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ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND EMERGING ANTIBIOGRAM TREND OF *ACINETOBACTER BAUMANNII* ISOLATES FROM A TERTIARY CARE HOSPITAL

Nazia Khursheed[1], Qurat-ul-Ain[1], Maria Mushtaq Ali[2] and Fareeha Adnan[1*]

[1]*Department of Microbiology, Indus Hospital and Health Network*
[2]*Indus Hospital Research Center, Indus Hospital and Health Network*
Email addresses: nazia.khursheed@tih.org.pk; fareeha.adnan@tih.org.pk; qurat.zahid@tih.org.pk; maria.hasnain@tih.org.pk
***Corresponding author**: fareeha.adnan@tih.org.pk; 03003548803
Postal address: The Indus Hospital, Plot C-76, Sector 31/5, Opposite Darussalam Society, Korangi Crossing, Karachi. +92 (21) 35112709-17, **Fax:**+92 (21) 35112718

ABSTRACT

Emergence of multidrug-resistant (MDR) bacteria has become a global problem because infections due to MDR are hard to treat due to resistance to two or more classes of drugs, limiting the therapeutic options. The present study was undertaken to analyze the susceptibility pattern of *Acinetobactor baumannii (A. baumanni*) isolated from January 2016 to December 2020. The isolates were tested for the susceptibility to ceftazidime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, co-trimoxazole, piperacillin/ tazobactam, and colistin by standard disc diffusion technique and assessed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. A total of 1332 *A. baumannii* isolates were collected which were mostly retrieved from blood samples (29.3%). The frequency of multi-drug resistance (MDR) was 69.4%. Overall the high resistance was observed for Co-trimoxazole and Ceftazidime (77%), followed by Fluoroquinolones (74%), Piperacillin/tazobactam (73%), Carbapenem (73%), Amikacin (66.6%), and Gentamycin (65%). In contrast, only 1.2% of the tested isolates were resistant to colistin. Increase in resistance against commonly used antibiotics were alarmingly high. Our data accentuate the need for comprehensive national control programs to control antibiotic resistance and to adopt the best therapeutic option for *A. baumanni* infections.

Keywords: *Acinetobacter baumannii*, Antibiotic Resistance, Fluoroquinolone, Carbapenem, Aminoglycoside, Beta-lactam, Colistin.

INTRODUCTION

Acinetobacter baumannii, gram-negative cocci, was previously considered as commensal of humans, but later has emerged as a troublesome pathogen in hospital environment where it may cause severe infections, including endocarditis, respiratory infections, meningitis, and septicemia (Bergogne-Berezin and Towner, 1996). The World Health Organization (WHO) has placed this pathogen in the list of "priority microorganisms" that pose the greatest threat to human health owing to its potential to develop drug resistance and consequently scarce options of efficient drugs to treat *A. baumanni* associated infections (Khaledi *et al*., 2017). It is accountable for the high morbidity and mortality rate extending from 27% to 91% particularly in immunocompromised patients (Uwingabiye *et al*., 2016). The patients infected with MDR *Acinetobacter* strains have longer stay in the hospital and ICU as compared to the patients infected with susceptible *Acinetobacter* strains (Nguyen and Joshi, 2021). Therefore, constant monitoring of antimicrobial resistance (AMR) patterns provides important data which benefits microbiologists and infectious disease professionals in understanding the spread of resistance mechanisms and to strengthen antibiotic stewardship programs. Both regional and national surveillance systems are deficient in Pakistan. Thus, in this study we assessed *in vitro* activity of clinically relevant antibiotics against *A. baumannii* isolated from the Indus Hospital and Health Network, Karachi, during 2016 to 2020.

METHODS

This descriptive cross sectional study evaluated the susceptibility profile of *A. baumannii* and their resistance trend from January 2016 to December 2020 at Department of Microbiology, Indus Hospital and Health Network. Patient data such as age, gender, and source of specimen were recorded and compiled. A number of clinical samples collected and processed aseptically during routine diagnostic work from both, in-patient and out-patient wards.

Multiple isolates from the same patient and from same specimen within a month were treated as a single "unique isolate".

Acinetobacter species was isolated on chocolate agar, 5% sheep blood agar and MacConkey agar. Identification of bacterial isolates were done by grams staining, colony morphology and biochemical reactions. *Acinetobacter* was recognized as gram negative, non-lactose fermenting, non-motile, oxidase negative coccobacilli. Identification was confirmed by analytical profile index (API 20 E). Antibiotic susceptibility was determined by the Kirby Bauer disc diffusion method (Biemer and Science, 1973). The bacterial suspension of each sample was made and compared with 0.5 McFarland turbidity standard. Inoculations were performed on Mueller-Hinton agar plates, incubated at 35 °C for 18 h, and the diameter of the zones of inhibition were evaluated as recommended by Clinical and Laboratory Standards Institute (CLSI) 2019 guidelines (Smiline Girija, 2019). The antibiotics including Amikacin, Ceftazidime, Ciprofloxacin, Colistin, Gentamycin, Imipenem, Meropenem, Piperacillin/Tazobactam and Co- trimoxazole were tested. Antibiotic disks were obtained from Oxoid.

Acinetobacter isolates that were resistant to three or more antibiotic classes were referred as MDR (Magiorakos *et al*., 2012).

The continuous variable like age was calculated as mean (± STD). While all the categorical variables like antibiotic susceptibility, multidrug resistance, specimen and gender were represented numerically along with percentage. Data (was entered and analyzed using SPSS version 24.0.

RESULTS

Bacterial isolates

A total of 1332 *A. baumannii* isolates were collected from 2016 to 2020. Most of the isolates (28.5%) were retrieved from blood samples (Table 1). Of these samples, 57.8% were from male and 42.1% were from female with a mean age of 35.4±0.6 years.

Percentage of MDR *A. baumannii*

Susceptibility pattern of *A. baumannii* strains was evaluated using 9 antibiotics by the disk diffusion technique. The results revealed that 69.4% of the *A.baumannii* isolates were MDR strains (**Fig. 1**).

Table 1. Types and percentage of various clinical specimens.

Specimen	Number (%)
Blood	378 (28.5)
Respiratory	328 (24.6)
Urine	173 (12.9)
Pus	171 (12.8)
Others	106 (7.9)
Tissue	79 (5.9)
Wounds	58 (4.3)
Sterile body fluid	39 (2.9)
Total	1332

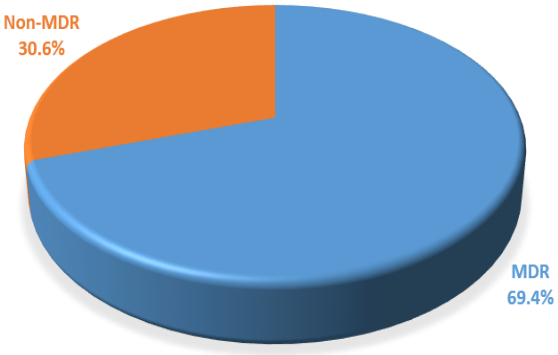

Fig. 1. Proportion of MDR *Acinetobacter baumannii* in clinical isolates.

DISCUSSION

Over the last decade, MDR *A. baumannii* has emerged as one of the most strenuous pathogen, with limited therapeutic options. The present study signifies a surveillance update on antimicrobial resistance status and trends among *A. baumannii* in a tertiary care hospital in 5-year duration. It will help to trace the changes in the antimicrobial drug resistance pattern among *A. baumannii* isolates and to modify treatment guidelines and antibiotic prescription practices.

MDR *A. baumannii* is a major ventilator associated pathogen found to be frequently isolated from respiratory tract specimens but interestingly in our study preeminent number of *A. baumannii* strains were recovered from blood samples (28.5%), this value is far greater than reported by Cisneros and Rodriguez where only 1.3% septicemia was observed due to *A. baumannii*. Moreover, on the contradictory to the present study respiratory tract was reported as the prime site of *A. baumannii* infections by Ben Haj Khalifa (Namiganda *et al*., 2019).

The essential finding of the study is the high resistance to the antibiotics that are generally considered as the best treatment option for *A. baumannii*. Among various clinical specimens, an average of 70% of the isolates were MDR which is in accordance with the past studies carried out in Kathmandu (Amatya and Acharya, 2015), India (Banerjee *et al*., 2018), Lebanon (Kanafani *et al*., 2018) and Nepal (Raut *et al*., 2020). The global data indicated that community and hospital-acquired infection (HAIs) and the prevalence of institutional outbreaks due to *A. baumannii* has significantly increased in the past years particularly in ICU (Howard, O'Donoghue, Feeney, and Sleator, 2012). *A. baumannii* is responsible for 0.7–4.6% of all healthcare associated infections with rates of MDR ranging from 47% to 93% according to SMART surveillance program 2016 (Lob *et al.*, 2016).

Table 2. Antibacterial susceptibility of *Acinetobacter baumannii* between 2016 and 2020.

Antibiotics	Year									
	2016 (n=178)		2017 (n=241)		2018 (n=367)		2019 (n=237)		2020 (n=309)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
AK	49.5	50.5	35.6	64.4	42.4	57.6	24.5	75.5	15.8	84.2
CAZ	25.5	74.5	20.6	79.4	35.6	64.4	4.2	75.8	26.8	73.2
CIP	53.5	46.5	26.2	73.8	48.1	51.9	21.2	78.8	20.7	79.3
CT	96.8	3.2	98.6	1.4	98.8	1.2	100	0	99.5	0.48
G	36.8	63.2	31.4	68.6	41	59.0	34.5	65.5	29.6	70.4
IPM	36.8	63.2	34.2	65.8	36.3	63.7	16.8	83.2	9.6	90.4
MEM	37.3	62.7	34.2	65.8	36.2	63.8	17.8	82.2	9.2	90.8
SXT	20.3	79.7	20.5	79.5	27.8	72.2	24.4	75.6	16.5	83.5
TZP	35.1	64.9	31.2	68.8	35.5	64.5	316	84	11	89.0

Carbapenems serves as a first-line therapy for *A. baumannii* infection (Labarca *et al.*, 2016). Globally, there is a rapid increase in development of carbapenem resistant *A. baumannii* (CRAB). In the developing and developed countries more than 55% of *A. baumannii* isolates are carbapenem resistant (Mera *et al.*, 2010; Rosenthal *et al*., 2012). The major cause of carbapenem resistance in *A. baumannii* is the production of carbapenemases (Manchanda, Sanchaita, and Singh, 2010) making it the most difficult pathogens. The majority of the patients suffered from CRAB infections are severely ill and are susceptible to other infections, prolonged diseases, and invasive life support measures. The overall resistance against carbapenem is 73% and is found to be increased from 63% to 90% during our study. Current surveillance studies in Latin America revealed that *Acinetobacter* isolates have 8% to 63% of susceptibility to this class of antibiotic (Labarca *et al*., 2016). Furthermore, in the recent years many studies from different countries confirmed the increase in carbapenem resistance ranging from 25 to 90% (Shareek *et al.*, 2012) (Chang *et al*., 2012); (Nazmul, Jamal, and Fazlul, 2012). Owing to the increase in carbapenem resistance during the last decade, this antibiotics class offers limited cure for the *Acinetobacter* infections.

Closely following carbapenem, fluoroquinolones were found to be the second most common antibiotics to which high resistance was exhibited by the understudy organism with an increase of 7% in the entire study span. The present study reported ciprofloxacin resistance in approximately 74% of the isolates which showed similarity to the resistance reported by Chinese antimicrobial resistance surveillance system in 2018 i.e. 75% (24). Concordant findings were also reported by European Antimicrobial Resistance Surveillance Network reports of 2012 and 2013 (16) as well Middle Eastern countries. The observation of high resistance could be attributed to inappropriate administration of the antibiotic for the management of common flu and other bacterial infections.

The present study also reported the resistance of β-lactam antibiotics including piperacillin /tazobactam combination and ceftazidime against *A. baumannii* infection. A steady rise in the resistance to piperacillin /tazobactam was observed from 65% to 89% in the study span with an average of 73.3% isolates being found

resistant which was similar to a European study (36% -75%) but comparatively less than the prevalence reported by Nazmul *et al*. (Nazmul *et al*., 2012) and Badave *et al* (Badave *et al.*, 2015) i.e. 77.5% and 84.7%, respectively. On the other hand, the trend observed in case of ceftazidime was undulating with a rise to 84.7% in 2018 from 74% at the initiation of the study followed by a decrease to 73.3% at end of study. On an average, 77.5% of the isolates were found to be resistant to the antibiotic which was comparably higher (52%) than reported by Ben Haj Khalifa (Namiganda *et al.,* 2019).

Resistance to aminoglycoside has also increased particularly for amikacin from 50.6% to 84.2% during 2016 to 2020. Overall, non–susceptibility to amikacin was 66.5% which is considerably lower than observed in Iran (83.9%) (Rashvand *et al*., 2021). On the other hand, the resistant rate against Gentamycin was marginally increased from 63.2% to 70.5% as compared to other antibiotics, the overall resistance rate was as 65%. This observation is comparable to the results obtained in a study conducted in India (Rani *et al.*, 2015), where the gentamycin was reported as most effective therapeutic option after colistin for *A. baumannii* infections.

Resistance to co-trimoxazole increased non-significantly from 79% to 83% in *A. baumannii* isolates during the study time. However, the total rate of resistance to this antibiotic was 78%. In a study conducted in the United States, the rate of resistance to co-trimoxazole was 55.3% (Zilberberg *et al.*, 2016). Even though co-trimoxazole has not usually been suggested for the treatment of MDR *Acinetobacter* infections, it might be considered in combination with other agents in absence of adequate therapeutic options (Falagas *et al.*, 2015)

The only drug for which we observed 99% sensitivity is colistin. Many studies have reported 80 to 100% sensitivity of *A. baumanni* against colistin (Tewari *et al.,* 2018). Our results are in correlation with the study of Jaggi *et al* (Jaggi *et al.*, and Diseases, 2012), where 1.2% resistance was reported towards colistin. Additionally, Vakili and colleagues (Vakili *et al*., 2014) from Iran reported 11.6% resistance to this drug. Contradictory, increased occurrence of *Acinetobacter* resistance for colistin is reported in some countries (Sohail *et al*., 2016). In Greece 7.9% of *A. baumannii* clinical isolates were resistant to colistin (Maraki *et al*., 2016). According to the Surveillance System in the United States, the susceptibility of *A. baumannii* isolates to polymyxin B is 95.4% (Manchanda *et al*., 2010). Numerous studies have reported 57 –77% cure rates with colistin in severely ill patients due to MDR *Acinetobacter* species infections. Since colistin is the only treatment options for the management of most of the infections instigated by understudy microorganism, increasing frequency of colistin resistance could be troublesome. Therefore, cautious prescribing practices will be essential in conserving the effectiveness of these 'last resort' antimicrobial agents.

There are certain limitations in this study. As this study is retrospective cross sectional in nature and many aspects including variations in length of hospital stay and shift of patients from the ICU to the ward could not be determined while evaluating the trends.

CONCLUSION

Our findings revealed the high burden of MDR *A. baumannii* in our setting. Hence, we firmly advised the implementation of a nationwide adequate surveillance system. More judicious use of antibiotics and clinical trials of various combinations of existing antibiotics are required immediately. This would facilitate the effective monitoring of resistance frequency, discriminating antibiotic resistance trends and prevalence to develop effective tools in antibiotic treatment programs. This would ultimately help to preclude the prevalence of drug-resistant variants.

DECLARATIONS

Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Patients consent

This is a cross sectional study, electronic medical records have been reviewed for a five years period. It was not be possible to take consent from the patients. However, all possible efforts are made to ensure data confidentiality.

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