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

Sabrina Amiri¹,², Samia Hammami², Kamel Amoura³, Mazouz Dekhil¹, Ilhem Boutiba-Ben Boubaker²,³

¹Laboratory of Microbiology, University Hospital of Annaba, Algeria, ²Laboratory of Antimicrobial Resistance, Faculty of Medicine of Tunis-Tunisia University of Tunis El Manar, Tunies, ³Laboratory of Microbiology of Charles Nicolle Hospital, Boulevard April 9, 1006 Tunis, Tunisia

Corresponding author: Sabrina Amiri, Laboratory of Microbiology, University Hospital of Annaba, Algeria

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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 blaOXA-23-like, blaOXA-24-like, blaOXA-51-like and blaOXA-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 remained susceptible to colistin. MBL production was negative for all isolates. blaOXA-51-like was detected in all strains and blaOXA-23-like in 23 strains. However, blaOXA-58-like and blaOXA-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-dived 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 blaOXA-51-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: blaOXA-24/26-ics, blaOXA-23-Ics and blaOXA-58-ics 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 Apal (Invitrogen) restriction endonuclease [14]. The Apal 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≥2048 µg/mL), ticarcillin-clavulanic acid (MIC≥1024 µg/mL), aztreonam (MIC≥256 µg/mL), ceftazidim (MIC≥2048 µg/mL), imipenem (MIC≥256 µg/mL), meropenem (MIC≥128 µg/mL) and cefepime (MIC≥128 µg/mL) (Table 2). All strains showed a negative EDTA disk synergy
test. PCR experiments for the naturally-occurring blaOXA-51-like gene gave positive results for all strains tested. The blaOXA-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 blaOXA-24-like or blaOXA-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**

*Acinetobacter 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 antibiotic therapy based on cephalosporin 3rd generation (N=11), amoxicillin/clavulanic 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 trimethoprimsulfamethoxazol. 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 ISAb1 were inserted upstream of blaAmpC and blaOXA-51-like genes, the strains become resistant to ceftazidime and to carpenems, respectively [27, 28]. The blaOXA-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 ISAb1 transposase [30]. Since then, the IS-OXA23 structure has been found among Acinetobacter isolates from various countries [31, 32]. The blaOXA-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, blaOXA-23-like and blaOXA-58-like were not detected in any of ours strains. In the 4 strains, where only blaOXA-51-like was detected, resistance can be explained by non enzymatic mechanisms or insertion of ISAb1 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 blaNDM-1 in *A. baumannii* isolated from Algerian patients evacuated to France.
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). Nosocomial clonal diffusion of multidrug resistant *A. baumannii* has been reported from various regions of the world. 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. 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 *bla*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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| References | Age [year]/ sex | Site of isolation | Date of isolation | length 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       | -                | Died          |
| R8898 Tu   | 45/M Blood     | 27/02/2009       | 25               | Died          |
| 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               | Died          |
| 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  | 128  | 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 | 128  | 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.