Genetic Analysis of Antibiotic Resistance Profiles of Acinetobacter baumannii Using Whole Genome Sequencing

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Introduction

- Acinetobacter spp. is a gram-negative coccobacilli involved in many healthcare-associated infections (HAIs), especially in the critically ill hospitalized patients.
- These infections are increasingly difficult to treat as they are known to quickly develop resistance to commonly used antibiotics. Therefore, surveillance studies are important and provide a snapshot of prevalent antibiotic resistance profiles in an area.
- In this study, we sought to characterize antibiotic resistance patterns in clinical Acinetobacter isolates by performing whole genome sequencing (WGS) and whole genome multi-locus sequence typing (wgMLST) analysis, and in silico analysis for antibiotic resistance genes.



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Methods

- The Acinetobacter isolates were provided from two tertiary Detroit area hospitals from patients admitted to 16 different intensive care units (ICU) and non-ICU wards between 2017-2019.
- Samples were subjected to WGS using the NextSeq instrument (Illumina). The contigs were *de novo* assembled using SPAdes (v3.7.1) and wgMLST analysis was performed using BioNumerics software v7.6.
- The genomic sequence for each isolate was uploaded in ResFinder 3.2 and known antibiotic resistance genes were analyzed.





U.S. Department of Veterans Affairs



	Unit A	ST2	ST15 ST36			ST119			ST406		
ς.	Unit B	ST2	ST36	ST49	ST93		ST212		ST406	ST667	
(Unit C	ST2	ST15								
									ST40C		
-	E Unit E								51406		
	Unit F								ST406		
	Unit G	ST2						ST214	ST406		
(_	Unit H	ST2	ST15					ST268	S	T427	
(Unit I	ST2							ST395 S	T427	
2	Unit J	ST2									
	Linit K	ST2									
		<u>ст</u> э									
	Unit L	512									
Ta	able 1: Sequenc	e Types (ST) arranged by hos	pital and by	/ unit. Geo	ographic ro	oom location	s are not represe	nted here; arranged for vis	ual clarity only.	
	Hospital 1							Hospital 2			
Sequence	ce Total No. of Resistance to Antibiotic Drugs					Sequence Total No. of Provision to Antibiotic Drugs					
Type (ST)	Isolates		Resistance to A		rugs		Type (ST)	Isolates	Resistance to A	Antibiotic Drugs	
ST2	11	β-lactar	n, aminoglycoside	e, sulphona	mide,		ST2	20	β-lactam, aminoglycosi	de, sulphonamide,	
	2	phenico	ol, macrolide, tetracycline				ST15	1 B	phenicol, macrolide, ter	tracycline de sulphonamide	
ST15 ST26	2	p-lactar	n, aminogiycoside m	e, suipnona	miae		5115	1	B-lactam aminoglycosi	de sulphonamide	
ST49	2	B-lactar	am				ST20	1	phenicol, macrolide, te	tracycline	
ST43 ST93	5	β-lacta	actam				ST214	1	β-lactam		
ST119	9 2 β-lactam						ST268	1	β-lactam		
ST212	Γ_{212} 1 β-lactam						ST395	1	β-lactam		
ST406	T4064β-lactam, aminoglycoside, sulphonamide						ST406	1	β-lactam, aminoglycosi	de, sulphonamide	
ST667	ST667 1 β-lactam						ST427	2	β-lactam		
Table 2: ST a	and genetic resi	istance e	xpression in Hospita	nl 1			Table 3: ST a	nd genetic resista	nce expression in Hospita	12	
									Llocation 2.		
-		Hospital 1:				Hospital 2: % of isolatos with					
			Antibiotic	% of i	% of isolates wit	vith	Antib	iotic Drugs	Antibiotic	Antibiotic	
Antibiotic Drugs		Resistance Gene	es Division	Antibiotic				Resistance Genes	Resistance Genes		
				Resist	ance Ge	nes			aadA1	30	
			aadA1		72				aadA2	15	
			aadA2		9				armA	70	
			armA		72				ant(2'')-la	15	
			$ant(2^{\prime\prime})$ -la		9		Amin	oglycoside	aph(3'')-lb	80	
Am	inoglycoside		ahii(2)-in		0				aph(3')-Via	40	
			anh(3')-Via		_				anh(2!) la	70	
			aph(3')-Via aph(3')-la		81				aph(3')-la	70 80	
			aph(3')-Via aph(3')-Ia aph(6)-Id		81 81				aph(3')-la aph(6)-ld aac(3)-lla	70 80 5	
			aph(3')-Via aph(3')-Ia aph(6)-Id aac(3)-Ia		81 81 9				aph(3')-Ia aph(6)-Id aac(3)-IIa aac(6')-Ib3	70 80 5 25	
			aph(3')-Via aph(3')-Ia aph(6)-Id aac(3)-Ia aac(6')-Ib3		81 81 9 63		Aminoglyc	oside/Quinolor	aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb-cr	70 80 5 25 30	
Aminogly	/coside/Quine	olone	aph(3')-Via aph(3')-Ia aph(6)-Id aac(3)-Ia aac(6')-Ib3 aac(6')-Ib-cr		81 81 9 63 63		Aminoglyc P	oside/Quinolor henicol	<pre>aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb-cr catB8</pre>	70 80 5 25 30 30	
Aminogly	/coside/Quino Phenicol	olone	aph(3')-Via aph(3')-Ia aph(6)-Id aac(3)-Ia aac(6')-Ib3 aac(6')-Ib-cr catB8		81 81 9 63 63 63		Aminoglyco P	oside/Quinolor henicol	<pre>aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb3 aac(6')-lb-cr catB8 mph(E)</pre>	70 80 5 25 30 30 80	
Aminogly	/coside/Quino Phenicol	olone	aph(3')-Via aph(3')-Ia aph(6)-Id aac(3)-Ia aac(6')-Ib3 aac(6')-Ib-cr catB8 mph(E)		81 81 9 63 63 63 63 72		Aminoglyco P M	oside/Quinolor henicol acrolide	<pre>aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb3 aac(6')-lb-cr aac(6')-lb-cr ancatB8 mph(E) msr(E)</pre>	70 80 5 25 30 30 30 80 80	
Aminogly	/coside/Quino Phenicol Macrolide	olone	aph(3')-Via aph(3')-Ia aph(6)-Id aac(3)-Ia aac(6')-Ib3 aac(6')-Ib-cr catB8 mph(E) msr(E)		81 81 9 63 63 63 72 72 72		Aminoglyco P M	oside/Quinolor henicol acrolide	<pre>aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb3 aac(6')-lb-cr aac(6')-lb-cr aactB8 mph(E) msr(E) sul1</pre>	70 80 5 25 30 30 30 80 80 80 40	
Aminogly	/coside/Quino Phenicol Macrolide	olone	aph(3')-Via aph(3')-la aph(6)-ld aac(3)-la aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) msr(E) sul1		81 81 9 63 63 63 63 72 72 72 90		Aminoglyco P M Sulp	oside/Quinolor henicol acrolide honamide	<pre>aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb3 aac(6')-lb-cr aac(6')-lb-cr aactB8 mph(E) msr(E) sul1 sul2</pre>	70 80 5 25 30 30 30 30 80 80 80 40 40 10	
Aminogly	/coside/Quino Phenicol Macrolide Iphonamide	olone	aph(3')-Via aph(3')-la aph(6)-ld aac(3)-la aac(6')-lb3 aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) msr(E) sul1 sul2		81 9 63 63 63 72 72 90 90 9		Aminoglyco P M Sulp Tet	oside/Quinolor henicol acrolide honamide racycline	 aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb-cr aac(6')-lb-cr catB8 mph(E) msr(E) sul1 sul2 tet(B) 	70 80 5 25 30 30 30 80 80 40 10 80	
Aminogly I Su Te	/coside/Quine Phenicol Macrolide Iphonamide etracycline	olone	aph(3')-Via aph(3')-la aph(6)-ld aac(3)-la aac(6')-lb3 aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) msr(E) sul1 sul1 sul2		81 81 9 63 63 63 72 72 72 90 9 81		Aminoglyco P M Sulp Tet	oside/Quinolor henicol acrolide honamide racycline	aph(3')-Ia aph(6)-Id aac(3)-Ila aac(6')-Ib3 aac(6')-Ib-cr catB8 mph(E) msr(E) sul1 sul2 tet(B) blaADC-25	70 80 5 25 30 30 30 80 80 40 10 80 40	
Aminogly F Su B	/coside/Quine Phenicol Macrolide Iphonamide etracycline Betalactam	olone	aph(3')-Via aph(3')-la aph(6)-ld aac(3)-la aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) msr(E) sul1 sul1 sul2 tet(B) blaADC-25		81 81 9 63 63 63 72 72 72 90 90 9 81 100 27		Aminoglyco P M Sulp Tet Be	oside/Quinolor henicol acrolide honamide racycline talactam	 aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) msr(E) sul1 sul2 tet(B) blaADC-25 blaOXA-223 	70 80 5 25 30 30 30 80 80 40 40 10 80 40 10 80 5	
Aminogly G Su Te B	/coside/Quine Phenicol Macrolide Iphonamide etracycline Betalactam	olone	aph(3')-Via aph(3')-la aph(6)-ld aac(3)-la aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) sul1 sul1 sul2 blaADC-25 blaADC-25		81 9 63 63 63 72 72 72 90 90 90 91 100 27 81		Aminoglyco P M Sulp Tet Be	oside/Quinolor henicol acrolide honamide racycline talactam	 aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) msr(E) sul1 sul2 tet(B) blaADC-25 blaOXA-223 blaOXA-23 blaOXA-23 	70 80 5 25 30 30 30 80 80 40 40 40 40 10 80 40 5 80 5 80 20	
Aminogly Gul Su B	/coside/Quine Phenicol Macrolide Iphonamide etracycline Betalactam	olone	aph(3')-Via aph(3')-la aph(6)-ld aac(3)-la aac(6')-lb3 aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) sul2 sul1 sul2 sul2 blaADC-25 blaOXA-23 blaOXA-23		81 9 63 63 63 63 72 72 72 90 90 90 90 90 91 100 27 81 100 27 81		Aminoglyco P M Sulp Tet Be	oside/Quinolor henicol acrolide honamide racycline talactam	aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) msr(E) sul1 sul2 tet(B) blaADC-25 blaOXA-233 blaOXA-66 blaOXA-82	70 80 5 25 30 30 30 80 80 40 40 40 40 10 80 40 5 80 5 80 5 80 40 100	
Aminogly G Su Te B	/coside/Quine Phenicol Macrolide Iphonamide etracycline Setalactam	olone	aph(3')-Via aph(3')-Ia aph(6)-Id aac(3)-Ia aac(6')-Ib3 aac(6')-Ib-cr aac(6')-Ib-cr catB8 mph(E) msr(E) sul1 sul1 sul2 sul2 tet(B) blaADC-25 blaOXA-23 blaOXA-23 blaOXA-66 blaOXA-82		81 81 9 63 63 63 72 90 91 63 72 90 91 92 81 100 27 81 18 72		Aminoglyco P M Sulp Tet Be	oside/Quinolor henicol acrolide honamide racycline talactam	aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) msr(E) sul1 sul2 sul2 tet(B) blaADC-25 blaOXA-223 blaOXA-23 blaOXA-23 blaOXA-66 blaOXA-82 blaTEM-1D	70 80 5 25 30 30 30 80 80 40 40 40 10 40 10 80 40 5 80 5 80 5 80 5 80 20 15 35	
Aminogly Gul Su B	vcoside/Quino Phenicol Macrolide Iphonamide etracycline Betalactam	olone	aph(3')-Via aph(3')-Ia aph(6)-Id aac(3)-Ia aac(6')-Ib3 aac(6')-Ib-cr catB8 mph(E) sul1 sul2 sul2 tet(B) blaADC-25 blaOXA-23 blaOXA-23 blaOXA-66 blaOXA-82	present in H	81 81 9 63 63 63 72 72 72 90 9 81 100 27 81 100 100 27 81 100 100 27 81 100 100 27 81 100 100 100 100 100 100 100		Aminoglyce P M Sulp Tet Be	oside/Quinolor henicol acrolide honamide racycline talactam	aph(3')-la aph(6)-ld aac(3)-lla aac(6')-lb3 aac(6')-lb-cr catB8 mph(E) msr(E) sul1 sul2 tet(B) blaADC-25 blaOXA-223 blaOXA-233 blaOXA-23 blaOXA-666 blaOXA-82 blaTEM-1D	70 80 5 25 30 30 30 80 80 40 40 40 10 80 40 10 80 5 80 5 80 5 80 20 100 5 80 100 5 5 80 100 5 5 80 100 5 5 80 100 5 5 80 100 5 5 80 100 100 5 5 80 100 100 5 5 80 100 100 100 100 100 100 100 100 100	







presented in Table 1.

- resistant. See tables 2 and 3.

- practices and clinical practice.

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Results

ST2 was the most prevalent sequence type (ST) accounting for 52% in both hospitals. Please check poster by *Chatterjee et al.* and as

The most common resistance gene found is blaADC-25 conferring resistance to beta-lactams across all STs. See tables 2 and 3.

Sulfonamide (sul1) and macrolide resistance (mphE/msrE) among STs were also common. See tables 4 and 5.

ST2 in H2 exhibited the presence of the maximum number of resistance genes including resistance to aminoglycosides, macrolides (2), tetracyclines (tetB), beta-lactams, fluoroquinolones (aac(6')-lb-cr), sulphonamides (sul1, sul2). See table 5.

ST2 had a slightly different resistance profile of beta-lactams in H1 when compared to H2. See tables 4 and 5.

ST406 and ST15 exhibited similar antibiotic profiles in both hospitals and a single isolate of ST20 from H2 is highly antibiotic

Conclusions

Acinetobacter infection is a known HAI problem which has necessitated emerging new treatments.

We provide a snapshot of resistance genes identified. All the hospital isolates demonstrated resistance to beta-lactams and some with similar antibiotic resistance profiles.

No colistin resistance genes were identified by *in silico* analysis.

In future practices, monitoring for genetic subtypes and encoded genetic resistance may prove useful for better infection control

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