Original Article

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

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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. baumanni*i isolates were used for the investigation. Bacteria isolation was by basic microbiological techniques. Organisms identification and antibiogram susceptibility testing was by the BioMerieux VITEK 2 compact automated system (BioMerieux, Marcy I'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$_{OXA-23}$, bla$_{OXA-40}$, and bla$_{OXA-51}$, 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 21st century. *Acinetobacter baumannii* has been listed among the most difficult antimicrobial resistant Gram-negative bacilli.[1,2] *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.[3] Researchers[4-8] have carried out extensive investigations into various aspects of this bacteria supper 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[8-10] Outbreaks of MDR *A. baumannii* in critical care units have also been reported in various regions of the world.[5,11] Furthermore, colonization with this bacterium in immunocompromised patients have been reported by researchers[6,12] with diabetes being shown to be a significant risk factor for *A. baumannii* nosocomial infections.[6,13,14] Other risk factors which have been associated with patient colonization by *A. baumannii* are exposure to ICUs as well as prolonged hospital stays.[1] Patients at risk could be through exposure to mechanical ventilation, recent surgery, invasive procedures[15,16] as well as items used in the care of patients.[11,17]

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.[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.[20] The clones harbored by different *A. baumannii* isolates have been shown to differ.[20] Differences have been indicated between the isolates diabetic and nondiabetic patients[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 I'Etoile France), according to the manufacturer's guidelines. For further confirmation, PCR was used to detect the intrinsic bla_{OXA-51} gene.[6]

Antimicrobial susceptibility testing

Pure samples grown overnight on MacConkey agar were used for the antibiogram 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, gentamicin, 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_{OXA-23}, bla_{OXA-40}, bla_{OXA-51}) among the isolates were detected using multiplex PCRgenes using primers pairs.[8,21-23] The primers used for the amplification and sequencing are shown in Table 1.

The PCR protocol is as described by Marco *et al*.[21] Samples were initially denatured for 5 min at 94°C, followed by amplification cycles and elongation steps as described by Marco *et al*.[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*.[8] (2008)
Oxa-23-R	ATT TCT GAC CGC ATT TCC AT'	Qi *et al*.[8] (2008)
OXA-40-F	GGT TAG TTG GCC CCC TTA AA	Qi *et al*. (2008)
OXA-40-R	AGT TGA GCG AAA AGG GGA TT	Qi *et al*. (2008)
OXA-51-F	TAA TGC TTT GAT CGG CCT TG	Woodford *et al*.[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 vales 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
3	L47M	ET	+	+	-	≥32	≥32	0.25	≥256
4	48M	ETA	+	-	-	≥32	≥32	0.125	≥256
5	50M	ABDT	+	+	-	≥32	0.25	0.25	≥256
6	51M	Throat	+	+	-	≥32	≥32	0.5	≥256
7	52M	Catheter	+	+	-	0.25	0.032	0.38	0.016
9	43M	ET	+	+	-	0.25	0.25	0.125	2
9	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

Table 3: Antibiogram of multidrug resistance clinical *Acinetobacter baumannii* isolates from Intensive Care Units

LID	Isolate number	Specimen	CRO	CTX	CAZ	FEP	GM	AK	IMP	MEM	CIP	LEV	TZP	CS	TG	SXT
27M	1	Sputum	R	R	R	I	**	I	S	S	**	S	S	**	R	**
47M	3	ET	**	**	R	R	R	**	R	R	R	R	R	S	I	S
48M	4	ETA	**	**	R	R	R	**	R	R	R	R	R	S	I	S
50M	5	AB. Tissue	**	**	R	R	S	**	R	R	R	R	R	S	I	R
51M	6	Throat	**	**	R	R	S	**	R	R	R	I	R	S	S	R
52M	7	Catheter	**	**	R	R	S	**	R	R	R	I	R	S	S	R
43M	8	ET	**	**	S	S	S	**	**	S	S	S	S	S	**	R
54M	9	Sputum	**	**	S	S	S	**	S	S	S	S	S	S	S	S

**Not given. LID: Laboratory ID; S: Sensitive; I: Intermediate; R: Resistant; CRO: Ceftriaxone; CTX: Cefotaxime; CAZ: Ceftazidime; FEP: Cefepime; GM: Gentamicin; AK: Amikacin; IMP: Imipenem; MEM: Meropenem; CIP: Ciprofloxacin; LEV: Levofloxacin; TZP: Piperacillin-tazobactam; CS: Colistin; TG: Tigecycline; SXT=Sulphamethoxazole/trimethoprim; ET: Endotrachea; ETA: Endotrachea aspirates; ABDT: Abdominal tissue

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 *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 *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 *A. baumannii* is further highlighted in the results of the present investigation. The isolation of MDR *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

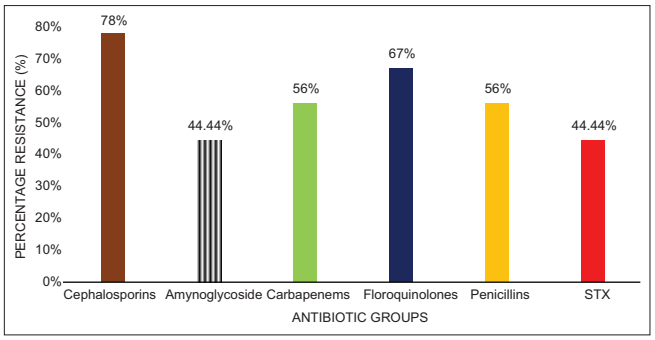

Figure 1: Percentage resistance of *Acinetobacter baumannii* to anti-microbial categories. STX = Sulphamethoxazole/trimethoprim

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 *A. baumannii* isolates has been reported in different regions of the world. Nageeb *et al*.[22] reported a 100% MDR and 80% XDR among *A. baumannii* isolates obtained from ICU in Egypt, whereas in Pakistan, Evans *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 *et al*.,[25] reported a 72% and 58% MDR and XDR, respectively, among *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 *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 *et al*.[22]

Furthermore, the resistance by more than half of the isolates to the carbapenems and other β-lactams as seen in the present investigation indicates that carbapenem resistance

Table 4: Comparison between resistance and sensitivity against the tested antibiotics by Intensive Care Unit isolates of *Acinetobacter baumannii*

Isolates number	R (%)	S (%)	Total (%)	*P*
Isolates 1	5 (50)	5 (50)	10 (100)	1
Isolates 3	8 (80)	2 (20)	10 (100)	0.00736*
Isolates 4	8 (80)	2 (20)	10 (100)	0.00736*
Isolates 5	8 (80)	2 (20)	10 (100)	0.00736*
Isolates 6	7 (70)	3 (30)	10 (100)	0.07346
Isolates 7	9 (75)	3 (25)	12 (100)	0.01428*
Isolates 8	8 (47.06)	9 (52.94)	17 (100)	0.72786
Isolates 9	8 (38.1)	13 (61.90)	21 (100)	0.12356

*The result is significant at $P<0.05$. S: Sensitive; R: Resistant

Table 5: Relationship between intermediate and resistance to tested antibiotics among Intensive Care Unit *Acinetobacter baumannii* isolates

Isolates number	R	I	Total	*P*
Isolates 1	5 (55.56)	4 (44.44)	9 (100)	0.63836
Isolates 3	8 (88.89)	1 (11.11)	9 (100)	0.00096**
Isolates 4	8 (88.89)	1 (11.11)	9 (100)	0.00096**
Isolates 5	8 (88.89)	1 (11.11)	9 (100)	0.00096**
Isolates 6	7 (87.5)	1 (12.5)	8 (100)	0.00278*
Isolates 7	9 (90)	1 (10)	10 (100)	0.00034**
Isolates 8	8 (88.89)	1 (11.11)	9 (100)	0.00096**
Isolates 9	8 (100)	0	8 (100)	6E-05***

*The result is significant, **Results are highly significant, ***Results are extremely significant. The result is significant at $P<0.05$. I: Intermediate; R: Resistant

remains a problem. Al-Sultan *et al*.[6] had reported that carbapenem resistance was very high among *A. baumannii* hospitals isolates in Saudi Arabia. The OXA-51- and OXA-23-like genes were the encountered genes responsible for carbapenem resistance in the *A. baumannii* isolates in the present study as shown in Figure 2. Yaowen *et al*.[26] observed OXA-23 gene to be the major carbapenemase responsible for resistance in their isolates from a teaching hospital. The findings are also consistent with those of Merino *et al*.[27] and Zowawi *et al*.[28] Observed in these carbapenem-resistant ICU isolates was the carriage of both the OXA-23 and OXA-51 genes. Similar findings had been reported by Al-Sultan *et al*.,[6] in 2013 on *A. baumannii* isolates from diabetic patients. The presence of both genes was however detected in one isolate which was susceptible to both imipenem and meropenem. This isolate was also susceptible to other β-lactams, fluoroquinolone and intermediate to aminoglycoside. Similar to this finding is that of Yaowen *et al*.,[26] that reported an *A. baumannii* isolate which had been susceptible to both imipenem and meropenem but was found to be carrying the bla_{OAX-72} gene with no other resistant gene. This isolate was reported as susceptible to other β-lactams, aminoglycosides, and fluoroquinolones among other antibiotics.[29] The authors concluded however that there was a need for more study

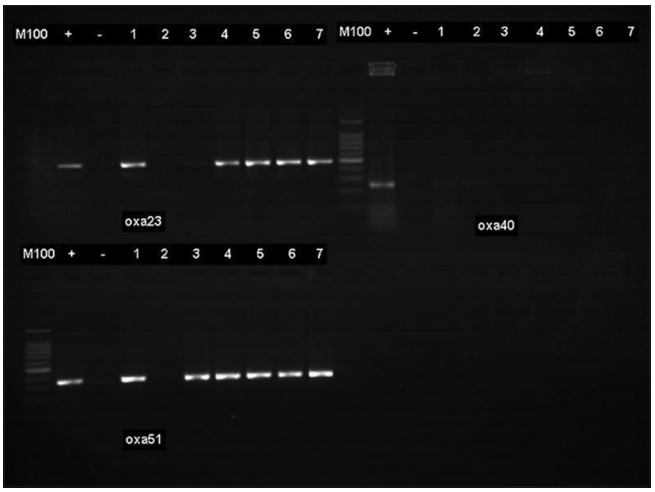

Figure 2: Characterisation of carbapenemase genes. The OXA-23 enzyme was detected in 5 (56%) of the isolates. All the isolates were negative to OXA-40

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 carbapenems 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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