Role of plasmid carrying blaNDM in mediating antibiotic resistance among Acinetobacter baumannii clinical isolates from Egypt

Alaa Abouelfetouh1 · Aisha S. Torky1 · Elsayed Aboulmagd1

Received: 30 October 2019 / Accepted: 28 February 2020 / Published online: 16 March 2020
© King Abdulaziz City for Science and Technology 2020

Abstract
We investigated antibiotic resistance levels among blaNDM-positive (n=9) and -negative (n=65) A. baumannii clinical isolates collected in 2010 and 2015 from Alexandria Main University Hospital, Egypt using disc diffusion and minimum inhibitory concentration (MIC) determination. Plasmids from blaNDM-positive isolates were transformed into a carbapenem-susceptible A. baumannii (CS-AB) isolate to assess the role of plasmid transfer in mediating carbapenem resistance. Imipenem, meropenem, and ertapenem MIC90 values against blaNDM-positive isolates were 128, > 256, and 256 µg/mL, respectively. Plasmid isolation and polymerase chain reaction revealed that blaNDM was plasmid mediated. The plasmids were electroporated into the cells of a CS-AB isolate at an efficiency of 1.3 × 10–8 to 2.6 × 10–7, transforming them to blaNDM-positive carbapenem-resistant cells with an imipenem MIC increase of 256-fold. In addition to carbapenem resistance, the blaNDM-positive isolates also exhibited higher levels of cephalosporins, tetracycline, aminoglycosides, fluoroquinolones, and colistin resistance than the blaNDM-negative isolates. Acquisition of blaNDM-carrying plasmids dramatically increased imipenem resistance among A. baumannii isolates. Intriguingly, blaNDM-positive isolates also showed a high degree of resistance to antibiotics of different classes. The potential co-existence of different resistance determinants on A. baumannii plasmids and their possible transfer owing to the natural competence of the pathogen are especially alarming. More effective infection control and antibiotic stewardship programs are needed to curb the spread and treat such infections in both hospital and community settings.

Keywords Metallo-beta-lactamases · Transformation · Antimicrobial resistance · Imipenem

Introduction
Acinetobacter baumannii (A. baumannii) is considered one of the most challenging pathogens for researchers and clinicians in medical settings all over the world. The threat posed by A. baumannii infections stems from the rapid and unchecked spread of this pathogen (Gerischer 2008) and its naturally low susceptibility to many antimicrobials (Lee et al. 2011). Moreover, A. baumannii can cause different types of infections, including ventilator associated pneumonia, skin and soft tissue infections, urinary tract infections, wound and bloodstream infections, and meningitis (Dijkshoorn et al. 2007; Fernández et al. 2012; McConnell et al. 2013; Rajamohan et al. 2009; Wisplinghoff et al. 2004). These infections are mainly hospital related, especially among intensive care unit patients (Eveillard et al. 2013), and in particular immunocompromised ones (Krahn et al. 2016). Moreover, the microorganism is also capable of causing community-acquired infections, albeit to a lesser extent (Wang et al. 2003). The propensity of A. baumannii to acquire resistance genes (Corbella et al. 2000), in addition to the excessive use of antibiotics in many health care settings caused the emergence of multidrug resistant (MDR) strains (Peleg et al. 2008) leading to the ineffectiveness of many antibiotics including the life-saving carbapenems (Gao et al. 2017).

Carbapenem-resistant A. baumannii (CR-AB) strains have been reported globally (Perez et al. 2007). The mechanisms involved in carbapenem resistance are diverse, including change in permeability of porins in the microorganism outer membrane, efflux pumps, and alteration in the affinity of...
penicillin binding proteins (Abbott et al. 2013). However, the most relevant mechanism is mediated by the acquisition of carbapenem hydrolyzing β-lactamases, mainly metallo-
β-lactamases (MBL): VIM, IMP, SPM, and NDM, and the carbapenem-hydrolyzing class D β-lactamases (CHDLs): OXA-23, OXA-24/40, OXA-58, and OXA-143 and less importantly class A (Evans and Amyes 2014; Palzkill 2013). Most of these genes are carried on plasmids of A. baumannii (Naas et al. 2008). NDM is one of the most recently discovered β-lactamases (Nordmann et al. 2011), being first reported in 2008 in New Delhi, India from Klebsiella Pneumoniae (Yong et al. 2009). It was then detected among Escherichia coli isolates (Kumarasamy et al. 2010), and later in A. baumannii and Pseudomonas aeruginosa (Johnson and Woodford 2013). NDM dissemination was originally confined to the Indian subcontinent, then it spread worldwide in diverse Gram-negative isolates not necessarily epidemiologically linked to the Indian subcontinent (Johnson and Woodford 2013). Despite being first discovered among members of the Enterobacteriaceae, it is thought that blaNDM evolved in Acinetobacter from the fusion of another metallo-
β-lactamase and aphA6, a gene encoding aminoglycoside resistance, then was transferred to other Gram-negative bacteria (Toleman et al. 2012).

In Acinetobacter spp., the blaNDM gene is mainly carried on plasmids belonging to the pNDM-BJ01-like family (Hu et al. 2012). These plasmids are usually conjugative which helps in the complex transmission of the gene between strains belonging to different genera (Espinal et al. 2011; Johnson and Woodford 2013). This makes A. baumannii harboring blaNDM a threatening and serious pathogen worldwide (Chen et al. 2011). The natural competency feature of A. baumannii further aggravates the issue (Traglia et al. 2014), rendering the study of plasmid transfer a focal issue to hinder the outbreaks caused by A. baumannii, especially in hospitals (Saranathan et al. 2014). This study aimed to establish the role played by plasmid harbored blaNDM in mediating carbapenem resistance relative to resistance to other commonly used antibiotics among A. baumannii isolates obtained from patients presenting to Alexandria Main University Hospital (AMUH), the largest tertiary hospital in Alexandria, Egypt, in 2010 and 2015.

Materials and methods

Bacterial isolates

In the present study, 74 CR-AB clinical isolates were collected from Alexandria Main University Hospital (AMUH) from different clinical specimens in 2010 and 2015. The isolates were previously identified by conventional methods such as colony shape and aerobic growth at 44 °C on MacConkey’s agar as well as the Vitek system (Biomerieux, UK). The identity was further confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS) (Bruker Daltonik, USA) and PCR amplification of the chromosomally intrinsic blaOXA-51 gene. Nine of the A. baumannii isolates were shown by PCR and sequence analysis to carry blaNDM (GenBank accession numbers: MN395910, MN395911, MN395912, MN395913, MN395914, MN395915, MN395916, MN395917, and MN395918). K. pneumoniae ATCC 10031 was used as a reference susceptible strain (Abouelfetouh et al. 2019).

Antimicrobial susceptibility testing of the isolates

The susceptibility of all 74 isolates towards 17 different antibiotics was determined using the standard disc diffusion technique and the results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute 2018 (CLSI 2018). The antibiotics used were imipenem, meropenem, aztreonam, piperacillin, piperacillin/tazobactam, ampicillin/subbactam, ceftazidime, cefepime, cefotaxime, ceftriaxone, tetracycline, doxycycline, amikacin, gentamicin, ciprofloxacin, levofloxacin, and sulphamethoxazole/trimethoprim (Oxoid Ltd, England).

Determination of the antibiotics minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of imipenem, meropenem, ertapenem, amikacin, and levofloxacin against the tested isolates was determined using agar dilution technique, whereas MIC of colistin was determined using broth microdilution technique and the results were interpreted according to CLSI, 2018. The antibiotic powders/solutions of pharmaceutical grade were purchased from the Egyptian market as Tienam® (Merck Sharp & Dohme B.V.), Meronem® (Astrazenca, UK), Invanz® (Merck Sharp & Dohme Corp.), Amikacin® (Amoun Pharmaceutical Co.) and Tavantic® (Sanofi-Aventis Ireland Ltd. T/A Sanofi), respectively. Colistin was obtained as colistin sulphate (Sigma Aldrich). Twofold serial dilutions (1–512 µg/mL for all antibiotics, except colistin: 0.25–512 µg/mL) were freshly prepared on the day the experiment was done.

Plasmid extraction and characterization

Plasmids were isolated from the nine isolates harboring blaNDM using the plasmid isolation kit “GeneJET™ Plasmid Miniprep Kit #K0502” (Thermo Scientific, USA) according to the manufacturer’s instructions. The plasmid profiles were analyzed by 1% agarose gel electrophoresis in presence of 1 Kbp DNA ladder (Thermo Scientific, USA). All nine plasmid preps were used as DNA template for PCR.
amplification of $bla_{NDM}$ using primers NDM-F (5′-CACCTC ATGTGTGAAATTCG-3′) and NDM-R (5′-CTCTGTTCAC ATCGAAATTCG-3′) and amplification conditions of: initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C/40 s, 54 °C/1 min and 72 °C/2 min, and final extension at 72 °C for 5 min (Poirel et al. 2010). The PCR products were resolved on 1% agarose gel in TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA, pH 8.3) at 100–120 V, in the presence of 100 bp DNA ladder (New England Biolabs, UK).

Transformation of carbapenem-susceptible A. baumannii cells with plasmids harboring $bla_{NDM}$

A. baumannii cells are naturally competent (Biswas 2015), which eliminated the need to prepare electrocompetent cells. The cells of the carbapenem-susceptible A. baumannii (CS-AB) isolate A20, selected as the recipient, were transformed with the nine plasmids harboring $bla_{NDM}$. Briefly, 10 mL of LB broth were inoculated with a single colony of the isolate A20 (MIC = 0.25 μg/mL and $bla_{NDM}$-negative). After overnight incubation at 37 °C, the culture was used to aseptically inoculate 100 mL LB broth and was allowed to grow with vigorous shaking until optical density at 600 nm (OD600) reached 2.7. Then, 50 μL aliquots of the culture at stationary phase were diluted with 50 μL fresh LB broth and 5 μL of each plasmid preparation was, in turn, electroporated at 1850 v (BTX Harvard Apparatus, USA), followed by incubation at 37 °C for 1 h. Eighty microliters were then plated onto LB agar plates containing 2 μg/mL of imipenem (Huang et al. 2015). The plates were incubated at 37 °C overnight then checked for transformants. Transformation efficiency was calculated by dividing the number of transformants by the initial count of recipient cells (Harding et al. 2013). Carbapenem resistance was confirmed in the transformants by determination of imipenem MIC by agar dilution technique. In addition, PCR amplification of $bla_{NDM}$ in the transformants was performed as previously explained.

Results

Antibiotic susceptibility testing

All $bla_{NDM}$ harboring isolates were shown by susceptibility testing and MIC values to be resistant to imipenem, meropenem and ertapenem, while 100% of $bla_{NDM}$-negative isolates were found to be resistant to meropenem and ertapenem only. Fifty nine (92.2%) $bla_{NDM}$-negative isolates were imipenem resistant as evidenced by either susceptibility testing and/or MIC values (Tables 1, 2). Moreover, all $bla_{NDM}$-positive isolates were also resistant to aztreonam, piperacillin, all tested cephalosporins, tetracycline, amikacin, and ciprofloxacin. On the other hand, only aztreonam was totally ineffective against the $bla_{NDM}$-negative isolates (Table 1). These findings were mostly confirmed by MIC results (Table 2). MIC$_{50}$ of imipenem, ertapenem, and levofloxacin was fourfold higher against the $bla_{NDM}$-positive isolates, relative to the $bla_{NDM}$-negative ones. In addition, the majority of the $bla_{NDM}$-positive isolates were inhibited by higher concentrations of imipenem, as evidenced in 77.7% of the isolates being inhibited ≥ 64 μg/mL versus 64.6% of the $bla_{NDM}$-negative isolates. The same can be said about meropenem and ertapenem where 77.7% and 100%, respectively of the $bla_{NDM}$-positive isolates were inhibited by ≥ 64 μg/mL of the antibiotics, relative to 56.9% and 90.8% of the $bla_{NDM}$-negative isolates, respectively. Furthermore, 33.3% of $bla_{NDM}$-positive isolates were only inhibited by ≥ 64 μg/mL of colistin, compared to 12.3% of the $bla_{NDM}$-negative isolates. Likewise, 128 μg/mL of levofloxacin were needed to inhibit 66.7% of $bla_{NDM}$-positive isolates, whereas only 26.2% of $bla_{NDM}$-negative isolates needed as much levofloxacin. However, 16.9% of the $bla_{NDM}$-negative isolates were only inhibited at 256 μg/mL. On the other hand, 77.8% of the $bla_{NDM}$-positive group versus 64.6% of the

| Antibiotic | $bla_{NDM}$-positive (n=9) | $bla_{NDM}$-negative (n=65) |
|------------|----------------------------|----------------------------|
| IMP        | 9 (100%)                   | 60 (92.3%)                 |
| MEM        | 9 (100%)                   | 64 (98.5%)                 |
| AZT        | 9 (100%)                   | 65 (100%)                 |
| PRL        | 9 (100%)                   | 64 (98.5%)                 |
| TZP        | 8 (88.9%)                  | 64 (98.5%)                 |
| SAM        | 9 (100%)                   | 63 (96.9%)                 |
| CAZ        | 9 (100%)                   | 64 (98.5%)                 |
| FEP        | 9 (100%)                   | 64 (98.5%)                 |
| CTX        | 9 (100%)                   | 64 (98.5%)                 |
| CRO        | 9 (100%)                   | 64 (98.5%)                 |
| TE         | 9 (100%)                   | 53 (81.5%)                 |
| DO         | 2 (22.2%)                  | 20 (30.8%)                 |
| AK         | 9 (100%)                   | 54 (83.1%)                 |
| CN         | 7 (77.8%)                  | 46 (70.8%)                 |
| CIP        | 9 (100%)                   | 64 (98.5%)                 |
| LEV        | 8 (88.9%)                  | 59 (90.8%)                 |
| SXT        | 7 (77.8%)                  | 59 (90.8%)                 |

IMP imipenem, MEM meropenem, AZT aztreonam, PRL piperacillin, TZP piperacillin/tazobactam, SAM ampicillin/sublactam, CAZ ceftazidime, FEP cefepime, CTX cefotaxime, CRO ceftriaxone, TE tetracycline, DO doxycycline, AK amikacin, CN gentamicin, CIP ciprofloxacin, LEV levofloxacin, SXT sulphamethoxazole/trimetoprim
blaNDM-negative group were inhibited by amikacin concentrations ≥ 512 µg/mL (Table 2).

### Table 2  Distribution and ranges of the minimum inhibitory concentrations of tested antibiotics among the blaNDM-positive and blaNDM-negative isolates

|                       | IMP | MEM | ERTA | CL  | LEV  | AK   |
|-----------------------|-----|-----|------|-----|------|------|
| **blaNDM-positive isolates (n = 9)** |     |     |      |     |      |      |
| MIC range (µg/mL)     | 8-128 | 32 to > 256 | 64–512 | 2–256 | 16–128 | 128 to > 512 |
| MIC<sub>50</sub> (µg/mL) | 64 | 64 | 256 | 2 | 128 | > 512 |
| MIC<sub>90</sub> (µg/mL) (%) | 128 | > 256 | 256 | 256 | 128 | > 512 |
| MIC (µg/mL) (%)       | 8 (11.1) | 32 (22.2) | 64 (22.2) | 2 (55.6) | 16 (22.2) | 128 (11.1) |
|                       | 16 (11.1) | 64 (33.3) | 128 (22.2) | 4 (11.1) | 32 (11.1) | 256 (11.1) |
|                       | 32 (22.2) | 128 (11.1) | 256 (44.4) | 256 (33.3) | 128 (66.7) | 512 (22.2) |
|                       | 64 (33.3) | > 256 (33.3) | 512 (11.1) |      |      | > 512 (55.6) |
| **blaNDM-negative isolates (n = 65)** |     |     |      |     |      |      |
| MIC range (µg/mL)     | 4-64 | 16–256 | 32–256 | < 0.5 to 256 | 8–256 | 16 to > 512 |
| MIC<sub>50</sub> (µg/mL) | 8 | 64 | 64 | 2 | 32 | > 512 |
| MIC<sub>90</sub> (µg/mL) (%) | 64 | 64 | 128 | 256 | 256 | > 512 |
| MIC (µg/mL) (%)       | 4 (9.2) | 16 (6.2) | 32 (9.2) | < 0.5 (1.5) | 8 (6.2) | 16 (1.5) |
|                       | 8 (50.8) | 32 (36.9) | 64 (53.8) | 1 (3.1) | 16 (23.1) | 64 (18.5) |
|                       | 16 (20) | 64 (55.4) | 128 (27.7) | 2 (46.2) | 32 (23.1) | 128 (12.3) |
|                       | 32 (7.7) | 256 (1.5) | 256 (9.2) | 4 (36.9) | 64 (4.6) | 256 (3.1) |
|                       | 64 (12.3) | 256 (12.3) | 128 (26.2) | 512 (9.2) | 256 (16.9) | > 512 (55.4) |

**IMP** imipenem, **MEM** meropenem, **ERTA** ertapenem, **CL** colistin, **LEV** levofloxacin, **AK** amikacin

<sup>a</sup>MIC<sub>50</sub>: MIC in µg/mL of the antimicrobial agent required to inhibit the growth of 50% of the clinical isolates

<sup>b</sup>MIC<sub>90</sub>: MIC in µg/mL of the antimicrobial agent required to inhibit the growth of 90% of the clinical isolate

Plasmid profiling/characterization

The extracted plasmids exhibited different profiles, ranging from 1.5 to about > 10 kbp (Fig. 1). PCR amplification of blaNDM and the subsequent resolution of the products on agarose gel showed bands at the expected size of 984 bps, confirming that all nine plasmids carried the gene.

Transformation of blaNDM carrying plasmids into carbapenem-susceptible A. baumannii

Transformation of a CS-AB isolate, A20, harboring no blaNDM (recipient), with the nine plasmid preparations harboring blaNDM was performed by electroporation. Successful
transformants were selected on imipenem plates, with transformation efficiencies that ranged from $1.3 \times 10^{-8}$ to $2.6 \times 10^{-7}$. MIC values of imipenem were determined against the transformants and were found to be $> 64 \mu g/mL$, which is 256-fold higher than the original MIC of the recipient A. baumannii (MIC 0.25 μg/mL). Moreover, plasmids were isolated from all nine transformants and used as templates to amplify bla\textsubscript{NDM} gene that was detected in all transformants except one, from plasmid preparation number 6, a representative is shown in Supplementary Fig. 1.

**Discussion**

*Acinetobacter baumannii* is a Gram-negative pathogen that is common in the hospital environment (Cerqueira and Peleg 2011). In addition, it has a broad diversity of resistance determinants and is capable of acquiring more resistance phenotypes via horizontal gene transfer (Fournier et al. 2006; Imperi et al. 2011; Perez et al. 2007). These factors, together with the high survival rate of the microorganism on dry surfaces made A. baumannii infections a major healthcare concern, especially among intensive care and immunocompromised patients (Fournier et al. 2006; García-Garmendia et al. 2001; Pogue et al. 2013). As a result of the increasing antibiotic resistance among A. baumannii isolates in the last decades, carbapenems became last option drugs to treat such infections (Meletis 2016). However, CR-AB isolates have emerged (Meletis 2016) and are extensive drug resistant (XDR) in most instances (Viehman et al. 2014), moreover, infection with carbapenem-resistant strains was associated with mortality in 16 to 76% of the cases relative to 5 to 53% for infections due to carbapenem-susceptible ones. This is largely attributed to the more severe nature of infection with resistant strains and the initial delay in proper antimicrobial therapy administration (Lemos et al. 2014).

Carbapenem-resistant Acinetobacter isolates have been reported at different rates from around the world. The rates ranged from 84% in a national surveillance study in Switzerland between 2005 and 2016 (Ramette and Kronenberg 2018) and 95% in Turkey between 2011 and 2012 (Cicek et al. 2014). In the Middle East, the rate in the last two decades was 45% in Tunisia (Ben Othman et al. 2007), 65% in Saudi Arabia (Al-Agamy et al. 2014), 19.14% in Kuwait (Al-Sweih et al. 2012), and 47.9% in Algeria (Bakour et al. 2013). In Cairo, Egypt, one study conducted between 2011 and 2012 (Foud et al. 2013) showed imipenem and meropenem resistance rates of 74% and 100%, respectively among A. baumannii clinical isolates, while a second study between 2012 and 2013 (Abdel Hamid et al. 2016) found that 95.1% of the tested isolates were resistant to carbapenems. Moreover, a more recent study carried out in Mansoura, Egypt reported extensive drug resistance among 100% of the A. baumannii isolates obtained from patients suffering from nosocomial infections. These isolates were simultaneously resistant to penicillins, cephalosporins, fluoroquinolones, aminoglycosides, carbapenems and tigecycline (Elsayed et al. 2019).

One of the main mechanisms driving carbapenem resistance among A. baumannii is the production of carbapenemases that could be acquired or intrinsic (Viehman et al. 2014). NDM is one of the most important acquired metallo-β-lactamases because of its wide substrate specificity and its current dissemination in various regions of the world since its first discovery in India (Chen et al. 2011; Decousser et al. 2013; Palzkill 2013).

The current study included 74 CR-AB clinical isolates, including nine bla\textsubscript{NDM} positive ones, collected from AMUH. Susceptibility testing showed that overall antibiotic resistance was higher among the bla\textsubscript{NDM}-positive isolates, than the bla\textsubscript{NDM}-negative ones. Moreover, MIC ranges for imipenem, meropenem, ertapenem, colistin, levofloxacin, and amikacin were generally at least twofold higher among the bla\textsubscript{NDM}-positive group. Among the tested antibiotics, colistin displayed highest activity, being active against 52.3% of bla\textsubscript{NDM}-negative isolates versus 55.6% of bla\textsubscript{NDM}-positive ones. These results corroborate the previously reported finding that bla\textsubscript{NDM}-positive strains are also resistant to all β-lactams, except for aztreonam (Yong et al. 2009). Nevertheless, both bla\textsubscript{NDM}-positive and -negative isolates reported here were also aztreonam resistant which could be attributed to other resistance determinants as previously reported (Rodriguez-Martinez et al. 2010). In addition, a bla\textsubscript{NDM}-positive A. baumannii recovered in Brazil in 2013 was also resistant to meropenem, imipenem, all cephalosporins, aztreonam, aminoglycosides, tetracyclines, and sulphonamethoxazole/trimethoprim (Pillonetto et al. 2014). A study from Egypt commented on the concomitant resistance to the carbapenems and quinolones, trimethoprim/sulphamethoxazole, and aminoglycosides in a collection of A. baumannii isolates that were 30% bla\textsubscript{NDM} positive (Benmahmod et al. 2019).

In Acinetobacter, bla\textsubscript{NDM} is usually carried in a Tn\textsubscript{25} composite transposon on pNDM-BJ01-like plasmids which are highly conserved (Chen et al. 2015; Hu et al. 2012). The genetic environment of bla\textsubscript{NDM} is also conserved in other plasmid families found among non-Acinetobacter (Partridge and Iredell 2012). Besides bla\textsubscript{NDM}, the plasmid also harbors aph\textsubscript{A6} upstream of Tn\textsubscript{25}, a gene that encodes aminoglycoside resistance (Jones et al. 2015), which could explain the higher range of amikacin MIC against bla\textsubscript{NDM}-positive isolates in the current study. Moreover, it is believed that bla\textsubscript{NDM} is a chimeric gene that originated from a recent fusion event between aph\textsubscript{A6} and an older metallo-β-lactamase (Toleman et al. 2019).
et al. 2012). Previous studies have described the presence of aminoglycoside-modifying enzymes as a “main” reason for aminoglycoside resistance (Peleg et al. 2008). A study investigating the transfer of blaNDM-carrying plasmids from Acinetobacter isolates revealed the acquisition of both carbapenem and aminoglycoside resistance in the resultant E. coli transconjugants (Huang et al. 2015), which highlights the link between carbapenem and aminoglycoside resistance determinants on blaNDM-carrying plasmids from Acinetobacter.

In A. baumannii, blaNDM is mostly plasmid mediated, an exception lies in the European isolates where the gene is chromosomal (Hu et al. 2012; Pfeifer et al. 2011). Since these plasmids also carry other resistance determinants (Kumarasamy et al. 2010), it was important to study plasmid transfer among our cohort of A. baumannii isolates. Plasmids were isolated from the nine clinical isolates harboring blaNDM. Profiles of the isolated plasmids were analyzed after agarose gel electrophoresis and ranged from 1.5 to > 10 kbp which agrees with the results reported by an earlier study (Saranathan et al. 2014) in which 2 to > 25 kbp plasmids were isolated from CR-AB. blaNDM presence on the isolated plasmids was confirmed by PCR using the different plasmid preparations as templates. These findings were in accordance with a previous study which reported carriage of blaNDM on different plasmids (Chen et al. 2011). The nine plasmid preparations were electroporated into CS-AB cells. Transformation efficiency ranged from $1.3 \times 10^{-8}$ to $2.6 \times 10^{-7}$. A previous study (Huang et al. 2015) reported an average conjugation frequency in A. baumannii of $7.69 \times 10^{-6}$ and $7.09 \times 10^{-7}$ and an even higher frequency among non-pathogenic Acinetobacter spp. which points these strains as potential reservoirs for the transfer of resistance determinants.

An important difference between the two studies is the use of conjugation in the previous study (Huang et al. 2015), whereas the current work relied on electroporation, a method recommended for transfer of foreign DNA into A. baumannii (Thompson and Yildirim 2019). Imipenem MIC values against the obtained transformants in the current study were 256-fold higher than the recipient A. baumannii strain. All the obtained transformants, except one (number 6), were shown to carry blaNDM by PCR. This indicated the successful transfer of the plasmids harboring blaNDM. Transfer of other plasmids conferring carbapenem resistance other than the one carrying blaNDM may have contributed to carbapenem resistance in the absence of blaNDM in the odd transformant.

Conclusions

blaNDM was plasmid mediated in the tested CR-AB isolates from Alexandria, Egypt. The increased resistance of these isolates to other antibiotic classes coupled with the natural competence of A. baumannii which facilitates plasmid transfer to CS-AB isolates point to potential loss of the effectiveness of invaluable antimicrobial agents. This warrants further investigation of the genetic context of blaNDM on the plasmids using genomic techniques, towards the design of effective antibiotic stewardship and infection control policies in Egyptian hospitals and the community.

Acknowledgements The authors would like to thank Dr. Mervat Kassem and Sylvia Danial for their help in isolate collection and Dr. Mohammed Bahey-El-Din for donating colistin sulphate powder.

Author contributions AA and EA were in charge of the conceptualization and design of the study. AT performed the experiments. All authors participated in the interpretation of the data. AA took an active role in manuscript drafting with the help of AT. All authors reviewed and approved of the final version of the manuscript.

Funding This work was supported by the Egyptian Science and Technology Development Fund (STDF) (Grant no. 25368). The funding body had no role in the study design and collection, analysis, and interpretation of data or in the writing of the manuscript.

Compliance with ethical standards
Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data transparency All data are available from the corresponding author upon reasonable request.

References

Abbott I, Cerqueira GM, Bhuiyan S, Peleg AY (2013) Carbapenem resistance in Acinetobacter baumannii: laboratory challenges, mechanistic insights and therapeutic strategies. Expert Rev Anti Infect Ther 11:395–409

Abdel Hamid R, Hassan S, El-Mahallawy H, Saber M (2016) Molecular characterization of carbapenem resistant Acinetobacter baumannii in cancer patients. Int J Curr Microbiol Appl Sci 5:637–647

Abouelfetouh A, Torky A, Aboulmagd E (2019) Phenotypic and genotypic characterization of carbapenem resistant Acinetobacter baumannii isolates from Egypt. Antimicrob Resist Infect Control 8:185

Al-Agamy MH, Shibil AM, Ali MS, Khoubnani H, Radwan HH, Livermore DM (2014) Distribution of beta-lactamases in carbapenem-nonsusceptible Acinetobacter baumannii in Riyadh. Saudi Arabia J Glob Antimicrob Resist 2:17–21

Al-Sweih NA, Al-Hubail M, Rotimi VO (2012) Three distinct clones of carbapenem-resistant Acinetobacter baumannii with high diversity of carbapenemases isolated from patients in two hospitals in Kuwait. J Infect Public Health 5:102–108

Bakour S, Touati A, Sahli F, Ameur AA, Haouchine D, Rolain JM (2013) Antibiotic resistance determinants of multidrug-resistant Acinetobacter baumannii clinical isolates in Algeria. Diagn Microbiol Infect Dis 76:529–531. https://doi.org/10.1016/j.diagimicrobio.2013.04.009

Ben Othman A, Zribi M, Masmoudi A, Abdellatif S, Ben Lakhal S, Fendri C (2007) Phenotypic and molecular epidemiology of...
Acinetobacter baumannii strains isolated in Rabta Hospital. Tunis Arch Inst Pasteur Tunis 84:11–19
Benmahmod AB, Said HS, Ibrahim RH (2019) Prevalence and mechanisms of carbapenem resistance among Acinetobacter baumannii clinical isolates in Egypt. Microb Drug Resist (Larchmont, NY) 25:480–488
Biswas I (2015) Genetic tools for manipulating Acinetobacter baumannii genome: an overview. J Med Microbiol 64:657–669
Cerqueira GM, Peleg AY (2011) Insights into Acinetobacter baumannii pathogenicity. JIBMB Life 63:1055–1060. https://doi.org/10.1002/jibb.533
Chen Y, Zhou Z, Jiang Y, Yu Y (2011) Emergence of NDM-1-producing Acinetobacter baumannii in China. J Antimicrob Chemother 66:1255–1259
Chen Z et al (2015) NDM-1 encoded by a pNDM-BJ01-like plasmid p3SP-NDM in clinical Enterobacter aerogenes. Front Microbiol 6:294
Cicek AC, Saral A, Iraz M, Ceylan A, Duzgun A, Peleg A, Sandalli C, Cicek AC, Saral A, Iraz M, Ceylan A, Duzgun A, Peleg A, Sandalli C (2014) OXA-and GES-type β-lactamases predominate in extensively drug-resistant Acinetobacter baumannii isolates from a Turkish University Hospital. Clin Microbiol Infect 20:410–415
CLSI (2018) Performance standards for antimicrobial susceptibility testing, 28th edn. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne
Corbella X et al (2000) Emergence and rapid spread of carbapenem resistance during a large and sustained hospital outbreak of multi-resistant Acinetobacter baumannii. J Clin Microbiol 38:4086–4095
Decousser J et al (2013) Outbreak of NDM-1-producing Acinetobacter baumannii in France, January to May 2013. Eurosurveillance 18:1–4
Dijkshoorn L, Nemec A, Seifert H (2007) An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol 5:939–951
Elsayed E, Elarabi MA, Sheriff DA, Elmoshered M, El-Mashad N (2019) Extensive drug resistant Acinetobacter baumannii: a comparative study between non-colistin-based combinations. Int J Clin Pharm 19:1–9
Espinal P et al (2011) Dissemination of an NDM-2-producing Acinetobacter baumannii clone in an Israeli rehabilitation center. Antimicrob Agents Chemother 55:5396–5398
Evans BA, Amyes SG (2014) OXA beta-lactamases. Clin Microbiol Rev 27:241–263
Eveillard M, Kempf M, Belmonte O, Paillorhès H, Joly-Guillou M-L (2013) Reservoirs of Acinetobacter baumannii outside the hospital and potential involvement in emerging human community-acquired infections. Int J Infect Dis 17:e802–e805
Fernández J et al (2012) Prevalence and risk factors of infections by multiresistant bacteria in cirrhosis: a prospective study. Hepatology 55:1551–1561
Foud M, Attia AS, Tawakkol WM, Hashem AM (2013) Emergence of carbapenem-resistant Acinetobacter baumannii harboring the OXA-23 carbapenemase in intensive care units of Egyptian hospitals. Int J Infect Dis 17:1252–1254
Fournier PE, Richet H, Weinstein RA (2006) The epidemiology and control of Acinetobacter baumannii in health care facilities. Clin Infect Dis 42:692–699
Gao L, Lyu Y, Li Y (2017) Trends in drug resistance of Acinetobacter baumannii over a 10-year period: nationwide data from the China Surveillance of Antimicrobial Resistance Program. Chin Med J 130:659–664. https://doi.org/10.4103/0366-6999.201601
Garcia-Garmendia J-L, Ortiz-Leyba C, Garancho-Montero J, Jiménez-Jiménez F-J, Pérez-Paredes C, Barrero-Almodóvar AE, Mínner MG (2001) Risk factors for Acinetobacter baumannii nosocomial bacteremia in critically ill patients: a cohort study. Clin Infect Dis 33:939–946
Gerischer U (2008) Acinetobacter molecular biology. Horizon Scientific Press, Poole
Harding CM, Tracy EN, Carruthers MD, Rather PN, Actis LA, Munsen RS (2013) Acinetobacter baumannii strain M2 produces type IV pilus which play a role in natural transformation and twitching motility but not surface-associated motility. MBio 4:1–10
Hu H et al (2012) Novel plasmid and its variant harboring both a blaNDM-1 gene and type IV secretion system in clinical isolates of Acinetobacter iwoffii. Antimicrob Agents Chemother 56:1698–1702
Huang T-W et al (2015) Effective transfer of a 47 kb NDM-1-positive plasmid among Acinetobacter species. J Antimicrob Chemother 70:2734–2738
Imperi F, Antunes LC, Blom J, Villa L, Iacono M, Visca P, Carattoli A (2011) The genomics of Acinetobacter baumannii: insights into genome plasticity, antimicrobial resistance and pathogenicity. JIBMB Life 63:1068–1074
Johnson AP, Woodford N (2013) Global spread of antibiotic resistance: the example of New Delhi metallo-β-lactamase (NDM)-mediated carbapenem resistance. J Med Microbiol 62:499–513
Jones LS et al (2015) Characterization of plasmids in extensively drug-resistant Acinetobacter strains isolated in India and Pakistan. Antimicrob Agents Chemother 59:923–929
Krahn T et al (2016) Intraspecies transfer of the chromosomal Acinetobacter baumannii blaNDC households. Antimicrob Agents Chemother 60:3032–3040
Kumarasamy KK et al (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 10:597–602
Lee K, Yong D, Jeong SH, Chong Y (2011) Multidrug-resistant Acinetobacter spp.: increasingly problematic nosocomial pathogens. Yonsei Med J 52:879–891
Lemos E, de la Hoz F, Einarson T, McGhan W, Quevedo E, Castaneda C, Kawai K (2014) Carbapenem resistance and mortality in patients with Acinetobacter baumannii infection: systematic review and meta-analysis. Clin Microbiol Infect 20:416–423
McCannell MJ, Actis L, Pachón J (2013) Acinetobacter baumannii: human infections, factors contributing to pathogenesis and animal models. FEMS Microbiol Rev 37:130–155
Meletis G (2016) Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Dis Inf Dis 5:1–10
Naas T, Curzon G, Villegas M-V, Lartigue M-F, Quinn JP, Nordmann P (2008) Genetic structures at the origin of acquisition of the β-lactamase blaKPC gene. Antimicrob Agents Chemother 52:1257–1263
Nordmann P, Poirel L, Walsh TR, Livermore DM (2011) The emerging NDM carbapenemases. Trends Microbiol 19:588–595
Palzkill T (2013) Metallo-β-lactamase structure and function. Ann N Y Acad Sci 1277:91–104
Partridge SR, Ireddell JR (2012) Genetic contexts of blaNDM-1. Antimicrob Agents Chemother 56:6065–6067
Peleg AY et al (2006) Acinetobacter baumannii bloodstream infection while receiving tigecycline: a cautionary report. J Antimicrob Chemother 59:128–131
Peleg AY, Seifert H, Paterson DL (2008) Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev 21:538–582
Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA (2007) Global challenge of multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 51:3471–3484
Pfeifer Y et al (2011) Molecular characterization of blaNDM-1 in an Acinetobacter baumannii strain isolated in Germany in 2007. J Antimicrob Chemother 66:1998–2001
Pillonetto M, Arend L, Vespori EC, Pelisson M, Chagas TPG, Carvalho-Assef APDA, Asensi MD (2014) First report of
NDM-1-producing *Acinetobacter baumannii* sequence type 25 in Brazil. Antimicrob Agents Chemother 58:7592–7594

Pogue JM, Mann T, Barber KE, Kaye KS (2013) Carbapenem-resistant *Acinetobacter baumannii*: epidemiology, surveillance and management. Expert Rev Anti Infect Ther 11:383–393

Poirel L, Hornbrouck-Alet C, Frenaux C, Bernabeu S, Nordmann P (2010) Global spread of New Delhi metallo-β-lactamase 1. Lancet Infect Dis 10:832

Rajamohan G, Srinivasan V, Gebreyes WA (2009) Biocide-tolerant multidrug-resistant *Acinetobacter baumannii* clinical strains are associated with higher biofilm formation. J Hosp Infect 73:287–289

Ramette A, Kronenberg A (2018) Prevalence of carbapenem-resistant *Acinetobacter baumannii* from 2005 to 2016 in Switzerland. BMC Infect Dis 18:1–6

Rodríguez-Martínez J-M, Nordmann P, Ronco E, Poirel L (2010) Extended-spectrum cephalosporinase in *Acinetobacter baumannii*. Antimicrob Agents Chemother 54:3484–3488

Saranathan R, Sudhakar P, Karthika RU, Singh SK, Shashikala P, Kanungo R, Prashanth K (2014) Multiple drug resistant carbapenemases producing *Acinetobacter baumannii* isolates harbours multiple R-plasmids. Indian J Med Res 140:262–270

Thompson MG, Yildirim S (2019) Transformation of *Acinetobacter baumannii*: electroporation. Methods Mol Biol 1946:69–74

Toleman M, Spencer J, Jones L, Walsh TR (2012) *bla*<sub>NDM-1</sub> is a chimera likely constructed in *Acinetobacter baumannii*. Antimicrob Agents Chemother 56:2773–2776

Traglia GM, Chua K, Centron D, Tolmasky ME, Ramirez MS (2014) Whole-genome sequence analysis of the naturally competent *Acinetobacter baumannii* clinical isolate A118. Genome Biol Evol 6:2235–2239. https://doi.org/10.1093/gbe/evu176

Viehman JA, Nguyen MH, Doi Y (2014) Treatment options for carbapenem-resistant and extensively drug-resistant *Acinetobacter baumannii* infections. Drugs 74:1315–1333

Wang S et al (2003) Healthcare-associated outbreak due to pan-drug resistant *Acinetobacter baumannii* in a surgical intensive care unit. J Hosp Infect 53:97–102

Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB (2004) Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 39:309–317

Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR (2009) Characterization of a new metallo-β-lactamase gene, *bla*<sub>NDM-1</sub>, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother 53:5046–5054