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Short Communication

Comparative genomic analysis of clinical *Acinetobacter nosocomialis* isolates from Terengganu, Malaysia led to the discovery of a novel tetracycline-resistant plasmid

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ABSTRACT

Objectives: To analyse the genome sequences of four archival *Acinetobacter nosocomialis* clinical isolates (designated AC13, AC15, AC21 and AC25) obtained from Terengganu, Malaysia in 2011 to determine their genetic relatedness and basis of antimicrobial resistance.
Methods: Antimicrobial susceptibility profiles of the *A. nosocomialis* isolates were determined by disk diffusion. Genome sequencing was performed using the Illumina NextSeq platform.
Results: The four *A. nosocomialis* isolates were cefotaxime resistant whereas three isolates (namely, AC13, AC15 and AC25) were tetracycline resistant. The carriage of the $bla_{ADC-255}$-encoded cephalosporinase gene is likely responsible for cefotaxime resistance in all four isolates. Phylogenetic analysis indicated that the three tetracycline-resistant isolates were closely related, with an average nucleotide identity of 99.9%, suggestive of nosocomial spread, whereas AC21 had an average nucleotide identity of 97.9% when compared to these three isolates. The tetracycline-resistant isolates harboured two plasmids: a 13476 bp Rep3-family plasmid of the GR17 group designated pAC13-1, which encodes the *tetA(39)* tetracycline-resistance gene, and pAC13-2, a 4872 bp cryptic PriCT-1-family plasmid of a new *Acinetobacter* plasmid group, GR60. The *tetA(39)* gene was in a 2 001 bp fragment flanked by XerC/XerD recombination sites characteristic of a mobile p*dif* module. Both plasmids also harboured mobilisation/transfer-related genes.
Conclusions: Genome sequencing of *A. nosocomialis* isolates led to the discovery of two novel plasmids, one of which encodes the *tetA(39)* tetracycline-resistant gene in a mobile p*dif* module. The high degree of genetic relatedness among the three tetracycline-resistant *A. nosocomialis* isolates is indicative of nosocomial transmission.

1. Introduction

Bacteria of the genus *Acinetobacter* are taxonomically diverse, ranging from environmental isolates with bioremediation potential to clinical isolates that cause substantial morbidity and mortality [1]. In the List of Prokaryotic Names with Standing in Nomenclature (https://lpsn.dsmz.de/genus/acinetobacter) [2], there are currently 78 listed *Acinetobacter* species (including synonyms, as of 21 July 2022). *A. baumannii* overshadows the rest of the *Acinetobacter* clan because of its importance as a nosocomial pathogen and its ability to accumulate antimicrobial resistance (AMR) determinants have brought carbapenem-resistant *A. baumannii* to the top of the World Health Organisation's priority pathogen list in ur-

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gent need of new antimicrobials [3]. Despite the clinical prominence of *A. baumannii*, other non-*baumannii* acinetobacters have also been associated with nosocomial infections and these include *A. nosocomialis, A. pittii, A. seifertii* and *A. dijkshoorniae*/*A. lactucae* which, along with *A. baumannii* and the soil bacterium *A. calcoaceticus*, are often grouped together as the *A. baumannii-A. calcoaceticus* (Abc) complex because of difficulties in identifying these bacteria by routine phenotypic methods [4,5]. Molecular methods such as matrix-assisted laser desorption-time of flight mass spectrometry (MALDI-TOF MS), *rpoB* or *gyrB* sequencing, and particularly whole genome sequencing (WGS), are required for the reliable species identification of these non-*baumannii* acinetobacters [6]. WGS has become an essential tool for the molecular surveillance of bacterial pathogens, in particular AMR strains, by providing detailed insights into their epidemiology, their genetic basis of resistance, as well as the evolution and population dynamics of the pathogen [7].

We previously investigated 153 non-repeat *Acinetobacter* spp. clinical isolates obtained from the main public tertiary hospital in Terengganu, Malaysia in 2015 and showed that most of the isolates (n = 128; 83.7%) were *A. baumannii*. Among the non-*baumannii* isolates, *A. nosocomialis* was the most prevalent (n = 16; 10.5%) [8]. One of the *A. nosocomialis* isolates from 2015 that was carbapenem- and multidrug-resistant, designated AC1530, was completely sequenced and was found to harbour two carbapenemase genes, $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-58}}$ on a large (174 kb), potentially transmissible plasmid, pAC1530, that also encode several other resistance determinants [9]. Intriguingly, a similar plasmid was found on an *A. baumannii* isolate, AC1633, from the same hospital but from the following year, raising the possibility of *A. nosocomialis* AC1530 being the source of transmission of a multidrug resistant plasmid within the same hospital [9]. Despite their importance as a critical priority nosocomial pathogen, there have been very few published detailed reports on the genomes of acinetobacters, particularly the non-*baumannii* isolates from Malaysia. In this study, we sequenced four *A. nosocomialis* clinical isolates obtained from the same tertiary hospital but from 2011, which is the earliest in our collection, to gain further insights into their genomic characteristics and genetic basis of antimicrobial resistance. We show that three of the four isolates, which were resistant to tetracycline, were very closely related, which is likely indicative of a past nosocomial spread. We also show that these three *A. nosocomialis* isolates harboured a plasmid-encoded *tetA(39)* tetracycline-resistant gene flanked by XerC/XerD recombination sites in a characteristic potentially mobile p*dif* module [10] and that an identical module was found on a different plasmid in *A. baumannii* AC1633 that was isolated five years later. Characteristics of a novel PriCT-family cryptic plasmid found in the three tetracycline-resistant *A. nosocomialis* isolates are also presented.

Part of this work was presented as a poster (PGN-006) at the 13$^{\text{th}}$ International Symposium on Antimicrobial Agents and Resistance (ISAAR 2021) [11].

2. Materials and methods

2.1. Bacterial isolates and their identification

In 2011, four non-repeat *Acinetobacter* spp. isolates were obtained from the Microbiology Laboratory, Department of Pathology, Hospital Sultanah Nur Zahirah (HSNZ), Kuala Terengganu. The isolates were identified as *Acinetobacter* spp. by the hospital laboratory using traditional biochemical methods. Further identification of the isolates to the species level was done by sequencing of the *rpoB* gene as previously described [8]. Further characteristics of the four isolates are in Supplementary Table S1.

2.2. Antimicrobial susceptibility profiles

Antimicrobial susceptibility profiles of the *A. nosocomialis* isolates were determined by disk diffusion (Oxoid Ltd., Basingstoke, UK) on Mueller-Hinton agar using a panel of 16 antibiotics encompassing 6 classes of antimicrobials: aminoglycosides (amikacin, gentamicin, and tobramycin), β-lactam/β-lactamase inhibitor combination (ampicillin/sulbactam and piperacillin/tazobactam), carbapenems (meropenem, imipenem, and doripenem), cephalosporins (cefotaxime, ceftriaxone, ceftazidime, and cefepime), fluoroquinolones (ciprofloxacin and levofloxacin), and tetracyclines (tetracycline and doxycycline). Susceptibility to a seventh class of antimicrobials, the polymyxins (i.e. colistin and polymyxin B), was determined by obtaining the MIC values by the agar diffusion method. Carbapenem susceptibility was validated by determining the MIC values for imipenem, meropenem, and doripenem using M.I.C. Evaluator strips (Oxoid Ltd., Basingstoke, UK). Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [12].

2.3. DNA extraction, WGS, sequence assembly and annotation, and bioinformatics analyses

Genomic DNA for WGS was prepared using the Geneaid Presto mini gDNA bacteria kit (Geneaid, Taipeh, Taiwan) following the manufacturer's recommended protocol. The quality of the extracted DNA was evaluated using a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA). Genome sequencing was performed on the Illumina NextSeq (Illumina Inc., San Diego, CA) platform by a commercial service provider (Novogene, Beijing, China). Sequences were assembled using UniCycler v0.4.7 [13] whereas annotation was carried out using the National Center for Biotechnology Information's Prokaryotic Annotation Pipeline during submission. Multilocus sequence typing (MLST) was performed via the *A. baumannii* MLST database at PubMLST (https://pubmlst.org/organisms/acinetobacter-baumannii). Antibiotic resistance genes were identified using ResFinder (https://cge.food.dtu.dk/services/ResFinder/) and the Comprehensive Antibiotic Resistance Database (https://card.mcmaster.ca/). Pan genome analysis for the *A. nosocomialis* isolates was determined using rapid large-scale prokaryote pan genome analysis, with core genomes identified using the criteria of amino acid sequence identities of > 95% and presence in 99% of genomes [9,14]. The derived core genome alignments were then used to infer maximum-likelihood trees using FastTree [15] with 100 bootstraps under the generalised time-reversible (GTR) model.

The resulting phylogenetic tree was visualised using iTOL v5 (https://itol.embl.de/) [16]. Average nucleotide identity (ANI) was calculated using the ANI calculator at https://www.ezbiocloud.net/tools/ani. SnapGene 6.0.4 (GSL Biotech LLC., San Diego, CA) was used to visualise and manipulate the sequences studied. Genetic maps of the plasmids were drawn to scale using EasyFig 2.2.2 (http://mjsull.github.io/Easyfig) [17]. The presence of p*dif* sites in pAC13-1 and related plasmids was determined by BLASTN screening using known XerC/XerD and XerD/XerC sites and manually examining hits that were 75–80% identical in sequence [10].

2.4. Conjugation assays

Conjugation assays were performed to investigate the transmissibility of pAC13-1 from its tetracycline-resistant hosts, *A. nosocomialis* AC13, AC15, and AC25 to the tetracycline-susceptible and azide-resistant variant of *A. baumannii* ATCC 19606 [9], which was used as the recipient strain. Conjugation assays were performed on solid media (Luria-Bertani agar) as previously described [9].

2.5. Data availability

The draft genome sequences of *A. nosocomialis* AC13 were deposited in GenBank under accession no. JACGEE010000000; AC15, accession no. JACGEF010000000; AC21, accession no. JACGED010000000; and AC25, accession no. JACGEG000000000.

3. Results and discussion

All four *A. nosocomialis* isolates were resistant to cefotaxime, while three of the four isolates (namely AC13, AC15, and AC25) were resistant to tetracycline but susceptible to doxycycline. The *A. nosocomialis* isolates were susceptible to the other tested antimicrobials.

The assembled draft genome statistics for the four *A. nosocomialis* isolates are presented in Supplementary Table S1. All four isolates have undetermined novel sequence types (STs). A maximum-likelihood phylogenetic tree of the core genomes of the isolates along with other related *A. nosocomialis* isolates in GenBank (Supplementary Table S2) indicate that these four isolates are closely related (Fig. 1). *A. nosocomialis* AC13, AC15, and AC25 have ANI values of 99.9% whereas AC21, when compared with the other three isolates, has an ANI value of 97.9%. This is strongly suggestive of nosocomial transmission as all four isolates were obtained from different patients in the medical ward of the same hospital in April and June 2011 (Supplementary Table S1). These four isolates were, however, not so closely related to another *A. nosocomialis* strain, AC1530, that was obtained from the same hospital but in 2015 [9] (Fig. 1) with ANI values of 97.2% (for AC13) and 97.5% (for AC21).

All four 2011 *A. nosocomialis* isolates harboured the ADC-255 cephalosporinase-encoded gene, $bla_{\text{ADC-255}}$, which is likely responsible for cefotaxime resistance in these isolates. The three tetracycline-resistant isolates, i.e. AC13, AC15, and AC21, harboured a *tetA(39)* tetracycline resistance gene along with its associated *tetR(39)* regulatory gene that is divergently transcribed from *tetA(39)*. The *tetA(39)* gene is known to confer resistance to tetracycline but not to doxycycline [18]. The *tetA(39)-tetR(39)* genes were located in an identical 13 476 bp plasmid in all three isolates. This plasmid was designated pAC13-1 (accession no. JACGEE010000027) in AC13, pAC15-1 (JACGEF010000028) in AC15, and pAC25-1 (JACGEG010000028) in AC25. For the rest of this article, this plasmid will be referred to as pAC13-1. pAC13-1 is a Rep3-family plasmid that belongs to the GR17 group of *Acinetobacter* plasmids, and the *repB* replicase gene is preceded by four iterons of 22 bp direct repeats (Fig. 2) [19]. Two transfer-related genes, *trbJ* and *trbL*, were found in pAC13-1, indicating its potential mobility or transmissibility. The *tetA(39)-tetR(39)* genes were located in a 2001 bp fragment flanked by XerC/XerD recombination sites in inverse orientation (i.e. XerC-XerD and XerD-XerC, with a 6 bp spacer in between the 11 bp XerC/D recognition sites; see Fig. 2B), characteristic of a mobile p*dif* module [10,18,19]. The *tet(39)* p*dif* module was first reported in a 18.2 kb *A. baumannii* plasmid pS30-1 [18], which belonged to the GR3 group of *Acinetobacter* plasmids. We had previously found an identical *tet(39)* p*dif* module in a 12 kb GR23 group plasmid, pAC1633-2 from *A. baumannii* AC1633, which was isolated from the same hospital in Terengganu but in 2016 [9]. The discovery of the *tet(39)* p*dif* module in various plasmid backbones (Fig. 2A) in other *Acinetobacter* isolates worldwide strongly suggests the genetic mobility of this module [10,18]. Interestingly, comparison of the flanking p*dif* se-

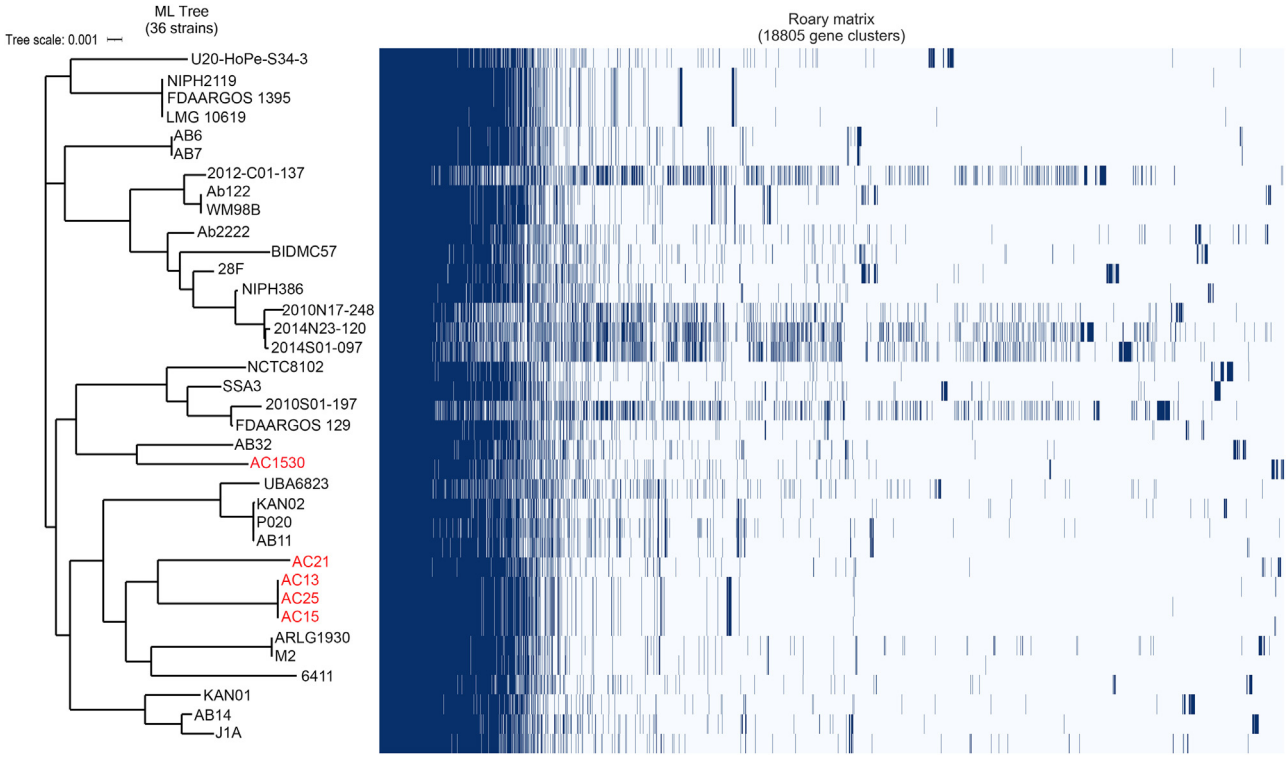
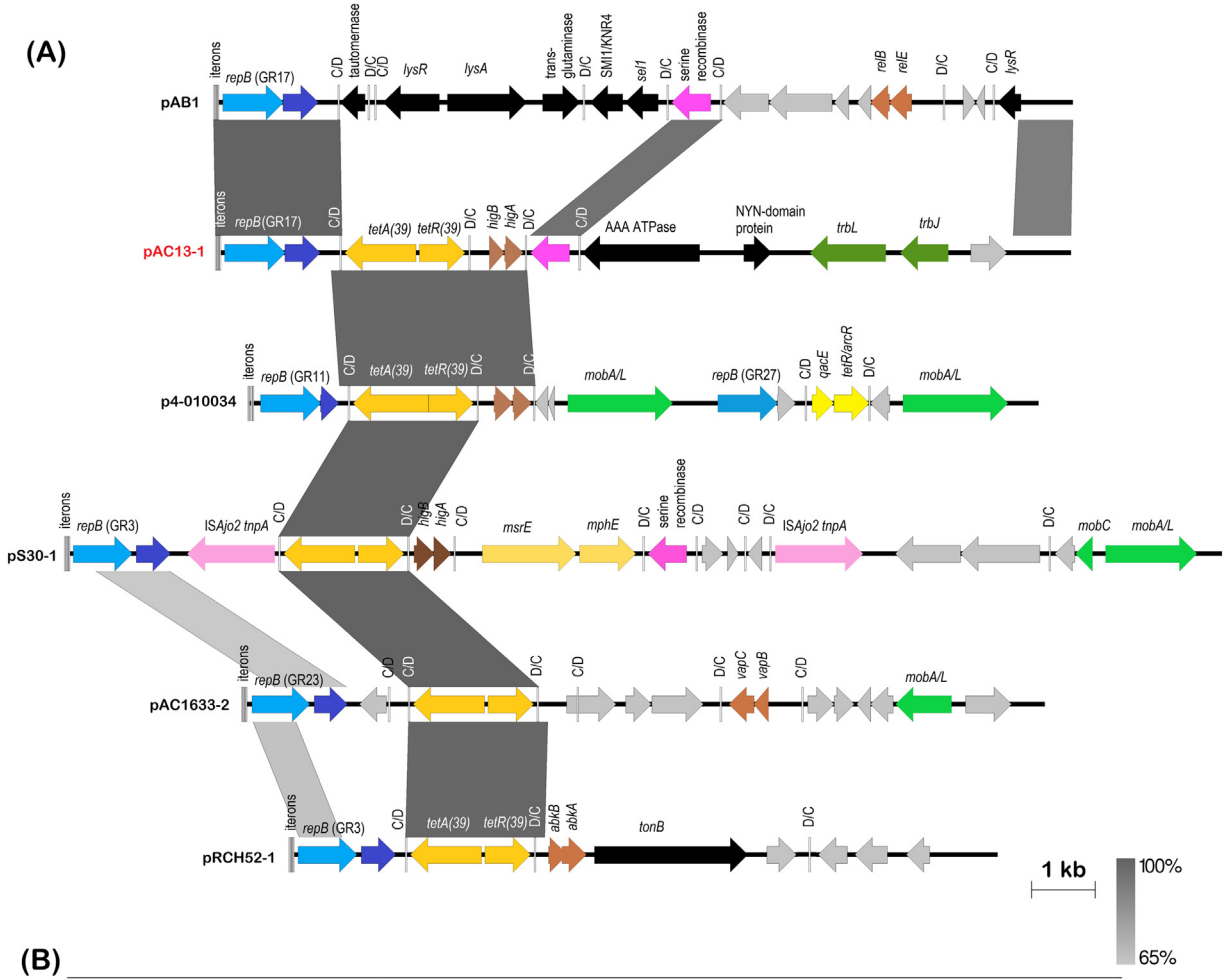


Fig 1. Core genome Maximum-likelihood phylogenetic tree of *Acinetobacter nosocomialis* AC13, AC15, AC21, and AC25 in comparison with other *A. nosocomialis* genomes in the NCBI database. The list of the *A. nosocomialis* genomes used to construct the phylogenetic tree is in Supplementary Table S2. In the Roary matrix, genomes are shown as rows and homologous gene clusters are depicted as columns. The presence of a gene cluster in a genome is indicated by blue. Core gene clusters that are found in all genomes are shown on the left side of the matrix. The *A. nosocomialis* core genome consisted of 1088 genes from a total of 18 805 genes. The *A. nosocomialis* isolates AC13, AC15, AC21, and AC25 presented in this study are labelled in the phylogenetic tree in bold red fonts, as is the other *A. nosocomialis* isolate AC1530 that was obtained from the same hospital but in 2016 and was previously described in Alattraqchi et al. [9].



(B)

Plasmid	Size of *tet39* p*dif* (bp)	Left p*dif* (XerC/XerD) sequence	Right p*dif* (XerD/XerC) sequence
pAC13-1	2,001	ATTTCGAATAAGGTGTATTATGTTAATT	ATTTAACATAATAGAGGTTATACGAAAC
pAC1633-2	2,001	AATTCGTATAACGTGTATTATGTTAATT	ATTTAACATAATAGAGCTTATACGAAAT
pS30-1	2,001	ATTTCGTATAAGGTGTATTATGTTAATT	ATTTAACATAATGGCTGTTATGCGAAAC
p4-010034	2,001	ATTTCGTATAAGGTGTATTATGTTAATT	ATTTAACATAATAGAGCTTATACGAAAC
pRCH52-1	2,001	ATTTCGTATAAGGTGTATTATGTTAATT	ATTTAACATAATGGATATTATACGAAGT

Fig. 2. (A) Linear map of plasmid pAC13-1 compared to other *Acinetobacter* plasmids containing the *tet(39)* p*dif* module. Arrows indicate the extent and direction of genes and open reading frames (ORFs). Antibiotic resistance genes are shown as yellow arrows; plasmid replication initiation or replicase genes of the Rep3 family are shown as blue arrows and labelled as *repB* with darker blue arrows immediately downstream of *repB* depicting ORFs that encode putative helix-turn-helix DNA-binding proteins that had been previously misannotated as *repA* [19]; mobilisation or transfer-related genes are shown as green arrows; transposase genes as light pink arrows; serine recombinase genes as pink arrows; and toxin-antitoxin genes are depicted as brown arrows. Black arrows are for ORFs with known domains or functions whereas light grey arrows are for ORFs encoding hypothetical proteins. The *tetA(39)-tetR(39)* genes are labelled and p*dif* sites are depicted as vertical lines labelled either as C/D for XerC-XerD sites, or D/C for XerD-XerC sites. Horizontal bars upstream of the *repB* gene and labelled as "iterons" are the four 22 bp iterons that are likely the *oriV* site for each plasmid. Grey shaded areas are regions with >65% DNA sequence identities with darker shades of grey indicating higher nucleotide sequence identities as shown in the vertical bar at the bottom right side of the Figure. (B) Sequences of the p*dif* sites flanking the *tetA(39)-tetR(39)* genes with bases in red fonts indicating sequences that differ from the p*dif* sequences of pAC13-1. The accession numbers and the original host of the plasmids used in this analysis are as follows: pAB1 (accession no. CP039030) from *A. baumannii* ATCC 17978; p4-010034 (accession no. CP032272) from *Acinetobacter* spp. WCHAc010034; pS30-1 (accession no. KY617771) from *A. baumannii* SGH0823 [18]; pAC1633-2 (accession no. CP059303) from *A. baumannii* AC1633 [9]; and pRCH52-1 (accession no. KT346360) from *A. baumannii* RCH52.

quences of several *tet(39)* modules indicated higher sequence conservation in the left XerC-XerD sequence (i.e. next to the *tetA(39)* gene) whereas the right XerD-XerC sequence (next to the *tetR(39)* gene) showed sequence conservation in the XerD recognition site but up to 3 nt differences in the 6 bp spacer and 1–2 nt differences in the 11 bp XerC recognition site (Fig. 2B). Besides *tet(39)*, other antimicrobial resistance genes, including bla_{OXA-24} and bla_{OXA-40}-encoded carbapenemases, have been found in similar p*dif* modules in *Acinetobacter* spp. The mobility of these p*dif* modules is likely by Xer-mediated site-specific recombination and thus contributes to the spread of antimicrobial resistance genes in *Acinetobacter* spp. [10]. Two other p*dif* modules are found in pAC13-1: one which contained a *higAB* toxin-antitoxin system, and another which harboured a resolvase/serine recombinase gene (Fig. 2). Similar p*dif* modules were previously reported in pS30-1 [18].

The transmissibility of pAC13-1 was investigated by performing conjugation assays as previously outlined [9] using *A. nosocomialis* AC13, AC15, and AC25 as donor strains and an azide-resistant *A. baumannii* ATCC 19606 as the recipient strain. However, no transconjugants were obtained that were able to grow on the

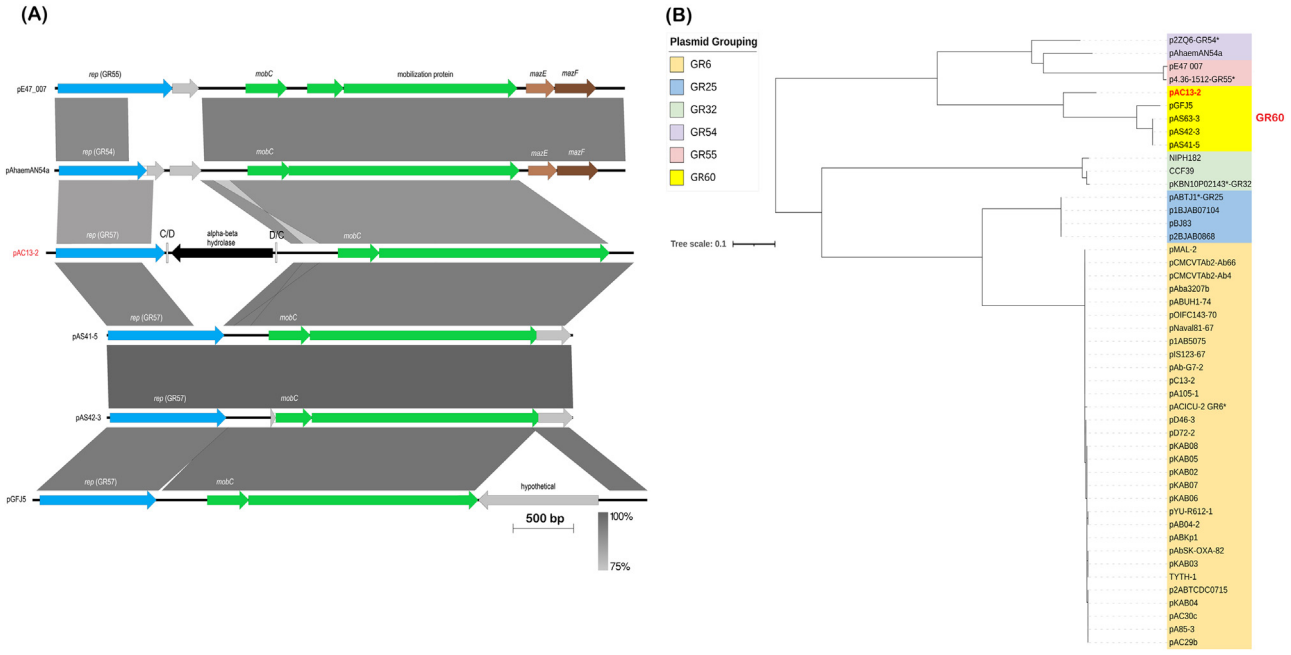


Fig. 3. (A) Linear map of plasmid pAC13-2 in comparison to other similar *Acinetobacter* plasmids. Arrows indicate the extent and direction of genes and open reading frames (ORFs). The plasmid replication initiation or replicase genes are shown as blue arrows and labelled as *rep* with the *Acinetobacter* plasmid grouping indicated in parenthesis; mobilisation or transfer-related genes are shown as green arrows; brown arrows are toxin-antitoxin genes; ORFs with known domains are shown as black arrows; light grey arrows depict ORFs encoding hypothetical proteins. Grey shaded areas are regions with >75% nucleotide sequence identities with darker shades of grey indicating higher sequence identities as depicted in the vertical bar at the bottom right side of the Figure. p*dif* sites in pAC13-2 flanking the alpha-beta hydrolase fold protein-encoding gene are depicted as vertical lines labelled either as C/D for XerC-XerD sites, or D/C for XerD-XerC sites. The accession nos. and the original hosts of the plasmids used in this analysis are as follows: pE47_007 (accession no. CP042563) from *A. baumannii* E47; pAhaemAN54a (accession no. CP041228) from *Acinetobacter haemolyticus* AN54; pAS41-5 (accession no. CP061639) from *Acinetobacter seifertii* AS41; pAS42-3 (accession no. CP061831) from *A. seifertii* AS42; and pGFJ5 (accession no. CP016901) from *Acinetobacter soli* GFJ2. (B) Unrooted maximum-likelihood phylogenetic tree of the PriCT-1 family of *Acinetobacter* plasmids obtained using the Rep amino acid sequences and labelled with the updated *Acinetobacter* plasmid GR homology grouping with different shades of colour [21]. The pAC13-2-encoded Rep is indicated in bold red font in the phylogenetic tree. The Rep protein sequences were obtained from Castro-Jaimes et al. [21] with the accession numbers of the Rep proteins of the proposed new GR60 group as follows: pGFJ5 (accession no. APV37792); pAS41-5 (accession no. QNX70686); pAS42-3 (accession no. QOD75433); and pAS63-3 (accession no. QNX21880). The tree was built from a CLUSTAL-Omega alignment of all the PriCT-1 Rep protein sequences using MEGA-X (https://www.megasoftware.net/) and visualised using iTOL v5.

selection plates (Luria-Bertani agar supplemented with 300 µg/mL sodium azide and 5 µg/mL tetracycline). In the absence of a full suite of conjugative transfer genes in pAC13-1 as well as the chromosomes of these *A. nosocomialis* isolates, it is likely that transfer of pAC13-1 will require the presence of a helper conjugative plasmid or a conjugative element, which were not found in the donor *A. nosocomialis* strains.

Besides pAC13-1, the three tetracycline-resistant *A. nosocomialis* isolates also harboured a 4862 bp plasmid designated pAC13-2 in AC13 (accession no. JACGEE010000028; pAC15-2 in AC15, accession no. JACGEF010000029; and pAC25-2 in AC25, accession no. JACGEG010000029). pAC13-2 encodes a PriCT-1 family replicase, and comparison with other *Acinetobacter* replicases in a recent update on *Acinetobacter* plasmid groupings [20,21] showed that the pAC13-2-encoded replicase belongs to a new group that we designate GR60 (Fig. 3). Like pAC13-1, pAC13-2 is also potentially mobilisable, as it encodes a *mobC* gene, and its downstream gene (locus tag: H2O10_RS18095) encodes a 634-amino acid residue protein of the relaxase superfamily (pfam03432). pAC13-2 also encodes an alpha-beta hydrolase fold protein of unknown function (locus tag: H2O10_RS18105) that was not found in comparison with other similar *Acinetobacter* plasmids (Figure 3). Interestingly, this gene is likely in a p*dif* module as it is flanked by inversely oriented XerC/XerD sites.

In the absence of a selective marker for pAC13-2, we were unable to determine if conjugative transfer of the plasmid had occurred during conjugation assays that were carried out using its host *A. nosocomialis* AC13 (as well as AC15 and AC25) as donor strains. However, this seems unlikely without the presence of a helper conjugative plasmid, as pAC13-2 only encodes two transfer-related genes. Nevertheless, the discovery of similar GR60-type plasmids in several *A. seifertii* clinical strains from Taiwan (with 94% sequence identity and 72% coverage) and an *A. soli* strain GF52 from Thailand (95.5% identity with 73% coverage; accession no. CP016901.1) (Fig. 3) is indicative of possible past transfer events. Notably, the *A. seifertii* GR60 cryptic plasmids were found in 11 of 42 *A. seifertii* strains that were isolated from several hospitals in Taiwan between January 2010 and March 2017 [22]. These *A. seifertii* plasmids were nearly identical (Supplementary Fig. S1), and the presence of large conjugative plasmids in some of these strains [22] could have enabled the transmission of these GR60 plasmids to other related strains.

4. Conclusions

In this study, WGS of four archived *A. nosocomialis* hospital isolates from Terengganu, Malaysia showed the likelihood of nosocomial transmission, particularly for three tetracycline-resistant isolates (AC13, AC15, and AC25) that were nearly identical with an ANI of 99.7%. These three isolates also harboured novel identical plasmids designated pAC13-1 and pAC13-2. The *tetA(39)* tetracycline resistance gene was found on a 2001 bp p*dif* module in pAC13-1 and was identical to the *tet39* p*dif* module found elsewhere [10,18] as well as on pAC1633-2, a 12 kb plasmid in *A. baumannii* AC1633, which was isolated from the same hospital but five years later in 2016 [9]. On the other hand, pAC13-2 is a cryptic plasmid with a PriCT-1 replicase of a new *Acinetobacter* plasmid group designated GR60. This study has given us further insights

into the genomes of *A. nosocomialis* from Malaysia, for which there has been a dearth of published data available. Along with our previously published work [9,23], this would form the basis for our continual genome sequencing efforts to enable us to track and monitor antimicrobial resistance genes and to determine if there are certain Acinetobacter clones and plasmids that are still circulating in the hospital. This, in turn, will help us gain a better understanding of the genomic characteristics of Malaysian isolates of *Acinetobacter* spp., thereby contributing to the global surveillance of AMR pathogens.

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval

Ethical approval for the collection of *Acinetobacter* spp. isolates was obtained from the Malaysian Ministry of Health's Medical Research Ethics Council (approval no. NMRR-14-1650-23625-IIR).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.08.019.

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