Editor: Edited by Stefania Stefani

Journal Pre-proof

Tn7382, a novel transposon harboring bla_{NDM-1} and aphA6 in Acinetobacter baumannii

Samira M. Hamed , Amira F.A. Hussein , Mohamed H. Al-Agamy , Hesham H. Radwan , Mai M. Zafer

PII: S2213-7165(22)00191-6
DOI: https://doi.org/10.1016/j.jgar.2022.08.001
Reference: JGAR 1927

To appear in: *Journal of Global Antimicrobial Resistance*

Received date: 21 March 2022
Revised date: 1 August 2022
Accepted date: 2 August 2022

Please cite this article as: Samira M. Hamed , Amira F.A. Hussein , Mohamed H. Al-Agamy , Hesham H. Radwan , Mai M. Zafer , Tn7382, a novel transposon harboring bla_{NDM-1} and aphA6 in Acinetobacter baumannii, *Journal of Global Antimicrobial Resistance* (2022), doi: https://doi.org/10.1016/j.jgar.2022.08.001

Tn*7382*, a novel transposon harboring *bla*$_{\text{NDM-1}}$ and *aphA6* in *Acinetobacter baumannii*

Samira M. Hamed[1]*, Amira F.A. Hussein[2], Mohamed H. Al-Agamy[3,4], Hesham H. Radwan[3], and Mai M. Zafer[5]

[1]Department of Microbiology and Immunology, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, Egypt;

[2]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt;

[3]Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia;

[4]Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt;

[5]Department of Microbiology and Immunology, Faculty of Pharmacy, Ahram Canadian University, Cairo, Egypt.

***Corresponding author:**

Mai M Zafer

Tel: +201111112472

E-mail: maizafer@acu.edu.eg ;

Abstract

Objectives: Co-transfer of carbapenem and amikacin resistance might contribute to the evolution of extensively drug resistant (XDR) *Acinetobacter baumannii*. The current study is aimed at *in silico* investigating the potential mobility of a novel composite transposon co-harboring $bla_{\text{NDM-1}}$ and *aphA6* using bioinformatic tools. **Methods:** The transposon, named here Tn*7382*, was recently described in the chromosomes of two XDR *A. baumannii* isolates (M02 and M11) from Egypt. The draft genomes of M02 and M11 were generated by Illumina sequencing. Nucleotide homology of Tn*7382* and flanking regions was analyzed using the BLASTN tool. **Results:** Tn*7382* is derived from Tn*125* and encompasses seven orfs [*aphA6*, IS*Aba125* transposase-coding gene, $bla_{\text{NDM-1}}$, *ble*, *iso*, *tat*, *cutA*] enclosed by two direct copies of IS*Aba14*. While described for the first time, Tn*7382* was found in the chromosomes of five *A. baumannii* strains deposited in the NCBI database. Using Artemis Comparison Tool, the potential mobility of Tn*7382* was demonstrated *in silico* by comparative genomic analysis of two *A. baumannii* strains (TP1 and TP2) retrieved from the NCBI database. The transposon was acquired by TP2 at the same location as an IS*Aba14* element in the ancestral variant TP1 isolated from the same patient in USA 11 days earlier. **Conclusions:** Here, we present the characteristics of Tn*7382*, a composite transposon flanked by IS*Aba14* and harboring the *aphA6* and $bla_{\text{NDM-1}}$ resistance genes. *In silico* analysis inferred the potential mobility of Tn*7382* but experimental validation is still required.

Keywords: *Acinetobacter baumannii*, whole genome sequencing, WGS, transposon, Tn*7382*, $bla_{\text{NDM-1}}$, *aphA6*, IS*Aba14*, carbapenem resistance, amikacin resistance, homologous recombination

1. Introduction

Co-transfer of amikacin and carbapenem resistance genes can greatly contribute to the emergence of extensively drug resistant (XDR) *Acinetobacter baumanni* (*A. baumannii*) for which very few treatment options are available [1]. The gene $bla_{\text{NDM-1}}$ is a widespread resistance gene that encodes New Delhi Metallo-β–lactamase-1 (NDM-1) capable of hydrolyzing penicillins, cephalosporins and carbapenems. One of the amikacin resistance genes commonly identified in *A. baumannii* is *aphA6* that encodes the aminoglycoside-3′-phosphotransferase type

VI (Aph(3′)-VI). Both genes are horizontally transferable through conjugative plasmids [2, 3]. Chromosomal copies of the genes were also identified within the mobilizable transposons Tn*125* and Tn*aphA6* harboring bla_{NDM-1} and *aphA6*, respectively [4, 5].

In a previous study [6], we identified both bla_{NDM-1} and *aphA6* enclosed by two copies of IS*Aba14* in a composite transposon not previously described. The current study is aimed at further characterization of this transposon, designated Tn*7382*, and to investigate its potential mobility through comparative genomics.

2. Materials and Methods

2.1. *A. baumannii* isolates and bioinformatic analysis of Tn*7382*

M02 and M11 are XDR *A. baumannii* strains isolated from patients admitted to Kasr Al-Ainy hospital in Cairo, Egypt during 2020 in a previous study in which the whole genome sequences of the isolates were generated by an Illumina MiSeq Sequencer (Illumina Inc., San Diego, CA, United States) and analyzed as described before [6]. The nucleotide sequence homology of the transposon found in both M02 and M11 was analyzed using the nucleotide Basic Local Alignment Search Tool (BLASTN) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the NCBI (National Center for Biotechnology Information) non-redundant nucleotide (nr/nt) database. The same analysis was done for the nucleotide sequences flanking the transposon in both M02 and M11. BLASTN tool was also used for analyzing the diversity of bacterial species and genetic compartments carrying IS*Aba14* elements. Identification of insertion sites and target site duplications of Tn*7382* and IS*Aba14* elements was aided by SnapGene viewer v5.1.3.1 (from Insightful Science; available at snapgene.com).

Comparative genomic analysis of *A. baumannii* TP2 (GenBank accession: CP060011.1) carrying Tn*7382* and its ancestral variant TP1 (GenBank accession: CP056784.2) lacking Tn*7382* was done using the Artemis Comparison Tool (www.sanger.ac.uk Software). Comparisons were illustrated using EasyFig v2.2.5 [7].

2.2. GenBank accession numbers

The draft genomes of M02 and M11 were submitted to the NCBI genome database with the accession numbers JAESHR000000000 and JAESHK000000000, respectively. GenBank accession numbers and chromosomal locations of Tn*7382* are shown in Table 1.

3. Results and Discussion

As reported before [6], M02 and M11 were XDR strains retaining susceptibility only to tigecycline and colistin (minimum inhibitory concentration of ≤0.125 µg/ml). Multilocus sequence typing showed that both M02 and M11 belonged to the sequence types ST85 (Pasteur scheme) and ST1089 (Oxford scheme) known to belong to international clone (IC) 9, as described by Mueller et al. [8]. In addition to the intrinsic resistance genes *aadA*, *bla*$_{OXA\text{-}94}$ (*bla*$_{\text{OXA-51-like}}$) preceded by IS*Aba1*, and *bla*$_{ADC}$, acquired resistance genes were identified in the genomes of the two isolates. These included the β-lactam resistance gene *bla*$_{\text{NDM-1}}$; the aminoglycoside resistance genes *aadB* and *aphA6*; the macrolide resistance genes *mphE* and *msrE*; and the sulfonamide resistance gene *sul2*. In addition, the two isolates were found to be equipped by a wide range of efflux pumps including AdeABC, AdeIJK, AdeFGH, AmvA, AbaF, AbaQ, FloR, AbeM, AbeS, and MacAB. Missense mutations were identified in the quinolone resistance determining regions of the genes *gyrA* and *parC* causing the amino acid alterations S83L and S80L, respectively.

Analysis of the context of *bla*$_{\text{NDM-1}}$ in M02 and M11 showed that it resided in an IS*Aba14*-interrupted Tn*125* first described by Bonnin et al. [9]. In their study, Bonin et al. failed to locate the right arm of the transposon that was thus described as truncated and was given the designation ΔTn*125*. The same structure was later identified by other authors [10, 11] in *A. baumannii* of the sequence type ST85 using WGS. Vijayakumar et al. [10] have also highlighted an IS*Aba125* element forming the right arm of the transposon that could not be previously located by Bonnin and his colleagues [9]. On the other hand, a composite transposon element bracketed by IS*Aba14* closely similar to Tn*7382* but carrying *bla*$_{\text{NMD-6}}$ was reported by Xanthopoulou et al. [12]. The transposon was carried by *A. baumannii* AbBAS-1 (GenBank accession: (CP065392.1) that was isolated from a patient from Maghreb in Spain in 2019. However, the authors did not focus on that transposon but rather their focus was on a nucleotide sequence duplication containing the gene *bla*$_{\text{NDM-6}}$. In our initial study [6], we could localize an *aphA6* preceded by a second copy of *ISAba14* upstream to the IS*Aba14*-interrupted transposon. Our hypothesis was that the insertion of IS*Aba14* in ΔTn*125* together with the upstream region [IS*Aba14-aphA6*] formed a new IS*Aba14*-bracketed composite transposon, named here Tn*7382*. The transposon is about 7955 bp long and carries seven open reading frames enclosed by two

copies of IS*Aba14* oriented in the same direction (Fig. 1). Alternative to Tn*125* that only carried a carbapenem resistance gene, Tn*7382* carries resistance genes to both carbapenems and amikacin which are two of the last-resort treatment options for infections caused by multidrug resistant *A. baumannii*. The transposon might, thus, contribute to the evolution of XDR *A. baumannii* strains.

GenBank homology search of Tn*7382* revealed that it was carried by the chromosomes of three *A. baumannii* strains that belong to the international clones IC2 and IC9. The same transposon was also found to carry $bla_{\text{NDM-6}}$ in the chromosome of AbBAS-1, as shown in Table 1. Notably, Tn*7382*-like transposon interrupted by seven direct copies of Tn*aphA6* was identified in the chromosome of *A. baumannii* strain Cl300 (GenBank accession: CP082952.1). In all mentioned Tn*7382*-positive strains, the transposon interrupted the same gene (ATP-binding protein) generating a 3-bp (TAT) target site duplication as discussed below. Figure 2 shows the context of the transposon in all positive strains.

BLAST analysis of the flanking sequences of the transposon carried by both M02 and M11 showed significant similarity to the chromosome of *A. baumannii* strain TP1 that did not carry Tn*7382*. Interestingly, TP1 was found to be isolated from the same patient as TP2 and TP3 (described in Table 1). All were recovered from the pancreatic drainage of a patient suffering from necrotizing pancreatitis complicated by a multidrug resistant *A. baumannii* infection in USA by Schooley *et al.* [13]. TP1, TP2 and TP3 were reported to be isolated on the 10th, 21st, and 23rd of March 2016, respectively.

Comparative genomic analysis of the three strains revealed that Tn*7382* was carried by TP2 and TP3 but not TP1, as shown in Supplementary Figure 1. As Tn*7382* and flanking sequences were identical in TP2 and TP3, all subsequent *in silico* analysis were done using the genome of TP2. Interestingly, comparative genomic analysis of TP1 and TP2 revealed another genetic event in which the gene *aphA1* (APH(3')-Ia-coding gene) was integrated into the *Acinetobacter baumannii* Genomic Resistance Island 3 (AbGRI3) carried by TP1. As a result, TP2 acquired a larger version of the genomic island enclosing an additional resistance gene (Supplementary Figure 2), as described before [14]. Together with the acquisition of Tn*7382*, such genetic events demonstrate the potential of *A. baumannii*, particularly IC2, to accumulate resistance genes within transposable elements to be transmitted as a single unit.

Analysis of the insertion site of Tn*7382* in TP2 showed that it was inserted in the same location as an IS*Aba14* element carried by its ancestral variant TP1. Both interrupted a gene encoding ATP-binding protein that was also interrupted by an IS4 element. We propose that Tn*7382* was inserted in a two-step process. IS*Aba14* was first inserted into the ATP-binding protein-coding gene generating a 3-bp (TAT) target site duplication as a transposition signature (Fig 1). This was followed by insertion of the transposon by homologous recombination between the IS*Aba14* elements.

IS*Aba14* is a 1282-bp element that belongs to IS3 family transposases. It was previously identified in *A. baumannii* as part of the composite transposon Tn*2114* harboring $bla_{\text{RTG-5}}$ [15]. BLAST analysis of IS*Aba14* against the NCBI nr/nt database revealed 99% or more identity to 180 accessions (accessed in November 28, 2021) shown in Supplementary Table 1. Most commonly, IS*Aba14* was carried by other *Acinetobacter* species (105/180, 58.3%) followed by *A. baumannii* (39/180, 21.7%) then members of the family *Enterobacteriaceae* (32/180, 17.8%). Up to 32 copies per chromosome were identified in *Acinetobacter* species. IS*Aba14* was also identified in the chromosomes of two *Pseudomonas aeruginosa* strains and a plasmid carried by the marine bacterium *Psychrobacter maritimus*. It is noteworthy that IS*Aba14* was identified both in the chromosomes and plasmids of *Acinetobacter* species, including *A. baumannii*, as well as *Proteus mirabilis*. Meanwhile, IS*Aba14* elements carried by other *Enterobacteriaceae* were confined to plasmids. In a unique organization, the two IS*Aba14*-positive *P. aeruginosa* strains carried two direct copies of IS*Aba14* enclosing an *aphA6* gene (Supplementary Figure 3). The mobility of this structure is yet to be investigated. Owing to the abundance of IS*Aba14* in *Acinetobacter* species and members of the family *Enterobacteriaceae* and as a hotspot for Tn*7382* insertion, interspecies transmission of Tn*7382* is potentially anticipated.

Analysis of the flanking sequences of IS*Aba14* elements found in the NCBI nr/nt database showed great diversity in their insertion sites. However, most commonly IS*Aba14* was found to be inserted in the upstream region of *aphA6* gene that in turn preceded intact copies or remnants of Tn*125*. Insertion of IS*Aba14* was also found to be accompanied by target site duplication for 314 out of 464 (67.6%) IS*Aba14* elements identified in the NCBI nr/nt database. Despite the diversity of IS*Aba14* insertion sites, the one identified here (ATP-binding protein-coding gene) was unique for TP1 and Tn*7382*-positive sequences. BLAST analysis of the ATP-binding protein-coding gene returned only ten hits of 99% or more identity, shown in

Supplementary Table 2 (accessed in November 28, 2021). In addition to the disrupted forms carried by TP1 and the Tn*7382*-positive sequences, intact copies were found in the chromosomes of two *A. baumannii* strains. These included *A. baumannii* strain 11W359501 (GenBank accession: CP041035.1) which had the sequence types ST1(Pasteur) and ST1604/231(Oxford) and *A. baumannii* strain AF-401 (GenBank accession: CP018254.1) with the sequence type ST79(Pasteur)/942(Oxford). Uninterrupted ATP-binding protein-coding gene was also carried in the chromosome of *Acinetobacter radioresistens* strain DD78 (GenBank accession: CP038022.1) and the plasmid pGX7carried by *Acinetobacter towneri* strain GX7. Such locations might present target sites for *de novo* insertion of Tn*7382* or for IS*Aba14* insertion.

4. Conclusion

The current study presents a new composite transposon (Tn*7382*) made up of two direct copies of IS*Aba14* flanking the genes *aphA6* and $bla_{\text{NDM-1}}$ that encode for amikacin and carbapenem resistance, respectively. While found in previously published *A. baumannii* chromosomes, the IS*Aba14*-bracketed transposon was described for the first time. The potential mobility of the transposon was evidenced by bioinformatic analysis of homologous sequences in the NCBI database. A mechanism for the mobility of Tn*7382* was also proposed. Interspecies transmission is potentially anticipated.

Declarations

Funding: The authors thank the Deanship of Scientific Research at King Saud University for funding this work through Project No. RGP-038.

Authors contributions:

SH, AH, MA, HR, and MZ participated to the study design, conducting the experiments, and data analysis. SH completed the bioinformatic analysis. All authors read and approved the final version of manuscript.

Competing Interests: None to declare.

Ethical Approval: Not required.

5. References

[1] Piperaki ET, Tzouvelekis LS, Miriagou V, Daikos GL. Carbapenem-resistant Acinetobacter baumannii: in pursuit of an effective treatment. Clin Microbiol Infect. 2019;25:951-7.

[2] Lambert T, Gerbaud G, Courvalin P. Transferable amikacin resistance in Acinetobacter spp. due to a new type of 3'-aminoglycoside phosphotransferase. Antimicrob Agents Chemother. 1988;32:15-9.

[3] Rolain JM, Parola P, Cornaglia G. New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect. 2010;16:1699-701.

[4] Bonnin RA, Poirel L, Naas T, Pirs M, Seme K, Schrenzel J, et al. Dissemination of New Delhi metallo-beta-lactamase-1-producing Acinetobacter baumannii in Europe. Clin Microbiol Infect. 2012;18:E362-5.

[5] Nigro SJ, Post V, Hall RM. Aminoglycoside resistance in multiply antibiotic-resistant Acinetobacter baumannii belonging to global clone 2 from Australian hospitals. J Antimicrob Chemother. 2011;66:1504-9.

[6] Zafer MM, Hussein AFA, Al-Agamy MH, Radwan HH, Hamed SM. Genomic Characterization of Extensively Drug-Resistant NDM-Producing Acinetobacter baumannii Clinical Isolates With the Emergence of Novel blaADC-257. Front Microbiol. 2021;12:736982.

[7] Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. Bioinformatics. 2011;27:1009-10.

[8] Müller C, Stefanik, D., Wille, J., Hackel, M., Higgins, P. G., and Siefert, H. . Molecular epidemiology of carbapenem-resistant Acinetobacter baumannii clinical isolates and identification of the novel international clone IC9: results from a worldwide surveillance study (2012–2016). Paper presented at the ECCMID 2019: Proceeding of the 29th European Congress of Clinical Microbiology & Infectious Diseases. Amsterdam, Netherlands April 13–16, 20192019.

[9] Bonnin RA, Cuzon G, Poirel L, Nordmann P. Multidrug-resistant Acinetobacter baumannii clone, France. Emerg Infect Dis. 2013;19:822-3.

[10] Vijayakumar S, Wattal C, J KO, Bhattacharya S, Vasudevan K, Anandan S, et al. Insights into the complete genomes of carbapenem-resistant Acinetobacter baumannii harbouring bla OXA-23, bla OXA-420 and bla NDM-1 genes using a hybrid-assembly approach. Access Microbiol. 2020;2:acmi000140.

[11] Salloum T, Tannous E, Alousi S, Arabaghian H, Rafei R, Hamze M, et al. Genomic mapping of ST85 blaNDM-1 and blaOXA-94 producing Acinetobacter baumannii isolates from Syrian Civil War Victims. Int J Infect Dis. 2018;74:100-8.

[12] Xanthopoulou K, Urrutikoetxea-Gutierrez M, Vidal-Garcia M, Diaz de Tuesta Del Arco JL, Sanchez-Urtaza S, Wille J, et al. First Report of New Delhi Metallo-beta-Lactamase-6 (NDM-6) in a Clinical Acinetobacter baumannii Isolate From Northern Spain. Front Microbiol. 2020;11:589253.

[13] Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, et al. Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant Acinetobacter baumannii Infection. Antimicrob Agents Chemother. 2017;61.

[14] Blackwell GA, Holt KE, Bentley SD, Hsu LY, Hall RM. Variants of AbGRI3 carrying the armA gene in extensively antibiotic-resistant Acinetobacter baumannii from Singapore. J Antimicrob Chemother. 2017;72:1031-9.

[15] Bonnin RA, Poirel L, Nordmann P. A novel and hybrid composite transposon at the origin of acquisition of bla(RTG-5) in Acinetobacter baumannii. Int J Antimicrob Agents. 2012;40:257-9.

Table 1: *Acinetobacter baumannii* strains carrying Tn*7382* retrieved from the NCBI nucleotide database

Strain	GenBank Accession	Chromosomal location	Country	Date	ST(Pasteur)	ST(Oxford)	IC
TP2	CP060011.1	110080 - 118034	USA	2016	ST570	ST1578/2055	IC2
TP3	CP060013.1	110078 - 118032	USA	2016	ST570	ST1578/2055	IC2
ACN21	CP038644.1	2097047 - 2105010	India	2018	ST85	ST1089	IC9
AbBAS-1[a]	CP065392.1	1261231 - 1269172	Spain	2019	ST85	ST957	IC9
Cl300[b]	CP082952.1	109,440 – 131,644	Lebanon	2015	ST85	ST1089	IC9

ST, sequence type.
IC, international clone.
[a]The transposon carries bla_{NDM-6} rather than bla_{NDM-1}.
[b]Tn*7382* is interrupted by seven direct copies of Tn*aphA6*.

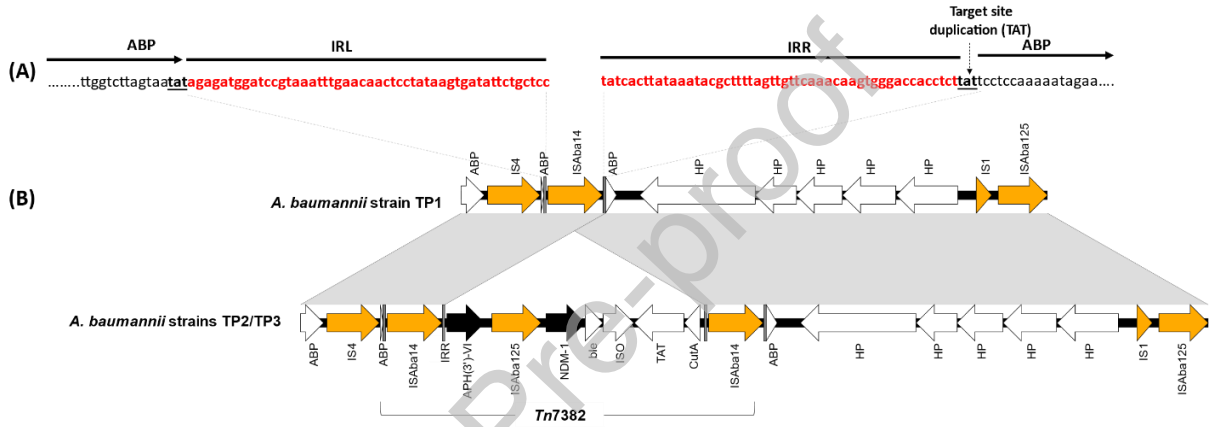

Fig. 1. (A) Depiction of Tn*7382* insertion site showing 3 bp (TAT) target site duplication (B) Comparative analysis showing the acquisition of Tn*7382* by TP2 and TP3 compared to TP1 recovered from the same patient in an earlier time. The comparison included *A. baumannii* strain TP1 (Genbank: CP056784, Region: 108360..120537) and *A. baumannii* strain TP2 (Genbank: CP060011.1, Region: 108364..127214) / TP3 (GenBank: CP060013.1, Region: 108362..127212). ORFs orientation is indicated by arrows. Resistance genes are represented by black arrows while orange arrows represent insertion sequences. Grey bands between panels indicate sequences with 100% similarity. Genes are labelled by their protein products; ABP, ATP-binding protein; IS4, IS4 family transposase; IS*Aba14*, IS3-like element IS*Aba14* family transposase; APH(3')-VI, APH(3')-VI family aminoglycoside O-phosphotransferase; IS*Aba125*, IS30-like element IS*Aba125* family transposase; NDM-1, subclass B1 metallo-beta-lactamase NDM-1; Ble, bleomycin resistance protein; ISO, phosphoribosylanthranilate isomerase; TAT, twin-arginine translocation pathway signal sequence protein; CutA, divalent cation tolerance protein; HP, hypothetical protein; IS1, IS1 family transposase. IRR and IRL denote inverted right and left repeats of IS*Aba14*, respectively. The figure was created using EasyFig v2.2.5.

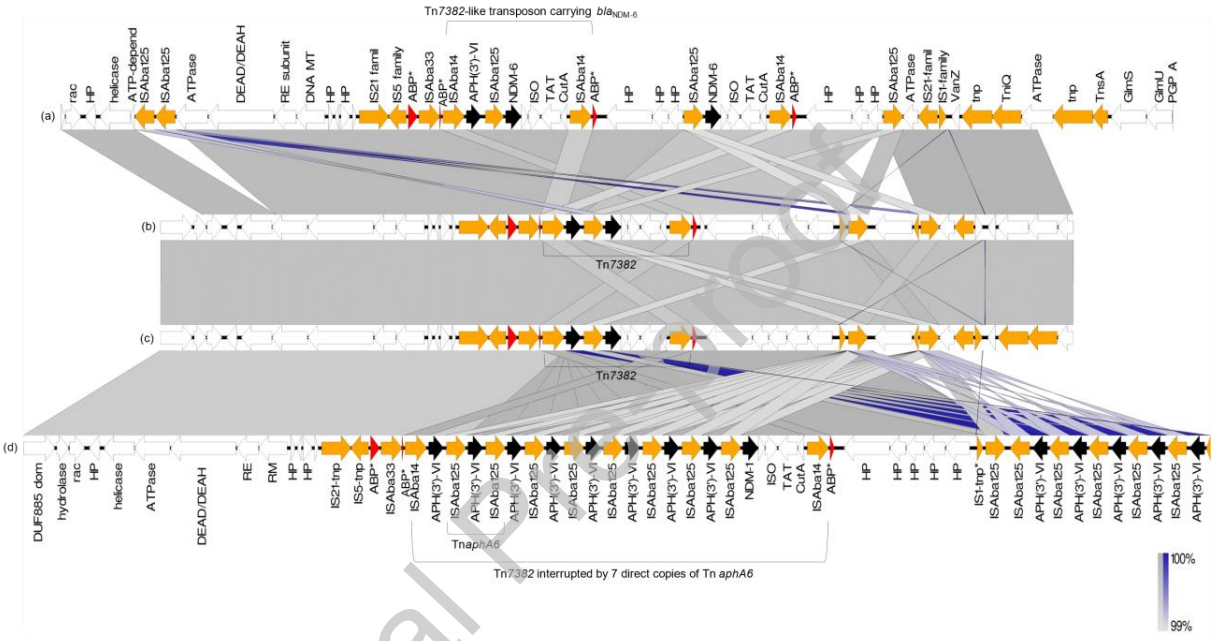

Fig. 2. Gene maps showing the genetic context of Tn*7382* in *A. baumannii* strains AbBAS-1 (GenBank: CP065392.1) (a), TP2/TP3 (GenBank: CP060011.1/CP060013.1) (b), ACN21 (GenBank: CP038644.1) (c), and Cl300 (GenBank: CP082952.1) (d). ORFs orientation is indicated by arrows. Resistance genes are represented by black arrows while orange arrows represent insertion sequences. The gene interrupted by Tn*7382* (ABP) is represented by red arrows. Grey bands between panels indicate sequences with at least 99% similarity while blue panels denote inverted sequences of at least 99% similarity. Genes are labelled by their protein products. Tn*7382* carries the gene $bla_{\text{NDM-1}}$ in all strains except in AbBAS-1 in which the transposon carries the gene bla_{NDM-6}. The figure was created using EasyFig v2.2.5.