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Original Research Article

Genetic diversity of *A. baumannii* sequence types isolated from VAP patients: A retrospective study from a tertiary care hospital in Vadodara, Gujarat

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Abstract

Introduction: *A. baumannii* is a Gram-negative, lactose-nonfermenting, oxidase-negative nosocomial pathogen. *A. baumannii* is one of the ESKAPE organisms that pose a global threat to humans due to constantly increasing resistance and limited treatment options. The aim of this study was to explore the distribution of sequence types (STs) among *A. baumannii* isolates from our tertiary care hospital, their epidemiological significance, and relationship with global trends.

Materials and Methods: A total of 59 MDRAB isolates, obtained from blood and respiratory samples of VAP patients, were included in the study. The isolates were identified using VITEK2 and MALDI-TOF MS further confirmed by WGS. MLST was performed on the isolates using a bioinformatics tool to determine their sequence types.

Result: Out of 59 isolates of *A. baumannii*, ST2 was found in 33 (55.9%), ST10 in 21 (35.6%), and ST10*, ST25, ST525, ST575 and an unknown ST were found in single isolates.

Conclusion: Our findings indicate that ST2 and ST10 sequence types are predominant in *A. baumannii* in our ICUs.

Keywords: *A. baumannii*, Sequence type, Ventilator associated pneumonia.

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1. Introduction

A. baumannii is a Gram-negative, lactose-nonfermenting, oxidase-negative nosocomial pathogen. The increasing rate of antimicrobial-resistant pathogens in developing countries such as India poses significant health issues.[1]

Infections caused by multi-drug resistant, extremely drug-resistant and pan-drug resistant organisms are challenging to manage due to limited treatment options.[1] The spread of multidrug-resistant (MDR) non-lactose-fermenting Gram-negative pathogens, such as *A. baumannii, P. aeruginosa, B. cepacia,* and *S.maltophilia* in healthcare settings is widespread and continues to increase in many countries.[1,2]

A. baumannii has become a significant nosocomial pathogen, known for causing multiple serious infections such as ventilator-associated pneumonia (VAP), septicemia, surgical site infections, and meningitis particularly among immunodeficient individuals and patients with underlying conditions, especially in ICUs.[3,4]

MDRAB is one of the ESKAPE organisms that pose a global threat to humans due to constantly increasing resistance and limited treatment options.[2] The WHO Bacterial Priority Pathogens List was updated in May 2024, highlighting the serious public health issues caused by carbapenem-resistant *A. baumannii* (CRAB), which remains in the critical priority category.[5] This classification indicates the urgent need for innovative treatments and interventions to

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combat this resilient and harmful pathogen, which continues to threaten effective infection control in healthcare settings worldwide.[3,5]

VAP is a leading cause of hospital-acquired infections. Despite progress in aseptic techniques, supportive patients care, and antibiotic treatments, VAP continues to be a major source of morbidity and mortality among patients in ICUs.[6,7] Incidence rates vary from 2 to 16 episodes per 1,000 ventilator days, influenced by diagnostic criteria, patient factors, preventative measures, and geographical variations.[6,7]

A. baumannii over time has evolved into a significant nosocomial pathogen with intrinsic resistance to multiple antibiotics, and some strains have acquired antibiotic resistance, making it a significant treatment challenge.[3,8,9] The acquires antibiotic resistance is through mobile genetic elements, including insertion sequences, plasmids, and antibiotic-resistant islands, which facilitate the transfer of resistance genes.[9,10]

Multilocus sequence typing (MLST) is an essential bioinformatics tool for detecting sequence types, which helps in understanding the epidemiology of pathogens. MLST identifies sequence types (STs) by analysing the allelic profiles of housekeeping genes,[11,12] providing essential insights into the genetic relationships among various isolates.[11]

MLST identifies unique sequence types (STs) of pathogens that provide insights into bacterial transmission, evolution, and population dynamics.[13] This information is crucial for tracking outbreaks, developing targeted therapeutic interventions, and informing public health policy. MLST helps researchers and clinicians better understand the complex dynamics of bacterial pathogens.[14]

This study aims to detect the distribution of STs among isolates of *A. baumannii*. By analysing these STs, we are able to understand their epidemiological significance and how they relate to patterns observed in global trends. This investigation will provide valuable insights into the genetic diversity of *A. baumannii* and its implications for public health and infection control strategies.

2. Materials and Methods

A prospective study was carried out at a tertiary care hospital in Vadodara, Gujarat, India. A 59 MDRAB were collected during the study periods included in this study.

Respiratory samples, comprising endotracheal secretions, tracheal secretions, and blood samples were obtained from mechanically ventilated patients. The collected samples were subsequently cultured on MacConkey agar, Nutrient agar and Blood agar to isolate and identify the pathogens. The MDR status of isolates decided based on WHO criteria.[15]

2.1. Identification by VITEK 2 compact system

All clinical isolates were identified using the Gram-negative identification (GN ID) card. The expected identification rate was up to 90%.

2.2. Identification by MALDI TOF MS: (Bruker MALDI BIOTYPER, version 4.1.100)

A small sample from a freshly grown bacterial colony on nutrient agar was placed on a MALDI target plate and added 1 µL of CHCA (α-cyano-4-hydroxycinnamic acid) matrix solution on top.[16] After it dried, the target plate was kept into the mass spectrometer for analysis. The Bruker MALDI Biotyper software, version 4.1.100, was used to identify the bacteria.

2.3. Whole genome Sequencing (WGS)

Bacterial DNA was extracted manually using the cetyltrimethylammonium bromide (CTAB) method.[17] The quality of the DNA was verified through gel electrophoresis, and once confirmed, the DNA was used to prepare libraries following the protocol of the Illumina Nextera XT kit. The quality of the prepared libraries was then assessed using the QIAXEL instrument. Finally, the libraries were sequenced using the Illumina NextSeq platform, which provided raw sequences.

Genomic annotation and assembly were conducted using BVBVRC version 3.35.5.[18] Isolate identification was done by the PubMLST software[19] which identified isolates based on their matches with reference sequence data in the NCBI database.

2.4. MLST typing

MLST was utilized to analyse *A. baumannii* genomes, leveraging the online MLST v2.0 tool hosted by the Center for Genomic Epidemiology.[12] The Pasteur scheme was applied, focusing on seven housekeeping genes: Recombinase A (recA), Ribosomal Protein L2 (rplB), Chaperonin 60 (cpn60), Citrate Synthase (gltA), Elongation Factor G (fusA), CTP Synthase (pyrG), and RNA Polymerase Beta Subunit (rpoB).[14] Sequencing these genes enabled the characterization of genetic diversity among A. baumannii isolates.

3. Result

Out of 236 isolates of *A. baumannii* obtained from patients with VAP. We could perform WGS on first 59 isolates and the same has been included in this study. Table 1 shows the number of isolates obtained from different clinical specimens. Specifically, 28 *A. baumannii* isolates were recovered from blood specimens, 24 from endotracheal secretions, and 7 from tracheal secretions. (Table 1)

All isolates were identified using VITEK2 and MALDI-TOF MS, and further confirmation was obtained through WGS.

All sequence reads from isolates obtained through WGS are analysed using MLST bioinformatics tools. The molecular typing of *A. baumannii* isolates reviled a diversity in the distribution of sequence types, highlighting the complexity of this pathogen. A total of 59 multidrug-resistant *A. baumannii* (MDRAB) isolates were characterized using MLST, a technique that provides insights into the genetic relationships among bacterial strains.

The pie chart (**Figure 1**) showed that the majority of *A. baumannii* isolates were clustered into two predominant sequence types: ST2 and ST10.

Table 2 shows that ST2 was identified in 33 (55.9%) isolates, and ST10 was identified in 21 (35.6%) isolates of *A. baumannii*. Beyond the dominant ST2 and ST10, various other sequence types were also identified, including ST25, ST10*, unknown type, ST525, and ST575, each represented by a single isolate. (**Table 2**)

The distribution of sequence types among *A. baumannii* isolates varied by specimen type, as represented in Table 3. ST2 was predominantly found in blood (18/59 isolates) and endotracheal secretions (12/59 isolates), and fewer in tracheal secretions (3/59). In contrast, ST10 was more frequently isolated from endotracheal secretions (12/59) and blood (6/59), and also from tracheal secretions (3/59). Other sequence types, such as ST25, were detected in a single tracheal secretion isolate, whereas ST10*, ST525, unknown type, and ST575 were each identified in a single blood isolate. (**Table 3**)

A phylogenetic tree in Figure 2 illustrates the relationships of 59 isolates of *A. baumannii*, using *A. baumannii* BJAB0715 as a reference genome. (**Figure 2**)

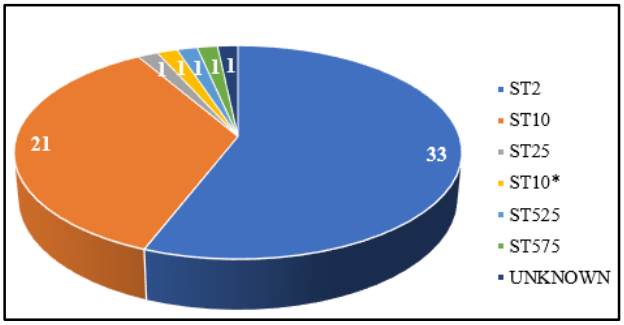

Figure 1: Distribution of Sequence type in *A.baumannii* isolates

Table 1: No. of isolates obtained from different clinical specimens

S.No.	Specimen	No. of isolates
1	Blood	28
2	Endotracheal secretion	24
3	Tracheal secretion	7

Table 2: Distribution of Sequence type in *A.baumannii* isolates

S.No.	Sequence type	No. of Isolates
1	ST2	33
2	ST10	21
3	ST25	1
4	ST10*	1
5	ST525	1
6	ST575	1
7	Unknown	1

Table 3: Distribution of sequence type in clinical specimen

S.No.	Sequence type	Specimen		
		Blood	**Endotracheal secretion**	**Tracheal secretion**
1	ST2	18	12	3
2	ST10	6	12	3
3	ST25	0	0	1
4	ST10*	1	0	0
5	ST525	1	0	0
6	ST575	1	0	0
7	Unknown	1	0	0
Total		28	24	7

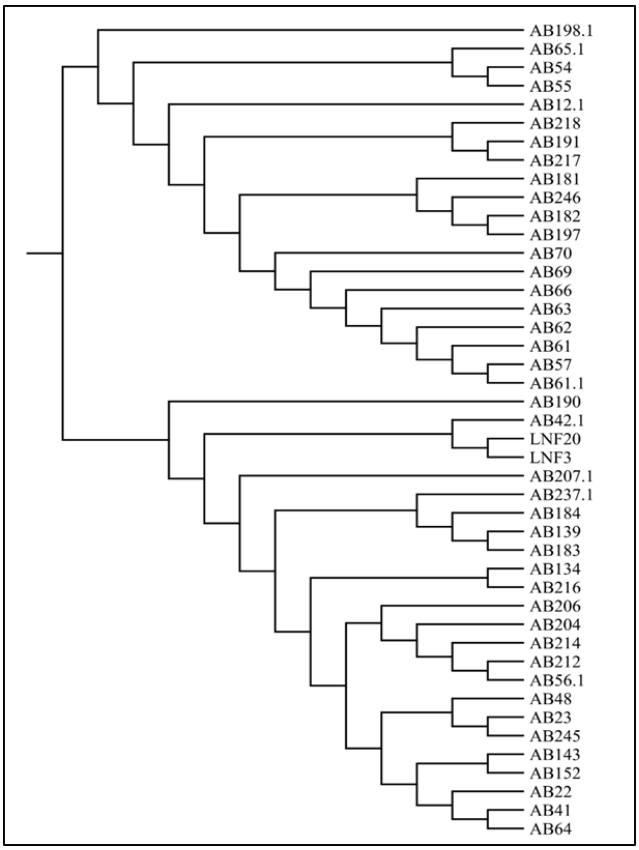

Figure 2: A phylogenetic tree illustrating the relationships of *A.baumannii* isolates using references genome of *A.baumannii* BJAB0715.

4. Discussion

A. baumannii is inherently resistant to a wide range of antibiotics and has the ability to acquire additional resistance through mechanisms such as horizontal gene transfer or genetic mutation.[9] This remarkable adaptability makes it a formidable challenge in the realm of infectious diseases.

The rise of *A. baumannii* as a prominent hospital-acquired pathogen is typically attributed to three main factors: its ability to show multidrug resistance through various mechanisms, the expression of multiple virulence factors, and its capacity to acquire or disseminate resistance genes via mobile genetic elements.[9,20,21]

In India, most studies focus on phenotypic and epidemiological characterization. Antimicrobial surveillance studies show that *Acinetobacter* species are the second most commonly isolated pathogens (45%), following *Pseudomonas* species (52%).[20] This report highlights that over 70% of *A. baumannii* isolates demonstrate non-susceptibility to most tested antibiotics, with the exception of colistin.[21]

MLST is a key method for determining genetic relationships and conducting molecular epidemiological studies of *A. baumannii* (14,21). Multi-drug-resistant clones of *A. baumannii* from various STs are present worldwide. ST types aid in epidemiological investigations, tracking the spread of resistant strains, identifying potential outbreaks, helps in preparation of treatment strategies based on resistance pattern and monitors prevalence or emergence of high-risk clones. This study's findings highlight the dominance of ST2 and ST10 sequence types among *A. baumannii* isolates, consistent with previous reports from various regions.[22]

In our study, 59 isolates were subjected to MLST analysis using the Pasteur Scheme, revealing ST2 as the predominant sequence type, followed by ST10. Notably, ST2, which is part of the international clone 2, has been reported as a dominant sequence type globally, consistent with both Indian and worldwide trends.[23]

Previous research has documented the prevalence of specific sequence types among *A. baumannii* isolates. Kumar et al. found that a significant proportion of isolates belonged to the ST2 genotype, specifically within the IC2 lineage.[24] In a separate study, Choudhary et al. employed MLST (Pasteur Scheme) to type 181 isolates of *A. baumannii*, identifying total 23 ST types, ST149 as the most prevalent sequence type, followed by ST2(11) and out of 23 STs found 4 new STs, ST2125, 2126 2128 and 2131 were all either single or double locus variants of ST2(11). Globally, ST2, associated with international clone 2, is a predominant sequence type among *A. baumannii* isolates, and this trend is mirrored in both Indian and international contexts.

The study by Pearl et al on genomic landscape on nosocomial *A. baumannii* showed that ST2 was most prevalent and associated with carbapenems resistance that highlights their epidemiological important.[23]

The study revealed significant clonal diversity in *A. baumannii* strains, with ST2 (Pasteur scheme) being the most prevalent sequence type. ST2 has been globally associated with CRAB outbreaks, highlighting its epidemiological importance. ST2 emerge as most prevalent ST type clone in most samples, suggesting their hypervirulent potential.[24]

A comparative analysis of sequence type profiles (Pasteur scheme) across different geographical regions revealed that ST2 is the predominant type, with a significant presence in Asia (65.2%), the Americas (39.3%), and Europe (32.6%).[23]

Research from North India identified ST-146, ST-110, ST-69, ST-103, ST-194, ST-108, and ST-188 as the most prevalent sequence types, whereas a study in South India found ST-538, ST-539, ST-103, and ST-576 in *A. baumannii* to be the dominant sequence types in their respective regions.[20] The high variability of STs suggests that exposure of selection pressure of antibiotics use in different clinical settings.

In Europe, ST2 is the predominant sequence type in *A. baumannii*, accounting for 83.9% of isolates (360/429), followed by ST1 (2.8%, 12/429) and ST636 (1.4%, 6/429).[25]

Research conducted by Baleivanualala in Fiji revealed seven distinct clusters of CRAB that corresponded to five sequence types: ST1, ST2, ST25, ST107, and ST1112. Notably, CRAB IC II/ST2 was the most prevalent strain in the region(25).Similarly, ST2 was also found to be common in our study, suggesting its widespread distribution.

The sequence types ST525 and ST575 are relatively rare among *A. baumannii* isolates. The distinct sequence types of *A. baumannii* exhibit unique allelic profiles in MLST genes, distinguishing them within the bacterial population.[26] Notably, a study in Argentina observed a surge in *A. baumannii* isolates producing NDM-1, all of which belonged to ST25.[27] In contrast, our findings revealed only one isolate, from a blood specimen, belonging to ST25.

A. baumannii has remarkable genomic plasticity. This bacterium can rapidly undergo genetic mutations, rearrangements, and acquire new traits through the integration of external genetic material, facilitated by mobile genetic elements.[28]

Strains of *A. baumannii* acquire antibiotic resistance due to the misuse or overuse of antibiotics, resulting in antibiotic selection pressure that contributes to antimicrobial resistance (AMR) and leads to the dissemination of resistant strains in the hospital environment.[29]

Specific sequence types (STs) linked to globally disseminated clones, such as those within the Global Clone 2 group (like ST2), frequently display extensive resistance to multiple antibiotics. This multidrug resistance enables these strains to thrive in healthcare settings.[29]

According to the antimicrobial surveillance data from the Indian Council of Medical Research (ICMR), blaOXA-23-like was the predominant carbapenemase, accounting for 95% of carbapenem resistance in *A. baumannii*, with Sequence Type 2 (ST2) emerging as a major clonal lineage associated with this resistance mechanism.[30]

The variation in sequence types of *A. baumannii* in our study suggests that there may be regional variability in the distribution of these sequence types.

Our study focused on the molecular epidemiology of *A. baumannii* sequence types isolated primarily from ventilated patients' specimens and explored their relationship with global trends.

Further studies are needed on the antibiotic resistance profiles and virulence of ST2 and ST10 isolates, as well as other sequence types. This information will aid in infection prevention and control, developing effective treatment strategies, and antimicrobial stewardship programs.

5. Conclusion

According to our study ST2 and ST10 sequence types are predominant in *A. baumannii* in our ICUs. Analyzing these sequence types, helped us better understand their distribution. Based on their Virulence factors and antibiotic resistance pattern better surveillance strategies of hospital-infection - control could be made. We can also suggest and incorporate new treatment changes into our Hospital Antibiotic Policy. Furthermore, tracking specific sequence types allows healthcare teams to detect outbreaks, understand the dynamics of *A. baumannii* transmission within healthcare settings, and implement precise interventions to curb its spread.

6. Authors' Contribution

Corresponding author: Conceptualization, Writing - original draft, Writing - review & editing.
First author: Conceptualization, Writing - original draft, Writing - review & editing,
Second author: Data curation, Investigation, Methodology.
Third author: Data analysis, Methodology.

7. Ethical Approval Statement

Approving body: Parul University Institutional Ethics Committee for Human Research (PU-IECHR).
Approval number: PUIECHR/PIMSR/00/081734/5812.
No direct experimentation was done on Humans or Animals.

8. Informed Consent Statement

No direct experimentation was done on Humans or Animals.

9. Data Availability

All data generated and analysed are included within this research article.

10. Conflict of Interest

None.

11. Source of Funding

None.

12. Acknowledgment

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