Molecular characterization of carbapenemases of clinical *Acinetobacter baumannii*–*calcoaceticus* complex isolates from a University Hospital in Tunisia

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Abstract

The aim of this study was to identify the carbapenemases from clinical carbapenem-resistant *Acinetobacter baumannii*–*calcoaceticus* complex (CRABC) isolates and to assess their potential dissemination by conjugation and natural transformation. CRABC ($n = 101$) were collected consecutively from inpatients of the University Hospital of Monastir, Tunisia, from 2013 to 2016. Antimicrobial susceptibility was determined by the disk diffusion method and E-test. Carbapenemase-encoding genes were screened by PCR. Genotyping was performed by Pasteur MLST scheme. Isolates were resistant to all beta-lactams, fluoroquinolones and aminoglycosides while 80 and 90% were susceptible to tigecycline and colistin, respectively. Resistance and intermediate resistance to imipenem were 87 and 13%, respectively. The genes *bla*_{OXA-24}-like, *bla*_{OXA-58}-like, *bla*_{OXA-143}-like, *bla*_{OXA-48}-like, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{KPC} were not found. The *bla*_{OXA-51}-like and *bla*_{OXA-23}-like genes were present in 100 and 82.17% isolates, respectively. One isolate (< 1%) carried *bla*_{NDM-1} and *bla*_{OXA-51}-like and belonged to Sequence Type 85 (ST85). Absence of transconjugants suggests a chromosomal location of NDM-1 determinant. The *bla*_{NDM-1} gene was inserted in a truncated form of Tn125, which may explain the absence of *bla*_{NDM-1} carrier-transformants. To our knowledge, this is the first report of the finding of NDM-positive *A. baumannii* in Tunisian territory. The study shows that despite the low prevalence and potential spread of NDM-1 enzyme among CRABC, continuous regional antimicrobial resistance surveillance and improved infection control measures are required in Tunisia to prevent further dissemination.

Keywords *Acinetobacter* · Tn125 · Epidemiology · Carbapenemases · Natural transformation · Antimicrobial resistance

Introduction

Acinetobacter baumannii–*calcoaceticus* complex are non-fermentative Gram-negative bacilli that includes *A. baumannii*, an opportunistic and emerging pathogen associated with nosocomial infections worldwide. It causes ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound infections and meningitis, especially in critically ill patients (Dijkshoorn et al. 2007).

Acinetobacter baumannii isolates are often resistant to many antimicrobial classes, including carbapenems. Carbapenems have been the mainstay of treatment for *A. baumannii* infections, but the widespread of carbapenem-resistant *A. baumannii* (CRAB) isolates has become an important health problem worldwide, leading to limited therapeutic options. Different mechanisms are responsible for carbapenem resistance in *A. baumannii*, namely reduced permeability of outer-membrane, efflux, and enzymatic hydrolysis.

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In *A. baumannii*, carbapenem resistance is mostly related to the production of carbapenem-hydrolyzing β -lactamases of Ambler class D (CHDLs) that include the oxacillinases families OXA-23, OXA-24/40, OXA-58, OXA-143, OXA-235, and the intrinsic oxacillinase *bla*_{OXA-51}-like associated with *ISAbal*. It can also be mediated by Ambler class A β -lactamases (GES, KPC) and class B metallo- β lactamases (MBLs) (IMP, VIM and NDM) (Da Silva and Domingues 2016).

Among the MBLs, the NDM-type β -lactamases are emergent carbapenemases. NDM-1 determinant was initially identified in *Klebsiella pneumoniae* and *Escherichia coli* isolates recovered from a Swedish patient who was previously hospitalized in India (Yong et al. 2009). The *bla*_{NDM-1} rapidly disseminated in the world but India remains the major reservoir of NDM producers (Nordmann et al. 2011). *Enterobacteriaceae* are usually referred to as the main NDM-producers (van Duin 2017). Since the first description of NDM-1, 18 variants of the *bla*_{NDM} have been identified (Bacterial Antimicrobial Resistance Reference Gene Database—<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047>. Accessed on 6 July 2017). To date, only NDM-1 and NDM-2 were reported in *A. baumannii* (Da Silva and Domingues 2016).

In Tunisia, the dissemination of carbapenemases of Ambler class D in *A. baumannii* has been reported in a few studies (Mansour et al. 2008; Hammami et al. 2011, 2017; Charfi-Kessiss et al. 2014; Chihi et al. 2016). In 2013, Bonnin et al. reported the finding of an *A. baumannii* isolate carrying the NDM-1 determinant isolated from a Tunisian patient hospitalized in France (Bonnin et al. 2013). Otherwise, and to our knowledge, the carbapenemase NDM-1 or other variants have never been reported in clinical *A. baumannii* in Tunisian territory. The main goals of the present study were to identify the carbapenemases in clinical isolates of the complex *A. baumannii*–*calcoaceticus* with reduced susceptibility to imipenem and to evaluate their potential dissemination by conjugation and natural transformation.

Materials and methods

Bacterial isolates and identification

A total of 101 non-duplicate *A. baumannii*–*calcoaceticus* complex non-susceptible to imipenem isolates were collected from clinical samples in the Laboratory of Microbiology at the UHMT, which is a tertiary care hospital with 858 beds. The clinical samples were obtained between 2013 and 2016 from patients hospitalized in different wards. Isolates were identified in the laboratory, using Api20E (BioMérieux), and identified as belonging to complex *A. baumannii*–*A. calcoaceticus*. PCR amplification of the *bla*_{OXA-51}-like

gene was used for presumptive identification of *A. baumannii* (Turton et al. 2006). The conserved *rpoB* gene of isolate Ab51 was amplified and sequenced (Stabvida) to further confirm the species (La Scola et al. 2006).

Susceptibility testing and screening for MBL-producing strains

Antibiotic susceptibility was determined on Mueller–Hinton agar using the standard disk diffusion procedure. The following antibiotics were tested: ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ceftazidime, cefpirome, imipenem, ciprofloxacin, gentamicin, tobramycin, amikacin, tigecycline and colistin. Results were interpreted according to the recommendations of the *Comité de l'antibiogramme de la Société Française de Microbiologie* (CA-SFM) (2013) (<http://www.sfm-microbiologie.org>). For tigecycline, as there are no guidelines for the interpretation of the susceptibility to this antibiotic in *A. baumannii*, we followed the criteria previously described by Jones et al. (2007). The minimum inhibitory concentrations (MICs) of imipenem were determined using an E-test (AB BioMérieux) and the MIC values were interpreted according to the guidelines of CA-SFM guidelines (2013). All isolates were screened for metallo- β -lactamases production using the EDTA double disk synergy test and combined disc-test. Isolates were considered MBL positive if there was an increase of ≥ 7 mm in the zone of inhibition around the imipenem + EDTA disc as compared to imipenem disk alone (Yong et al. 2002).

Screening of carbapenemase genes by PCR

The carbapenemase-encoding genes of the Ambler classes A, B and D were screened by multiplex and single PCR, using specific primers for *bla*_{OXA-23}-like, *bla*_{OXA-24}-like, *bla*_{OXA-58}-like, *bla*_{OXA-143}-like, *bla*_{OXA-48}-like, *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM} and *bla*_{KPC} as previously described (Yigit et al. 2001; Da Silva et al. 2002; Woodford et al. 2006; Poirel et al. 2011; Erturk et al. 2014; Cardoso et al. 2002). Presence of the *ISAbal* insertion sequence upstream the *bla*_{OXA-23}-like and *bla*_{OXA-51}-like genes was also assessed by end-point PCR, combining one primer that binds in *ISAbal* (Heritier et al. 2006) and one binding in the *bla*_{OXA-23}-like (Woodford et al. 2006) and *bla*_{OXA-51}-like (Turton et al. 2006) genes, respectively. All the primers used in this study are shown in Table 1.

Identification of the *bla*_{NDM} variant, genetic environment and plasmid typing

The *bla*_{NDM} gene is usually associated with the Tn125 transposon, where one copy of the *ISAbal25* is located upstream

Table 1 Primers used in the amplification of selected genes

Gene(s)	Nucleotide sequences (5'→3')	Product size (bp)	References
<i>bla</i> _{OXA-51} -like	F: TAATGCTTTGATCGGCCTTG R: TGGATTGCACTTCATCTTGG	353	Turton et al. (2006)
<i>bla</i> _{OXA-23} -like	F: GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTCCAT	501	Woodford et al. (2006)
<i>bla</i> _{OXA-24} -like	F: GGTTAGTTGGCCCCCTTAAA R: AGTTGAGCGAAAAGGGGATT	246	Woodford et al. (2006)
<i>bla</i> _{OXA-58} -like	F: AAGTATTGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	599	Woodford et al. (2006)
<i>bla</i> _{OXA-143} -like	F: CAGGAAGTACAACGCATCCA R: TGCTGGACTTGAGGATCAAA	158	Higgins et al. (2009)
<i>bla</i> _{OXA-48} -like	F: TTGGTGGCATCGATTATCGG R: GAGCACTTCTTTGTGATGGC	733	Poirel et al. (2004)
<i>bla</i> _{NDM}	F: GTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621	Poirel et al. (2011)
<i>rpoB</i>	Ac1055F: GTGATAARATGGCBGGTCGT Ac1598R: CGBGCRTGCATYTTGTCTRT	450	La Scola et al. (2006)
<i>bla</i> _{VIM}	F: ACTCACCCCATGGAGTTTT R: ACGACTGAGCGATTGTGTG	815	Cardoso et al. (2002)
<i>bla</i> _{IMP}	F: CTACCGCAGCAGAGTCTTTG R: AACCAGTTTTGCCTTACCAT	587	Da Silva et al. (2002)
<i>bla</i> _{KPC}	F: TATCGCCGTCTAGTTCTGCTGCT R: ACTGCCCGTTGACGCCCAAT	871	Yigit et al. (2001)
<i>ISAbal-bla</i> _{OXA-23} -like	F: AATGATTGGTGACAATGAAG R: ATTTCTGACCGCATTTCCAT	1433	Heritier et al. (2006); Woodford et al. (2006)
<i>ISAbal-bla</i> _{OXA-51} -like	F: AATGATTGGTGACAATGAAG R: TGGATTGCACTTCATCTTGG	1252	Heritier et al. (2006); Turton et al. (2006)
<i>ISAbal25-bla</i> _{NDM}	IPIS_F: AGATGTATATTCTGTGACC NDM-R: CGGAATGGCTCATCACGATC	1104	This study; Poirel et al. (2011)
<i>Tn125</i>	IPIS_F: AGATGTATATTCTGTGACC TRANSIS_R: AAACAACGGATCGCTTCAAC	8517	This study; Pfeifer et al. (2011)
Δ <i>Tn125</i>	IPIS_F: AGATGTATATTCTGTGACC ISAbal4_F: AACTCAGCACTCAATTCAGC NDM-F: GTTTGGCGATCTGGTTTTC	3832 or 3349 ^a	This study; Poirel et al. (2011)

^aObtained with IPIS_F + ISAbal4_F or NDM-F + ISAbal4_F, respectively

the carbapenemase gene (Poirel et al. 2012). We designed some primers and combined them with described primers to analyse the genetic environment. The primers IPIS_F and NDM-R (Table 1), binding in *ISAbal25* and *bla*_{NDM}, respectively, were used for PCR amplification in the *bla*_{NDM}-positive isolate. The amplicon was purified with Illustra ExoStart (GE Healthcare), following manufacturer's instructions, and sequenced at Stabvida. Presence of the *Tn125* was checked with primers IPIS_F and TRANSIS_R (Pfeifer et al. 2011), binding in the *ISAbal25*, located on both ends of the transposon. Since a truncated isoform of *Tn125* was also described in *A. baumannii* (Bonnin et al. 2012), the Δ *Tn125* was screened by PCR with primers IPIS_F and ISAbal4_F and with NDM-F plus ISAbal4_F (Table 1). All these PCRs were performed with the Phusion polymerase (ThermoFisher Scientific). Cycling conditions were: initial denaturation cycle at 98 °C for 30 s, followed by 30 cycles

of denaturation at 98 °C for 10 s, annealing at 58 °C for 10 s, extension at 72 °C for 30 s per 1 kb of expected PCR product, and final extension at 72 °C for 5 min.

Plasmid typing was performed in the NDM-positive strain to identify the most common plasmid Inc groups, namely FIA, FIB, FIC, HI1, HI2, I1-I γ , L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA (Carattoli et al. 2005).

Multilocus sequence typing

The multilocus sequence typing (MLST) technique was performed for the NDM-positive *A. baumannii* (Diancourt et al. 2010), using the internal fragments of seven housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB* and *rpoB*), according to the schemes available at Institute Pasteur's MLST website (<http://www.pasteur.fr/mlst>). The PCR products were purified with Illustra ExoStart (GE Healthcare)

sequenced at Stabvida. The sequences of the seven house-keeping genes were analyzed by comparing them to the MLST database (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html>).

Conjugation and natural transformation assays

Conjugation assays were performed in duplicate, with NDM-positive *A. baumannii* Ab51 as the donor cell and *E. coli* J53 (azide resistant) as the recipient by liquid mating broth method. Cultures of donor and recipient cells (1:10) were added to 5 ml of fresh Trypticase soy broth (TSB) and incubated overnight at 37 °C. Transconjugants were selected on Trypticase soy agar (TSA) plates containing sodium azide (150 µg/ml; Sigma) and cefotaxime (1.25 µg/ml; Sigma) to select for plasmid-encoded resistance. Acquisition of the *bla*_{NDM} gene by natural transformation was tested with total free DNA (naked DNA) from *A. baumannii* Ab51 as donor and the clinical naturally competent strain *A. baumannii* A118 as the recipient (Ramirez et al. 2010), following the Wilharm and colleagues protocol (Wilharm et al. 2013). Selection of transformants was performed in Luria–Bertani (LB; Fluka) agar (Liofilchem) plates supplemented with cefotaxime 30 µg/ml or imipenem (Sigma) 4 µg/ml. Positive and negative controls were performed with homologous DNA and water, respectively. Two independent transformation assays were performed in triplicate.

Results

Origin and identification bacterial isolates

Of the 101 carbapenem-resistant *Acinetobacter baumannii*–*calcoaceticus* complex (CRABC) isolates, 51.48% were isolated from tracheal aspirate, 7.92% from bronchial

aspirate and 3.96% from sputum. Other samples were from skin wounds (10.89%), 5.94% from catheters, 5.94% from blood, 3.96% from drains, 2.97% from urine, 2.97% from other non-specified sources, 0.99% from amniotic fluid, 0.99% from genital tract, 0.99% from liquid puncture (ascites, pleural effusion, pericardial effusion) and 0.99% from cerebrospinal fluid. Isolates were recovered from patients in various hospital units, including the intensive care unit (ICU) (70.29%), surgery (11.88%), internal medicine (9.9%), followed by gynecology and emergency (2.97% each), pediatric and from other departments (0.99% each).

All 101 isolates were identified at the hospital as belonging to the complex *A. baumannii*–*A. calcoaceticus* and carried the *bla*_{OXA-51}-like, suggesting that they were *A. baumannii* (Turton et. 2006) since it is the most common multiresistant species found at hospital settings, which is assumed by the majority of the studies with numerous isolates. Isolates with specific characteristics, like carrying a metallo-β-lactamase gene, should be identified by sequencing conserved genes. For the *bla*_{NDM}-positive strain, further confirmation was done by sequencing of the conserved *rpoB* gene since it has been shown that rarely some *A. non-baumannii* strains may carry *bla*_{OXA-51}-like as well (Lee et al. 2009).

Antibiotic susceptibility testing

The results of antibiotic susceptibility testing revealed that the isolates were resistant to almost all antibiotics tested that included β-lactams, fluoroquinolones and aminoglycosides (Table 2). Ninety per cent and 80% were susceptible to colistin and tigecycline, respectively. The MICs of imipenem ranged from 4 to 32 mg/L; 87.13% of the isolates were resistant to imipenem and the remaining were classified as intermediate. These latter were considered as CRABC. In addition, the activity of β-lactamase was inhibited by EDTA

Table 2 Antibiotic susceptibility pattern of 101 *A. baumannii*–*calcoaceticus* complex isolates

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Ticarcillin	0 (0)	0 (0)	101 (100)
Ticarcillin/clavulanic acid	0 (0)	0 (0)	101 (100)
Piperacillin	0 (0)	0 (0)	101 (100)
Piperacillin /tazobactam	0 (0)	0 (0)	101 (100)
Ceftazidime	0 (0)	0 (0)	101 (100)
Cefpirome	0 (0)	0 (0)	101 (100)
Imipenem	0 (0)	13 (12.87)	88 (87.13)
Ciprofloxacin	0 (0)	0 (0)	101 (100)
Gentamicin	11 (10.89)	0 (0)	90 (89.11)
Tobramycin	42 (41.58)	0 (0)	59 (58.42)
Amikacin	8 (7.92)	7 (6.93)	86 (85.15)
Tigecycline	81 (80.2)	8 (7.92)	12 (11.88)
Colistin	91 (90.1)	0 (0)	10 (9.9)

solution in one strain (Ab51), suggesting the production of a MBL by this strain.

Identification of the carbapenem resistance genes

The genes *bla*_{OXA-24}-like, *bla*_{OXA-58}-like, *bla*_{OXA-143}-like, *bla*_{OXA-48}-like, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{KPC} were not detected. The *bla*_{OXA-51}-like was present in all the isolates (100%) and 82.17% carried the gene *bla*_{OXA-23}-like. The *ISAbal* was located upstream the gene in 19.8% and 55.4% of the *bla*_{OXA-51}-like and the *bla*_{OXA-23}-like-carrying isolates, respectively. The gene *bla*_{NDM} was detected in only one isolate, Ab51. This isolate did not carry any other tested acquired carbapenemase genes except *bla*_{OXA-51}-like.

Characterization of the *bla*_{NDM}-positive isolate

The sequencing results identified the *bla*_{NDM-1} variant in *A. baumannii* Ab51. This strain was isolated from a bronchial aspirate in a patient hospitalized in the ICU ward. The imipenem MIC was > 32 mg/L.

One plasmid was identified belonging to the replicon type IncW. Conjugation and natural transformation failed to produce transconjugants or transformants with *bla*_{NDM}, respectively, as confirmed by PCR, both on cefotaxime or imipenem selection plates.

Figure 1 shows the genetic environment of the *bla*_{NDM} gene and the strategy used for its detection. The *bla*_{NDM-1} gene was found embedded into Δ Tn/25. Genotyping by MLST revealed that the *bla*_{NDM-1} positive strain belongs to the sequence type 85 (ST85).

Discussion

Acinetobacter baumannii clinical isolates are usually multidrug resistant (MDR) and carbapenems have been used as one of the last therapeutic resort for the treatment of these MDR infections. The emergence of CRAB have been observed worldwide, including in countries of the Mediterranean basin (Mathlouthi et al. 2016). However, in Tunisia there is

a limited knowledge in the epidemiology of clinical CRAB producing carbapenemases.

During the 4 years (2013–2016) of the study, 101 non-repetitive CRABC were collected from different patients at the UHMT. The majority of CRABC isolates were collected from inpatients of the ICU and from respiratory tract samples, but only a small percentage was isolated from blood samples. Our results differ from a previous study conducted in Tunisia (Hammami et al. 2011) that showed that CRAB strains were mostly isolated from blood cultures (32%), followed by pulmonary specimens (26%), with the majority of the samples collected from surgery ward (60%) and ICU (22%).

All the isolates were resistant or showed reduced susceptibility to imipenem. The frequency of resistance to amikacin, gentamicin, and ciprofloxacin was high and similar to previous findings in Tunisia (Hammami et al. 2011; Erturk et al. 2014). Amikacin is often reported as the aminoglycoside that shows better activity against MDR *A. baumannii*; yet, in Tunisia its efficacy may be compromised by the high-resistance rate. Tigecycline and colistin have been used for the treatment of infections caused by CRAB. However, we found already that 12 and 10% of the isolates were resistant to tigecycline and colistin, respectively. Previous studies in Tunisia, Libya and Turkey showed that all tested *A. baumannii* strains were susceptible to colistin (Mansour et al. 2008; Hammami et al. 2011; Erturk et al. 2014; Mathlouthi et al. 2016), which suggests the recent emergence of extended-drug resistant (XDR) strains.

In this study, OXA-23-like was found in the majority of CRABC, which is in accordance with the previous epidemiological studies performed in Tunisia (Mansour et al. 2008; Hammami et al. 2011, 2017; Charfi-Kessiss et al. 2014). In Charles Nicolle Hospital of Tunisia, five *A. baumannii* isolates carried the OXA-23-like in association with GES-11 or GES-12 genes, but none of them harbored OXA-48-like or NDM determinants (Hammami et al. 2017). The *ISAbal*, associated with expression of the carbapenemase, was found located upstream the *bla*_{OXA-23}-like in more than half the isolates. Other isolates carried the *ISAbal* upstream *bla*_{OXA-51}-like and/or may have other resistance mechanisms that contribute to carbapenem resistance. Here, we report for the first time the finding of the *bla*_{NDM-1} gene in *A. baumannii* in Tunisian territory. The frequency was rather low (1 out of 101 isolates during 4 years). In this country, the NDM-1 enzyme has only been reported in *Enterobacteriaceae* (Messaudi et al. 2017). Nonetheless, NDM-1 has been found in other North African countries, such as Algeria (Boulanger et al. 2012), Libya (Mathlouthi et al. 2016) and Egypt (El-Sayed-Ahmed et al. 2015). This carbapenemase was also reported in six CRAB strains isolated in France from four patients with origin in Algeria, one in Tunisia and one in Egypt (Bonnin et al. 2013). However, in Tunisia

Fig. 1 Schematic representation of the genetic environment of the *bla*_{NDM} gene, as determined by the primer pairs shown

the circulation of NDM-1-producing *A. baumannii* strains is unknown. Our work includes a considerable number of isolates ($n = 101$), and shows that, at least in this hospital, the isolation of NDM-1-producing *A. baumannii* strains is rare since only one isolate was identified. Despite the resistance of the strain, the infection could be controlled and the patient was discharged. No direct link could be established with CRAB NDM-producers of geographically close regions since the patient did not travel abroad. The NDM-1 producer Ab51 belongs to ST85, the same ST of the NDM-1 producer isolate recovered from a Tunisian patient in France in 2012 (Bonnin et al. 2013). Although ST2 is the most frequent ST in North Africa countries (Mathlouthi et al. 2016; El-Sayed-Ahmed et al. 2015; Bakour et al. 2015), a recent study in Libya showed a clonal diversity among CRAB. However, only two isolates belonged to ST85: one producing the NDM-1/OXA-23 (like the CRAB strain identified in France), and the other expressing only the NDM-1 metallo- β -lactamase (Mathlouthi et al. 2016), as the Ab51 of the present study.

The *bla*_{NDM} have been found in diverse genera located in different replicon type plasmids, explaining their expanded dissemination (Da Silva and Domingues 2016). However, conjugation assays failed to originate transconjugants, suggesting a chromosomal location for *bla*_{NDM-1}. Indeed, NDM-1/2 determinants have been found in the chromosome inserted in the composite transposon Tn125, supporting the hypothesis that Tn125 is the major vehicle of NDM dissemination (Poirel et al. 2012; Bontron et al. 2016). The movement of carbapenemase genes among *A. baumannii* populations is complex and it cannot be often explained by conjugation. Recently, transduction has been suggested as an intraspecies lateral mechanism for *bla*_{NDM-1} spread (Krahn et al. 2016). However, to our knowledge, natural transformation has never been tested for horizontal transfer of *bla*_{NDM}. Only one study reports the transfer by natural transformation of a carbapenemase gene embedded in an integron (Domingues et al. 2012). This mechanism is dependent on the competence of the recipient cells. So far, only two studies report that clinical *A. baumannii* strains may express natural competence (Ramirez et al. 2010; Wilharm et al. 2013). Therefore, this mechanism cannot be ruled out and we hypothesized that *bla*_{NDM-1} could be transferred by natural transformation. Transformants were obtained in selection plates; however, none of the tested transformants harbored the *bla*_{NDM-1}. The analysis of the genetic environment of *bla*_{NDM-1} in Ab51 confirmed its location in the truncated form of Tn125, as described previously in ten ST85 CRAB NDM-1-producers recovered in France between 2011 and 2012, being nine from North African patients (Bonnin et al. 2013, 2012). The absence of NDM-positive transformants might be explained by the presence of this truncated Tn125 in the Ab51, which prevents transposition to the genome

of the recipient cells, as previously demonstrated for other functional transposons (Domingues et al. 2012). Based on previous results (Domingues et al. 2012), we may speculate that the non-truncated composite transposon Tn125 could transpose to competent *A. baumannii* recipient cells after uptake of DNA by natural transformation.

In conclusion, this study reports, to the best of our knowledge, the first occurrence of a *A. baumannii* NDM-1 producer in Tunisian territory, and reinforces the wide dissemination of OXA-23-like in this country. The low spread of *bla*_{NDM-1} among *A. baumannii* isolates might be related to a low mobilization due to its insertion in the truncated form of Tn125. This study allowed the implementation of more efficient control measures in this hospital. The travel of people across North Africa, South Europe, and East Mediterranean countries may contribute to the CRAB spread. In addition, the existence of ST85 *A. baumannii* strains carrying *bla*_{NDM-1} in North African neighbor countries, suggests possible clonal spread, probably by people cross-border dissemination. Therefore, it is imperative to develop antimicrobial stewardship initiatives and maintain antimicrobial resistance surveillance system in North Africa countries, as well as to screen patients in hospital admission to allow early detection and to prevent the high-dissemination of CRAB with application of convenient control measures.

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Compliance with ethical standards

Conflict of interest No conflict of interest.

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