Lineage-Linked Biofilm Formation and Widespread Multidrug Resistance among Indian *Acinetobacter baumannii* Clinical Isolates

Vaishnavi Margoli Subbaraya[1], Varun Shamanna[1,2], Kavitha Arakalgud Kumar[1], Geetha Nagaraj[1], Harshitha Gangaiah Krishnappa[1], Madhushree Ravi[1], David Aanensen[3,4], Ravikumar Kadahalli Lingegowda[1] and NIHR Global Health Research Unit on genomic surveillance - India consortium

[1]Central Research Laboratory, Kempegowda Institute of Medical Sciences, Bengaluru, India

[2]Department of Biotechnology, NMAM Institute of Technology, Nitte University, Nitte, Udupi, India

[3]Centre for Genomic Pathogen Surveillance, Pandemic Sciences Institute, University of Oxford, Oxford, United Kingdom

[4]WHO Collaborating Center

Corresponding Author:

Ravikumar Kadahalli Lingegowda: klravikumar@gmail.com

Abstract

Aims: This study aimed to investigate the diversity and determinants of biofilm formation among clinical *Acinetobacter baumannii* Indian isolates and assess their relationship with antimicrobial resistance profiles, biofilm-associated genes, and genetic lineages revealed through whole-genome analysis.

Methods and Results: 230 *A. baumannii* clinical isolates across India (2015–2022) were tested for antibiotic susceptibility using the VITEK 2 system. Biofilm formation was quantified via the Tissue Culture Plate method. Whole genome sequencing (Illumina MiSeq) and bioinformatic analysis were performed to identify biofilm-associated genes, antimicrobial resistance genes and sequence types. Statistical associations were assessed using Kruskal-Wallis, Spearman's, and Fisher's tests. 85.22% of isolates were multidrug-resistant (MDR), and 100% exhibited biofilm formation, with 52.17% strong, 39.57% moderate, and 8.26% weak biofilm producers. Genes including ompA, bfmR, pgaA, pgaB, and pgaD were universally present. No significant association was observed between biofilm formation and antibiotic resistance ($P = 0.55$), specimen type ($P = 0.54$), or the presence of specific biofilm-related genes ($P > 0.05$). 21 sequence types (STs) were identified, with ST2 being the most prevalent (51.73%). Strong biofilm formation was more common in ST164, ST1, and ST575.

Conclusions: This study demonstrates a high prevalence of MDR and strong biofilm-forming *A. baumannii* isolates in India. Biofilm formation appeared independent of resistance or gene carriage but showed lineage-linked variation across sequence types.

Impact Statement: These findings underscore the need for enhanced surveillance and targeted strategies against infections caused by strong biofilm-forming *A. baumannii*, which may exhibit lineage-linked persistence and resistance.

Keywords: *Acinetobacter baumannii*, Biofilm formation, Antibiotic resistance, Whole genome sequencing, Biofilm-associated genes

Introduction

Acinetobacter baumannii is a significant opportunistic pathogen in humans, capable of causing various infections, including ventilator-associated pneumonia, meningitis, bacteremia, wound and soft-tissue infections, peritonitis, and urinary tract infections (Zeighami et al. 2019). In recent years, there has been a notable increase in multidrug resistance (MDR) and extensive drug resistance (XDR) among *A. baumannii*, contributing significantly to nosocomial infections (Gharaibeh et al. 2024). In India, national genomic surveillance and in-silico analyses have reported similarly high MDR/XDR rates, with broad diversity in resistance determinants and mobile genetic elements among clinical isolates (Kumkar et al. 2022). The bacterium exhibits broad-spectrum antibiotic resistance to cephalosporins, penicillins, carbapenems, fluoroquinolones, and aminoglycosides (Babapour et al. 2016). Carbapenem-resistant *A. baumannii* is ranked as a critical priority pathogen on the World Health Organization's priority list. Designated as a critical concern, it demands urgent management, continual public health surveillance, and proactive preventive measures (Piperaki et al. 2019). Furthermore, there are reports of several *A. baumannii* nosocomial isolates that display resistance to colistin and tigecycline, which are considered last-resort antibiotics prescribed in treatment guidelines, including those set forth by the Clinical and Laboratory Standards Institute (CLSI) (Asaad et al. 2021). A key factor contributing to the chronicity, persistence of infections, and antibiotic resistance in *A. baumannii* is its ability to colonize and form biofilms on both biotic and abiotic surfaces (Thummeepak et al. 2016). The rate of biofilm formation in *A. baumannii* is reported to be between 80% and 91%, surpassing that of other species, which typically range from 5%

to 24% (Zeighami et al. 2019). However, despite multiple reports of MDR/XDR *A. baumannii* from India, comprehensive studies that integrate phenotypic biofilm assessment with whole-genome sequencing remain relatively scarce in the Indian setting (Gedefie et al. 2023).

Biofilm formation in *A. baumannii* involves several virulence factors. For instance, the biofilm-associated protein (Bap) is crucial for biofilm attachment, maturation, and maintenance (Amin et al. 2019). The csuABCDE gene cluster synthesizes pili, essential for biofilm adhesion (Luo et al. 2015). The BfmR-BfmS system regulates pili synthesis and secretion, affecting biofilm formation via the csuABCDE cluster (Tomaras et al. 2008). The pgaABCD locus produces PNAG, vital for biofilm formation (Choi Alexis H. K. et al. 2009). The abaI and abaR genes are key in quorum sensing, crucial for biofilm maturation (Niu et al. 2008). Outer Membrane Protein A (OmpA) plays a crucial role in *A. baumannii* by facilitating adhesion, invasion, and biofilm formation (Nie et al. 2020). The biofilm growth-associated repressor (BigR) regulates genes related to cell adhesion and biofilm formation (Walsh et al. 2020). The beta-lactamase blaPER-1 gene enhances biofilm formation and attachment to respiratory cells (Thummeepak et al. 2016).

The Tissue Culture Plate (TCP) method for detecting biofilm production, stands out as the most reliable and is considered the gold standard method. It is a quantitative method available for assessing biofilm formation (de Castro Melo et al. 2013), (Harika et al. 2020), (Harika et al. 2020).

The study examined antibiotic susceptibility profiles, biofilm formation capacities, and biofilm-associated genes in 230 *A. baumannii* clinical isolates. Associations between biofilm formation capacities, specimen types, and antibiotic resistance were explored. Additionally, biofilm-associated genes' influence on biofilm formation capacity and antibiotic resistance was

evaluated. Phylogenetic analysis was performed to determine potential connections between specific sequence types (STs) and biofilm formation capacity.

Materials and Methods

Bacterial isolates and antibiotic susceptibility testing (AST)

The study was conducted at the Central Research Laboratory, Kempegowda Institute of Medical Sciences (KIMS), Bengaluru. A total of 230 retrospective isolates of *A. baumannii* were included for analysis. These isolates were originally collected as part of the Global Health Research Unit (GHRU) study on antimicrobial resistance (AMR), which generated a larger archive of 650 clinical *A. baumannii* isolates from multiple medical colleges and hospitals across India between 2015 and 2022. For the present study, a subset was selected based on sample-size calculations using the formula $n = Z^2 p(1-p) / d^2$ (Z = 1.96, p = 0.85 from published prevalence (Zeighami et al. 2019) , d = 0.05), which indicated a minimum requirement of approximately 196 isolates. We therefore selected 230 isolates to ensure adequate statistical power for genomic and phenotypic comparisons. The Ethical approval for the study was obtained from the KIMS ethical committee with the study number KIMS/IEC/27/2017. The strain details are provided in Supplementary Table 1.

The isolates were preserved at −80 °C in 15 % glycerol until processing. The antibiotic susceptibility data of all the clinical isolates of *A.baumannii* were retrieved from the VITEK 2 (bioMérieux, Marcy-l'Étoile, France) compact system. The AST-N406 card was utilized to test a total of 12 CLSI-recommended antibiotics. Prior to inoculation, a pure overnight colony was suspended in sterile saline, adjusted to ~0.5 McFarland using a DensiCHEK™ meter, and then loaded into the AST card module according to manufacturer instructions. Considering the

definitions provided by Magiorakos et al. 2012 (Magiorakos et al. 2012) and using AMR (for R) (https://msberends.github.io/AMR/), the isolates were characterized as MDR if the isolates were non-susceptible to ≥1 agent in ≥ 3 antibiotic categories, XDR if the isolates were non-susceptible to ≥1 agent in ≥ 5 antibiotic categories and susceptible if the isolates were non-susceptible to ≥1 agent in 0-2 antibiotic categories.

Biofilm quantification using the Tissue culture plate (TCP) method

Biofilm detection and quantification were carried out using the tissue culture plate method following the procedure outlined by Christensen et al. 1985 (Christensen et al. 1985). Frozen test isolates stored at −80 °C were revived by streaking onto blood agar plates and incubating overnight at 37 °C. Single colonies from these freshly grown cultures were inoculated into 5 mL of Trypticase soy broth (TSB) and then incubated for 24 hours at 37°C. Subsequently, the culture density was adjusted to 0.5 McFarland after 24 hours of incubation and further diluted to 1:100 using fresh Trypticase soy broth. A sterile 96-well polystyrene plate was utilized, with each well filled with 0.2 mL of the bacterial inoculum and incubated for 24 hours at 37 °C. The strong biofilm-forming strain *Pseudomonas aeruginosa* ATCC 27853 and the sterile TSB were used as positive and negative controls, respectively. Following incubation, the bacterial suspension was removed from all wells by gentle tapping, and the wells were washed twice with phosphate-buffered saline (pH 7.2) before being incubated for 1 hour at 37°C. Subsequently, the wells were stained with 0.2 mL of 0.1% Crystal Violet for 10 minutes, excess stain was removed by washing with distilled water, and the plates were allowed to dry. Then, 0.2mL of 33% glacial acetic acid was added to each well, and the optical density at a wavelength of 570 nm was determined using a Microtiter plate reader, Spectramax ABA Plus, Molecular Devices (Softmax pro 7.1.1). The isolates were characterized as weak, moderate and strong

biofilm producers according to the classification recommended by Stepanović et al. 2007 (Stepanović et al. 2007). The criteria for interpretation are detailed in Table 1.

Genomic Characterisation of Biofilm & AMR Determinants, Lineages and Phylogeny

Genomic DNA was extracted from *A.baumannii* isolates grown overnight on blood agar plates. A single colony was harvested, suspended in buffer, and processed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's spin-column protocol for Gram-negative bacteria. Briefly, after lysis with protease and Buffer AL at ~56 °C, the sample was ethanol-precipitated, passed through a silica membrane, washed with Buffer AW1 and AW2, and eluted in 200 µL Buffer AE. DNA concentration and purity of the extracted DNA was measured with the Qubit dsDNA kit (ThermoScientific, Massachusetts, USA). For sequencing, libraries with a 450 bp insert size were prepared using the NEB ultraFS-II kit (New England Biolabs, London, United Kingdom). The quality of these libraries was assessed using an Agilent Tapestation (Santa Clara, California, USA). Sequencing was then carried out on the Illumina MiSeq platform (Illumina, San Diego, California, USA), producing paired-end reads of 250 bp.

The bioinformatic analysis was conducted utilizing Nextflow pipelines developed as part of the Genomic Surveillance of Antimicrobial Resistance AMR project, available at protocols.io. The Quality control and Assembly were performed using the assembly pipeline (https://gitlab.com/cgps/ghru/pipelines/dsl2/pipelines/assembly). The raw reads are trimmed for low-quality reads, and sequencing error correction was performed before assembling the reads into contigs using the SPAdes assembler v3.12 (Prjibelski et al. 2020).

The assembled contigs were annotated using Bakta v1.8.2 with a v5.0 database (Schwengers et al. 2021). To specifically target biofilm-associated genes, the Virulence Factor Database (VFDB) http://www.mgc.ac.cn/VFs/ was scanned using the ariba tool v2.14.7 (Hunt et al. 2017). The biofilm-related genes, including *abaI*, *abaR*, *adeF*, *adeG*, *adeH*, *csuA_B*, *csuB*, *csuC*, *csuD*, *csuE*, *pgaA*, *pgaB*, *pgaC*, *pgaD*, and *bap,* were detected using the VFDB. Additionally, the annotations from the Bakta were screened for additional biofilm-related genes such as *ompA*, *bidR*, *bfmR*, *ptk*, *bmfS*, *epsA*, and $bla_{\text{PER-1}}$. A custom Python script was developed and employed to extract information regarding the presence or absence of the identified biofilm-related genes in the bacterial isolates.

Additionally, Sequence types (STs) were determined using PubMLST (https://pubmlst.org/), a widely used scheme for sequence-based typing of microbial isolates, which utilizes curated databases and standardized typing schemes to assign STs based on allelic profiles of specific genetic loci.

Panaroo v1.4.2 (Tonkin-Hill et al. 2020) was utilised to construct the pangenome from the annotations, and the core genome alignment was used for phylogenetic tree construction. The SNPs were extracted from the core-gene alignment using SNP-sites v2.5.1 (Page et al. 2016) and the maximum likelihood tree was constructed using the General Time Reversible model (GTR) using IQ-Tree v2.2.6 (Minh et al. 2020). The phylogenetic tree was visualized on Microreact (https://microreact.org/) (Argimón et al. 2016) for interactive exploration of bacterial isolate relationships.

All the WGS data from this study were uploaded to the European Nucleotide Archive (ENA) under the Bioproject numbers PRJEB29740 and PRJEB50614.

Statistical Analysis

The Kruskal-Wallis rank sum test was utilized to analyze the associations between biofilm formation capacities and the specimen types of clinical isolates of *A. baumannii.* Additionally, Spearman's rank correlation test was employed to examine the relationship between biofilm formation capacity and antibiotic resistance in these clinical isolates. To further investigate the association between biofilm-related genes and biofilm formation, Fisher's Exact test was conducted. All statistical analyses were performed using RStudio v2023.12.0.The code snippet has been uploaded to figshare and is available at this link (https://doi.org/10.6084/m9.figshare.25930018.v1).

Results

The study included 230 clinical isolates of *A. baumannii* obtained from 150 male and 80 female patients, aged between 8 days and 90 years (mean age: 46.27 ± 19.77 years). The majority of isolates were from respiratory samples (56.52%), followed by wound and soft tissue (27.39%), blood (7.39%), urine and catheter samples (5.64%), and cerebrospinal and other body fluids (3.04%). Detailed specimen-wise distribution for each isolate is provided in Supplementary Table 1.

Biofilm-Forming Capacity of *A. baumannii* and Its Distribution Across Clinical Specimens

The adherence ability of each *A. baumannii* isolate, based on OD_{570} measurements and ODc calculations, was categorized as none, weak, moderate, or strong biofilm producers. The mean

OD_{570} (± SD) of the reference strain *Pseudomonas aeruginosa* ATCC 27853 (positive control) was 2.36 ± 0.11, and that of sterile TSB medium (negative control) was 0.22 ± 0.006. The OD_{570} values for the clinical isolates ranged from 0.54 ± 0.01 to 3.98 ± 0.2. All the isolates (100%) could form biofilm, of which 52.17% (120/230) were strong biofilm producers, 39.57% (91/230) were moderate biofilm producers, and 8.26% (19/230) of the isolates were weak biofilm producers. The distribution of OD_{570} for each of the categories is represented in Figure 1. No isolate was found to be a non-biofilm former.

When stratified by specimen type, 72.5% (29/40) of isolates from pus samples and 52.45% (32/61) from tracheal aspirates were strong biofilm producers. In contrast, 52.38% (33/63) of sputum-derived isolates were moderate biofilm producers (Table 2). However, statistical analysis using the Kruskal–Wallis rank sum test did not reveal any significant association between specimen type and biofilm formation strength ($\chi^2 = 1.2018$; $P = 0.54$).

Antimicrobial Resistance Profiles and Association Between Biofilm-Forming Capacity

The antibiotic susceptibility test results for 12 antibiotics are shown in Figure 1. Most of the isolates (206/230, 89.56%) were resistant to all three tested carbapenems: Imipenem, Meropenem, and Doripenem, Piperacillin-tazobactam, Ticarcillin-clavulanic acid and Cefepime, followed by Ciprofloxacin and Ceftazidime (205/230 89.13%), Gentamicin (199/230 86.52%), Levofloxacin (198/230 86.08%), Trimethoprim-sulfamethoxazole (188/230 81.73%) and Colistin (16/230 6.95%). Out of the 230 studied *A. baumannii* isolates, 196 (85.22%) were MDR and 11 (4.78%) were XDR. 23 (10%) of the isolates showed a susceptible pattern.

The Spearman's rank correlation analysis revealed no significant correlation between the

antibiotic resistance groups (MDR, XDR and Susceptible) and biofilm formation capacities (rs=-0.039, P=0.55) (Table 3). Among the 206 carbapenem-resistant *A. baumannii* isolates, the majority (52.91%) were strong biofilm producers (SBP), followed by moderate biofilm producers (MBP) at 38.35%, and weak biofilm producers (WBP) at 8.74%. Correlation analysis between biofilm formation and resistance to each antibiotic showed no significant association (P > 0.05) (Supplementary Table 2).

Distribution of Biofilm-Associated Genes, Their Associations with Biofilm Formation Capacities, Antibiotic Resistance Groups, and Multilocus sequence types (MLST)

Among the 22 biofilm-related genes tested, the most commonly detected genes were *ompA*, *bfmR*, *pgaA*, *pgaB*, and *pgaD*, found in all isolates (230/230, 100%). This was followed by *adeF* (229/230, 99.56%), *adeH* and *pgaC* (228/230, 99.13%), *adeG* (222/230, 96.52%), *abaI* (208/230, 90.43%), *csuA_B*, *csuC*, and *csuE* (205/230, 89.13%), *csuB* (204/230, 88.69%), *csuD* (201/230, 87.39%), and *abaR* (189/230, 82.17%). Less commonly detected genes included *bap* (66/230, 28.69%) and *ptk* (7/230, 3%). The genes *bigR*, *bmfS*, *epsA*, and *blaPER-1* were not detected in any isolates.

Analysis using Fisher's Exact test revealed no significant associations between the biofilm-associated genes and biofilm formation capacities (P>0.05) (Figure 3B & Supplementary Table 3). Additionally, despite the *ptk* positive isolates comprising only 3% of the samples, all of them exhibited strong biofilm formation (Figure 3B). The carrying rates of biofilm-associated genes across the three antibiotic resistance groups (MDR, XDR, and susceptible), as shown in Figure 3A, did not indicate any observable association between gene presence and resistance profile.

Multilocus sequence typing (MLST) analysis identified 21 distinct STs among the *A. baumannii*

isolates. The most frequently identified were ST2 (119/230, 51.73%), ST149 (26/230, 11.30%), ST1 (17/230, 7.39%), ST10 (9/230, 3.91%), and ST575 (7/230, 3.04%). ST2 was the most predominant among all isolates. Furthermore, the study uncovered associations between specific STs such as ST164 (5/5, 100%), ST1 (16/17, 94.11%) and ST575 (6/7, 85.71%), with strong biofilm formation capacities. The relationship between phylogenetic relatedness, sequence types, and biofilm-forming capacities is depicted in Figure 4.

Discussion

Drug-resistant *A. baumannii* has demonstrated a remarkable capacity to spread globally, with rates of MDR and XDR escalating from under 4% in 2000 to exceeding 60% more recently and reaching nearly 90% in certain regional hospital environments (Wong et al. 2017, (Ghahramani et al. 2024).The National Institutes of Health and the Centre for Disease Control and Prevention estimate that between 65% and 80% of human infections are attributable to bacteria that form biofilms (Wolcott and Dowd 2011).

This study highlights the persistent challenges posed by *A. baumannii* within clinical settings, characterized by high rates of MDR and robust biofilm formation. Our findings resonate with global trends of escalating antibiotic resistance among *A. baumannii* isolates, which complicate treatment strategies and increase healthcare burdens (Peleg, Seifert and Paterson 2008).The majority (90%) of isolates displayed MDR profiles, including resistance to carbapenems and other critical antimicrobials, mirroring patterns observed globally (Perez et al. 2007).

The high carbapenem resistance (89.56% for imipenem, meropenem, and doripenem) is particularly concerning given their importance as last-resort drugs. Although resistance was not stratified geographically, isolates originated from multiple tertiary hospitals across India,

suggesting broad dissemination. Increased reliance on polymyxin B and tigecycline has been reported (Falagas et al. 2014), yet toxicity and inconsistent outcomes highlight the need for novel therapies and stronger antibiotic stewardship.

Biofilm formation remains a key virulence factor enhancing persistence and antibiotic tolerance in *A. baumannii*. In our study, all 230 clinical isolates exhibited the ability to form biofilms, with over half (52.17%) classified as strong biofilm formers. This is in line with prior research of Zeighami et al., 2019, (Zeighami et al. 2019), where a high prevalence of biofilm formation was reported. The propensity for robust biofilm formation by *A. baumannii* complicates clinical management due to biofilms' inherent resistance to antimicrobial agents and immune evasion capabilities (Rodríguez-Baño et al. 2008). The lack of a significant association between biofilm formation capacities and specimen types suggests that biofilm formation in *A. baumannii* is consistent across different clinical sources. This indicates that biofilm formation is likely an intrinsic characteristic of the isolates, not significantly influenced by the site of infection (Zeighami et al. 2019)(Sanchez et al. 2013). Currently, there remains no consistent pattern in the relationship between biofilm formation capabilities and bacterial antibiotic resistance. Findings across various studies continue to be contradictory (Qi et al. 2016), (Badave and Kulkarni 2015). Our study did not find any significant correlation between antibiotic susceptibility and biofilm formation capabilities in the clinical isolates ($P>0.05$), aligning with earlier research findings (Seleim et al. 2023) & (Donadu et al. 2022), where no significant correlations were found. This lack of correlation suggests that the ability of bacteria to form biofilms may not directly influence their resistance to antibiotics. This observation supports the notion that biofilm formation and antimicrobial resistance may be independently regulated traits within bacterial populations.

The analysis of biofilm-associated genes in this study provides essential insight into the persistence and pathogenic potential of *A. baumannii*. Our study's results, which showed that the genes *ompA*, *bfmR*, *pgaB*, and *pgaD* were present in all 230 samples (100%), are consistent with findings from previous research. Li et al., 2021, (Li et al. 2021) noted *bfmR,* which is involved in regulating biofilm formation and crucial for bacterial adherence and colonization, as the most frequent gene, appearing in 94.2% of their samples. Similarly, research by Badmasti et al., 2015 (Badmasti et al. 2015) identified *ompA*, crucial for bacterial cell integrity and immune evasion, as being present in all samples (100%). Zeighami et al., 2019 (Zeighami et al. 2019) reported a high prevalence of *pgaB*, part of the operon responsible for biofilm matrix production in 98% of their samples. The universal presence of these genes among diverse clinical isolates suggests that they are part of the core genome and essential for biofilm formation and survival in clinical environments. A particularly interesting finding in our study was the identification of *ptk* in only 3% of isolates — all of which were strong biofilm producers. *Ptk* encodes a protein tyrosine kinase involved in exopolysaccharide biosynthesis, a critical process in biofilm maturation. To our knowledge, this is the first report from India correlating *ptk* presence specifically with strong biofilm production. This highlights *ptk* as a promising candidate for future anti-biofilm strategies or as a biomarker for hyper-virulent strains.

The lack of significant correlations between individual biofilm-associated genes and biofilm formation capacities implies the involvement of complex regulatory networks and genetic interactions in governing biofilm formation in *A. baumannii* (Gedefie et al. 2023). Further studies elucidating the regulatory mechanisms underlying biofilm gene expression are warranted. However, some studies have found associations. For instance, Li et al., 2021 (Li et al. 2021) observed that the presence of certain genes, like *csuD*, was significantly correlated with stronger biofilm formation. This discrepancy might be due to differences in genetic

backgrounds, environmental conditions, or specific regulatory pathways influencing gene expression.

No association between the carrying rates of biofilm-related genes and antimicrobial resistance profiles in our study underscores the complexity of the relationship between genetic determinants of biofilm formation and antimicrobial resistance in *A. baumannii.* This observation aligns with findings from several other studies that have also reported a lack of significant associations between the two categories. For instance, a study by Habib Zeighami et al., 2019 (Zeighami et al. 2019) similarly found no significant correlation between the presence of biofilm-associated genes and antimicrobial resistance profiles in *A. baumannii* isolates. Furthermore, the regulatory networks governing biofilm formation and antimicrobial resistance in *A. baumannii* are complex and interconnected (Zeighami et al. 2019). Genetic interactions and epistatic effects may contribute to the observed lack of correlation between biofilm-related genes and antimicrobial resistance (Silva et al. 2023). However, some studies have found associations. For example, Li et al., 2021 (Li et al. 2021) observed that the presence of certain biofilm-associated genes like csuA and csuD was significantly correlated with higher resistance in MDR strains, whereas non-MDR strains had more ompA genes and formed stronger biofilms. This discrepancy might be due to differences in genetic backgrounds, environmental conditions, or specific regulatory pathways influencing gene expression. Understanding these intricate regulatory mechanisms requires further investigation through functional genomics and systems biology approaches.

The observed prevalence of ST2 in the phylogenetic analysis aligns with previous epidemiological studies highlighting its prominence among *A. baumannii* clinical isolates. Additionally, the identified associations between ST1, ST164, and ST575 with robust biofilm formation capacities substantiate the role of genetic lineages in shaping biofilm phenotypes,

suggesting potential genetic determinants driving biofilm formation in *A. baumannii.* This finding, to our knowledge, has not been previously reported in Indian isolates. These findings provide valuable insights into the genetic diversity and biofilm-related traits of *A. baumannii* strains, which can inform targeted intervention strategies and therapeutic approaches to combat biofilm-associated infections (Wiradiputra et al. 2023).

Further, the core genome SNP-based phylogenetic analysis revealed five major clades corresponding to the predominant sequence types (ST2, ST149, ST1, ST164, and ST575). Isolates within each clade displayed close genetic relatedness (typically <50 SNPs), whereas inter-clade distances exceeded 1,000 SNPs, confirming distinct evolutionary trajectories. The predominance of ST2 and ST149 across multiple institutions and collection years suggests their successful adaptation and persistence in Indian healthcare settings. Although no patient-level epidemiological data were available to infer direct transmission, the clustering pattern indicates the potential for regional dissemination of these high-risk clones. These results underscore the importance of integrating genomic and epidemiological surveillance to better track the evolution and spread of multidrug-resistant *A. baumannii* in India.

Overall, the novelty and significance of this study lie in its multi-centric design, nationwide coverage, and integration of phenotypic and genomic data. By linking biofilm phenotypes, genetic lineages, and resistance profiles across isolates collected from multiple regions of India, this study provides one of the most comprehensive insights into the biofilm-genotype landscape of *A. baumannii* in the country. The detection of lineage-specific biofilm strengths (e.g., ST164, ST575) and the limited influence of gene carriage on biofilm phenotype highlight the need to explore epigenetic and regulatory mechanisms that could be driving persistence in clinical environments. Collectively, these findings establish a genomic and epidemiological framework for future surveillance and development of targeted anti-biofilm strategies.

Despite its comprehensive analysis, this study has some limitations. Firstly, the study focused on a set of biofilm-associated genes, and further research may explore additional genes that could provide additional insights. Additionally, the proportions of MDR, XDR, and susceptible isolates were not balanced, with MDR isolates comprising the majority, potentially impacting the generalizability of the findings.

Conclusion

This study unveils the intricate interplay among biofilm formation, antibiotic resistance, and genetic diversity in clinical isolates of *A. baumannii*. By shedding light on these relationships, the findings underscore the enduring hurdles presented by this pathogen in clinical contexts, emphasising the critical need to unravel the underlying mechanisms governing biofilm formation and antibiotic resistance. This understanding paves the way for future research endeavours aimed at developing precise intervention strategies and innovative therapeutics to effectively tackle biofilm-associated infections attributed to *A. baumannii.*

Acknowledgements.

Members of the NIHR Global Health Research Unit on Genomic Surveillance of Antimicrobial Resistance: Dr. Sophia David, Dr. Monica Abrudan, Dr. Julio Diaz Caballero, Ms. Emmanuelle Kumaran, Mrs. Georgina Lewis-Woodhouse, Dr. Khalil Abudahab and Dr. Ben Pascoe of the Centre for Genomic Pathogen Surveillance, Pandemic Sciences Institute, University of Oxford, Old Road Campus, Oxford, UK; Dr. Pilar Donado-Godoy of the Colombian Integrated Program for Antimicrobial Resistance Surveillance, Coipars, CI Tibaitatá, Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Tibaitatá, Mosquera, Cundinamarca, Colombia;

Mrs. M.R. Shincy, Ms. D. Sravani, Mr. K. N. Ravishankar of the Central Research Laboratory, Kempegowda Institute of Medical Sciences, Bengaluru, India; Dr. Iruka N. Okeke, Mr. Anderson O. Oaikhena of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Oyo State, Nigeria; Dr. Sonia Sia, Dr. Celia Carlos, Mrs. Marietta L. Lagrada and Mr. June M. Gayeta of the Antimicrobial Resistance Surveillance Reference Laboratory, Research Institute for Tropical Medicine, Muntinlupa, the Philippines; Dr. John Stelling, The Brigham and Women's Hospital, Boston, MA, USA; and Dr. Carolin Vegvari, Imperial College London, London, UK. From NIHR Global Health Research Unit on genomic surveillance - India consortium: Dr. Anuradha Sharma, AIIMS, Jodhpur, Rajasthan, India; Dr. Ujjwayini Ray, Apollo Multi Scpeciality Hospital, Kankurgachi, Kolkata, West Bengal, India; Dr. Manick Das, Apollo Medical College, Hyderabad, India; Dr. Maneesha Sahu, BALCO Medical Center, Raipur, Chhattisgarh, India; Dr. Aruna Poojary, Breach Candy Hospitals, Mumbai, Maharashtra, India; Dr. Lakshmi, Biocare Research Lab, Gandhinagar, Gujarat, India; Dr. Shwetha, Bangalore Medical College, Bangalore, Karnataka, India; Dr. Shubranshu mandal, Calcutta Medical Research Institute, Kolkata, West Bengal, India; Dr. Frincy, Excel Health care, Guwahati, Assam, India; Dr. Anitha, Government medical college, Trichy, Tamil Nadu, India; Dr. Varsha Gupta, GMC, Chandigarh, India; Dr. Namrata Rai, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India; Dr. Bhattacharyya, All India Institute of Hygiene & Public Health, Kolkata, West Bengal, India; Dr. Naveena, Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bangalore, Karnataka, India; Dr. Sujatha, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, Pondicherry, India; Dr. Sheetal Verma, King George's Medical University, Lucknow, Uttar Pradesh, India; Dr. Shrikala baliga, Kasturba Medical College, Mangalore, Karnataka, India; Dr. Lakshmi, Kamineni Hospital, Hyderabad, Telangana, India; Dr. Shalab Malik, Dr Lal PathLabs, Delhi, India; Dr. Vishwanath, Mediquest Diagnostics, Hyderabad, Telangana, India;

Dr. Sohan Lal, Malabar Institute of Medical Sciences, Kozhikode, Kerala, India; Dr. veenakumari, NIMHANS, Bangalore, Karnataka, India; Dr. Milan, Neugen Laboratories, Rajkot, Gujarat, India; Dr. Venkatraman Kandi, Prathima Institute of Medical sciences, Karimnagar, Telangana, India; Dr. Smita Sood, Rukmani Birla Hospital, Jaipur, Rajasthan, India; Dr. Keerthi Lakshmi, RajaRajeswari Medical College, Bangalore, Karnataka, India; Dr. Jyothi EK, SCTIMST, Thiruvananthapuram, Kerala, India; Purna chandra, Kolar medical college, Kolar, Karnataka, India; Dr. Vaidehi, Sundaram Medical Foundation, Chennai, Tamil Nadu, India; Dr. Jagatheeswary, Saveetha Medical College, Kuthambakkam, Tamil Nadu, India; Dr. B G Vishwanath, Sreekar Lab, Hyderabad, Telangana, India; Dr. Shruthi Uppoor, Siddiah Referral Hospital, Bangalore, Karnataka, India; Dr. Chitra rajalakshmi and Dr. Vallab Ganesh Bharadwaj, Trichy SRM Medical College Hospital and Research Centre, Trichy, Tamil Nadu, India; Dr. Mohit Bhatia, AIIMS, Rishikesh, Uttarakhand, India; Dr. Sowmusharee, VIMSAR, Burla, Odisha, India; Dr. Malini Sahriff, Vallabhbhai Patel Chest Institute, Delhi, India. We would like to express our gratitude to Dr. Shirshendu Mukherjee and Dr. Richa Vashishtha, who supported this research through the Biotechnology Industry Research Assistance Council (BIRAC) of the Government of India.

Conflict of interest

No potential conflict of interest was reported by the authors.

References

Amin, Mansour, Navidifar, Tahereh, Shooshtari, Farkhondeh Saleh, Rashno, Mohammad, Savari, Mohammad, Jahangirmehr, Fatemeh, et al., ‘Association Between Biofilm

Formation, Structure, and the Expression Levels of Genes Related to Biofilm Formation and Biofilm-Specific Resistance of Acinetobacter Baumannii Strains Isolated from Burn Infection in Ahvaz, Iran', *Infection and Drug Resistance*, 12 (2019), 3867–81

Argimón, Silvia, Abudahab, Khalil, Goater, Richard J. E., Fedosejev, Artemij, Bhai, Jyothish, Glasner, Corinna, et al., 'Microreact: Visualizing and Sharing Data for Genomic Epidemiology and Phylogeography', *Microbial Genomics*, 2/11 (2016), e000093

Asaad, Ahmed Morad, Ansari, Shamshul, Ajlan, Soma Elsayed, and Awad, Samah Mohammed, 'Epidemiology of Biofilm Producing Acinetobacter Baumannii Nosocomial Isolates from a Tertiary Care Hospital in Egypt: A Cross-Sectional Study', *Infection and Drug Resistance*, 14 (2021), 709–17

Babapour, Ebrahim, Haddadi, Azam, Mirnejad, Reza, Angaji, Seyed-Abdolhamid, and Amirmozafari, Nour, 'Biofilm Formation in Clinical Isolates of Nosocomial Acinetobacter Baumannii and Its Relationship with Multidrug Resistance', *Asian Pacific Journal of Tropical Biomedicine*, 6/6 (2016), 528–33

Badave, Gitanjali Kailas, and Kulkarni, Dhananjay, 'Biofilm Producing Multidrug Resistant Acinetobacter Baumannii: An Emerging Challenge', *Journal of Clinical and Diagnostic Research: JCDR*, 9/1 (2015), DC08–10

Badmasti, Farzad, Siadat, Seyed Davar, Bouzari, Saeid, Ajdary, Soheila, and Shahcheraghi, Fereshteh, 'Molecular Detection of Genes Related to Biofilm Formation in Multidrug-Resistant Acinetobacter Baumannii Isolated from Clinical Settings', *Journal of Medical Microbiology*, 64/Pt 5 (2015), 559–64

de Castro Melo, Poliana, Ferreira, Luciano Menezes, Filho, Antônio Nader, Zafalon, Luiz Francisco, Vicente, Hinig Isa Godoy, and de Souza, Viviane, 'Comparison of Methods for

462 the Detection of Biofilm Formation by Staphylococcus Aureus Isolated from Bovine
463 Subclinical Mastitis', *Brazilian Journal of Microbiology: [publication of the Brazilian*
464 *Society for Microbiology]*, 44/1 (2013), 119–24

465 Choi Alexis H. K., Slamti Leyla, Avci Fikri Y., Pier Gerald B., and Maira-Litrán Tomás,
466 'The pgaABCD Locus of Acinetobacter Baumannii Encodes the Production of Poly-β-1-
467 6-N-Acetylglucosamine, Which Is Critical for Biofilm Formation', *Journal of*
468 *Bacteriology*, 191/19 (2009), 5953–63

469 Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton,
470 D. M., et al., 'Adherence of Coagulase-Negative Staphylococci to Plastic Tissue Culture
471 Plates: A Quantitative Model for the Adherence of Staphylococci to Medical Devices',
472 *Journal of Clinical Microbiology*, 22/6 (1985), 996–1006

473 Donadu, Matthew Gavino, Ferrari, Marco, Mazzarello, Vittorio, Zanetti, Stefania,
474 Kushkevych, Ivan, Rittmann, Simon K-M R., et al., 'No Correlation between Biofilm-
475 Forming Capacity and Antibiotic Resistance in Environmental Staphylococcus Spp.: In
476 Vitro Results', *Pathogens*, 11/4 (2022) <http://dx.doi.org/10.3390/pathogens11040471>

477 Falagas, Matthew E., Lourida, Panagiota, Poulikakos, Panagiotis, Rafailidis, Petros I., and
478 Tansarli, Giannoula S., 'Antibiotic Treatment of Infections due to Carbapenem-Resistant
479 Enterobacteriaceae: Systematic Evaluation of the Available Evidence', *Antimicrobial*
480 *Agents and Chemotherapy*, 58/2 (2014), 654–63

481 Gedefie, Alemu, Alemayehu, Ermiyas, Mohammed, Ousman, Bambo, Getachew Mesfin,
482 Kebede, Samuel Sahile, and Kebede, Berhanu, 'Prevalence of Biofilm Producing
483 Acinetobacter Baumannii Clinical Isolates: A Systematic Review and Meta-Analysis',
484 *PloS One*, 18/11 (2023), e0287211

Ghahramani, Ali, Naghadian Moghaddam, Mohammad Mahdi, Kianparsa, Joben, and Ahmadi, Mohammad Hossein, 'Overall Status of Carbapenem Resistance among Clinical Isolates of Acinetobacter Baumannii: A Systematic Review and Meta-Analysis', *The Journal of Antimicrobial Chemotherapy*, 79/12 (2024), 3264–80

Gharaibeh, Mohammad H., Abandeh, Yaman M., Elnasser, Ziad A., Lafi, Shawkat Q., Obeidat, Haneen M., and Khanfar, Malak A., 'Multi-Drug Resistant Acinetobacter Baumannii: Phenotypic and Genotypic Resistance Profiles and the Associated Risk Factors in Teaching Hospital in Jordan', *Journal of Infection and Public Health*, 17/4 (2024), 543–50

Harika, Kala, Shenoy, Vishnu Prasad, Narasimhaswamy, Nagalakshmi, and Chawla, Kiran, 'Detection of Biofilm Production and Its Impact on Antibiotic Resistance Profile of Bacterial Isolates from Chronic Wound Infections', *Journal of Global Infectious Diseases*, 12/3 (2020), 129–34

Hassan, Afreenish, Usman, Javaid, Kaleem, Fatima, Omair, Maria, Khalid, Ali, and Iqbal, Muhammad, 'Evaluation of Different Detection Methods of Biofilm Formation in the Clinical Isolates', *The Brazilian Journal of Infectious Diseases: An Official Publication of the Brazilian Society of Infectious Diseases*, 15/4 (2011), 305–11

Hunt, Martin, Mather, Alison E., Sánchez-Busó, Leonor, Page, Andrew J., Parkhill, Julian, Keane, Jacqueline A., et al., 'ARIBA: Rapid Antimicrobial Resistance Genotyping Directly from Sequencing Reads', *Microbial Genomics*, 3/10 (2017), e000131

Kumkar, Shital N., Kamble, Ekta E., Chavan, Nikeeta S., Dhotre, Dhiraj P., and Pardesi, Karishma R., 'Diversity of Resistant Determinants, Virulence Factors, and Mobile Genetic Elements in Acinetobacter Baumannii from India: A Comprehensive in Silico Genome

Analysis', *Frontiers in Cellular and Infection Microbiology*, 12 (2022), 997897

Li, Zhaoyinqian, Ding, Zixuan, Liu, Yao, Jin, Xinrui, Xie, Jingling, Li, Tingting, et al., 'Phenotypic and Genotypic Characteristics of Biofilm Formation in Clinical Isolates of Acinetobacter Baumannii', *Infection and Drug Resistance*, 14 (2021), 2613–24

Luo, Li-Mei, Wu, Li-Juan, Xiao, Yu-Ling, Zhao, Dan, Chen, Zhi-Xing, Kang, Mei, et al., 'Enhancing Pili Assembly and Biofilm Formation in Acinetobacter Baumannii ATCC19606 Using Non-Native Acyl-Homoserine Lactones', *BMC Microbiology*, 15 (2015), 62

Magiorakos, A-P, Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., et al., 'Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance', *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 18/3 (2012), 268–81

Minh, Bui Quang, Schmidt, Heiko A., Chernomor, Olga, Schrempf, Dominik, Woodhams, Michael D., von Haeseler, Arndt, et al., 'IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era', *Molecular Biology and Evolution*, 37/5 (2020), 1530–34

Nie, Dan, Hu, Yue, Chen, Zhou, Li, Mingkai, Hou, Zheng, Luo, Xiaoxing, et al., 'Outer Membrane Protein A (OmpA) as a Potential Therapeutic Target for Acinetobacter Baumannii Infection', *Journal of Biomedical Science*, 27/1 (2020), 26

Niu, Chen, Clemmer, Katy M., Bonomo, Robert A., and Rather, Philip N., 'Isolation and Characterization of an Autoinducer Synthase from Acinetobacter Baumannii', *Journal of Bacteriology*, 190/9 (2008), 3386–92

Page, Andrew J., Taylor, Ben, Delaney, Aidan J., Soares, Jorge, Seemann, Torsten, Keane, Jacqueline A., et al., 'SNP-Sites: Rapid Efficient Extraction of SNPs from Multi-FASTA Alignments', *Microbial Genomics*, 2/4 (2016), e000056

Peleg, Anton Y., Seifert, Harald, and Paterson, David L., 'Acinetobacter Baumannii: Emergence of a Successful Pathogen', *Clinical Microbiology Reviews*, 21/3 (2008), 538–82

Perez, Federico, Hujer, Andrea M., Hujer, Kristine M., Decker, Brooke K., Rather, Philip N., and Bonomo, Robert A., 'Global Challenge of Multidrug-Resistant Acinetobacter Baumannii', *Antimicrobial Agents and Chemotherapy*, 51/10 (2007), 3471–84

Piperaki, E-T, Tzouvelekis, L. S., Miriagou, V., and Daikos, G. L., 'Carbapenem-Resistant Acinetobacter Baumannii: In Pursuit of an Effective Treatment', *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 25/8 (2019), 951–57

Prjibelski, Andrey, Antipov, Dmitry, Meleshko, Dmitry, Lapidus, Alla, and Korobeynikov, Anton, 'Using SPAdes De Novo Assembler', *Current Protocols in Bioinformatics / Editoral Board, Andreas D. Baxevanis ... [et Al.]*, 70/1 (2020), e102

Qi, Lihua, Li, Hao, Zhang, Chuanfu, Liang, Beibei, Li, Jie, Wang, Ligui, et al., 'Relationship between Antibiotic Resistance, Biofilm Formation, and Biofilm-Specific Resistance in Acinetobacter Baumannii', *Frontiers in Microbiology*, 7 (2016), 483

Rodríguez-Baño, Jesús, Alcalá, Juan C., Cisneros, Jose M., Grill, Fabio, Oliver, Antonio, Horcajada, Juan P., et al., 'Community Infections Caused by Extended-Spectrum Beta-Lactamase-Producing Escherichia Coli', *Archives of Internal Medicine*, 168/17 (2008), 1897–1902

554 Sanchez, Carlos J., Jr, Mende, Katrin, Beckius, Miriam L., Akers, Kevin S., Romano,
555 Desiree R., Wenke, Joseph C., et al., 'Biofilm Formation by Clinical Isolates and the
556 Implications in Chronic Infections', *BMC Infectious Diseases*, 13 (2013), 47

557 Schwengers, Oliver, Jelonek, Lukas, Dieckmann, Marius Alfred, Beyvers, Sebastian,
558 Blom, Jochen, and Goesmann, Alexander, 'Bakta: Rapid and Standardized Annotation of
559 Bacterial Genomes via Alignment-Free Sequence Identification', *Microbial Genomics*,
560 7/11 (2021) <http://dx.doi.org/10.1099/mgen.0.000685>

561 Seleim, Shaimaa, Abd El-Massih, Mona, Saleh, Mohamed, and El-Wakil, Doaa, 'Biofilm
562 Formation by Acinetobacter Species Isolated from Intensive Care Units: Unveiling the
563 Impact on Antibiotic Resistance', *Egyptian Journal of Medical Microbiology*, 32/4 (2023),
564 0–0

565 Silva, Adriana, Silva, Vanessa, López, María, Rojo-Bezares, Beatriz, Carvalho, José
566 António, Castro, Ana Paula, et al., 'Antimicrobial Resistance, Genetic Lineages, and
567 Biofilm Formation in Pseudomonas Aeruginosa Isolated from Human Infections: An
568 Emerging One Health Concern', *Antibiotics (Basel, Switzerland)*, 12/8 (2023)
569 <http://dx.doi.org/10.3390/antibiotics12081248>

570 Stepanović, Srdjan, Vuković, Dragana, Hola, Veronika, Di Bonaventura, Giovanni,
571 Djukić, Slobodanka, Cirković, Ivana, et al., 'Quantification of Biofilm in Microtiter Plates:
572 Overview of Testing Conditions and Practical Recommendations for Assessment of
573 Biofilm Production by Staphylococci', *APMIS: Acta Pathologica, Microbiologica, et*
574 *Immunologica Scandinavica*, 115/8 (2007), 891–99

575 Thummeepak, Rapee, Kongthai, Phattaraporn, Leungtongkam, Udomluk, and Sitthisak,
576 Sutthirat, 'Distribution of Virulence Genes Involved in Biofilm Formation in Multi-Drug

Resistant Acinetobacter Baumannii Clinical Isolates', *International Microbiology: The Official Journal of the Spanish Society for Microbiology*, 19/2 (2016), 121–29

Tomaras, Andrew P., Flagler, Michael J., Dorsey, Caleb W., Gaddy, Jennifer A., and Actis, Luis A., 'Characterization of a Two-Component Regulatory System from Acinetobacter Baumannii That Controls Biofilm Formation and Cellular Morphology', *Microbiology*, 154/Pt 11 (2008), 3398–3409

Tonkin-Hill, Gerry, MacAlasdair, Neil, Ruis, Christopher, Weimann, Aaron, Horesh, Gal, Lees, John A., et al., 'Producing Polished Prokaryotic Pangenomes with the Panaroo Pipeline', *Genome Biology*, 21/1 (2020), 180

Walsh, Brenna J. C., Wang, Jiefei, Edmonds, Katherine A., Palmer, Lauren D., Zhang, Yixiang, Trinidad, Jonathan C., et al., 'The Response of Acinetobacter Baumannii to Hydrogen Sulfide Reveals Two Independent Persulfide-Sensing Systems and a Connection to Biofilm Regulation', *mBio*, 11/3 (2020) <http://dx.doi.org/10.1128/mBio.01254-20>

Warner, Wayne A., Kuang, Shan N., Hernandez, Rina, Chong, Melissa C., Ewing, Peter J., Fleischer, Jen, et al., 'Molecular Characterization and Antimicrobial Susceptibility of Acinetobacter Baumannii Isolates Obtained from Two Hospital Outbreaks in Los Angeles County, California, USA', *BMC Infectious Diseases*, 16 (2016), 194

Wiradiputra, Made Rai Dwitya, Thirapanmethee, Krit, Khuntayaporn, Piyatip, Wanapaisan, Pagakrong, and Chomnawang, Mullika Traidej, 'Comparative Genotypic Characterization Related to Antibiotic Resistance Phenotypes of Clinical Carbapenem-Resistant Acinetobacter Baumannii MTC1106 (ST2) and MTC0619 (ST25)', *BMC Genomics*, 24/1 (2023), 689

600 Wolcott, Randall, and Dowd, Scot, 'The Role of Biofilms: Are We Hitting the Right
601 Target?', *Plastic and Reconstructive Surgery*, 127 Suppl 1 (2011), 28S – 35S

602 Wong, Darren, Nielsen, Travis B., Bonomo, Robert A., Pantapalangkoor, Paul, Luna,
603 Brian, and Spellberg, Brad, 'Clinical and Pathophysiological Overview of Acinetobacter
604 Infections: A Century of Challenges', *Clinical Microbiology Reviews*, 30/1 (2017), 409–
605 47

606 Zeighami, Habib, Valadkhani, Fatemeh, Shapouri, Reza, Samadi, Elham, and Haghi,
607 Fakhri, 'Virulence Characteristics of Multidrug Resistant Biofilm Forming Acinetobacter
608 Baumannii Isolated from Intensive Care Unit Patients', *BMC Infectious Diseases*, 19/1
609 (2019), 629

610

Author Contribution

V.M.S. and V.S. conceptualised this study. V.M.S. performed the biofilm assay. V.S. performed the genomic analysis of the samples collected in this study. V.M.S. and V.S. were involved in statistical analysis, table, and figure generation. KLR guided the manuscript preparation and reviewed the manuscript. K.A.K. critically reviewed the manuscript. G.N. and H.G.K. were involved in the sample collection. H.G.K., M.R. and K.A.K. performed the microbiology part of the analysis, and G.N. did the sequencing. Funding for the study was provided through grants to K.L.R. and D.A. All authors reviewed the manuscript and suggested improvements.

Funding

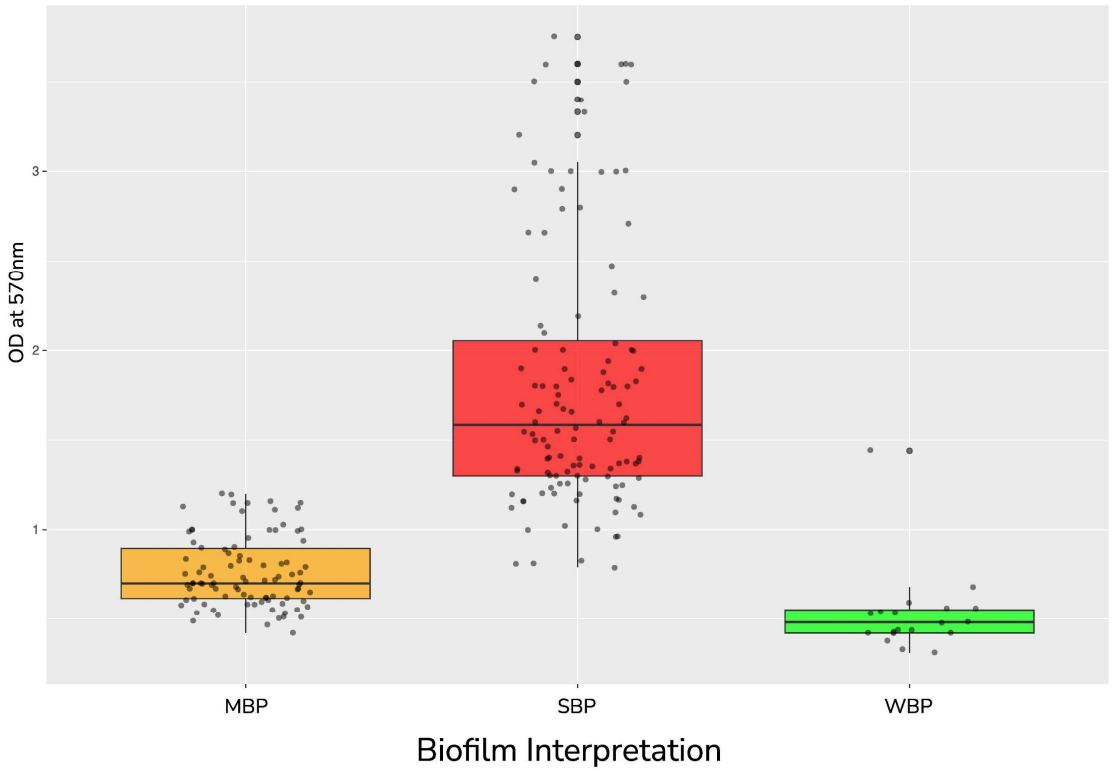
The sample collection and whole genome sequencing work was supported by the Official Development Assistance (ODA) funding from the National Institute for Health Research [grant number 16_136_111] and the Welcome Trust grant number 206194. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research, or the Department of Health.

Data Availability

All original data generated and analyzed during this study are included within the article and its supplementary materials.

632

Figure 1



633

634 **Figure 1**: Biofilm detection in *A. baumannii* isolates using the Tissue Culture Plate (TCP)
635 method: Box-and-whisker plot showing OD_{570} values for strong, moderate, and weak biofilm-
636 forming categories. Boxes represent interquartile ranges, horizontal lines indicate medians, and
637 whiskers show minimum and maximum values

638 Abbreviations: MBP: Moderate biofilm producer; SBP: Strong biofilm producer; WBP: Weak
639 biofilm producer

640

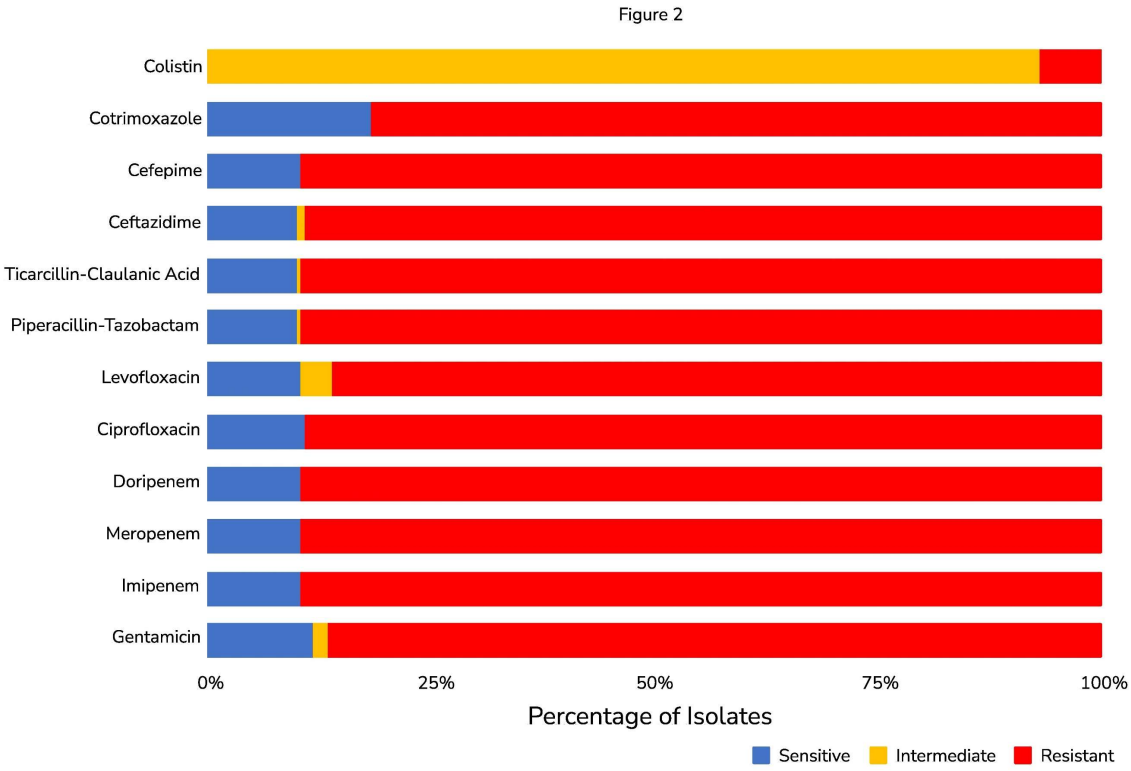


Figure 2: Antibiotic susceptibility profiles of *A. baumannii* clinical isolates. The figure shows a horizontal stacked bar plot where each bar represents a specific antibiotic. The y-axis lists the antibiotics tested, and the x-axis shows the percentage distribution of isolates. Each bar is stacked to 100% and divided into sensitive, intermediate, and resistant.

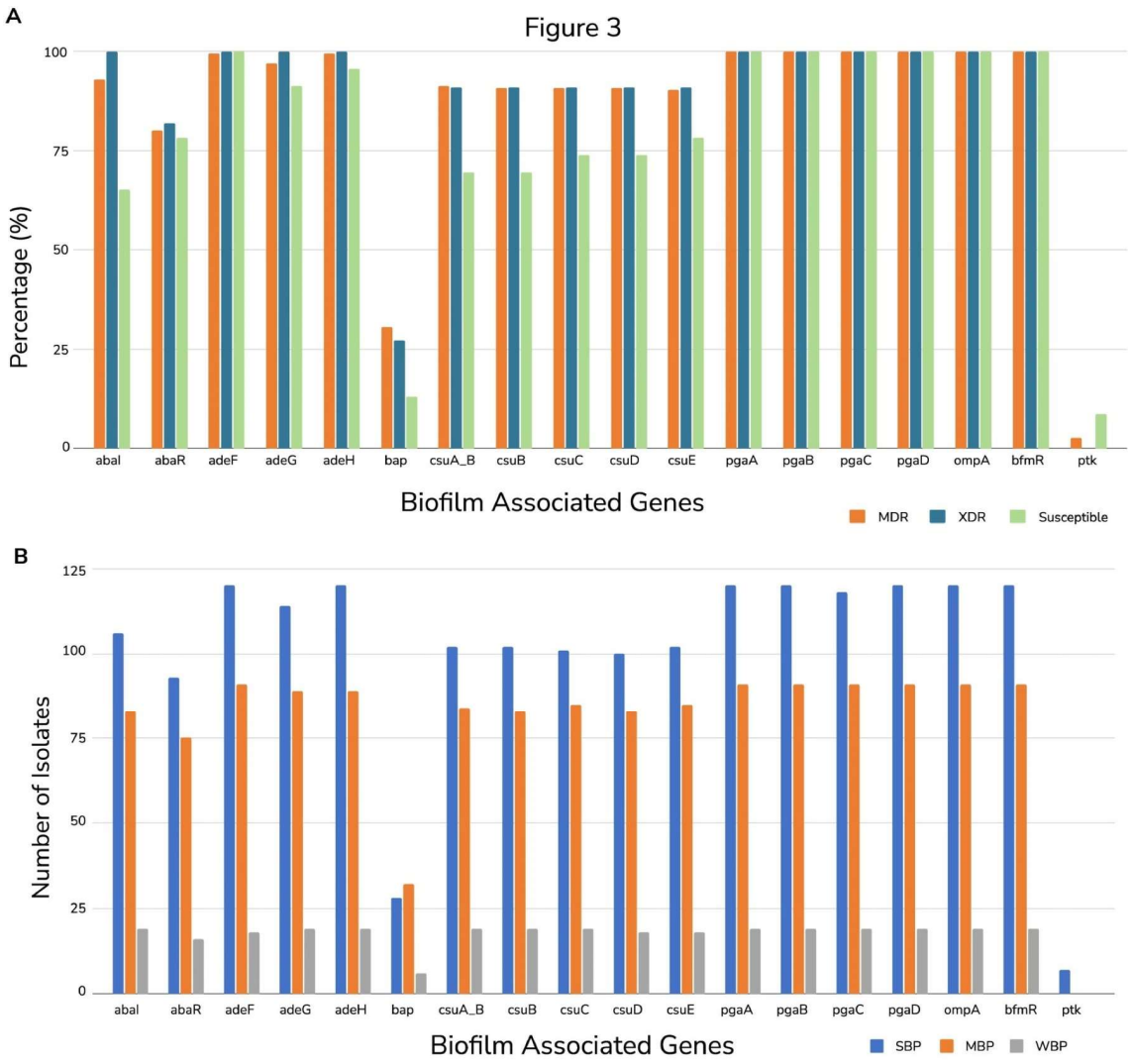


Figure 3: A) Bar chart showing the percentage of *A. baumannii* isolates carrying specific biofilm-associated genes across three antibiotic resistance categories: MDR, XDR, and Sensitive. The y-axis represents the percentage of gene-positive isolates.

B) Bar chart representing the number of isolates carrying each biofilm gene among SBP, MBP, and WBP. The y-axis shows the count of gene-positive isolates.

Figure 4: A) Circular phylogenetic tree view of the same dataset, offering an alternative visualization for comparison and clarity. The outer rings display metadata layers for biofilm formation phenotypes and the presence of selected biofilm-associated genes (ompA, bfmR, bap,

pgaABCD, etc.). This view enhances the interpretation of lineage-specific traits and highlights clusters of strong biofilm-forming STs, particularly ST1, ST164, and ST575.

B) Rectangular phylogenetic tree view showing the relationships among 230 A. baumannii isolates based on core genome SNPs. Each isolate is annotated with its corresponding sequence type (ST), biofilm formation capacity (color-coded as strong, moderate, or weak), and the presence or absence of key biofilm-associated genes. Sequence types are indicated along the branches, allowing visualization of the genetic clustering of isolates and their biofilm-related characteristics.

Table 1: Interpretation of biofilm formation using the tissue culture plate (TCP) method.

Mean OD value	Degree of Adherence	Biofilm formation
OD ≤ ODc*	-	None
ODc < OD ≤ 2 ODc	+	Weak
2 ODc < OD ≤ 4 ODc	++	Moderate
4 ODc < OD	+++	Strong

*Optical density cut-off value (ODc) was considered as three standard deviations above the mean optical density (OD) of the negative control

678

679 **Table 2:** Distribution of biofilm formation capacities among the specimen types

Clinical Specimen Type	Biofilm formation capacities (No of isolates/total number (percentage))		
	Strong	**Moderate**	**Weak**
Sputum	25/63 (39.68)	33/63 (52.38)	5/63 (7.94)
Pus	29/40 (72.5)	11/40 (27.5)	-
TA	32/61 (52.45)	22/61 (36.06)	7/61 (11.47)
Wound	13/18 (72.22)	3/18 (16.67)	2/18 (11.11)
Blood	5/17 (29.41)	10/17 (58.82)	2/17 (11.77)
Urine	3/8 (37.5)	5/8 (62.5)	-
BAL	2/6 (33.33)	3/6 (50)	1/6 (16.67)
Catheter	4/5 (80)	1/5 (20)	-
Tissue	3/5 (60)	1/5 (20)	1/5 (20)
CSF	1/3 (33.33)	1/3 (33.34)	1/3 (33.33)
Fluid	2/3 (66.66)	1/3 (33.34)	-

HVS	1/1 (100)	-	-

Abbreviations: TA: Tracheal aspirate; BAL: Bronchoalveolar Lavage; CSF: Cerebrospinal fluid; HVS: High vaginal swab

Table 3: Biofilm formation capacities of the *A. baumannii* clinical isolates with different Antibiotic resistance groups

Antibiotic resistance groups	**Number of isolates**	**OD_{570} (IQR)**	**r_S**	**P value**
Susceptible	23	1.35 (0.60-1.8)	-0.039	0.55
MDR	196	1.28 (0.7-1.6)		
XDR	11	0.78 (0.49-0.735)		

Abbreviations: MDR: multiple drug resistant; XDR: extensively drug-resistant; r_s=Spearman's rank correlation coefficient.