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SHORT COMMUNICATION

Genome of a Carbapenem Resistant *Acinetobacter baumannii* Isolate of a new Sequence Type ST1724 from Saudi Arabia

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SUMMARY

Background: **The World Health Organization has classified carbapenem-resistant *Acinetobacter baumannii* as a pathogen of critical priority that poses a serious threat to human health. We report a draft genome sequence of colistin and carbapenem-resistant *A. baumannii* strain AB134 of a new sequence type ST1724.**
Methods: ***A. baumannii*** **strain AB34 was isolated from a tracheal aspirate specimen from a patient diagnosed with atherosclerotic disease and treated at a hospital in Saudi Arabia. Antimicrobial susceptibility was determined via microdilution using a VITEK 2 system. Genome sequencing was performed using a HiSeq 2500 platform (Illumina Inc., USA).**
Results: ***A. baumannii*** **strain AB134 was classified as a new sequence type (ST1724) comprised of 3,763 predicted genes and a guanine-cytosine content of 38.8%. The isolate was phenotypically resistant to 16 clinically important antibiotics, and 33 antimicrobial resistance genes were detected, including the beta-lactamase genes of *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-66}}$, *bla*$_{\text{ADC-25}}$, *and bla*$_{\text{TEM-1D}}$. It carries the colistin resistance gene *lpsB*. In addition, 49 genes associated with virulence factors (biofilm formation, adherence, quorum sensing, iron uptake, and two-component system), and seven insertion sequences were detected in the AB134 genome.**
Conclusions: **This report presents the first draft genome of ST1724 of carbapenem-resistant and extensively drug-resistant *A. baumannii*. The findings facilitate further understanding of the genomics of this pathogen, which is a leading cause of healthcare-associated infections worldwide.**
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KEYWORDS

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INTRODUCTION

In the modern healthcare system, *Acinetobacter baumannii* is among the most widespread pathogens involved in hospital-acquired infections [1]. *A. baumannii* can survive on abiotic surfaces, and it is resistant to multiple drugs [1]. The mortality rate for *A. baumannii*

infections is estimated from 8% to 35%, depending on the type of infection and duration [2]. Increasing evidence from around the world indicates extensive spread of multidrug-resistant *A. baumannii* strains [2-4]. For example, around 70% of *A. baumannii* isolates from the Middle East are considered multidrug resistant [5]. Increasing resistance to antibiotics, such as carbapenem, is considered a major factor in hospital-acquired infections, leading to high mortality rates, especially in intensive care units [5]. In this study, genome sequencing of carbapenem-resistant and extensively drug-resistant *A. baumannii* strain AB134 was performed of a novel sequence type isolated from a patient in the ICU at a hospital in Saudi Arabia.

MATERIALS AND METHODS

Strain AB134 was isolated from a tracheal aspirate specimen from a patient diagnosed with atherosclerotic disease. The strain was obtained from the clinical microbiology laboratory at a healthcare facility in Jeddah, Saudi Arabia. The isolate was identified via the MALDI-TOF technique using VITEK® MS (BioMerieux, France). Antimicrobial susceptibility testing was performed using an automated VITEK 2 system (bioMerieux, France) with a specific AST-N291 card. A genomic library was prepared using a Nextera DNA Flex Library Preparation Kit (Illumina, Inc., USA), and sequencing was performed using 2 x 150-bp chemistry with a HiSeq 2500 platform (Illumina, Inc.). Genomic analysis was performed as described previously [6]. Briefly, de novo genome assembly was completed using SPAdes 3.1 (https://github.com/ablab/spades), and Prokka software for genome annotation. Antimicrobial resistance genes were identified using ResFinder 4.1 (https://cge.food.dtu.dk/services/ResFinder/) and CARD 3.1.4 (https://card.mcmaster.ca/analyze/rgi). The OrthoANI (Orthologous Average Nucleotide Identity) values were calculated and plotted using OrthoANI software (https://help.ezbiocloud.net/orthoani-genomic-similarity/). BPGA (bacterial pan-genome analysis) pipeline (https://iicb.res.in/bpga/) was used for pan genomic analyses, clusters of orthologous group (COG), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Genome sequence data of AB134 strain was deposited to GenBank under accession no. JAMDLP000000000.

RESULTS

The draft genome of *A. baumannii* strain AB134 contained 157 contigs and a guanine-cytosine content of 38.8%, with 3,882 coding sequences, 57 tRNAs, and three rRNAs identified. The OrthoANI value of *A. baumannii* strain AB134 was 99.74% similar to *A. baumannii* strain CUVET MIC596 (Figure 1A), and all other *A. baumannii* strains presented greater than 97% similar OrthoANI values, confirming classification of the AB134 strain as *A. baumannii*. Using multilocus typing analysis, strain AB194 was classified as a new sequence type (ST1724) based on new combinations of known alleles *gltA* 1, *gyrB* 185, *gdhB* 3, *recA* 2, *cpn*60 2, *gpi* 102, and *rpoD* 3.

To better understand the bacterial evolution and phylogenetic relationship, we analyzed core, accessory, and unique genes in the related *A. baumannii* AB134 strains. We identified 2,638 core genes, 806 accessory genes, and 32 unique genes, as well as seven exclusively absent genes in the strain AB134. The highest number of accessory genes (888) was found in strain VB35575, and the lowest (43) in strain ACN21. The *A. baumannii* strain ABF9692 genome contained the highest number of unique genes (390). Compared to the core-phylogenic tree (Figure 1B), the pan-phylogenic tree (Figure 1C) presented complex branches. In the core-phylogenic tree, strains AB134 and CUV-MIC596 were present in the same clade. The pan and core genome of *A. baumannii* (Figure 1D) suggested an "open" pan-genome: the pan-genome increased in size with the addition of each genome, whereas the core genome decreased in size. These results indicate that genomes of *A. baumannii* species are remarkably distinct. In the COG functional distribution (Figure 1E) and KEGG distribution (Figure 1F), the most common functions were associated with metabolism.

Isolate AB134 was determined to be infectious in humans based on the pathogenicity linkage of gene families identified using PathogenFinder 1.1. In total, 49 genes associated with virulence in *A. baumannii* were identified in the analyzed genome, including those for outer membrane protein (*ompA*), biofilm formation (AdeFGH efflux pump), two-component system (*bfmR/S*), serum resistance (*pbpG*), and *acinetobactin* (*bau/bas*) (Table S1). Among the 17 tested antibiotics, the isolate was susceptible only to nitrofurantoin (Table 1). In total, 33 antimicrobial resistance genes (conferring resistance to eight different drug classes) and multidrug resistance genes were found in the genome sequence. Among the β-lactamase genes, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-66}}$, $bla_{\text{ADC-25}}$, and $bla_{\text{TEM-1D}}$ were detected in strain AB134 (Table 1). The *lpsB* gene was detected in AB134 strain genome, which encodes a glycosyltransferase involved in lipopolysaccharide synthesis (LPS) and produced colistin resistance. Moreover, *lpsB* is considered critical for pathogenesis in the lung.

Seven insertion sequences, mainly of ISAba26, ISVsa3, ISEc29, and IS26, were detected in the AB134 genome. The coding region BLAST map showed that *A. baumannii* strain AB134 shared the greatest sequence similarity with *A. baumannii* strains VB33071, VB35575, and CUVET MIC596 (Figure S1). The DNA BLAST map showed sequence similarities among all ten *A. baumannii* strains (Figure S2).

Table 1. Antimicrobial resistance profile of *Acinetobacter baumannii* strain AB134.

Antibiotic class	Resistance genes	AMR phenotype
Aminoglycoside	***ant(3'')-IIc, aph(3'')-Ib, aph(6)-Id, aph(3')-Ia, aph(3')-Via, armA***	**Gentamicin (R), Tobramycin (R)**
Beta-lactam	***bla*$_{\text{OXA-66}}$, *bla*$_{\text{ADC-25}}$, *bla*$_{\text{TEM-1D}}$, *bla*$_{\text{OXA-23}}$**	**Ampicillin (R), Ceftazidime (R), Ceftriaxone (R), Cefepime (R), Aztreonam (R), Imipenem (R), Meropenem (R), Piperacillin / Tazobactam (R)**
Fluoroquinolone	***gyrA, parC, abaQ***	**Ciprofloxacin (R), Levofloxacin (R)**
Fosfomycin	***abaF***	**NT**
Macrolide	***amvA, mphE, abeS***	**NT**
Peptide antibiotic	***lpsB***	**Colistin (R)**
Sulfonamide	***sul2***	**Trimethoprim / Sulfamethoxazole (R)**
Tetracycline	***tetB, tetR, adeC, adeA, adeR***	**Minocycline (R), Tigecycline (I)**
Fluoroquinolone; Tetracycline	***adeH, adeF, adeG, adeL***	**NT**
Multi drug resistant	***adeI, adeJ, adeK, adeN, msrE***	**NT**

AMR - antimicrobial resistance, R - resistant, I - intermediate-resistant, NT - not tested.

DISCUSSION

The primary infection sites for *A. baumannii* are the urinary and respiratory tracts, bloodstream, and surgical sites [1,7]. While infective endocarditis caused by *A. baumannii* is rare, it represents a severe complication due to the pathogen's increasing antimicrobial resistance [8]. In line with prior studies, we identified an *A. baumannii* strain AB134 of a novel sequence type from an endocarditis patient that showed resistance to 16 antibiotics, including carbapenems, cephalosporins, aminoglycosides, fluoroquinolones, tetracyclines, and colistin [9]. Similarly, an earlier study we observed 55.6% of *A. baumannii* isolates with carbapenem resistance, predominantly carrying *bla*$_{\text{OXA-23-like}}$ genes [10]. Other studies from Saudi Arabia also documented *A. baumannii* resistance to imipenem and meropenem [3,11].

Strain AB134 was also resistant to colistin, a last-resort treatment option for multidrug-resistant *A. baumannii* infections, used primarily in severe cases [12]. Studies from Saudi Arabia reported varied colistin resistance rates in *A. baumannii* isolates. For example, Ibrahim et al. noted low resistance, with only 4% of isolates showing non-susceptibility [13]. In contrast, Al-Agamy et al. found a 30% colistin resistance rate [14]. Globally, colistin resistance is notably higher in Southeast Asia and the Eastern Mediterranean, with a prevalence of 11.2% (e.g., Germany 0.2%, UK 2.3%, India 8.2%, China 11.8%, Lebanon 17.5%) [4].

Consistent with previous findings, we identified 33 resistance genes in the strain AB134 genome, including ESBL genes (*bla*$_{\text{ADC-25}}$, *bla*$_{\text{TEM-1D}}$, *bla*$_{\text{OXA-23}}$), aminoglycoside resistance genes (*aph(3'')-Ib*, *aph(6)-Id*, *aph(3')-Ia*), and tetracycline resistance genes (*tetB*, *tetR*, *adeC*) [3,9,11]. Over the past two decades, MDR phenotypes have significantly increased in *A. baumannii*, driven by horizontal gene transfer and recombination due to its highly adaptable genome [1]. Overall, this study highlights the emergence of a new sequence type of extensively drug-resistant *A. baumannii* strain within healthcare settings in Saudi Arabia, underscoring the risk it poses to public health.

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Ethical Approval:
The study was approved by the ethics committee of the Faculty of Medicine at King Abdulaziz University, reference no. 235-15.

Declaration of Interest:
The authors declare no conflict of interest.

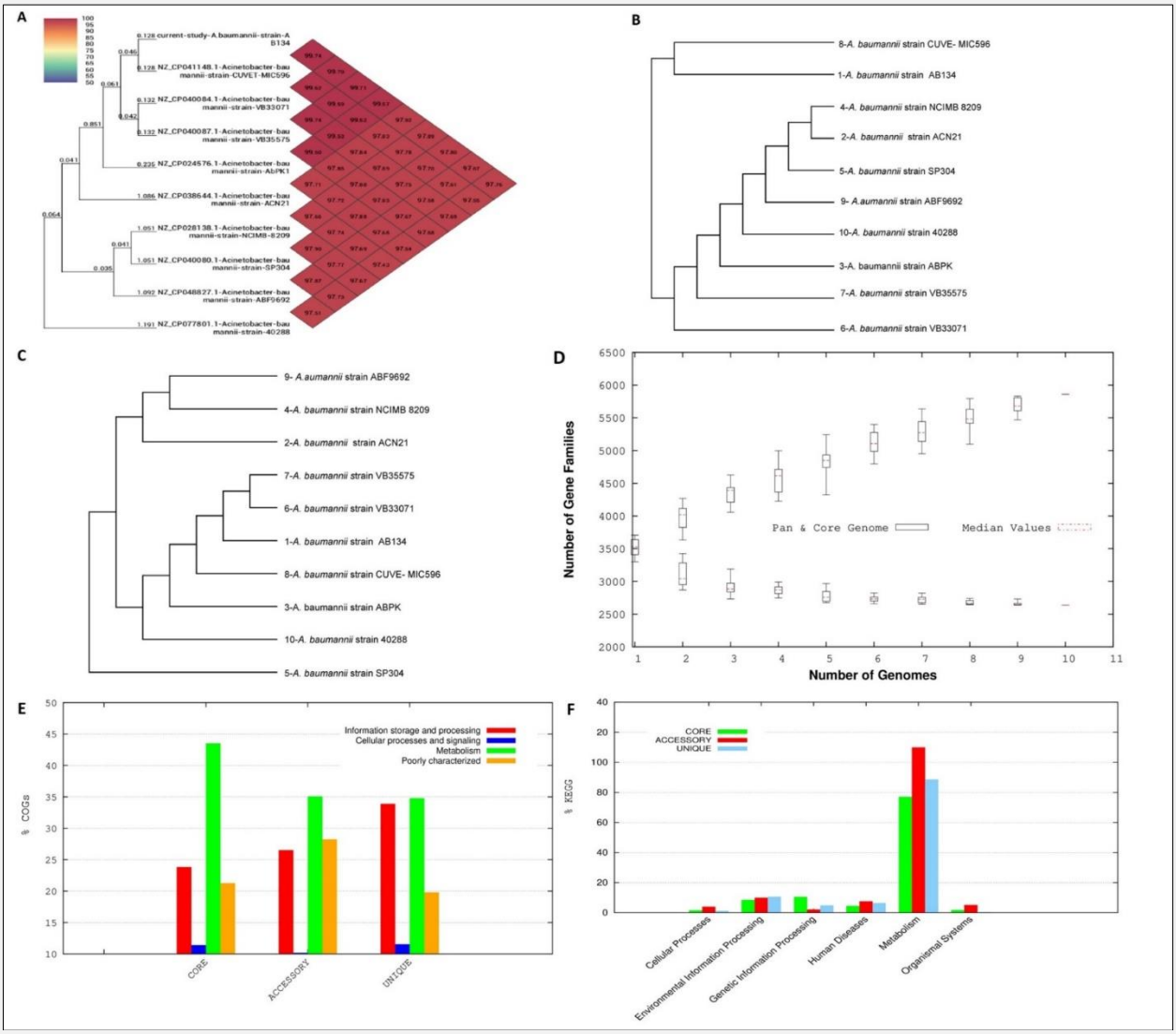

Figure 1. Genomic analysis of *Acinetobacter baumannii* AB134 and closely related strains.

A - OrthoANI values calculation, B - core-phylogenic tree, C - pan-phylogenic tree, D - pan and core genome, E - COG functional distribution, and F - KEGG distribution.

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