



Comparing the Bioinformatic and Experimental approaches for Identifying Antibiotic Resistance Genes on Plasmids of *Acinetobacter baumannii*



Munok Hwang, MS¹; Hosoon Choi, PhD¹; Chetan Jinadatha, MD, MPH¹; Jing Xu, MS¹; Thanuri Navarathna, BS¹; Landon Ashby, BS¹; Morgan Bennett, BS¹; Keith S. Kaye MD²; Sorabh Dhar MD^{3, 4}; Piyali Chatterjee, MS, PhD¹
¹Central Texas Veterans Health Care System, ²University of Michigan, ³Wayne State University, ⁴John D Dingell VAMC



Introduction

- Antimicrobial resistance (AMR) genes of bacteria can be found in chromosomes or plasmids.
- AMR genes on plasmids can be easily spread through horizontal transfer from one bacterium to another (Figure 1).

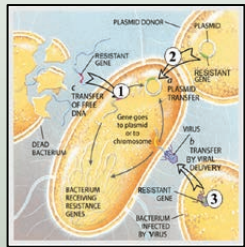


Figure 1. Mechanism of resistance gene transfer through horizontal gene transfer. Image copied from <https://amrls.umn.edu/microbiology>

- However, distinguishing whether AMR genes are from plasmids or chromosomes is difficult.
- Here, we assessed two different approaches, a bioinformatic approach using PlasmidSPAdes, or using SPAdes to assemble isolated plasmid sequence data for identifying AMR genes on the plasmids of *A. baumannii*.

Reference

Dmitry Antipov, Nolan Hartwick, Max Shen, Mikhail Raiko, Alla Lapidus, Pavel A. Pevzner, plasmidSPAdes: assembling plasmids from whole genome sequencing data, *Bioinformatics*, Volume 32, Issue 22, 15 November 2016, Pages 3380-3387

Acknowledgements

Funding: This project is supported by 1R03HS027667 grant to P. Chatterjee. Support was also provided by the Central Texas Veterans Healthcare System (CTVHCS) facility during the study period.

Methods

- Two approaches were applied to identify which AMR genes were on plasmids of 30 *A. baumannii* isolates (Figure 2):
 - A. assemble plasmid by PlasmidSPAdes, a bioinformatic tool, that distinguishes the plasmid sequences from whole genome sequence data based on the copy number.
 - B. assemble plasmid by SPAdes using sequence data of plasmid DNA isolated by plasmid preparation kit
- AMR genes from both approaches were identified using the open-source AMR database, ResFinder.
- The AMR gene data obtained from plasmids utilizing each approach were compared based on information from whole genome sequencing (WGS) data.

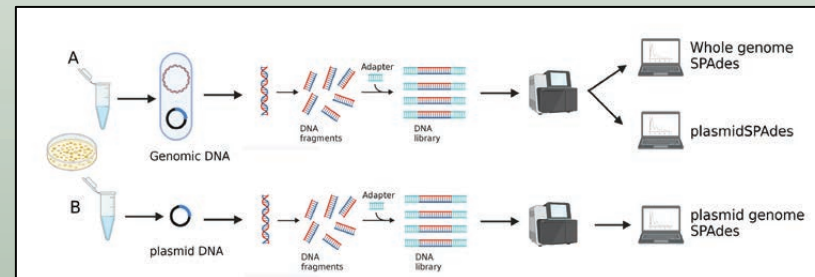


Figure 2. Illustration of two approaches applied:
A. An informatics approach-whole genomic DNA isolation and assemble plasmid using PlasmidSPAdes
B. Experimental approach-Isolate plasmid DNA using plasmid preparation kit

Conclusions

- The information obtained by the two approaches for identifying resistance genes on plasmids mostly agreed with some exceptions.
- The AMR genes identified in both (*su12*) could be on plasmids while most AMR genes not identified from both approaches (ex. *bla*TEM-ID, *tet* B) could be on chromosomes. (Figure 3)
- Carbapenem resistance gene, *bla*OXA-23 could be both plasmid-mediated and chromosome-mediated (Table 1 & 2).
- PlasmidSPAdes was a useful tool to discriminate plasmid sequence from whole genome without isolation of plasmid DNA.

Results

Table 1. Comparison of informatics and experimental approaches in studying AMR genes on plasmids in *A. baumannii*

Carbapenem resistance (AST)	<i>bla</i> OXA-23 gene	PlasmidSPAdes (Informatics)		Isolated Plasmid DNA (Experimental)			
		No. of contig	Largest Contig Size	No. of contig	Largest Contig Size	<i>bla</i> OXA-23 position	
R	Yes	4	65615	Node 2	506	61480	Node 2
R	Yes	9	107022	Node 4	508	107022	Node 3
R	Yes	8	5464	Node 2	1038	8753	Node 3
R	Yes	18	107130	Node 4	2317	35587	Node 2
R	Yes	7	67481	Node 1	1891	48570	Node 2
R	Yes	29	107043	Node 5	404	36030	Node 5
R	Yes	4	60482	Node 2	1450	54691	Node 2
R	Yes	7	35676	Node 4	2454	33576	Node 5
R	Yes	11	39850	Node 5	218	35576	Node 3
R	Yes	19	36569	Node 3	1543	35626	Node 3
R	Yes	22	107044	Node 4	315	66942	Node 5
R	Yes	0	0	X	686	9318	X
R	Yes	13	57126	X	44	57082	X
R	Yes	2	8808	X	5	8753	X
R	Yes	13	57126	X	29	57082	X
R	Yes	1	8808	X	778	8786	X
R	Yes	1	8809	X	91	8754	X
R	Yes	13	257173	Node 11	83	37647	X
R	Yes	0	0	X	2538	8414	Node 72
R	Yes	1	19731	X	32	50505	Node 3
R	Yes	8	107044	X	1217	35586	Node 5
R	Yes	13	107044	X	445	107022	Node 3
R	No	3	9332	X	1422	9310	X
S	No	2	5799	X	942	7046	X
S	No	2	5799	X	808	5909	X
S	No	3	46319	X	2229	8537	X
S	No	2	126104	X	903	6267	X
S	No	2	5799	X	1078	4695	X
S	No	2	5799	X	2361	7745	X
S	No	3	12159	X	2407	5910	X

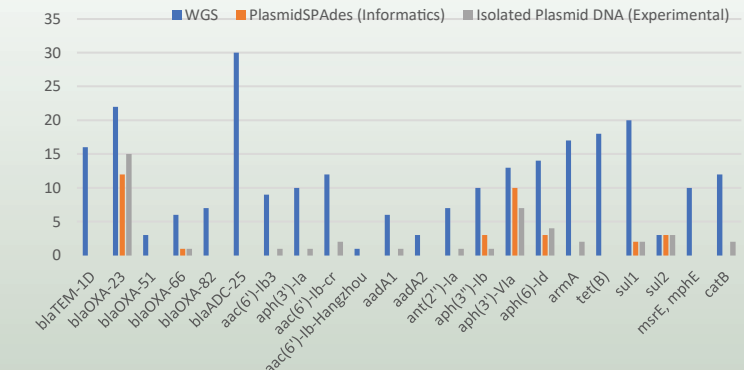


Figure 3. Comparison of number of AMR genes detected in WGS, PlasmidSPAdes (Informatics), and isolated plasmid DNA (Experimental)

Table 2. AMR genes and corresponding antibiotics detected from *A. baumannii* isolates

Antibiotics by Class	Generic antibiotics name	Resistance genes
Aminoglycosides	Amikacin, Gentamicin, Tobramycin, Kanamycin	<i>aac(6)-Ib3</i> , <i>aac(6)-Ib-cr</i> , <i>aac(6)-Ib-Hangzhou</i> , <i>aadA1</i> , <i>aadA2</i> , <i>ant(2'')-Ia</i> , <i>aph(3')-Ia</i> , <i>aph(3'')-Ib</i> , <i>aph(3)-Ia</i> , <i>aph(6)-Ia</i> , <i>aph(6)-Id</i> , <i>armA</i>
Carbapenems	Ertapenem, Doripenem, Imipenem, Meropenem	<i>bla</i> OXA-23, <i>bla</i> OXA-51, <i>bla</i> OXA-66, <i>bla</i> OXA-82
Penicillin	Piperacillin, Ampicillin, Amoxicillin, Piperacillin/tazobactam	<i>bla</i> TEM-1D
Quinolones	Ciprofloxacin, Levofloxacin, Nalidixic acid	<i>aac(6)-Ib-cr</i>
Macrolides	Erythromycin	<i>mphE</i> , <i>msrE</i>
Sulfonamides	Sulfamethoxazole	<i>su1</i> , <i>su2</i>
Tetracyclines	Doxycycline, Tetracycline	<i>tetB</i>
Chloramphenicol	Chloramphenicol	<i>catB</i>

Legend:
 Both detected
 Both Not detected
 Informatics only
 Experimental only