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Emerging Drug Resistance in *Acinetobacter* species: A Study on Isolation, Speciation, and Antimicrobial Susceptibility Patterns in a Tertiary Care Hospital

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Acinetobacter species are emerging pathogens in healthcare settings, responsible for many infections, including bacteremia, pneumonia, meningitis, peritonitis, and wound infections. Their ability to develop multidrug resistance through various resistance determinants poses significant challenges for treatment. This study aimed to isolate and speciate Acinetobacter using simple phenotypic tests, determine their antibiotic susceptibility profiles, and conduct molecular characterization of resistance genes. This prospective cross-sectional study was conducted from January 2023 to December 2023 to isolate and identify Acinetobacter isolates from confirmed infections using phenotypic methods, followed by antibiotic susceptibility testing. Among 108 confirmed cases of Acinetobacter infection, 73 were male patients, 49 were over 50 years old, and 49 were from the Intensive Care Unit (ICU). The most common specimens were endotracheal aspirates (36.1%) and sputum (34.2%). Levofloxacin and meropenem were the most effective antibiotics, with 81.5% sensitivity each, while ceftriaxone (81.4%), gentamicin (71.3%), cefepime (77%), and ceftazidime (70.3%) exhibited high resistance rates.All the isolates were sensitive to colistin and tigecycline. In terms of speciation, Acinetobacterbaumannii was predominant, accounting for 97.2% of isolates. Notably, Acinetobacter species were more frequently isolated from ICU patients (38%) than other wards. These findings underscore the critical need for vigilant infection control practices and tailored antibiotic stewardship programs in healthcare settings.

Keywords: Acinetobacterbaumannii; Acinetobacter infections; Antibiotic susceptibility test; Colistin; Ceftazidime-avibactam+aztreonam; ICU; MDR; Synergistic effect.

Acinetobacter species, particularly *Acinetobacter baumannii*(*A .baumannii*), have emerged as significant pathogens in both community and healthcare settings. These bacteria are highly adaptable and able to survive on both dry and moist surfaces, which allows them to persist in hospital environments and cause a variety of opportunistic infections. These infections range from pneumonia, often associated with endotracheal tubes, to bacteremia, meningitis, urinary tract infections, endocarditis, and wound and soft tissue infections.[1] Various risk factors contribute to these infections, including climatic conditions, diabetes mellitus, smoking, alcohol use, and chronic obstructive pulmonary disease (COPD).[2]

Globally, the prevalence of *A. baumannii* infections is increasing, particularly in hospital settings.[3] The World Health Organization (WHO) has classified carbapenem-resistant *A. baumannii* as one of the most critical antibiotic-resistant pathogens.[4] The prevalence rates of multidrug-resistant (MDR) *A. baumannii* are particularly concerning, with studies indicating that resistance rates are 65% to 80% in many countries worldwide.[5]

In India, the situation is particularly dire, with studies showing a significant rise in *A. baumannii* infections. According to Systemic review studies, the prevalence rate of MDR species ranges from 8.9 % to 73.2 % in different healthcare settings.[6,7]

This rise in resistance is largely due to factors such as the overuse and misuse of antibiotics, inadequate infection control practices, and the absence of stringent antibiotic stewardship programs. Even more concerning is the growing resistance to colistin, an antibiotic often used as a last resort. Studies indicate that the global resistance to colistin is around 4%, and similar trends are being observed in India, further limiting the treatment options for these infections. In response to the high level of antibiotic resistance and the variability in resistance profiles across different geographical areas, this study aims to achieve objectives such as (1) to isolate and identify the various *Acinetobacter* species 2) to determine the antibiotic susceptibility profiles of *Acinetobacter* ~~isolates~~ species, focusing on understanding the resistance patterns to critical antibiotics such as carbapenem, colistin, and other commonly used antibiotics 3) to investigate the synergistic effects of ceftazidime-avibactam and aztreonam combination therapy using the E-strip method.

MATERIALS AND METHODS

Study Design and Sample Collection: This study was a prospective cross-sectional study conducted in a tertiary care center in the Department of Microbiology over a year (January 2023 to December 2023). The study was approved by our Institutional Human Ethics Committee (SBMCH/002/SBMCH/IEHC/1828).

Sample Types and Collection

Clinical samples such as blood, pus, wound swabs, endotracheal (ET) aspirates, bronchoalveolar lavage (BAL), and sputum were collected from different wards within the hospital. Around 108 non-duplicate *Acinetobacter* isolates were isolated from the clinical samples.

Isolation: The samples collected were processed using standard microbiological techniques. Initially, the samples inoculated onto appropriate culture media, such as MacConkey agar (Hi media), blood agar (Hi media), and Nutrient agar (Hi media).[8]

Identification by Standard Biochemical Test

Once the colonies suspected to be *Acinetobacter* were isolated, they were identified using Gram Staining and standard biochemical tests. Common biochemical tests for *Acinetobacter* include oxidase, catalase, motility, carbohydrate utilization test, oxidation of glucose, beta hemolysis at 37°C and 42°C, and arginine hydrolysistests.[9] These tests help differentiate *Acinetobacter* from other Gram-negative bacilli.

Confirmation of Species by Vitex-2MALDI-TOF

The isolates were chosen and smeared over the sample locations on the target slide using loops. After that, the sample was covered with 1 ì of VITEK MS-CHCA matrix, and it was allowed to air dry until the matrix and sample co-crystallized. The VITEK MS (BioMérieux) system was then used to load the target slide containing all of the prepared samples to obtain the mass spectra of each sample's entire bacterial cell protein, primarily ribosomal protein.In the end, the mass spectra obtained for every sample were compared to those already known and recorded in the database. Based on how well the collected spectra matched the mass spectra in the database, a confidence score was assigned.[10]

Kirby-Bauer Disk Diffusion Method

The antibiotic susceptibility of the isolated *Acinetobacter* strains was determined using the Kirby-Bauer disk diffusion method. This method involves placing antibiotic-impregnated paper disks on an agar plate inoculated with the isolated bacteria. The antibiotics used were:Gentamicin (30 ìg) (Himedia), Amikacin (30 ìg) (Himedia), Ciprofloxacin (5 ìg) (Himedia), Levofloxacin (5 ìg) (Himedia), ceftazidime (ìg) (Himedia), Ceftriaxone (30 ìg) (Himedia), Cefepime (30 ìg) (Himedia), Piperacillin-tazobactam (100/20 ìg) (Himedia), Meropenem (30 ìg) (Himedia). After incubation, the zone of inhibition around each disk is measured

to determine the susceptibility of the bacteria to each antibiotic.The interpretation was made using CLSI guidelines 2023.[11]

Minimum Inhibitory Concentration (MIC) Determination

The MIC for colistin and tigecycline was determined using the VITEK-2 system (Biomerieux) using AST Card 406.

Detection of Ceftazidime-Avibactam and Aztreonam synergy using E strip

This method was done for only 5 MDR *Acinetobacter* strains isolated from ICU patients. Lawn cultures ofMDR *Acinetobacter* were done on the Mueller Hinton agar plate, and aztreonam-containing E-test strips were placed and diffused. The first E-test strip (Aztreonam) was removed after 10 minutes of incubation. The Ceftazidime-Avibactam E-strip was placed over the impression of the Aztreonam E-strip. The aztreonam strip was again placed over the ceftazidime/avibactam strip by gradient stacking. Then it was incubated for 16–18 hours after which the MIC value was noted.[12]

RESULTS

A total of 108 non-duplicate *Acinetobacter* isolates were obtained from various clinical samples. Of these, 103 (95.3%) were identified as *Acinetobacter baumannii* and 5 (4.6%) as *Acinetobacter lwoffii* (Figure 1). The distribution of isolates across wards showed that ICU patients contributed the highest number of isolates (42.59%, 46/108), followed by the surgical (19.4%, 21/108) and medical (15.7%, 17/108) wards (Table 1). The *Acinetobacter* infections were more prevalent in male patients (67.6%) compared to females (32.4%), as shown in Table 2.

Table 1. Department-wise distribution of the *Acinetobacter* species isolates

S.No	Department/ward	Isolation of isolates in Males	Isolation of isolates in Females	Total
1	ICU	38	8	46 (42.59 %)
2	Surgical ward	11	10	21 (19.4 %)
3	Medicine ward	14	3	17 (15.7 %)
4	Paediatric ward	1	4	5 (4.6 %)
5	Orthopaediatrics ward	9	9	18 (16.7 %)
6	Gynecology	-	1	1 (0.9 %)

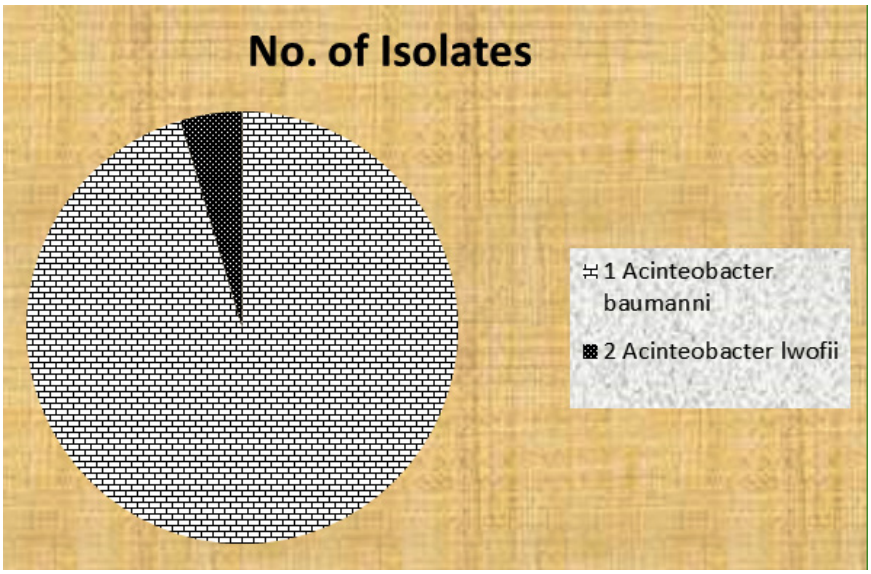

Fig. 1. Species-wise distribution of *Acinetobacter* spp

Age Distribution

The majority of patients with *Acinetobacter* infections were over 50 years old (37.9%, 41/108), followed by those aged 41-50 (10.2%, 11/108), and 31-40 (9.25%, 10/108) (Table 3). Only 0.9% of isolates were from patients aged 1-10 years, indicating that older adults were significantly more affected by these infections.

Gender Distribution by Sample Type

Table 4 highlights the gender-specific distribution of isolates from different sample types. Endotracheal (ET) aspirates yielded the highest number of isolates (36.1%, 39/108), with 30 from male patients and 9 from females. Sputum samples were the second most common source (34.2%, 37/108), with 24 from males and 13 from females. Pus samples accounted for 21.3% (23/108) of isolates, showing a similar gender trend, while blood and bronchoalveolar lavage (BAL) samples contributed to a smaller percentage of the isolates.

Duration of Hospitalization and Co-morbid Illness

Patients hospitalized for more than 7 days represented 54.6% (59/108) of the total cases, indicating that prolonged hospital stays were associated with a higher incidence of *Acinetobacter* infections (Table 2). Additionally, 54.6% of the patients had comorbid illnesses, further suggesting that *Acinetobacter* infections are more prevalent in patients with pre-existing health conditions.

Antibiotic Susceptibility Test

Antibiotic resistance patterns revealed significant resistance to commonly used antibiotics. Resistance rates for cephalosporins (cefepime,

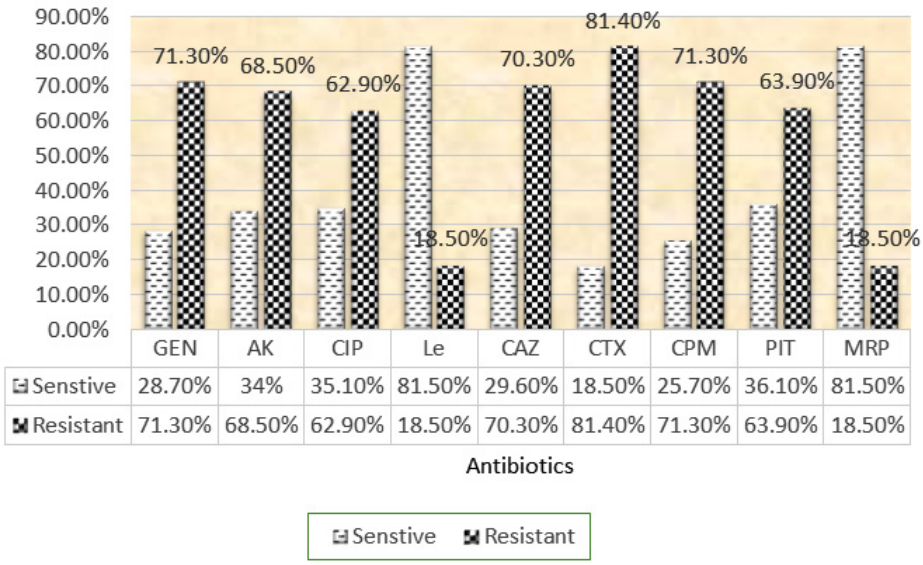

Fig. 2. Antibiotic sensitivity pattern of *Acinetobacter* isolates

Table 2. Risk factor analysis

Age	> 50 years	67 (62 %)
	< 50 years	41 (38%)
Gender	Male	73 (67.6%)
	Female	35 (32.4%)
Duration of hospitalisation	< 7days	49 (45.4%)
	> 7 days	59 (54.6%)
Co-morbid Illness	Present	59 (54.6%)
	Absent	49 (45.4%)

Table 3. Age-wise distribution of *Acinetobacter* isolates

S.No	AGE (in Years)	Number of Isolates
1	1-10	1 (0.9 %)
2	11-20	4 (3.7 %)
3	21-30	8 (7.4 %)
4	31-40	10 (9.25 %)
5	41-50	11(10.2 %)
6	> 50	41 (37.9 %)
	Total	108

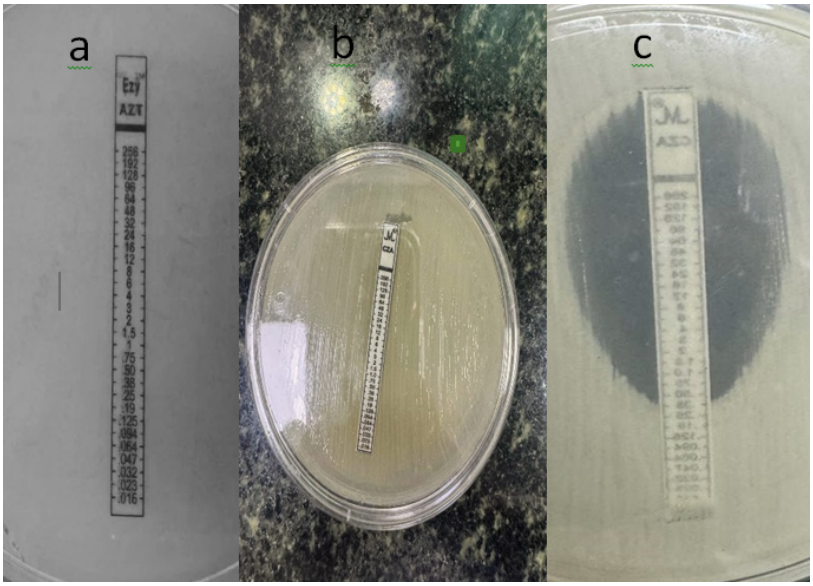

Fig. 3. Synergy testing with ceftazidime-avibactam and aztreonam E-strip
a) Aztreonam E-strip - No zone of inhibition seen, b) ceftazidime E-Strip -No zone of inhibition c) synergy testing with ceftazidime-avibactam and aztreonam E-strip - Zone of inhibition seen.

Table 4. Gender-wise distribution of the *Acinetobacter* spp., isolates in different samples

S. No	Sample	Isolation of isolates in Males	Isolation of isolates in Females	Total
1	ET aspirate	30	9	39 (36.1%)
2	Sputum	24	13	37 (34.2%)
3	Pus	14	9	23 (21.3%)
4	Blood	4	3	7 (6.5%)
5	BAL	1	1	2 (1.9%)
		73	35	108

cefotaxime, ceftazidime) ranged from 70.3% to 81.4%, while 71.3% of isolates were resistant to gentamicin, and 77% were resistant to cefepime (Figure 2). Ciprofloxacin and amikacin also exhibited high resistance rates at 62% and 68%, respectively. In contrast, levofloxacin and meropenem demonstrated the highest sensitivity, with 81.5% of isolates susceptible to these antibiotics. Notably, all isolates were sensitive to colistin and tigecycline, making them the most reliable options for treating *Acinetobacter* infections in this study.

Synergy Testing

The results of synergy testing with ceftazidime-avibactam and aztreonam (Figure 3) were encouraging, as all tested multidrug-resistant (MDR) isolates showed reduced minimum inhibitory concentrations (MICs), indicating that this combination therapy could be a promising option for treating MDR *Acinetobacter* infections. This finding is particularly significant given the high resistance to other commonly used antibiotics.

DISCUSSION

Acinetobacter infections have emerged as a significant threat in healthcare settings, particularly in intensive care units.[13] These infections commonly manifest as ventilator-associated pneumonia, bacteremia, and urinary tract infections.[14] The

prevalence of *Acinetobacter* pneumonia is higher in Asian and European hospitals compared to the United States.[13] Multidrug-resistant strains pose a significant challenge, with resistance rates varying geographically.[13,15] The mortality rate associated with *Acinetobacter* infections is high, reaching 45% in some studies.[14]

In our study,the majority of the isolates were *A .baumannii (95.3 %),* and 5 were Acinetobacter lwoffii (4.6 %), similar to a study.[16] The high prevalence of A .baumannii has highlighted the prominence of this species in hospital-acquired infections. Infections in our study were more prevalent in males (67.6) than females, similar to many studies.[6, 17,18] The predominance of male patients may reflect underlying health behaviors or greater exposure to healthcare interventions, such as mechanical ventilation, that predispose to infection.

In the present study, the infections were more common among the age group > 50 years, and patients with *Acinetobacter* infections tend to have longer hospital stays than those without infections. This demographic distribution is consistent with previous research, which suggests that older adults,[6] particularly those with underlying comorbidities,[19] are more susceptible to *A. baumannii* infections.

Acinetobacter infections are growing in intensive care units (ICUs), particularly in Asia and Europe.[13] The prevalence of *Acinetobacter* infections in ICUs ranges from 11.7% to 30.8%.[19- 20] Out of 108 isolates studied, 41(38 %) were from ICU patients. *A. baumannii* infection was more pronounced in the ICU than in other wards, this is in concordance with other studies.[6,9] Most of the patients in the ICU have an immunocompromised state, due to this, it's associated with increased length of stay in ICUs.[19] *Acinetobacter* species were recovered from respiratory samples (ET aspirate (36.1%) and sputum (34.2%) followed by wound samples, indicating a high prevalence of respiratory infections similar to some studies.[21] In this study, the isolation rates of *Acinetobacter* in ET aspirate and sputum are 36.1% and 34.2% similar to another study,[22] with isolation rates ranging from 31.3% to 46%. These nosocomial infections primarily affect the respiratory tract of intubated patients, with medical patients being more susceptible to lung infections, especially late-onset ventilator-associated pneumonia.[23] The high prevalence of *A. baumannii* in ICU patients underscores the need for stringent infection control measures, particularly in respiratory care settings where ventilator-associated pneumonia is a significant concern. Adopting rigorous hand hygiene, equipment sterilization protocols, and antimicrobial stewardship programs is essential to limit the spread of MDR strains .

Multidrug-resistant strains are common, with high resistance to penicillins, cephalosporins, and even extended-spectrum antibiotics.[19] Previously multidrug resistance was found in 60–80% of isolates, with strong resistance to carbapenems, cephalosporins, and other widely used antibiotics.[6,24] While carbapenems were traditionally the treatment of choice, increasing resistance has led to the reintroduction of polymyxins (colistin and polymyxin B) and the use of tigecycline. The retained sensitivity to levofloxacin (81.5%) and meropenem (81.5%) is a positive finding, but the increasing reports of carbapenem resistance in other regions suggest that continued surveillance is essential. Studies have shown varying susceptibility rates to these antibiotics and reported high susceptibility to colistin (99.2%) and polymyxin B (100%), with 94% susceptibility to tigecycline.[25] One of the most significant findings of this study is the complete sensitivity of all isolates to colistin and tigecycline. However, the global rise of colistin-resistant *A. baumannii* warrants caution, as resistance to this last-resort antibiotic could severely limit treatment options. To combat this threat, strict infection control practices and appropriate antibiotic policies must be implemented in ICUs.[26,27]

The ability of ceftazidime-avibactam+aztreonam to reduce the MICs of *Acinetobacter baumanii* and MBL-producing *Pseudomonas aeruginosa* is a potentially promising therapeutic option when faced with growing antimicrobial resistance which is evident from present study. All MDR isolates subjected to the ceftazidime-avibactam+aztreonam synergy test were sensitive. In case of the limited options available for MDR infections, this combination should be further explored in clinical trials to validate its efficacy.

CONCLUSION

This study confirms the predominance of *A. baumannii* as a major pathogen in ICU settings, with a significant proportion of isolates exhibiting multidrug resistance. The high resistance to commonly used antibiotics highlights the need for alternative therapeutic strategies. The sensitivity of all isolates to colistin and tigecycline supports their continued use in managing MDR infections, while the potential of combination therapies with ceftazidime-avibactam and aztreonam warrants further investigation. Future research should explore the genetic mechanisms driving resistance in *A. baumannii* and evaluate the efficacy of novel combination therapies in clinical settings. Such infection and resistance development to antibiotics can be prevented by hospital infection control practices, strengthening of antimicrobial stewardship program, and implementation of antimicrobial surveillance strategies.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

The study was approved by our Institutional Human Ethics Committee (SBMCH/002/SBMCH/IEHC/1828).

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Permission to reproduce material from other sources

Not Applicable

Authors Contributions

Dr.Bindu: Conceptualised and designed the study, and drafted the article; Dr.Chitralekha Saikumar: reviewed the article; Dr.Risha Mynooah collected and interpreted the data including the pictures

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