The road less travelled: Exploring the genomic characteristics and antimicrobial resistance potential of *Acinetobacter baumannii* from the indigenous Orang Asli community in Peninsular Malaysia

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Abstract

Acinetobacter baumannii is widely recognized as a multidrug-resistant pathogen, although most public genome datasets are biased toward hospital-derived isolates. Little is known about *A. baumannii* isolates from healthy individuals from the community. This study analyzed genome sequences from nine *A. baumannii* isolates obtained from the upper respiratory tract of the indigenous Orang Asli in their rural community in Peninsular Malaysia. Genomic analysis revealed genetic diversity, including three new Pasteur sequence types (STs) and six novel Oxford STs. One isolate, *A. baumannii* 19064, belonged to Global Clone 8 (GC8), a lineage linked to clinical infections. Core genome phylogeny showed these community isolates interspersed with clinical isolates from a nearby hospital, indicating potential pathogenicity under suitable conditions. All isolates carried intrinsic $bla_{\text{OXA-51-like}}$ carbapenemase and bla_{ADC} cephalosporinase genes but remained susceptible to meropenem. Two isolates, *A. baumannii* 19053 and 19062, were tetracycline-resistant but minocycline-susceptible, and harbored the *tet(39)-tetR* gene pair within a mobile p*dif* module on distinct Rep_3-type plasmids. Only one isolate, *A. baumannii* 19055, is plasmid-free; the rest mainly harbored cryptic plasmids, often

containing identifiable p*dif* modules. These findings highlight the clinical relevance of *A. baumannii* strains residing in healthy individuals, particularly in isolated communities that are seldom accessible to public health. Despite their remote origins, these isolates possess virulence factors and resistance genes similar to those in hospital settings. This underscores the importance of genomic surveillance of commensal pathogens, as exploring these less-studied isolates can yield insights into broader epidemiological trends and guide future public health strategies.

Keywords: indigenous community *A. baumannii*, Orang Asli, antibiotic resistance genes, virulence factors, p*dif* modules, plasmids

1 Introduction

Carbapenem-resistant *Acinetobacter baumannii* is recognized by the World Health Organization as the top critical priority pathogen, posing the highest threat to public health due to limited treatment options (World Health Organization, 2024). Unsurprisingly, the majority of reported *A. baumannii* genomes and those deposited in public databases, such as NCBI Genomes and the PubMLST genome collection, are mainly of hospital origin. These isolates are obtained from patients with hospital-acquired infections (HAIs), such as ventilator-associated pneumonia (VAP), meningitis, blood stream infections and urinary tract infections (Bian et al., 2021; Cui et al., 2023; Shelenkov et al., 2024). As a result, the public *A. baumannii* genome datasets are heavily skewed toward dominant hospital-associated clones, notably members of the notorious Global Clone 2 (GC2) lineage (Shelenkov et al., 2023). While keeping track of hospital-related *A. baumannii* is essential due to their formidable antimicrobial resistance (AMR) and clinical relevance, they only represent a subset of the species. These strains often differ significantly from those found in the normal flora of healthy populations (Muzahid et al., 2023). *A. baumannii* from healthy communities remain largely understudied, more so for indigenous communities that are isolated from urban populations.

The Orang Asli are indigenous people in Peninsular Malaysia comprising of several ethnic subgroups who retained their aboriginal language, customs and lifestyle (Mahmud et al., 2022). They are only a minor population in Malaysia (0.8% of the population in Peninsular Malaysia based on the year 2020 census) and often fall behind national socioeconomic, education and healthcare improvement plans. Despite government resettlement programs, many Orang Asli

communities remain in rural areas due to their lifestyle preferences (Pah Rokiah Syed Hussain et al., 2017; Mahmud et al., 2022). Some of these isolated communities are difficult to reach, limiting their access to modern medicines such as antibiotics and vaccines (Mohd Rosman et al., 2020; Chew et al., 2022). This restricted interaction with urban communities and healthcare has led to the formation of what we term here as "genomic capsules" – distinct microflora genomes unique to the Orang Asli community and their respective tribes. As a result, opportunistic pathogens such as *A. baumannii* harbored by the Orang Asli may differ from strains commonly present in urban hospitals.

A. baumannii from hospitals have been well-studied over the past two decades, gaining their notoriety due to their multidrug resistance (MDR), extensive drug resistance (XDR) and pan-drug resistance (PDR) characteristics (Shi et al., 2024). The Malaysian Ministry of Health has published annual National Surveillance of Antibiotic Resistance (NSAR) Reports since 2003. Beginning in the 2010s, more than 50% of *A. baumannii* isolates have been reported to be resistant to carbapenems (i.e., imipenem and meropenem), which are the drugs of choice for treatment. However, the NSAR dataset is limited to participating hospitals which are mainly in the urban and suburban areas of Malaysia (Ministry of Health, 2023). While the World Health Organization's (WHO) Tracking AMR Country Self-Assessment Survey (TrACSS) Country Report emphasizes the importance of addressing AMR at the community level to enhance infection prevention and control (IPC) efforts (WHO, 2022), there remains a significant knowledge gap regarding AMR profiles and genomic characteristics of bacterial pathogens within the indigenous communities in Malaysia. Notably, a recent study investigating *A. baumannii* isolates from human fecal samples in a community in Segamat, Malaysia, revealed phylogenomic clustering of four community-derived strains with two isolates from the town's main tertiary hospital. This finding suggests the potential persistence and circulation of certain *A. baumannii* strains across both community and healthcare settings (Muzahid et al., 2023). In this study, we aim to provide a genomic snapshot of *A. baumannii* that were isolated during an all-age, upper respiratory tract microbial carriage study undertaken among two rural Orang Asli communities in the state of Terengganu, located in the eastern coast of Peninsular Malaysia in 2017. A previous investigation of *Klebsiella pneumoniae* isolates from a broader indigenous cohort revealed the predominance of ST23 which is commonly associated with clinical *K. pneumoniae* infections, and of concern, a proportion of these isolates harbored genes that categorized them as hypervirulent (Das et al., 2024). Here, we present the genomic analysis of *A. baumannii* isolates recovered from the upper respiratory tract of the Orang Asli and show the genetic diversity of this hitherto unexplored *A. baumannii* "genomic capsule". Our findings

offer insights into strains of novel STs, their patchwork of unique and shared mobile genetic elements, antimicrobial resistance and virulence genes.

2 Materials and Methods

2.1 Sampling and isolation of A. baumannii from Orang Asli communities

Swabs were taken from two Orang Asli villages, namely Kampung Sungai Pergam and Kampung Berua, in the state of Terengganu on the east coast of Peninsular Malaysia. Nasal swabs and nasopharyngeal swabs were taken from each participant, as described (Cleary et al., 2021). Conventional bacteriology of the samples were carried out using Columbia Blood Agar (CBA), CHOC agar (CBA with chocolated horse blood), CNA agar (CBA with colistin and naladixic acid), BACH (CBA with chocolated horse blood and Bacitracin) and GC agar (Lysed GC selective agar) (all culture media from Oxoid, UK) (Cleary et al., 2021). Preliminary identification of presumptive *Acinetobacter* spp. isolates was done using the MALDI Biotyper (Bruker, UK) at the Portsmouth Microbiology Laboratories, UK.

2.2 Antibiotic susceptibility tests

A. baumannii was spread over Mueller-Hinton agar plates (MH; Oxoid, UK). Susceptibilities to the antibiotics meropenem and ciprofloxacin were determined by disk diffusion using the appropriate antibiotic disk (meropenem, 10 µg; ciprofloxacin, 5 µg) (Oxoid, UK) whereas tetracycline and doxycycline susceptibilities were determined by placing Minimum inhibitory concentration (MIC) E-test strips (bioMérieux, France) onto the surface of the agar. All agar plates were incubated at 35°C ± 1°C for 18 h ± 2 h. Susceptibility was determined against the EUCAST Clinical Breakpoint guidelines (2024).

2.3 Genome Sequencing and Assemblies

Genomic DNA of the nine *A. baumannii* isolates were extracted using the QIAmp DNA Mini extraction kit (Qiagen, UK) per the manufacturer's instructions. Concentration of genomic DNA was determined using Qubit 2.0 fluorometer (Thermo-Fisher, UK). Whole genome sequencing was performed on a MiSeq (Illumina, UK) short-read platform at a commercial sequencing provider (MicrobesNG, UK) using the 500 cycle v2 reagent kit to generate 2×150 bp paired-end reads. Raw reads obtained were then quality assessed and trimmed using fastp (available from https://github.com/OpenGene/fastp; (Chen, 2023)). Genome assemblies were carried out using Unicycler (available from https://github.com/rrwick/Unicycler; (Wick et al., 2017), followed by evaluation using Quast (available from https://github.com/ablab/quast).

2.4 Bioinformatics Analyses

Average nucleotide identity (ANI) of the assembled genomes to reference genomes were determined using fastANI (available from https://github.com/ParBLiSS/FastANI; (Jain et al., 2018). Annotation of the genomes was performed using Prokka (available from https://github.com/tseemann/prokka; (Seemann, 2014)). Conventional multilocus sequence typing (MLST) profiles of the assembled genomes were determined using mlst (available from https://github.com/tseemann/mlst) and matched to the PubMLST database (https://pubmlst.org/organisms/acinetobacter-baumannii). MLST profiles determined from the two available schemes, namely the Oxford and Pasteur schemes, were used to identify the corresponding Global Clones (GC). Serotyping based on *A. baumannii* surface polysaccharide loci, namely capsule K loci (KL) and lipo-oligosaccharide OC loci (OCL), were carried out using Kaptive v3.0.0b6 (available from https://github.com/klebgenomics/Kaptive; (Wyres et al., 2020; Cahill et al., 2022)).

Genotypic resistance profiles of the genomes were determined using AMRFinderPlus (available from https://github.com/ncbi/amr; (Feldgarden et al., 2021)) and ABRicate (available from https://github.com/tseemann/abricate), whereby databases from CARD (Alcock et al., 2023) and ResFinder (Zankari et al., 2012) were utilized to the latter approach. Virulome of the assembled genomes were determined using ABRicate, utilizing database from Virulence Factor DataBase (VFDB) (Liu et al., 2022). Findings were then compared to the results obtained from VFAnalyzer (available from https://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi). MGEs such as plasmids, insertion sequence (IS) elements and resistance island (RI) hotspots were also determined from the genomes. Plasmids were identified using PlasmidFinder (available from https://github.com/genomicepidemiology/plasmidfinder; (Carattoli et al., 2014)), whereas classification of the plasmid replication protein (Rep) was performed using an in-house built script, pREPonly (https://github.com/lean-SS/pREP-only) utilizing the AcinetobacterPlasmidTyping database (available from https://github.com/MehradHamidian/AcinetobacterPlasmidTyping; (Lam et al., 2023)). p*dif* sites in the plasmids found were identified using a combination of p*dif* finder (https://github.com/mjshao06/pdifFinder) (Shao et al., 2023) and manual search as outlined by (Ambrose and Hall, 2024). Toxin-antitoxin (TA) systems were determined using the TADB 3.0 database (https://bioinfo-mml.sjtu.edu.cn/TADB3/index.php) (Guan et al., 2024). IS elements were screened using ISEScan (available from https://github.com/xiezhq/ISEScan; (Xie and Tang, 2017)) and ISfinder-sequences database through the Prokka *–protein* option (available

from https://github.com/thanhleviet/Isfinder-sequences; (Siguier et al., 2006)), whereas the *comM* resistance island (RI) hotspot (Hamidian and Hall, 2017) was determined through local BLAST.

2.5 Pangenome and Phylogenetic Analysis

The pangenomes of the nine Orang Asli *A. baumannii* isolates were compared to other community *A. baumannii* genomes using the Anvi'o platform (available from https://github.com/merenlab/anvio; (Delmont and Eren, 2018; Eren et al., 2021)). Through the use of the *anvi-pan-genome* program, the pangenomes were determined and then visualized through *anvi-display-pan*, which links to the Anvi'o server. Core genome phylogenetic analysis of *A. baumannii* genomes were performed using Roary (available from https://sanger-pathogens.github.io/Roary/; (Page et al., 2015)) and VeryFastTree with the GTR+CAT model, which combines the General Time Reversible (GTR) nucleotide substitution model with a Constant Rate Across Sites (CAT) approximation (Piñeiro et al., 2020; Piñeiro and Pichel, 2024), and visualized with iTOL v7 (Letunic and Bork, 2024).

3.0 Results and Discussions

3.1 Preliminary Genomic Analysis of A. baumannii from the Orang Asli

A total of thirteen presumptive *Acinetobacter* spp. isolates were obtained from the Orang Asli carriage studies at Kampung Sungai Pergam (n = 3) and Kampung Berua (n = 10). These were from the nasopharyngeal swabs of a total of 130 participants (of which 68 were from Kampung Sungai Pergam and 62 from Kampung Berua) (Cleary et al., 2021). Whole genome sequencing was performed on all thirteen isolates, out of which nine were shown to be *A. baumannii* (**Table 1**). The remaining four isolates were determined to be *A. nosocomialis*.

Genome assemblies of the nine *A. baumannii* Orang Asli isolates showed total genome sizes that ranged from ~3.7 Mbp to 3.9 Mbp (**Table 1**). Average nucleotide identity (ANI) of the genomes revealed >97% nucleotide identities to *A. baumannii* genomes including those from Thailand (SAMEA104305267, SAMEA104305309, SAMEA104305313 and SAMEA104305269), Vietnam (5577STDY7716391, 5577STDY7716201 and 5577STDY7716392) and Malaysia (SAMN03174920 and SAMN03174917), as listed in **Table 1**. *In silico* epidemiological typing of the assembled genomes, which includes traditional MLST and surface polysaccharide loci typing, showed that each genome is distinct with its own

sequence type (ST), KL and OCL types. MLST profiles based on the Oxford scheme revealed six new STs, with only *A. baumannii* 19056, 19060 and 19064 that were identified as preexisting $ST3415_{Oxf}$, $ST871_{Oxf}$ and $ST585_{Oxf}$, respectively (**Table 1**). The new Oxford STs were submitted and assigned by the PubMLST curators as $ST3540_{Oxf}$, $ST3541_{Oxf}$, $ST3542_{Oxf}$, $ST3543_{Oxf}$, $ST3543_{Oxf}$, $ST3544_{Oxf}$ and $ST3545_{Oxf}$ (**Table 1**). Additionally, the Pasteur MLST scheme identified three novel STs from the nine *A. baumannii* genomes, and these were assigned as $ST2832_{Pas}$, $ST2833_{Pas}$, and $ST2844_{Pas}$. The six remaining genomes each belonged to different preexisting Pasteur STs (**Table 1**). The identification of new STs suggests the possible emergence of genetically distinct lineage within the Orang Asli community. The *A. baumannii* isolates with the new STs, although currently mostly susceptible, are capable of acquiring new resistance and virulence genes. Their presence in a community setting is noteworthy as they could represent a new lineage with the potential to spread and evolve, similar to how the hospital-associated GCs such as GC2 have emerged (Hamidian and Nigro, 2019).

A. baumannii surface polysaccharide loci typing based on the K loci (KL), which were responsible for the production of acinetamic acid (Lam et al., 2022), showed that each of the nine Orang Asli *A. baumannii* genomes belonged to distinct KL types (**Table 1**). Notably, four novel capsule types (i.e., KL170, KL202, KL172 and KL183) were detected, indicating previously unreported characteristics and underscoring the uniqueness of these Orang Asli *A. baumannii* isolates. In contrast, analysis of the outer core lipo-oligosaccharide loci (OCL) showed OCL2 ($n = 4$) to be the most common type, followed by OCL4 ($n = 2$) and OCL6 ($n = 2$) (**Table 1**). Nevertheless, *A. baumannii* 19055 was identified as the lesser-studied OCL13 type, which was originally described in *A. baumannii* strains associated with community-acquired pneumonia in the Northern Territories, Australia (Meumann et al., 2019). The *A. baumannii* capsule plays a crucial role in its ability to cause disease, primarily by protecting the bacterium from the host immune system and desiccation (Rakovitsky et al., 2021; Talyansky et al., 2021). Novel capsule types could therefore have a significant impact on the bacteria's survival and virulence.

Table 1: General information of the source and the genome characteristics of the *A. baumannii* (*n* = 9) isolates from the upper respiratory tract of the Orang Asli in this study.

***Acinetobacter* isolate**	**19053**	**19055**	**19056**	**19058**	**19060**	**19061**	**19062**	**19063**	**19064**
Location/source[§]	KSP/N	KSP/NP	KB/N	KB/N	KB/NP	KB/N	KB/NP	KB/N	KB/N
Species identification	*Acinetobacter baumannii*	*Acinetobacter baumannii*	*Acinetobacter baumannii*	*Acinetobacter baumannii*	*Acinetobacter baumannii*	*Acinetobacter baumannii*	*Acinetobacter baumannii*	*Acinetobacter baumannii*	*Acinetobacter baumannii*
Accession no.	JBNPBH000000000	JBNPBI000000000	JBNPBJ000000000	JBNPBK000000000	JBNPBL000000000	JBNPBM000000000	JBNPBN000000000	JBNPBO000000000	JBNPBP000000000
Closest neighbor (fastANI)	*Acinetobacter baumannii* SAMEA 104305267 (98.1849%)	*Acinetobacter baumannii* 5577 STDY7716391 (99.8833%)	*Acinetobacter baumannii* 5577 STDY7716201 (97.9228%)	*Acinetobacter baumannii* 5577 STDY7715392 (98.7137%)	*Acinetobacter baumannii* SAMEA 104305309 (98.8467%)	*Acinetobacter baumannii* SAMEA 104305313 (98.0632%)	*Acinetobacter baumannii* SAMN 03174920 (98.6532%)	*Acinetobacter baumannii* SAMN 03174917 (97.9238%)	*Acinetobacter baumannii* SAMEA 104305269 (99.8239%)
Total genome size (bp)	3,821,374	3,697,509	3,748,932	3,904,855	3,767,717	3,865,738	3,844,544	3,828,269	3,664,686
No. of contigs	62	166	32	44	126	35	149	47	47
No. of plasmids	2	0	1	1	1	1	2	1	2
GC content (%)	38.91	39.04	38.97	38.87	38.95	38.75	38.99	38.81	38.78
N_{50}	478,783	67,766	458,370	254,121	77,219	477,098	73,853	369,580	195,600
N_{90}	68,035	15,343	166,877	88,568	17,967	165,195	17,420	116,120	67,830
L_{50}	3	17	3	6	16	2	14	3	7
L_{90}	11	61	8	18	49	8	53	10	20
No. of CDS	3,579	3,428	3,497	3,661	3,582	3,573	3,674	3,576	3,490
$MLST_{Oxf}$	ST3542*	ST3543*	ST3415	ST3541*	ST871	ST3544*	ST3545*	ST3540*	ST585
$MLST_{Pas}$	ST2832*	ST2114	ST2522	ST2834*	ST2700	ST470	ST2635	ST2833*	ST10
K loci	KL121	KL96	KL170[#]	KL202[#]	KL112	KL172[#]	KL81	KL183[#]	KL108
OC loci	OCL2	OCL13	OCL2	OCL4	OCL6	OCL4	OCL6	OCL2	OCL2

[§]**Abbreviations used for location/source:** KSP, Kampung Sungai Pergam; KB, Kampung Berua; N, nasal swab; NP, nasopharyngeal swab.

Notes: $MLST_{Oxf}$ and $MLST_{Pas}$ represent the Oxford and Pasteur schemes available in PubMLST, respectively. ST represents sequence type and those marked with an asterisk (*) are new STs that were identified in this study; KL represents K loci with those labelled with [#] representing K loci that has not been reported; OCL represents lipo-oligosaccharide OC loci.

3.2 Genotypic AMR observation revealed non-mainstream β-lactamases

The presence of genes encoding β-lactamases (*bla*) has become the hallmark of *A. baumannii*, not only as key determinants of carbapenem resistance (Shi et al., 2024), but also through the intrinsic *bla*$_{\text{OXA-51-like}}$ genes, which have been adopted as one of the typing methods for *A. baumannii* over the past two decades (Shelenkov et al., 2023). The recent categorization of global clones (GCs) also incorporated various *bla* genes as characteristic traits observed in certain GCs, for example, members of the GC8 lineage commonly harbor both *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-68}}$ (Shelenkov et al., 2023). Although *bla* genes such as *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-51}}$ and *bla*$_{\text{OXA-66}}$, are widespread among *A. baumannii* globally (Li et al., 2023; Shelenkov et al., 2023; Shi et al., 2024), a different spectrum of *bla* genes were found from the Orang Asli *A. baumannii* in this study (**Table 2**).

The *bla*$_{\text{OXA-51}}$ family (or *bla*$_{\text{OXA-51-like}}$) is comprised of the well-characterized *bla*$_{\text{OXA-51}}$ gene and its numerous variants, such as *bla*$_{\text{OXA-64}}$, *bla*$_{\text{OXA-66}}$ and others (Li et al., 2023). In this study, several variants were detected in the Orang Asli *A. baumannii* genomes including *bla*$_{\text{OXA-68}}$, *bla*$_{\text{OXA-98}}$, *bla*$_{\text{OXA-120}}$, *bla*$_{\text{OXA-377}}$, *bla*$_{\text{OXA-424}}$, *bla*$_{\text{OXA-510}}$, and *bla*$_{\text{OXA-555}}$. Only two genomes (i.e., *A. baumannii* 19058 and 19061) carried *bla*$_{\text{OXA-51}}$ itself while the remaining seven harbored distinct variants of the *bla*$_{\text{OXA-51}}$ family (**Table 2**). The spectrum of genes of the *bla*$_{\text{OXA-51}}$ family aligns with the findings of (Muzahid et al., 2023) who reported that community-derived *A. baumannii* isolates from Segamat, Peninsular Malaysia also carried a wide range of *bla*$_{\text{OXA-51}}$ variants. In the Segamat *A. baumannii* isolates, *bla*$_{\text{OXA-120}}$ was the most prevalent variant, followed by *bla*$_{\text{OXA-441}}$, *bla*$_{\text{OXA-510}}$, *bla*$_{\text{OXA-69}}$, *bla*$_{\text{OXA-98}}$ and *bla*$_{\text{OXA-412}}$. None of these variants were found among the *A. baumannii* isolates from the Orang Asli community.

However, the presence of the intrinsic *bla*$_{\text{OXA-51}}$ family of genes is not considered a definite marker for carbapenem resistance in *A. baumannii* due to the low affinities of the OXA-51 family of β-lactamases to meropenem and imipenem as well as the very low expression levels of the *bla*$_{\text{OXA-51-like}}$ genes. In certain cases, the presence of IS*Aba1* directly upstream of the *bla*$_{\text{OXA-51-like}}$ gene provides a strong promoter which increases its expression level, leading to carbapenem resistance but this also depends on the *bla*$_{\text{OXA-51}}$ variant that is being overexpressed (Nigro and Hall, 2018). All nine Orang Asli *A. baumannii* isolates were phenotypically carbapenem susceptible, and none of their genomes contained IS*Aba1* (or related elements) upstream of the *bla*$_{\text{OXA-51-like}}$ genes. None of the nine Orang Asli *A. baumannii* isolates also

harbored acquired *bla*$_{\text{OXA}}$-encoded carbapenemase genes such as *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, and *bla*$_{\text{OXA-58}}$, which have been directly implicated in carbapenem resistance, particularly in clinical *A. baumannii* isolates (Hamidian and Nigro, 2019). This was similarly reported by (Muzahid et al., 2023) where the acquired carbapenemase gene *bla*$_{\text{OXA-23}}$ were only identified in their Segamat hospital isolates which are carbapenem resistant. Likewise, our recent study of a 10-year collection of *A. baumannii* from the main tertiary hospital in Terengganu also revealed the predominance of the *bla*$_{\text{OXA-23}}$ gene among the carbapenem-resistant isolates (Din et al., 2025).

Another class of intrinsic β-lactamase found in *A. baumannii* genomes is the AmpC cephalosporinase variants which are designated *Acinetobacter*-derived cephalosporinases (ADCs). Overproduction of ADCs resulting from insertion of IS*Aba1* or similar IS elements upstream of the *bla*$_{\text{ADC}}$ gene has been shown to be responsible for the development of resistance towards extended-spectrum cephalosporins and in some cases, carbapenems (Tian et al., 2011; Bhattacharya et al., 2014; Shi et al., 2024). Seven different variants of ADCs were detected from the genomes of the nine Orang Asli *A. baumannii* isolates (**Table 2**) and in all cases, IS*Aba1* or similar elements were absent upstream of the encoding gene, suggesting that these genes were either not expressed or were expressed at low levels in their hosts. ADC-25 were identified in two of the Orang Asli *A. baumannii* isolates (i.e., 19053 and 19061; **Table 2**) and this variant was found to be the 7$^{\text{th}}$ most prevalent ADC variant among *A. baumannii* isolates globally (Mack et al., 2025). Four of the eight ADC variants identified here (i.e., ADC-99 in *A. baumannii* 19062, ADC-238 in 19060, ADC-279 in 19055, and ADC-312 in 19058) were listed by (Mack et al., 2025) as variants that were rarely found in *A. baumannii*. By comparison, the *A. baumannii* isolates from the Segamat community also presented a different spectrum of *bla*$_{\text{ADC}}$ genes where they mainly harbored *bla*$_{\text{ADC-154}}$ and *bla*$_{\text{ADC-156}}$ (Muzahid et al., 2023), but both variants were absent in our Orang Asli isolates. However, *bla*$_{\text{ADC-238}}$ which was found in *A. baumannii* C-65 from the Segamat community, was also found in *A. baumannii* 19060 form our Orang Asli collection. The ADC-238 variant was listed as a less-frequently encountered variant (Mack et al., 2025) and its singular presence in both these community-based studies supports this finding. Intriguingly, hospital isolates from Segamat (Muzahid et al., 2023) and Terengganu (Din et al., 2025) showed a uniform pattern of *bla*$_{\text{ADC-73}}$ being the most prevalent, agreeing with the analysis presented by (Mack et al., 2025) which revealed *bla*$_{\text{ADC-73}}$ as the most prevalent ADC variant in *A. baumannii* isolates globally, with the exception of isolates from North America.

Hence, in terms of the class C (ADC) and class D (OXA) β-lactamases, the intrinsic variants harbored by the *A. baumannii* community isolates showed diversity with little in common although both studies (i.e., this study and the Segamat study) were in Peninsular Malaysia. Even between communities, the AMR profiles varies (Meumann et al., 2019; Muzahid et al., 2023) and thus, there are more to be learned about *A. baumannii* from the Orang Asli community, which is further elaborated in the following sections.

3.3 Other resistance genes in the Orang Asli community A. baumannii isolates

Two out of the nine Orang Asli *A. baumannii* isolates (i.e., 19053 and 19062) were found to harbor the *tet(39)* tetracycline resistance gene (Table 2), which encodes a tetracycline efflux pump of the major facilitator superfamily (MFS) (Agersø and Guardabassi, 2005). This differs from the *A. baumannii* community isolates from Segamat in which no tetracycline resistance genes were detected. Both *A. baumannii* 19053 and 19062 were phenotypically tetracycline resistant (with MIC values of 64 µg/mL and 128 µg/mL, respectively) but doxycycline susceptible (both with MIC values of 4 µg/mL). In contrast, Malaysian *A. baumannii* hospital isolates predominantly carried the *tet(B)* gene (n = 60/126 from HSNZ (Din et al., 2025); n = 12/15 from Segamat Hospital (Muzahid et al., 2023)), with only one isolate from HSNZ harboring *tet(A)* and 10/126 carrying *tet(39)* (Din et al., 2025). All *A. baumannii* hospital isolates harboring the *tet(39)* and *tet(A)* genes were tetracycline resistant and minocycline susceptible whereas those that harbored the *tet(B)* gene were mostly resistant to tetracycline but showed intermediate susceptibility to minocycline (Din et al., 2025). Meumann et al. (2019) reported two *A. baumannii* isolates from community-onset pneumonia in Australia which harbored both the *tet(B)* and *tet(39)* genes but phenotypic susceptibility testing for tetracyclines was not performed in their study. The two tetracycline-resistant Orang Asli *A. baumannii* isolates, 19053 and 19062, harbored the *tet(39)* gene on plasmids, which will be elaborated in a later section.

Resistance to aminoglycosides in *A. baumannii* is mainly mediated by the possession of genes encoding aminoglycoside acetyltransferase (*aac*), nucleotidyltransferase (*ant*) and/or phosphotransferase (*aph*) (Shi et al., 2024). Five out of the nine *A. baumannii* Orang Asli isolates carried the *ant(3")-IIa* gene (Table 2) whereas Muzahid et al. (2023) reported the presence of this gene in all twelve of their *A. baumannii* strains that were isolates from the community in the town of Segamat. Nevertheless, only two of the twelve Segamat community

isolates showed resistance to amikacin, and all were gentamicin susceptible, suggesting that some of these aminoglycoside resistance genes were either not expressed or expressed at a very low level. Phenotypic resistance to aminoglycosides was, however, not tested for the Orang Asli *A. baumannii* isolates in this study.

Sulfonamide resistance in *A. baumannii* is usually mediated by *sul1* and/or *sul2* genes (Köld, 2001), with *sul2* predominantly reported from Southeast Asian countries and the Asia-Pacific region (Bian et al., 2021; Brito et al., 2022; Din et al., 2025). Only one Orang Asli isolate, *A. baumannii* 19062, was found to harbor the *sul2* gene (**Table 2**), which was absent in the Segamat community isolates (Muzahid et al., 2023). In contrast, nearly 50% (61/126) of the *A. baumannii* hospital isolates from HSNZ, Terengganu, harbored the *sul2* gene (Din et al., 2025) whereas in Hospital Segamat, the gene was identified in 2/15 of the *A. baumannii* isolates. The significance of the carriage of the *sul2* gene in the solitary Orang Asli *A. baumannii* isolate is currently unknown, but *sul2* is known to be present on mobile elements such as plasmids and transposons (Jeon et al., 2023). Plasmid analysis appeared to rule out the carriage of *sul2* in either of the two plasmids found in *A. baumannii* 19062 (see subsequent section 3.7) but this does not rule out its location on other mobile elements such as transposons or genomic islands in the chromosome.

Table 2: Distribution of antimicrobial resistance determinants across the Malaysian Orang Asli *A. baumannii* genomes (*n* = 9) and their corresponding phenotypic resistance profiles.

Antimicrobial resistance genes/phenotype	***Acinetobacter baumannii* isolate**								
	19053	**19055**	**19056**	**19058**	**19060**	**19061**	**19062**	**19063**	**19064**
Beta-lactams: • **Resistance gene(s)**	*bla*$_{\text{ADC-25}}$, *bla*$_{\text{OXA-377}}$	*bla*$_{\text{ADC-279}}$, *bla*$_{\text{OXA-120}}$	*bla*$_{\text{ADC-169}}$ variant, *bla*$_{\text{OXA-555}}$	*bla*$_{\text{ADC-312}}$ variant, *bla*$_{\text{OXA-51}}$	*bla*$_{\text{ADC-238}}$, *bla*$_{\text{OXA-510}}$	*bla*$_{\text{ADC-25}}$, *bla*$_{\text{OXA-51}}$	*bla*$_{\text{ADC-99}}$, *bla*$_{\text{OXA-98}}$	*bla*$_{\text{ADC-158}}$ variant, *bla*$_{\text{OXA-424}}$	*bla*$_{\text{ADC-76}}$, *bla*$_{\text{OXA-68}}$
• **Meropenem (10 µg)***	22.3 (S)	26.6 (S)	24.2 (S)	29.1 (S)	28.6 (S)	25.7 (S)	21.0 (S)	27.0 (S)	24.6 (S)
Aminoglycoside resistance gene(s)	*ant(3")-IIa*	*ant(3")-IIa*	-	-	*ant(3")-IIa*	-	*ant(3")-IIa*	-	*ant(3")-IIa*
Tetracyclines: • **Resistance gene(s)**	*tet(39)*	-	-	-	-	-	*tet(39)*	-	-
• **Tetracycline**[#]	64.0 (R)	12.0 (S)	4.0 (S)	6.0 (S)	6.0 (S)	4.0 (S)	128.0 (R)	4.0 (S)	6.0 (S)
• **Doxycycline**[#]	4.0 (S)	4.0 (S)	4.0 (S)	4.0 (S)	4.0 (S)	4.0 (S)	4.0 (S)	4.0 (S)	4.0 (S)
Sulfonamide resistance genes(s)	-	-	-	-	-	-	*sul2*	-	-
Fluroquinolones: • **Mutation(s) in QRDRs of *gyrA* and *parC*** • **Ciprofloxacin (5 µg)***	-	-	-	-	-	-	-	-	-
	23.5 (S)	25.2 (S)	24.7 (S)	28.3 (S)	28.0 (S)	28.0 (S)	21.7 (S)	26.8 (S)	24.8 (S)
Efflux pumps	*amvA abeS, abeM, adeA, adeF, adeG, adeH, adeI, adeJ, adeK, adeL, adeN*	*amvA abeS, abeM, adeF, adeG, adeH, adeI, adeJ, adeK, adeL, adeN, adeS, adeR*	*amvA abeS, abeM, adeA, adeB, adeF, adeG, adeH, adeI, adeJ, adeK, adeL, adeN, adeS,adeR*	*amvA abeS, abeM, adeA, adeB, adeF, adeG, adeH, adeI, adeJ, adeK, adeL, adeN, adeS,adeR*	*amvA abeS, abeM, adeA, adeB, adeF, adeG, adeH, adeI, adeJ, adeK, adeL, adeN, adeS, adeR*	*amvA abeS, abeM, adeA, adeB, adeF, adeG, adeH, adeI, adeJ, adeK, adeL, adeN, adeS,adeR*	*amvA abeS, abeM, adeF, adeG, adeH, adeI, adeJ, adeK, adeL, adeN*	*amvA abeS, abeM, adeA, adeB, adeC, adeF, adeG, adeH, adeI, adeJ, adeK, adeL, adeN*	*amvA abeS, abeM, adeB, adeC, adeF, adeG, adeH, adeI, adeJ, adeK, adeL, adeN*

*values indicate zone of inhibition (in mm) of respective antibiotic discs with interpretations of resistance (R) or susceptibility (S) following EUCAST (2024) breakpoints.

[#]values indicate minimum inhibitory concentrations (in µg/ml) measured using MIC E-test strips with interpretations of resistance (R) or susceptibility (S) following EUCAST (2024) breakpoints.

3.4 The virulome of A. baumannii from the Orang Asli community

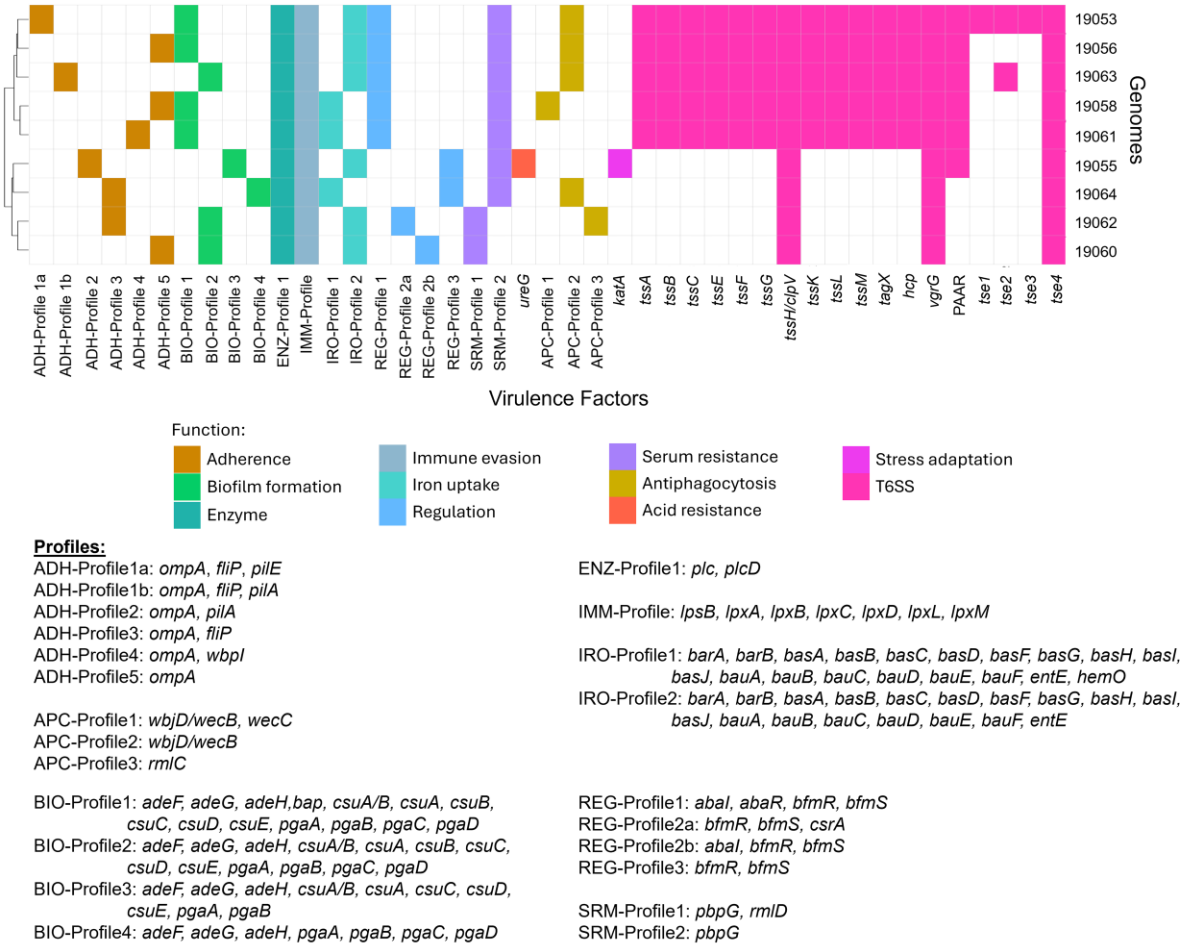
The isolation of *A. baumannii* from diverse sources, including clinical settings, soil, and wastewater, highlights its ability to persist across various environmental niches, facilitating its widespread dissemination, colonization, and pathogenicity (Harding et al., 2018). This persistence, evidenced by stress resistance and biofilm formation, is likely supported by the acquisition and/or inheritance of multiple virulence factors (VFs). In this study, the majority of identified VFs are associated with adhesion, biofilm formation, and quorum sensing regulation (**Figure 1**). These findings are in agreement with Muzahid et al. (2023), who reported similar virulome profiles in *A. baumannii* isolates from the Segamat community, including genes related to adhesion (e.g., *ompA*, *fliP*, *pilA*, *pilE*), biofilm formation (e.g., *adeFGH*, *bap*, *csuA/B*, *csuABCDE*, *pgaABCD*), and quorum sensing (e.g., *abaI*, *abaR*) (Choi et al., 2009; Lannan et al., 2016; Ahmad et al., 2023). However, slight variations in VF combinations were observed between these two Malaysian community studies. Notably, the biofilm gene combination we termed BIO-Profile 1 (**Figure 1**) was dominant in our isolates but was absent in those from the Segamat community. Despite these differences, the presence of numerous shared VFs underscores their potential role in supporting the persistence of the *A. baumannii* isolates in their niche and their capacity to cause infection.

Survival of *A. baumannii* in harsh environmental conditions requires mechanisms for the acquisition of micronutrients, such as iron scavenging through the production of acinetobactin in iron-limiting environments (Lannan et al., 2016; Harding et al., 2018). The presence of iron uptake genes is thus a signature VF in *A. baumannii* and the combination of iron acquisition genes designated IRO-Profile2 (**Figure 1**) was also observed in the Segamat community isolates along with isolates from the Australian Northern Territory community (Meumann et al., 2019; Muzahid et al., 2023).

All nine *A. baumannii* isolates displayed the full complement of the *lps-lpx* genes (designated IMM-Profile; **Figure 1**), which are tagged as virulence factors that function in immune evasion. Deficiency in *lpxC* has been shown to cause the loss of the LPS layer in *A. baumannii* leading to the development of colistin resistance (Kamoshida et al., 2020). The full suite of the *lps-lpx* genes were found in the Segamat *A. baumannii* isolates, and this included hospital isolates that were identified as resistant to colistin (n = 2) and polymyxin B (n = 4); nevertheless, a more

detailed analysis of possible mechanisms for polymyxin resistance, including mutations in the *lps-lpx* genes, was not presented (Muzahid et al., 2023).

The Type-6 Secretion System (T6SS) is utilized by *A. baumannii* to release toxic effector proteins into the neighboring environment, offering a competitive advantage to the pathogen in multispecies environments (Carruthers et al., 2013) and also allowing *A. baumannii* to spread, invade and resist host immune responses (Shadan et al., 2023). The T6SS main cluster (T6MC), which encompasses the *tssA*, *tssB*, *tssC*, *tssD*/*hcp*, *tssE*, *tssF*, *tssG*, *tssH*/*clpV*, *tssK*, *tssL*, *tagX*, *vgrG* and PAAR genes (Fitzsimons et al., 2018; Lewis et al., 2019), was identified in five of the nine Orang Asli *A. baumannii* isolates (**Figure 1**). These genes are responsible for T6SS apparatus assembly, whereby TssA functions as the priming protein (also known as the cap), TssBC forms the sheath, TssD/Hcp the secretion tube with VgrG and PAAR proteins as the spike. The spike (i.e., VgrG and PAAR) teams with the wedge (i.e., TssK and TssEFG) to form the baseplate (Fitzsimons et al., 2018; Marazzato et al., 2022). The structure was then supported by the membrane complex formed by TssJ, TssM and TssL proteins connecting between inner and outer membrane (Fitzsimons et al., 2018; Marazzato et al., 2022). The T6SS found in the five *A. baumannii* genomes (**Figure 1**) was further identified as T6SS-1A (i.e., 19053, 19056 and 19063) and T6SS-1B (i.e., 19058 and 19061), according to the classification of (Repizo et al., 2019). These five *A. baumannii* isolates also encode the Tse4 effector (**Figure 1**), which function as an amidase (Lewis et al., 2019; Repizo et al., 2019). Other effectors were also present in the Orang Asli isolates, with *A. baumannii* 19063 and 19053 encoding an additional Tse2 (predicted to function as a DNase), while *A. baumannii* 19053 also encodes additional Tse1 (predicted lipase producer), and Tse3 (effector of unknown function) (Lewis et al., 2019; Repizo et al., 2019). Conversely, only one of the Segamat community isolate, *A. baumannii* C-98, harbored the complete T6SS whereas the hospital isolates contained the full suite of T6SS genes (Muzahid et al., 2023). Majority of the *A. baumannii* hospital isolates from Terengganu also harbored the full T6MC (n = 94/126; or 74.6%) (Din et al., 2025). This suggests that the Orang Asli *A. baumannii* isolates may be better adapted to survival in a multispecies environment with also the capacity to invade and colonize their host, should the opportunity arise; however, such possibilities would require further experimental validation.



Profiles:

ADH-Profile1a: *ompA, fliP, pilE*
ADH-Profile1b: *ompA, fliP, pilA*
ADH-Profile2: *ompA, pilA*
ADH-Profile3: *ompA, fliP*
ADH-Profile4: *ompA, wbpI*
ADH-Profile5: *ompA*

APC-Profile1: *wbjD/wecB, wecC*
APC-Profile2: *wbjD/wecB*
APC-Profile3: *rmlC*

BIO-Profile1: *adeF, adeG, adeH,bap, csuA/B, csuA, csuB, csuC, csuD, csuE, pgaA, pgaB, pgaC, pgaD*
BIO-Profile2: *adeF, adeG, adeH, csuA/B, csuA, csuB, csuC, csuD, csuE, pgaA, pgaB, pgaC, pgaD*
BIO-Profile3: *adeF, adeG, adeH, csuA/B, csuA, csuC, csuD, csuE, pgaA, pgaB*
BIO-Profile4: *adeF, adeG, adeH, pgaA, pgaB, pgaC, pgaD*

ENZ-Profile1: *plc, plcD*

IMM-Profile: *lpsB, lpxA, lpxB, lpxC, lpxD, lpxL, lpxM*

IRO-Profile1: *barA, barB, basA, basB, basC, basD, basF, basG, basH, basI, basJ, bauA, bauB, bauC, bauD, bauE, bauF, entE, hemO*
IRO-Profile2: *barA, barB, basA, basB, basC, basD, basF, basG, basH, basI, basJ, bauA, bauB, bauC, bauD, bauE, bauF, entE*

REG-Profile1: *abaI, abaR, bfmR, bfmS*
REG-Profile2a: *bfmR, bfmS, csrA*
REG-Profile2b: *abaI, bfmR, bfmS*
REG-Profile3: *bfmR, bfmS*

SRM-Profile1: *pbpG, rmlD*
SRM-Profile2: *pbpG*

Figure 1: Profiles of various virulence factors (VFs) identified from the Orang Asli *A. baumannii* genomes (n = 9). The profiles were abbreviated according to their biological functions: adhesion (ADH), anti-phagocytosis (APC), biofilm formation (BIO), phospholipase enzyme (ENZ), immune evasion (IMM), iron uptake (IRO), regulation (REG) and serum resistance (SRM); singleton genes were labelled as is.

3.5 The Orang Asli A. baumannii isolates were genetically diverse

Community isolates of *A. baumannii* represents a pool of unexplored genomes when compared to the more well-studied clinical isolates. Therefore, many *A. baumannii* isolates presented in community studies belonged to novel STs that have yet to be classified into any clonal complexes (CCs). We pooled together the *A. baumannii* genomes obtained from the Orang Asli in this study along with the genomes obtained from fecal samples of the community in the town of Segamat (Muzahid et al., 2023) for pangenome analysis. The analysis showed genetic diversity among these community isolates from the 5,277 cloud genes identified (53.84%) when compared to the 2,273 core genes (23.19%) (**Figure 2**). The lower ratio of core genes indicated low homogeneity between the *A. baumannii* community isolates, highlighting the uniqueness of the bacterium in each population. Even within the Segamat population itself, the higher diversity of the community *A. baumannii* isolates was apparent, as compared to the hospital isolates from the same town (Muzahid et al., 2023). Of interest, there did not seem to be any apparent geographical clustering between the two populations (**Figure 2**), and this was evident when examining the core genome phylogenetic tree that was generated using both community and clinical isolates of *A. baumannii* from Malaysia (**Figure 3**). Segamat is a township in the state of Johor and is approximately 390 km to the south of the state of Terengganu where the sampling was carried out in the Orang Asli rural settlements.

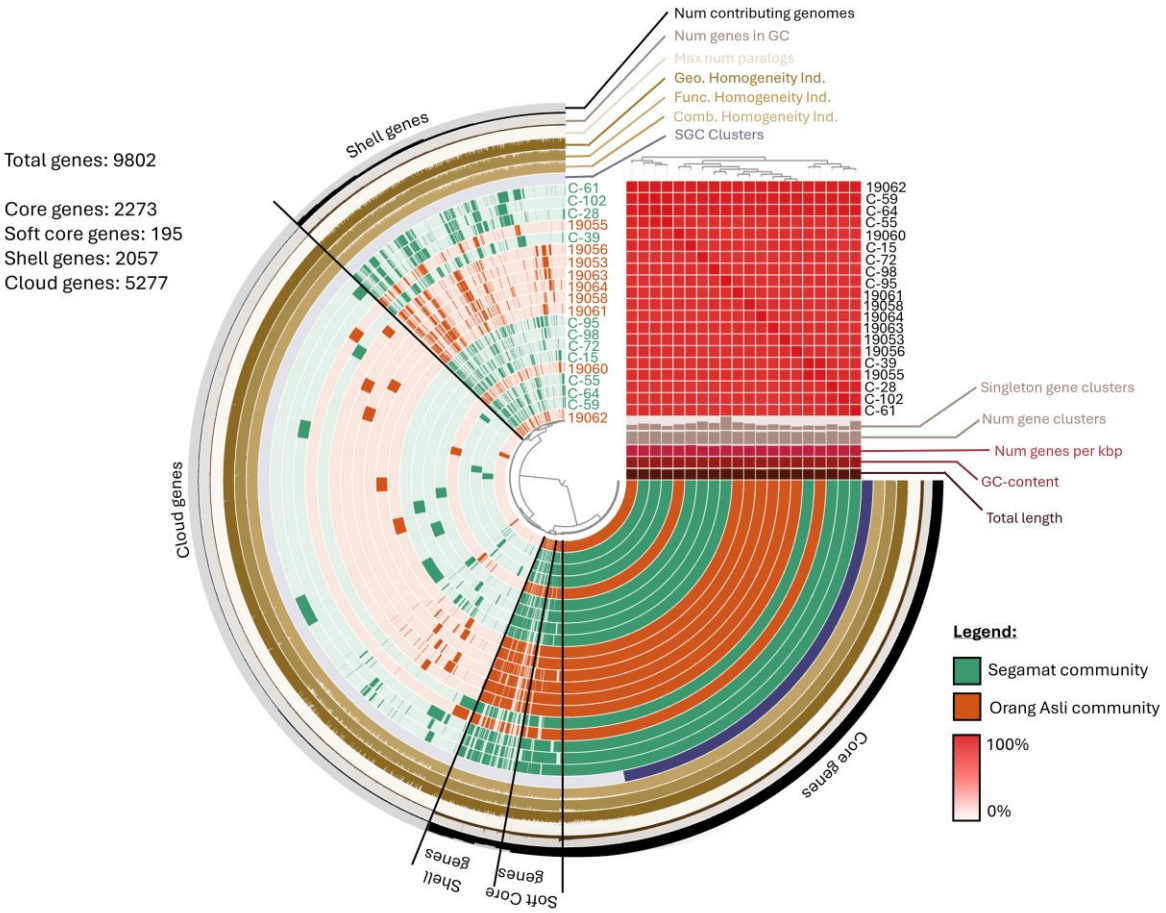


Figure 2: Pangenome analysis of community *A. baumannii* isolates from Malaysia that are currently available in the databases (n = 20). The orange-colored tracks represent the Orang Asli community *A. baumannii* from this study (n = 9), whereas the green-colored tracks represent the Segamat community strains (n = 11; isolates with the prefix "C") that were previously published (Muzahid et al., 2023). Heatmap on the top right corner presents the average nucleotide identity (ANI) of *A. baumannii* from both communities, with all of them having >97% identity.

A maximum-likelihood phylogenetic tree was generated from the core genome alignment of 199 Malaysian *A. baumannii* genomes (**Figure 3**) and these included the genomes from this study, the Segamat study (both community and hospital isolates) (Muzahid et al., 2023), 126 genomes from a ten-year collection of isolates from Hospital Sultanah Nur Zahirah (HSNZ), the main tertiary hospital in Terengganu (Din et al., 2025), and other clinical isolates of *A. baumannii* obtained from the PubMLST database (refer to **Appendix 1** for the list of genomes). Hospital isolates of *A. baumannii* were predominantly $ST2_{Pas}$ which were categorized under the Global Clone 2 (GC2) lineage, and this is clearly evident in the phylogenetic tree where they are clustered in a distinct clade (**Figure 3**). GC2 is the predominant *A. baumannii* lineage

globally (Nigro and Hall, 2018) but the basis of this predominance is the overwhelming majority of sequenced isolates were from the hospitals (Shelenkov et al., 2023). As mentioned earlier, the Orang Asli *A. baumannii* genomes were scattered throughout the phylogenetic tree, as were the Segamat community isolates whereas most of the Segamat hospital isolates were clustered together in the GC2 clade, much like the HSNZ isolates. Interestingly, one Orang Asli isolate, *A. baumannii* 19064 ($ST10_{Pas}$; $ST585_{Oxf}$), was found to be a member of the GC8 lineage. Similarly, Muzahid et al. (2023) had reported that one of their community isolates from Segamat was identified as a member of the GC1 lineage. Apart from these exceptional cases, none of the community *A. baumannii* isolates belonged to any GC clusters. Additionally, the Orang Asli *A. baumannii* was also distinct from the Segamat community isolates with only *A. baumannii* 19062 distantly grouped with C-72 (**Figure 3**). Although majority of the Orang Asli isolates did not belong to any of the major Global Clones, we observed that they were interleaved with a few non-GC clinical isolates from HSNZ in neighboring branches (**Figure 3**). It is possible that these community *A. baumannii* isolates were able to cause infections whenever the opportunity arises (i.e., potential pathogenicity) and thus, we see the relatively close genetic relationship between some of the Orang Asli isolates and the non-GC hospital isolates. However, without specific infection data and proven clinical relevance, this remains speculative. Nevertheless, genomic data can inform future research on *A. baumannii*, particularly with broader longitudinal carriage studies, contract tracing or case control analysis with clinical samples, any transmission between the community and hospital isolates could be detected and proven.

Apart from the distinct clustering of various GCs (**Figure 3**), the Malaysian clinical *A. baumannii* isolates also presented a different catalogue of dominant *bla* genes, which varies from the community *A. baumannii* resistome described earlier. The presence of bla_{TEM-1} (Ambler Class A), bla_{NDM-1} (Ambler Class B), bla_{ADC-73} ($bla_{ADC-1-like}$; Ambler Class C), bla_{OXA-23} and bla_{OXA-66} (Ambler Class D) were recorded from almost all the Malaysian GC2 genomes, and likewise for the tetracycline resistance gene *tet(B)* (**Figure 3**). One distinctive feature of *A. baumannii* hospital isolates is the presence of large AbaR resistance islands that were often inserted within the chromosomal *comM* gene (Meumann et al., 2019). The intactness of the *comM* gene was investigated for all the Malaysian *A. baumannii* genomes presented here. Not surprisingly, the *comM* gene was interrupted in all the GC2 isolates whereas the proportion was 87.5% for GC1, 71.4% for GC7, and 66.7% for GC11 isolates (**Figure 3**). The non-GC *A. baumannii* genomes have a much lower percentage of interrupted *comM*, and within the nine Orang Asli isolates, only *A. baumannii* 19060 was identified with disruption of the *comM* gene

(**Figure 3**). Nevertheless, the genes that were inserted within *comM* in *A. baumannii* 19060 were not associated with antimicrobial resistance but rather those associated with metabolism, regulatory genes and hypothetical proteins, much like what was described for the community-onset isolates from the Australian Northern Territory (Meumann et al., 2019).

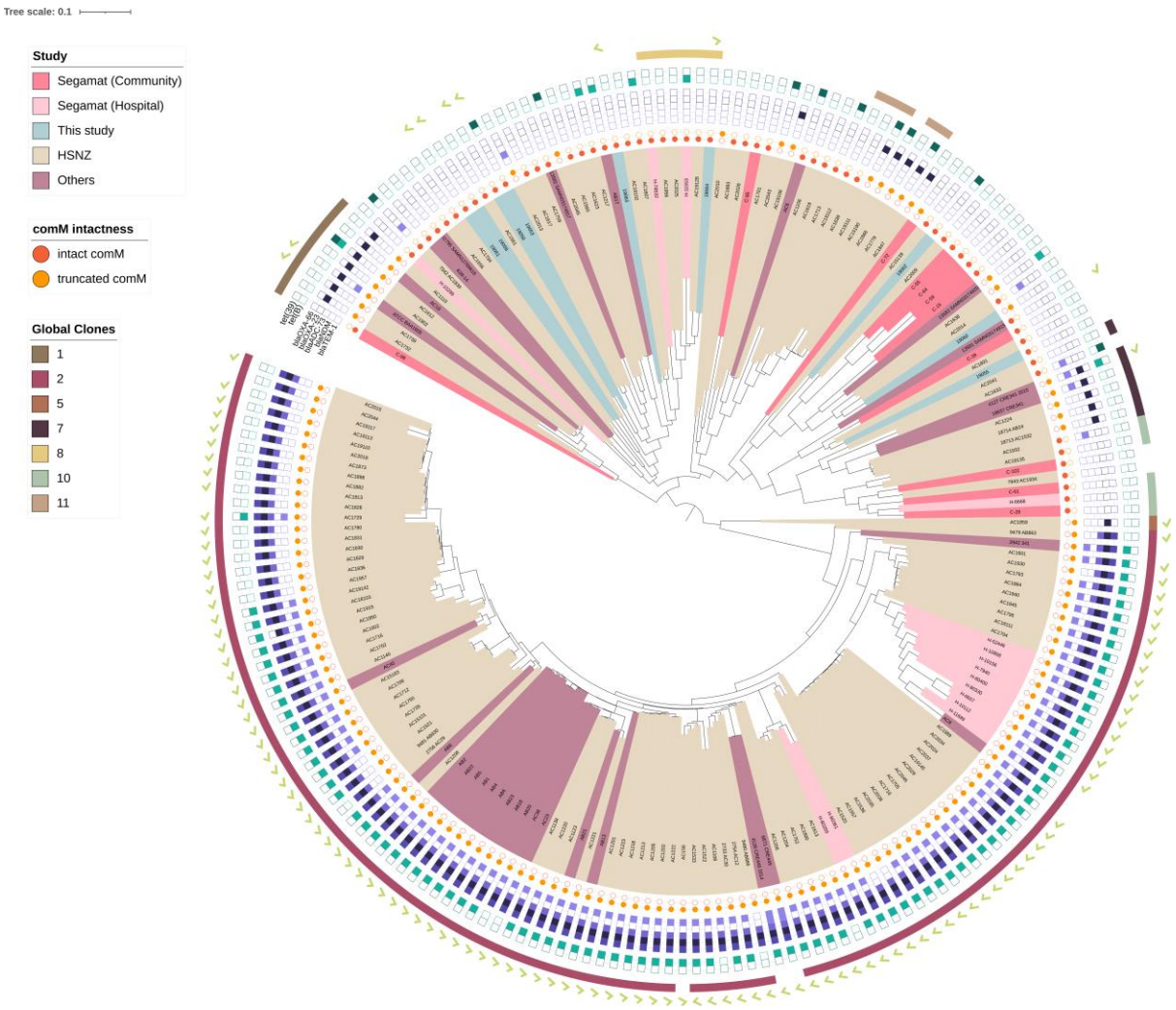


Figure 3: Midpoint-rooted maximum likelihood phylogenetic tree of all Malaysian *A. baumannii* genomes that are published [i.e., the current Orang Asli isolates, the Segamat hospital and community isolates described by (Muzahid et al., 2023), and the ten-year HSNZ isolates reported by (Din et al., 2025)] along with *A. baumannii* genomes originating from Malaysia that are found in PubMLST (as of 30 January 2025). Also shown are their categorization into the various Global Clone (GC) lineages, the presence/absence of predominant carbapenemase (purple boxes) and tetracycline resistance genes (green boxes), the intactness of the *comM* gene (orange circles) and presence of plasmids of the Rep_3 family (light green tick marks).

3.6 Insertion sequence (IS) elements

A total of 17 ISs belonging to five families, namely IS*3*, IS*5*, IS*91*, IS*630* and IS*L3*, were identified from the Orang Asli *A. baumannii* genomes (**Appendix 2**). IS*Aba43* of the IS*L3* family (Cameranesi et al., 2020) was found in all nine *A. baumannii* genomes, with at least one copy of the IS in each genome (**Appendix 2**). Other IS families were found in fewer numbers

with IS*Aba40*, IS*Aba57*, and IS*Aba63* of the IS*3* family found in one genome each, and the remaining IS elements identified found only in *A. baumannii* 19062 (**Appendix 2**). There were few reports for most of these IS elements except IS*1006* which was identified in *A. baumannii* 19062 and is well-known for its association with plasmid-borne antimicrobial resistance regions (Harmer and Hall, 2021; Varani et al., 2021; Hall, 2022). However, the identified copy of IS*1006* in *A. baumanii* 19062 was not found to be associated with any resistance genes.

3.7 Carriage of plasmids in A. baumannii from the Orang Asli community

Plasmids play an important role in the evolution of *A. baumannii*, being a major vehicle for the dissemination of antibiotic resistance genes (Lam and Hamidian, 2024; Tobin et al., 2025). In a comprehensive survey of 439 mostly complete *A. baumannii* genomes, more than half (52%) contained one plasmid, 27% harbored two plasmids while seven genomes contained six to eleven plasmids (Lam and Hamidian, 2024). Plasmids of the Rep_3 family were by far, the most predominant with more than half of these plasmids harboring antibiotic resistance genes (Lam et al., 2023; Lam and Hamidian, 2024; Tobin et al., 2025). Among the nine Orang Asli *A. baumannii* genomes, only one isolate, *A. baumannii* 19055, was without any detectable plasmids; three isolates (i.e., 19053, 19062 and 19064) harbored two plasmids each, while the remaining five isolates contained a plasmid each (**Appendix 3**). Eight of these plasmids were small plasmids (i.e., <10 kb; (Lean and Yeo, 2017)), ranging in size from 2,178 bp to 8,837 bp. Out of these eight small plasmids, only one plasmid, p19053a, belonged to the Rep_1 family, specifically the R1-T6 type. p19053a was only 2,178 bp and harbored the *rep* gene and two other hypothetical open reading frames (ORFs) (**Appendix 4**). Lam and Hamidian (2024) noted that the R1-type plasmids are typically 2 – 3 kb in size and encode only the replication initiation protein along with one or two hypothetical proteins. None of these plasmids harbored AMR genes and p19053a follows the characteristics of the prototypical R1-type plasmid.

The remaining seven small plasmids were of the Rep_3 family, specifically the R3-T5 (n = 4), R3-T13 (n = 2) and R3-T64 (n = 1) types (**Appendix 3 & 4**). Two of these R3 types (i.e., R3-T5 and R3-T13) were reportedly among the most abundant R3 types globally, but their distribution were mainly linked to minor STs (Lam and Hamidian, 2024). Plasmid p19064a from the R3-T5 type was found in an $ST10^{Pas}$ host (*A. baumannii* 19064), which was the only isolate from this study that belonged to the one of the known Global Clone lineages, GC8, but did not harbor antibiotic resistance genes. Among these small plasmids, only p19053b, which was also of the R3-T5 type, harbored the *tet(39)* tetracycline resistance gene (**Figure 4**). In

557 contrast, *A. baumannii* clinical isolates from Malaysia, particularly those of the GC2 lineage,
558 prevalently harbored an 8,731 bp plasmid we initially designated pAC12a (Lean et al., 2014;
559 Lean and Yeo, 2017; Din et al., 2025) and which is identical to the pA1-1 plasmid (accession
560 no. CP010782) that is harbored in a GC1 isolate obtained in 1982. This plasmid was previously
561 typed as GR2 in an older scheme (Bertini et al., 2010) but has since been typed as R3-T1, and
562 almost half of the members of this group were identical or nearly identical to pA1-1 (Lam et
563 al., 2023). This supports the long-standing association of this plasmid with the GC1 and GC2
564 lineages but isolates of other STs have also been found that harbor this plasmid albeit at a much
565 lower frequency (Lam et al., 2023; Din et al., 2025). A characteristic feature of this plasmid is
566 the presence of mobile p*dif* modules containing the *sel1*, *abkBA* toxin-antitoxin genes, and a
567 gene encoding a TonB-dependent receptor (Lean and Yeo, 2017; Lam et al., 2023). Notably,
568 this plasmid type was absent from all *A. baumannii* isolates obtained from the Orang Asli
569 population and the Segamat community, suggesting a possible specific association with clinical
570 strains.
571 Lam and Hamidian (2024) reported that almost half of the R3-type plasmids were not
572 associated with AMR determinants. The genetic structures of the R3-T5 and R3-T13-type
573 plasmids identified in this study ($n = 6$; **Figure 4** and **Appendix 4**) showed the absence of AMR
574 genes from five of them, except for the *tet(39)-tetR* genes in p19053b. The *tet(39)-tetR* genes
575 are located within a mobile p*dif* module usually found in *Acinetobacter* plasmids (Blackwell
576 and Hall, 2017; Lean and Yeo, 2017). A p*dif* module typically comprises one or two related
577 genes flanked by p*dif* sites, which resemble the chromosomal *dif* site involved in site-specific
578 recombination. These 28 bp p*dif* sites contain binding regions for the recombinases XerC and
579 XerD, similar to the chromosomal *dif* site near the bacterial chromosome terminus. The term
580 p*dif* was used to differentiate these plasmid-associated sites from chromosomal *dif* sites
581 (Blackwell and Hall, 2017; Castillo et al., 2017; Balalovski and Grainge, 2020). Three other
582 p*dif* modules were uncovered from p19053b, and these carry a nucleotide-binding protein-
583 encoding gene, *higBA* toxin-antitoxin genes, and a *sel1* gene along with a gene encoding the
584 SMI1/KNR4-family protein (**Figure 4; Appendix 5**). Interestingly, the gene arrangement of
585 the *higBA*, and *sel1*-SMI1/KNR4-family p*dif* modules were observed in almost all the R3-T5-
586 type plasmids identified in this study and also extends to the R3-T13-type plasmids (i.e.,
587 p19058 and p19063) (**Figures 4** and **5; Appendix 4**).
588 Two plasmids of the R3-T5-type, namely p19056 and p19061, were nearly identical, with both
589 at 7,146 bp (**Figure 4**). They were closely related to the pD1279779 plasmid (accession no.
590 CP003968.1) which was slightly larger at 7,416 bp with an additional hypothetical protein

found downstream of the SMI1/KNR4-family gene (**Figure 4**). Plasmid p19064a also appeared to be closely related to pD1279779 but the p19064a sequence was actually stitched together from two separate contigs (**Figure 4**), and whether there are additional genes that are lost in between these two contigs is not known. Interestingly, the host for pD1279779 was *A. baumannii* D1279779, which was isolated from a community-acquired bacteremia patient in Northern Australia and typed as $ST267_{Pas}$/$ST942_{Oxf}$ (Farrugia et al., 2013). Based on the collection of *Acinetobacter* plasmids curated by (Lam et al., 2023), the R3-T5-type plasmids identified in this study were related to pPM194229-3 and pDETAB6, besides pD1279779. The p19053b plasmid, which harbored the *tet(39)-tetR* genes, is thus the sole R3-T5-type plasmid which encode AMR determinants. This plasmid is more closely related to pDETAB6, which harbored two hypothetical ORFs instead of the *tet(39)-tetR* genes in p19053b (**Figure 4**).

AMR genes are also a rarity among the R3-T13-type plasmids. A search in the *Acinetobacter* plasmid repository posted by (Lam et al., 2023) led to the discovery of p29FS20-2 (accession no. CP044521.1) as the sole carrier of *tet(39)-tetR*, and the aminoglycoside resistance genes *aph(3")-Ib* and *aph(6)-Id* in this plasmid type. Two of the three R3-T13-type plasmids identified in this study, p19058 and p19063, were not associated with AMR determinants; however, p19062b was the only R3-T13-type plasmid that harbored the *tet(39)-tetR* gene pair (**Figure 5**). Similar to p19053b, the *tet(39)-tetR* genes in p19062b was also located within a p*dif* module, a feature that was also observed in p29FS20-2 (**Figure 5**). Plasmids p19058 and p19063 were more closely related to another R3-T13-type plasmid, p6200-9.327kb, but this plasmid contained additional genes encoding sulphate permease (*sulP*) and a universal stress protein (*usp*) (**Figure 5**). One of the interesting observations of note regarding these plasmids is the almost universal presence of the *rep_3-orfX* backbone, the *higBA*, and *sel1*-SMI1/KRN4 family modular arrangement of genes, covering also the R3-T5-type plasmids (**Figures 4** and **5**). The sole exception to this is plasmid p19062b where the *sel1*-SMI1/KRN4 p*dif* module was absent, and the putative toxin-antitoxin module (identified as *relBE* by TADB 3.0) was distantly related to the *higBA* module usually found in the other similar plasmid-types (**Figure 5**).

The other plasmids identified in the Orang Asli *A. baumannii* genomes are p19062a of the R3-T64 type, p19060 of R3-T26, and p19064b of R3-T27 type (**Appendix 4**). These plasmid types are rarely encountered when compared to the R3-T5 and R3-T13 types (Lam et al., 2023). None of these plasmids harbor AMR determinants.

Even though only nine *A. baumannii* genomes were investigated in this study, the diversity of plasmids found is reflective of the diversity reported for the genus *Acinetobacter* (Lam and Hamidian, 2024). Given the limited direct clinical antibiotic exposure in the studied

community, it is plausible that plasmid-mediated resistance (in particular, tetracycline resistance) is maintained and disseminated through environmental and ecological interactions. Local environmental sources (such as soil and water contaminated with antimicrobial residues) and animals (including livestock and wildlife) can act as reservoirs of resistance plasmids and provide opportunities for gene exchange between commensal, environmental, and pathogenic bacteria. Such interactions may help explain the persistence and spread of resistance determinants in these isolates despite low human antibiotic usage (Davies and Davies, 2010; Aminov, 2009). This is particularly true for tetracycline resistance in which the antibiotic has been extensively used for decades in both human and veterinary medicine as well as in agriculture and aquaculture. This historical use could have created an initial selective pressure which led to the persistence of the resistance gene(s) in the bacterial population (Aminov, 2009). Even if the exposure to tetracycline is now limited, the fitness cost of carrying the resistance gene might be low, which enables them to persist by being integrated into mobile genetic elements such as p*dif* modules and plasmids that are easily transmitted between bacteria. The *tet(39)* p*dif* module along with p*dif* modules harboring carbapenem and macrolide resistance genes have indeed been reported in diverse *Acinetobacter* spp. isolated from aquatic environments in South Australia (Tobin et al., 2024).

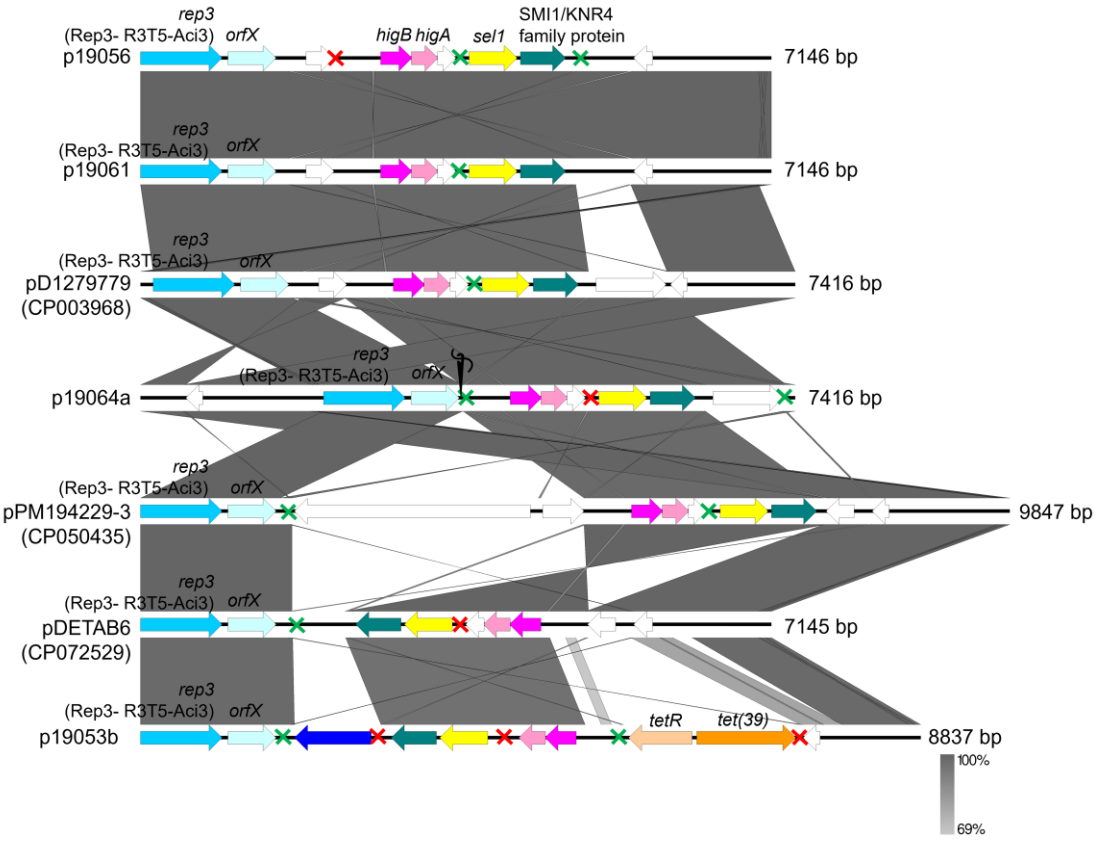


Figure 4: Comparisons of the R3-T5-type plasmids found in the Orang Asli *A. baumannii* isolates with similar R3-T5-type plasmids in the database curated by (Lam et al., 2023). Needle and thread icon shown for the p19064a plasmid map indicated that the plasmid was a composite that was stitched together from two separate contigs. The p*dif* sites are marked with green and red crosses representing XerC/D and XerD/C recognition sites, respectively.

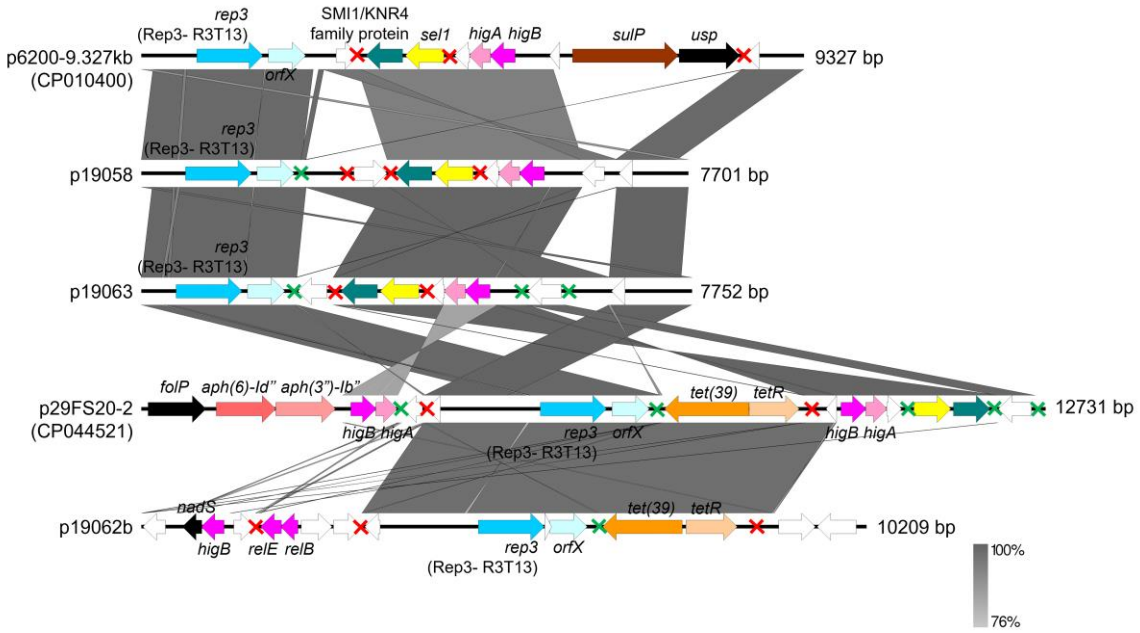


Figure 5: Comparative linear map of R3-T13-type plasmids identified from the Orang Asli *A. baumannii* with closely related R3-T13-type plasmids found in the plasmid database presented by (Lam et al., 2023). Red-green crosses representing p*dif* sites were labelled as indicated in the previous Figure.

3.8 Perspectives, Limitations and Suggestions for Future Studies

By conducting genomic analysis of *A. baumannii* strains from a previously understudied population, our work addresses a key WHO priority in its Global Action Plan on AMR (WHO, 2015). The identification of resistance genes and their prevalence in this unique community provides new surveillance data from a region where such information is limited. This is essential for informing and strengthening national and regional AMR strategies, thereby contributing to the global surveillance network. Our findings on the presence of antimicrobial-resistant *A. baumannii* in asymptomatic carriers from a community setting also underscore the importance of community-level surveillance and infection prevention. WHO recognizes that AMR is not just a hospital problem (WHO, 2015). For *A. baumannii*, there has been scarce data on the genomic characteristics of community-origin isolates worldwide, resulting in the

overwhelming prevalence of hospital-origin GC2 genomes in the public databases (Lam and Hamidian, 2024; Shelenkov et al., 2023; Hamidian and Nigro, 2019). Our study also highlights the need for targeted public health interventions and awareness campaigns to mitigate the spread of resistant bacteria in non-clinical settings. This information is vital for developing effective community-based strategies to limit the transmission of AMR.

A major limitation in this study is that these *A. baumannii* genomes were sequenced using the Illumina short-read platform, which does not allow for complete genome assembly. Therefore, some of the plasmid architecture presented here should be taken with caution as we could not ascertain if there are genes or genetic elements that are lost in the assembly of the draft genomes. This limitation is particularly relevant for plasmid p19064a which was assembled across two separate contigs. Nevertheless, the fact that some of these plasmids have similar counterparts from clinical *A. baumannii* isolates is of concern as they could serve as vehicles for the dissemination of AMR genes. The discovery of the *tet(39)-tetR* gene pair within a mobile p*dif* module in two distinct plasmids, p19053b and p19062b, underlines this likelihood and highlights the importance of continued genomic surveillance particularly among the community.

Another limitation of this study is the small number of *A. baumannii* isolates that were obtained and analyzed (n = 9), thus necessitating future broader longitudinal studies for a better representation of the population. We also phenotypically tested a limited number of antimicrobials in this study (i.e., meropenem, ciprofloxacin, tetracycline, and doxycycline). Hence, increasing the number of antibiotics by including classes such as the aminoglycosides would be useful for a more comprehensive phenotypic resistance profile, more so as the aminoglycoside resistance gene, *ant(3")-IIa*, was detected in five isolates. The expression of these resistance genes can be validated by using quantitative real-time reverse transcriptase PCR (qRT-PCR), particularly for isolates in which the resistance gene is detected but the isolate remains phenotypically susceptible. Future studies should also focus on functional assays to determine the role of the new STs and capsule types in biofilm formation, resistance to environmental stresses and host immune evasion. This will provide a clearer understanding of how these novel types might influence the ability of the bacteria to cause disease and resist antibiotics in clinical settings.

4 Conclusions

Acinetobacter baumannii and its antimicrobial resistance mechanisms have long been central in the global fight against superbugs, as understanding these traits is essential for improving treatment strategies. Over the decades, numerous reports have highlighted the alarming rise in carbapenem-resistant, MDR and even PDR *A. baumannii* strains in clinical settings (Akeda, 2021; Chen et al., 2023), suggesting that that treatment practices themselves may contribute to the development of resistance (De Blasiis et al., 2024). In contrast, *A. baumannii* isolates from remote communities, such as the indigenous Orang Asli population studied here, exhibited lower levels of antibiotic resistance. However, data on community-derived isolates remain scarce when compared to clinical isolates (Lam and Hamidian, 2024).

Despite their relative antibiotic susceptibility, these community-associated *A. baumannii* isolates still harbor a range of virulence factors (VFs) and mobile genetic elements, including plasmids and insertion sequences (Meumann et al., 2019; Muzahid et al., 2023), features well-documented in hospital-derived strains. Notably, one isolate from this study belonged to the Global Clone 8 (GC8) clinical lineage, while (Muzahid et al., 2023) previously identified a Global Clone 1 (GC1) isolate in a more urbanized community setting in Malaysia. Moreover, the phylogenetic interleaving of these community isolates with certain non-GC hospital strains suggests that they possess the capacity to cause infections and acquire resistance traits, akin to their clinical counterparts. The presence of shared genomic features in *A. baumannii* from a presumptive antibiotic-naïve environment underscores the pathogen's inherent capacity for persistence and colonization, even in healthy individuals. Of concern is the detection of two *A. baumannii* isolates from this study that harbors tetracycline resistance genes in mobile p*dif* modules located in distinct plasmids with similarities to those isolated from clinical strains. This finding suggests the potential for further acquisition of resistance determinants and serves as a cautionary signal against the unregulated introduction of antibiotics into vulnerable populations, highlighting the need for a targeted public health policy that guides the use of antibiotics in these communities. Healthcare practitioners working with remote communities can use these insights to make more informed treatment decisions, adopting rapid diagnostic tests, if possible, to ensure that antibiotics are prescribed only when clinically necessary. This minimizes unnecessary antibiotic exposure, thereby potentially reducing the emergence and spread of AMR (Yau et al., 2021). Therefore, expanding genomic surveillance to include community-derived *A. baumannii* strains, even from remote indigenous tribes is indeed a useful endeavor. Although this is a road less taken, the knowledge obtained particularly tracking the

shifts in known and novel STs, will be invaluable for understanding the pathogen's broader epidemiological dynamics and informing future public health strategies.

Ethics approval and consent to participate

Ethical approval for isolates taken in Peninsular Malaysia was provided by Universiti Sultan Zainal Abidin (UniSZA) Ethics Committee: approval no. UniSZA/C/1/UHREC/628–1(85) dated 27 June 2016, the Department of Orang Asli Affairs and Development (JAKOA): approval no. JAKOA/PP.30.052Jld11[42], and by the University of Southampton Faculty of Medicine Ethics Committee (Submission ID: 20831). Written informed consent was taken with parents/guardians providing consent for those < 18 years old.

Conflict of interest disclosure statement

None declared

Data availability

The nine *Acinetobacter baumannii* genomes have been deposited in the National Center for Biotechnology Information (NCBI)'s Genomes database under BioProject no. PRJNA1258958.

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Author contributions

Soo Sum Lean: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (equal); methodology (lead); software (lead); visualization (lead); writing – original draft (lead); writing – review & editing (equal). **Denise E. Morris:** investigation (equal); methodology (equal); project administration (equal); resources (equal). **Rebecca Anderson:** investigation (equal); methodology (equal); project administration (equal); resources (equal). **Ahmed Ghazi Alattraqchi:** investigation (equal); methodology (equal); resources (equal); software (equal). **David W. Cleary:** conceptualization (equal); formal analysis (equal); funding acquisition (equal); resources (equal); software (equal); supervision (equal); writing – review & editing (equal). **Stuart C. Clarke:** conceptualization (equal); funding acquisition (lead); supervision (lead); resources (lead); writing – review & editing (equal). **Chew Chieng Yeo:** conceptualization (equal); formal analysis (equal); resources (equal); supervision (equal); validation (equal); writing – original draft (equal); writing – review & editing (equal).

Consent for publication

All authors have provided consent for publication.

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APPENDIX 1: List of *Acinetobacter baumannii* genomes from Malaysia used to construct the phylogenetic tree

Strain ID	Location	Latitude	Longitude	Country	Year	ST (Oxford)	ST (Pasteur)	Global Clone	K locus	O locus	R3 Plasmid	*comM* intactness
19053	Kg. Sungai Pergam	4.0471	103.2859	Malaysia	2017	3542	2832	NA	KL121	OCL2	Present	Intact
19055	Kg. Sungai Pergam	4.0471	103.2859	Malaysia	2017	3543	2114	NA	KL96	OCL13		Intact
19056	Kg. Sungai Pergam	4.0471	103.2859	Malaysia	2017	3415	2522	NA	KL170	OCL2	Present	Intact
19058	Kg. Sungai Pergam	4.0471	103.2859	Malaysia	2017	3541	2834	NA	KL202	OCL4	Present	Intact
19060	Kg. Sungai Pergam	4.0471	103.2859	Malaysia	2017	871	2700	NA	KL112	OCL6		Truncated
19061	Kg. Sungai Pergam	4.0471	103.2859	Malaysia	2017	3544	470	NA	KL172	OCL4	Present	Intact
19062	Kg. Sungai Pergam	4.0471	103.2859	Malaysia	2017	3545	2635	NA	KL81	OCL6		Intact
19063	Kg. Sungai Pergam	4.0471	103.2859	Malaysia	2017	3540	2833	NA	KL183	OCL2	Present	Intact
19064	Kg. Sungai Pergam	4.0471	103.2859	Malaysia	2017	585	10	8	KL108	OCL2	Present	Intact
11795 SAMN12769618	Unknown	4.2105	101.9758	Malaysia	2017	2098	46	NA	KL28	OCL1		Intact
13581 SAMN03174915	Unknown	4.2105	101.9758	Malaysia	2012	-	739	NA	KL229	OCL6	Present	Intact
13582 SAMN03174917	Unknown	4.2105	101.9758	Malaysia	2012	2596	374	NA	KL50	OCL6		Intact
13583 SAMN03174920	Unknown	4.2105	101.9758	Malaysia	2012	-	360	NA	KL52	OCL6		Truncated
18713 AC1532	Unknown	4.2105	101.9758	Malaysia	2015	229	25	7	KL14	OCL6		Truncated
18714 AB24	Unknown	4.2105	101.9758	Malaysia	2012	229	25	7	KL14	OCL6		Truncated
18837 CRE341	Unknown	4.2105	101.9758	Malaysia	2015	1947	25	7	KL139	OCL6		Truncated
2733 AC30	Terengganu	5.3283	103.1412	Malaysia	2011	195	2	2	KL3	OCL1	Present	Truncated
2754 AC12	Terengganu	5.3283	103.1412	Malaysia	2011	195	2	2	KL3	OCL1	Present	Truncated
2756 AC29	Terengganu	5.3283	103.1412	Malaysia	2011	195	2	2	KL3	OCL1	Present	Truncated
2942 341	Unknown	4.2105	101.9758	Malaysia	2013	-	2	2	KL210	OCL1		Truncated
4127 CRE341 2015	Kuala Lumpur	3.1319	101.6841	Malaysia	2015	1947	25	7	KL139	OCL6	Present	Truncated
4128 CRE449 2014	Kuala Lumpur	3.1319	101.6841	Malaysia	2014	-	-	NA	KL3	OCL1	Present	Truncated
6871 CRE449	Pahang	3.9743	102.4381	Malaysia	2014	-	-	NA	KL3	OCL1	Present	Truncated
7942 AC1839	Terengganu	5.3283	103.1412	Malaysia	2018	-	-	NA	KL120	OCL1		Intact
7943 AC1934	Terengganu	5.3283	103.1412	Malaysia	2019	-	-	NA	KL176	OCL5		Intact

9479 AB863	Unknown	4.2105	101.9758	Malaysia	2014	-	2	2	KL210	OCL1	Present	Truncated
9480 AB889	Unknown	4.2105	101.9758	Malaysia	2014	-	2	2	KL3	OCL1	Present	Truncated
9481 AB930	Unknown	4.2105	101.9758	Malaysia	2014	-	2	2	KL3	OCL1	Present	Truncated
AB1	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB13	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB17	Segamat	2.5035	102.8208	Malaysia	2012	2006	1230	NA	KL54	OCL2		Intact
AB18	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB2	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB20	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB21	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB22	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB23	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB4	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB5	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB6	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AB8	Segamat	2.5035	102.8208	Malaysia	2012	-	2	2	KL3	OCL1	Present	Truncated
AC1106	Terengganu	5.3283	103.1412	Malaysia	2011	1000	638	NA	KL47	OCL7		Intact
AC1108	Terengganu	5.3283	103.1412	Malaysia	2011	195	2	2	KL3	OCL1	Present	Truncated
AC1119	Terengganu	5.3283	103.1412	Malaysia	2011	207	1	1	KL4	OCL3		Truncated
AC1123	Terengganu	5.3283	103.1412	Malaysia	2011	195	2	2	KL3	OCL1	Present	Truncated
AC1138	Terengganu	5.3283	103.1412	Malaysia	2011	195	2	2	KL3	OCL1	Present	Truncated
AC1140	Terengganu	5.3283	103.1412	Malaysia	2011	938	2	2	KL210	OCL1	Present	Truncated
AC1201	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1202	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1204	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1205	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1206	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1208	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated

AC1213	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1217	Terengganu	5.3283	103.1412	Malaysia	2012	2006	1230	NA	KL54	OCL2		Intact
AC1218	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1220	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1221	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1222	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1223	Terengganu	5.3283	103.1412	Malaysia	2012	195	2	2	KL3	OCL1	Present	Truncated
AC1224	Terengganu	5.3283	103.1412	Malaysia	2012	229	25	7	KL14	OCL6		Truncated
AC15101	Segamat	2.5035	102.8208	Malaysia	2015	-	2	2	KL2	OCL1	Present	Truncated
AC15139	Segamat	2.5035	102.8208	Malaysia	2015	1201	721	NA	KL81	OCL6		Intact
AC15163	Segamat	2.5035	102.8208	Malaysia	2015	-	2	2	KL210	OCL1	Present	Truncated
AC15190	Segamat	2.5035	102.8208	Malaysia	2015	1418	164	NA	KL47	OCL5		Truncated
AC1520	Segamat	2.5035	102.8208	Malaysia	2015	-	2	2	KL2	OCL1	Present	Truncated
AC1521	Segamat	2.5035	102.8208	Malaysia	2015	-	2	2	KL2	OCL1	Present	Truncated
AC1522	Terengganu	5.3283	103.1412	Malaysia	2015	195	2	2	KL3	OCL1	Present	Truncated
AC1533	Segamat	2.5035	102.8208	Malaysia	2015	-	2	2	KL3	OCL1	Present	Truncated
AC1538	Segamat	2.5035	102.8208	Malaysia	2015	-	2	2	KL210	OCL1	Present	Truncated
AC1556	Terengganu	5.3283	103.1412	Malaysia	2015	2021	504	NA	KL118	UK		Intact
AC1557	Terengganu	5.3283	103.1412	Malaysia	2015	208	2	2	KL2	OCL1	Present	Truncated
AC156	Terengganu	5.3283	103.1412	Malaysia	2015	192	2	2	KL2	OCL1	Present	Truncated
AC1560	Segamat	2.5035	102.8208	Malaysia	2015	2022	77	NA	KL33	OCL2		Intact
AC1561	Segamat	2.5035	102.8208	Malaysia	2015	1320	265	NA	KL109	OCL4		Intact
AC1601	Terengganu	5.3283	103.1412	Malaysia	2016	NA	2	2	UK	OCL1	Present	Truncated
AC1602	Terengganu	5.3283	103.1412	Malaysia	2016	938	2	2	KL210	OCL1	Present	Truncated
AC1607	Terengganu	5.3283	103.1412	Malaysia	2016	1980	1093	NA	UK	OCL2		Intact
AC1612	Terengganu	5.3283	103.1412	Malaysia	2016	207	1	1	KL4	OCL3		Truncated
AC1613	Terengganu	5.3283	103.1412	Malaysia	2016	547	2	2	KL14	OCL1	Present	Truncated
AC1617	Terengganu	5.3283	103.1412	Malaysia	2016	942	267	NA	KL9	OCL2		Intact

AC1619	Terengganu	5.3283	103.1412	Malaysia	2016	1209	727	NA	KL47	OCL6		Intact
AC1623	Terengganu	5.3283	103.1412	Malaysia	2016	2090	1147	NA	KL9	OCL2		Intact
AC1633	Terengganu	5.3283	103.1412	Malaysia	2016	2089	126	NA	KL14	OCL6		Intact
AC1638	Terengganu	5.3283	103.1412	Malaysia	2016	2024	1102	NA	UK	OCL6		Truncated
AC1639	Terengganu	5.3283	103.1412	Malaysia	2016	234	164	11	KL106	OCL8		Intact
AC1701	Terengganu	5.3283	103.1412	Malaysia	2017	514	103	NA	KL24	OCL7		Intact
AC1702	Terengganu	5.3283	103.1412	Malaysia	2017	451	2	2	KL120	OCL1	Present	Truncated
AC1704	Terengganu	5.3283	103.1412	Malaysia	2017	208	2	2	KL2	OCL1	Present	Truncated
AC1705	Terengganu	5.3283	103.1412	Malaysia	2017	208	2	2	KL2	OCL1	Present	Truncated
AC1709	Terengganu	5.3283	103.1412	Malaysia	2017	547	2	2	KL14	OCL1	Present	Truncated
AC1712	Terengganu	5.3283	103.1412	Malaysia	2017	547	2	2	KL14	OCL1	Present	Truncated
AC1713	Terengganu	5.3283	103.1412	Malaysia	2017	3387	1131	NA	KL26	OCL9		Intact
AC1716	Terengganu	5.3283	103.1412	Malaysia	2017	451	2	2	KL120	OCL1	Present	Truncated
AC1718	Terengganu	5.3283	103.1412	Malaysia	2017	208	2	2	KL2	OCL1	Present	Truncated
AC1729	Terengganu	5.3283	103.1412	Malaysia	2017	195	2	2	KL3	OCL1	Present	Truncated
AC1732	Terengganu	5.3283	103.1412	Malaysia	2017	231	1	1	KL1	OCL1		Truncated
AC1738	Terengganu	5.3283	103.1412	Malaysia	2017	2199	1	1	KL4	OCL3	Present	Truncated
AC1739	Terengganu	5.3283	103.1412	Malaysia	2017	547	2	2	KL14	OCL1	Present	Truncated
AC1750	Terengganu	5.3283	103.1412	Malaysia	2017	547	2	2	KL14	OCL1	Present	Truncated
AC1752	Terengganu	5.3283	103.1412	Malaysia	2017	547	2	2	KL14	OCL1	Present	Truncated
AC1759	Terengganu	5.3283	103.1412	Malaysia	2017	3388	729	NA	KL6	OCL2		Intact
AC1763	Terengganu	5.3283	103.1412	Malaysia	2017	684	2	2	KL155	OCL1	Present	Truncated
AC1779	Terengganu	5.3283	103.1412	Malaysia	2017	1418	164	11	KL47	OCL5		Truncated
AC1780	Terengganu	5.3283	103.1412	Malaysia	2017	195	2	2	KL32	OCL1	Present	Truncated
AC1794	Terengganu	5.3283	103.1412	Malaysia	2017	3389	960	NA	KL26	OCL11		Intact
AC1795	Terengganu	5.3283	103.1412	Malaysia	2017	684	2	2	KL155	OCL1	Present	Truncated
AC18102	Terengganu	5.3283	103.1412	Malaysia	2018	1980	1093	NA	KL49	OCL2		Intact
AC18103	Terengganu	5.3283	103.1412	Malaysia	2018	684	2	2	KL155	OCL1	Present	Truncated

AC18111	Terengganu	5.3283	103.1412	Malaysia	2018	684	2	2	KL155	OCL1	Present	Truncated
AC1813	Segamat	2.5035	102.8208	Malaysia	2013	-	2	2	KL32	OCL1	Present	Truncated
AC1828	Segamat	2.5035	102.8208	Malaysia	2018	-	2	2	KL32	OCL1		Truncated
AC1829	Segamat	2.5035	102.8208	Malaysia	2018	-	2	2	KL32	OCL1	Present	Truncated
AC1830	Terengganu	5.3283	103.1412	Malaysia	2018	195	2	2	KL32	OCL1	Present	Truncated
AC1831	Terengganu	5.3283	103.1412	Malaysia	2018	195	2	2	KL32	OCL1	Present	Truncated
AC1836	Terengganu	5.3283	103.1412	Malaysia	2018	195	2	2	KL32	OCL1	Present	Truncated
AC1845	Terengganu	5.3283	103.1412	Malaysia	2018	684	2	2	KL155	OCL1	Present	Truncated
AC1847	Terengganu	5.3283	103.1412	Malaysia	2018	1418	164	NA	KL47	OCL5		Truncated
AC1859	Terengganu	5.3283	103.1412	Malaysia	2018	3390	79	5	KL32	OCL10	Present	Truncated
AC1866	Terengganu	5.3283	103.1412	Malaysia	2018	1418	164	11	KL47	OCL5		Truncated
AC1873	Terengganu	5.3283	103.1412	Malaysia	2018	195	2	2	KL32	OCL1	Present	Truncated
AC1882	Terengganu	5.3283	103.1412	Malaysia	2018	195	2	2	KL32	OCL1	Present	Truncated
AC1883	Terengganu	5.3283	103.1412	Malaysia	2018	753	1620	NA	KL112	OCL9		Intact
AC1884	Terengganu	5.3283	103.1412	Malaysia	2018	684	2	2	KL155	OCL1	Present	Truncated
AC1890	Terengganu	5.3283	103.1412	Malaysia	2018	547	2	2	KL14	OCL1	Present	Truncated
AC1891	Terengganu	5.3283	103.1412	Malaysia	2018	1088	338	NA	UK	UK		Intact
AC1898	Terengganu	5.3283	103.1412	Malaysia	2018	195	2	2	KL32	OCL1	Present	Truncated
AC19	Segamat	2.5035	102.8208	Malaysia	2011	-	1	1	KL4	OCL3		Truncated
AC1902	Terengganu	5.3283	103.1412	Malaysia	2019	207	1	1	KL4	OCL3		Truncated
AC19106	Terengganu	5.3283	103.1412	Malaysia	2019	514	103	NA	KL24	OCL7		Truncated
AC19110	Terengganu	5.3283	103.1412	Malaysia	2019	195	2	2	KL32	OCL1	Present	Truncated
AC19111	Terengganu	5.3283	103.1412	Malaysia	2019	1418	164	11	KL47	OCL5		Truncated
AC19112	Terengganu	5.3283	103.1412	Malaysia	2019	2294	164	11	KL111	OCL8		Intact
AC19113	Terengganu	5.3283	103.1412	Malaysia	2019	195	2	2	KL32	OCL1	Present	Truncated
AC19117	Terengganu	5.3283	103.1412	Malaysia	2019	195	2	2	KL32	OCL1	Present	Truncated
AC19128	Terengganu	5.3283	103.1412	Malaysia	2019	585	10	8	KL108	OCL2		Intact
AC19135	Terengganu	5.3283	103.1412	Malaysia	2019	3391	132	10	UK	OCL6		Intact

AC19142	Terengganu	5.3283	103.1412	Malaysia	2019	208	2	2	KL2	OCL1	Present	Truncated
AC19145	Terengganu	5.3283	103.1412	Malaysia	2019	208	2	2	KL2	OCL1	Present	Truncated
AC1919	Terengganu	5.3283	103.1412	Malaysia	2019	684	2	2	KL155	OCL1	Present	Truncated
AC1930	Terengganu	5.3283	103.1412	Malaysia	2019	451	2	2	KL120	OCL1	Present	Truncated
AC1932	Terengganu	5.3283	103.1412	Malaysia	2019	1496	1405	10	KL30	OCL5		Intact
AC1940	Terengganu	5.3283	103.1412	Malaysia	2019	684	2	2	KL155	OCL1	Present	Truncated
AC1950	Terengganu	5.3283	103.1412	Malaysia	2019	684	2	2	UK	OCL1	Present	Truncated
AC1956	Terengganu	5.3283	103.1412	Malaysia	2019	447	10	8	KL49	OCL2		Intact
AC1957	Terengganu	5.3283	103.1412	Malaysia	2019	195	2	2	KL32	OCL1	Present	Truncated
AC1989	Terengganu	5.3283	103.1412	Malaysia	2019	208	2	2	KL2	OCL1	Present	Truncated
AC2009	Terengganu	5.3283	103.1412	Malaysia	2020	3392	139	NA	KL116	OCL5		Intact
AC2010	Terengganu	5.3283	103.1412	Malaysia	2020	2271	1112	NA	KL51	OCL6		Truncated
AC2013	Terengganu	5.3283	103.1412	Malaysia	2020	942	267	NA	KL9	OCL2		Truncated
AC2014	Terengganu	5.3283	103.1412	Malaysia	2020	3393	142	NA	KL59	OCL5		Intact
AC2015	Terengganu	5.3283	103.1412	Malaysia	2020	195	2	2	KL32	OCL1	Present	Truncated
AC2018	Terengganu	5.3283	103.1412	Malaysia	2020	195	2	2	KL32	OCL1	Present	Truncated
AC2024	Terengganu	5.3283	103.1412	Malaysia	2020	208	2	2	KL2	OCL1	Present	Truncated
AC2025	Terengganu	5.3283	103.1412	Malaysia	2020	585	10	8	KL108	OCL2		Intact
AC2028	Terengganu	5.3283	103.1412	Malaysia	2020	2827	2177	NA	KL106	OCL8		Intact
AC2029	Terengganu	5.3283	103.1412	Malaysia	2020	208	2	2	KL2	OCL1	Present	Truncated
AC2034	Terengganu	5.3283	103.1412	Malaysia	2020	208	2	2	KL2	OCL1	Present	Truncated
AC2035	Terengganu	5.3283	103.1412	Malaysia	2020	208	2	2	KL2	OCL1	Present	Truncated
AC2036	Terengganu	5.3283	103.1412	Malaysia	2020	208	2	2	KL2	OCL1	Present	Truncated
AC2037	Terengganu	5.3283	103.1412	Malaysia	2020	208	2	2	KL2	OCL1	Present	Truncated
AC2041	Terengganu	5.3283	103.1412	Malaysia	2020	884	113	7	KL10	OCL5		Intact
AC2043	Terengganu	5.3283	103.1412	Malaysia	2020	1000	638	NA	KL47	OCL7		Truncated
AC2044	Terengganu	5.3283	103.1412	Malaysia	2020	195	2	2	KL32	OCL1		Truncated
AC2045	Terengganu	5.3283	103.1412	Malaysia	2020	1324	374	NA	KL50	OCL2		Truncated

AC2046	Terengganu	5.3283	103.1412	Malaysia	2020	208	2	2	KL2	OCL1	Present	Truncated
AC23	Segamat	2.5035	102.8208	Malaysia	2011	-	2	2	KL3	OCL1	Present	Truncated
AC38	Segamat	2.5035	102.8208	Malaysia	2011	-	2	2	KL3	OCL1	Present	Truncated
AC40	Segamat	2.5035	102.8208	Malaysia	2011	-	2	2	KL210	OCL1	Present	Truncated
AC6	Segamat	2.5035	102.8208	Malaysia	2011	1000	638	NA	KL47	OCL7		Intact
AC8	Segamat	2.5035	102.8208	Malaysia	2011	-	2	2	KL3	OCL1		Truncated
ATCC BAA1605	Segamat	2.5035	102.8208	Malaysia	2020	-	1	1	KL15	OCL3	Present	Truncated
C-102	Segamat	2.5035	102.8208	Malaysia	2018	2235	2108		KL223	OCL5		Intact
C-15	Segamat	2.5035	102.8208	Malaysia	2018	2230	216	NA	KL75	OCL6		Intact
C-28	Segamat	2.5035	102.8208	Malaysia	2018	1463	284	10	KL223	OCL13		Intact
C-39	Segamat	2.5035	102.8208	Malaysia	2018	485	338	NA	KL197	OCL14		Intact
C-55	Segamat	2.5035	102.8208	Malaysia	2018	503	336	NA	KL125	OCL5		Intact
C-59	Segamat	2.5035	102.8208	Malaysia	2018	2236	-	NA	KL32	OCL3		Intact
C-61	Segamat	2.5035	102.8208	Malaysia	2018	2232	1411	10	KL52	OCL6		Intact
C-64	Segamat	2.5035	102.8208	Malaysia	2018	1912	331	NA	KL61	OCL6		Intact
C-72	Segamat	2.5035	102.8208	Malaysia	2018	128	49	NA	KL11	OCL8		Truncated
C-95	Segamat	2.5035	102.8208	Malaysia	2018	2234	-	NA	KL47	OCL6		Intact
C-98	Segamat	2.5035	102.8208	Malaysia	2018	231	1	1	KL1	OCL1		Intact
H-10112	Segamat	2.5035	102.8208	Malaysia	2018	684	2	2	KL155	OCL1	Present	Truncated
H-10156	Segamat	2.5035	102.8208	Malaysia	2018	208	2	2	KL2	OCL1	Present	Truncated
H-10299	Segamat	2.5035	102.8208	Malaysia	2018	2238	-		KL97	OCL4		Intact
H-10858	Segamat	2.5035	102.8208	Malaysia	2018	208	2	2	KL2	OCL1	Present	Truncated
H-11553	Segamat	2.5035	102.8208	Malaysia	2018	447	10	8	KL49	OCL2		Intact
H-11699	Segamat	2.5035	102.8208	Malaysia	2018	208	2	2	KL2	OCL1		Truncated
H-52446	Segamat	2.5035	102.8208	Malaysia	2018	208	2	2	KL2	OCL1	Present	Truncated
H-6657	Segamat	2.5035	102.8208	Malaysia	2018	684	2	2	KL155	OCL1	Present	Truncated
H-6668	Segamat	2.5035	102.8208	Malaysia	2018	2237	459	10	KL128	OCL2		Intact
H-7940	Segamat	2.5035	102.8208	Malaysia	2018	208	2	2	KL2	OCL1	Present	Truncated

H-79532	Segamat	2.5035	102.8208	Malaysia	2018	2241	23	8	KL8	OCL2		Intact
H-80330	Segamat	2.5035	102.8208	Malaysia	2018	208	2	2	KL2	OCL1	Present	Truncated
H-80359	Segamat	2.5035	102.8208	Malaysia	2018	547	2	2	KL14	OCL1	Present	Truncated
H-80361	Segamat	2.5035	102.8208	Malaysia	2018	547	2	2	KL14	OCL1	Present	Truncated
H-80400	Segamat	2.5035	102.8208	Malaysia	2018	207	2	2	KL2	OCL1	Present	Truncated
K09-14	Segamat	2.5035	102.8208	Malaysia	2017	2098	46		KL28	OCL1		Intact

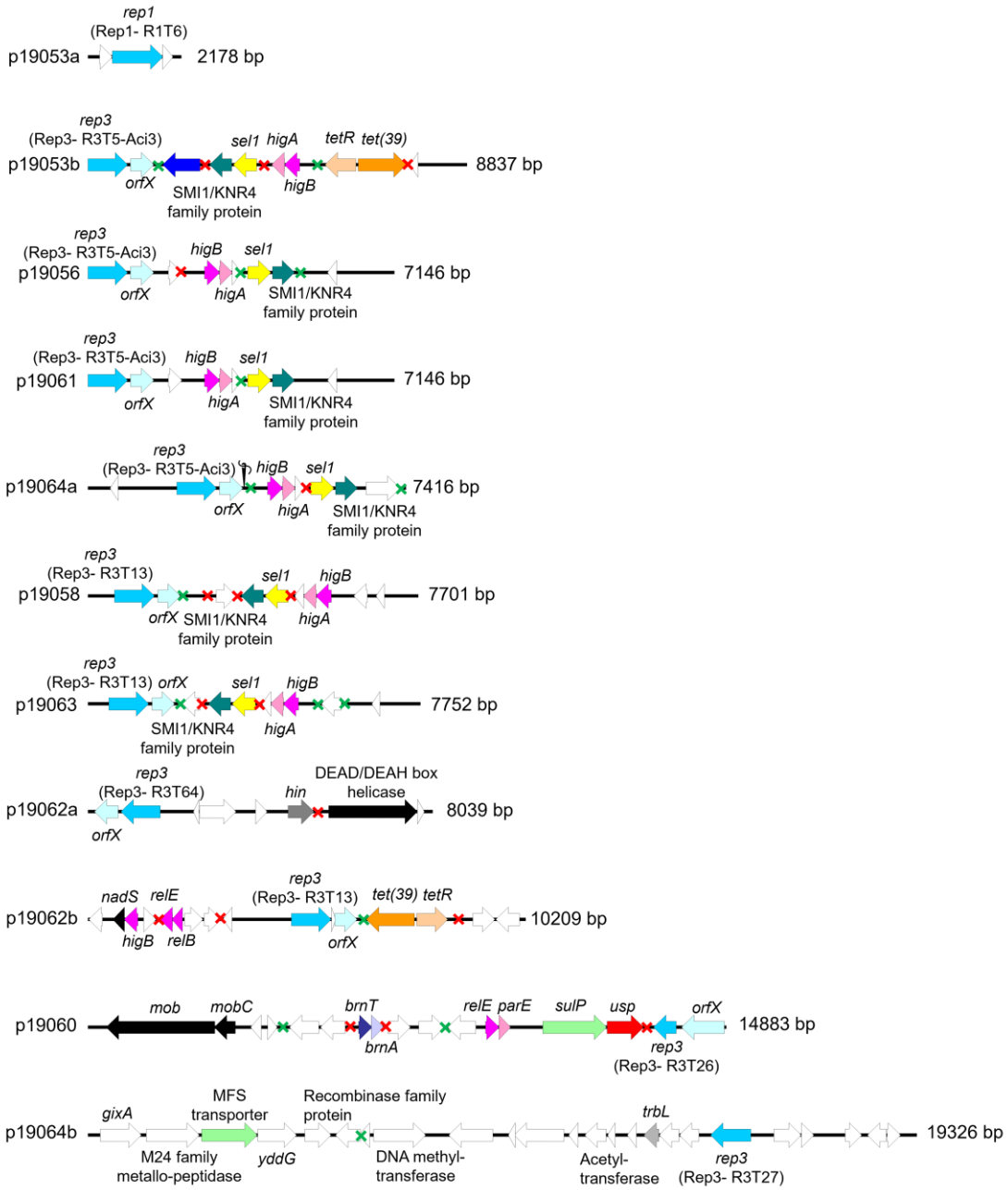
APPENDIX 2: List of insertion sequence (IS) elements found in the Orang Asli *A. baumannii* genomes

***A. baumannii* isolate ID**	**IS elements (family)**
19053	ISAba43 (ISL3), ISAba57 (IS3), ISAba40 (IS3)
19055	ISAba43 (ISL3)
19056	ISAba43 (ISL3)
19058	ISAba43 (ISL3)
19060	ISAba43 (ISL3)
19061	ISAba43 (ISL3)
19062	ISAba43 (ISL3), ISAba68 (IS5), IS1006 (IS91), ISAba44 (IS630)
19063	ISAba43 (ISL3)
19064	ISAba43 (ISL3), ISAba63 (IS3)

APPENDIX 3: List of plasmids identified from the genomes of the Orang Asli *A. baumannii* isolates.

***A. baumannii* strain ID**	**Plasmids (*n*)**	**Plasmid name**	**Location**	**Size (bp)**	**Rep family**	**Coverage (x)**
19053	2	p19053a	contig29	2,178	R1-T6	198
		p19053b	contig20	8,837	R3-T5	20.1
19055	0	-	-	-	-	-
19056	1	p19056	contig15	7,146	R3-T5	81
19058	1	p19058	contig24	7,701	R3-T13	29.7
19060	1	p19060	contig56	14,883	R3-T26	26.7
19061	1	p19061	contig14	7,146	R3-T5	39.2
19062	2	p19062a	contig51	8,039	R3-T64	562.6
		p19062b	contig51	10,209	R3-T13	562.6
19063	1	p19063	contig16	7,752	R3-T13	330.2
19064	2	p19064a	contig34, 35	7,416	R3-T5	201.7
		p19064b	contig27	19,328	R3-T27	20.9

APPENDIX 4: Linear genetic maps of all plasmids identified in the Orang Asli community *A. baumannii* isolates. Majority of the plasmids were of the Rep_3 family with only plasmid, p19053a, belonging to the Rep_1 family. The *rep*-encoded replication initiation protein gene is depicted as blue-colored arrows with its Rep type labelled while its downstream gene, recently designated *orfX* (Lam et al., 2023), is shown as light blue arrows. White colored arrows represent genes encoding hypothetical proteins. Other colored arrows are as in the legends to **Figures 4** and **5** in the main text. p*dif* sites are depicted as green (XerC/D) and red (XerD/C) crosses. Note that plasmid p19064a is a composite of two contigs that were stitched together as signified by the needle and thread icon above its linear map.



APPENDIX 5: List of p*dif* sequences identified in the Orang Asli *A. baumannii* plasmids

Plasmid	Start	End	Sequence (5'→3')*	Type§
p19053a	ND#	ND#	-	-
p19053b	1696	1723	ATTTCGTATAA GGCGTA TTATGTTAATT	C|D
	2727	2754	ATTTAACATAA TGGGCG TTATATGAAAT	D|C
	4048	4075	ATTTAACATAG AAACTC TTATGCGAATC	D|C
	5445	5472	ATTTCGGATAA CGCCCA TTATGTTAAAT	C|D
	7474	7501	AATTAACATAA TACGCC TTATGCGAAGC	D|C
p19056	2187	2214	ATTTAACATAA TGGGCG TTATCCGAAAT	D|C
	3582	3609	GATTCGCATAA GAGTTT CTATGTTAAAT	C|D
	4902	4929	ATTTCATATAA CGCCCA TTATGTTAAAT	C|D
p19058	2293	2320	ATTTCGTATAA GGTGTA TTATGTTAATT	C|D
	2945	2972	ATTTAACATAA AATCTC TTATTCGAAAT	D|C
	3466	3493	ATTTAACATAA TGGGCG TTATATGAAAT	D|C
	4786	4813	ATTTAACATAG AAACTC TTATGCGAATC	D|C
p19060	4641	4668	ATTTCGTATAA GGCGTA TTATGTTAATT	C|D
	6088	6115	ATTTAACATAA TGGGCG TTATACGAAAC	D|C
	6933	6960	GTATAAGGTGT ATTATG TTAATTTTAGA	D|C
	8372	8399	GCTTCACATAA GAGATT TTATGTTAAAT	C|D
	13010	13037	AATTAACATAA TACACC TTATACGAAAT	D|C
p19061	3582	3609	GATTCGCATAA GAGTTT CTATGTTAAAT	C|D
p19062a	2685	2712	ATTTAACATAA AATCTC TTATGTGAAGC	D|C
p19062b	1623	1650	AATTAACATAA TACACC TTATACGAAGG	D|C
	3109	3136	TCTAAAATTAA CATAAT ACACCTTATAC	D|C
	6421	6448	GTATAAGGTGT ATTATG TTAATTTTAGA	C|D
	8445	8472	ATTTAACATAA TGGGCG TTATCCGAAAT	D|C
p19063	2160	2187	ATTTCGTATAA GGTGTA TTATGTTAATT	C|D
	2701	2728	ATTTAACATAA TGGGCG TTATATGAAAT	D|C
	4021	4048	ATTTAACATAG AAACTC TTATGCGAATC	D|C
	5413	5440	ATTTCGGATAA CGCCCA TTATGTTAAAT	C|D
	5934	5961	ATTTCGAATAA GAGATT TTATGTTAAAT	C|D
p19064a	3750	3777	ATTTCGTATAA GGCGTA TTATGTTAATT	C|D
	5992	6019	ATTTAACATAG AAACTC TTATGCGAATC	D|C
	7387	7414	ATTTCGGATAA CGCCCA TTATGTTAAAT	C|D
p19064b	5705	5732	GCTTCACATAA GAGATT TTATGTTAAAT	C|D

*p*dif* sites are presented with underlined fonts representing the 6 bp spacer that is flanked by the XerC/XerD binding sites.

§C|D refers to the XerC-spacer-XerD orientation while D|C refers to the XerD-spacer-XerC orientation of the p*dif* site

#ND = not detected