# Current Trends of Drug Resistance Patterns of *Acinetobacter baumannii* Infection in Blood Transfusion-dependent Thalassemia Patients

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## Abstract

**Objective:** The present study aimed to evaluate the current trends of drug resistance patterns of *Acinetobacter baumannii* infection in blood transfusion-dependent thalassemia patients. **Study Design:** This study was a cross sectional study, conducted at the Liaquat University of Medical and Health Sciences, Jamshoro/Hyderabad, Sindh, Pakistan from October 2014 to January 2016. **Subjects and Methods:** Of 921 blood samples, *A. baumannii* strains were isolated from 100 blood samples. Blood samples were processed for the isolation, identification, and drugs sensitivity as per the Clinical and Laboratory Standards Institute. *A. baumannii* strains were identified by microbiological methods and Gram's staining. API 20 E kit (Biomeriuex, USA) was also used for identification. Data were analyzed on Statisti × 8.1 (USA). **Results:** Mean ± standard deviation age was 11.5 ± 2.8 years. Nearly 70% were male and 30% were female ($P = 0.0001$). Of 921 blood transfusion-dependent thalassemia patients, 100 (10.8%) patients showed growth of *A. baumannii*. Drug resistance was observed against the ceftazidime, cefixime, cefepime, imipenem, meropenem, amikacin, minocycline, tigecycline, and tazocin except for the colistin. **Conclusion:** The present study reports drug-resistant *A. baumannii* in blood transfusion-dependent thalassemia patients. National multicenter studies are recommended to estimate the size of the problem.

**Keywords:** *Acinetobacter baumannii*, blood transfusion, Sindh, thalassemia

## INTRODUCTION

*Acinetobacter baumannii* is a Gram-negative obligate aerobic bacterium. *A. baumannii* genus includes more than thirty species. They are catalase positive, peroxidase negative, nonfermenting, and nonmotile coccobacilli.[1,2] *A. baumannii* is contracted through skin wounds, burn wounds, mucosal surfaces, intravenous, and urinary catheters.[2,3] *A. baumannii* is one of the causes of nosocomial infections transmitted by infusion pumps, resuscitation apparatus, and fomites.[4] Nowadays, the *A. baumannii* has emerged a worldwide cause of nosocomial infections.[5,6] The bacteremia, septicemia, lung infections, infective endocarditis, and urinary tract infections have been reported with *A. baumannii*.[7] Community-acquired infections have been reported.[1,5] Virulence factors include adherence to epithelial surfaces, adhesion to solids, iron chelation, skin colonization, biofilm formation, and production of gelatinase and proteases for pathogenicity.[8] Biofilm

production by *A. baumannii* strains is stimulated by iron deficiency in the media, for example, by the iron chelator 2, 2'-dipyridyl;[9] hence, iron is essential for growth.[5] Iron overload overwhelms in patients with thalassemia who receive blood transfusions continuously, particularly in those without iron chelation therapy.[10] Thalassemia is a genetic disorder of hemoglobin, presenting clinically with severe anemia requiring regular blood transfusions for patient survival and results in iron accumulation in the body.[10,11] Thalassemia is prevalent in the Mediterranean and Middle East regions and Southeast Asia. The β-thalassemia accounts for most of the

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cases in the developing countries requiring multiple blood transfusions.[12] Documentary registries lack in Pakistan for the prevalence of thalassemia, but estimates show approximately 5000–9000 infants are born with β-thalassemia yearly. Carrier rate is estimated 5%–7% with total 9.8 million carriers.[10,13]

Geographically, Pakistan is located in Southeast Asia and carries a large number of patients with thalassemia who are blood transfusion dependent. Blood transfusion causes iron load which is a notorious predisposing factor for the bacterial infections. The present study was designed to estimate the frequency of *A. baumannii* infection in blood transfusion-dependent thalassemia patients and drug susceptibility and resistance patterns at a tertiary care hospital of Sindh.

## Subjects and Methods

The present cross-sectional study was conducted at the Liaquat University of Medical and Health Sciences Jamshoro/Hyderabad, Sindh, Pakistan from October 2014 to January 2016. Liaquat University Hospital is a tertiary care hospital which caters millions of patients each year. Diagnostic and Research (DR) Laboratory is attached with Liaquat University Hospital. It is well equipped with modern facilities of blood testing. The Liaquat University Hospital and DR Laboratory are open for both indoor and outdoor patients. DR has a widespread network of collection points making it a pool of blood samples and bacterial isolates from a variety of patients. Blood transfusion-dependent diagnosed cases of thalassemia were the inclusion criteria. Nine hundred and twenty-one blood samples of blood transfusion-dependent thalassemia patients were screened for *A. baumannii*. Clinical presentation, duration and frequency of blood transfusions, previous laboratory investigations, and potential risk factors were noted. Samples included the blood, pus, sputa, stool, urine, and/or any other body fluids, which were inoculated on the culture media. Of 921 samples, *A. baumannii* were isolated from 100 blood samples.

### Bacterial isolation and antibiotic susceptibility testing
Blood samples were processed for the isolation, identification, and drugs sensitivity as per the Clinical and Laboratory Standards Institute (CLSI).
- MacConkey and Blood Agar Media (Oxoid Ltd., Cambridge, UK) were used for bacterial growth
- The isolates were identified and characterized using standard microbiological methods such as colony morphology and Gram's staining
- API 20 E kit (Biomeriuex, USA) was also used for identification purpose[14]
- Automated microbiology system (Phoenix; BD) was used for the determination of drug sensitivity and resistance pattern
- Antimicrobial agents were tested by the minimum inhibitory concentration (MIC) method with Phoenix System: ceftazidime, cefixime, cefepime, colistin, imipenem, meropenem, amikacin, minocycline, tigecycline, and tazocin (piperacillin/tazobactam)
- Kirby–Bauer disc diffusion method (Oxoid, UK) was used for antibiotic susceptibility, if intermediate sensitivity or resistance noted, then *E*-test was performed (AB Biodisk, Sweden)
- CLSI criteria were used for the results of the antimicrobial susceptibility tests[15]
- Quality control was ensured by testing the antibiotics against reference bacterial strains.

### Ethical approval and patients consent
Approval for conducting the study was taken from the Institute's Committee of Research Ethics. Informed written consent was necessary for the study protocol.

### Data analysis
Data were analyzed on Statistix 10.0 software (Tallahassee, FL 32317, USA). Student's *t*-test and Chi-square test were used for the continuous and categorical variables analysis at 95% confidence interval ($P \leq 0.05$).

## Results
Mean ± standard deviation age of study participants was 11.5 ± 2.8 years. Nearly 70% were male and 30% were female ($P = 0.0001$). First blood transfusion age was 3.5 ± 0.9 years, and total years of blood transfusion were 9.5 ± 1.3 years. Almost 65% of patients gave a history of two transfusions a week ($P = 0.0001$). Of 921 blood transfusion-dependent thalassemia patients, bacterial growth of *A. baumannii* was observed in 100 (10.8%) patients. MIC concentrations were categorized as sensitive, intermediate sensitive, and resistant for *E*-test and disc diffusion technique. Drug resistance of *A. baumannii* for ceftazidime was 97%, cefixime - 90%, cefepime - 91%, colistin - 0%, imipenem - 71%, meropenem - 69%, amikacin - 24%, minocycline - 89%, tigecycline - 89%, and for tazocin was 76%.

## Discussion
*A. baumannii* is a globally emerging pathogen targeting the critically sick patients.[16] The present study is first of its design conducted in blood transfusion-dependent thalassemia patients at our tertiary care hospital. It is the first study being reported on frequency and drug susceptibility patterns of *A. baumannii* in blood transfusion-dependent thalassemia patients. Of 921, 100 patients showed growth of *A. baumannii* showing frequency of 10.8%. Drug resistance of *A. baumannii* was found as ceftazidime was 97%, cefixime - 90%, cefepime - 91%, colistin - 0%, imipenem - 71%, meropenem - 69%, amikacin - 24%, minocycline - 89%, tigecycline - 89%, and tazocin was 76%. To the best of our knowledge, this is the first study being reported from Sindh. Patients with thalassemia acquire the *A. baumannii* infection by intravenous catheters.[17] Out of 921 samples, *A. baumannii* was isolated from 100 (10.8%) specimens in the present study. The

finding, i.e., 10.8% is comparable finding to a previous study which reported 1.6 bacterial infections/100 patient-years;[18] however, the true incidence and prevalence of *A. baumannii* infection in blood transfusion-dependent thalassemia are not known. The results of the present study are in keeping with previous studies.[17,18] The finding of *A. baumannii* infection in blood transfusion-dependent thalassemia patients is a unique study being reported from a tertiary care hospital of Sindh. The iron abundance is one of the predisposing factors facilitating bacterial infections in thalassemia.[9,19] Sequestration of iron occurs by the siderophore proteins of *A. baumannii*.[20-22] Previous studies reported 82.2% bacteremia in adults and 15.8% in children caused by *A. baumannii* in intensive care unit.[16,23,24] The findings of above studies are inconsistent with the present study findings because of different study populations. *A. baumannii* has now acquired drug resistance, particularly for the carbapenems,[25,26] and the findings are consistent with the present study. Multidrug-resistant *A. baumannii* had acquired extended drug resistance against the aminoglycosides and cephalosporins.[25-27] Yadegarynia *et al*.[27] have recently reported on the resistance patterns of *A. baumannii*. They reported high drug resistant against cefepime, gentamicin, meropenem, tigecycline, and imipenem. Another recent study[28] reported that the frequent use of carbapenems is causing the drug-resistant *A. baumannii* against imipenem and meropenem. They also reported high drug resistance against ceftazidime, cefepime, amikacin, tazocin, and levofloxacin.[28] The findings support the drug resistance of *A. baumannii* observed in the present study. A previous study[29] reported 100% susceptibility of *A. baumannii* to imipenem and approximately 69% for the ceftazidime and gentamicin. The findings are in contradistinction to the present study. Susceptibility of *A. baumannii* in the present study for imipenem, amikacin, and ceftazidime was noted as 19%, 76%, and 0%, respectively. However, a study[30] reported 74.4% cephalosporin and 38.3% imipenem resistance, which are consistent with the present study. A study from Jordan[31] reported high drug resistant similar to the present study. They reported *A. baumannii* strains exhibited approximately 70% resistance for imipenem and meropenem. The findings are consistent with the present study as shown in Table 1. Our findings are also consistent with previous studies.[32,33] Other previous studies[34,35] reported 72% of *A. baumannii* isolates were drug resistant. The findings support the present study. However, one study has demonstrated that high resistance rates to imipenem and carbapenems were significantly correlated with resistance rates to β-lactams, aminoglycosides, and fluoroquinolones.[28] In conclusion, *A. baumannii* has become already drug resistant in blood transfusion-dependent thalassemia patients, and there is a risk of emergence of multidrug resistance and extensively drug-resistant strains for which strategies should be planned in advance. This study highlights the alarming current drug resistance patterns of *A. baumannii* in our population with thalassemia. A controlled and restrictive use of antibiotics is recommended after culture and sensitivity to overcome the problem of drug resistance.

**Table 1: Antibiotic Drug Sensitivity Testing of *Acinetobacter baumannii* by *E*-test and Disc Diffusion Technique**

| Antibiotics | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Ceftazidime | 0 | 3 | 97 |
| Cefixime | 3 | 7 | 90 |
| Cefepime | 7 | 2 | 91 |
| Colistin | 100 | 0 | 0 |
| Imipenem | 19 | 10 | 71 |
| Meropenem | 21 | 8 | 69 |
| Amikacin | 76 | 0 | 24 |
| Minocycline | 19 | 0 | 81 |
| Tazocin | 15 | 9 | 76 |
| Tigecycline | 89 | 16 | 5 |

## CONCLUSION

The present study reports drug-resistant *A. baumannii* thalassemia patients who are blood transfusion dependent; this is an alarming situation. Drug resistance was observed for the ceftazidime, cefixime, cefepime, imipenem, meropenem, amikacin, minocycline, tigecycline, and tazocin except for the colistin. National multicenter studies are recommended to estimate the size of problem and strategies be implemented urgently on the issue. This will help in establishing national antibiotic and infection control in our hospitals.

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## Conflicts of interest
There are no conflicts of interest.

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