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In silico Detection of Acquired Antimicrobial Resistance Genes in 110 Complete Genome Sequences of *Acinetobacter baumannii*

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Abstract

Multidrug-resistant (MDR) *Acinetobacter baumannii* is one of the most common nosocomial pathogens posing a serious health threats to patients around the world. The present study aims at the in silico detection of the acquired antimicrobial resistance (AMR) genes in *A. baumannii*. In this study,110 complete whole genome sequences (WGS) of *A. baumannii* obtained from the NCBI database are analyzed. The comprehensive ResFinder 3.1 tool is used to identify the AMR genes among the *A. baumannii* sequences. The core genome of *A. baumannii* in fasta format was created using Spine program. Furthermore, the Pasteur MLST scheme was performed for the 110 complete WGS of *A. baumannii* using the MLST 2.0 web server. Resistance genes against beta-lactam antimicrobial agents were detected in all *A. baumannii* strains (n=110; 100%), followed by resistance genes against aminoglycoside (n=87; 79 %), sulphonamide (n=84 ; 76%), phenicol (n=52; 47 %), tetracycline (n=52; 47 %), fluoroquinolone (n=42; 38 %), MLS (n=43; 39 %), trimethoprim (n=4; 3.6 %), and rifampicin (n=2; 1.8%). Moreover, the blaADC-25 gene encoding beta-lactam resistance was found in all of the *A. baumannii* strains (n=110; 100 %), followed by blaOXA-66 (n=61; 55.4%), blaOXA-23 (n=50; 45%) and blaTEM-1D (n=36; 32 %) as the most predominant genes. These results suggest that bioinformatics tools such as ResFinder can be utilized for the detection of AMR genes in *A. baumannii* and other pathogens.

Keywords: *In silico*, Resistance genes, Genome sequences, *Acinetobacter baumannii*

1. Introduction

The current emergence of multidrug-resistant (MDR) pathogens is a major area of concern. The World Health Organisation (WHO) has published its first list ever of antibiotic-resistant "priority pathogens" which includes twelve families of bacteria that pose serious threats to human health (WHO, 2017). Among these families *Acinetobacter baumannii* is an organism of serious concern due to the increased emergence of carbapenems-resistant isolates. Hence, *A. baumannii* has been designated as "critical" and occupies the top of the list (WHO, 2017). In the 1970s, it was thought that *A. baumannii* strains were sensitive to most antimicrobial agents. Today *A. baumannii* strains have been found resistant to most antibiotic classes, including beta-lactams, aminoglycosides, fluoroquinolones, and tetracyclines (Perez *et al*., 2007; Pleg *et al*., 2008; Wong *et al*., 2016). Many recent studies have shown that *A. baumannii* strains are still considered emerging health threats to patients around the world, due to their ability to develop multidrug-resistance (MDR) (Fang *et al*., 2015; Michiels *et al*., 2016; Almaghrabi *et al.*, 2018; Nasser *et al*, 2018).

The disk diffusion test and the broth micro-dilution test are the routine methods utilized in diagnostic laboratories to probe for antibiotic susceptibility of bacterial clinical isolates (Rolinson and Russell 1972; Diene and Rolain 2013). These techniques are markedly influenced by a number of variables including inoculum size, depth of agar, conditions of incubation, and medium composition. Although these parameters may influence antibacterial susceptibility testing results, these parameters have been standardized; hence, they are no longer an area of concern. Furthermore, the obtained results usually cannot be replicated among different laboratories for the same antimicrobial susceptibility test (Huys *et al*., 2005; Baltekin *et al*., 2017).

In several recent studies, the whole genome sequencing technology has been applied to identify bacteria and their antimicrobial gene patterns from different sources (Stoesser *et al.*, 2013; Tyson *et al*., 2015; Ruppé *et al*., 2017). The presence of antimicrobial resistance (AMR) genes within the bacterial whole genome sequences can be detected using dedicated databases of antibiotic gene sequences such as ResFinder (Zankari *et al*., 2012), CARD (McArthur *et al.*, 2013), and ARG-ANNOT (Gupta *et al*. 2014). The ResFinder reference gene database has been developed by the Centre for Genomic Epidemiology (CGE) (www.genomice pidemiology.org) for *in silico* analysis of bacterial whole genome sequences (WGS) including *A. baumannii*. ResFinder is based on a database of more than 2,000 resistance genes covering twelve types of antimicrobial agents (aminoglycosides, beta-lactams, fluoroquinolones, fosfomycin, fusidic acid, glycopeptides,

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macrolide-lincosamide-streptogramin B (MLS), phenicol, rifampicin, sulphonamide, tetracycline, and trimethoprim).

In the present study, 110 complete WGS of *A. baumannii* that were deposited in the National Centre for Biotechnology Information (NCBI) were utilized for the detection of the antimicrobial resistance (AMR) genes and agents for each isolate using the ResFinder tool. Additionally, the multiple locus sequence typing (MLST) was performed for the 110 *A. baumannii* genome sequences to classify the strains based on their antimicrobial profiles.

2. Materials and Methods

2.1. The Complete Whole Genome Sequences of A. baumannii

Because the main goal of this study is to identify acquired AMR genes in *A. baumannii*, only the 110 complete WGS of *A. baumannii* (https://www.ncbi. nlm.nih. gov/genome/? term= acinetobacter) were used for *in silico* AMR genes identification. The WGS of *A. baumannii* were obtained from the National Center for Biotechnology Information (NCBI) public database as of December, 2018. Draft genomes, scaffolds, and contigs were excluded from this study.

2.2. Detection of Antimicrobial Resistance Agents and Genes in the Genomes of A. baumannii

A web version of the ResFinder 3.1 tool (Zankari *et al*., 2012) was used to identify AMR agents and genes in the 110 *A. baumannii* sequences. The ResFinder tool uses BLAST (Basic Local Alignment Search Tool) (Altschul *et al*., 1990) for the detection of acquired AMR genes in the whole genome database. The fasta (.fasta) format of the input genome sequences was used. The threshold for reporting a match between a gene in the ResFinder database and the input sequence was set at a 50 % identity with a minimum length of 60 %.

2.3. Detection of Antimicrobial Resistance Genes in the Core Genome of A. baumannii

The core genome of *A. baumannii* was constructed from the 110 complete WGS of *A. baumannii* used in this study. Spine software program (Ozer *et al.*, 2014) was used to create the core genome of *A. baumannii* in a fasta format file. For the detection of AMR genes in the core genome of *A. baumannii*, the constructed sequence file was uploaded on the ResFinder database and the input sequence was set at a 50 % identity with the minimum length of 60 %.

2.4. Multiple Locus Sequence Typing (MLST)

MLST was performed from the 110 complete WGS of *A. baumannii* using the MLST 2.0 web server (www.genomicepidemiology.org). The Pasteur MLST Scheme (Jolley *et al*., 2010) which is based on seven housekeeping genes (*cpn60, fusA, gltA, pyrG, recA, rplB* and *rpoB*) of *A. baumannii* (http://pubmlst.org/ abaumannii) was used to classify the strains based on the sequence types.

3. Results

3.1. Detection of Antimicrobial Resistance Agents

The RestFinder 3.1 analysis of the 110 *A. baumannii* strains indicate that the strains harboured genes mediating resistance to nine antimicrobial agent groups, namely aminoglycosides, beta-lactams, fluoroquinolone, macro-lide-lincosamide-streptogramin B (MLS), phenicol, rifampicin, sulphonamide, tetracycline, and trimethoprim (Figure 1). The AMR genes against beta-lactams were found in all strains (n=110; 100 %), followed by those against aminoglycosides (n=87; 79 %), sulphonamide (n=84 ; 76 %), phenicol (n=52; 47 %), tetracycline (n=52; 47 %), fluoroquinolones (n=42; 38 %), MLS (n=43; 39 %), trimethoprim (n=4; 3.6 %), and rifampicin (n=2; 1.8 %).

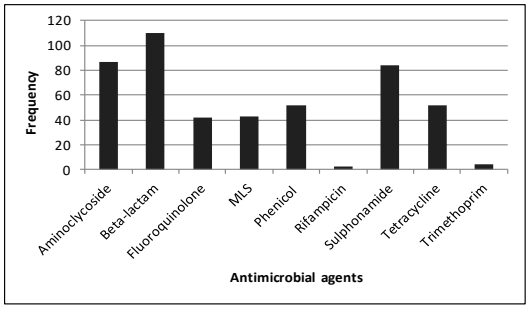

Figure 1. Antimicrobials' resistance genes harboured within the 110 WGS of *A. baumannii* strains obtained from the NCBI database as of December 2018, as detected by the ResFinder 3.1 tool.

3.2. Detection of Antimicrobial Resistance (AMR) Genes

A total of fifty-six unique resistance genes were identified in the 110 *A. baumannii* sequences (Table 1), most of which are genes encoding beta-lactam and aminoglycoside resistance. The blaADC-25 gene encoding beta-lactam resistance was found in all *A. baumannii* strains (n=110; 100 %), followed by blaOXA-66 (n=61; 55.4 %), blaOXA-23 (n=50; 45 %) and blaTEM-1D (n=36; 32 %), as the most predominant genes (Table 1). The BlaOXA-66 and blaADC-25 genes were also found in the predicted core genome of the 110 *A. baumannii* sequences used in this study, with identities of 100 % and 99.9 %, respectively. In addition to that, twenty-six genes encoding beta-lactam resistance were also detected in *A. baumannii* using ResFinder (Table 1).

As for the aminoglycoside resistance, fourteen distinct aminoglycoside resistance genes were detected, with twelve different gene types. The most predominant aminoglycoside resistance genes in the 110 *A. baumannii* strains were aadA1 (n=63; 57 %), aph (3")-Ib (n=59; 53 %) and aph (6)-Id (n=57; 52 %), followed by aph (3')-Ia (46; 42%) and aac (6')-Ib3 (40; 36 %) (Table 1).

As for fluoroquinolone, only the aac (6')-Ib-cr resistance gene was detected in *A. baumannii* (n=40; 36 %). As for MLS resistance, msr (E) (n=43; 39 %) and mph (E) (n=43; 39 %) resistance genes were detected. As for phenicol resistance, three resistance genes were detected, with the most common being catB8 (n=37; 33.6 %), followed by catA1 (n=7; 6 %) and cmlA1 (n=2; 1.8 %) (Table 1).

Sulphonamide resistance was predominantly encoded by sul1 (n=64; 58 %) and sul2 (n=43; 39 %) (Table 1).

Tetracycline resistance was encoded predominantly by tet(B) (n=42; 38 %), followed by tet(A) (n=7; 6.3 %) and tet(G) (n=3; 2.7 %).

Rifampicin resistance was encoded by ARR-2 (n=; 1.8 %). Trimethoprim resistance was encoded by dfrB1, dfrA1, dfrA10 and dfrA12 which were encountered at less than 2 % of the strains, each (Table 1).

Table 1. Resistance genes detected in the 110 whole genome sequences of *A. baumannii* by ResFinder with ID ≥99.00 %.

Resistance gene	Frequency (N=110)	Percentage (100%)	Predicted Phenotype	Accession No.
*aad*A1	63	57	Aminoglycoside resistance	JQ414041
*arm*A	48	43		AY220558
aph(3")-Ib	59	53		AF321550
aph(6)-Id	57	52		M28829
aac(6')-Ian	8	7.2		AP014611
aph(3')-Ia	46	42		X62115
aph(3')-Via	10	9		X07753
aac(6')-Ib3	40	36		X60321
aac(3)-IIa	6	5.4		X51534
*aad*A11	1	0.9		AY144590
ant(2")-Ia	2	1.8		X04555
*aad*A2	1	0.9		JQ364967
*bla*TEM-1D	36	32	Beta-lactam resistance	AF188200
*bla*OXA-23	50	45		AY795964
*bla*OXA-66	61	55.4		AY750909
*bla*OXA-65	8	7.2		AY750908
*bla*OXA-239	1	0.9		JQ837239
*bla*OXA-109	1	0.9		EF650035
*bla*PER-1	6	5.4		GU944725
*bla*OXA-94	1	0.9		DQ519088
*bla*OXA-64	7	6.3		AY750907
*bla*OXA-235	4	3.6		JQ820240
*bla*OXA-69	13	11.8		AY859527
*bla*TEM-1B	4	3.6		AY458016
*bla*OXA-2	1	0.9		DQ112222
*bla*OXA-68	6	5.4		AY750910
*bla*OXA-92	1	0.9		DQ335566
*bla*OXA-70	1	0.9		AY750912
*bla*ADC-25	110	100		EF016355
*bla*OXA-51	1	0.9		DQ385606
*bla*OXA-20	1	0.9		AY307114
*bla*OXA-100	1	0.9		AM231720
*bla*PER-7	1	0.9		HQ713678
*bla*OXA-259	1	0.9		KF057028
*bla*OXA-10	1	0.9		J03427
*bla*OXA-106	1	0.9		EF650032
*bla*OXA-68	6	5.4		AY750910
*bla*OXA-180	2	1.8		HM570036
*bla*NDM-1	4	3.6		FN396876
*bla*VEB-1	1	0.9		DQ393569
aac(6')-Ib-cr	40	36	Fluoroquinolone resistance	EF636461
msr(E)	43	39	MLS resistance	EU294228
mph(E)	43	39		DQ839391
*cat*B8	37	33.6	Phenicol resistance	AF227506
*cat*A1	7	6.3		V00622
*cml*A1	2	1.8		AB212941
*sul*1	64	58	Sulphonamide resistance	U12338
*sul*2	43	39		AY034138
tet(B)	42	38	Tetracycline resistance	AP000342
tet(G)	3	2.7		AF133140
tet(A)	7	6.3		AJ517790
ARR-2	2	1.8	Rifampicin resistance	HQ141279
*dfr*B1	1	0.9	Trimethoprim resistance	U36276
*dfr*A1	2	1.8		X00926
*dfr*A10	1	0.9		L06418
*dfr*A12	1	0.9		AM040708

3.3. Multilocus Sequence Typing (MLST)

Using Pasteur MLST scheme, twenty-five different MLST types and one unknown ST were observed in the 110 *A. baumannii* strains (Table 2). The most prevalent MLST types were ST2 (n=56; 51 %) with a resistance profile against aminoglycosides, beta-lactams, fluoroquinolones, MLS, phenicol, sulphonamide and tetracycline, and ST1 (n=12; 11 %) having a resistance profile against aminoglycosides, beta-lactams, fluoroquinolones, MLS, phenicol, rifampicin, sulphonamide, tetracycline and trimethoprim. The remaining MLST types were represented by less than 5 % of the strains, and had different resistance profiles (Table 2).

Table 2. Multilocus Sequence Typing (MLST) of the 110 *A. baumannii* complete whole genome sequences according to the Pasteur MLST Scheme.

MLST (Pas)	Frequency	Resistance agents profile
ST1	12	Aminoglycoside, Beta-lactam, Fluoroquinolone, MLS, Phenicol, Rifampicin, Sulphonamide, Tetracycline, Trimethoprim
ST2	56	Aminoglycoside, Beta-lactam, Fluoroquinolone, MLS, Phenicol, Sulphonamide, Tetracycline
ST10	4	Aminoglycoside, Beta-lactam, Sulphonamide, Tetracycline
ST20	1	Aminoglycoside, Beta-lactam, MLS, Phenicol, Sulphonamide, Tetracycline, Trimethoprim
ST23	2	Aminoglycoside, Beta-lactam, Sulphonamide, Tetracycline
ST25	3	Aminoglycoside, Beta-lactam,
ST32	1	Beta-lactam, Sulphonamide
ST52	1	Beta-lactam, Sulphonamide
ST79	5	Aminoglycoside, Beta-lactam, Phenicol, Sulphonamide,
ST81	1	Beta-lactam
ST126	2	Beta-lactam
ST138	1	Beta-lactam

MLST (Pas)	Frequency	Resistance agents profile
ST156	1	Aminoglycoside, Beta-lactam, Phenicol, Sulphonamide,
ST187	1	Aminoglycoside, Beta-lactam, Fluoroquinolone, MLS, Phenicol, Sulphonamide,
ST215	1	Aminoglycoside, Beta-lactam, Fluoroquinolone, MLS, Phenicol, Sulphonamide, Tetracycline
ST229	1	Aminoglycoside, Beta-lactam, Fluoroquinolone, MLS, Sulphonamide, Trimethoprim
ST267	2	Beta-lactam
ST422	2	Aminoglycoside, Beta-lactam, Phenicol, Sulphonamide, Tetracycline
ST437	2	Beta-lactam, Sulphonamide
ST464	1	Beta-lactam
ST622	1	Beta-lactam
ST638	1	Aminoglycoside, Beta-lactam, Sulphonamide
ST639	1	Beta-lactam, Sulphonamide, Tetracycline
ST880	1	Aminoglycoside, Beta-lactam, Fluoroquinolone, MLS, Phenicol, Sulphonamide, Tetracycline
ST922	1	Aminoglycoside, Beta-lactam, MLS, Sulphonamide, Tetracycline
STUnknown	5	Aminoglycoside, Beta-lactam, Fluoroquinolone, MLS, Phenicol, Sulphonamide

4. Discussion

This study is focused on the detection of resistance genes and antimicrobial resistance profiles of 110 WGS of *A. baumannii* strains submitted to the NCBI from different laboratories across the world. Several studies have assessed the power of WGS to detect antimicrobial resistance genes in several bacterial genomes (Ruppé *et al*., 2017; Almaghrabi *et al*., 2018; Kagambèga *et al*., 2018). The results of this study have shown that the 110 *A. baumannii* strains were resistant to more than one antimicrobial agent. Among these antimicrobial agents nine were detected using the ResFinder tool; they included: aminoglycosides, beta-lactams, fluoroquinolone, macrolide-lincosamide-streptogramin B (MLS), phenicol, rifampicin, sulphonamide, tetracycline, and trimethoprim (Zankari *et al*., 2012). However, the wide spectrum of multidrug-resistance of *A. baumannii* can be mediated by all of the major resistance mechanisms known to exist in bacteria (Peleg *et al*., 2008; Mihu and Martinez 2011).

Analysing the core genome of the 110 A. *baumannii* WGS revealed that *bla*ADC-25 gene (GenBank accession number EF016355 with 99.9 % identity) is an intrinsic gene in *A. baumannii*. The presence of *bla*ADC-25 gene in *A. baumannii* genome can be correlated with resistance to cephalosporins (Srinivasan *et al*., 2008). However, only isolates having an insertion sequence (IS), such as IS*Aba1* or IS*Aba9*, upstream from *blaADC-25*, can display a high-level β-lactamase resistance to cephalosporins (Périchon *et al*., 2014; Evans and Amyes 2014).

Within the large diversity of OXA-type carbapenemase genes identified by the ResFinder tool, the genes for *bla*OXA-66 and *bla*OXA-23 types were the most predominant. Studies have shown that *A. baumannii* strains harbouring one or both of these genes are resistant to all β-lactam antibiotics, including carbapenems (Woodford *et al*., 2006; Figueiredo *et al*., 2009; Diene and Rolain 2013). Moreover, multidrug-resistant *A. baumannii* strains, showing resistance to carbapenems, have emerged worldwide, which raises serious concerns about the limited number or antimicrobial treatment options available (Pogue *et al*., 2013; WHO, 2017). Furthermore, the *bla*OXA-66 and *bla*OXA-23 genes have been associated with IS*Aba1* or IS*Aba4* upstream sequences that enhance their expression (Corvec *et al*. 2007).

Other resistance genes attributed to cephalosporin-resistant isolates, including those encoding extended-spectrum β-lactamases (ESBLs) (*bla*TEM, *bla*PER and *bla*VEB) (Diene and Rolain 2013), and carbapenemase-encoding gene (*blaNDM*) (Cornaglia *et al*., 2011), were also detected in this study. *Bla*NDM is the newest metallo-β-lactamase. *Bla*-NDM-1 gene, first reported in an MDR *Klebsiella pneumoniae* clinical isolate from a patient of an Indian origin, confers resistance to all β-lactams, including carbapenems (Yong *et al*., 2009). However, *Bla*NDM-1 was also identified in clinical isolates of *A. baumannii* in several continents including Asia, North America, and Australia (Cornaglia *et al*., 2011).

Various genes encoding aminoglycoside-modifying enzymes and 16S rRNA methylases that confer resistance to aminoglycosides were detected in this study. These genes are known to contribute to resistance to streptomycin, gentamycin, kanamycin and amikacin (Ramirez and Tolmasky 2010). However, among the aminoglycosides, amikacin and tobramycin are genes having the greatest activity against many *A. baumannii* isolates (Fishbain and Peleg 2010).

The presence of *aac* (6')-Ib-cr gene in forty (36 %) of the strains confers resistance to fluoroquinolones. Moreover, genes confering MLS *(msr*(E) and *mph*(E)), phenicol (*cat*B8, *cat*A1 and *cml*A1), sulphonamide (*sul*1 and *sul*2) and tetracycline (*tet*(B), *tet*(G) and *tet*(A)) resistances were detected in ≥33.6 % of isolates. These results are in agreement with other findings (Ramirez and Tolmasky 2010; Diene and Rolain 2013; Nowak *et al*., 2014; Wong *et al*., 2016; Wang *et al*., 2017), which indicate that *A. baumannii* has a wide variety of genes contributing to resistance against a wide variety of antimicrobials.

Notably, only two (1.8 %) and four (3.4 %) of the isolates were found to carry genes that confer resistance to rifampicin and trimethoprim, respectively. Interestingly, rifampicin has been used successfully in combination with colistin for the treatment of *A. baumannii* (Lee *et al*., 2013). Also, trimethoprim-sulfamethoxazole in combination with colistin has shown synergistic activity against *A. baumannii* clinical isolates (Nepka *et al*., 2016).

No genes mediating colistin resistance were identified in the current study. Colistin, alone or in combination with other agents, has been used as a potential therapeutic option for the treatment of MDR Gram-negative bacteria (Fishbain and Peleg 2010) including carbapenem-resistant *A. baumannii* (Gounden *et al*., 2009; Velkov *et al*., 2013). However, colistin resistance in the *A. baumannii* isolates has been recently reported in many parts of the world (Lee *et al*., 2014; Choi *et al*., 2016; Dortet *et al*., 2018).

Among the 110 strains, the ST2 was the most dominant sequence type followed by ST1. Studies have shown that isolates having these two sequence types are associated with *A. baumannii* nosocomial outbreaks and antimicrobial MDR phenotypes (Diancourt *et al*., 2010; Matsui *et al*., 2014) according to a scheme developed by the Pasteur

Institute (Jolley *et al*., 2010). Moreover, ST1 and ST2 are known to be endemic clinical strains that have emerged worldwide (Rafei *et al*., 2014; Kim *et al*., 2016).

5. Conclusions

This study has shown that the RestFinder bioinformatics tool can serve as accurate *in silico* genetic analysis tool to identify acquired AMR genes in the genomes of *A. baumannii*. In this study, fifty-six known resistance genes were identified in the 110 complete WGS of *A. baumannii* available in the NCBI database. Most detected genes were associated with resistance to beta-lactam and aminoglycoside agents. Furthermore, the *bla*OXA-66 and *bla*ADC-25 genes were found in all *A. baumannii* sequences which suggest that these two genes are within the intrinsic genes in *A. baumannii*. Also, no genes contributing to colistin resistance were detected in this study. Finally, the current study demonstrates that ST2 has been the most dominant sequence type in *A. baumannii* according to the Pasteur MLST scheme.

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