ORIGINAL ARTICLE

Antibiotic Susceptibility Pattern of Carbapenem Resistant Acinetobacter Baumannii Isolated from Clinical Specimen

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ABSTRACT

Objective: To determine the antibiotic susceptibility pattern of Carbapenem-resistant *Acinetobacter baumannii* isolated from the different clinical samples.

Study Design: It was a cross-sectional study.

Place and Duration of Study: Department of Microbiology, Khyber Medical University, and the Department of Microbiology, Armed Forces Institute of Pathology (AFIP) from January 2024 to December 2024.

Materials and Methods: After obtaining ethical approval, Various samples from the intensive care units and wards were collected. It included pus, tissue, non-directed bronchial lavage, blood, bronchoalveolar lavage, sputum, fluid, urine, nasopharyngeal swab, drain, bone, and Cerebrospinal fluid, which were processed in the laboratory. All the samples were inoculated on appropriate culture media, and the bacteria were identified by using Gram stain, motility, colony morphology, and biochemical tests. Antibiotic susceptibility was performed by using the Kirby disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI 2024). Isolates showing resistance or intermediate sensitivity to meropenem and imipenem were considered resistant. Susceptibility pattern of the rest of the antibiotics was noted according to CLSI 2024. SPSS version 26 was used for the data analysis.

Result: Out of 57 Carbapenem-resistant *Acinetobacter baumannii* 52 (91.2%) were sensitive to minocycline, 39(68.4%) were sensitive to tigecycline, 4(7%) to gentamicin, 3(5.2%) were sensitive to cefepime, 2(3.5%) to ceftazidime, 1(1.8%) to levofloxacin, tazo-pipracillin and ceftriaxone and showed no sensitivity to amikacin, ciprofloxacin and ampicillin-sulbactam. Tetracycline was tested in urine samples, and Carbapenem-resistant *Acinetobacter baumannii* showed 100% sensitivity towards it.

Conclusion: Carbapenem-resistant *Acinetobacter baumannii* showed very low susceptibility to maximum antibiotics. The highest was towards minocycline.

Keywords: *Carbapenem Resistant Acinetobacter baumannii (CRAB), Clinical and Laboratory Standards Institute (CLSI), Disk Diffusion Technique.*

Introduction

Acinetobacter baumannii is a Gram-negative, aerobic, non-motile coccobacillus that does not ferment carbohydrates, shows pleomorphic characteristics, and is known for causing hospital-acquired opportunistic infections.[1] Until 1971, the genus *Acinetobacter* was not considered a definitive human pathogen and was therefore often disregarded when isolated from clinical specimens.[2] *Acinetobacter baumannii* is now frequently isolated from a variety of clinical specimens, particularly from hospitalized patients. Colonization rates are notably higher among patients in Intensive Care Units (ICUs), particularly on mechanical ventilation.[3,4] It is considered a significant nosocomial pathogen responsible for serious infections such as ventilator-associated pneumonia, bloodstream infections, urinary tract infections, meningitis, and wound infections, especially in immunocompromised patients [5] *Acinetobacter baumannii* can withstand harsh environmental conditions, including desiccation and extreme pH levels, making infection

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control really difficult in intensive care and burn units. Its remarkable resistance to drying allows it to persist on inanimate surfaces for prolonged periods, facilitating transmission within hospital environments and contributing to both outbreaks and long-term endemic presence.[5,6]

In the mid-20th century, *Acinetobacter baumannii* was believed to retain at least intermediate susceptibility to third- and fourth-generation cephalosporins, fluoroquinolones, semisynthetic aminoglycosides, and carbapenems, with nearly 100% of isolates remaining sensitive to imipenem. However, during the late 1980s and 1990s, the global emergence and spread of imipenem-resistant *Acinetobacter* strains significantly limited treatment options. By the late 1990s, carbapenems (Doripenam, Ertapenam & Meropenem) remained the primary and often the only effective antimicrobials against severe *Acinetobacter* infections.[7]

During the last two decades, Carbapenem-resistant *Acinetobacter baumannii* (CRAB) has been designated as a top-priority critical pathogen by the World Health Organization, highlighting the urgent need for new antibiotic research and development. It has also been recently classified by the CDC as a serious and immediate public health threat [5]. WHO also included it among the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* species) a group of bacteria known for their ability to evade the effects of multiple antibiotics, thereby significantly limiting treatment options.[8]

This global health dilemma is due to the overuse of antibacterial agents, which has led to the development of resistance in *Acinetobacter* species against many commonly used antibiotics. A strong positive correlation has been identified between antibiotic resistance rates and antibiotic usage, accompanied by an increasing trend in antimicrobial resistance.[4] Multidrug resistance in *Acinetobacter baumannii* is mediated through multiple mechanisms, such as modification of target sites, enzymatic inactivation of antibiotics by production of carbapenamase, reduced drug uptake due to decreased permeability or increased efflux pump activity, and the ability to form biofilms.[9]

When referring to Southeast Asia, it is observed that Carbapenem resistance is notably high among Gram-negative bacteria in hospitals across South and Southeast Asia, particularly in *Acinetobacter baumannii* isolates. South Asia is considered the likely origin of the New Delhi metallo-β-lactamase-1 (NDM-1) gene, which encodes one of the most widely disseminated carbapenemases across various bacterial species and geographic regions[10]. Oxa-23 is the most prevalent carbapenemase enzyme found in *Acinetobacter baumannii*[11]

As one of the major Southeast Asian countries, Pakistan faces a substantial infectious disease burden caused by various bacterial species, among which *Acinetobacter* species have emerged as prominent pathogens.[7] This situation continues to cause serious threats and challenges to the country's healthcare system. According to research conducted so far, more than 60% of *Acinetobacter baumannii* isolates in Pakistan are resistant to carbapenems[7]

The objective of our study was to determine the sensitivity of Carbapenem-resistant *Acinetobacter baumannii* to a panel of antibiotics according to the Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines.

Materials and Methods

After getting ethical approval KMU/IPDM/IEC/202335 on 18 Dec 2023, the study was conducted at the Department of Microbiology, Khyber Medical University, and the Department of Microbiology, Armed Forces Institute of Pathology (AFIP) from January 2024 to December 2024. It was a cross-sectional study; the sample size was calculated by following the formula used for descriptive studies.[12] (Formula: $n= z^2pq/e^2$), Where n is the sample size, z is the standard normal variate (value is 1.96 at a 5%margin of error),p is the expected proportion in the population based on previous studies[13] (3.5% overall average prevalence, q is equal to 1-p, and e is the margin of error (0.05). The estimated sample size calculated was 52. Non-probability convenience sampling was used. Samples were received from patients admitted to the medical, surgical ICUs, NICU, and wards. Carbapenem-resistant *Acinetobacter baumannii* isolated from different clinical specimens were included in the study. Duplicate isolates from the same patient and *Acinetobacter baumannii* sensitive to carbapenem

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were excluded from the study. Samples were collected from the department of microbiology, Armed Forces Institute of Pathology (AFIP), and processed in the laboratory by inoculating on Blood, Chocolate, and MacConkey agar, and for urine culture, Cystine Lactose Electrolyte Deficient (CLED) agar was used. The culture plates were incubated at 35°C ±2°C for 20-24 hours. Initial identification was done by colony morphology, lactose fermentation, and Gram staining. API 10S and 20E were used to identify up to the genus and species level. According to CLSI 2024, antibiotic susceptibility test of imipenem (10µg) and meropenem (10µg), ampicillin-sulbactam (20µg), ciprofloxacin (5µg), levofloxacin (5µg), Ceftazidime (30µg), Gentamicin (10µg), Minocycline (30µg), Cotrimoxazole(25µg), Tazo-pipracillin (110µg), Cefepime (30µg), amikacin(30µg), tetracycline(30µg), ceftriaxone (30µg) was done by Kirby Bauer disk diffusion method. Isolates showing resistance or intermediate sensitivity to meropenem and imipenem were considered resistant. Susceptibility pattern of the rest of the antibiotics was noted according to CLSI 2024. SPSS version 26 was used for the data analysis. For qualitative analysis, frequencies and percentages were used, and for quantitative analysis, means ± SD were used.

Results

During the study period, a total of 848 samples were positive; out of these positive samples, Gram-positive cocci were 190(22.4%), and Gram-negative rods were 658(77.6%). Out of Gram-negative rods 213(32.3%) were *Escherichia coli*, 141(21.4%) were *Klebsiella pneumoniae,*126(19.1%) were *Pseudomonas*, 59(8.9%) were *Acinetobacter baumannii*, 33(5%) were *Proteus,* 27(4.1%) were *Serratia,*17(2.5%) were *Enterobacter*,14(2.1%) were *Salmonella typhi*, 12(1.8%) were *Burkholderia* and 6(0.9%) were *Salmonella paratyphi.*

Out of 59 *Acinetobacter baumannii,* 57(97%) were carbapenem-resistant, and these were included in the study. samples received from male patients were 42(73.7%), and 15(26.3%) were from female patients. The mean age of the patients was 43.46. The minimum age was newborn, and the maximum was 83 years.

Samples yielded carbapenem resistant *Acinetobacter baumannii* were from pus 14(24.6%), tissue 12(21.1%), non-directed bronchial lavage 10(17.5%), blood 5(8.8%), bronchoalveolar lavage 4(7%), sputum 3(5.3%), fluid 3(5.3%) urine 2(3.5%) and 1 (1.8%) sample of each nasopharyngeal swab, drain, bone and Cerebrospinal fluid as shown in figure 1

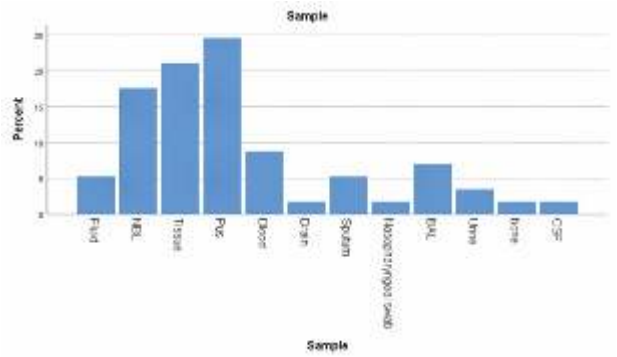

Figure 1: Samples Yielded Growth of Carbapenem-Resistant *Acinetobacter Baumannii*

Out of 57 carbapenem resistant *Acinetobacter baumannii* 52 (91.2%) were sensitive to minocycline, 39(68.4%) were sensitive to tigecycline, 4(7%) to gentamicin 3(5.2%) were sensitive to cefepime, 2(3.5%) to ceftazidime, 1(1.8%) to levofloxacin, tazo-pipracillin and ceftriaxone and showed zero sensitivity to amikacin, ciprofloxacin and ampicillin-sulbactam. Tetracycline was tested in urine samples, and CRAB showed 100% sensitivity towards it, as shown in Figure 2 and Table I.

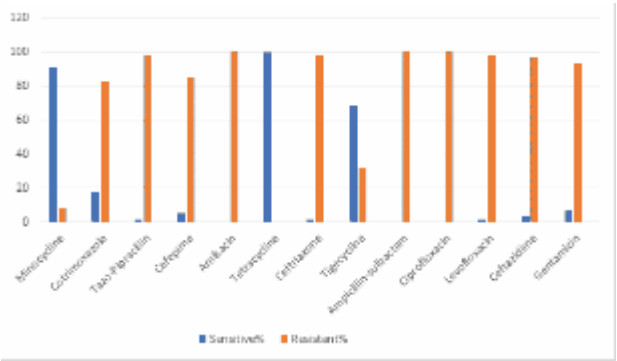

Fig 2: Antimicrobial Resistance Pattern of Antimicrobials against CRAB

Discussion

According to the WHO, Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is recognized globally as a priority one, critical pathogen due to its extensive drug resistance and its association with nosocomial outbreaks, especially in intensive care units. In our study, CRAB isolates exhibited an

Table I: CRAB Susceptibility Profile

Antibiotics	Sensitive n(%)	Resistant n(%)
Ampicillin-Sulbactam	0	57(100)
Ciprofloxacin	0	57(100)
Levofloxacin	1(1.8)	56(98.2)
Ceftazidime	2(3.5)	55(96.5)
Gentamicin	4(7)	53(93)
Minocycline	52(91.2)	5(8.8)
Cotrimoxazole	10(17.5)	47(82.4)
Tazo-Pipracillin	1(1.8)	56(98.2)
Cefepime	3(5.2)	54(84.8)
Amikacin	0	57(100)
Tetracycline	2(100)	00
Ceftriaxone	1(1.8)	56(98.2)
Tigecycline	39(68.4)	18(31.6)

alarming resistance pattern, with 100% resistance to Ampicillin-Sulbactam, Ciprofloxacin, and Amikacin. These results are consistent with recent surveillance data that report similar high resistance rates across many regions, making these antibiotics largely ineffective against CRAB infections.[14,15]

The resistance to fluoroquinolones Levofloxacin (98.2%) and Ciprofloxacin (100%) is particularly concerning. Resistance mechanisms in *A. baumannii* include mutations in the *gyrA* and *parC* genes and the overexpression of efflux pumps, which have been well documented in recent literature.[16] High resistance to third-generation cephalosporins Ceftazidime (96.5%), Cefepime (84.8%), and Ceftriaxone (98.2%)—is also consistent with previous findings, often attributed to the widespread production of OXA-type carbapenemases and ESBLs[17]

Aminoglycoside resistance was notable, with complete resistance to Amikacin and 93% resistance to Gentamicin, similar to the results reported by Sajerli et al., who found high levels of aminoglycoside-modifying enzymes in clinical CRAB isolates.[18.] Furthermore, Tazo-Piperacillin resistance (98.2%) reflects the inefficacy of β-lactam/β-lactamase inhibitor combinations against CRAB, as previously reported in other studies.[19]

Amongst all the tested anti-microbial agents, **Minocycline** demonstrated the highest sensitivity rate (91.2%), followed by **Tigecycline** (68.4%). These results are consistent with various meta-analyses and clinical studies conducted around the world, highlighting the continued effectiveness of Minocycline against CRAB, even when resistance to other tetracyclines is present [20,21] Tigecycline, although less effective than Minocycline, remains a valuable option, particularly for initial treatment.[22]

Conversely, Gentamicin displayed low sensitivity (7%), confirming its limited role against CRAB compared to Minocycline. Cotrimoxazole showed 17.5% sensitivity, which, while limited, might still offer a role in combination therapy in certain clinical contexts, as noted by Al-Sheboul.[23]

These findings emphasize the critical need for robust antimicrobial stewardship, ongoing resistance surveillance, and the development of new agents, such as Cefiderocol, which has shown promise against CRAB in recent trials [24]. In the absence of effective therapeutic options, infection control practices and targeted therapy based on susceptibility data remain key pillars in managing CRAB infections.

Conclusion

CRAB showed very low sensitivity towards maximum antibiotics. Maximum sensitivity was shown towards minocycline (91.2%) and then tigecycline (68.4%). However, tetracycline showed good sensitivity if used in UTI patients. So, the treatment of the patient depends on pathogen-directed susceptibility. For empirical treatment, continuous surveillance of antibiotic susceptibility is needed.

Conflict of Interest

There is no conflict of interest declared by any author.

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Data Availability

The data that support the findings are available on request from the corresponding authors.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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