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Genome Note

Genome sequence of a carbapenemase-encoding *Acinetobacter baumannii* isolate of the sequence type 231 isolated from hospital wastewater in South Africa

Emmanuel C. Eze[a], Mohamed E. El Zowalaty[b,c,*], Linda Falgenhauer[d], Manormoney Pillay[a]

[a] Department of Medical Microbiology, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa
[b] Department of Medical Biochemistry and Microbiology, Zoonosis Science Center, Uppsala University, Uppsala, Sweden
[c] Department of Microbiology and Immunology, Faculty of Pharmacy, El Salehya El Gadida University, New Salhia City, Ash Sharqia, Egypt
[d] Institute of Hygiene and Environmental Medicine, German Center for Infection Research, Site Giessen- Marburg-Langen and Hessian University Competence Center for Hospital Hygiene, Justus Liebig University Giessen, Germany

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ABSTRACT

Objectives: The resistome, virulome, mobilome and phylogenetic relationship of the *Acinetobacter baumannii* isolate FG121 depicting the multilocus sequence type (ST) 231 isolated from hospital effluent water in South Africa was determined using whole-genome sequence analysis.
Method: *A. baumannii* FG121 was isolated on Leed *Acinetobacter* Medium (LAM) agar and the bacterial isolate was identified using the VITEK®2 platform. Antibiotic susceptibility testing was performed using Kirby–Bauer Disk diffusion method. A whole genome sequencing library was constructed from DNA extracted from the isolate using the Illumina Nextera XT library preparation kit and was sequenced using the Illumina NextSeq500 platform. Generated reads were de novo assembled using SpAdes v.3.9. The assembled contigs were annotated, and multilocus sequence type, antimicrobial resistance, and virulence genes were identified.
Results: The resistome was consistent with the resistance phenotype of the isolate with resistance determinants for beta-lactams, aminoglycosides, and tetracycline (bla_{ADC-25}, bla_{OXA-23}, bla_{OXA-51}, bla_{NDM-1}, *aph[3']-VIa* and *tet[B]*). Global phylogenomic analysis using BacWGSTdb revealed that the isolate belonged to the multilocus sequence type ST-231, similar to previously reported isolates from South Africa, the United States, and related to the invasive KR3831 isolate identified from Oman in 2012, suggesting the isolate might be imported from abroad. Virulome analysis predicted both virulence and biofilm-determinants of *A. baumannii,* which may help to establish infections in adverse conditions.
Conclusion: This is the first report on a carbapenemase-encoding *A. baumannii* ST-231 isolated from hospital effluent water. Our data will offer insight into the global phylogenetic, pathogenicity and distribution of *A. baumannii* in South Africa.

Acinetobacter baumannii is recognized globally as a recalcitrant pathogen that contains resistance determinants to a variety of antibiotics [1,2]. Numerous research groups have reported the isolation of *A. baumannii* from human patients with few reports from the aquatic environment (effluent water). Here, we present the global phylogenetic relatedness of an *A. baumannii* strain isolated from hospital effluent water in KwaZulu-Natal Province in South Africa in 2017 depicting the multilocus sequence type (ST) 231 with an invasive potential.

Leed *Acinetobacter* Agar medium (HiMedia™ Laboratories, India) was used to isolate *A. baumannii* strain FG121 from effluent hospital sample through filtration using a 0.45 µm membrane filter (Millipore, Billerica, MA, USA) as was described previously [3]. Preliminary species identification was performed using an oxidase test, growth at 44°C, and confirmation was performed using the VITEK®2 automated system (bioMérieux, Marcy-l'Étoile, France) [3]. The antimicrobial susceptibility profile of strain FG121 to ampicillin/sulbactam (10/10 μg), piperacillin/tazobactam (100/10 μg), ticarcillin/clavulanic acid

* Corresponding author at: Zoonosis Science Center, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, SE 75 123, Sweden.
E-mail address: elzow005@gmail.com (M.E. El Zowalaty).

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Table 1
Multilocus sequence type and the detected antimicrobial resistance genes and associated predicted resistance phenotype of *A. baumanni* strain FG121 isolated from hospital wastewater in South Africa

Bacterial strain	Multilocus sequence type (Oxford scheme)	Antimicrobial resistance gene	Predicted resistance phenotype
FG121	231	$bla_{\text{ADC-25-like}}$, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-69}}$	β-lactams
		aac(3)-I-like, aadA1,aph(3′)-Ic,armA,strA-like, strB-like	Aminoglycosides
		tet(B)	Tetracyclines
		mph(E)	Macrolide
		msr(E)	Macrolide, Lincosamide and Streptogramin B
		cmlA1-like	Phenicol
		ARR-2	Rifampicin
		sul1,sul2	Sulphonamide
		dfrA1	Trimethoprim

(75/10 μg), ceftazidime (30 μg), cefepime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), imipenem (10 μg), doripenem (10 μg), meropenem (10 μg), gentamicin (10 μg), tobramycin (10 μg), amikacin (30 μg), doxycycline (30 μg), minocycline (30 μg), tetracycline (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg) and piperacillin (100 μg) was determined using the Kirby–Bauer disk diffusion method (Oxoid Ltd., UK) as was previously reported [3]. The results were interpreted according to Clinical and Laboratory Standard Institute guidelines [4]. The minimum inhibitory concentrations (MICs) of imipenem, ciprofloxacin, cefotaxime, ceftazidime, and tetracycline were determined using the agar dilution method as was previously reported, and *A. baumannii* ATCC 19606 was used as a reference strain [3]. DNA was extracted from overnight culture using the cetyltrimethylammonium bromide protocol as was previously reported [5]. A paired-end sequencing library was prepared using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA, USA) and sequenced using an Illumina NextSeq 500 machine (Illumina, San Diego, USA) with 2 × 150 nt read length. Sample preparation and sequencing were performed by Admera Health, LLC (South Plainfield, NY, USA). Quality control of derived raw sequence reads was performed using the FastQC v.0.11.9 [6] and was trimmed using Sickle v.1.33 (https://github.com/najoshi/sickle). A sequencing depth of 50 × and an average read length of 147 nt was achieved. De novo assembly was performed using the SPAdes v.3.9 pipeline, available at the Centre for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/SPAdes/) [7]. All contig sequences were then submitted to GenBank, where gene annotation was implemented using the NCBI Prokaryotic Genome Annotation Pipeline v.5.1 (PGAP, https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) [8]. The genome of FG121 features an assembly length of 4 052 782 bp, an N_{50} of 126 625 bp, a total of 3907 coding sequences and 77 RNA genes. The average GC content accounts for 40.7%.

The analysis of the multilocus sequence type (Oxford scheme) [9] using the MLST v.2.0 tool available from the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services) indicated that the strain belongs to the multilocus sequence type (ST) 231 (Table 1). The virulence factor analysis of strain using the VFanalyzer tool implemented at the virulence factor database (VFDB) (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi?func=VFa-nalyzer) [10] predicted a total of 63 different virulence-encoding factors (Table 2) belonging to biofilm-forming determinants (*ade*FGH efflux pumps, Csu pili, polysaccharide poly-N-acetylglucosamine), phospholipase C (two slightly different copies) and D, evasive capsule, lipopolysaccharides, acinetobactin, heme utilisation factors, quorum sensing genes, two-component systems, serum resistance gene *pbpG*. Differential expression of the predicted virulence factors provides a competitive advantage among *A. baumannii* strains, promoting success in establishing infection and resistance to environmental factors [2,11].

Resistome analysis was performed using Resfinder v.3.1 [12], and results showed that FG121 harboured antibiotic resistance determinants encoding resistance to β-lactams ($bla_{\text{OXA-23}}$, $bla_{\text{ADC-25}}$, $bla_{\text{OXA-69}}$, $bla_{\text{NDM-1}}$), aminoglycosides (*aph[3']-VIa*) and tetracyclines (*tet[B]*) as shown in Table 1. The resistome was consistent with the resistance phenotype of the isolate, where the isolate was found resistant to meropenem, ticarcillin-clavulanic acid, piperacillin, ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, cefepime, cefotaxime, ceftriaxone, doripenem, imipenem, gentamicin, tobramycin, amikacin, doxycycline, minocycline, and tetracycline. MICs of imipenem, cefotaxime, ciprofloxacin, ceftazidime, and tetracycline were 128, 128, 2, 128 and 32 µg/ml, respectively [3]. The presence of $bla_{\text{OXA-23}}$ and $bla_{\text{NDM-1}}$ is of particular concern, as these β-lactamase genes confer resistance to carbapenems, last-line drugs used to treat highly resistant bacteria.

Because strain FG121 was reported earlier to contain a plasmid [3], plasmid replicon typing of strain FG121 was performed in silico using the *A. baumannii* typing scheme by Bertini et al [13]. For this, a tblastN search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the replicase protein sequences depicted in the above-mentioned article in the assembled contigs of strain FG121 was performed. According to this analysis, FG121 depicts the rep group GR2.

Analysis of the insertion sequences using MobileElementFinder [14] showed that the assembled genome harboured several insertion sequences (IS*1006* in contig NODE_84; IS*Ec29* and IS*Ec28* in contig NODE_49) and a unit transposon, Tn*6018* (in contig NODE_52) depicting 99.58% homology to the Tn*6018* found in the *A. baumannii* GC1 clone (GenBank accession number FJ172370).

Analysis of the genetic relatedness of FG121 to *A. baumannii* global isolates was performed using the 'single genome analysis' module of the global phylogenetic analysis tool BacWGSTdb (http://bacdb.cn/BacWGSTdb/analysis_single.php) [15]. For detection of close relatives of FG121, a cgMLST threshold of ≤200 cgMLST allele differences was selected. Fifty-five global *A. baumannii* isolates were detected within this threshold (Supplementary Table S1). The phylogenetic tree (Figure 1) was annotated using iTOL v.5 [16] to include information on country, source, and year of isolation (Supplementary Table S1). The most closely related isolates were four $bla_{\text{NDM-1}}$-positive *A. baumannii* human isolates from South Africa (Aci00879, Aci00880, Aci00881, Aci00882; 9-14 cgMLST allele differences to FG121; Figure 1, Supplementary Table S1) and one isolate from the United States (AR_0083, difference of 17 cgMLST alleles). Moreover, results indicated that *A. baumannii* FG121 was closely related to the invasive *A. baumannii* isolate KR3831 from Muscat, Oman [17], which was isolated in 2012 (cgMLST allele difference to FG121 n = 31; Figure 1, Supplementary Table S1) with similar virulome and was reported to be involved in invasive diseases among hospital patients. Although there is no evidence to show that *A. baumannii* strain FG121 was imported from Oman to South Africa, the close relationship could demonstrate the diversity, evolution and dissemination of *A. baumannii* worldwide.

Table 2
Detected virulence genes and their function, class, contig location and BLAST results of *A. baumannii* strain FG121

Detected genes	Virulence factor	Virulence factor class	Contig location	Position in contig		Coverage	Identity
				Start	Stop		
ompA	Outer membrane protein	Adherence	NZ_JAGSHW010000008.1	95396	96457	100.00	94.60
adeF	AdeFGH efflux	Biofilm	NZ_JAGSHW010000016.1	91418	92638	100.00	97.95
adeG	pump/transport	formation	NZ_JAGSHW010000016.1	92645	95824	100.00	97.61
adeH	autoinducer		NZ_JAGSHW010000016.1	95837	97285	100.00	98.97
csuA/B	Csu pili		NZ_JAGSHW010000016.1	6406	5870	100.00	98.51
csuA			NZ_JAGSHW010000016.1	5795	5247	100.00	97.81
csuB			NZ_JAGSHW010000016.1	5241	4723	100.00	98.84
csuC			NZ_JAGSHW010000016.1	4729	3896	100.00	98.32
csuD			NZ_JAGSHW010000016.1	3899	1401	100.00	98.12
csuE			NZ_JAGSHW010000016.1	1404	385	100.00	95.88
pgaA	PNAG (Polysaccharide		NZ_JAGSHW010000020.1	63228	65666	100.00	98.48
pgaB	poly-N-		NZ_JAGSHW010000020.1	65666	67495	100.00	98.03
pgaC	acetylglucosamine)		NZ_JAGSHW010000020.1	67495	68742	100.00	98.48
pgaD			NZ_JAGSHW010000020.1	68739	69203	100.00	97.42
bap	Biofilm-associated protein		NZ_JAGSHW010000014.1	106107	105187	15.18	86.88
plc (first copy)	Phospholipase C	Enzymes	NZ_JAGSHW010000013.1	91566	89399	99.95	96.22
plc (second copy)			NZ_JAGSHW010000028.1	16153	13925	100.00	98.61
plcD	Phospholipase D		NZ_JAGSHW010000040.1	3562	5187	100.00	99.02
ACICU_RS00395	Capsule	Immune	NZ_JAGSHW010000013.1	81691	83874	100.00	85.11
ACICU_RS00405		evasion	NZ_JAGSHW010000013.1	80139	81239	100.00	82.31
tviB			NZ_JAGSHW010000013.1	79772	78762	79.53	78.40
galU			NZ_JAGSHW010000013.1	67210	66336	99.89	90.29
pgi			NZ_JAGSHW010000013.1	64959	63296	99.76	89.44
ACICU_RS00500			NZ_JAGSHW010000013.1	61646	63010	99.56	95.39
lpxM	LPS		NZ_JAGSHW010000004.1	195692	196675	100.00	98.88
lpxL			NZ_JAGSHW010000010.1	28552	27617	100.00	96.37
lpsB			NZ_JAGSHW010000010.1	27351	26251	100.00	96.91
lpxA			NZ_JAGSHW010000012.1	51059	51847	100.00	99.62
lpxB			NZ_JAGSHW010000001.1	85354	84201	98.13	98.96
lpxC			NZ_JAGSHW010000003.1	127512	126610	100.00	98.56
lpxD			NZ_JAGSHW010000012.1	49500	50570	100.00	99.72
bauF	Acinetobactin	Iron uptake	NZ_JAGSHW010000031.1	9469	8609	100.00	97.21
basA			NZ_JAGSHW010000031.1	9867	11714	100.00	95.29
basB			NZ_JAGSHW010000031.1	13814	11785	99.95	95.76
bauD			NZ_JAGSHW010000031.1	14446	15373	100.00	96.44
bauC			NZ_JAGSHW010000031.1	15387	16334	100.00	96.41
bauE			NZ_JAGSHW010000031.1	16331	17101	100.00	98.70
bauB			NZ_JAGSHW010000031.1	17108	18076	100.00	98.14
bauA			NZ_JAGSHW010000031.1	18162	19483	58.30	77.39
basC			NZ_JAGSHW010000031.1	21839	20529	100.00	97.71
basD			NZ_JAGSHW010000031.1	24829	21887	100.00	96.91
entE			NZ_JAGSHW010000031.1	24991	26619	100.00	97.12
basF			NZ_JAGSHW010000031.1	26637	27506	100.00	98.05
basG			NZ_JAGSHW010000031.1	27624	28775	100.00	98.09
barA			NZ_JAGSHW010000031.1	29021	30631	100.00	96.65
barB			NZ_JAGSHW010000031.1	30628	32223	100.00	97.62
basH			NZ_JAGSHW010000031.1	32295	33029	100.00	97.55
basI			NZ_JAGSHW010000031.1	33040	33795	100.00	96.03
basJ			NZ_JAGSHW010000031.1	33920	35089	100.00	98.21
ACICU_RS04565	Heme utilisation		NZ_JAGSHW010000034.1	12572	13462	100.00	95.17
ACICU_RS04570			NZ_JAGSHW010000034.1	13599	14120	100.00	97.51
ACICU_RS04575			NZ_JAGSHW010000034.1	14121	15137	100.00	95.67
ACICU_RS04580			NZ_JAGSHW010000034.1	15346	18450	100.00	95.30
ACICU_RS04585			NZ_JAGSHW010000034.1	18643	19434	100.00	96.47
ACICU_RS04590			NZ_JAGSHW010000034.1	19545	21010	99.93	95.84
ACICU_RS04595			NZ_JAGSHW010000034.1	21021	21941	100.00	94.26
hemO			NZ_JAGSHW010000034.1	21974	22573	100.00	98.17
ACICU_RS04605			NZ_JAGSHW010000034.1	22602	23042	100.00	100.00
ACICU_RS04610			NZ_JAGSHW010000034.1	23320	23045	100.00	99.64
abaI	Quorum sensing	Regulation	NZ_JAGSHW010000013.1	18732	19298	100.00	98.06
abaR			NZ_JAGSHW010000013.1	17493	16777	100.00	98.47
bfmR (ACICU_RS03720)	Two-component		NZ_JAGSHW010000005.1	87611	86895	100.00	99.44
bfmS (ACICU_RS03725)	system		NZ_JAGSHW010000005.1	86862	85213	100.00	98.49
pbpG	PbpG	Serum resistance	NZ_JAGSHW010000026.1	19929	20975	100.00	98.47

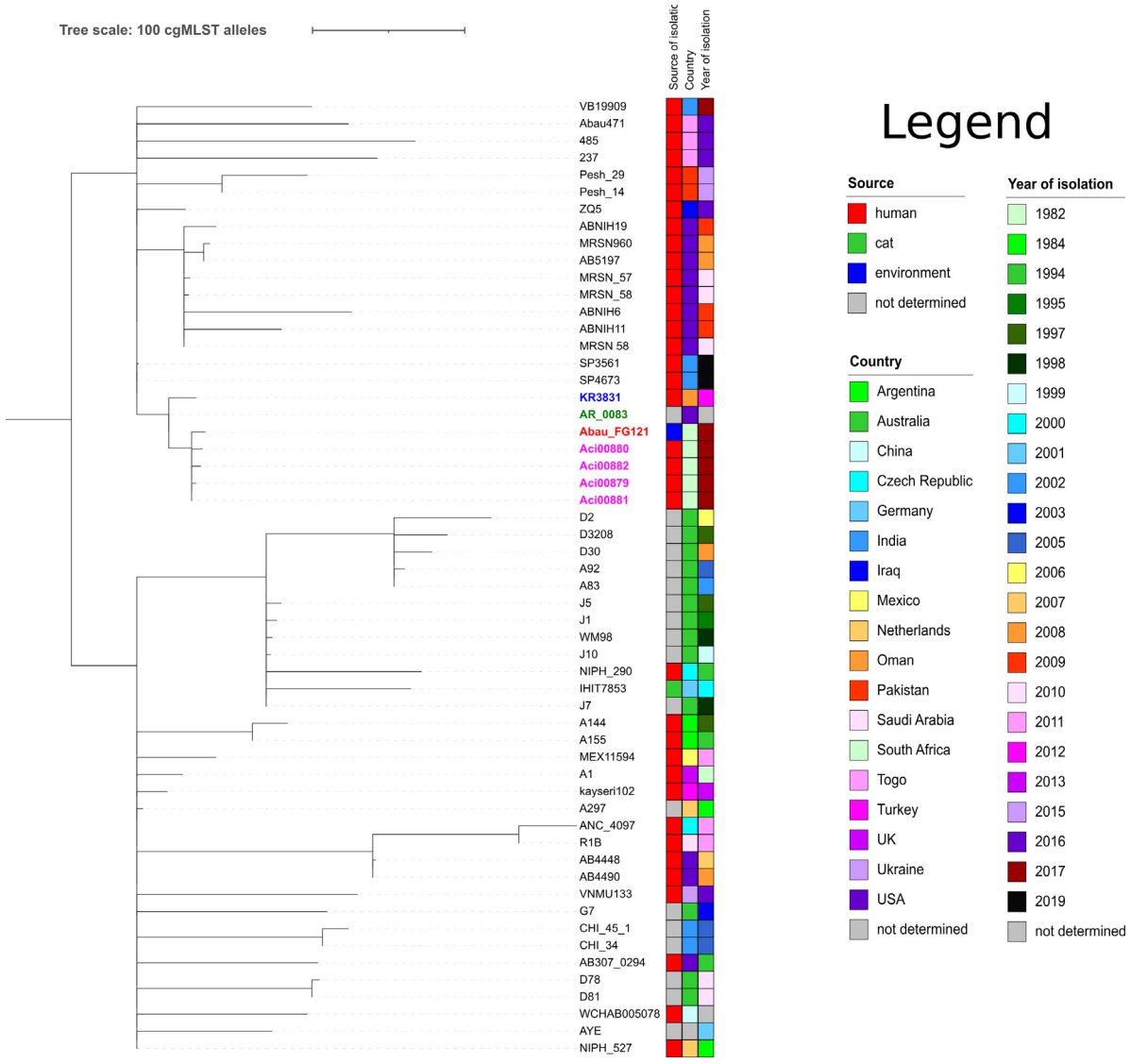

Fig. 1. Global phylogenomic tree showing the ancestral relationship between *A. baumannii* FG121 (coloured red) and other *A. baumannii* isolates of international origin. The four pink-coloured isolates are *A. baumannii*, previously isolated in South African hospitals, the green-coloured isolates in the United States (AR_0083), while the KR3831 (coloured blue) isolate originates from Muscat, Oman. The tree was annotated using iTOL v.5 [14] to include information on country, source and year of isolation (for more information, see Supplementary Table S1).

The present report of *A. baumannii* FG121 isolated from hospital wastewater effluent in South Africa is of high significance and provides insight into the pathogenome and global evolutionary relatedness of *A. baumannii* from Africa to other international isolates. Our finding is of particular clinical and public health relevance, as it reports on the first detection of a carbapenemase-producing *A. baumannii* ST-231 isolate in environmental sources. Its detection in hospital effluent water is alarming, as it gives rise to the assumption that pollution of the environment with highly resistant carbapenemase-producer pathogens might be mediated through this path.

CRediT authorship contribution statement

Emmanuel C. Eze: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Visualization, Validation, Writing – original draft, Writing – review & editing. **Mohamed E. El Zowalaty:** Conceptualization, Formal analysis, Data curation, Investigation, Visualization, Validation, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration, Supervision. **Linda Falgenhauer:** Formal analysis, Methodology, Visualization, Data curation, Software, Validation, Writing – original draft, Writing – review & editing, Funding acquisition. **Manormoney Pillay:** Supervision, Project administration, Writing – review & editing.

Conflict of interest

None declared.

Data availability

This whole genome shotgun project was deposited at NCBI (https://www.ncbi.nlm.nih.gov/) under the BioProject number PRJNA722597, BioSample accession number SAMN18781032, GenBank accession number JAGSHW000000000. The version described in this article is the first version JAGSHW000000000.1. The raw read sequences were submitted to the Sequence Read Archive (SRA) under the accession number SRR14455652.

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Appendix A. Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.02.019.

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