3 Biotech (2020) 10:170
https://doi.org/10.1007/s13205-020-2157-y

ORIGINAL ARTICLE

Role of plasmid carrying bla_{NDM} in mediating antibiotic resistance among *Acinetobacter baumannii* clinical isolates from Egypt

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Received: 30 October 2019 / Accepted: 28 February 2020 / Published online: 16 March 2020

Abstract

We investigated antibiotic resistance levels among bla_{NDM}-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 bla_{NDM}-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 bla_{NDM}-positive isolates were 128, > 256, and 256 µg/mL, respectively. Plasmid isolation and polymerase chain reaction revealed that bla_{NDM} 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 bla_{NDM}-positive carbapenem-resistant cells with an imipenem MIC increase of 256-fold. In addition to carbapenem resistance, the bla_{NDM}-positive isolates also exhibited higher levels of cephalosporins, tetracycline, aminoglycosides, fluoroquinolones, and colistin resistance than the bla_{NDM}-negative isolates. Acquisition of bla_{NDM}-carrying plasmids dramatically increased imipenem resistance among *A. baumannii* isolates. Intriguingly, bla_{NDM}-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

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-020-2157-y) contains supplementary material, which is available to authorized users.

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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 bla_{NDM} 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 bla_{NDM} 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 bla_{NDM} 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 bla_{NDM} 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 bla_{OXA-51} gene. Nine of the *A. baumannii* isolates were shown by PCR and sequence analysis to carry bla_{NDM} (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/sulbactam, 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 Tavanic® (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 bla_{NDM} 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 ATGTTTGAATTCGCC-3′) and NDM-R (5′-CTCTGTCAC ATCGAAATCGC-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 by concentrations ≥ 32 µg/mL versus 20% 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 256 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

Table 1 Antimicrobial susceptibility of the bla_{NDM}-positive and bla_{NDM}-negative isolates

Antibiotic	Number of resistant isolates (%)	
	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/sulbactam, *CAZ* ceftazidime, *FEP* cefepime, *CTX* cefotaxime, *CRO* ceftriaxone, *TE* tetracycline, *DO* doxycycline, *AK* amikacin, *CN* gentamicin, *CIP* ciprofloxacin, *LEV* levofloxacin, *SXT* sulphamethoxazole/trimethoprim

Table 2 Distribution and ranges of the minimum inhibitory concentrations of tested antibiotics among the bla_{NDM}-positive and bla_{NDM}-negative isolates

	IMP	MEM	ERTA	CL	LEV	AK
bla_{NDM}-positive isolates ($n=9$)						
MIC range (µg/mL)	8–128	32 to > 256	64–512	2–256	16–128	128 to > 512
MIC_{50}[a] (µg/mL)	64	64	256	2	128	> 512
MIC_{90}[b] (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)
	128 (22.2)					
bla_{NDM}-negative isolates ($n=65$)						
MIC range (µg/mL)	4–64	16–256	32–256	< 0.5 to 256	8–256	16 to > 512
MIC_{50}[a] (µg/mL)	8	64	64	2	32	> 512
MIC_{90}[b] (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

[a]MIC_{50}: MIC in µg/mL of the antimicrobial agent required to inhibit the growth of 50% of the clinical isolates

[b]MIC_{90}: MIC in µg/mL of the antimicrobial agent required to inhibit the growth of 90% of the clinical isolate

bla_{NDM}-negative group were inhibited by amikacin concentrations ≥ 512 µg/mL (Table 2).

Plasmid profiling/characterization

The extracted plasmids exhibited different profiles, ranging from 1.5 to about > 10 kbp (Fig. 1). PCR amplification of bla_{NDM} 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 bla_{NDM}-carrying plasmids into carbapenem-susceptible *A. baumannii*

Transformation of a CS-AB isolate, A20, harboring no bla_{NDM} (recipient), with the nine plasmid preparations harboring bla_{NDM} was performed by electroporation. Successful

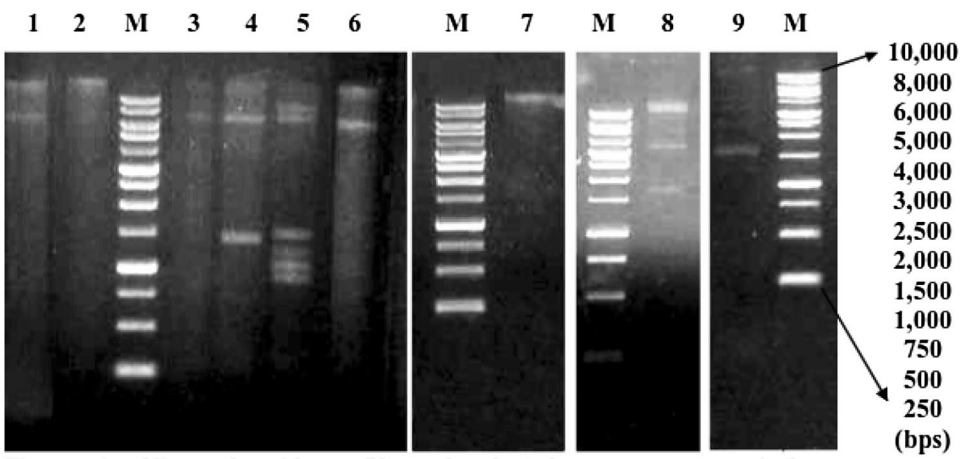

Fig. 1 Profiles of the nine plasmids extracted from the bla_{NDM}-positive isolates. Lanes 1–9 represent the nine plasmid preparations and lane M the DNA ladder. Different profiles were obtained, with a size range between 1.5 and > 10 kbp

transformants were selected on imipenem plates, with transformation efficiencies that ranged from 1.3×10^{-8} to 2.6×10^{-7}. MIC values of imipenem were determined against the transformants and were found to be > 64 µg/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_{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), which leaves the second-line treatment options, that are usually more toxic or of controversial efficacy, e.g., colistin and tigecycline (Peleg et al. 2006; 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 (Fouad 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_{NDM}-positive ones, collected from AMUH. Susceptibility testing showed that overall antibiotic resistance was higher among the bla_{NDM}-positive isolates, than the bla_{NDM}-negative ones. Moreover, MIC ranges for imipenem, meropenem, ertapenem, colistin, levofloxacin, and amikacin were generally at least twofold higher among the bla_{NDM}-positive group. Among the tested antibiotics, colistin displayed highest activity, being active against 52.3% of bla_{NDM}-negative isolates versus 55.6% of bla_{NDM}-positive ones. These results corroborate the previously reported finding that bla_{NDM}-positive strains are also resistant to all β-lactams, except for aztreonam (Yong et al. 2009). Nevertheless, both bla_{NDM}-positive and -negative isolates reported here were also aztreonam resistant which could be attributed to other resistance determinants as previously reported (Rodríguez-Martínez et al. 2010). In addition, a bla_{NDM}-positive *A. baumannii* recovered in Brazil in 2013 was also resistant to meropenem, imipenem, all cephalosporins, aztreonam, aminoglycosides, tetracyclines, and sulphamethoxazole/trimethoprim (Pillonetto et al. 2014). A study from Egypt commented on the concomitant resistance to the carbapenems and quinolones, trimethoprim/sulfamethoxazole, and aminoglycosides in a collection of *A. baumannii* isolates that were 30% bla_{NDM} positive (Benmahmod et al. 2019).

In Acinetobacter, bla_{NDM} is usually carried in a Tn*125* composite transposon on pNDM-BJ01-like plasmids which are highly conserved (Chen et al. 2015; Hu et al. 2012). The genetic environment of bla_{NDM} is also conserved in other plasmid families found among non-Acinetobacter (Partridge and Iredell 2012). Besides bla_{NDM}, the plasmid also harbors *aphA6* upstream of Tn*125*, a gene that encodes aminoglycoside resistance (Jones et al. 2015), which could explain the higher range of amikacin MIC against bla_{NDM}-positive isolates in the current study. Moreover, it is believed that bla_{NDM} is a chimeric gene that originated from a recent fusion event between *aphA6* and an older metallo-β-lactamase (Toleman

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 bla_{NDM}-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 bla_{NDM}-carrying plasmids from Acinetobacter.

In *A. baumannii*, bla_{NDM} 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 bla_{NDM}. 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 kb plasmids were isolated from CR-AB. bla_{NDM} presence on the isolated plasmids was confimed by PCR using the different plasmid preparations as templates. These findings were in accordance with a previous study which reported carriage of bla_{NDM} on different plasmids (Chen et al. 2011). The nine plasmid preparations were electroporated into CS-AB cells. Transformation efficiency ranged from 1.3×10^{-8} to 2.6×10^{-7}. A previous study (Huang et al. 2015) reported an average conjugation frequency in *A. baumannii* of 7.69×10^{-6} and 7.09×10^{-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 bla_{NDM} by PCR. This indicated the successful transfer of the plasmids harboring bla_{NDM}. Transfer of other plasmids conferring carbapenem resistance other than the one carrying bla_{NDM} may have contributed to carbapenem resistance in the absence of bla_{NDM} in the odd transformant.

Conclusions

bla_{NDM} 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 bla_{NDM} 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.

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