RESEARCH ARTICLE

Phenotypic detection of extended-spectrum beta-lactamase in multidrug-resistant acinetobacter baumannii isolated in Fauji Foundation Hospital Rawalpindi

Asim Ali Shah,[1] Yasir Ali,[2] Ayesha Maqbool,[3] Shahid Ahmad Abbasi[4]

Abstract

Objective: To evaluate the phenotypic detection of extended-spectrum betalactamase in multidrug-resistant *acinetobacter baumannii.*

Methods: The cross-sectional study was conducted at the Department of Microbiology, Fauji Foundation Hospital, Rawalpindi, Pakistan, from August 2018 to April 2019, after the ethical approval from the Institutional Review Committee. Consecutive Non- probability sampling technique was used, and comprised clinical specimens, including pus, blood, sputum, urine, tracheal tubes and canula double lumen, which were processed using standard protocols. Colonies of *acinetobacter baumannii* were identified by gram staining and Analytical Profile Index-20E kit. Combination disc method was used for the identification of extended-spectrum beta-lactamse. Clinical and Laboratory Standards Institute guidelines were used for antimicrobial susceptibility. Data was analysed using SPSS 22 and Sample size was calculated by using earlier study with 5 % margin of error and 95 % confidence level.

Results: Of the 78 isolates, 58(74.4%) related to females and 20(25.6%) to males. There was no extended-spectrum beta-lactamse producer. Imipenem, meropenem, cefotaxime, ampicillin and ceftazidime showed 100% resistance, while colistin and polymyxin B were sensitive to all strains. The incidence rate was high in samples isolated from tracheal tubes 47(60.3%), followed by pus 21(26.9%). Age was not found to be a significant factor ($p>0.05$).

Conclusion: *Acinetobacter baumannii* showed a high resistance to multiple drugs and was not confined to any specific age group. Colistin and polymyxin B were found to be better choices.

Keywords: Acinetobacter baumannii, Antimicrobial susceptibility, Extended-spectrum beta-lactamase.

(JPMA 71: 1144; 2021) **DOI: https://doi.org/10.47391/JPMA.1265**

Introduction

Acinetobacter (A.) baumannii is a gram-negative, non-motile coccobacilli, oxidase-negative bacterium and shows a wide range of infections, including urinary tract infection, pneumonia and burn infections.[1] It is responsible for nosocomial infections worldwide.[2] Multidrug resistant (MDR) strains of *A. baumannii* are a great concern and cause increased death rates in intensive care unit (ICU) patients.[3] Some bacterial strains produce extended spectrum beta-lactamase (ESBL) enzymes which hydrolyze beta-lactam antibiotics, including third and fourth generation of cephalosporins (ceftazidime, cefixime, cefotaxime, cefodizime, ceftizoxime, ceftriaxone and cefepime), penicillin and monobactams.[4] Clavulanic acid is used as an inhibitor of beta-lactamase.[5]

Carbapenems were considered the drug of choice while treating *A. baumannii* infections.[6] Due to increased used of carbapenems, carbapenem-resistant *A. baumannii* strains were isolated which worsened the situation, especially in ICU patients.[7] ESBLs are isolated from different organisms of the *Enterobacteriaceae* family, including *Escherichia (E.) coli, proteus spp., Klebsiella (K.) pneumoniae, Serratia (S.) marcescens, providencia spp. and citrobacter spp.*[8]

ESBLs are reported in *A. baumannii* strains isolated in different countries, such as the United Kingdom, the United States, South Korea, Belgium, France and India.[9] *A. baumannii* can remain for a long period of time on the equipment surface and human skin in ICUs and burns wards.[10]

Polymerase chain reaction (PCR) technology is widely used in different hospitals to study ESBL at the molecular level which is more accurate and highly reliable.[11] The current study was planned to evaluate the phenotypic detection of ESBL in MDR *A. baumannii.*

Materials and Methods

The cross-sectional study was conducted at the Department of Microbiology, Fauji Foundation Hospital, Rawalpindi, Pakistan, from August 2018 to April 2019, after the ethical approval from the Institutional Review Committee. Consecutive Non-probability sampling technique was used, and comprised clinical samples collected from different wards and ICUs related to

[1,4]Department of Microbiology, Fauji Foundation Hospital, Rawalpindi, [2]Department of Biology, [3]Department of Molecular Biology, Virtual University of Pakistan Rawalpindi campus, Pakistan.

Correspondence: Yasir Ali. Email: yasirswati85@gmail.com

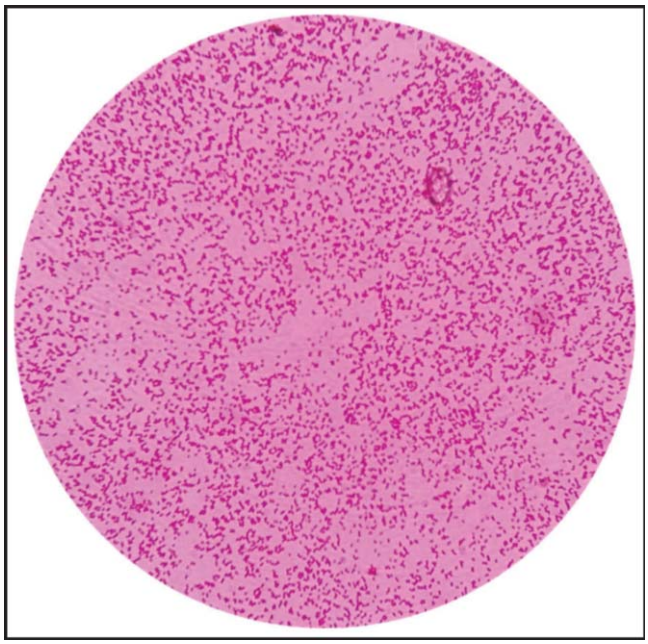

Figure-1: Microscopic view of *acinetobacter baumannii* after gram staining.

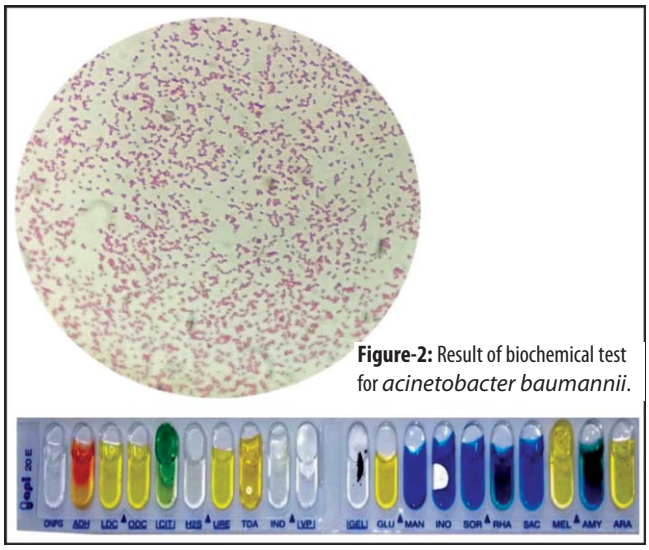

Figure-2: Result of biochemical test for *acinetobacter baumannii*.

patients aged 1-100 years. Samples from the out-patient department (OPD) and from patients aged <1 year were excluded. The specimens, including tracheal tubes (TT), pus, blood, sputum, urine and canula double lumen (CDL), were inoculated on MacConkey and blood agar, and incubated at 37°C for 24 hours. Colonies were then confirmed by gram staining (Figure-1) and Biomeriuex (USA) Analytical Profile Index (API) 20E kit (Figure-2). Combination disc method was used for phenotypic detection of ESBL in ceftazidime-resistant *A. baumannii* strains. Ceftazidime (30µg) disk alone or in combination with clavulanic acid (ceftazidime 30µg + clavulanic acid 10µg) was placed centre-to-centre at 20mm distance on Mueller Hinton agar plate streaked with suspension. The plates were than incubated at 37°C, for 24h. After incubation, the zone was observed of both discs, alone and in combination with clavulanic acid. The test was considered positive for ESBL when the zone diameter in the presence of clavulanic acid was >5mm larger than in the absence of clavulanic acid. Kirby Bauer disc diffusion method was performed for antibiotic sensitivity testing on Mueller Hinton agar plates as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.[12] The disks used were imipenem (10µg), meropenem (10µg), cefotaxime (30µg), ampicillin (10µg), gentamicin (10µg), ciprofloxacin (5µg), ceftazidime (30µg), amikacin (30µg), doxycycline (30µg), minocycline (30µg), colistin (10µg) and polymyxin b (300 U).

Data was analysed using SPSS 22. Chi-square test was performed for *A. baumannii* incidence in different age groups and $p<0.05$ was considered statistically significant.

Sample size was determined by using previous study with 95% confidence level and margin of error 5%.[13]

Results

Of the 78 isolates, 58(74.4%) related to females and 20(25.6%) to males, and 55(70.51%) of the samples related to ICU patients (Figure-3). There was no ESBL producer

Table-1: Acinetobacter baumannii specimen distribution.

Specimen	No. of Isolates	Among Different Age Group Patients (Years)				
		1-20	21-40	41-60	61-80	81-100
TT	47 (60.3%)	10	2	24	9	2
Pus	21 (26.9%)	3	5	5	7	1
Blood	5 (6.4%)	2	1	1	1	0
Sputum	2 (2.6%)	0	0	0	2	0
Urine	2 (2.6%)	1	0	1	0	0
CDL	1 (1.3%)	0	0	1	0	0
Total	78	16(20.5%)	8(10.3%)	32(41%)	19(24.4%)	3(3.8%)

TT: Tracheal tube. CDL: Canula double lumen.

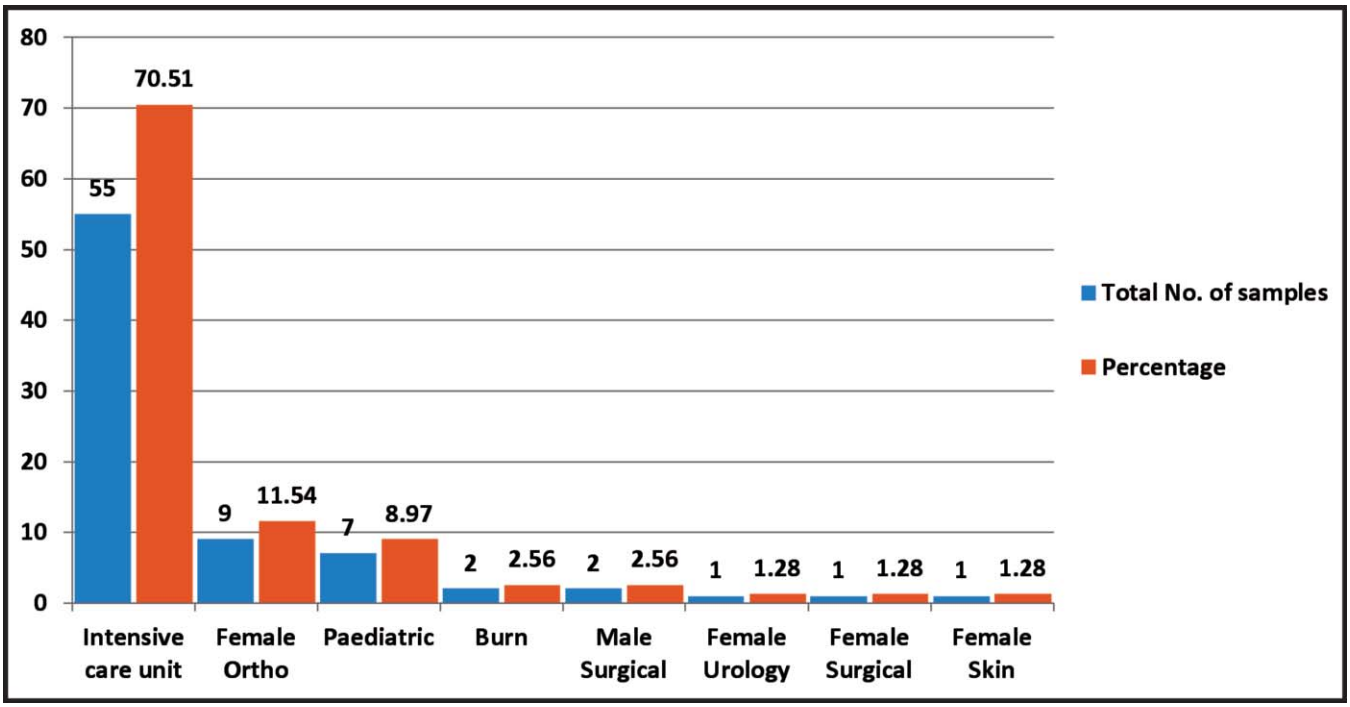

Figure-3: Specimen distribution isolated from different wards.

Table-2: Drug Resistance pattern of acinetobacter baumannii.

Antibiotic	Resistance (%)
Imipenem	78 (100)
Meropenem	78 (100)
Cefotaxime	78 (100)
Ampicillin	78 (100)
Ceftazidime	78 (100)
Gentamicin	77 (98.72)
Ciprofloxacin	76 (97.44)
CoTrimoxazole	75 (96.15)
Sulzone	74 (94.87)
Amikacin	72 (92.31)
Doxycline	58 (74.36)
Minocycline	50 (64.10)
Colistin	0
Polymyxin B	0

among the smaples. The incidence rate was high in TT samples 47(60.3%), followed by pus 2 (26.9%), blood 5(6.4%), sputum 2(2.6%), urine 2(2.6%) and CDL 1(1.3%). *A. baumannii* incidence was high 32(41%) in those aged 41-60 years, followed by 19(24.4%) aged 61-80 years, 16(20.5%) aged 01-20 years, 08(10.3%) aged 21-40 years and 3(3.8%) aged 81-100 years (Table-1). Age had no significant correlation with *A. baumannii* incidence ($p>0.05$). Among all the antibiotics, colistin and polymyxin B were sensitive to all strains (Table-2, Figure-4).

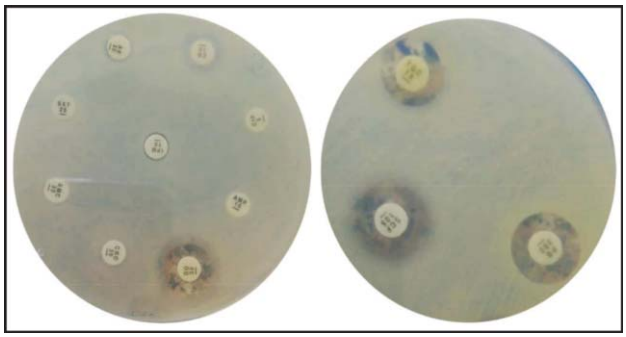

Figure-4: Antibiotic sensitivity plates.

Discussion

In the current study, no single strain of *A. baumannii* was positive for ESBL, which is in line with studies conducted in Iran and Pakistan.[14,15] Low ESBL production 32(27.5%), 7(35%), 28(25%) was seen in *A. baumannii* isolates in certain studies.[16-18]

In the current study none of the *A. baumannii* strains was resistant to polymyxin B and colistin, which was also reported earlier.[19] Two studies found 100% sensitivity of polymyxin B.[20,21] The ratio of antibiotic resistance reported earlier also support the findings of the current study.[22,23] The ratio of *A. baumannii* was high in respiratory samples isolated in a Pakistani study.[24] *A. baumannii* caused infection in both male and female

patients and the incidence was high in ICU specimens.[25,26] A study in Iran showed highest isolation of *A. baumannii* from ICU, which correlates with the current findings.[13]

The single-centre orientation and cross-sectional design are limitations of the current study which also had a high numbers of TT isolates from the ICU. A multi-centre study with equal number of samples from different wards is recommended.

Conclusion

MDR was found in *A. baumannii* strains, and no strain was positive for ESBL. The incidence rate was high in TT samples isolated from the ICU.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Qureshi ZA, Hittle LE, O'Hara JA, Rivera JI, Syed A, Shields RK, et al. Colistin-resistant Acinetobacter baumannii: beyond carbapenem resistance. Clin Infect Dis. 2015; 60:1295-303.
2. Abdar MH, Taheri-Kalani M, Taheri K, Emadi B, Hasanzadeh A, Sedighi A, et al. Prevalence of extended-spectrum beta-lactamase genes in Acinetobacter baumannii strains isolated from nosocomial infections in Tehran, Iran. GMS Hyg Infect Control. 2019; 14:Doc02.
3. Khatun N, Farzana R, Lopes BS, Shamsuzzaman SM. Molecular characterization and resistance profile of nosocomial Acinetobacter baumannii in intensive care unit of tertiary care hospital in Bangladesh. Bangladesh Med Res Counc Bull. 2015; 41:101-7.
4. Kaur A, Singh S. Prevalence of Extended Spectrum Betalactamase (ESBL) and Metallobetalactamase (MBL) Producing Pseudomonas aeruginosa and Acinetobacter baumannii Isolated from Various Clinical Samples. J Pathog. 2018; 2018:6845985.
5. Litake GM, Ghole VS, Niphadkar KB, Joshi SG. Phenotypic ESBL detection in Acinetobacter baumannii: a real challenge. Am J Infect Dis 2015; 11:48-3.
6. Nath H, Barkataki D. Prevalence of ESBL and MBL producing Acinetobacter Isolates in Clinical Specimens in Tertiary Care Hospital, Assam, India. Int J Curr Microbiol Appl Sci. 2016; 5:515-22.
7. Owlia P, Azimi L, Gholami A, Asghari B, Lari AR. ESBL-and MBL-mediated resistance in Acinetobacter baumannii: a global threat to burn patients. Infez Med. 2012; 20:182-7.
8. Moosavian M, Sirous M, Shams N. Phenotypic and Genotypic Detection of Extended Spectrum β-lactamase and Carbapenemases Production Including bla TEM, bla PER and bla NDM-1 Genes Among Acinetobacter baumannii Clinical Isolates. Jundishapur J Microbiol. 2017; 10:e58206.
9. Batra P, Khurana S, Govindaswamy A, Aravinda A, Bajpai V, Ayyanar M, et al. Antibiotic resistance profile and co-production of extended spectrum beta lactamases and AmpC in Acinetobacter spp. in a level 1 trauma center from India. J Lab Physicians.2019; 11:128-32.
10. Al-Hasnawy HH, Saleh RH, Hadi BH. Existence of a ESBL genes in Escherichia coli and Acinetobacter baumannii isolated from different clinical specimens. J Pharm Sci Res. 2018; 10:1112-7.
11. Salimizand H, Modarresi F, Azizi O, Mansouri S, Rahmati M, Barkhordari K. Antimicrobial Profile and Phenotypic Metallo-β-Lactamase Detection of Acinetobacter baumannii Isolated From Clinical and Environmental Specimens. Zahedan J Res Med Sci. 2015;17.
12. CLSI. Performing standards for antibiotic susceptibility. 27th edition supplement M100. Wayne PA. CLSI; 2016.
13. Abdar MH, Taheri-Kalani M, Taheri K, Emadi B, Hasanzadeh A, Sedighi A, et al. Prevalence of extended-spectrum beta-lactamase genes in Acinetobacter baumannii strains isolated from nosocomial infections in Tehran, Iran. GMS Hyg Infect Control. 2019; 14:Doc02.
14. Zarifi E, Eslami G, Khaledi A, Vakili M, Vazini H, Zandi H. Prevalence of ESBLs in Acinetobacter baumannii isolated from intensive care unit (ICU) of Ghaem hospital, Mashhad, Iran. J Pure Appl Microbiol. 2017; 11:811-9.
15. Begum S, Hasan F, Hussain S, Shah AA. Prevalence of multi drug resistant Acinetobacter baumannii in the clinical samples from Tertiary Care Hospital in Islamabad, Pakistan. Pak J Med Sci. 2013; 29:1253-8.
16. Kaur A, Singh S. Prevalence of Extended Spectrum Betalactamase (ESBL) and Metallobetalactamase (MBL) Producing Pseudomonas aeruginosa and Acinetobacter baumannii Isolated from Various Clinical Samples. J Pathog. 2018; 2018:6845985.
17. El-Baky ARM, Farhan SM, Ibrahim RA, Mahran KM, Hetta HF. Antimicrobial resistance pattern and molecular epidemiology of ESBL and MBL producing Acinetobacter baumannii isolated from hospitals in Minia, Egypt. Alexandria J Med. 2020; 56:4-13.
18. Smail SB, AL-Otrachi KI. Phenotypic Characterization of Extended-Spectrum Beta-Lactamases and Metallo-Beta-Lactamase of Multi Drug Resistant Acinetobacter baumannii Causing Nosocomial Infections in Erbil City. Al-Mustansiriyah J Sci. 2020; 30:51-6.
19. Goudarzi H, Azad M, Seyedjavadi SS, Azimi H, Chirani AS, Omrani VF, et al. Characterization of integrons and associated gene cassettes in Acinetobacter baumannii strains isolated from intensive care unit in Tehran, Iran. J Acute Dis. 2016; 5:386-92.
20. Subramaniyan A, Nair S, Joseph NM, Kanungo R. Profile of multidrug resistant Acinetobacter baumannii infections among hospitalized patients. J Med Sci Clin Res. 2017; 5:23111-5.
21. Kooti S, Motamedifar M, Sarvari J. Antibiotic resistance profile and distribution of oxacillinase genes among clinical isolates of Acinetobacter baumannii in Shiraz teaching hospitals, 2012-2013. Jundishapur J Microbiol. 2015; 8:e20215.
22. Amer RR, Alzomaili BT, AlTuwaijri RM, AlZahrani RS, AlHarbi SH, AlThubaiti A, et al. Prevalence of Antimicrobial Resistance Among Gram-Negative Isolates in an Adult Intensive Care Unit at a Tertiary Care Center in Saudi Arabia (2010-2014). Ann Saudi Med.2010; 30:364-9.
23. Safari M, Nejad AS, Bahador A, Jafari R, Alikhani MY. Prevalence of ESBL and MBL encoding genes in Acinetobacter baumannii strains isolated from patients of intensive care units (ICU). Saudi J Biol Sci. 2015; 22:424-9.
24. Sohail M, Rashid A, Aslam B, Waseem M, Shahid M, Akram M, et al. Antimicrobial susceptibility of Acinetobacter clinical isolates and emerging antibiogram trends for nosocomial infection management. Rev Soc Bras Med Trop. 2016; 49:300-4.
25. Lusignani LS, Starzengruber P, Dosch V, Assadian O, Presterl E, Diab-Elschahawi M. Molecular epidemiology of multidrug-resistant clinical isolates of Acinetobacter baumannii. Wien Klin Wochenschr 2017; 129:816-22.
26. Ren G, Zhou M, Ding N, Zhou N, Li Q. Analysis on distribution features and drug resistance of clinically isolated Acinetobacter baumannii. Exp Ther Med. 2016; 12:1715-8