High frequency of carbapenem-resistant *Acinetobacter baumannii* in patients with Diabetes Mellitus in Saudi Arabia

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Abstract

Carbapenem resistant *Acinetobacter baumannii* is becoming increasingly prevalent in patients with diabetes mellitus in the Middle East. We examined the relationship between these bacteria and their resistance mechanisms with the diabetic disease status of patients. Susceptibilities of 271 isolates to carbapenems, tigecycline and colistin were determined, followed by detection of carbapenemase genes. A bla_{VIM} gene was detected in ~95% isolates; $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-40}}$ genes were also prevalent. Diabetic patients were significantly more likely to carry carbapenem-resistant isolates. Carbapenem resistant *A. baumannii* is a serious problem in diabetics, and molecular detection of resistance mechanisms in these isolates is required.

Introduction

One of the greatest threats to modern medicine is the increasing prevalence of antibiotic-resistant bacteria, particularly Gram-negative bacteria (Boucher *et al.*, 2009). One of these bacteria, *Acinetobacter baumannii*, has risen to prominence due to the international dissemination of multidrug-resistant lineages resistant to the carbapenem antibiotics (Higgins *et al.*, 2009; Towner *et al.*, 2008; Turton *et al.*, 2007). *A. baumannii* can become resistant to the carbapenems through a number of mechanisms including expression of OXA-type carbapenemases and expression of an acquired metallo-β-lactamase (Evans *et al.*, 2013; Turton *et al.*, 2006). Recent studies have shown that the prevalence of carbapenem-resistant *A. baumannii* can be incredibly high in the Middle East (Al Johani *et al.*, 2010; Mugnier *et al.*, 2009).

Another growing health concern particularly prominent in the Middle East is the increase in the number of people with diabetes mellitus (Danaei *et al.*, 2011). Diabetes mellitus has been shown to be a significant risk factor in the acquisition of serious hospital-acquired infections with *A. baumannii* (Metan *et al.*, 2009; Michalopoulos *et al.*, 2011; Prata-Rocha *et al.*, 2012). The combination of increasing prevalence of diabetic patients and of carbapenem-resistant *A. baumannii*, to which these patients appear to be particularly susceptible, presents a worrying scenario of a rapidly rising number of difficult-to-treat infections. In order to begin to understand the nature of this problem, we investigated the prevalence of carbapenem-resistant *A. baumannii* and the β-lactamase genes they carry in both diabetic and non-diabetic patients from hospitals across Saudi Arabia.

Methods

A total of 271 isolates preliminarily identified as *A. baumannii* obtained from patients from intensive care units (ICUs) in hospitals in Saudi Arabia between 2008 and 2011 were selected

for inclusion in the study. Isolates were confirmed as *A. baumannii* using the Vitek compact II system and detection of a $bla_{\text{OXA-51-like}}$ gene by PCR (Woodford *et al.*, 2006). These 271 isolates comprised two distinct groups. One group of 196 isolates were selected for inclusion due to initial identification as being carbapenem resistant, and of these, 84 were obtained from patients with diabetes mellitus. Patients were defined as being diabetic if they were insulin users and being treated for diabetes under the care of a hospital. The remaining 75 of the 271 isolates were selected for inclusion at random without any prior knowledge about the antimicrobial sensitivity of the isolates or the diabetic status of the patients they were obtained from, with the exception that all isolates included in the study came from different patients. Of the group of 75 randomly selected isolates, 20 were obtained from diabetic patients. Detection of β-lactamase genes and insertion sequences was performed as described previously (Ellington *et al.*, 2007; Poirel & Nordmann, 2006; Woodford *et al.*, 2006). Antibiotic MICs were determined by the plate doubling dilution method according to British Society for Antimicrobial Chemotherapy (BSAC) guidelines (Andrews, 2010). As no breakpoint for tigecycline exists for *A. baumannii*, the breakpoint for Enterobacteriacea was used. For statistical analyses, isolates showing an intermediate level of antibiotic resistance were grouped with resistant isolates. Analyses were performed in SPSS v.20 (SPSS Inc, Chicago, IL, USA), and where appropriate were corrected for multiple testing (Benjamini & Hochberg, 1995).

Results and Discussion

The isolates included in the study came from a total of 37 hospitals and a wide range of samples including blood, respiratory, urine and wound. Of the 24 hospitals contributing more than one isolate, 9 contributed isolates from both diabetic and non-diabetic patients. Analyses were first performed on the data by dividing it into the isolates selected because they were

carbapenem resistant, and those selected randomly (Table 1). Combining the datasets for analysis was not possible as the method of isolate selection would bias the results.

Determination of antibiotic MICs confirmed that carbapenem resistance was found in almost all isolates selected for this trait, with only 6 of the 196 isolates (3%) having lost resistance to both imipenem and meropenem during storage. Carbapenem resistance was also prevalent in the randomly selected isolates, with a patient being more likely to carry a carbapenem-resistant isolate than not (chi squared test, $\chi^2 = 7.053$, d.f. = 1, $p = 0.008$). A high proportion of isolates (99%) carried genes for at least one acquired carbapenem-hydrolysing β-lactamase, with bla_{VIM} sequences particularly prevalent. Of the 32 carbapenem-susceptible isolates, 29 (91%) were positive for a bla_{VIM} gene.

The data were then analysed with respect to the disease status of the patient. Overall there is a significantly higher proportion of diabetic patients in the carbapenem-resistant isolate group than in the randomly selected isolate group (44% and 28% respectively, chi squared test, $\chi^2 = 5.787$, d.f. = 1, $p = 0.016$). The data were then sub-divided into those isolates that were obtained from patients with diabetes, and those obtained from non-diabetics. Analyses were performed on all categories for which there were enough data (Table 2). Amongst the randomly selected isolates, diabetic patients were significantly more likely to carry an isolate with a $bla_{\text{OXA-23}}$ gene or for it to be carbapenem-resistant, consistent with the previous finding above that diabetics are over-represented in the carbapenem-resistant isolate group. In the carbapenem-resistant isolate group, diabetic patients were significantly more likely to carry an isolate with an IS*Aba2* or IS*Aba3* insertion sequence, and significantly less likely to carry a tigecycline-resistant isolate. A similar result was found in the randomly selected isolates. It was also noted that there was a higher proportion of isolates carrying more than one gene of $bla_{\text{OXA-23}}$, $bla_{\text{OXA-40}}$ or IS*Aba1* upstream of $bla_{\text{OXA-51}}$ in non-diabetic patients than in those with diabetes (10% and 1% respectively, chi squared test, $\chi^2 = 9.085$, d.f. = 1, $p = 0.003$).

In the present study we find that carbapenem resistance is very prevalent in *A. baumannii* isolates from hospitals in Saudi Arabia, with isolates much more likely to be resistant than not. Worryingly, a very high percentage of isolates carry a VIM-type metallo-β-lactamase alongside high levels of carriage of the acquired carbapenemases $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-40}}$. As has been noted previously for metallo-β-lactamases (Franklin *et al.*, 2006; Peleg *et al.*, 2005), the number of isolates carrying a gene for a VIM-type enzyme is much higher than the number of isolates phenotypically resistant to carbapenems, reinforcing the need for molecular diagnostics particularly in regions where there is a known problem with such organisms.

Another health issue that is particularly acute in the Middle East in countries such as Saudi Arabia is the increasing number of patients diagnosed with diabetes mellitus (Danaei *et al.*, 2011). Previous studies have shown that diabetes is a significant risk factor in acquiring a serious infection with *A. baumannii* (Metan *et al.*, 2009; Michalopoulos *et al.*, 2011; Prata-Rocha *et al.*, 2012). To our knowledge, here we show for the first time that diabetic patients with an *A. baumannii* infection are more likely than non-diabetic patients to carry a carbapenem-resistant isolate. With the prevalence of diabetes increasing, this represents a real healthcare problem as it amplifies the risk posed to diabetics from infection with *A. baumannii*. Previously we demonstrated that particular *A. baumannii* clones were associated with diabetic patients from Saudi Arabia (Alsultan *et al.*, 2009). In the present study there are also indications that the isolates harboured by diabetics and non-diabetics differ. The further characterisation of these isolates through typing and β-lactamase gene sequencing will allow us to establish whether there are particular bacterial clones that are associated with diabetic patients.

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Characteristic	Carbapenem resistant		Random	
	No.	%	No.	%
Total isolates	196	100	75	100
No. diabetics	84	43	20	27
bla $_{\text{OXA-23}}$	108	55	42	56
bla $_{\text{OXA-40}}$	59	30	9	12
bla $_{\text{OXA-58}}$	0	0	0	0
IS*Aba1* -*bla* $_{\text{OXA-51}}$	7	4	0	0
IS*Aba1*	178	91	57	76
IS*Aba2*	9	5	1	1
IS*Aba3*	19	10	0	0
IS*18*	0	0	0	0
bla $_{\text{VIM}}$	182	93	72	96
Imipenem resistant	181	92	49	65
Meropenem resistant	186	95	49	65
Tigecycline resistant	25	13	9	12
Colistin resistant	2	1	0	0

Table 1: Presence of genotypic and phenotypic characteristics amongst isolates.

Carbapenem resistant	Diabetic		Non-diabetic		Chi Squared		
	No.	%	No.	%	statistic	d.f.	*p* value*
Total	84	43	112	57	-	-	-
bla $_{OXA-23}$	46	55	62	57	0.086	1	0.9228
bla $_{OXA-40}$	25	30	31	28	0.04	1	0.8410
IS*Aba1*	78	93	97	89	0.839	1	0.5400
IS*Aba2*	9	11	0	0	12.25	1	**0.0028**
IS*Aba3*	16	19	3	3	14.193	1	**0.0020**
bla $_{VIM}$	79	94	100	92	0.374	1	0.7213
Imipenem resistant	80	95	98	90	1.88	1	0.2914
Meropenem resistant	83	99	100	92	4.822	1	0.0672
Tigecycline resistant	5	6	20	18	6.465	1	**0.0440**
Random							
Total	20	28	55	72	-	-	-
bla $_{OXA-23}$	16	76	26	47	5.14	1	**0.0460**
bla $_{OXA-40}$	0	0	9	16	3.719	1	0.0720
IS*Aba1*	16	80	41	75	0.239	1	0.6250
Imipenem resistant	20	100	29	53	14.471	1	**0.0006**
Meropenem resistant	20	100	29	53	14.471	1	**0.0006**
Tigecycline resistant	0	0	9	16	3.898	1	0.0768

Table 2: Comparison of genotypic and phenotypic traits in diabetic and non-diabetic patients. *Corrected *p* values according to Benjamini and Hochberg False Discovery Rate (Benjamini & Hochberg, 1995), values in bold are significant at the 5% level.