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Phenotypic Extended-Spectrum Beta-Lactamase (ESBL) Burden, Multidrug Resistance, and Beta-Lactamase Inhibitor Failure Across Clinical Acinetobacter Species

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Abstract

Background

Extended-spectrum beta-lactamase (ESBL) production in *Acinetobacter* species severely impairs first-line antimicrobial therapy and complicates routine phenotypic diagnostic identification. Moreover, conventional beta-lactamase inhibitors frequently fail to restore parent beta-lactam susceptibility due to multi-layered enzymatic and non-enzymatic resistance mechanisms. This study evaluated the phenotypic prevalence of ESBL production, defined the multidrug resistance (MDR) architecture, and quantified beta-lactamase inhibitor failure across clinical *Acinetobacter* species.

Materials and methods

A cross-sectional observational study was carried out on 147 nonduplicate clinical isolates of *Acinetobacter* species collected at a tertiary care hospital. Speciation was established through standardized phenotypic and biochemical profiling. In vitro susceptibility against 12 antimicrobial agents was evaluated using Kirby-Bauer disk diffusion per CLSI M100 standards. Phenotypic ESBL production was screened via the double-disc synergy test (DDST). Inhibitor failure was determined by comparing cephalosporin and penicillin susceptibility with and without clavulanic acid and tazobactam. Multidrug resistance was defined per international consensus criteria (non-susceptibility to one or more agent in three or more antimicrobial categories).

Results

Among the 147 isolates, *A. baumannii* complex accounted for 88.4% (n = 130), *A. lwoffii* for 8.8% (n = 13), *A. junii* for 2.0% (n = 3), and *A. haemolyticus* for 0.7% (n = 1). The overall phenotypic ESBL positivity rate via DDST was 94.6% (n = 139/147), comprising 96.9% (n = 126/130) in *A. baumannii* complex, 92.3% (n = 12/13) in *A. lwoffii*, and 100.0% (n = 1/1) in *A. haemolyticus*. MDR was documented in 95.2% (n = 140/147) of isolates, with 77.6% (n = 114/147) exhibiting resistance to six or more distinct antimicrobial categories. Profound inhibitor inefficacy was confirmed ceftazidime resistance alone was 92.5% (n = 136/147), and the addition of clavulanic acid yielded an identical 92.5% resistance rate (n = 136/147, 0.0% net restoration), while piperacillin/tazobactam exhibited 89.1% resistance (n = 131/147) compared to 68.7% (n = 101/147) for piperacillin alone ($p < 0.0001$).

Conclusion

Phenotypic ESBL production and multidrug resistance are hyperendemic across clinical *Acinetobacter* isolates, spanning both *A. baumannii* and non-*baumannii* species. Classical beta-lactamase inhibitors fail completely to restore antimicrobial activity, rendering empirical beta-lactam/inhibitor combinations unreliable. Implementation of rigorous phenotypic screening and targeted stewardship is essential.

Categories: Infectious Disease
Keywords: acinetobacter species, clavulanic acid, diagnostic microbiology, double disc synergy test, extended-spectrum beta-lactamases (esbl), inhibitor failure, multidrug resistance (mdr), tazobactam

Introduction

The genus *Acinetobacter* comprises ubiquitous, non-fermenting Gram-negative coccobacilli that have emerged as paramount opportunistic nosocomial pathogens worldwide [1]. Designated by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) among the highest-priority antimicrobial-resistant threats, these organisms present substantial therapeutic challenges in healthcare facilities [1,2].

Among the diverse mechanisms mediating resistance, the elaboration of extended-spectrum beta-lactamases (ESBLs) - principally Ambler Class A enzymes (such as TEM, SHV, CTX-M, PER, and VEB) and Class D oxacillinases - plays a pivotal role in hydrolyzing oxyimino-cephalosporins (ceftazidime, cefotaxime, cefepime) and monobactams [3-5]. In clinical therapeutics, beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam are conventionally paired with beta-lactams to protect the core molecule against enzymatic degradation [6]. However, in *Acinetobacter*, inhibitor combinations often exhibit severe failure in vitro and in vivo [7,8]. This failure is mediated by concurrent overexpression of intrinsic chromosomal cephalosporinases (AmpC/ADC), cephalosporinase hyperproduction, class D oxacillinases, altered penicillin-binding proteins (PBPs), and upregulated multidrug efflux pumps [9-11].

Although *Acinetobacter baumannii* complex represents the primary clinical focus, non-*baumannii* species such as *Acinetobacter lwoffii, Acinetobacter junii* , and *Acinetobacter haemolyticus* are increasingly isolated from inpatient blood cultures, urine, and surgical site infections [12,13]. Historically dismissed as low-virulence skin commensals, these non-*baumannii* species are now recognized as active reservoirs for transferable resistance genes [14]. Defining the precise burden of phenotypic ESBL production, the architecture of multidrug resistance (MDR), and the extent of inhibitor failure across clinical isolates is imperative [15-18]. This study investigates the phenotypic profile of ESBL production, antimicrobial non-susceptibility across seven major drug classes, and the clinical efficacy of beta-lactamase inhibitors in clinical *Acinetobacter* isolates.

Materials And Methods

Study setting and ethical approval

This cross-sectional, laboratory-based study was conducted in the Department of Microbiology at Krishna Hospital and Medical Research Centre, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad, India. During the one-year study period (May 2025 to May 2026), a total of 7,640 clinical specimens submitted from hospitalized patients with suspected bacterial infections were processed, yielding 5,644 culture-positive samples (including 2,913 Gram-negative isolates) and 1,996 sterile cultures. From the Gram-negative cohort, 370 *Acinetobacter* isolates were initially recovered. To distinguish clinically significant infections from colonization or contamination, specimens were evaluated using physician-initiated clinical requisition details alongside standard microbiological criteria: direct Gram smear correlation (presence of polymorphonuclear leukocytes and compatible Gram-negative coccobacilli), quantitative urine culture thresholds ($\geqslant 10^5$ CFU/mL), semi-quantitative moderate-to-heavy predominant growth in respiratory secretions, and strict aseptic isolation from blood and sterile body fluids. After excluding 223 repeat isolates from the same patient and probable superficial colonizers, 147 clinically significant, non-duplicate *Acinetobacter* isolates were included in the final analysis. The study protocol was approved by the Institutional Ethics Committee (Ref. No. KVV/IEC/03/2025, Protocol Number: 689/2024-2025). Written informed consent was waived as the investigation utilized anonymized bacterial isolates obtained during routine diagnostic workflows.

Bacterial isolation and speciation

Clinical specimens including lower respiratory tract secretions (sputum, endotracheal aspirates), blood, urine, pus/wound swabs, and sterile body fluids were processed using standard microbiological protocols. Isolates were plated onto 5% sheep blood agar and MacConkey agar plates and incubated aerobically at 37°C for 24-48 hours. Colonies demonstrating non-lactose fermenting morphology, Gram-negative coccobacillary morphology, negative oxidase reaction, positive catalase reaction, and lack of motility were provisionally identified as *Acinetobacter*. Species-level differentiation into *A. baumannii* complex, *A. lwoffii, A. junii,* and *A. haemolyticus* was established using standardized biochemical tests: Hugh-Leifson oxidation-fermentation test for glucose, growth temperature profiling at 44°C and 37°C, Simmons citrate utilization, Christensen's urease test, 10% lactose utilization, beta-hemolysis on 5% sheep blood agar (specifically identifying *A. haemolyticus*), and gelatin liquefaction. Standard reference control strains were included to validate biochemical reactions. Because conventional phenotypic and biochemical profiling cannot resolve individual genomic species within the *Acinetobacter calcoaceticus-baumannii* (Acb) complex, glucose-oxidizing, citrate-positive isolates growing at 44°C were grouped collectively as *A. baumannii* complex in accordance with standard clinical microbiology conventions. No automated mass spectrometry (MALDI-TOF MS) or molecular genotypic assays were performed for species-level confirmation.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar using the standardized Kirby-Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) M100 guidelines (34th edition, 2024) [3]. Zone diameters for all tested *Acinetobacter* species (*A. baumannii* complex*, A. lwoffii, A. junii,* and *A. haemolyticus*) were interpreted using CLSI M100 section 2B-2 interpretive criteria for *Acinetobacter* spp. The antimicrobial discs tested included piperacillin (100 µg), piperacillin/tazobactam (100/10 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), and minocycline (30 µg). Because CLSI section 2B-2 does not define independent therapeutic clinical

breakpoints for ceftazidime/clavulanate (30/10 µg) in *Acinetobacter* spp., this combination disc was utilized primarily for phenotypic ESBL confirmation (defined per CLSI criteria as a ⩾ 5 mm increase in zone diameter compared to ceftazidime 30 µg alone), while comparative in vitro susceptibility restoration was evaluated using the corresponding CLSI section 2B-2 ceftazidime (30 µg) zone diameter breakpoints (⩾ 18 mm susceptible, 15-17 mm intermediate, ⩽ 14 mm resistant). Quality control for routine disc diffusion and ESBL combination testing was performed concurrently using *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and ESBL-positive *Klebsiella pneumoniae* ATCC 700603.

Phenotypic screening for ESBL (double disc synergy test)

Phenotypic ESBL detection was performed using the double disc synergy test (DDST) and the combined disc diffusion test (CDDT; ceftazidime 30 µg versus ceftazidime/clavulanate 30/10 µg, with a ⩾ 5 mm zone diameter increase considered positive). Because CLSI M100 phenotypic ESBL confirmatory criteria are formally standardized for *Enterobacterales* rather than *Acinetobacter* species, both assays were applied in tandem as complementary phenotypic screening and confirmatory tools in accordance with established epidemiological surveillance practices for non-fermenting Gram-negative bacilli. Quality control for phenotypic ESBL detection was verified using *Klebsiella pneumoniae* ATCC 700603 (ESBL-positive) and *Escherichia coli* ATCC 25922 (ESBL-negative). No molecular genotypic assays (e.g., PCR for *bla*TEM, *bla*SHV, *bla*CTX-M, *bla*PER, or *bla*VEB) or AmpC-inhibitor suppression tests were performed to confirm the underlying genetic determinants.

Definitions of MDR and inhibitor failure

MDR and extensive drug resistance (XDR) were classified in accordance with the international consensus criteria proposed by Magiorakos et al. [19], adapted to the seven antimicrobial categories evaluated in our routine Kirby-Bauer disc diffusion panel (aminoglycosides, antipseudomonal carbapenems, antipseudomonal fluoroquinolones, antipseudomonal penicillins plus beta-lactamase inhibitors, extended-spectrum cephalosporins, folate pathway inhibitors, and tetracyclines). MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Because polymyxins (colistin, which requires broth microdilution rather than disc diffusion per CLSI M100) and ampicillin/sulbactam were excluded from the routine disc diffusion panel, XDR was defined using a panel-adapted criterion as non-susceptibility to at least one agent in all but two or fewer of the seven tested categories (i.e., non-susceptibility across five to seven tested categories).

Statistical analysis

Data were entered into Microsoft Excel (Redmond, WA, USA) and analyzed using standard statistical software. Categorical variables were summarized as frequencies and percentages. Because the same clinical isolates (n = 147) were evaluated across paired antimicrobial regimens (beta-lactam agent alone versus beta-lactam/beta-lactamase inhibitor combination), paired nominal comparisons of resistance proportions were analyzed using McNemar's test (with exact binomial p-values for small discordant cell counts). Independent-sample chi-square or Fisher's exact tests were reserved strictly for unpaired comparisons across independent isolate groups. A two-tailed p-value < 0.05 was considered statistically significant.

Results

A total of 147 non-duplicate clinical *Acinetobacter* isolates were evaluated. Speciation identified *Acinetobacter baumannii* complex as the dominant organism (n = 130, 88.4%), followed by *Acinetobacter lwoffii* (n = 13, 8.8%), *Acinetobacter junii* (n = 3, 2.0%), and *Acinetobacter haemolyticus* (n = 1, 0.7%).

Phenotypic ESBL positivity across species

The overall prevalence of phenotypic ESBL production via DDST was 94.6% (n = 139/147). ESBL positivity was 96.9% (n = 126/130) in *A. baumannii* complex, 92.3% (n = 12/13) in *A. lwoffii*, and 100.0% (n = 1/1) in *A. haemolyticus*. All isolates of *A. junii* (n = 3, 100.0%) were negative for ESBL production via DDST (Table *1*, Figure *1*).

Acinetobacter Species	Total Isolates (N=147)	ESBL Positive (DDST) n (%)	ESBL Negative n (%)	Multidrug Resistant (MDR) n (%)
Acinetobacter baumannii complex	130 (88.4%)	126 (96.9%)	4 (3.1%)	126 (96.9%)
Acinetobacter lwoffii	13 (8.8%)	12 (92.3%)	1 (7.7%)	13 (100.0%)
Acinetobacter junii	3 (2.0%)	0 (0.0%)	3 (100.0%)	0 (0.0%)
Acinetobacter haemolyticus	1 (0.7%)	1 (100.0%)	0 (0.0%)	1 (100.0%)
Total Cohort	147 (100.0%)	139 (94.6%)	8 (5.4%)	140 (95.2%)

TABLE 1: Phenotypic ESBL Production (DDST) and Multidrug Resistance Across Acinetobacter Species (N = 147)

Values are expressed as absolute numbers and percentages, n (%). DDST: Double Disc Synergy Test, ESBL: Extended-Spectrum Beta-Lactamase, MDR: Multidrug Resistance.

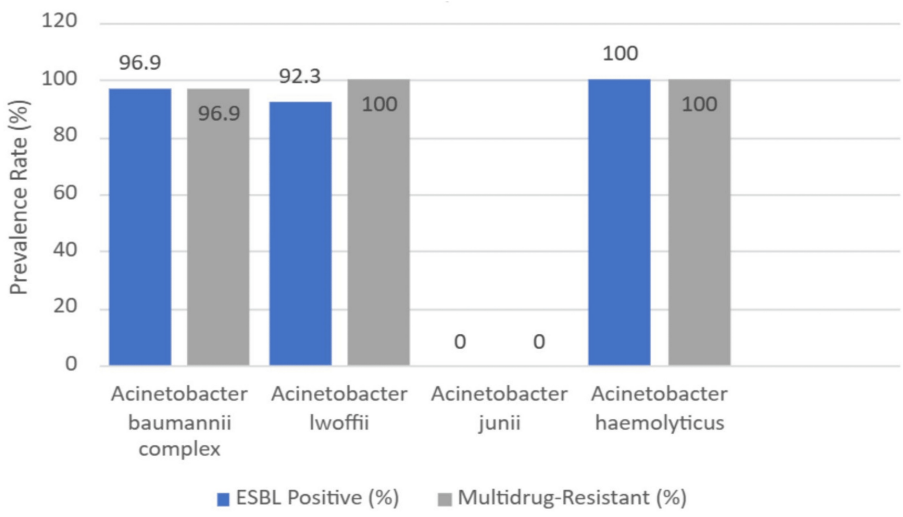

FIGURE 1: ESBL Positivity and MDR Prevalence Across Acinetobacter Species (N = 147)

Bar chart showing the percentage prevalence of ESBL production (via DDST) and MDR across identified *Acinetobacter* species. ESBL: Extended-Spectrum Beta-Lactamase; DDST: Double Disc Synergy Test; MDR: Multidrug Resistance.

Evaluation of beta-lactamase inhibitor efficacy

Analysis of paired isolate-level susceptibility transitions between standalone beta-lactam agents and beta-lactam/beta-lactamase inhibitor combinations demonstrated profound inhibitor failure across the cohort (n = 147) (Table *2*, Figure *2*). For ceftazidime versus ceftazidime/clavulanate, aggregate resistance remained identical at 92.5% (n = 136/147), comprising 134 (91.2%) concordantly resistant isolates (R → R), nine (6.1%) concordantly non-resistant isolates (Non-R → Non-R), two (1.4%) isolates restored from resistant to non-resistant (R → Non-R), and two (1.4%) isolates shifting from non-resistant to resistant (Non-R → R; exact McNemar's test, p = 1.0000). For piperacillin versus piperacillin/tazobactam, aggregate resistance increased from 68.7% (n = 101/147) to 89.1% (n = 131/147), consisting of 100 (68.0%) concordantly resistant isolates (R → R), 15 (10.2%) concordantly non-resistant isolates (Non-R → Non-R), only one (0.7%) isolate showing phenotypic restoration (R → Non-R), and 31 (21.1%) isolates shifting from non-resistant (predominantly intermediate) to resistant (Non-R → R; McNemar's test, $\chi^2 = 28.13$, df = 1, exact $p < 0.0001$).

Regimen Comparison	Agent Alone Resistant n (%)	With Inhibitor Resistant n (%)	Concordant Resistant (R → R) n (%)	Restored (R → Non-R) n (%)	Shifted to Resistant (Non-R → R) n (%)	Concordant Non-Resistant (Non-R → Non-R) n (%)	McNemar's p-value
Ceftazidime vs. Ceftazidime / Clavulanate	136 (92.5%)	136 (92.5%)	134 (91.2%)	2 (1.4%)	2 (1.4%)	9 (6.1%)	1
Piperacillin vs. Piperacillin / Tazobactam	101 (68.7%)	131 (89.1%)	100 (68.0%)	1 (0.7%)	31 (21.1%)	15 (10.2%)	< 0.0001

TABLE 2: Paired Transition Analysis of Beta-Lactam Susceptibility With and Without Beta-Lactamase Inhibitors (N = 147)

Values are presented as isolate count and percentage, n (%). R: Resistant; Non-R: Non-resistant (susceptible or intermediate). Paired transitions depict isolate-level category shifts from the standalone beta-lactam agent to the corresponding beta-lactam/beta-lactamase inhibitor combination: R → R (concordant resistance), R → Non-R (in vitro susceptibility restoration), Non-R → R (discordant shift to resistance), and Non-R → Non-R (concordant non-resistance). Paired comparisons were evaluated using McNemar's test.

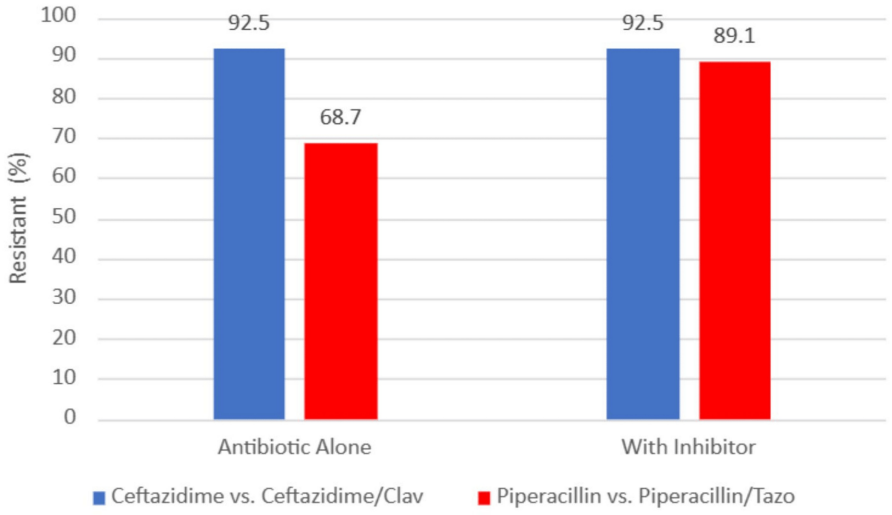

FIGURE 2: Beta-Lactamase Inhibitor Inefficacy in Clinical Acinetobacter (N = 147)

In vitro resistance rates comparing parent β-lactam antibiotics alone versus β-lactamase inhibitor combinations (ceftazidime vs. ceftazidime/clavulanate (Clav) and piperacillin vs. piperacillin/tazobactam (Tazo)).

MDR architecture

According to international consensus definitions, 95.2% (n = 140/147) of all isolates met criteria for multidrug resistance (resistant to one or more agent in three or more antimicrobial categories). Extensively drug-resistant patterns were prominent: 77.6% (n = 114/147) were resistant to six or more categories, and 27.9% (n = 41/147) showed non-susceptibility to all seven tested categories (Table *3*, Figure *3*).

Antimicrobial Class	Antimicrobial Agent	Total Cohort (N=147)		A. baumannii complex (n=130)		A. lwoffii (n=13)		A. junii (n=3)		A. haemolyticus (n=1)	
		Resistant, n (%)	Sensitive, n (%)	Resistant, n (%)	Sensitive, n (%)	Resistant, n (%)	Sensitive, n (%)	Resistant, n (%)	Sensitive, n (%)	Resistant, n (%)	Sensitive, n (%)
Carbapenems	Meropenem	136 (92.5%)	11 (7.5%)	126 (96.9%)	4 (3.1%)	10 (76.9%)	3 (23.1%)	0 (0.0%)	3 (100.0%)	0 (0.0%)	1 (100.0%)
Carbapenems	Imipenem	134 (91.2%)	13 (8.8%)	124 (95.4%)	6 (4.6%)	10 (76.9%)	3 (23.1%)	0 (0.0%)	3 (100.0%)	0 (0.0%)	1 (100.0%)
Fluoroquinolones	Ciprofloxacin	136 (92.5%)	11 (7.5%)	122 (93.8%)	8 (6.2%)	13 (100.0%)	0 (0.0%)	0 (0.0%)	3 (100.0%)	1 (100.0%)	0 (0.0%)
Fluoroquinolones	Levofloxacin	129 (87.8%)	18 (12.2%)	119 (91.5%)	11 (8.5%)	10 (76.9%)	3 (23.1%)	0 (0.0%)	3 (100.0%)	0 (0.0%)	1 (100.0%)
Cephalosporins	Ceftazidime	136 (92.5%)	11 (7.5%)	123 (94.6%)	7 (5.4%)	12 (92.3%)	1 (7.7%)	0 (0.0%)	3 (100.0%)	1 (100.0%)	0 (0.0%)
Cephalosporins	Cefepime	132 (89.8%)	15 (10.2%)	123 (94.6%)	7 (5.4%)	8 (61.5%)	5 (38.5%)	0 (0.0%)	3 (100.0%)	1 (100.0%)	0 (0.0%)
Cephalosporins	Ceftriaxone	123 (83.7%)	24 (16.3%)	110 (84.6%)	20 (15.4%)	11 (84.6%)	2 (15.4%)	1 (33.3%)	2 (66.7%)	1 (100.0%)	0 (0.0%)
Beta-lactamase Inhibitor Combinations	Ceftazidime / Clavulanate	136 (92.5%)	11 (7.5%)	123 (94.6%)	7 (5.4%)	12 (92.3%)	1 (7.7%)	0 (0.0%)	3 (100.0%)	1 (100.0%)	0 (0.0%)
Beta-lactamase Inhibitor Combinations	Piperacillin / Tazobactam	131 (89.1%)	16 (10.9%)	119 (91.5%)	11 (8.5%)	11 (84.6%)	2 (15.4%)	0 (0.0%)	3 (100.0%)	1 (100.0%)	0 (0.0%)
Penicillins	Piperacillin	101 (68.7%)	46 (31.3%)	90 (69.2%)	40 (30.8%)	10 (76.9%)	3 (23.1%)	0 (0.0%)	3 (100.0%)	1 (100.0%)	0 (0.0%)
Aminoglycosides	Gentamicin	126 (85.7%)	21 (14.3%)	114 (87.7%)	16 (12.3%)	10 (76.9%)	3 (23.1%)	1 (33.3%)	2 (66.7%)	1 (100.0%)	0 (0.0%)
Tetracyclines	Minocycline	70 (47.6%)	77 (52.4%)	64 (49.2%)	66 (50.8%)	4 (30.8%)	9 (69.2%)	1 (33.3%)	2 (66.7%)	1 (100.0%)	0 (0.0%)

TABLE 3: Antimicrobial Resistance Rates Across Seven Drug Classes Stratified by Acinetobacter Species (N = 147)

Susceptibility testing was performed via Kirby-Bauer disk diffusion according to CLSI M100 standards [3]. Values are presented as number of isolates and percentage, n (%). R: Resistant, S: Sensitive/Susceptible, CLSI: Clinical and Laboratory Standards Institute.

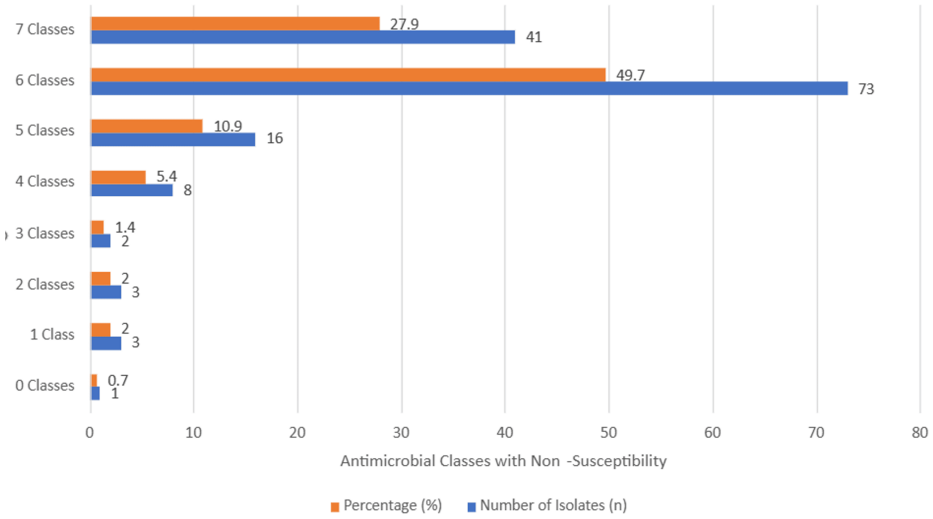

FIGURE 3: Distribution of Resistance Extent Across Seven Drug Classes (N = 147)

Architecture of multidrug non-susceptibility illustrating the number of isolates (n) and percentage (%) resistant to zero through seven antimicrobial classes.

Anatomical distribution of ESBL-producing isolates

As illustrated in Figure 4, lower respiratory secretions represented the largest anatomical reservoir for phenotypic ESBL-producing *Acinetobacter* isolates (n = 69, 49.6%), followed by pus, wound, and tissue swabs (n = 28, 20.1%), blood (n = 16, 11.5%), and urine (n = 15, 10.8%).

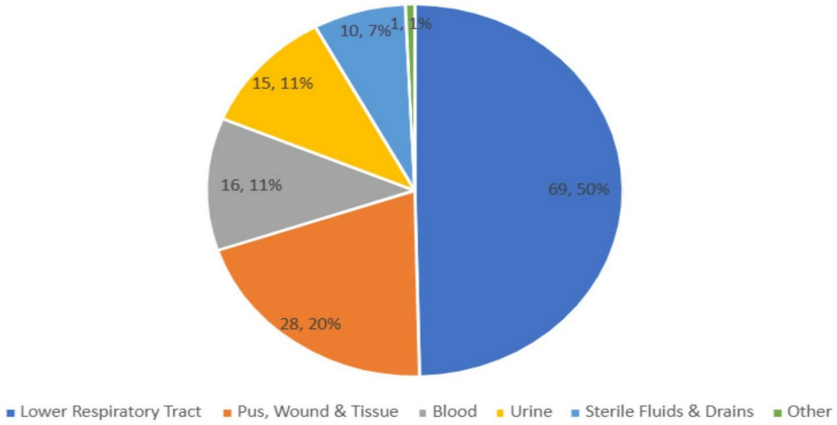

FIGURE 4: Clinical Specimen Distribution of ESBL-Producing Isolates (n = 139)

Proportional distribution of phenotypic ESBL-positive Acinetobacter isolates across anatomical specimen categories. ESBL: Extended-Spectrum Beta-Lactamase.

Anatomical specimen sourcing and resistance profiling

The anatomical specimen distribution and associated resistance patterns are detailed in Table 4, showing that lower respiratory tract samples predominated (n = 72, 49.0%) with a 95.8% ESBL positivity rate, while blood isolates demonstrated the highest ESBL prevalence at 100.0% (n = 16/16).

Specimen Category	Total Isolates (N=147)	ESBL Positive (DDST) n	MDR Positive n	ESBL Positivity Rate (%)
Lower Respiratory Tract (Sputum, ETT, TT)	72	69	70	95.8%
Pus, Wound & Tissue Swabs	29	28	28	96.6%
Blood (Bloodstream Infections)	16	16	14	100.0%
Urine (Catheter & Midstream)	16	15	16	93.8%
Sterile Body Fluids & Drains (Pleural, CSF, Ascitic)	13	10	11	76.9%
Other (Nasal, High Vaginal Swab)	1	1	1	100.0%
Total Cohort	147	139	140	94.6%

TABLE 4: Anatomical Specimen Distribution of ESBL Production and Multidrug Resistance (N = 147)

DDST: Double Disc Synergy Test, ESBL: Extended-Spectrum Beta-Lactamase, ETT: Endotracheal Tube aspirate, TT: Tracheostomy Tube aspirate, CSF: Cerebrospinal Fluid, MDR: Multidrug Resistance.

Discussion

The rapid escalation of ESBL production and MDR in *Acinetobacter* presents a profound clinical dilemma [1,4]. In our investigation, 94.6% of clinical isolates tested positive for ESBL via the DDST, and 95.2% satisfied the consensus criteria for multidrug resistance [4,10]. These numbers reflect an exceptional degree of cumulative antimicrobial selective pressure within the tertiary hospital environment [2,5].

Diagnostic value and limitations of DDST in *Acinetobacter*

While the DDST is a standard phenotypic screening assay for *Enterobacterales*, its application in *Acinetobacter* is clinically intricate [8,10]. The presence of baseline chromosomal cephalosporinases (AmpC/ADC enzymes) and co-existing oxacillinases can mask classical synergy zones [6,11]. In our cohort, combined phenotypic screening with ceftazidime and cefepime against central clavulanate discs detected presumptive ESBL-like synergy in 94.6% of clinical isolates [10]. While phenotypic synergy testing offers a practical, low-cost epidemiological screening approach in resource-constrained microbiology laboratories lacking routine molecular multiplex PCR systems, positive phenotypic findings in *Acinetobacter* spp. should be interpreted cautiously as presumptive rather than definitive in the absence of genotypic confirmation [3,17].

The biological basis of inhibitor inefficacy

A crucial finding of this study is the near-total failure of beta-lactamase inhibitors to restore antimicrobial efficacy [8]. The addition of clavulanic acid to ceftazidime yielded an identical 92.5% resistance rate, producing a negligible 1.4% net restoration, while piperacillin/tazobactam exhibited 89.1% resistance [8]. Although molecular genotypic or targeted enzyme-inhibition assays were not performed in the present study, this phenotypic refractoriness is consistent with well-documented resistance mechanisms in *Acinetobacter* spp. compared with *Enterobacterales* [9,12]. Specifically, co-production of Ambler Class D carbapenem-hydrolyzing oxacillinases (such as OXA-23 or upregulated OXA-51-like enzymes), Class B metallo-beta-lactamases (such as NDM), or hyperproduction of intrinsic chromosomal AmpC cephalosporinases-none of which are effectively neutralized by clavulanic acid or tazobactam-as well as outer membrane porin loss and efflux pump overexpression, could plausibly account for the observed lack of synergy [9,12]. However, in the absence of molecular characterization, these mechanisms remain plausible literature-supported hypotheses rather than confirmed determinants in our study isolates. Consequently, empiric administration of piperacillin/tazobactam or cefoperazone/sulbactam in patients suspected of harboring ESBL-producing *Acinetobacter* carries a high risk of therapeutic failure unless guided by validated in vitro susceptibility testing [7,15].

Species diversification and reservoir expansion

Although *A. baumannii* complex constituted 88.4% of isolates, opportunistic non-*baumannii* species displayed equally alarming resistance [13,14]. Notably, *A. lwoffii* exhibited 92.3% ESBL positivity and 100.0% multidrug resistance, accompanied by 100.0% resistance to ciprofloxacin and 92.3% resistance to ceftazidime [13]. Furthermore, *A. haemolyticus* showed multidrug resistance with high cephalosporin failure while remaining carbapenem susceptible [14]. Traditionally viewed as benign skin commensals, these non-*baumannii* species clearly function as potent horizontal reservoirs for conjugative plasmids carrying ESBL determinants, transposons, and integrons [13,16]. Clinicians and clinical microbiologists must no longer dismiss non-*baumannii* isolates as contaminants when recovered from deep tissue, invasive lines, or sterile fluids [14].

Extensive co-resistance architecture

Analysis of resistance across seven drug categories revealed that 77.6% of isolates were resistant to six or more antimicrobial classes [4]. Non-beta-lactam agents were compromised, including ciprofloxacin (92.5%) and gentamicin (85.7%) [6]. Among tested agents, minocycline demonstrated the highest preserved susceptibility, retaining in vitro activity in 52.4% of isolates overall and 69.2% in *A. lwoffii* [18]. This highlights minocycline as an important candidate for carbapenem- and beta-lactam-sparing treatment regimens [18].

Study limitations

A recognized limitation of this study is the low numerical representation of specific non-*baumannii* species, namely *A. junii* (n = 3) and *A. haemolyticus* (n = 1), which naturally limits generalized statistical inferencing for these minor taxa [13,14]. Additionally, because this was a laboratory-based study utilizing anonymized diagnostic isolates, formal retrospective chart reviews or standardized bedside surveillance definitions (such as CDC/NHSN criteria) were not performed to independently adjudicate clinical outcomes or colonization in non-sterile sites.

Furthermore, species identification relied exclusively on conventional phenotypic and biochemical methods without confirmatory MALDI-TOF MS or molecular sequencing (such as *bla*OXA-51-like PCR or *rpoB* gene analysis); consequently, cryptic sibling species within the *Acinetobacter calcoaceticus-baumannii* complex could not be differentiated individually.

Additionally, because our routine disc diffusion panel evaluated seven of the nine Magiorakos et al. [19] antimicrobial categories for *Acinetobacter* spp. (excluding colistin, which requires broth microdilution, and ampicillin/sulbactam), XDR classification was based on a panel-adapted definition rather than full nine-category testing.

Finally, phenotypic ESBL detection methods (DDST and CDDT) lack formal CLSI validation for *Acinetobacter* species and were not supplemented with molecular genotypic confirmation (such as PCR for *bla*CTX-M, *bla*TEM, *bla*SHV, *bla*PER, or *bla*VEB) or AmpC-inhibitor (e.g., cloxacillin or boronic acid) co-testing. In *Acinetobacter* spp., the hyperproduction of intrinsic chromosomal AmpC cephalosporinases (*bla*ADC) or co-existing carbapenemases both of which are refractory to clavulanic acid inhibition, can mask clavulanate-mediated synergy, potentially underestimating true ESBL carriage and reducing phenotypic assay specificity.

Conclusions

This study establishes that phenotypic ESBL production (94.6%) and MDR (95.2%) are hyper-endemic across both *Acinetobacter baumannii* and opportunistic non-*baumannii* species such as *A. lwoffii* and *A. haemolyticus*. Standard beta-lactamase inhibitors (clavulanic acid, tazobactam) fail completely to restore antimicrobial activity, rendering conventional beta-lactam/inhibitor combinations unsuitable for empiric monotherapy. Clinical laboratories must routinely employ phenotypic synergy testing to unmask ESBL and co-resistance patterns. Stringent hospital antimicrobial stewardship and infection control precautions are urgent priorities to prevent the further clonal dissemination of these pan-resistant pathogens.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Acquisition, analysis, or interpretation of data: Pranay R. Ullengala, Ravindra V. Shinde, Satish Patil

Drafting of the manuscript: Pranay R. Ullengala, Ravindra V. Shinde, Satish Patil

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Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Krishna Vishwa Vidyapeeth (Deemed to be University), Karad issued approval KVV/IEC/03/2025. The study protocol was reviewed and granted formal ethical clearance by the Institutional Ethics Committee, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad (Ref. No. KVV/IEC/03/2025, Protocol Number: 689/2024-2025) on 15/04/2025. The research was approved under the titled project: "Phenotypic Detection and Antimicrobial Resistance Profiling of ESBL and Carbapenemase Producing Acinetobacter Species Isolated from Clinical Samples in a Tertiary Care Hospital" conducted by Mr. Ullengala Pranay under the guidance of Dr. R. V. Shinde, Professor, Department of Microbiology, Krishna Institute of Medical Sciences, Karad. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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