) enzymes and the chromosomally encoded OXA-51.\cite{20} The clones harbored by different A. baumannii isolates have been shown to differ.\cite{20} Differences have been indicated between the isolates diabetic and nondiabetic patients\cite{6} with no specifics indicating if there are clones associated with diabetes. The present work looks into A. baumannii isolates from Intensive Care Unit (ICU) samples with a view of providing an insight in the prevalent OXA β-lactamase associated with ICU isolates. This would help provide information on the genes associated with ICU isolates as there is the need for constant surveillance in the microbial susceptibility of bacteria isolates in this era of evolving bacteria superbugs.

**Materials and Methods**

**Sample collection and processing**

Clinical isolates on A. baumannii obtained from ICU samples were used for the study. Samples had been isolated from sputum, throat, abdomen, catheter and endotracheal aspirates and plated out on MacConkey agar. A. baumannii isolation and identification was carried out in hospitals using basic microbiological and biochemical techniques.

Isolation was through basic microbial techniques while confirmation of isolates was carried out using BioMerieux VITEK 2 compact automated system (BioMerieux, Marcy l’Etoile France), according to the manufacturer’s guidelines. For further confirmation, PCR was used to detect the intrinsic bla\textsubscript{OXA-51} gene.\cite{6}

**Antimicrobial susceptibility testing**

Pure samples grown overnight on MacConkey agar were used for the anti-biogram testing. The antibiotic susceptibility testing was carried out using the VITEK 2 compact automated system according to manufacturer’s guidelines. Isolates were tested against the GN cards with the following antibiotics: cefazolin, imipenem, meropenem, gentamycin, tobramycin, ciprofloxacin, levofloxacin, tigecycline, and sulfamethoxazole/trimethoprim. Only isolates which showed resistance to imipenem and meropenem were used for the investigations. The minimum inhibitory concentration values were determined using the ETest strips (AB Biodisks) following the manufacturer’s guidelines. The test experiments were repeated in triplicates.

**Molecular analysis for carbapenem-resistant genes**

The presence of carbapenemase encoding resistant gene (bla\textsubscript{OXA-23}, bla\textsubscript{OXA-40}, bla\textsubscript{OXA-51}) among the isolates were detected using multiplex PCR genes using primers pairs.\cite{21,23} The primers used for the amplification and sequencing are shown in Table 1.

The PCR protocol is as described by Marco et al.\cite{21} Samples were initially denatured for 5 min at 94°C, followed by amplification cycles and elongation steps as described by Marco et al.\cite{21} Resulting PCR products were visualized after agarose gel electrophoresis, staining with ethidium bromide.

**Statistical analysis**

Excel software, 2013 version was used to analyze the obtained data, with student’s T-test used for comparison between groups. Statistically, significance level was set at $P < 0.05$.

**Results**

A total of eight nonrepetitive confirmed isolates of A. baumannii collected from ICUs in the study region were used for the investigation. The isolate source and the minimum inhibitory concentration (MIC) values are shown in Table 2. The result on the susceptibility of these isolates against tested antibiotics is presented in Table 3. The isolates are seen to exhibit varying degrees of resistance to the different antibiotics with 56% of them being resistant to imipenem and meropenem. However, all (100%) the isolates were sensitive to colistin, One (12.5%) isolate

### Table 1: Sequence of primers used for the study

| Primer name | Nucleotide sequence (5’-3’ ) | Reference |
|-------------|-----------------------------|-----------|
| Oxa-23-F    | GAT CGG ATT GGA GAA CCA GA’| Qi et al.\cite{8} (2008) |
| Oxa-23-R    | ATT TCT GAC CGC ATT TCC AT’| Qi et al.\cite{8} (2008) |
| OXA-40-F    | GGT TAG TTG GCC CCC TTA AA | Qi et al. (2008) |
| OXA-40-R    | AGT TGA GCC AAA AGG GGA TT | Qi et al. (2008) |
| OXA-51-F    | TAA TGC TTT GAT CGG CCT TG | Woodford et al.\cite{23} (2006) |
| OXA-51-R    | TGG ATT GCA CTT CAT CTT GG | Woodford et al. (2006) |

### Table 2: Source of isolation and the minimum inhibitory concentration values against tested antibiotics

| Isolates number | LID | Source | OXAs | MICs (µg/ml) |
|-----------------|-----|--------|------|--------------|
|                 |     | 51     | 23   | 40 | IMP | MP | CS | TG |
| 1               | L27M| Sputum | +    | +  | -  | 0.125 | 0.25 | 0.25 | 0.36 |
| 2               | L47M| ET     | +    | +  | -  | ≥32 | 32  | 0.25 | ≥256 |
| 3               | 48M | ETA    | +    | -  | -  | ≥32 | ≥32 | 0.125 | ≥256 |
| 4               | 50M | ABDT   | +    | +  | -  | ≥32 | 0.25 | 0.25 | ≥256 |
| 5               | 15M | Throat | +    | +  | -  | ≥32 | 32  | 0.5  | ≥256 |
| 6               | 52M | Catheter | +   | +  | -  | 0.25 | 0.032 | 0.38 | 0.016 |
| 7               | 43M | ET     | +    | +  | -  | 0.25 | 0.25 | 0.125 | 2 |
| 8               | 54M | NG     | +    | -  | -  | 0.125 | 0.125 | 0.19 | 16 |

LID: Laboratory ID; IMP: Imipenem; MP: Meropenem; CS: Colistin; TG: Tigecycline; MIC: Minimum inhibitory concentration; ET: Endotrachea; ETA: Endotrachea aspirates; ABDT: Abdominal tissue; NG: Not given; +: Positive; -: Negative
was resistant to tigecycline, whereas 33.33% of the isolates were intermediate to tigecycline. Based on their resistance against the tested antibiotic groupings, 67% of the isolates are MDR. Figure 1 shows the results of the resistance of these isolates to the antibiotic categories. Highest resistance was against the cephalosporins (78%) followed by fluoroquinolones with a 67% resistance by the isolates. Fifty-six percent of the \textit{A. baumannii} were each resistant to the carbapenems and penicillin, whereas 44% of them were each resistant to the aminoglycosides and trimethoprim/sulfamethoxazole.

A comparison between the number of antibiotics that the isolates were resistant and sensitive to is shown in Table 4. For isolate 1, there was about a 50% resistance against the tested antibiotics. The difference between the number of antibiotics that the isolate was resistant or sensitive to is not statistically significant. However, for isolates 3, 4, 5, and 7, there was a 75% to 80% resistance against the tested antimicrobials, inclusive of the carbapenems. The results are statistically significant ($P < 0.05$). Isolates 8 and 9 were more susceptible to most of the tested drugs and the difference against the drugs to which they were resistant to was not statistically significant as shown in Table 4.

On the other hand, for the comparison between the antibiotics to which isolates were resistant or intermediate to, results showed that there is a statistically significant difference ($P < 0.05$) with the exception of isolate 1 that recorded no statistical difference [Table 5]. Also, the results on the prevalent genes seen to be associated with the ICU \textit{A. baumannii} isolates is shown in Figure 2. The OXA-23 was present in 56% of the isolates while OXA 40 was not encountered in any of the isolates.

**Discussion**

The health-care problem caused by MDR \textit{A. baumannii} is further highlighted in the results of the present investigation. The isolation of MDR \textit{A. baumannii} from ICU samples as seen in the present study is not unexpected as such isolations had been reported earlier by researchers.$^{[7,15,16,22]}$ Furthermore, the evolvement of both MDR and XDR A. baumannii has been reported by Kempf and Rolain in 2012.$^{[23]}$

The results from the present study also showed majority of the isolates to be MDR, whereas >50% of them showed XDR against the tested antibiotic groups. The exhibition of high levels of MDR and XDR by \textit{A. baumannii} isolates has been reported in different regions of the world. Nageeb \textit{et al.}$^{[22]}$ reported a 100% MDR and 80% XDR among \textit{A. baumannii} isolates obtained from ICU in Egypt, whereas in Pakistan, Evans \textit{et al.}$^{[24]}$ encountered 82.4% MDR and 65% XDR among the isolates in their study. Both findings differ from those of the present investigation. However, Dent \textit{et al.}$^{[25]}$ reported a 72% and 58% MDR and XDR, respectively, among \textit{A. baumannii} in their study Nashville General Hospital and this finding is similar to those of the present study. While being certain that all the \textit{A. baumannii} ICU isolates in the present study showed both MDR and XDR against the tested antibiotic groups, the observed difference between researches could be attributed to a number of contributing factors one of which is geographical regional isolate differences, as well as the total number of isolates being used for the investigations by the different researchers as had been indicated by Nageeb \textit{et al.}$^{[22]}$

Furthermore, the resistance by more than half of the isolates to the carbapenems and other \beta-lactams as seen in the present investigation indicates that carbapenem resistance
to find out why the presence of OXA-72 did not produce β-lactam resistance. Furthermore, El-Shazy et al.,[29] in 2015 reported that not all the A. baumannii isolates in their study harboring the ISAbla1 gene were resistant to the carbapenems. Evans et al.,[24] found the OXA-51-like enzyme expressed in a sensitive A. baumannii isolates and explained that the enzyme demonstrated the capability to dramatically increase the MIC to carbapenem for an isolate. They also indicated that the OXA-51-like enzyme demonstrated only a weak hydrolytic activity against carbapenemase. These might therefore explain the reason for the results recorded with one of the isolate in the present study. However, there is need for further study on the nonconferring of resistance in the presence of OXA-like gene as earlier suggested.[27]

### Conclusion

From the results of the present investigation, it can be concluded that the predominant carbapenem resistant in the ICU A. baumannii isolates in the present investigation, are those of the OXA-23. There is however need for more investigation for both monitoring and further updating.

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### Conflicts of interest

There are no conflicts of interest.

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