Clonal diversity of *Acinetobacter baumannii* from diabetic patients in Saudi Arabian hospitals

Abdulrahman A. Alsultan[1], Elsayed Aboulmagd[1,2,*] Benjamin A. Evans[3], Sebastian G.B. Amyes[4]

[1] Department of Medical Microbiology, College of Medicine, King Faisal University, Kingdom of Saudi Arabia

[2] Department of Microbiology, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt

[3] Department of Life Sciences, Faculty of Science and Technology, Anglia Ruskin University, East Road, Cambridge CB1 1PT, UK

[4] Medical Microbiology, University of Edinburgh, The Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, UK

Abstract

The emergence of carbapenem-resistant *Acinetobacter baumannii* represents a major problem in the heath settings. Infections with such pathogens are often associated with high rate of morbidity and mortality. The aim of the present study was to investigate the clonal relatedness associated with genes encoding β-lactamases and metallo-β-lactamases associated with carbapenem resistance in clinical isolates of *A. baumannii* from diabetic patients from different regions in Saudi Arabia. A total of 64 non-repetitive carbapenem resistant *A.*

baumannii clinical isolates were collected from sixteen different regions in Saudi Arabia from patients in intensive care units. Isolates were identified phenotypically by Vitek 2 compact system and genotypically by amplification of intrinsic blaOXA-51-like gene by PCR. The target sequences were amplified by PCR and the clonal diversity of the isolates was explored by PFGE. The resistance pattern of the tested isolates was determined by Vitek 2 compact system and the minimum inhibitory concentrations of imipenem, meropenem, tigecycline and colistin were determined by Etest strips. The results of the current study revealed that the prevalence of imipenem and meropenem resistance was 92% and 96%, respectively, while the vast majority of the isolates were susceptible to tigecycline and colistin (97% each). In addition, the prevalence of $bla_{\text{OXA-23}}$, $bla_{\text{OXA-40}}$, bla_{VIM} and bla_{SPM} was 53.1%, 29.7%, 92.2% and 28.1, respectively, while bla_{IMP}, bla_{SIM} and bla_{GIM} were not detected. Moreover, IS*Aba1*, IS*Aba2* and IS*Aba3* were amplified from 58 (90.6%), 6 (9.4%) and 13 (20.3%) of the tested isolates, respectively. PFGE results showed that the tested isolates were clustered in thirteen groups. Clone H was the dominant clone containing 20 isolates from four hospitals followed by clone C and F containing 11 isolates each from 3 and 6 hospitals, respectively. In conclusion, bla_{VIM} and $bla_{\text{OXA-23}}$ were the most prevalent genes in the carbapenem resistant *A. baumannii* isolates under investigation while IS*Aba1* was the most common insertion sequence. Early recognition of the epidemic clone is very helpful to prevent its dissemination by application of strict infection control measures.

KEYWORDS: *Acinetobacter baumannii;* Clonal diversity; Saudi Arabia; Diabetic patients

Introduction

Acinetobacter baumannii (*A. baumannii*) is an aerobic, non-motile, non-fermenting Gram-negative opportunistic pathogen that is playing a major role in nosocomial infections of immunecompromised patients.[1] It is considered one of the six most important multidrug-resistant microorganisms in the hospitals especially in the intensive care units. Infections with such pathogen are often associated with high rate of morbidity and mortality.[2] *A. baumannii* is intrinsically has low susceptibility to different antimicrobial agents.[3] In the last decade, many multi-drug resistant (MDR) and extensive-drug resistant *A. baumannii* were isolated globally[4], regionally[5,6] and locally[7,8]. Nowadays, MDR *A. baumannii* is among the most difficult

pathogens to treat.[9] Carbapenems represent the main therapy for the serious infections caused by such pathogens.[10,11] Unfortunately, a dramatic increase of carbapenem resistant *A. baumannii* isolates has been recorded in the recent years.[6,7]

Carbapenem resistance of *A. baumannii* is mainly mediated by: (i) changes in porin proteins, (ii) development of efflux pumps, (iii) modification of penicillin-binding proteins, and production of different types of β-lactamases.[12] According to Ambler classification, there are four classes of β-lactamases (A, B, C and D). Classes A, C and D are serine type enzymes while class B is metallo-type enzymes (metallo-β-lactamases, MBLs) which require zinc for their catalytic activity.[13] In *A. baumannii,* MBLs (class B) and OXA-type carbapenemases (class D) mainly mediate resistance to carbapenems and to a lesser extent class A e.g. KPC.[1]

Since the discovery of the first OXA-type carbapenemases in 1993 in *A. baumannii* isolate from Scotland[14], many types were discovered and the number of OXA-type β-lactamases exponentially increases.[15] Five main groups of OXA carbapenemase are involved in the resistance of *A. baumannii*: OXA-23-like, OXA-40-like, OXA-51-like, OXA-58-like and OXA-143 enzymes.[1] The genes coding these enzymes are regulated by upstream insertion sequences (IS) specifically IS*Aba1*, IS*Aba2,* IS*Aba3* and IS*18*.[1,16,17] These insertion elements play a major role in the expression of such genes.[18,19] Chromosomally located $bla_{\text{OXA-51-like}}$ genes are intrinsically present in all *A. baumannii* stains.[20] In addition to OXA carbapenemases, three MBLs have been detected in *A. baumannii* namely IMP, VIM and SIM types.[17] In Saudi Arabia, the data regarding the mechanism of carbapenem resistance in *A. baumannii* is limited although some reports are recently published.[7,8]

The aim of the present study was to investigate the prevalence of various genes and the different insertion sequences associated with carbapenem resistance in *A. baumannii* clinical isolates from sixteen different regions in Saudi Arabia. In addition, the clonal relatedness of such clinical isolates was investigated.

Methods

Bacterial isolates

Sixty four non-repetitive *A. baumannii* clinical isolates were collected from different clinical specimens from patients in intensive care units. The isolates were collected from sixteen different regions in Saudi Arabia between January and November 2012. No ethical approval was required because samples were collected as a routine and standard patient care. Conventional microbiological methods were performed for preliminarily identification of the isolates and the identification was confirmed by the Vitek 2 compact system (BioMerieux, Marcy L'Etoile, France) according to the guidelines of the manufacturer. For molecular

confirmation of the Vitek 2 compact system identification, PCR was used to detect the intrinsic *bla*$_{\text{OXA-51-like}}$ gene. The primers used for amplification of such gene were depicted in Table 1.

Antimicrobial susceptibility testing

The resistance pattern of the tested isolates was determined by Vitek 2 compact system and confirmed by agar diffusion assay[21] against imipenem, meropenem, tigecycline and colistin. Antibiotic discs were purchased from Oxoid Ltd, UK. The minimum inhibitory concentrations (MICs) of the previously mentioned four antimicrobial agents were determined by Etest strips (AB Biodisk, Solna, Sweden) according to instructions of the manufacturer. All experiments were carried out in triplicate.

Detection of *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-40}}$ genes

Polymerase chain reaction (PCR) was used to amplify the genes encoding OXA-type carbapenemases (*bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-40}}$) and MBLs (*bla*$_{\text{VIM}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{GIM}}$, *bla*$_{\text{IMP}}$ *and bla*$_{\text{SPM}}$) as previously described[11,22-24] using the primers depicted in Table 1.

Molecular typing by PFGE

Sixty four *A. baumannii* clinical isolates were typed by PFGE analysis as previously described.[25] DNA obtained from bacteria was digested using Apa1 restriction endonuclease (Promega, Southampton, UK), and DNA fragments were separated on 1% agarose gel in 0.5× TBE buffer using the CHEF-RDII apparatus (Bio-Rad, UK). The conditions used were the following: pulse time 5-35s at field strength of 6v/cm for 24h at 37C. The gel was stained by ethidium bromide and then the digital images were captured by Gel doc2000 (Bio-Rad, UK). All isolates were analyzed using Bionumerics software version 6.5. Isolates that clustered together with a similarity of >85% were considered to belong to the same PFGE type.

Results

Sixty four non-repetitive *A. baumannii* clinical isolates (coded DM001 to DM064) were collected from sixteen different regions in Saudi Arabia. The isolates were cultured from different clinical specimens of diabetic patients admitted to ICUs. Thirty four patients (53%) were males while the female patients represented 47% (30/64). Most isolates were collected from respiratory tract clinical specimens (41 out of 64, 64%) while the rest of the isolates were obtained from infected blood, urine, abdomen and skin. Vitek II compact system was

used to identify the isolates and the identification was confirmed after amplification of the intrinsic *bla*$_{\text{OXA-51-like}}$ gene by PCR.

All isolates showed resistance to at lease one of the two tested carbapenems (imipenem and meropenem) as shown in Figure 1. No significant different between the prevalence of imipenem (59 out of 64, 92%) and meropenem (62 out of 64, 96%) resistance among the tested isolates as shown in Table 2. On the other hand, the majority of the isolates showed susceptibility to tigecycline and colistin, 97% each as mentioned Table 2.

The prevalence of the different genes coding two OXA carbapenemases (OXA-23 and OXA-40), five MBLs (VIM, IMP, SPM, SIM and GIM) and the different insertion sequences (ISs) was calculated after amplification of the target genes by PCR and the results were depicted in Table 3. Thirty four (53.1%) and nineteen (29.7%) isolates harbored *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-40}}$, respectively. Only two MBLs were detected in the isolates where *bla*$_{\text{VIM}}$ was significantly more prevalent than *bla*$_{\text{SPM}}$, 59/64 (92.2%) and 18/64 (28.1), respectively. On the other hand, *bla*$_{\text{IMP}}$, *bla*$_{\text{SIM}}$ and *bla*$_{\text{GIM}}$ were not detected in any of the tested isolates as shown in Table 3. Although IS*18* was completely absent from all the tested isolates, IS*Aba1*, IS*Aba2* and IS*Aba3* were amplified from 58 (90.6%), 6 (9.4%) and 13 (20.3%) of the tested isolates, respectively, as shown in Table 3.

PFGE was applied to study the clonal diversity and relatedness of the tested isolates. The discrimination power of PFGE technique was express by dice coefficient via BioNumerics software version 6.5. Figure 1 shows the clustering of thirteen groups from A to M of PFGE types comprise the ID number (isolate code), sex, locations, β-lactamase of OXA-23, OXA-40, insertion sequences (IS*Aba1*, IS*Aba2*, IS*Aba3* and IS*18*) and MBLs (VIM, SIM, GIM, IMP and SPM). All detected PFGE patterns demonstrate the genetic similarity coefficient ranged from 70% to 100%. Epidemic isolates that clustered together with a similarity of more than 85% were considered to present the same PFGE type. Clone C, F and H included 11, 11 and 20 PFGE types, respectively, with genetic similarity ranges from 92 to 100%, 88 to 100% and 89 to 100% respectively, and have shared 4, 3 and 6 cities, respectively, as shown if Figure 1. In addition, table 4 showed the diversity of the thirteen clones and the mechanism of resistance in relation to the cities from which the isolates were collected. Nine and five different clones were detected in 34 and 6 isolates from Riyadh and Almandine, respectively.

Discussion

The emergence and global distribution of carbapenem resistant *A. baumannii* represent a major problem in the health care setting specially ICUs. *A. baumannii* is a notorious opportunistic pathogen mainly associated with hospital-acquired infections.[26] This pathogen causes serious hospital acquired infections associated with high mortality rate particularly in immunocompromised patients. Moreover, very limited therapeutic options (e.g. colistin and tigecycline) are available for treatment of infection cause by such pathogens.[27]

In the present study, sixty four *A. baumannii* clinical isolates (based on the presence of $bla_{\text{oxa-51-like}}$ gene) were collected from tertiary care hospitals located in sixteen different cities in Saudi Arabia. These cities are distributed in many provinces of the Kingdom. The majority of the isolates were recovered from respiratory tract secretions of diabetic patients. In the last few years, emergence of carbapenem resistance in Gram negatives has been observed worldwide. This phenomenon is mostly related to the spread of different types of β-lactamases.[28] *A. baumannii* is an organism that appears to have the ability to develop antibiotic resistance very rapidly.[3] Carbapenems were the drug of choice for treating infections caused by MDR *A. baumannii*, however, resistance to such antimicrobial agents is now a common occurrence and pan-drug resistant strains are beginning to emerge.[1] In *A. baumannii*, the main carbapenem hydrolyzing β-lactamases are OXA-type carbapenemases (Ambler class D β-lactamases) and MBLs (class B β-lactamases).[28]

In the present study, the vast majority of the isolates showed susceptibility to colistin and tigecycline (97%). Higher tigecycline resistance rate (9.7%) was recently recorded in Riyadh region[29] while susceptibility to colistin in the current study is in accordance with different previously published reports.[29-32] In Iran, Shahcheraghi et al 2011[6] reported that 12% of *A. baumannii* isolates showed resistance to colistin while in another recently published article, no colistin resistance was observed in 104 clinical isolates.[10] Although many colistin and tigecycline resistant *A. baumannii* pathogens were recently isolated[33,34], these two antimicrobial agents remain the drug of choice for combating infections caused by carbapenem resistant *A. baumannii*.

The prevalence of OXA-23 in the current study was 53.1% which is comparable with recently published result from Egypt (50%)[32] and from India (47.9%)[35]. Higher prevalence of OXA-23 (100% and 80.4%) was detected in CRAB isolates from Riyadh[36] and the Eastern region of Saudi Arabia[8], respectively, from Taiwan (92.9%)[37], from Iran (84%[6] and 77.9%[10]) and from Columbia[38] (75%). On contrary, only two isolates carried $bla_{\text{OXA-23}}$ out of 40 (5%) isolates collected from Kuwait[5] while only one isolate out of 92 (1.1%) isolated from Taiwan harbored such gene.[39] On the other hand, OXA-40 was amplified from 29.7% of the tested

isolates in the present work. This carbapenemase was not detected in any of the tested isolates (n=253) from Riyadh.[36] While *bla*$_{OXA-40}$ was the most prevalent acquired gene (57.6%) in 59 isolates from Spain, none of such isolates harbored *bla*$_{OXA-23}$.[40] In addition, 7.5%, 19.2% and 22.9% of isolates from Egypt[32], Iran[10] and India[35], respectively, contained OXA-40. Co-existence of both *bla*$_{OXA-23}$ and *bla*$_{OXA-40}$ was not detected in any of the tested isolates. In contrast, 45% and 16.4% CRAB isolates collected from Egypt[32] and Iran[10], respectively, co-produced both enzymes OXA-23 and OXA-40.

Beside OXA carbapenemases, MBLs were detected in many of the tested isolates in the present work. VIM and SPM were the only two MBLs detected where their prevalence was 92.2% (59 out of 64) and 28.1% (18 out of 64), respectively, while the other three MBLs (IMP, GIM and SIM) were completely absent. Lower prevalence of MBLs (6%) was recorded in Iran[6] where only *bla*$_{SPM}$ was detected while *bla*$_{VIM}$ was completely absent. In addition, 46 carbapenem resistant *A. baumannii* (CRAB) clinical isolates from the Eastern region of Saudi Arabia harbored neither *bla*$_{VIM}$ nor *bla*IMP.[8] Moreover, MBL encoding genes were not amplified from any of 40 clinical isolates from Egypt.[32] On contrary, most CRAB isolates from Kuwait (72.5%, 29 out of 40 isolates) carried *bla* genes coding VIM and IMP MBLs.[5]

Our results revealed that IS*Aba1* was the most prominent (90.6%) insertion element in the tested isolates followed by IS*Aba3* (20.3%) while IS*Aba2* was detected in only 6 isolates out of 64 (9.4%). The prevalence of IS*Aba1*is comparable with that recorded in 59 isolates from Spain[40] (93.2%). Lower prevalence rate was recorded in Taiwan[39] (36%) and India[35] (33%). The presence of different insertion sequences renders the *A. baumannii* resistance to carbapenems.[3] Such insertion sequences located in the proximity of genes coding different OXA-types carbapenemases and involved in their overexpression.[41]

PFGE is one of the most important discriminatory method for *A. baumannii* and many other pathogens[42-44] and an efficient tool for determining the genetic relationship between strains isolated from epidemiological situation.[45] In the current work, the genetic similarity of PFGE types was very high (89 to 100%). The clonal diversity revealed two types of epidemic clones: monoclonal and polyclonal. The monoclonal model showed the most common clones appear in 12 out of 16 cities. These monoclonal outbreaks were caused by either one or more of epidemic PFGE type. The polyclonal model has affected four cites (Riyadh, Almadinah, Tabok and Kamis mosait). Riyadh is the capital city of Saudi Arabia, that affected by 9 different clones out of thirteen. Almadinah is the capital city of Almadinah province had affected by 5 different clones that both cities might cause an explosive outbreaks at different time. For Tabok and Kamis Mosait are coexistence clones were clearly in the minority and

that could reflect the coexistence of sporadic and epidemic clones.[46] Tabok had 2 clones (C2 and F2) and Kamis Mosait had (J and K) that might reflect low level of hospital infection control in these cities. Clone F has been detected in hospitals of Riyadh, Almadinah, Tabok, Abha Alrass and Alkafji. Therefore this study point to the transmission of an existent clones from one hospital to another one originating a new outbreak in later hospital or may be to the health care worker. The possibility of *A. baumannii* transmission is highly recognized[47-49] that meaning the reappearance of certain clones within these hospitals reflects endemic persistence of this pathogen in diabetic patients, hospital and environments which represent a risk factor in future outbreak. A further investigation of these diabetic isolates to be compared with non diabetic strains is highly recommended through typing and β-lactamase gene sequencing will allow us to establish whether there are particular bacterial clones that are associated with diabetic patients.

Conclusion

The result of the current study revealed that OXA-23 and VIM were the most common β-lactamases conferring carbapenem resistance to *A. baumannii*. In addition, IS*Aba1* was the most prevalent insertion sequence. Moreover, the current study provided significant data regarding the clonal diversity of carbapenem resistant *A. baumannii* in different cites in Saudi Arabia. Detection of the certain clone in different cities reflects the horizontal transmission of carbapenem resistance. Strict infection control measures should be applied to prevent such type of transmission.

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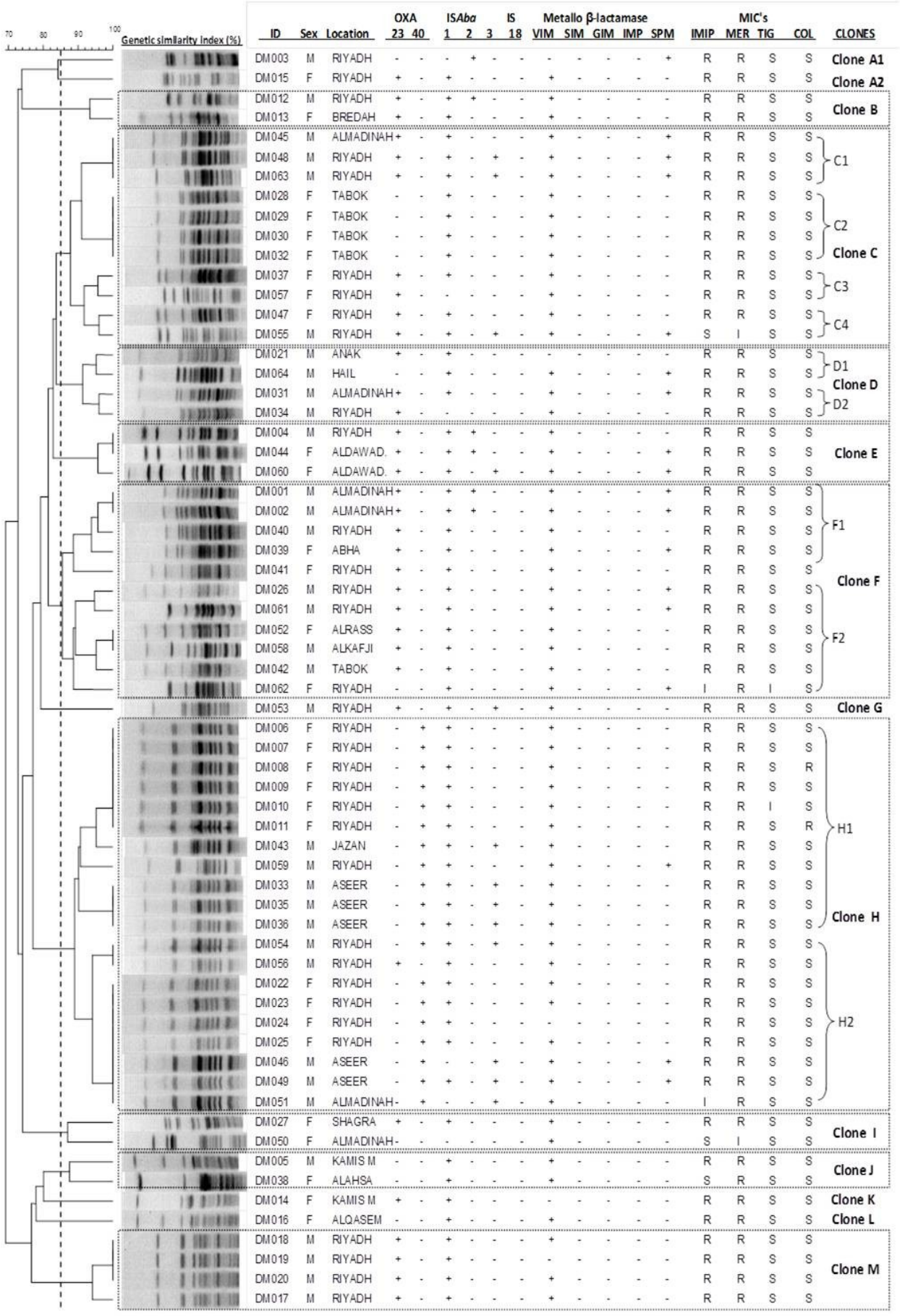

ID	Sex	Location	OXA 23	OXA 40	ISAba 1	ISAba 2	ISAba 3	IS 18	Metallo β-lactamase VIM	SIM	GIM	IMP	SPM	MIC's IMIP	MER	TIG	COL	CLONES
DM003	M	RIYADH	-	-	-	+	-	-	-	-	-	-	+	R	R	S	S	Clone A1
DM015	F	RIYADH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	Clone A2
DM012	M	RIYADH	+	-	+	+	-	-	+	-	-	-	-	R	R	S	S	Clone B
DM013	F	BREDAH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM045	M	ALMADINAH	+	-	+	-	-	-	+	-	-	-	+	R	R	S	S	C1
DM048	M	RIYADH	+	-	+	-	+	-	+	-	-	-	+	R	R	S	S	
DM063	M	RIYADH	+	-	+	-	+	-	+	-	-	-	+	R	R	S	S	
DM028	F	TABOK	-	-	+	-	-	-	+	-	-	-	-	R	R	S	S	C2
DM029	F	TABOK	-	-	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM030	F	TABOK	-	-	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM032	F	TABOK	-	-	+	-	-	-	+	-	-	-	-	R	R	S	S	Clone C
DM037	F	RIYADH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	C3
DM057	F	RIYADH	+	-	-	-	-	-	+	-	-	-	-	R	R	S	S	
DM047	F	RIYADH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	C4
DM055	M	RIYADH	+	-	+	-	+	-	+	-	-	-	+	S	I	S	S	
DM021	M	ANAK	+	-	+	-	-	-	-	-	-	-	-	R	R	S	S	D1
DM064	M	HAIL	-	-	+	-	-	-	+	-	-	-	+	R	R	S	S	
DM031	M	ALMADINAH	+	-	+	-	-	-	+	-	-	-	+	R	R	S	S	Clone D
DM034	M	RIYADH	+	-	-	-	-	-	+	-	-	-	-	R	R	S	S	D2
DM004	M	RIYADH	+	-	+	+	-	-	+	-	-	-	-	R	R	S	S	Clone E
DM044	F	ALDAWAD.	+	-	+	+	-	-	+	-	-	-	+	R	R	S	S	
DM060	F	ALDAWAD.	+	-	+	-	+	-	+	-	-	-	+	R	R	S	S	
DM001	M	ALMADINAH	+	-	+	+	-	-	+	-	-	-	+	R	R	S	S	F1
DM002	M	ALMADINAH	+	-	+	+	-	-	+	-	-	-	+	R	R	S	S	
DM040	M	RIYADH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM039	F	ABHA	+	-	+	-	-	-	+	-	-	-	+	R	R	S	S	
DM041	F	RIYADH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	Clone F
DM026	M	RIYADH	+	-	+	-	-	-	+	-	-	-	+	R	R	S	S	F2
DM061	M	RIYADH	+	-	+	-	-	-	+	-	-	-	+	R	R	S	S	
DM052	F	ALRASS	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM058	M	ALKAFJI	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM042	M	TABOK	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM062	F	RIYADH	-	-	+	-	-	-	+	-	-	-	+	I	R	I	S	
DM053	M	RIYADH	+	-	+	-	+	-	+	-	-	-	-	R	R	S	S	Clone G
DM006	F	RIYADH	-	+	+	-	-	-	+	-	-	-	-	R	R	S	S	H1
DM007	F	RIYADH	-	+	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM008	F	RIYADH	-	+	+	-	-	-	+	-	-	-	-	R	R	S	R	
DM009	F	RIYADH	-	+	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM010	F	RIYADH	-	+	+	-	-	-	+	-	-	-	-	R	R	I	S	
DM011	F	RIYADH	-	+	+	-	-	-	+	-	-	-	-	R	R	S	R	
DM043	M	JAZAN	-	+	+	-	+	-	+	-	-	-	-	R	R	S	S	
DM059	M	RIYADH	-	+	+	-	-	-	+	-	-	-	+	R	R	S	S	
DM033	M	ASEER	-	+	+	-	+	-	+	-	-	-	-	R	R	S	S	
DM035	M	ASEER	-	+	+	-	+	-	+	-	-	-	-	R	R	S	S	
DM036	M	ASEER	-	+	+	-	+	-	+	-	-	-	-	R	R	S	S	Clone H
DM054	M	RIYADH	-	+	+	-	+	-	+	-	-	-	-	R	R	S	S	H2
DM056	M	RIYADH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM022	F	RIYADH	-	+	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM023	F	RIYADH	-	+	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM024	F	RIYADH	-	+	+	-	-	-	-	-	-	-	-	R	R	S	S	
DM025	F	RIYADH	-	+	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM046	M	ASEER	-	+	-	-	+	-	+	-	-	-	+	R	R	S	S	
DM049	M	ASEER	-	+	+	-	+	-	+	-	-	-	+	R	R	S	S	
DM051	M	ALMADINAH	-	+	-	-	+	-	+	-	-	-	-	I	R	S	S	
DM027	F	SHAGRA	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	Clone I
DM050	F	ALMADINAH	-	-	-	-	-	-	+	-	-	-	-	S	I	S	S	
DM005	M	KAMIS M	-	-	+	-	-	-	+	-	-	-	-	R	R	S	S	Clone J
DM038	F	ALAHSA	-	-	+	-	-	-	+	-	-	-	-	S	R	S	S	
DM014	F	KAMIS M	+	-	+	-	-	-	-	-	-	-	-	R	R	S	S	Clone K
DM016	F	ALQASEM	-	-	+	-	-	-	+	-	-	-	-	R	R	S	S	Clone L
DM018	M	RIYADH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	Clone M
DM019	M	RIYADH	+	-	+	-	-	-	-	-	-	-	-	R	R	S	S	
DM020	M	RIYADH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	
DM017	M	RIYADH	+	-	+	-	-	-	+	-	-	-	-	R	R	S	S	

Figure (1): Dendrogram analysis of 64 clinical isolates from patient with diabetes showing the genetic diversity of *A. baumannii* in Saudi Arabian hospitals. The broken line corresponds to the cutoff level (85%) used to define single PFGE clones. Dotted squares mark the boundaries of clusters B to F, H to J and M. (DM) abbreviation of diabetes mellitus samples numbers. R, resistant; S, susceptible and I, intermediate.

435 **Table 1** Nucleotide Sequence of primers used in this study
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Primer name	Nucleotide Sequence (5`- 3`)	Reference
OXA-51-F	TAATGCTTTGATCGGCCTTG	22
OXA-51-R	TGGATTGCACTTCATCTTGG	22
OXA-23-F	GATCGGATTGGAGAACCAGA	11
OXA-23-R	ATTCTTGACCGCATTTCCAT	11
OXA-40-F	GGTTGTTGGCCCCCTTAAA	11
OXA-40-R	AGTTGAGCGAAAAGGGGATT	11
IS*Aba1*-F	GTGCTTTGCGCTCATCATGC	23
IS*Aba1*-R	CATGTAAACCAATGCTCACC	23
IS*Aba2*-F	AATCCGAGATAGAGCGGTTC	23
IS*Aba2*-R	TGACACATAACCTAGTGCAC	23
IS*Aba3*-F	CAATCAAATGTCCAACCTGC	23
IS*Aba3*-R	CGTTTACCCCAAACATAAGC	23
IS*18*-F	CACCCAACTTTCTCAAGATG	23
IS*18*-R	ACCAGCCATAACTTCACTCG	23
IMP-F	GGAATAGAGTGGCTTAAYTCTC	24
IMP-R	CCAAACYACTASGTTATCT	24
VIM-F	GATGGTGTTTGGTCGCATA	24
VIM-R	CGAATGCGCAGCACCAG	24
GIM-F	TCGACACACCTTGGTCTGAA	24
GIM-R	AACTTCCAACTTTGCCATGC	24
SPM-F	AAAATCTGGGTACGCAAACG	24
SPM-R	ACATTATCCGCTGGAACAGG	24
SIM-F	TACAAGGGATTCGGCATCG	24
SIM-R	TAATGGCCTGTTCCCATGTG	24

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445 **Table 2** Phenotypic characteristics of the tested *A. baumannii* clinical isolates
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Antimicrobial agents	Number of isolates (%) (*n=64*)		
	Sensitive (S)	Intermediate (I)	Resistant (R)
Imipenem	3 (4.7)	2 (3.1)	59 (92.2)
Meropenem	0 (0)	2 (3.1)	62 (96.9)
Tigecycline	62 (96.9)	2 (3.1)	0
Colistin	62 (96.9)	0	2 (3.1)

Table 3 Genotypic characteristics of the tested *A. baumannii* clinical isolates

Characteristic	Number of isolates (%) (*n=64*)
$bla_{\text{OXA-23}}$	34 (53.1)
$bla_{\text{OXA-40}}$	19 (29.7)
Co existence of both $bla_{\text{OXA-40}}$ and $bla_{\text{OXA-23}}$	0 (0)
Absence of both $bla_{\text{OXA-40}}$ and $bla_{\text{OXA-23}}$	11 (17.2)
bla_{VIM}	59 (92.2)
bla_{SPM}	18 (28.1)
Co existence of both bla_{VIM} and bla_{SPM}	17 (26.6)
bla_{IMP}	0 (0)
bla_{GIM}	0 (0)
Bla_{SIM}	0 (0)
IS*Aba1*	58 (90.6)
IS*Aba2*	6 (9.4)

IS*Aba3*	13 (20.3)
IS*18*	0 (0)
Co existence of both IS*Aba1* and IS*Aba2*	5 (7.8)
Co existence of both IS*Aba1* and IS*Aba3*	11 (17.2)

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498 **Table 4** Profiles of *A. baumannii* tested isolates

Hospital location (Area)	Clone diversity	Mechanism of resistant	No. of isolates (%) (*n=64*)
RIYADH	A1 A2 B C1 C3 C4 D2 E F1 F2 G H1 H2 M	OXA-23, IS*Aba1*, IS*Aba2*, IS*Aba3*, VIM and SPM	34 (53.1)
BREDAH	B	OXA-23, IS*Aba1* and VIM	1 (1.6)
ALMADINAH	C1 D2 F1 H2 I	OXA-23, IS*Aba1*, IS*Aba2*, SPM and VIM	6 (9.4)
TABOK	C2 F2	OXA-23, IS*Aba1* and VIM	5 (7.8)

ANAK	D1	OXA-23 and IS*Aba1*	1 (1.6)
HAIL	D1	IS*Aba1*, VIM and SPM	1 (1.6)
ALDAWADME	E	OXA-23, IS*Aba1*, IS*Aba2*, VIM and SPM	2 (3.1)
ABHA	F1	OXA-23, IS*Aba1*, VIM and SPM	1 (1.6)
ALRASS	F2	OXA-23, IS*Aba1* and VIM	1 (1.6)
ALKAFJI	F2	OXA-23, IS*Aba1* and VIM	1 (1.6)
JAZAN	H1	OXA-40, IS*Aba1*, IS*Aba3* and VIM	1 (1.6)
ASEER	H1	OXA-40, IS*Aba1*, IS*Aba3*, VIM and SPM	5 (7.8)
SHAGRA	I	OXA-23, IS*Aba*1 and VIM	1 (1.6)
KAMIS MOSAIT	J K	OXA-23, IS*Aba*1 and VIM	2 (3.1)
ALQASEEM	L	IS*Aba*1 and VIM	1 (1.6)
ALAHSA	J	IS*Aba*1 and VIM	1 (1.6)

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