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Research

Characterization of carbapenem resistant *Acinetobacter baumannii* isolated from intensive care units in two teaching hospitals from Algeria and Tunisia

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Abstract

Introduction: This study was conducted to identify the enzymatic mechanism of carbapenem resistance in *A. baumannii* isolated from intensive care units of 2 teaching hospitals (Charles Nicolle hospital of Tunis and University hospital of Annaba). **Methods:** Twenty seven non repetitive carbapenem-resistant *A. baumannii* were collected (7 strains in Algeria and 20 in Tunisia). Antibiotic susceptibility was performed by disk diffusion method. MICs were determined by agar dilution method. EDTA-disk synergy test was performed for metallo-β-lactamases (MBL) phenotypic detection. Detection of $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$ and $bla_{OXA-58-like}$ families was performed by PCR followed by sequencing. Genetic relatedness between strains was investigated by pulsed-field gel electrophoresis (PFGE). **Results:** Strains were recovered especially from respiratory tract specimens (n=12) and blood (n=11). All strains were co-resistant to all β-lactams, gentamicin, amikacin and ciprofloxacin, but remainded susceptible to colistin. MBL production was negative for all isolates. $bla_{OXA-51-like}$ was detected in all strains and $bla_{OXA-23-like}$ in 23 strains. However, $bla_{OXA-58-like}$ and $bla_{OXA-24-like}$ were not found in any isolate. Six major PFGE patterns were found in the Tunisian isolates. However, the Algerian strains were clustered in one clone. **Conclusion:** This study shows a high distribution of blaOXA-23 in imipenem-resistant *A. baumannii* isolated in Tunisia and Algeria. It demonstrated the epidemic diffusion of this multidrug resistant pathogen. Thus, strengthening of prevention measures are required to control further spread of carbapenemases in the two countries.

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Introduction

Acinetobacter baumannii has emerged as a leading cause of nosocomial infections, particularly among critically ill patients in intensive care units [1,2] *A. baumannii* clinical isolates are commonly resistant to multiple antimicrobial drug classes and have the ability to survive in the environment for prolonged periods of time, which facilitates their persistence in hospitals and make them a frequent cause of hospital outbreaks and an endemic healthcare associated pathogens [3]. Carbapenems have been widely used to treat these infections [4], but a trend of increasing resistance to this antibiotic class has been reported worldwide [5]; limiting drastically the range of therapeutic alternatives. Carbapenem resistance in *A. baumannii* results mainly from beta-lactamases production [6,7]. Metallo-beta-lactamases are prevalent in East Asia and Western Europe and confer resistance to all beta-lactams except aztreonam [1]. However, the OXA-type carbapenemases have emerged as the most widespread beta-lactamases with carbapenemase activity [8,9]. These enzymes can be sub-devised into 5 distinct groups: intrinsic OXA-51-like and acquired, OXA-23-like (OXA-23, OXA-27 and OXA-49), OXA-24-like (OXA-24, OXA-25, OXA-26, OXA-40 and OXA-72), OXA-58-like and OXA-143 [10-13]. In addition to beta-lactamases, carbapenem resistance in *A. baumannii* may also result from porin or penicillin-binding protein modifications [5]. The aim of this study was to determine the enzymatic mechanism of carbapenem resistance in *A. baumannii* isolates recovered from intensive care units of two teaching hospitals (Tunisia and Algeria) and to characterize nosocomial outbreaks by antibiotyping and pulsed-field gel electrophoresis (PFGE).

Methods

Bacterial isolates: This study analyzed 27 unduplicated carbapenem-resistant *A. baumannii* clinical isolates, collected from 2 intensive care units of 2 teaching hospitals [Charles Nicolle hospital of Tunis, (n= 20) and the university hospital of Annaba (n=7)], during 2009. Strains were isolated from respiratory tract specimens (n=12), blood (n=11), material (n=3) and urine (n=1) of 27 different patients aged from 17 to 78 years; 21 males and 6 females (sex ratio 3.3). Demographic and clinical characteristics of all patients were shown in Table 1. Strain identification was performed by conventional techniques and confirmed by PCR amplification of the endogen $bla_{OXA\text{-}51\text{-}like}$ gene [14].

Antimicrobial susceptibility testing: Antibiotic susceptibility testing was performed using the disk diffusion method on Mueller Hinton agar. The minimal inhibitory concentrations (MICs) values of imipenem, meropenem, ticarcillin, ticarcillin /clavulanic acid; ceftazidime, cefepime and aztreonam were determined by the agar dilution technique. Current quality control testing was performed using the following organisms: *E. coli* ATCC 25922 and *P. Aeruginosa* ATCC 27853. The interpretation of the results was referred to the guidelines defined by the Clinical and Laboratory Standards Institute [3].

Screening for MBL-producing strains: Detection of MBL was done by double disk synergy test using an imipenem disk placed 10 mm from a disk saturated with 5µl of EDTA (0.5 M pH 8). An enlargement of the inhibition zone of imipenem facing the disk of EDTA was considered as a positive test [15].

PCR amplification and sequencing: $Bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}23\text{-}like}$ and $bla_{OXA\text{-}58\text{-}like}$ genes were detected by PCR simplex assays as previously described [16]. DNA sequencing was performed by the dideoxy chain terminator method with Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analyzed using an ABI Prism 3100 genetic analyser (Applied Biosystems). Similarity searches and alignments of the nucleotide sequences were performed with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

Pulsed-field gel electrophoresis (PFGE): Molecular typing of isolates was carried out, as described previously by Pulsed-Field Gel Electrophoresis (PFGE) using *ApaI* (Invitrogen) restriction endonuclease [14]. The *Apa*I restriction profiles were compared by visual inspection according to the criteria proposed by Tenover et al [17].

Results

All strains were co-resistant to all β-lactams, gentamicin, amikacin, ciprofloxacin but remained susceptible to colistin. They showed high level of resistance to ticarcillin (MIC_{50}>2048 µg/mL), ticarcillin-clavulanic acid (MIC_{50}>1024 µg/mL), aztreonam (MIC_{50} ≥256 µg/mL), ceftazidim (MIC_{50}=2048 µg/mL), imipenem (MIC_{50}= 256 µg/mL), meropenem (MIC_{50}=128 µg/mL) and cefepime (MIC_{50}=128 µg/mL) (Table 2). All strains showed a negative EDTA disk synergy

test. PCR experiments for the naturally-occurring $bla_{OXA-51-like}$ gene gave positive results for all strains tested. The $bla_{OXA-23-like}$ was present in 23 strains (5 from Algeria and 18 from Tunisia) and the sequencing of the amplified fragments confirmed the presence of bla_{OXA-23} in all isolates, whereas no isolate harbored $bla_{OXA-24-like}$ or $bla_{OXA-58-like}$ genes. Six major PFGE patterns were found in the Tunisian isolates; named from A to F (Figure 1B). The genotype A was the most prevalent (12 strains) with 3 pulsotypes A1 (n=8), A2 (n=3) and A3 (n=1). However, the Algerian strains were clustered in one pattern G (Figure 1A).

Discussion

A. baumannii has been stealthily gaining ground as an agent of serious nosocomial infections, including bacteremia, pneumonia, urinary tract and wound infections [18]. Historically, it has been associated with opportunistic infections; the last two decades have seen an increase in both the incidence and seriousness of *A. baumannii* infection, with the main targets being immunocompromized patients in intensive-care units [19]. The increase in *A. baumannii* infections has paralleled the alarming development of resistance [20]. Actually, multidrug-resistant *A. baumannii* is recognized to be among the most difficult antimicrobial-resistant Gram-negative bacilli to control and treat, especially if isolates are resistant to the carbapenem class of antimicrobial agents [21]. This study aimed to investigate the enzymatic mechanism of 27 carbapenem resistant *A. baumannii* clinical strains causing nosocomial infections in 27 debilitated patients hospitalized in two intensive care units of 2 different hospitals. As it was previously reported they mainly caused pneumonia and bacteremia [22, 23]. The known risk factors for acquisition of *A. baumannii* infections are length of intensive care unit [6] stay, use of central venous catheters, antibiotic use especially extended spectrum β-lactams and fluoroquinolones, urinary catheters and comorbidities [24]. In the present study, for all patients' length of hospital stay exceeded 6 days (6 to 90 days) and 18 of them were previously hospitalized. Empiric antibiotherapy based on cephalosporin 3rd generation (N=11), amoxicillin/clavulanique acid (N=14) and imipenem (N=2) in association with aminosides or fluoroquinolones was noted in 11, 14 and 2 cases, respectively. Clinical outcome was favorable for only 1/3 of patients.

All strains were co-resistant to all β-lactams, gentamicin, amikacin, ciprofloxacin but 6 isolates remained susceptible to netilmicin, 2 isolates to tobramycin and 3 strains to trimethoprim-sulfamethoxazol. All strains were susceptible to colistin. Despite its renal toxicity, colistin has become useful antibiotic for treating infections caused by carbapenem resistant pathogens [25], but dissemination of *A. baumannii* resistant to colistin is worrying. In another side, many studies provide the activity of tigecycline against multidrug *A. baumannii* clinical isolates [26]. Antibiotic resistance in *A. baumannii* is frequently an interplay between several different mechanisms [5]. This bacteria produces naturally 2 intrinsic types of β-lactamases [5]. An AmpC type cephalosporinase expressed at a basal level and an oxacillinase represented by the OXA-51/69 variants [5]. When ISAba1 were inserted upstream of blaAmpC and $bla_{OXA-51-like}$ genes, the strains become resistant to ceftazidime and to carpenems, respectively [27, 28]. The $bla_{OXA-51-like}$ genes are chromosomally located in all of the *A. baumannii* isolates studied to date and their presence has been used to confirm identification of *A. baumannii* [29]. In addition to those naturally occurring β-lactamases, several acquired β-lactamases have been identified as a source of carbapenem resistance in *A. baumannii*. They belong to either class D (oxacillinases) or class B (metallo-β-lactamases [MBLs] [5]. The first carbapenem-hydrolysing oxacillinase was OXA-23 identified in Scotland in 1985 [30]. The gene encoding this enzyme, named ARI-1, was plasmid born and was associated with the IS*Aba1* transposase [30]. Since then, the IS-OXA23 structure has been found among Acinetobacter isolates from various countries [31, 32]. The bla_{OXA-23} genes have been identified as part of transposon structures, namely Tn2006 and Tn2007 [31]. Interestingly, the reservoir (natural producer) of this gene has been identified as being *A. radioresistens* [33]. This *Acinetobacter* species shares the same reservoir with *A. baumannii*, the skin flora in humans [31].

Our study revealed the presence of this carbapenemase in Tunisian (n=18) and Algerian (n=5) strains. However, $bla_{OXA-24-like}$ and $bla_{OXA-58-like}$ were not detected in any of ours strains. In the 4 strains, where only bla_{OXA-51} was detected, resistance can be explained by non enzymatic mechanisms or insertion of IS*Aba1* sequences [28, 34]. MBLs are powerful carbapenemases [35]. They have been identified worldwide in *A. baumannii* [36, 37]. Four groups were described in *A. baumannii*: IMP-like, VIM-like SIM-1 and NDM-1 enzymes [35]. MBL production was not detected in any of our strains. However, many studies describe bla_{NDM-1} in *A. baumannii* isolated from Algerian patients evacuated to France

[36, 38]. Resistance to carbapenems in *A. baumannii* may be also due to an association between several β-lactamases and other mechanisms of resistance, such as porin(s) loss, over expression of the naturally occurring AdeABC efflux pump, and rarely modification of penicillin-binding proteins (PBPs) [39]. Nosocomial clonal diffusion of multidrug resistant *A. baumannii* has been reported from various regions of the world [40]. Although antibiotyping may alert to the dissemination of a multiresistant *A. baumannii* strain, distinguishing between strains with slight differences in their resistance profiles may be difficult. Only genotypic methods including PFGE of chromosomal DNA restriction fragments have been used to investigate nosocomial *A. baumannii* outbreaks [14]. Tunisian *A. baumannii* isolates were clustered in 6 different molecular epidemiology patterns. However, Algerian strains were clustered in one clone G.

Conclusion

This study shows a high distribution of bla_{OXA-23} in imipenem-resistant *A. baumannii* isolated in Tunisia and Algeria. It demonstrated the epidemic diffusion of this multidrug resistant pathogen. Thus, strengthening of prevention measures are required to control further spread of carbapenemases in the two countries.

What is known about this topic

- Acinetobacter baumannii is a nosocomial pathogen;
- It has a high ability to develop antibiotic resistance and it has become a problematic challenge in the modern healthcare system;
- The molecular and genetic mechanisms of gaining multidrug resistance in ACB complex are well known.

What this study adds

- Resistance of A.baumannii to carbapenems is mostly associated with the gene OXA-23 in Algeria and Tunisia;
- The description of the genotypic epidemiology of Acinetobacter baumannii.

Competing interests

The authors declare no competing interests.

Authors' contributions

Sabrina Amiri, Samia Hammami, Kamel Amoura: participated in the identification of Acinetobacter baumannii, analysis and interpretation of the phenotypic and genotypic tests, writing and critically revising manuscript. Dekhil mazouz, Boutiba Ilhem: participated in the interpretation and writing of manuscript. All authors have read and agreed to the final version of this manuscript.

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Tables and figure

Table 1: Demographic and clinical characteristics of patients with carbapenem-resistant *Acinetobacter baumannii*

Table 2: The MICs, PCR results and PFGE clusters of the clinical isolates

Figure 1: PFGE patterns of carbapenem-resistant *A. baumannii*. PFGE patterns are indicated by the letters above the lanes numbers. Lane M: lambda ladder (Bio-Rad). A: PFGE of Algerian isolates. B: PFGE of Tunisian isolates

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Table 1: Demographic and clinical characteristics of patients with carbapenem-resistant *Acinetobacter baumannii*

References	Age [year]/ sex	Site of isolation	Date of isolation	lenght of stay	outcome
R3267 Tu	25/F	Blood	22/01/2009	6	Died
R4027 Tu	23/M	Material	27/01/2009	33	Died
R4035 Tu	21/M	Pulmonary	28/01/2009	13	Improved
R7950 Tu	45/M	Blood	21/02/2009	-	-
R8898 Tu	45/M	Blood	27/02/2009	25	improved
R8892 Tu	45/M	Material	27/02/2009	25	Died
R9495 Tu	29/M	Pulmonary	02/03/2009	90	Died
R10222 Tu	34/M	Blood	10/03/2009	19	Improved
R11433 Tu	67/M	Blood	16/03/2009	35	Died
R13761 Tu	71/M	Blood	31/03/2009	15	Died
R13787 Tu	78/M	Pulmonary	01/04/2009	19	Died
R15217 Tu	72/M	Pulmonary	10/04/2009	20	Died
R17020 Tu	69/F	Blood	20/04/2009	31	Died
R22573 Tu	23/M	Blood	20/05/2009	78	Died
R31908 Tu	68/F	Pulmonary	16/07/2009	15	Died
R34160 Tu	60/F	Urine	01/08/2009	13	Improved
R36120 Tu	17/M	Blood	18/08/2009	17	Improved
R37139 Tu	63 /M	Material	24/08/2009	41	Improved
R43146 Tu	28/M	Blood	06/10/2009	66	Died
R49528 Tu	20/M	Blood	14/03/2009	7	Died
R135 An	18/F	Pulmonary	17/11/2009	12	Died
R1824 An	27/M	Pulmonary	18/11/2009	10	Died
R1825An	34/M	Pulmonary	18/11/2009	7	improved
R189An	26/F	Pulmonary	03/12/2009	20	Died
R1716 An	72/M	Pulmonary	03/11/2009	8	Improved
R1711 An	55/M	Pulmonary	01/11/2009	19	Died
R1725 An	68/M	Pulmonary	29/10/2009	23	Died

Tu: Tunis, An: Annaba, M: male, F: female

Table 2: The MICs,PCR results and PFGE clusters of the clinical isolates

Strains	MER	IMP	CAZ	ATM	FEP	TIC	TCC	*Bla-OXA-23*	*Bla*-OXA-24	*Bla*-OXA-51	*Bla*-OXA-58	PFGE patterns
R3267	128	128	1024	256	128	>2048	>1024	+	-	+	-	B
R4027	256	512	2048	256	128	>2048	>1024	+	-	+	-	A1
R4035	128	512	>2048	128	128	>2048	>1024	+	-	+	-	A1
R7950	128	512	>2048	128	256	>2048	>1024	+	-	+	-	A1
R8898	128	512	>2048	128	128	>2048	>1024	+	-	+	-	A1
R8892	512	512	>2048	512	512	>2048	>1024	+	-	+	-	A1
R9495	128	512	2048	128	64	>2048	>1024	-	-	+	-	A3
R1022A	128	512	1024	128	64	>2048	>1024	+	-	+	-	A1
R11433	64	256	1024	64	128	>2048	>1024	+	-	+	-	A1
R13761A	512	512	>2048	256	512	>2048	>1024	+	-	+	-	A1
R13787	32	256	128	128	32	>2048	>1024	-	-	+	-	A2
R15217	32	128	>2048	>512	>512	>2048	>1024	+	-	+	-	C
R17020	64	256	>2048	256	256	>2048	>1024	+	-	+	-	A2
R22573	64	256	>2048	>512	>512	>2048	>1024	+	-	+	-	C
R31908	16	32	>2048	256	128	>2048	>1024	+	-	+	-	A2
R34160	64	256	512	512	128	>2048	>1024	+	-	+	-	F
R36120	128	512	512	512	256	>2048	>1024	+	-	+	-	D
R37139	128	512	512	512	256	>2048	>1024	+	-	+	-	D
R43146	32	64	1024	256	64	>2048	>1024	+	-	+	-	E
R49528	32	128	128	64	32	>2048	>1024	+	-	+	-	E
R135an	64	256	>256	1024	>521	>2048	>2048	+	-	+	-	G
R1824 an	128	256	>256	1024	>521	>2048	>2048	+	-	+	-	G
R1825an	128	256	>256	1024	128	>2048	>2048	-	-	+	-	G
R1892an	128	256	>256	1024	128	>2048	>2048	+	-	+	-	G
R1716 an	128	256	256	>1024	128	>2048	>2048	-	-	+	-	G
R1711 an	32	256	256	1024	128	>2048	>2048	+	-	+	-	G
R1725 an	128	256	256	>1024	128	>2048	>2048	+	-	+	-	G

TCC:ticarcillin/clavulanic acid , CAZ: Ceftazidime, FEP: cefepime, ATM: aztreonam , IMI: imipenem, MER :meropenem

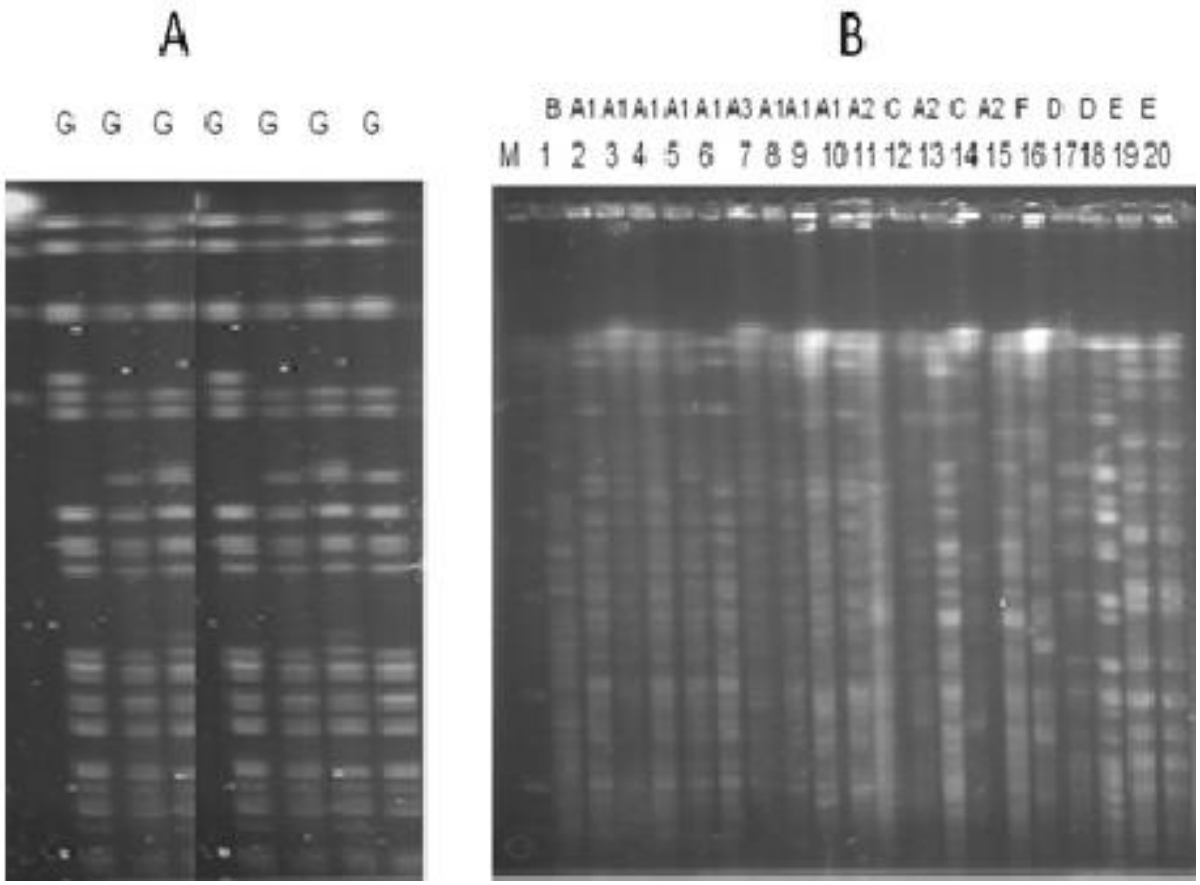

Figure 1: PFGE patterns of carbapenem-resistant *A. baumannii* .PFGE patterns are indicated by the letters above the lanes numbers. Lane M: lambda ladder (Bio-Rad). A: PFGE of Algerian isolates. B: PFGE of Tunisian isolates