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Data Article

Analysis of the genome data of *Acinetobacter baumannii* UOL-KIMZ-24, exhibiting multiple drug resistance through efflux pumps

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ABSTRACT

Acinetobacter baumannii is a well-known opportunistic pathogen, responsible for various nosocomial infections. *A. baumannii* UOL-KIMZ-24 was previously isolated from a clinical specimen, collected from Lahore General Hospital, Lahore (LGH), Pakistan, dated 3rd March, 2022. During the initial screening for antimicrobial susceptibility, the UOL-KIMZ-24 was found a multiple drug resistant (MDR) strain. However, the detailed genomic insights for genes e.g. responsible for exhibiting antibiotic resistance via efflux pumps, have not yet been reported from *A. baumannii* strains, recovered from LGH. The current research to fills this gap by isolating, whole genome sequencing and subsequent post-sequencing analysis for addressing and identifying the efflux pumps associated genes, responsible for multiple drug resistant in *A. baumannii*. In a hybrid approach, short reads were processed through Illumina platform, while long reads

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were sequenced by MinION MK1B sequencing technique. The assembled and annotated genome of the UOL-KIMZ-24 revealed that it has 4048631 bp genome size with 179 contigs, 38.9 % GC content, 3628 protein coding sequences, 80 tRNA and 7 rRNA. The analysis of antibiotic-resistance genes (AMR) depicted 27 genes. where the genes encoding efflux pumps such as *adeABC, adeRS, adeJK*, and *adeMN* were the more prominent. In addition, sequence typing (ST) study showed that UOL-KIMZ-24 strain lies in ST2, six prophage sequences and 73 virulence factors were also identified in the studied UOL-KIMZ-24. Such an all-inclusive study uncovered the genetic flexibility of UOL-KIMZ-24 genome for acquiring MDR against in-practice antibiotics.

Specifications Table

Subject	Biological Sciences
Specific subject area	Bacteriology, Clinical Genetics, Microbiology, Molecular Biology
Type of data	Whole genome sequencing data demonstrated in Figures and Tables
Data collection	The genomic DNA from UOL-KIMZ-24 strain was extracted by DNeasy blood and tissue kit. Good quality gDNA was sent to MicrobesNG United Kingdom for whole genome sequencing and subjected to Illumina Miseq platform for sequencing. The raw sequenced reads were passed through initial quality check by using FastQC tool. The resulting trimmed sequences were assembled into contigs by applying SPAdes. The functional annotation was performed through PATRIC database. The efflux pumps associated genes e.g. *adeABC* were studied by CARD. The prediction of antimicrobial resistant genes was done through ResFinder, mobile genetic elements by VPprofile, multi locus sequence tying through PubMLST.
Data source location	Pus wound swabs were collected from intensive care unit, General Hospital Lahore, Pakistan. https://lgh.punjab.gov.pk/
Data accessibility	The UOL-KIMZ-24 strain, a multi-drug resistant bacteria, a clinical isolate collected from General Hospital Lahore (Original data) NCBI. The complete sequence report of *A. baumanniis* UOL-KIMZ-24 can be found at NCBI/GenBank under the accession number JBGVUC000000000 (first version), BioProject number PRJNA1155329 and BioSample number SAMN43457898. Direct URL to data: https://www.ncbi.nlm.nih.gov/nuccore/JBGVUC000000000 https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1155329 https://www.ncbi.nlm.nih.gov/biosample/SAMN43457898
Related research article	None

1. Value of the Data

- This study reports the genomic features present in *A. baumannii*, UOL-KIMZ, 24 strain, isolated from pus swab, General Hospital Lahore by following next generation sequencing approach.
- The data provides information about important mechanisms of multi-drug resistance acquired by *A. baumannii.*
- The functional annotation of studied *A. baumanni* strain harbor a wide plethora of genes either involved in virulence, AM resistance, sequence typing or other primary and secondary metabolic pathways.
- The genomic data helped us to study phylogeny based on whole genome sequence in addition to 16S rRNA gene.

- The findings of this study will help Bioinformaticians and Medical Microbiologists to understand MDR traits in *A. baumannii* and development of more effective antibiotics.

2. Background

A. baumannii is a Gram negative aerobic coccobacillus, known as opportunistic pathogen concomitant with nosocomial infections, majorly restricted to the intensive care units [1,2]. The infection rates reach up to 1 million annually on global level, causing high mortality in severely ill patients [3–5]. The recent studies reported 45 % of all isolated *A. baumannii* as multidrug resistant (MDR), which peaks up to 70 % in Asia and Europe [6,7]. The clinical pertinent species are majorly limited to *A. calcoaceticus/A. baumannii* (ACB) complex but their correct identification and differentiation are very difficult as they share very close phenotypic and biochemical features [8]. Therefore, swift and precise identification of *A. baumannii* is always required for distinguishing it from other close related species. The available methods providing accurate identification include amplified fragment length polymorphism analysis [9], matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [10], multilocus variable-number tandem repeat analysis [11], multilocus sequence typing [12], pulsed-field electrophoresis [13], repetitive extragenic palindromic PCR [14] and whole-genome sequencing [15].

During last few decades, *A. baumannii*, one of the most prevalent infectious Gram negative bacteria, has acquired resistance against a broad spectrum of antibiotics at previously unforeseen rate [16]. For example, carbapenem resistance in *A. baumannii* was reported in range between 70 and 85 % in the Asia-Pacific region [17]. Therefore, *A. baumannii* is ranked as the most perilous antibiotic-resistant pathogen by the World Health Organization (WHO) in 2017, which requires new and actual antibiotic-based therapies [18]. So far, various factors have been identified which are responsible for antibiotic resistance in *A. baumannii*. The up-regulation of membrane efflux pumps is a key mechanism to develop multi-drug resistance in *A. baumannii*, additional to other factors such as biofilm formation, alterations in membrane permeability, target compounds and enzymes [19]. The *adeABC* efflux pumps, regulated by *adeRS*, senor-response regulator (two component system) play crucial role in developing MDR in *A. baumannii* [20], which usually showed highest expression rate in clinically isolates [21]. A *A. bumannii* strain UOL-KIMZ-24 was recovered from pus swab, collected from Lahore General Hospital (LGH), dated 3rd March, 2022. During the initial screening for antimicrobial susceptibility, *A. baumanni* showed highest resistance against piperacillin-tazobactum and ciprofloxacin antibiotics. Previous studies have shown that pathogenic bacteria which were susceptible to ciprofloxacin, gave high expression of *adeB* gene and vice versa [22,23]. However, the comprehensive genetic background underlying the MDR efflux system in clinical isolates of *A. baumannii* has not yet been fully elucidated. Hence, the current study provides genomic insights for correlating antibiotic resistance genes with *in-vitro* outcomes, taxonomic classification, virulence factors, mobile genetic elements and identification of phage sequences. This study will help to propose the strategy for formulating new antibiotics to minimize the health hazards posed by opportunistic *A. baumannii*.

3. Data Description

The current research elucidates the genomic basis of *A. baumannai* UOL-KIMZ-24 strain, proved as a MDR strain during the primary study of antimicrobial susceptibility testing. The genome features of the UOL-KIMZ-24 included 4048631 bp genome size with 179 contigs, 38.9 % GC content, 3628 protein coding sequences, 80 tRNA and 7 rRNA (Table 1). The functional annotation study revealed that overall metabolic activities in *A. baumannai* UOL-KIMZ-24 are classified into 419 subsystems and almost 1301 proteins are directly or indirectly associated with various subsystems as predicted through RAST and PATRIC databases.

During the taxonomic evaluation by TYGS, the UOL-KIMZ-24 showed maximum homology with *Acinetobacter baumannii* type strain and further confirmed by ANI outcomes which gave 97

Table 1
Genomic features predicted by PATRIC database.

Features	Acinetobacter baumannii UOL-KIMZ-24
Length	4048631 bp
Contig	179
GC Content	38.93284
Contig L50	10
Contig N50	139814
rRNA	7
tRNA	77
CDS	4068

% similarity index with reference *A. baumannii* genome retrieved from NCBI (Fig. 1). The genome was also mined to explore antimicrobial resistance genes through CARD database, which confirmed presence of the genes e.g. *adeA, adeB, adeG, adeH, adeI, adeK, adeM, adeN* encoding the efflux pumps and their regulator protein (*adeRS*) (Table 2). The ResFinder also demonstrated UOL-KIMZ-24 as MDR strain resistant to gentamicin, tobramycin, streptomycin, amikacin, isepamicin, netilmicin, imipenem, meropenem, erythromycin, azithromycin, tetracycline, doxycycline, minocycline, quinupristin and virginiamycin, respectively. Moreover, four mobile genetic elements (*ISEc35, ISEc29, ISAba24* and *IS15DI*) associated with AMR genes, were identified in UOL-KIMZ-24 strain belonging to IScluster/Tn, on chromosome between 20383 and 29290bp (Fig. 2). The multi locus sequence typing (MLST) was studied by adapting scheme via PubMLST, which inferred that UOL-KIMZ-24 strain harbors Sequence typing (ST2) (Table 3). There were 79 genes responsible for encoding the virulence factors, detected through Victors.

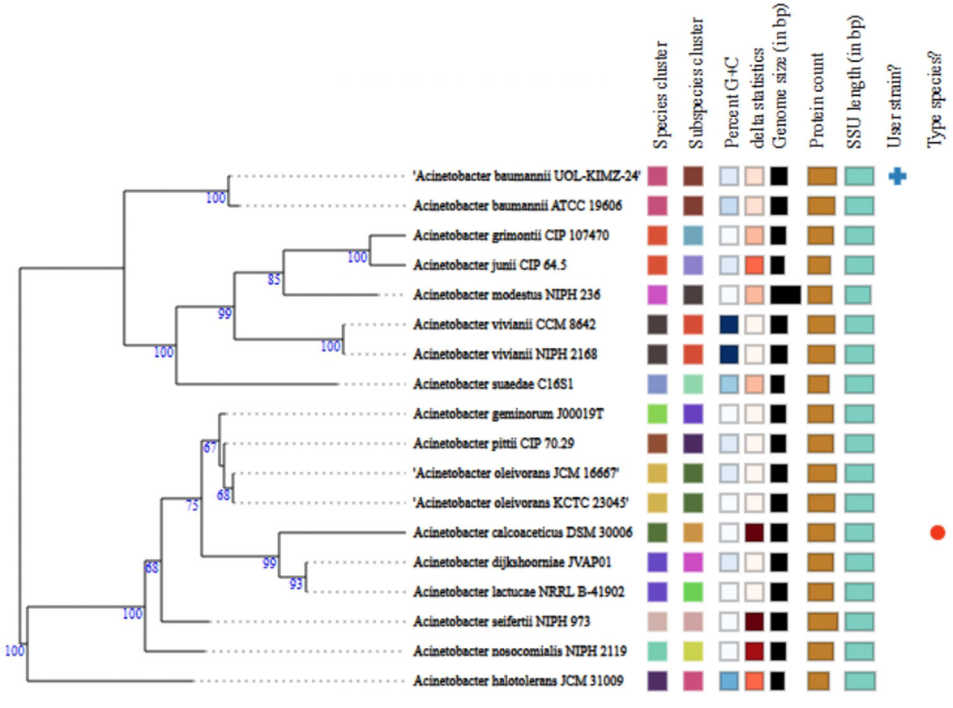

Fig. 1. Phylogram of UOL-KIMZ-24, generated through Type Strain Genome Server, based on 16S rRNA gene sequence similarity with closely related type strains.

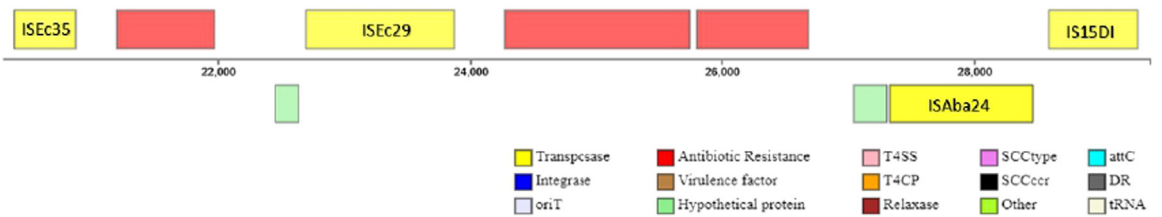

Fig. 2. Mobile genetic elements associated with antimicrobial resistance genes, predicted by VRprofile.

Table 2
Antimicrobial resistance genes associated with efflux pumps system predicted by CARD database.

Gene	Product	Classification
AdeN	Transcriptional regulator, AcrR family	Efflux pump conferring antibiotic resistance, gene modulating antibiotic efflux
adeG	RND efflux system, inner membrane transporter	Efflux pump conferring antibiotic resistance
adeJ	RND efflux system, inner membrane transporter	Efflux pump conferring antibiotic resistance
abeM	Multidrug efflux transporter MdtK/NorM (MATE family)	Efflux pump conferring antibiotic resistance
adeH	Efflux transport system, outer membrane factor (OMF) lipoprotein	Efflux pump conferring antibiotic resistance
adeS	Osmosensitive K+ channel histidine kinase KdpD	Efflux pump conferring antibiotic resistance, gene modulating antibiotic efflux
adeI	RND efflux system, membrane fusion protein	Efflux pump conferring antibiotic resistance
adeR	Two-component transcriptional response regulator, LuxR family	Efflux pump conferring antibiotic resistance, gene modulating antibiotic efflux
adeA	RND efflux system, membrane fusion protein	Efflux pump conferring antibiotic resistance
adeF	RND efflux system, membrane fusion protein	Efflux pump conferring antibiotic resistance
adeB	RND efflux system, inner membrane transporter	Efflux pump conferring antibiotic resistance
adeK	Efflux transport system, outer membrane factor (OMF) lipoprotein	Efflux pump conferring antibiotic resistance

Table 3
Sequence typing study of UOL-KIMZ-24 strain through PubMLST database.

Locus	Allele	Length	Start position	End position
Pas cpn60	2	405	27366	27770
Pas fusA	2	633	218661	219293
Pas gltA	2	483	240078	240560
Pas pyrG	2	297	92241	92537
Pas recA	2	372	18347	18718
Pas rplB	2	330	59225	59554
Pas rpoB	2	456	203873	204328

The genome was also subjected to PHASTEST database to find prophage sequences. A total of 6 prophage sequences (Fig. 3a & b) were highlighted in this analysis as follows; region 1 showed insertion of *PHAGE_Acinet_Bphi_B1251_NC_019541*, which spans from 2695740 to 2816585bp with 40.51 % GC content, the second phage sequence was found in between 2695740 and 2816585bp with 39.2 % GC content and showed sequence relatedness with *PHAGE_Acinet_ymc11/11R3177_NC_041866*, the third region was located between 3035813 and 3139317bp and predicted to be *PHAGE_Acinet_vB_AbaM_B09_Aci02_2_nc_048075* In addition, another prophage region (region 4) identified in UOL-KIMZ-24 strain was *PHAGE_Acinet_vB_AbaS_TRS1_NC_031098* with 27703bp in size and 39.17 % GC content, the 5th prophage sequence was found located from 3885211 to 3899367bp with the total length of 13485bp, identified as *PHAGE_Acinet_YMC11/11/R3177_NC_041866* and the last prophage sequence was present in between 3946179 and 3955671, predicted as *PHAGE_Acinet_Bphi_B1251_NC_019541,* respectively.

The annotation of UOL-KIMZ-24 genome helped us to look deep into genetic background related to *adeABC* efflux pumps, responsible for regulating the frequently evolving resistance against a broad spectrum of antibiotics in *A. baumannii* and urge to discover and exploit antibiotics to tackle the health hazards caused by this opportunistic pathogen.

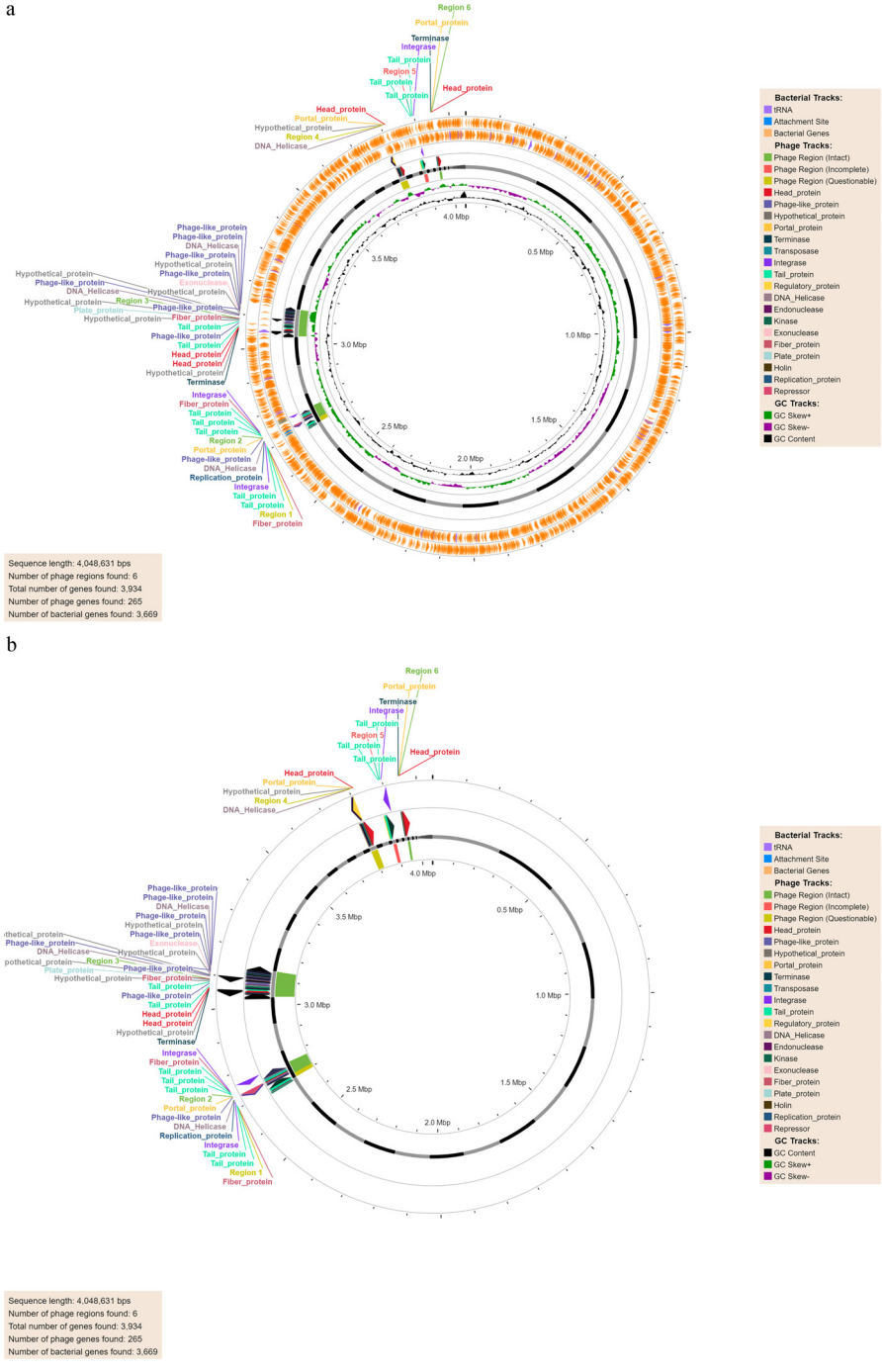

Fig. 3. a & b Prophage sequences in UOL-KIMZ-24 genome predicted by PHASTEST.

4. Experimental Design, Material and Methods

4.1. Sample Collection and Bacterial Isolation

The wound pus swabs were collected from intensive care unit within the premises of Lahore General Hospital (LGH), Lahore, Pakistan dated 3rd March, 2022 and the collected samples were used for isolation of frequent clinical isolates of *A. baumannii*. The serially diluted samples were spread on MacConkey agar, Blood agar and Chocolate agar and incubated overnight at 37 °C. The spread and pour plate methods were used for appropriate serial dilution in triplicate form. The pure cultures were stored in Tryptic Soya Broth (TSB) at -80 °C up till further processing.

4.2. Antibiotic Susceptibility Testing

The susceptibility testing of UOL-KIMZ-24 strain against various antibiotics was evaluated by Kirby Bauer disc diffusion method. The tested antibiotics were included amikacin (30 µg), ampicillin-sulbactam (10/10 µg), aztreonam (30 µg), cefepime (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (30 µg) and piperacillin-tazobactam (110 µg). The Whatman™ Antibiotic Assay Discs (6 mm diameter) were purchased from Merck Life Science UK Limited, United Kingdom. Briefly, culture suspension of UOL-KIMZ-24 was prepared and compared with 0.5 McFarland solution as turbidity standard. Mueller-Hinton agar (MHA) plates were inoculated with UOL-KIMZ-24 strain and antibiotic discs were placed inside the MHA plates. Then, plates were incubated at 37 °C for 24 h and zone of inhibition were observed and measured as per Clinical and Laboratory Standards Institute (CLSI) 2010 guidelines.

4.3. Extraction of Genomic DNA and Genome Sequencing

For the extraction of genomic DNA from UOL-KIMZ-24 strain, the steps mentioned in user manual of DNeasy blood and tissue kit were followed. After assessing the purity through Qubit 4.0 Fluorometer, the 50 µg, good quality gDNA was sent to MicrobesNG United Kingdom for whole genome sequencing.

The library was prepared by adapting hybrid approach, where short reads with 2 × 250bp paired end read length were developed by following steps devised by the manufacturer for Nextera XT library preparation kit and subjected to Illumina Miseq platform for sequencing. However, the long reads were generated by shearing, filtering and subsequent ligating through Ligation Sequencing Kit protocol (SQK-LSK109), afterward loaded in MinION FLO-MIN106 flow cell and submitted to MinION MK1B sequencing device (Oxford Nanopore Technologies). The resulting signal output was converted to fasta format by using Guppy v3.2.10; MinKnow v3.6.17 by selecting the base calling parameter.

The raw sequenced reads were passed through initial quality check assessment analysis by using FastQC tool [24] followed by trimming out the contaminated bases through Trimmomatic v0.36 tool [25]. The resulting trimmed sequences were assembled into contigs by applying SPAdes [26] tool available at Galaxy database. These assembled reads were deposited at GenBank under the accession number JBGVUC000000000 (first version).

4.4. Genomic Insights of A. baumanniis *UOL-KIMZ-24*

The study of functional annotation was performed through PATRIC database [27] and subsystems were predicted by using RAST server [28] and circular view was designed by CGview [29]. The genetic basis for the presence of efflux pumps especially *adeABC* were studied by CARD

[30] and subsequent pathways were predicted by using Kyoto encyclopedia genes and genomes (KEGG) database [31]. The taxonomic classification was done through Type strain genome server (TYGS) [32]. The genome was further dissected to find prophage sequences by submitting the sequence data in PHASTEST online tool [33]. The prediction of antimicrobial resistant genes was done through ResFinder [34], mobile genetic elements by VPprofile [35], multi locus sequence tying through PubMLST [36] and virulence factors were identified through VBDF database.

Limitations

None

Ethics Statement

The authors have read and followed the ethical requirements for publication in Data in Brief and confirmed that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

Data Availability

Analysis of the genome data of Acinetobacter baumannii UOL-KIMZ-24, exhibiting multiple drug resistance through efflux pumps (Original data) (GenebANK/NCBI.).

CRediT Author Statement

Kiran Fatima: Conceptualization, Methodology, Formal analysis, Writing – original draft; **Syed Zeeshan Haider Naqvi:** Conceptualization, Supervision, Project administration, Writing – review & editing; **Hazrat Ali:** Conceptualization, Supervision, Project administration, Writing – review & editing; **Anam Saqib:** Resources, Formal analysis, Methodology; **Farheen Ansari:** Formal analysis, Validation, Software; **Sidrah Saleem:** Formal analysis, Validation, Software; **Shah Jahan:** Resources, Formal analysis, Methodology; **Mushtaq Ahmad:** Resources, Formal analysis, Methodology.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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