

An Escalating Antimicrobial Resistance Crisis: Seven-Year Trends of *Acinetobacter baumannii* in a Tertiary Care Hospital in Pakistan

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Received: 📅 2026 Sep 21

Accepted: 📅 2026 Oct 11

Published: 📅 2026 Oct 20

Abstract

Purpose: *Acinetobacter baumannii* is a multidrug-resistant nosocomial pathogen classified as a critical priority organism by the WHO. Its persistence and adaptability pose serious treatment challenges, particularly in resource-limited settings such as Pakistan.

Methods: A retrospective registry-based study was conducted in the Department of Microbiology at a tertiary care hospital covering the period from January 2017–December 2023. A total of 1,085 clinical isolates from patients aged 10–90 years were identified using standard microbiological techniques and API 20NE® (bioMérieux). Antimicrobial susceptibility testing was performed using the modified Kirby-Bauer disc diffusion method, as outlined in the CLSI guidelines. Susceptibility to colistin polymyxin B was determined using the broth microdilution method, according to CLSI guidelines. Data were analyzed with Microsoft Excel 19.0.

Results: The highest number of isolates occurred in 2018 (22.0%), with the lowest in 2021 (4.8%), likely reflecting the COVID-19 pandemic's impact on healthcare utilization. ICU samples contributed nearly half of all isolates, highlighting the concentration of *Acinetobacter* infections in critical care. Resistance rates were alarmingly high: ciprofloxacin (88.8%), imipenem (88.1%), gentamicin (87.7%), and amikacin (84.5%). In contrast, colistin (97%), polymyxin B (96%), minocycline (70%), and tigecycline (71%) demonstrated the highest sensitivity. Infections were most frequent among patients aged 51–70 years.

Conclusion: *Acinetobacter baumannii* shows extremely high resistance to most conventional antibiotics, including carbapenems and cephalosporins, leaving few therapeutic options. The ICU predominance and alarming resistance patterns underscore the urgent need for robust infection control strategies, antimicrobial stewardship, and continuous surveillance to mitigate the growing threat posed by this pathogen.

Abbreviations

- **API** – Analytical Profile Index
- **API 20NE®** – Analytical Profile Index for Non-Enteric Gram-Negative Bacteria
- **APIweb™** – API Web Identification System
- **AST** – Antimicrobial Susceptibility Testing
- **CLSI** – Clinical and Laboratory Standards Institute
- **CSF** – Cerebrospinal Fluid
- **ESBLs** – Extended-Spectrum Beta-Lactamases
- **ESKAPE** – *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter species*
- **GNR** – Gram-Negative Rod
- **ICU** – Intensive Care Unit
- **IND** – Indole
- **MIC** – Minimum Inhibitory Concentration
- **MDR** – Multidrug Resistant
- **OPD** – Outpatient Department
- **OXA** – Oxacillinase
- **UTI** – Urinary Tract Infection
- **WHO** – World Health Organization

Keywords: Antimicrobial Resistance, Antimicrobial Stewardship, Bacteria, Pandemic, Antibiotics

1. Introduction

Acinetobacter is a rod-shaped, gram-negative bacterium belonging to the Moraxellaceae family. It has a typical gram-negative cell wall with lipopolysaccharides, outer membrane proteins, and phospholipases contributing to its virulence. It is highly resilient to harsh and generally uninhabitable environments like desiccation, high temperatures, low nutrition, and antibiotics. Naturally present in soil and water, *Acinetobacter* also inhabits the human microbiome. However, its true opportunistic nature emerges in hospital environments where it finds a breeding ground on ventilators, catheters, and ICU surfaces, flourishing as a nosocomial pathogen [1]. There are 50 to 60 species of *Acinetobacter*, with around 10 to 15 being clinically important. Among these, *Acinetobacter baumannii* is the most significant since it is the leading cause of hospital-acquired infections, particularly in ICUs, where it accounts for 34% of organisms isolated from these critical care units [2]. *Acinetobacter* synthesizes a range of enzymes, but a distinguishing enzyme is OXA-type carbapenemase, a beta-lactamase, conferring resistance to carbapenems and making it a major nosocomial pathogen [3]. The environmental persistence, the antibiotic resistance, along with the absence of identified host-damaging toxins in its genome, suggest that the virulence potential of *Acinetobacter baumannii* is based on a 'persist and resist' strategy [4]. This pathogen primarily spreads in healthcare environments, and it contributes to pneumonia, sepsis, urinary tract infections (UTIs), meningitis, and endocarditis, especially in hospitalized or immunocompromised patients. Owing to its resistance to last-resort antibiotics like colistin, tigecycline, and carbapenems, *Acinetobacter* has cemented its status among the most problematic ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*). According to a WHO priority list, *Acinetobacter* has been placed in the critical priority group, highlighting its status as a major threat due

to its multidrug resistance.

This acknowledgement emphasizes the pressing need for creative research and development to counter its ever-evolving resistance techniques. [5]. The emergence of this multidrug resistance is aided by both clonal transmission and horizontal gene transfer, driving the distribution of antibiotic resistance, which has largely resulted from recent misuse of medications and mistakes in treatment management [6]. *Acinetobacter baumannii* infections have been increasing worldwide, especially in hospitals with limited treatment options and increased antibiotic use. Studies done in the United States have shown that *Acinetobacter baumannii* causes 41,400 to 83,000 yearly cases, with carbapenem-resistant cases accounting for 15-75% of these infections. Globally, the number of cases ranges from 600,000 to 1,400,000, with deaths ranging between 750 to 22,500 [7]. The emergence of *Acinetobacter baumannii* resistant to carbapenems, a drug that has been effective against Gram-negative infections for decades, is particularly concerning. The surveillance data from the Global Antimicrobial Surveillance Program shows that carbapenem resistance has exceeded 50-80% in several regions [8]. This has made the pathogen a formidable challenge for medics. This situation is particularly aggravated in Asia, where some of the highest rates of resistant *Acinetobacter* strains are present. Countries like China, India, Pakistan, Bangladesh, Thailand, and Vietnam report over 70-90% carbapenem resistance in *Acinetobacter baumannii* isolates from hospitals [9]. The widespread misuse of antibiotics in both healthcare and agriculture has caused the emergence of resistant strains. In many Asian countries, colistin therapy in livestock production has resulted in the continuing presence of colistin-resistant *Acinetobacter* strains [10]. Additionally, inadequate hospital setup and infection control measures have made the situation of multi-drug-resistant *Acinetobacter* worse. Mirroring the global trend, Pakistan

also grapples with the major public health issue of the rising menace of antibiotic resistance. Contributing factors such as misuse of antibiotics, substandard infection control, and limited medical resources have all exacerbated the spread of antibiotic-resistant strains of many pathogens, including *Acinetobacter*. A study found that the resistant *Acinetobacter* samples accounted for 65% of those from several national hospitals [11]. Hence, a need for a study to establish the prevalence and resistance profile of *Acinetobacter*. High carbapenem resistance rates reported in Pakistan, coupled with noted regional trends, will be vital in generating treatment plans and enhancing infection control.

2. Methodology

Ethical approval was obtained from the ethical review committee at a tertiary care hospital in Rawalpindi, Pakistan to carry out this retrospective registry-based study in the Department of Microbiology, covering the period from January 2017 to December 2023. The study employed a consecutive non-probability sampling technique, involving male and female patients from various wards and outpatient departments, aged between 10 and 90 years. Patients taking antibiotics and those whose ages were <10 and >90 years were excluded from the study. During study period, all clinical samples (including tracheal tube for culture, pus culture, sputum culture, blood culture, tissue for culture, urine culture, cannula double lumen for culture, high vaginal swab culture, bronchial washing fluid for culture, fluid culture, ascitic fluid culture, cerebrospinal fluid culture) were collected aseptically as per standard microbiological methods from different departments of the hospital. A well-isolated colony of a Gram-negative rod (GNR) showing shiny, mucoid colonies on blood agar and non-lactose fermentation with pink/purple hue on MacConkey agar was selected after overnight incubation (18–24 hours). A standardized bacterial suspension equivalent to 0.5 McFarland turbidity was prepared in sterile distilled water. For biochemical identification, the suspension was inoculated into the API 20NE® strip (bioMérieux, France), which is designed for the identification of non-fermenting, aerobic, Gram-negative coccobacilli.

The strip was incubated at 35–37°C for 24 hours as per the manufacturer's instructions. After incubation, the IND reagent was added to the indole cupule, and NIT 1 and NIT 2 reagents were added to the nitrate reduction cupule; the ZN reagent was added when nitrate reduction was negative. The colour changes were interpreted using the API color chart, and a numerical code was obtained. This code was used to enter into the APIweb™ database to get the most probable or confirmed identification of the *Acinetobacter baumannii* [12]. The antimicrobial susceptibility testing of the samples was done on Mueller-Hinton agar plates by the modified Kirby-Bauer disc diffusion method [13]. The inoculum was evenly spread on Mueller-Hinton agar plates, and antibiotic discs which includes amikacin (30 µg), ampicillin (10 µg), amoxicillin-clavulanate (30 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cephadrine (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), colistin (10 µg or MIC), doxycycline (30 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), minocycline (30 µg), polymyxin B (300 units or MIC), sulbactam-cefoperazone (75/30 µg), piperacillin-tazobactam (100/10 µg), and tigecycline (15 µg) were applied according to CLSI guidelines [14]. Susceptibility testing for colistin and polymyxin B was performed only by broth microdilution, as disc diffusion is not recommended for these agents. Plates were incubated at 35–37°C for 16–18 hours, and zones of inhibition were measured to interpret susceptibility profiles. After susceptibility profiles, the collected data were analyzed using Microsoft Excel 19.0 to generate tables and charts.

3. Results

3.1. Annual Distribution of Isolates

A total of 1085 *Acinetobacter* isolates were identified from clinical samples between 2017 and 2023. The annual distribution showed the highest number of isolates in 2018 (22.03%), followed by 2019 (20.74%) and 2017 (16.87%). A notable decline was observed in 2021, with only 4.79% of the total isolates reported that year (Figure 1).

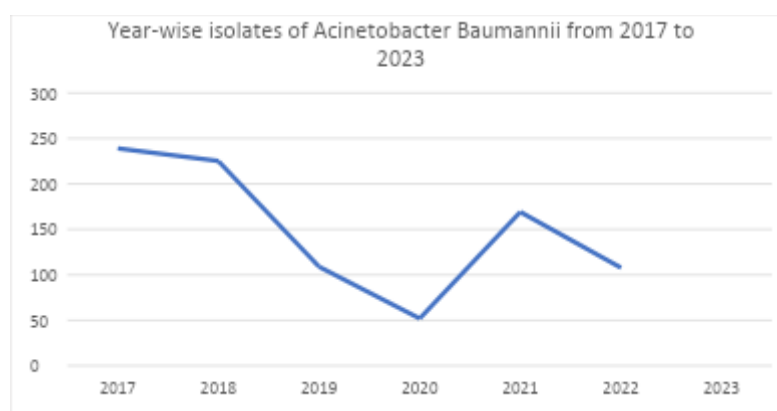


Figure 1: Year-Wise Isolates of *Acinetobacter baumannii* from 2017 to 2023

3.2. Hospital Unit Distribution

Department-wise analysis revealed the highest number of isolates from the Surgery Department (n=169), followed by the Medicine Department (n=131), Pulmonology (n=56), Orthopedics (n=65), Gynecology & Obstetrics (n=38),

Nephrology/Urology (n=36), and Neurology (n=24) (Figure 2). When comparing healthcare settings, isolates from the ICU accounted for a significant proportion, indicating a concentration of *Acinetobacter* infections in critical care units.

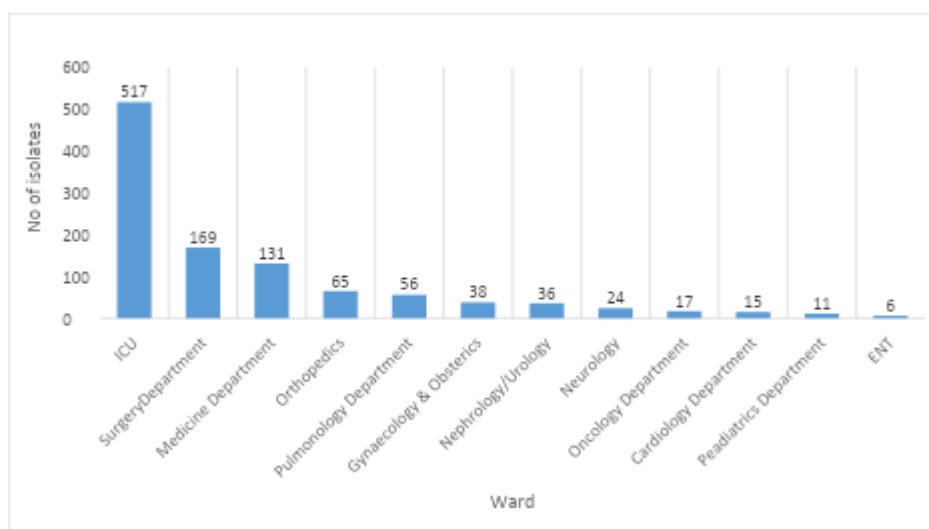


Figure 2: Ward-Wise Distribution of *Acinetobacter baumannii* from 2017 to 2023

The culture-wise distribution of *Acinetobacter baumannii* isolates obtained from various clinical specimens is summarized in Table 1.

Culture Type	Isolates n (%)
Tracheal Tube for Culture	427 (39)
Pus Culture	369 (34)
Sputum Culture	92 (8)
Blood Culture	74 (6)
Tissue for Culture	45 (4)
Urine Culture	19 (1)
Canula Double Lumen for Culture	14 (1)
HVS Culture	14 (1)
Bronchial Washing Fluid for Culture	14 (1)
Fluid Culture	7 (0.64)
Ascitic Fluid Culture	5 (0.46)
CSF Culture	5 (0.46)

Table 1: Culture-Wise Isolates of *Acinetobacter baumannii* from 2017 to 2023

3.3. Antibiotic Resistance Patterns

Antibiotic susceptibility patterns were alarming, with high resistance observed across most agents. The testing showed resistance rates of 88.82% for Ciprofloxacin, 88.10% for

Imipenem, 87.69% for Gentamicin, 84.50% for Amikacin, and 52.41% for Doxycycline. The corresponding sensitivity rates were 11.18% for Ciprofloxacin, 14.85% for Amikacin, and 46.78% for Doxycycline (Table 2).

Antibiotic	Sensitive (n)	Sensitive (%)	Intermediate (n)	Intermediate (%)	Resistant (n)	Resistant (%)	Total (n)
Ciprofloxacin	121	11	0	0	964	89	1085
Imipenem	127	12	3	0	956	88	1085
Gentamicin	129	12	4	0	951	88	1085
Doxycycline	508	47	9	1	569	52	1085
Amikacin	161	15	7	1	917	85	1085
Co-Trimoxazole	106	10	6	1	979	90	1085
Ampicillin	8	1	0	0	1070	99	1085
Meropenem	121	11	2	0	961	89	1085
Polymyxin B	883	96	0	0	38	4	921
Cefotaxime	31	4	2	0	803	96	836
Ceftazidime	63	9	1	0	660	91	724
Sulzone	254	33	23	3	477	63	754
Minocycline	487	70	3	1	197	28	691
Colistin	569	97	9	3	13	2	587
Ceftriaxone	31	6	0	0	445	94	476
Tigecycline	332	71	13	3	124	26	469
Aztreonam	14	3	0	0	408	97	422
Augmentin	11	3	0	0	362	97	373
Tazocin	47	24	0	0	146	76	193
Chloramphenicol	26	16	0	0	136	84	162
Cephadrine	11	8	0	0	125	92	136
Polymyxin B	75	91	0	0	7	9	82

Table 2: Antibiotic Resistance Pattern of *Acinetobacter baumannii* from 2017 to 2023

3.4. Inference

These findings underscore the grave resistance profile of *Acinetobacter* species, particularly *Acinetobacter baumannii*, and reinforce its status as a formidable nosocomial pathogen, especially within ICU settings.

4. Discussion

This study provides a comprehensive view of the antimicrobial susceptibility pattern of *Acinetobacter baumannii* isolated from a tertiary care hospital in Rawalpindi, Pakistan, highlighting significant trends across different age groups, clinical settings, and antimicrobial classes. These findings highlight the need for targeted intervention strategies to manage *Acinetobacter* infections more effectively. In this study, Colistin (97%) and Polymyxin B (96%) exhibited the highest in-vitro activity against *Acinetobacter baumannii*. This aligns with multiple studies conducted globally, which report Polymyxin and Colistin to be highly effective last resort drugs for MDR-*Acinetobacter* infections [15-18]. Additionally, prior research indicates combination regimes of Polymyxin and colistin or Polymyxin with tetracycline, like minocycline, are efficacious against the ventilator-associated MDR-*Acinetobacter* [19]. Literature suggests that Polymyxins retain exceptional efficacy against multidrug-resistant strains owing to their potent membrane-targeting

action and historically restricted clinical use owing to their nephrotoxicity, which has minimized resistance development [19,20]. Consequently, the low resistance rates of these antibiotics reflect their judicious clinical use in our hospital, which has curbed selective pressure and helped preserve their potency against the *Acinetobacter baumannii*.

Minocycline and Tigecycline demonstrated strong antimicrobial activity against *Acinetobacter baumannii* in our study, with sensitivity rates of 70% and 71%, respectively. These results are supported by findings from a study conducted in Saudi Arabia, which reported a 77% sensitivity rate for both antibiotics [21]. Similarly, a study from Jordan documented a Minocycline susceptibility rate of 62.2% [22]. The continued potency of these antibiotics is likely linked to their extensive antimicrobial spectrum and their proven ability to disrupt bacterial biofilms, rendering them highly effective against resilient and drug-resistant *Acinetobacter* strains [23]. Carbapenems—namely, imipenem and meropenem—have long been relied upon as cornerstone therapies for managing multidrug-resistant Gram-negative infections. Alarming, our study revealed resistance rates of 88% for imipenem and 89% for meropenem, reflecting a sharp decline in their clinical utility. These findings are consistent with regional data; a

multicenter analysis across several Asian nations reported an 82.5% resistance rate to carbapenems, while an Indian study found 81.5% of *Acinetobacter baumannii* isolates to be resistant [24,25]. Similarly, in China, the sensitivity rate was as low as 20%, reinforcing the critical concern regarding their diminishing efficacy against *Acinetobacter* [26]. The underlying mechanism is the persistent presence of carbapenem-hydrolyzing class D β -lactamases (particularly OXA-type enzymes), along with porin loss and efflux pump upregulation, which has significantly compromised the clinical effectiveness of these drugs against this pathogen [27]. High resistance rates were observed for β -lactam antibiotics, as 92% resisted cephadrine, while ceftriaxone and ampicillin showed 94% and 99% resistance, respectively. The organism's intrinsic low outer membrane permeability, together with the ability to produce β -lactamase enzymes

such as AmpC and ESBLs, is likely to account for these resistance patterns.

These mechanisms resist first- and third-generation cephalosporins and soluble penicillins [28]. Additionally, the prolonged and extensive use of these antibiotics in clinical practice has contributed to strong selective pressure, favoring the proliferation of resistant strains and significantly reducing their clinical effectiveness against *Acinetobacter baumannii*. The analysis of 1,085 *Acinetobacter* infection cases reveals clear age-related trends. The highest infection rates were observed in individuals aged 51–60 years (18.80%), followed closely by those aged 61–70 (18.34%) and 41–50 (14.19%). In contrast, the lowest proportion was seen in the 81–90 age group, accounting for just 5.62% of cases (Figure 3).

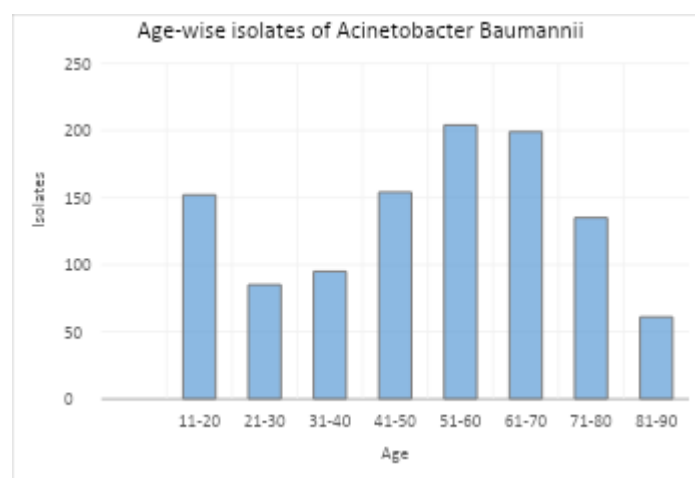


Figure 3: Age-Wise Distribution of *Acinetobacter baumannii* Isolates from 2017 to 2023

Adolescents aged 11–20 years made up 14.01% of the cases, with infection rates gradually rising through middle age. The increased incidence in the 51–70 age range may be attributed to factors such as prolonged healthcare exposure, occupational hazards, and age-related weakening of the immune system. Meanwhile, the lower prevalence in the oldest age group could be due to decreased hospital contact or survivorship bias. These patterns highlight the combined influence of environmental exposure and declining immune function in determining vulnerability to *Acinetobacter* infections. Analysis of incidence data from a sample of 1,085 cases revealed that 2018 had the highest proportion of resistant cases (22.03%), while 2021 had the lowest (4.79%). Post-2021, resistance rates rebounded to 15.58% in 2022 and 9.95% in 2023. The sharp decline in 2021 likely reflects disruptions from the COVID-19 pandemic, including altered hospital workflows and antibiotic stewardship practices. The subsequent recovery points to a return to pre-pandemic resistance patterns, potentially exacerbated by renewed antibiotic selection pressures in clinical settings. Almost half of the isolates were discovered from the ICU, contrasting with non-ICU departments, which comprised the other half [29]. This is per several studies conducted globally, which suggest *Acinetobacter* species to be a risk for infections in invasive

surgeries [30]. Patients in ICUs often require invasive procedures like ventilation and catheterization, which increase infection risk. Additionally, frequent antibiotic use in these settings promotes the survival of resistant strains.

5. Conclusion

In conclusion, this study highlights the alarming prevalence of multidrug-resistant *Acinetobacter baumannii*, particularly in critical care units such as the ICU and among older age groups, reflecting the pathogen's opportunistic nature and adaptability in hospital environments. The organism demonstrated high resistance to most commonly used antibiotics, including carbapenems and cephalosporins, with only a few agents, like colistin, polymyxin B, minocycline, and tigecycline, retaining notable effectiveness. These findings reinforce the serious threat posed by *A. baumannii* in healthcare settings, especially in regions with limited infection control and high antibiotic misuse. There is an urgent need for enhanced infection control practices, judicious antibiotic use, and continued surveillance to curb the spread of this formidable pathogen and to preserve the efficacy of the few remaining treatment options.

Competing Interest

No competing was declared by all authors.

Consent for publication

Consent for publication was taken from all authors.

Data Availability

None

Funding

No funding was obtained from any person or department for the study.

Acknowledgements

The authors would like to thank the administration of hospital for providing the facilities necessary to conduct this study.

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