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

Suhail Ahmed Almani, Ali Naseer, Sanjay Kumar Maheshwari, Pir Maroof, Raza Naseer, Haji Khan Khoharo

Department of Medicine, Liaquat University of Medical and Health Sciences, Jamshoro, 1Department of Medicine, Abbasi Shaheed Hospital, Karachi, 2Department of Medicine, Faculty of Medicine and Allied Medical Sciences, Isra University, Hyderabad, Sindh, Pakistan

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 (Biomerieux, 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, 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.[1,2] 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...
cases in the developing countries requiring multiple blood transfusions.\[12\] Documentaries 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 \textit{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 \textit{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, \textit{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 (Biomeriux, 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 \textit{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 \textit{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**

\textit{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 \textit{A. baumannii} in blood transfusion-dependent thalassemia patients. Of 921, 100 patients showed growth of \textit{A. baumannii} showing frequency of 10.8%. Drug resistance of \textit{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 \textit{A. baumannii} infection by intravenous catheters.\[17\] Out of 921 samples, \textit{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.\textsuperscript{[18]} however, the true incidence and prevalence of \textit{A. baumannii} infection in blood transfusion-dependent thalassemia are not known. The results of the present study are in keeping with previous studies.\textsuperscript{[17,18]} The finding of \textit{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.\textsuperscript{[9,19]} Sequestration of iron occurs by the siderophore proteins of \textit{A. baumannii}.\textsuperscript{[20-22]} Previous studies reported 82.2% bacteremia in adults and 15.8% in children caused by \textit{A. baumannii} in intensive care unit.\textsuperscript{[16,23,24]} The findings of above studies are inconsistent with the present study findings because of different study populations. \textit{A. baumannii} has now acquired drug resistance, particularly for the carbapenems,\textsuperscript{[23,26]} and the findings are consistent with the present study. Multidrug-resistant \textit{A. baumannii} had acquired extended drug resistance against the aminoglycosides and cephalosporins.\textsuperscript{[25-27]} Yadegarynia \textit{et al.}\textsuperscript{[27]} have recently reported on the resistance patterns of \textit{A. baumannii}. They reported high drug resistant against ceftepime, gentamicin, meropenem, tigecycline, and imipenem. Another recent study\textsuperscript{[24]} reported that the frequent use of carbapenems is causing the drug-resistant \textit{A. baumannii} against imipenem and meropenem. They also reported high drug resistance against ceftazidime, cefepime, amikacin, tazocin, and levofloxacin.\textsuperscript{[28]} The findings support the drug resistance of \textit{A. baumannii} observed in the present study. A previous study\textsuperscript{[29]} reported 100% susceptibility of \textit{A. baumannii} to imipenem and approximately 69% for the ceftazidime and gentamicin. The findings are in contradistinction to the present study. Susceptibility of \textit{A. baumannii} in the present study for imipenem, amikacin, and ceftazidime was noted as 19%, 76%, and 0%, respectively. However, a study\textsuperscript{[30]} reported 74.4% cephalosporin and 38.3% imipenem resistance, which are consistent with the present study. A study from Jordan\textsuperscript{[31]} reported high drug resistant similar to the present study. They reported \textit{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.\textsuperscript{[32,33]} Other previous studies\textsuperscript{[34,35]} reported 72% of \textit{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.\textsuperscript{[28]} In conclusion, \textit{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 \textit{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 baumanii by E-test and Disc Diffusion Technique**

| Antibiotics     | Sensitive | Intermediate | Resistant |
|-----------------|-----------|--------------|-----------|
| Cefazidime      | 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 \textit{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.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Al-Mously N, Hakawi A. \textit{Acinetobacter baumannii} bloodstream infections in a tertiary hospital: Antimicrobial resistance surveillance. Int J Infect Control 2013;vol.9:12.
2. Al-Anazi KA, Al-Jasser AM. Infections caused by \textit{Acinetobacter baumannii} in recipients of hematopoietic stem cell transplantation. Front Oncol 2014;4:186.
3. Obeidat N, Jawdat F, Al-Bakri AG, Shehabi AA. Major biologic characteristics of \textit{Acinetobacter baumanii} isolates from hospital environmental and patients’ respiratory tract sources. Am J Infect Control 2014;42:401-4.
4. Uwingabiye J, Frikh M, Lemmouer A, Bssaibis B, Belefquih B, Maleb A, \textit{et al.} \textit{Acinetobacter} infections prevalence and frequency of the antibiotics resistance: Comparative study of Intensive Care Units versus other hospital units. Pan Afr Med J 2016;23:191.
5. Gentile V, Frangipani E, Bonchi C, Minandri F, Runci F, Visca P. Iron and \textit{Acinetobacter baumannii} biofilm formation. Pathogens 2014;3:704-19.
6. Antunes LC, Visca P, Towner KJ. \textit{Acinetobacter baumannii}: Evolution of a global pathogen. Pathog Dis 2014;71:292-301.
7. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: Multidrug-resistant \textit{Acinetobacter baumannii}. Nat Rev Microbiol 2007;5:939-51.
8. Eveillard M, Kempf M, Belmonte O, Pailhories H, Joly-Guillou ML. Reservoirs of \textit{Acinetobacter baumanii} outside the hospital and potential involvement in emerging human community-acquired infections. Int J Infect Dis 2013;17:e802-5.
9. Tomaras AP, Dorsey CW, Edelmann RE, Actis LA. Attachment to and biofilm formation on abiotic surfaces by \textit{Acinetobacter baumanii}: Involvement of a novel chaperone-usher pil assembly system. Microbiology 2003;149(Pt 12):3473-84.
10. Ansari SH, Shamshi TS, Ashraf M, Bohray M, Farzana T, Khan MT, et al. Molecular epidemiology of β-thalassemia in Pakistan: Far reaching implications. Int J Mol Epidemiol Genet 2011;2:403-8.

11. Weatherall DJ, Clegg JB. The Thalassemia Syndromes. 4th ed. Oxford: Blackwell Science; 2001.

12. Angastiniotis M, Modell B. Global epidemiology of hemoglobin disorders. Ann N Y Acad Sci 1998;850:251-69.

13. Ahmed S, Saleem M, Modell B, Petrou M. Screening extended families for genetic hemoglobin disorders in Pakistan. N Engl J Med 2002;347:1162-8.

14. Koneman EW, Allen SD, Janda WM, Schereckenberger PC, Winn JW. Color Atlas and Text Book of Diagnostic Microbiology. 5th ed. Philadelphia, New York: Lippincott; 1997. p. 211-302.

15. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 2009;53:3471-84.

16. Yadegarynia D, Abedy SH, Gachkar L, Rahmati Roodsari S. Prevalence and drug resistance of Acinetobacter baumannii in ICU of teaching hospital. J Appl Environ Biol Sci 2013;3:22-7.

17. Al-Marzoqi AH, Shamran AR, Al-Hindi ZS. Bacterial and viral infections associated with thalassemia patients in Hillah City. Iraq Acad Sci J 2008;1:7-15.

18. Wang SC, Lin KH, Chen JP, Lu MY, Jou ST, Lin DT, et al. Severe bacterial infection in transfusion-dependent patients with thalassemia major. Clin Infect Dis 2003;37:984-8.

19. Lee HW, Koh YM, Kim J, Lee JC, Lee YC, Seol SY, et al. Capacity of multidrug-resistant clinical isolates of Acinetobacter baumannii to form biofilm and adhere to epithelial cell surfaces. Clin Microbiol Infect 2008;14:49-54.

20. Weinberg ED. Iron availability and infection. Biochim Biophys Acta 2009;1790:600-5.

21. Antunes LC, Imperi F, Towner KJ, Visca P. Genome-assisted identification of putative iron-utilization genes in Acinetobacter baumannii and their distribution among a genotypically diverse collection of clinical isolates. Res Microbiol 2011;162:279-84.

22. Zimbler DL, Penwell WF, Gaddy JA, Menke SM, Tomaras AP, Connerly PL, et al. Iron acquisition functions expressed by the human pathogen Acinetobacter baumannii. Biometals 2009;22:23-32.

23. Casal M, Rodríguez F, Johnson B, Garduno E, Tubau F, de Lejarazu RO, et al. Influence of testing methodology on the tigecycline activity profile against presumably tigecycline-non-susceptible Acinetobacter spp. J Antimicrob Chemother 2009;64:69-72.

24. Ko KS, Suh JY, Kwon KT, Jung SI, Park KH, Kang CI, et al. High rates of resistance to colistin and polymyxin B in subgroups of Acinetobacter baumannii isolates from Korea. J Antimicrob Chemother 2007;60:1163-7.

25. Feizabadi MM, Fathollahzadeh B, Taherikalani M, Rasoolinejad M, Sadeghfard N, Aligholi M, et al. Antimicrobial susceptibility patterns and distribution of blaOXA genes among Acinetobacter spp. Isolated from patients at Tehran hospitals. Jpn J Infect Dis 2008;61:274-8.

26. Ekrami A, Kalantar E. Bacterial infections in burn patients at a burn hospital in Iran. Indian J Med Res 2007;126:541-4.

27. Yadegarynia D, Khalili Azad M, Gachkar L, Rahmati Roodsari S, Arab-Mazar Z. Drug resistance of Acinetobacter in selected hospitals. Novel Biomed 2015;3:103-10.

28. Bayram A, Balci I. Patterns of antimicrobial resistance in a surgical Intensive Care Unit of a university hospital in Turkey. BMC Infect Dis 2006;6:155.

29. Li J, Nation RL, Owen RJ, Wong S, Spelman D, Franklin C. Antibiograms of multidrug-resistant clinical Acinetobacter baumannii: Promising therapeutic options for treatment of infection with colistin-resistant strains. Clin Infect Dis 2007;45:594-8.

30. Scott P, Deye G, Srinivasan A, Murray C, Moran K, Hulten E, et al. An outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus complex infection in the US military health care system associated with military operations in Iraq. Clin Infect Dis 2007;44:1577-84.

31. Petersen K, Riddle MS, Danko JR, Blazes DL, Hayden R, Taskar SA, et al. Trauma-related infections in battlefield casualties from Iraq. Ann Surg 2007;245:803-11.

32. Cunha BA. Optimal therapy for multidrug-resistant Acinetobacter baumannii. Emerg Infect Dis 2010;16:170.

33. Lin MF, Lan CY. Antimicrobial resistance in Acinetobacter baumannii: From bench to bedside. World J Clin Cases 2014;2:787-814.

34. Halachev MR, Chan JZ, Constantinidou CI, Cumley N, Bradley C, Smith-Banks M, et al. Genomic epidemiology of a protracted hospital outbreak caused by multidrug-resistant Acinetobacter baumannii in Birmingham, England. Genome Med 2014;6:70.

35. Curcio D, Fernández F. Acinetobacter spp. susceptibility to tigecycline: A worldwide perspective. J Antimicrob Chemother 2007;60:449-50.