GENOMIC ANALYSIS OF ACINETOBACTER BAUMANNII POST-PHAGE EXPOSURE REVEALS THE LOSS OF A CONJUGATIVE PLASMID CARRYING AN INTEGRON

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INTRODUCTION

- The rise in **antimicrobial resistant** (AMR) bacterial infections poses a growing threat to warfighter readiness as established antibiotic treatment lines become ineffective.
- **Bacteriophages** (phages) are viruses with specific host range at the species level that target bacteria leading to cell death. Phage mediated therapy provides an alternative venue for the treatment of AMR infections.
- **Phage therapy** often relies on phage cocktails, or mixes of phages which may have different modes of infecting the bacterial pathogen, reducing potential therapy failure due to evolved phage resistance. Finding adequate phage candidates for therapy has many considerations including assessment of the phage genomic safety and bacterial survival test.
- **Phage characterization assays** were carried out for *Acinetobacter baumannii* AB5075, a highly virulent multi-drug-resistant clinical isolate.
- **Plasmid dependent phages**[1,2] allow the targeting of non-essential conjugative plasmids driving the AMR phenotype, resulting in antibiotic susceptible bacteria. Therefore, phages targeting plasmids could re-sensitize pathogenic bacteria to established antibiotic treatments.
- Researching novel AMR therapies expands treatment options for medical personnel.

METHODS

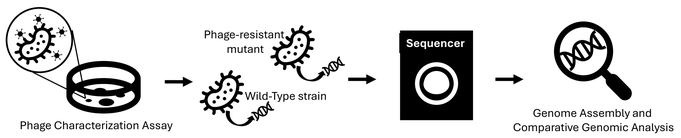

Figure 1: Methods schematic for isolation, sequencing, and characterization of phage-resistant mutants.

- Two *A. baumannii* AB5075 samples, a parent strain (Ab5075H) and a phage resistant strain (Ab5075HR1) were sequenced and analyzed for comparative genomic analysis (CGA).
- Bacterial gDNA was extracted and quality-controlled, yielding 300 µl of DNA at concentration 57-62 ng/µL (measured via Qubit fluorometer). Sequencing libraries were constructed using the PacBio HiFi Prep 96 kit (Cat. #102-009-200) and sequenced on a PacBio Vega instrument.
- Raw sequencing data were processed using the Circular Consensus Sequencing workflow in PacBio Vega SMRT Link[3] (v25.2.0.266456) to generate demultiplexed Hi-Fi reads. These reads were then analyzed using the SMRT Link Microbial Genome Analysis workflow for *de novo* assembly, read mapping, and coverage analysis. Assembly completeness was evaluated using CheckM2[4] (v1.0.2).
- Bacterial genome assemblies were annotated using Prokka[5] (v1.14.6). Comparative genomic analyses were performed using Bandage[6] (v0.8.1) and the Whole Genome Alignment workflow within CLC Workbench[7] (v25.0.3). Antimicrobial resistance (AMR) profiles were characterized using the Resistance Gene Identifier[8] (RGI, v6.0.3) tool against the Comprehensive Antibiotic Resistance Database[8] (CARD).

RESULTS

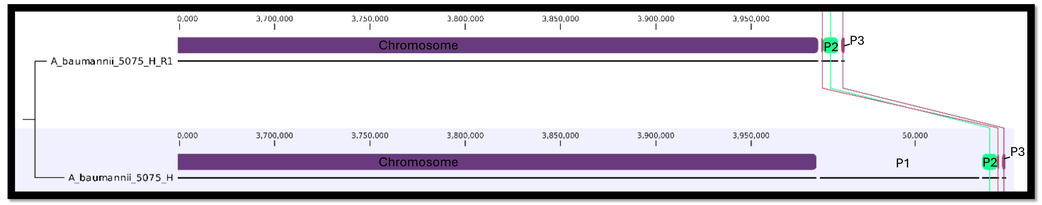

Figure 2. Zoomed view of the whole genome alignment between Ab5075H (WT) and Ab5075HR1 (Mutant), partial chromosome (purple), plasmid 2 (P2, green), and plasmid 3 (P3, maroon) alignments. Plasmid 1 (P1) in Ab5075H does not align to Ab5075HR1 (uncolored contig).

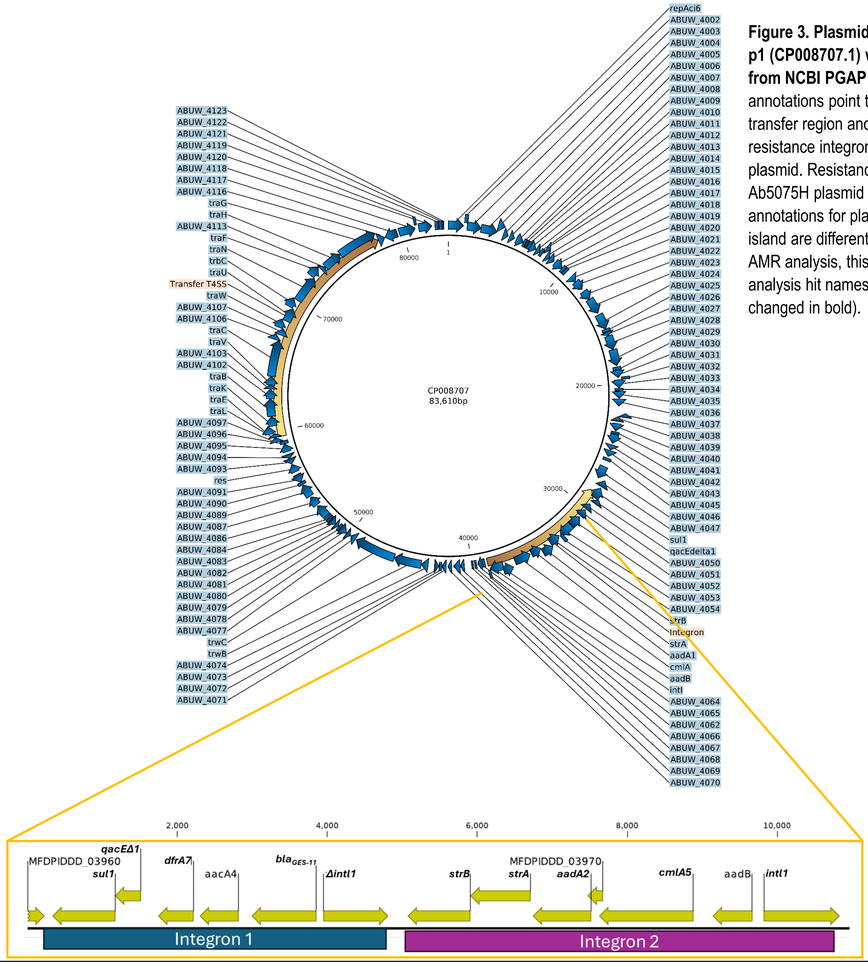

Figure 3. Plasmid map of AB5075-UW p1 (CP008707.1) with annotations from NCBI PGAP pipeline. Yellow annotations point to the potential T4SS transfer region and the antimicrobial resistance integron found in the plasmid. Resistance island contained in Ab5075H plasmid 1. Note the Prokka annotations for plasmid 1 resistance island are different than those in the AMR analysis, this figure uses the AMR analysis hit names (gene names changed in bold).

REFERENCES

1. Li, J., et al., *Male-specific bacteriophages and their potential on combating the spreading of T4SS-bearing antimicrobial resistance plasmids.* Crit Rev Microbiol, 2025. **51**(4): p. 639-650.
2. Penttinen, R., C. Given, and M. Jalasvuori, *Indirect Selection against Antibiotic Resistance via Specialized Plasmid-Dependent Bacteriophages.* Microorganisms, 2021. **9**(2)
3. PacBio. SMRT Link software tools cited 2025; Available from: https://www.pacb.com/smrt-link/.
4. Chklovski, A., Parks, D. H., Woodcroft, B. J., & Tyson, G. W. (2023). CheckM2: a rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. *Nature methods*, *20*(8), 1203–1212. https://doi.org/10.1038/s41592-023-01940-w
5. Seemann, T., *Prokka: rapid prokaryotic genome annotation.* Bioinformatics, 2014. **30**(14): p. 2068-9.
6. Wick, R.R., et al., *Bandage: interactive visualization of de novo genome assemblies.* Bioinformatics, 2015. **31**(20): p. 3350-2.
7. QIAGEN. *CLC Genomics Workbench 25.0.3, Whole Genome Alignment Module*. Available from: https://digitalinsights.qiagen.com.
8. Alcock et al. 2023. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Research, 51, D690-D699 [PMID 36263822].

RESULTS

- In support of **phage discovery**, phage characterization assays (**Figure 1**) were carried out for *Acinetobacter baumannii* AB5075, a highly virulent multi-drug-resistant clinical isolate. Assays resulted in *A. baumannii* phage-resistant mutant strain (AB5075HR1) with lower Minimum Inhibitory Concentration (MIC) to previously resistant antibiotics sulfamethoxazole and trimethoprim.
- PacBio Vega sequencing resulted in >940k HiFi reads per sample, with an average quality Phred score of 40, and average read length of 11,240 bp (**Table 1**). The assembly of sample Ab5075H resulting in four circularized contigs and Ab5075HR1 with three circularized contigs, both assemblies had a max contig size of 3.9 Mbp, the size expected for an *A. baumannii* chromosome (**Table 2**).

Table 1. Summary of HiFi reads per sample

	Total HiFi Reads	HiFi Read Length	Median Quality	Yield (bp)
A_baumannii_5075_H	942,477	11,161	40	10,519,875,214
A_baumannii_5075_H_R1	946,629	11,320	40	10,716,694,388

Table 2. Summary of assembly contigs

Sample Name	Contig	Length (bases)	Circular	Coverage (X)
A_baumannii_5075_H	Chromosome	3,984,310	yes	2600.60
	Plasmid 1	83,610	yes	916.41
	Plasmid 2	8,731	yes	1908.51
	Plasmid 3	1,967	yes	262.83
A_baumannii_5075_H_R1	Chromosome	3,985,151	yes	2685.37
	Plasmid 1	**Not detected**		
	Plasmid 2	8,731	yes	1843.42
	Plasmid 3	1,924	yes	324.08

- Whole genome comparison of the *de novo* assemblies showed that the parent strain, Ab5075H, has three plasmids, while the mutant strain, Ab5075HR1, is missing P1 plasmid, the 83,610 bp plasmid which was found to carry two integrons with multiple drug resistance genes including *drfA7* and *sul1* conferring resistance to sulfamethoxazole and trimethoprim, respectively (**Figure 2 and 3**). No AMR genes were found on plasmids P2 or P3.
- Analysis of plasmid P1 in Ab5075H shows that it contains 11 AMR genes. The structure of the integron region shows one class 1 integron with structure Δ*intI1-blaGES-11- aacA4-drfA7-qace*Δ*1-sul1*, where Δ*intI1* is a truncated class 1 integrase, immediately followed by a second class 1 integron with structure *intI1-aadB-cmlA5-aadA2-strA-str*B (**Figure 2**).
- The other well annotated region of P1 includes a type IV secretion transfer operon (*tra* genes) most likely involved in the mobilization of P1.

CONCLUSIONS

- The loss of plasmid P1 and the resulting phage resistant phenotype in the mutant AB5075HR1 post-phage exposure suggests the phage entry target is probably a plasmid encoded receptor.
- The loss of P1 also explains the antibiotic susceptibility seen in AB5075HR1, due to the loss of the multi-drug resistance encoding integrons found in P1.
- These findings suggest the characterized phage is a plasmid dependent phage.
- Plasmid dependent phages have been previously described and are of interest as phages targeting plasmids could re-sensitize pathogenic bacteria to established antibiotic treatments. Phage therapy with plasmid dependent phages could be used in co-treatment strategies and act as an adjuvant to established antibiotic therapies.

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