



Original Article

Genotypic Strain Determination of Multi-Drug Resistance (MDR) and Extended Drug Resistance (XDR) in *Acinetobacter Baumannii* from Tertiary Care Hospitals in LahoreMaryam Sheikh¹, Qurat Ul Ain Amjad¹, Seerat Fatima Tu Zahra^{2*}, Jalees Khalid Khan³ and Zahid Munir⁴¹Department of Pathology, King Edward Medical University, Lahore, Pakistan²Department of Pathology, Shalamar Medical and Dental College, Lahore, Pakistan³Department of Pathology, Lahore General Hospital, Lahore, Pakistan⁴Department of Community Medicine, Institute of Public Health, Lahore, Pakistan

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ABSTRACT

Antibiotic resistance in *Acinetobacter baumannii* poses a major future challenge, with a sharp rise in drug-resistant infections significantly limiting treatment options. **Objectives:** To determine the frequency of multidrug-resistant (MDR) and extended drug-resistant (XDR) *Acinetobacter baumannii* and the frequency of blaOXA-23, blaOXA-24, blaOXA-51, and blaOXA-58 among all other resistance genes responsible for resistance in *Acinetobacter baumannii*. **Methods:** A descriptive cross-sectional study was conducted in the Department of Pathology, King Edward Medical University, Lahore. Specimens were collected from the (Neonatal ICU, ICU, CCU) of Mayo Hospital, Lahore, and were processed at the hospital laboratory. *Acinetobacter baumannii* was identified on CHROMagar based on colony morphology and growth conditions, confirmed by biochemical tests. Antimicrobial sensitivity was assessed using the disc diffusion method, and PCR was performed for genotypic identification, with primer details in the data collection procedure. **Results:** Out of 67 samples, 19 (23.36%) were positive for *Acinetobacter baumannii*. Among these, blaOXA-23 (57.89%) was the most common resistance gene, followed by blaOXA-51 (47.36%). All 19 had the OXA-58 strain. The isolates showed 100% resistance to Penicillin, Cefazidime, Cefepime, Imipenem, Levofloxacin, Doxycycline, and Septran. The most effective antibiotics were Ampicillin + Sulbactam (42.11%) and Piperacillin + Tazobactam (26.32%). **Conclusions:** The frequency of *Acinetobacter baumannii* was 28.36%. according to the study results. Among these positive isolates, carbapenem-resistant genes blaOXA-23 (57.89%) and bla-OXA-58 (47.36%) were the most frequent drug-resistant genes which were isolated. Regarding antimicrobial susceptibility, very few drugs showed sensitivity for *Acinetobacter baumannii*, which poses a major challenge for clinicians.

INTRODUCTION

Morphology of *Acinetobacter baumannii* consists of the following characteristics: Gram-negative coccobacilli. It is immotile and has an aerobic nature. It can easily spread from one patient to another patient very easily especially in hospital settings. It can also persist in the environment for many days [1]. The intensive care units (ICUs) are the most common location for severe infections caused by *A. baumannii*. Ventilator-associated pneumonia (VAP),

secondary meningitis, endocarditis, septicemia, infections of the skin, soft tissues, urinary system, and infections resulting from prosthetic devices are among the many illnesses it causes [2]. *Acinetobacter baumannii* can enter the body through various routes as open wounds, mechanical ventilators and intravascular catheters [3]. *Acinetobacter baumannii* has become a major threat to intensive care units (ICUs) after *Pseudomonas aeruginosa*



and methicillin-resistant *Staphylococcus aureus* (MRSA). They are directly related to high morbidity and mortality as well as long hospital stays [4]. *Acinetobacter baumannii* is now being considered an emerging threat of outbreaks in hospital settings throughout the world. The Infectious Diseases Society of America (IDSA) has included *Acinetobacter baumannii* among the six deadliest pathogens. Among all clinically isolated, aerobic, and facultative gram-negative pathogens, the overall percentage of *A. baumannii* varies by region; it ranges from 0.7% in North America to 4.6% in the Middle East, according to reports [5]. Pakistan is one of the most affected nations in South and Southeast Asia by carbapenem-resistant *A. baumannii*. More than 60% of *A. baumannii* isolates in Pakistan were found to be carbapenem-resistant [6]. A number of recent studies have shown that the prevalence of *A. baumannii* infections is rising in various parts of Pakistan. According to one study, *A. baumannii* is the most common bacterium responsible for bloodstream infections (54.2%) and ventilator-related pneumonia (30%) in Karachi [7]. A major worry related to the isolates of drug-resistant *A. baumannii* isolates is their direct relation with various nosocomial infections [8]. Nowadays, the most commonly isolated nosocomial pathogen in clinical laboratories is *A. baumannii*, with a mortality rate being about 41% [9]. Broad-spectrum antibiotics are becoming more and more resistant to *A. baumannii* day by day, even the carbapenems are showing resistance [10]. Carbapenem resistance is frequently initiated by serine oxacillinases (OXAs). Different genes encode these enzymes, the most important being blaOXA₂₃, blaOXA₄₀, blaOXA₅₈, and metallo-β-lactamases (MBLs) genes of the Vimentin (VIM) and Imipenemase (IMP). Clavulanic acid does not inhibit OXAs. While these genes are present in most parts of the world, MBLs exhibit resistance to carbapenems and every other β-lactamase except aztreonam [11]. Carbapenem resistance is thought to involve a list of various interlinked mechanisms: hydrolysis by various beta-lactamases, change in the permeability of the outer cell membrane, antibiotic affinity for proteins that bind penicillin, and elevated efflux pump activity [12]. Owing to the increasing importance of drug-resistant genes, my research aims to determine the frequency of drug-resistant genes in *Acinetobacter baumannii* in our population, namely blaOXA-51, blaOXA-23, blaOXA-24, and blaOXA-58.

This study aims to determine the frequency of multidrug resistance (MDR) and extended drug resistance (XDR), and the frequency of blaOXA-51, blaOXA-23, blaOXA-24, and blaOXA-58 among all other resistant genes responsible for resistance in *Acinetobacter baumannii*.

METHODS

This descriptive cross-sectional study was conducted to assess the susceptibility to antibiotics and genotypic characterization of *Acinetobacter baumannii*. The samples were taken for six months, i.e. July 15, 2017, to January 15, 2018, following the approval of the research synopsis from the institutional review board (Ref # 464/RC/KEMU). Clinical specimens, including blood, sputum, urine, and pus, were obtained from the NICU, ICU, and CCU of tertiary care hospitals in Lahore. These were then processed at the Pathology Department of King Edward Medical University. The sample size was calculated by using the WHO calculator, taking a 95% confidence level, 8% margin of error, and a previously reported prevalence of 87.3% for the blaOXA-23-like gene [13]. A non-probability purposive sampling technique was employed. Informed consent was taken from patients whose specimens satisfied the inclusion criteria. Contaminated samples, defined by the growth of more than two organisms on culture plates, were excluded from the study. Collected clinical specimens were inoculated on Chromagar *Acinetobacter* for preliminary identification. Positive isolates were confirmed based on phenotypic characteristics such as colony morphology and growth patterns, followed by biochemical testing for oxidase, citrate utilization, motility, catalase, and oxidative carbohydrate utilization. Following the criteria of the Clinical and Laboratory Standards Institute (CLSI), isolates identified as *Acinetobacter baumannii* were tested for antibiotic susceptibility using the disc diffusion method. Seven classes of antibiotics were tested: carbapenems, aminoglycosides, fluoroquinolones, cephalosporins, penicillin, beta-lactamase inhibitors, and monobactams. A portion of each pure colony was reserved for molecular testing through PCR to detect specific genotypes. Genotypic analysis of *Acinetobacter baumannii* was conducted using PCR with specific primers targeting blaOXA-23, blaOXA-24, blaOXA-51, and blaOXA-58 genes. Primer sequences were selected to ensure specific amplification of each gene. Screening was performed following annexure 01, while antimicrobial susceptibility patterns were recorded following annexures 02 and 03. SPSS version 21.0 was used for data entry and statistical analysis. Qualitative variables like gender and resistance patterns were presented as frequencies and percentages, whereas quantitative data, like age, were represented as mean ± standard deviation.

RESULTS

A total of 67 patients were included in the study. The mean age of the patients was 41.98 ± 10.86 years, with the youngest patient being 25 years and the oldest 60 years. Of the total participants, 40 (59.70%) were male and 27 (40.30%) were female, indicating a male predominance in

the study population. *Acinetobacter baumannii* was isolated from 19 (28.36%) of the 67 clinical samples (Table 1).

Table 1: Demographic Characteristics and *Acinetobacter baumannii* Frequency among Patients (n=67)

Parameters	Value
Age (Years)	
Mean ± SD	41.98 ± 10.86
Minimum - Maximum	25 - 60
Gender Distribution	
Male, n (%)	40 (59.70%)
Female, n (%)	27 (40.30%)
Acinetobacter Baumannii Positive Cases	
Number of positive samples, n (%)	19 (28.36%)

Molecular characterization of these 19 isolates revealed the presence of various carbapenemase-encoding genes. The blaOXA-23 gene was detected in 11 (57.89%) isolates, while bla OXA-51 was identified in 9 (47.36%) isolates. Importantly, all 19 isolates (100%) harbored the blaOXA-58 gene. The blaOXA-24 gene was not detected in any of the isolates (Table 2).

Table 2: Frequency of Strains of *Acinetobacter Baumannii*

Parameters	Frequency (%)
blaOXA-23	11 (57.89%)
blaOXA-24	0 (0%)
blaOXA-51	9 (47.36%)
blaOXA-58	19 (100%)

All 19 positive samples of *Acinetobacter baumannii* were tested for antimicrobial susceptibility, with the results summarized. Complete resistance (100%) was observed against Penicillin, Ceftazidime, Cefepime, Imipenem, Levofloxacin, Doxycycline, and Trimethoprim-Sulfamethoxazole (Septran). Among the remaining antibiotics, Ampicillin + Sulbactam showed the highest sensitivity, with 8 isolates (42.11%) being susceptible. Piperacillin + Tazobactam and Amikacin each demonstrated sensitivity in 5 (26.32%) isolates, while Tobramycin was effective in 3 (15.79%) cases. Tigecycline showed the lowest susceptibility, with only 2 (10.53%) isolates being sensitive.

Table 3: Antimicrobial Susceptibility of *Acinetobacter Baumannii* (n=100)

Positive Samples of <i>Acinetobacter Baumannii</i>	Sensitive	Inter-mediate	Resistant	Total
Penicillin	0 (0%)	0 (0%)	19 (100%)	19
Ampicillin + Sulbactam	8 (42.11%)	1 (5.26%)	10 (52.63%)	19
Piperacillin+ Tazobactam	5 (26.32%)	2 (10.53%)	12 (63.16%)	19
Amikacin	3 (15.79%)	1 (5.26%)	15 (78.95%)	19
Tobramycin	3 (15.79%)	3 (15.79%)	13 (68.42%)	19
Ceftazidime	0 (0%)	0 (0%)	19 (100%)	19

Cefipime	0 (0%)	0 (0%)	19 (100%)	19
Imipenem	0 (0%)	0 (0%)	19 (100%)	19
Levofloxacin	0 (0%)	0 (0%)	19 (100%)	19
Doxycycline	0 (0%)	0 (0%)	19 (100%)	19
Tigecycline	2 (10.53%)	1 (5.26%)	16 (84.21%)	19
Septran	0 (0%)	0 (0%)	19 (100%)	19

DISCUSSION

Recently, a sharp rise in the occurrence of MDR *A. baumannii* percentage contributing to nosocomial infections, has been demonstrated [12]. Especially during the last few decades, due to the combined effect of features of antibiotic resistance, ability to sustain environmental pressures, and easy transmission within health care facilities, MDR *A. baumannii* phenotypes are directly assigned to morbidity and mortality. Mortality rate of MDR *A. baumannii* bacteremia was 21.2% [14]. Contributions of general wards were 5% and of ICUs were 54% [15]. In present study, 19 (23.36%) samples were positive for *A. baumannii*. Among these positive samples, the frequency of resistance genes as highest for bla OXA-23 (57.89%) and bla OXA-51 (47.36%), respectively. Sepavand *et al.* did a study which showed that the most frequent resistant gene among multidrug resistance class was blaOXA-51 (48.38%), then bla-OXA-23 (46.31%) and bla-OXA-58 (5.31%) respectively [16]. These results are comparable to the findings of this study regarding the pattern of multidrug resistance genes. But in this study, no sample was positive for the blaOXA-24 and blaOXA-58 genes. Another study found blaOXA-23 and blaOXA-58 in bacterial chromosomes, but they did not identify patient strains of blaOXA-24 [17]. Four carbapenemases—blaOXA-23, blaOXA-24, blaOXA-51, and blaOXA-58 are responsible for *Acinetobacter baumannii* resistance. In contrast to other *Acinetobacter*s, the OXA-51 is a naturally occurring organism [18]. One previous study reported that the main mechanisms of resistance to imipenem were due to blaOXA-51-like and blaOXA-23-like in *Acinetobacter baumannii* [1]. According to a different study conducted in an Indian community, the primary pathogens for carbapenem-resistant *Acinetobacter* are blaOXA51-like and blaOXA-23-like [5]. Nevertheless, other investigations did not present findings that were comparable to ours. All MDR *Acinetobacter baumannii* were found to have blaOXA-51-like and blaOXA58-like genes, according to a study conducted in a Spanish population [19]. Carbapenem-resistant *Acinetobacter baumannii* were shown to have an abundance of blaOXA-23-like genes in another study conducted in a Chinese population; the genes blaOXA-24 and blaOXA-58 follow in order [20]. Disparity in various studies is likely to be due to various factors like different sample selection criteria, different sample sizes and genetic variations in different populations due to slight

variation in genetic make-up. Our results propose that the CRAB resistance phenotype is mainly attributed to the blaOXA-23 gene, which is consistent with previous reports [21]. In current study, the resistance pattern showed that Penicillin, Ceftazidime, Cefipime, Imipenem, Levofloxacin, Doxycycline and Septran were the drugs who were showed 100% resistance for *Acinetobacter baumannii*. However, the most sensitive drugs for *Acinetobacter baumannii* was Ampicillin + Sulbactam (42.11%), followed by Piperacillin+ Tazobactam (26.32%), Amikacin (15.79%), Tobramycin (15.79%) and Tigecycline (10.53%). For *Acinetobacter baumannii*, all over the world, the pattern of drug resistance varies. Usually, the ability to tolerate various classes of antibiotics is related to the horizontal uptake of certain resistance genes. But the key mechanisms of tolerance to various disinfectants are not fully understood so far. Digging into the genetic traits as well as its morphological and physiological characteristics of *A. baumannii* clinical isolates will be an important starting point to explain the tactics of survival and tolerance of this opportunistic pathogen [22, 23]. Bahman Mirzaei's study reported that tobramycin, ceftazidime, ciprofloxacin, and imipenem were resistant [24]. Tobramycin and ceftazidime resistance was observed in 70% of *A. baumannii*, amikacin in 50% and imipenem in 80% of isolates, according to Kishk R's result [25]. 100% resistance for imipenem and 99.2% resistance for meropenem were noticed in all Dawiche F. isolates in another study [26]. In a cross-sectional study of Brazil in 2024, Freire MP, along with her colleagues, analyzed a steep rise in carbapenem-resistant *A. baumannii* from 30% to 70% [27]. Minocycline and tigecycline show sensitivity against all *A. baumannii* producing carbapenemases and metallo β -lactamases [28]. Comparable drug resistance patterns for *A. baumannii* isolates were found in another local study conducted in Pakistan. This multidrug resistance pattern exhibited the highest level of resistance to cephalosporins (98.75% for ceftazidime and cefepime, 97.5% for cefotaxime), 96.25% for trimethoprim-sulfamethoxazole, 88.75% for aztreonam, 86.25% for gentamicin, 77.5% for imipenem, 72.5% for piperacillin-tazobactam, and 72.05% for doxycycline [29]. Alamu J from Iran in his study showed that ciprofloxacin and imipenem were 100% resistant, piperacillin 99% and cefepime /levofloxacin/ ceftazidime were 97% resistant. Out of all isolates, complete resistance was noticed in 32% of isolates, while 91% were XDR. The prevailing resistance pattern was 32% with the sequence of resistance being "ampicillin/sulbactam-ceftazidime-imipenem-gentamicin-tobramycin-doxycycline-ciprofloxacin-levofloxacin-cotrimoxazole piperacillin-cefepime [30].

CONCLUSIONS

The frequency of *Acinetobacter baumannii* was 28.36%, based to this study results. Among these positive isolates, the highest frequency of multidrug resistance was seen for blaOXA-23 (57.89%) and bla-OXA-58 (47.36%). Regarding antimicrobial susceptibility, very few drugs showed sensitivity for *Acinetobacter Baumannii*, which is posing a major challenge for the health care authorities as well as for MDR and XDR *A. baumannii*.

Authors Contribution

Conceptualization: MS

Methodology: MS, QUAA, SFTZ

Formal analysis: SFTZ, JKK, ZM

Writing review and editing: QUAA, ZM

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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