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ORIGINAL ARTICLE

Distribution of MBL and serine-β-lactamase-producing pathogens in ventilator-associated pneumonia: insights into MDR and XDR strains

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ABSTRACT

Background and Objectives: Ventilator-associated pneumonia (VAP) caused by carbapenem-resistant Gram-negative bacteria is a serious ICU challenge. This study determined the prevalence, antimicrobial susceptibility profiles, and phenotypic carbapenemase resistance mechanisms of Gram-negative isolates from VAP patients in two tertiary hospitals in Karachi, Pakistan.

Materials and Methods: We included 104 consecutive cases of VAP (July 2021–January 2023). A total of 67 carbapenem-resistant Gram-negative isolates were identified and tested. Antibiotic susceptibility was assessed by disk diffusion and broth micro dilution, according to CLSI and EUCAST guidelines. Modified and enhanced carbapenem inactivation methods (mCIM/eCIM) were used to distinguish metallo-β-lactamase (MBL) and serine carbapenemase production.

Results: The mean age was 44.6 ± 18.3 years; 52.2% were male. Early-onset VAP accounted for 37.3% and late-onset for 62.7%. The most frequent pathogens were *Acinetobacter baumannii* (49.3%, 33/67) and *Klebsiella pneumoniae* (20.9%, 14/67). Notably, 67% of isolates produced MBLs, and 33% produced serine carbapenemases (phenotypically). The prevalence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug-resistant (PDR) phenotypes was 42.6%, 31.3%, and 19.4%, respectively.

Conclusion: VAP in our ICUs was dominated by *A. baumannii* and *K. pneumoniae* with high levels of MBL-mediated resistance. These findings highlight the urgent need for surveillance, stewardship, and new therapeutic options.

Keywords: Ventilator-associated pneumonia; Carbapenems; *Raoultella terrigena*; *Elizabethkingia meningoseptica*; Drug resistance; Bacterial

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INTRODUCTION

Ventilator-associated pneumonia (VAP) is a major cause of morbidity and mortality among mechanically ventilated patients in intensive care units (ICUs) (1). VAP is associated with prolonged ICU stays, increased healthcare costs, and a high risk of multi–organ failure, especially when caused by multidrug-resistant pathogens (2). Among the greatest challenges in treating VAP are infections caused by carbapenem-resistant Gram-negative bacteria (CR-GNB), which demonstrate resistance to broad-spectrum β-lactams, leaving clinicians with few effective antibiotics (3). The emergence and spread of carbapenem-resistant pathogens have been linked to antibiotic overuse and inadequate infection control practices (4). Notoriously, *Acinetobacter baumannii* and *Klebsiella pneumoniae* are among the most prevalent carbapenem-resistant pathogens in VAP, often associated with poor clinical outcomes and treatment failures (5). Other Gram-negative organisms – e.g., *Escherichia coli*, *Raoultella terrigena*, and *Elizabethkingia* species – have occasionally been identified in VAP cases, although their roles as primary VAP pathogens have been less established (6).

Carbapenem resistance mechanisms are multifactorial. Key among these are the production of carbapenem-hydrolyzing enzymes, such as metallo-β-lactamases (MBLs) and serine β-lactamases, as well as the overexpression of efflux pumps and the loss of porins (7). MBLs (e.g., NDM, VIM, IMP types) can confer high-level resistance to carbapenems and are increasingly reported in pathogens such as *A. baumannii* and *E. coli*, making infections extremely difficult to treat (8).

In parallel, the prevalence of MDR, XDR, and PDR Gram-negative bacteria in hospitals has been rising (9). Infections with such organisms are associated with higher mortality rates, longer hospital stays, and increased costs. An emerging concern is *Elizabethkingia meningoseptica*, which, though less common, has been reported in late-onset VAP and is intrinsically resistant to many drugs (due in part to biofilm formation and β-lactamase production) (10).

Previous studies in Pakistan have shown varying rates of carbapenem-resistant Gram-negative bacteria in different healthcare settings (11, 12). However, comparative analyses of resistance profiles and mechanisms across hospitals are limited. The impact of local factors, including patient demographics and hospital infection control practices, on the distribution of resistant pathogens in VAP remains poorly understood (13).

This study addresses these gaps by comparing the prevalence, phenotypic resistance mechanisms, and antibiograms of VAP-associated Gram-negative bacteria in two tertiary-care hospitals in Karachi. We specifically investigated the presence of MBL vs. serine-carbapenemase production via phenotypic tests, alongside the distribution of MDR, XDR, and PDR strains. By focusing on these aspects, this research aims to provide valuable insights into the epidemiology of CR-GNB in VAP, shedding light on the distribution of local resistance mechanisms and underscoring the need for improved surveillance and management strategies in ICU settings.

MATERIALS AND METHODS

Study design and setting. We conducted a descriptive, cross-sectional study at two tertiary-care hospitals in Karachi (Jinnah Postgraduate Medical Centre and Dow University of Health Sciences) between mid-2021 and early 2023. All eligible patients during the study period were included consecutively, adult (≥17 years) ICU patients who developed ventilator-associated pneumonia (VAP) ≥48 hours after intubation. VAP was confirmed clinically and microbiologically (Clinical Pulmonary Infection Score ≥6) (14). Patients with prior pneumonia, alternative infections (positive blood/urine cultures), polymicrobial or non–target organism growth (e.g. Gram-positives, Candida), or meropenem-sensitive isolates were excluded. Informed consent was obtained from patients or surrogates, and the study was approved by the institutional IRB (Dow UHS).

Sample collection and microbiological analysis. When VAP was suspected, an endotracheal aspirate was collected aseptically. Samples were plated quantitatively on MacConkey, blood, and chocolate agar. Growth of ≥10,000 colony-forming units (CFU) per mL was considered diagnostic of VAP (15). Bacterial colonies were identified to species by standard biochemical tests and API 20E/20NE systems (bioMérieux). Of the 104 aspirates, 67 yielded car-

bapenem-resistant Gram-negative isolates (the remainder had no growth or excluded pathogens). These resistant isolates were stored at -80°C in glycerol for further testing.

Antimicrobial susceptibility testing. All isolates underwent antibiotic susceptibility testing on Mueller–Hinton agar by the Kirby–Bauer disk diffusion method, interpreted using CLSI 2022 breakpoints. The panel included beta-lactams (piperacillin–tazobactam, ceftazidime, ceftriaxone, cefepime, aztreonam, meropenem), aminoglycosides (amikacin, gentamicin, tobramycin), fluoroquinolone (ciprofloxacin), and others (trimethoprim–sulfamethoxazole, tigecycline, colistin). Because all isolates were meropenem-resistant by disk testing, meropenem MICs were confirmed using Etest strips. Highly resistant isolates of *A. baumannii*, *K. pneumoniae*, and *Raoultella terrigena* were further tested by reference broth microdilution (Thermo Scientific Sensititre "Gram Negative EUMDR" panels) to obtain precise MICs for last-line agents. Interpretive criteria followed CLSI (with EUCAST or FDA provisional breakpoints as needed). Importantly, colistin MICs were determined by broth microdilution (the CLSI/EUCAST reference method) since agar-based diffusion methods are unreliable for polymyxins. We applied FDA provisional breakpoints as needed. In the absence of specific CLSI/EUCAST breakpoints for certain species (e.g., *Raoultella terrigena*), we interpreted the results using Enterobacterales breakpoints (based on *Klebsiella*/*E. coli* criteria) as recommended by CLSI.

Phenotypic carbapenemase detection. We applied the CLSI-recommended modified Carbapenem Inactivation Method (mCIM) and EDTA-modified CIM (eCIM) to all carbapenem-resistant isolates. In brief, for mCIM a meropenem disk was incubated in a suspension of the test organism and then placed on an *E. coli* lawn; a zone of inhibition ≤19 mm indicated carbapenemase production. For eCIM, the test was repeated with EDTA added to one meropenem disk. Restoration of a zone around the EDTA disk (i.e. positive eCIM) indicated a metallo-β-lactamase (MBL), whereas no change (negative eCIM) indicated a serine-carbapenemase. This combined mCIM/eCIM approach, per CLSI, allows detection of any carbapenemase and differentiation of MBL (mCIM+/eCIM+) from serine enzymes (mCIM+/eCIM–).

Definition of MDR/XDR/PDR. Isolates were classified as multidrug-resistant (MDR) if non-susceptible to ≥1 agent in ≥3 antimicrobial categories, extensively drug-resistant (XDR) if non-susceptible to ≥1 agent in all but ≤2 categories, and pan drug-resistant (PDR) if non-susceptible to all agents in all categories (16). These definitions were applied based on the panel of drugs tested.

Statistical analysis. Data were analyzed using SPSS v25. Continuous variables (e.g., age) were expressed as mean ± SD and compared by t-test; categorical variables were presented as frequencies/percentages and compared by chi-square test. A p-value <0.05 was considered significant. Multivariate analysis was not performed due to the sample size and descriptive focus. All P values are two-tailed.

Sample size. The sample size was determined by the number of available cases during the study period, with no a priori power calculation. Post hoc analysis indicated that the inclusion of 67 resistant isolates allows for the estimation of trait prevalence with a 95% confidence interval of approximately ± 10% for an attribute with a true incidence of 50% (17). This level of precision was considered acceptable for this exploratory analysis.

Ethical approval for the study was obtained as noted above, and we adhered to STROBE guidelines for reporting observational studies.

RESULTS

Patient demographics and clinical characteristics. A total of 104 VAP patients were included, with a mean age of 44.6 ± 18.3 years and 52.2% male (Table 1). The leading ICU admission diagnoses were renal failure (35.7%), CNS infection (20.9%), and cardiac disease (19.4%). Early-onset VAP (≤5 days) occurred in 37.3% of cases and late-onset VAP in 62.7%. These baseline characteristics show a diverse ICU population, with no single admission category overwhelmingly dominant.

Distribution of carbapenem-resistant pathogens by hospital and VAP onset. Of 104 endotracheal aspirates, 78 yielded Gram-negative growth. Sixty-seven carbapenem-resistant Gram-negative bacilli (CR-GNB) were recovered – 48 from Hospital A and 19

Table 1. Distribution of bacterial isolates from endotracheal aspirates (with carbapenem resistance profile by Hospital)

Organism	Hospital A (DUHS) (n, %)	Hospital B (JPMC) (n, %)	Total isolates (n)
Acinetobacter baumannii	25 (24.0%)	10 (9.6%)	35
Klebsiella pneumoniae	11 (10.6%)	9 (8.7%)	20
Raoultella terrigena	5 (4.8%)	2 (1.9%)	7
Elizabethkingia meningoseptica	4 (3.8%)	2 (1.9%)	6
Escherichia coli	5 (4.8%)	0 (0%)	5
Pseudomonas aeruginosa	2 (1.9%)	3 (2.9%)	5
No growth	3 (2.9%)	7 (6.7%)	10
Mixed growth (Polymicrobial)	2 (1.9%)	6 (5.8%)	8
Gram-positive bacteria	0 (0%)	2 (1.9%)	2
Candida species	5 (4.8%)	1 (1.0%)	6
Total samples (N=104)	52 (50.0%)	26 (25.0%)	104
Carbapenem-resistant GNB	48/52 (92.3%)	19/26 (73.1%)	67

from Hospital B. Overall, *A. baumannii* was the most frequent carbapenem-resistant pathogen, accounting for 35 isolates, of which 33 were meropenem resistant (49.3% of the total CR isolates), followed by *K. pneumoniae* (14 of 67, 20.9%). Other CR organisms included *Raoultella terrigena* (7 isolates), *Elizabethkingia meningoseptica* (6), *Escherichia coli* (5), and *Pseudomonas aeruginosa* (2). Notably, a higher fraction of Gram-negative isolates from Hospital A were carbapenem-resistant (92.3% of isolates) compared to Hospital B (73.1%). There were no significant differences in species distribution between early-onset and late-onset VAP, although all *E. meningoseptica* and 4/5 *E. coli* were from late-onset cases (ventilation >5 days).

Resistance phenotypes. All 67 CR-GNB were non-susceptible to carbapenems and virtually all β-lactams. As summarized in Table 2, 42.6% were multidrug-resistant (MDR), 31.3% extensively drug-resistant (XDR), and 19.4% pan-drug resistant (PDR). The PDR proportion (13/67 isolates) reflected strains resistant to every tested agent, including colistin and tigecycline, and these were mostly *A. baumannii* and *E. meningoseptica*. In fact, *A. baumannii* and *K. pneumoniae* together accounted for all PDR isolates, with *A. baumannii* having the highest absolute numbers of XDR and PDR. *Elizabethkingia meningoseptica* was uniformly XDR (100% of its cases) under our criteria. There was no significant difference in MDR/XDR rates between early-onset and late-onset VAP isolates (chi-square $p > 0.3$ for each category), indicating that timing of VAP did not predict.

Antibiotic susceptibility profiles. The detailed antibiograms are given in Table 3. In summary, *A. baumannii* (n=33) was almost completely resistant to all β-lactams, fluoroquinolones, and aminoglycosides. For example, 100% of *A. baumannii* isolates were resistant to piperacillin-tazobactam, ceftriaxone, cefepime, ciprofloxacin, and gentamicin. However, approximately 57% remained susceptible to colistin and 78% to tigecycline. *K. pneumoniae* (n=14) showed a similar multidrug-resistant profile: all were intrinsically resistant to ampicillin and uniformly resistant to cefuroxime and cefixime, but about 71% retained susceptibility to colistin and tigecycline. *R. terrigena* isolates were universally resistant to most β-lactams and tobramycin, yet 71% of them were tigecycline-susceptible and 57% were susceptible to minocycline. All *R. terrigena* had elevated carbapenem MICs (selected as CR strains) and most also showed elevated colistin MICs (consistent with polymyxin resistance). The five CR *E. coli* isolates were also highly resistant, but 80% remained susceptible to colistin and tigecycline. The two CR *P. aeruginosa* isolates retained some susceptibility: each was susceptible to ceftazidime and tobramycin (50% susceptible). Finally, *E. meningoseptica* (n=6) showed extreme resistance to aminoglycosides and β-lactams; 50% of isolates were intermediate to piperacillin-tazobactam and 33% to trimethoprim-sulfamethoxazole. All *E. meningoseptica* were resistant to carbapenems and aminoglycosides, consistent with its known MDR profile.

Table 2. Drug resistance phenotype by pathogen and VAP onset

Pathogen	Total (n)	MDR n (%)	XDR n (%)	PDR n (%)	Age (mean ± SD)	p (age)	Early-onset VAP n (%)	Late-onset VAP n (%)	p (VAP timing)
Acinetobacter baumannii	33 (49.3%)	19 (57.6%)	10 (30.3%)	4 (12.1%)	43.9 ± 18.9	0.31	15 (45.5%)	18 (54.5%)	0.37
Klebsiella pneumoniae	14 (20.9%)	9 (64.3%)	1 (7.1%)	4 (28.6%)	44.0 ± 19.1	–	5 (35.7%)	9 (64.3%)	–
Raoultella terrigena	7 (10.4%)	0 (0%)	4 (57.1%)	3 (42.9%)	48.4 ± 16.1	–	3 (42.9%)	4 (57.1%)	–
Elizabethkingia meningoseptica	6 (8.9%)	0 (0%)	6 (100%)	0 (0%)	44.8 ± 23.0	–	0 (0%)	6 (100%)	–
Escherichia coli	5 (7.5%)	2 (40%)	2 (40%)	1 (20%)	58.4 ± 10.3	–	1 (20%)	4 (80%)	–
Pseudomonas aeruginosa	2 (3.0%)	1 (50%)	0 (0%)	1 (50%)	45.0 ± 14.1	–	1 (50%)	1 (50%)	–
Total	67 100%)	31 (46.3%)	21 (31.3%)	13 (19.4%)	45.5 ± 18.3	–	25 (37.3%)	42 (62.7%)	0.82

Table 3. Antibiotic resistance and susceptibility profiles of pathogens isolated from ICU patients

Pathogens	*Acinetobacter baumannii* (33) (n= %)		*E. coli* (5) (n= %)		*Elizabethkingia meningoseptica* (6) (n= %)		*Klebsiella pneumonia* (14) (n= %)		*Klebsiella pneumonia* (14) (n= %)		*Raoultella terrigena* (7) (n= %)		*Pseudomonas aeruginosa* (5) (n= %)	
Antibiotics	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
TZP	33 (100)	-	5 (100)	-	3 (50)	(50)	13 (92)	1 (8)	13 (92)	1 (8)	7 (100)	-	1 (50)	1 (50)
CRO	33 (100)	-	5 (100)	-	NT		13 (92)	1 (8)	13 (92)	1 (8)	7 (100)	-	NT	
CAZ	26 (78)	7 (21)	2 (40)	3 (60)	6 (100)	-	8 (57)	6 (42)	8 (57)	6 (42)	6 (85)	1 (14)	1 (50)	1 (50)
CFM	33 (100)	-	5 (100)	-	NT		14 (100)	-	14 (100)	-	7 (100)	-	NT	
CXM	33 (100)	-	5 (100)	-	NT		14 (100)	-	14 (100)	-	7 (100)	-	NT	
GN	30 (90)	3 (9)	5 (100)	-	6 (100)	-	13 (92)	1 (8)	13 (92)	1 (8)	6 (85)	1 (14)	1 (50)	1 (50)
ERT	33 (100)	-	5 (100)	-	NT		13 (92)	1 (8)	13 (92)	1 (8)	7 (100)	-	2 (100)	-
FEP	33 (100)	-	5 (100)	-	6 (100)	-	13 (92)	1 (8)	13 (92)	1 (8)	7 (100)	-	1 (50)	1 (50)
CT	14 (42)	19 (57)	1 (20)	4 (80)	NT		4 (28)	10 (71)	4 (28)	10 (71)	4 (57)	3 (42)	1 (50)	1 (50)
MEM	33 (100)	-	5 (100)	-	(100)	-	(100)	-	(100)	-	(100)	-	(100)	-
AK	32 (96) 1 (4)		3 (60)	2 (40)	6 (100)	-	11 (78)	3 (21)	11 (78)	3 (21)	6 (85)	1 (14)	0 (0)	2 (100)
TOB	26 (78) (21)	7	4 (80)	1 (20)	NT		12 (85)	2 (14)	12 (85)	2 (14)	7 (100)	-	1 (50)	1 (50)
IMI	33 (100) -		5 (100)	-	6 (100)	-	13 (92)	1 (8)	13 (92)	1 (8)	7 (100)	-	2 (100)	-
SXT	31 (93)	2 (6)	5 (100)	-	4 (66)	2 (33)	13 (92)	1 (8)	13 (92)	1 (8)	6 (85)	1 (14)	NT	
DOR	33 (100)	-	5 (100)	-	6 (100)	-	13 (92)	1 (8)	13 (92)	1 (8)	7 (100)	-	2 (100)	-
AMC	33 (100)	-	5 (100)	-	NT		13 (92)	1 (8)	13 (92)	1 (8)	7 (100)	-	NT	
TGC	7 (21)	26 (78	1 (20)	4 (80)	NT		4 (28)	10 (71)	4 (28)	10 (71)	2 (28)	5 (71)	NT	
MH	2 (6)	21 (63)	3 (60)	2 (40)	NT		3 (21)	11 (78)	3 (21)	11 (78)	3 (42)	4 (57)	NT	

*Abbreviations: TZP, piperacillin/tazobactam; CRO, ceftriaxone; CAZ, ceftazidime; CFM, cefixime; CXM, cefuroxime; GN, gentamicin; ERT, ertapenem; FEP, cefepime; CT, cefotaxime; MEM, meropenem; AK, amikacin; TOB, tobramycin; IMI, imipenem; SXT, trimethoprim-sulfamethoxazole; DOR, doripenem; AMC, amoxicillin-clavulanate; TGC, tigecycline; MH, minocycline; NT, not tested. *

MIC profiles of *Klebsiella* and *Raoultella*. Table 4 presents MICs for representative carbapenemase-producing *K. pneumoniae* and *R. terrigena* strains. These MIC profiles confirm broad resistance: colistin was the most active agent, with MIC ≤2 µg/mL in 5 of 7 strains tested. Tigecycline was also active against several isolates (e.g. some *K. pneumoniae* had tigecycline MIC 0.5-1 µg/mL, within the FDA susceptible range). Fosfomycin showed limited efficacy (active only in two *K. pneumoniae*). In contrast, nearly all isolates had high MICs to amikacin and aztreonam, indicating resistance (only one *K. pneumoniae*, strain KP-4, had an amikacin MIC of 4 µg/mL, borderline susceptible). Overall, these data underscore that colistin and tigecycline were the only moderately active agents against these carbapenemase-positive Enterobacterales.

MIC profiles of *A. baumannii*. Table 5 shows that all tested *A. baumannii* isolates had uniformly elevated MICs (above CLSI breakpoints) for aztreonam, cefepime, fosfomycin and tobramycin, indicating complete resistance to these agents. Ceftolozane/tazobactam was likewise largely ineffective (MICs 16-32 µg/mL, except one isolate at 2 µg/mL). Amikacin activity was generally poor, though a few isolates had lower MICs (4-16 µg/mL) near the susceptibility threshold. Colistin retained partial activity: several isolates had low MICs (≤2 µg/mL) while others were resistant (MIC 8 µg/mL). Tigecycline was essentially inactive against this panel, with all strains exhibiting very high MICs (32-64 µg/mL).

Clinical condition association with pathogens. As shown in Table 6 pathogen distribution did not significantly vary by the primary ICU diagnosis (chi-square p=0.40). In other words, no particular organism was uniquely linked to a specific comorbidity. For example, *A. baumannii* and *K. pneumoniae* were both frequently isolated from patients with renal failure (30.3% and 42.9% of their cases, respectively), but neither dominated a single category. *E. meningoseptica* was most often seen in renal-failure patients (4 of 6 cases, 66.7%), and *P. aeruginosa* cases were split between COPD and CNS infection admissions. However, the lack of a significant p-value indicates no strong association between specific pathogens and admission diagnoses in this cohort.

Carbapenemase phenotypes by species. Phenotypic carbapenemase testing (mCIM/eCIM) revealed that 45 of 67 CR isolates (67.2%) were metallo-β-lactamase (MBL) producers and 22 (32.8%) produced serine carbapenemases. Table 7 details these results by species. For instance, *A. baumannii* was predominantly MBL-positive (26/33, 78.8%), whereas *K. pneumoniae* mostly harbored serine enzymes (9/14, 64.3% serine-positive and only 35.7% MBL-positive). Similarly, *R. terrigena* isolates were largely serine-positive (6/7, 85.7% serine phenotype). In contrast, all *E. coli* (5/5), *E. meningoseptica* (6/6), and *P. aeruginosa* (2/2) strains were MBL-producers. Thus, MBL production dominated in four of the six species (Table 7).

Table 4. Antimicrobial resistance profiles of *Klebsiella pneumoniae* and related strains against selected antibiotics

Strains	AMI		AZT		FEP		C/T		COL		FOS+		TGC		TOB	
	S(≤8)	R(>8)	S(≤1)	R(>4)	S(≤2)	R(>4)	S(≤2)	R(>2)	S(≤2)	(R>2)	S(≤32)	R(>32)	S(≤2)	R(>2)	S(≤2)	R(>2)
KP-4	4	-	-	32	2	-	1	-	0.5	-	-	16	0.5	-	-	4
KP-81	-	32	-	32	-	16	-	8	-	4	-	16	0.5	-	-	4
KP-85	-	32	-	32	-	16	-	8	2	-	-	64	1	-	-	4
RT-27	-	32	-	32	2	-	-	8	-	16	-	64	0.5	-	-	4
RT-35	-	32	-	32	-	16	-	8	-	0.5	32	-	1	-	-	4
RT-37	-	32	-	32	-	16	-	8	2	-	-	64	0.5	-	-	4
KP-92	-	32	-	32	-	16	-	8	-	16	-	64	1	-	-	4
E. coli	-	32	-	16	-	16	-	8	2	-	-	64	-	1	-	4

KP (*Klebsiella pneumoniae*) RT (*Raoultella terrigena*). Breakpoints for antimicrobial susceptibility and resistance are defined as per CLSI guidelines. Susceptible (S) and resistant (R) values are expressed in micrograms per milliliter (µg/mL). Antibiotics assessed include amikacin, aztreonam (AZT), cefepime (FEP), colistin, fosfomycin (FOS), tigecycline (TGC), and tobramycin (TOB).

Table 5. Antimicrobial rsistance profiles of *Acinetobacter baumannii* strains against selected antibiotics

Strains	AMI		AZT		FEP		C/T		COL		FOS		TGC		TOB	
	S(≤2)	R(>4)	S(≤2)	R(>2)	S(≤2)	R(>8)	S(≤2)	R(>2)	S≤2	R>2	S(≤)	R(>)	S(≤1)	R(>1)	S(≤2)	R(>2)
AB-04	-	8	-	8	-	16	-	8	-	16	-	32	1	-	-	4
AB-19	-	8	-	8	-	16	-	8	1	-	-	64	0.5	-	-	4
AB-20	-	8	-	8	-	16	-	8	-	16	-	64	0.5	-	-	4
AB-21	-	8	-	8	-	16	-	8	-	16	-	64	0.5	-	-	4
AB-32	-	8	-	8	-	16	-	8	-	16	-	64	1	-	-	4
AB-40	-	8	-	8	-	16	2	-	0.5	-	-	32	0.5	-	-	4
AB-50	-	8	-	8	-	16	-	8	-	16	-	64	1	-	-	4
AB-54	-	8	-	8	-	16	-	8	-	16	-	64	1	-	-	4
AB-56	-	8	-	8	-	16	-	8	-	16	-	64	1	-	-	4
AB-57	-	8	-	8	-	16	-	8	-	16	-	64	1	-	-	4
AB-70	-	8	-	8	-	16	-	8	-	16	-	64	1	-	-	4

AB (*Ascinetobacter baumannii*). Breakpoints for antimicrobial susceptibility and resistance are defined as per CLSI guidelines. Susceptible (S) and resistant (R) values are expressed in micrograms per milliliter (μg/mL). Antibiotics assessed include amikacin, aztreonam (AZT), cefepime (FEP), colistin, fosfomycin (FOS), tigecycline (TGC), and tobramycin (TOB). ceftolozane/tazobactam (C/T).

Table 6. Distribution of pathogens by primary ICU admission diagnosis

Pathogen (Total n)	Renal Disease n (%)	Septic Shock n (%)	COPD n (%)	Cardiac Disease n (%)	Epilepsy n (%)	CNS Infection n (%)	Liver Failure n (%)	Total (n)
Acinetobacter baumannii (33)	10 (30.3%)	3 (9.1%)	5 15.2%)	4 (12.1%)	1 (3.0%)	7 (21.2%)	3 (9.1%)	33
Klebsiella pneumoniae (14)	6 (42.9%)	0 (0%)	3 21.4%)	0 (0%)	0 (0%)	3 (21.4%)	2 (14.3%)	14
Raoultella terrigena (7)	3 (42.9%)	0 (0%)	1 14.3%)	1 (14.3%)	1 (14.3%)	1 (14.3%)	0 (0%)	7
Elizabethkingia meningoseptica (6)	4 (66.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (33.3%)	0 (0%)	6
Escherichia coli (5)	0 (0%)	0 (0%)	0 (0%)	1 (20.0%)	1 (20.0%)	2 (40.0%)	1 (20.0%)	5
Pseudomonas aeruginosa (2)	0 (0%)	0 (0%)	1 50.0%)	0 (0%)	0 (0%)	1 (50.0%)	0 (0%)	2

Table 7. Carbapenemase phenotypes of isolates (mCIM/eCIM results)

Pathogen	Total CR isolates N (%)	eCIM positive (MBL producer) N (%)	eCIM negative (Serine carbapenemase) N (%)
Acinetobacter baumannii	33 (49.3%)	26 (38.8%)	7 (10.4%)
Klebsiella pneumoniae	14 (20.9%)	5 (7.5%)	9 (13.4%)
Raoultella terrigena	7 (10.4%)	1 (1.5%)	6 (9.0%)
Escherichia coli	5 (7.5%)	5 (7.5%)	0 (0%)
Elizabethkingia spp. (*E. meningoseptica*)	6 (9.0%)	6 (9.0%)	0 (0%)
Pseudomonas aeruginosa	2 (3.0%)	2 (3.0%)	0 (0%)
Total	67 (100%)	45 (67.2%)	22 (32.8%)

Representative phenotypic assays are shown in Fig. 1. In particular, panel C illustrates a positive mCIM/eCIM result (growth within the carbapenem disk), confirming an MBL-producing isolate, and panel D shows the API 20E biochemical strip used for species identification.

Overall, these findings (Tables 1-7 and Fig. 1) highlight that *A. baumannii* and *K. pneumoniae* were the predominant CR pathogens, with very high rates of MDR/XDR/PDR phenotypes. Antibiotic susceptibility was extremely limited (nearly all β-lactams ineffective, and even colistin/tigecycline only variably active). Most isolates (especially *A. baumannii*, *E. coli*, *E. meningoseptica* and *P. aeruginosa*) produced MBL carbapenemases, whereas *K. pneumoniae* and *R. terrigena* often carried serine carbapenemases. These results underscore the critical resistance challenges in VAP-associated pathogens in our ICUs.

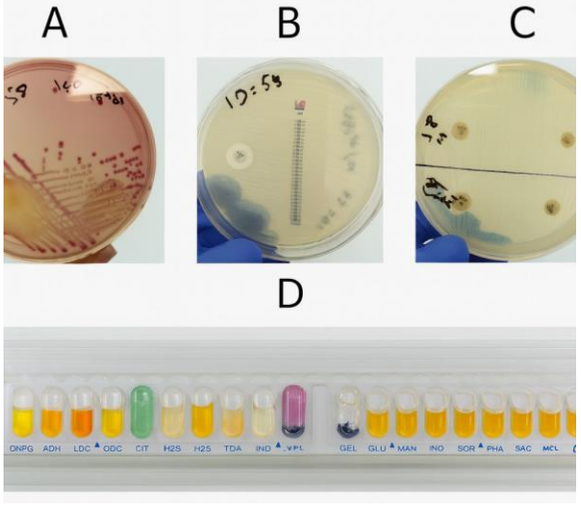

Fig. 1. Phenotypic and biochemical methods used for the characterization of carbapenem-resistant isolates
A: Growth of bacterial colonies on a MacConkey agar plate, showcasing a distinct colony morphology with characteristics indicating lactose fermentation B: Antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method. The zone of inhibition around antibiotic disks demonstrates the resistance of the bacteria to meropenam C. Modified carbapenem inactivation method (mCIM) and enhanced carbapenem inactivation method (eCIM) testing results. The image shows positive results for both mCIM and eCIM, indicating the presence of carbapenemase-producing bacteria (MBL-positive) as evidenced by growth within the antibiotic disk area. D: The API 20E strip showing multiple biochemical reactions, including tests for glucose, mannitol, indole, and others, used to identify and differentiate enteric bacteria

No statistically significant correlation was found between carbapenemase type (MBL vs. serine) and patient demographics, such as age or VAP onset (for example, in *A. baumannii*, MBL-producers were found in both early- and late-onset cases; $p = 0.37$). However, we observed that all *Elizabethkingia* and *Pseudomonas* isolates were MBL producers, consistent with known species tendencies. *K. pneumonia*'s tendency toward serine enzymes in our sample reflects the presence of likely KPC/OXA-48-like producers (18, 19).

DISCUSSION

Our study of 67 carbapenem-resistant Gram-negative isolates from VAP patients in two ICUs in Karachi provides several important insights. First, *Acinetobacter baumannii* emerged as the predominant pathogen (49% of isolates), followed by *Klebsiella pneumoniae* (20). This mirrors global ICU surveillance findings, where *A. Baumannii* consistently ranks as a leading cause of ventilator-associated infections (21). *A. baumannii*'s resilience in the hospital environment (e.g., ability to survive desiccation and disinfectants) and its propensity to accumulate resistance determinants contribute to its prevalence and persistence in ICUs (22). Our data also reinforces its significance in the local context.

We found that all our Gram-negative VAP isolates were resistant to carbapenems by selection, and indeed, most were resistant to multiple other classes of antibiotics. There was universal or near-universal resistance to penicillins and cephalosporins. Alarmingly, many isolates also showed reduced susceptibility to "last-resort" antibiotics: for instance, only ~57% of *A. baumannii* were susceptible to colistin and ~78% to tigecycline. Similarly, *K. pneumoniae* had ~71% susceptibility to those agents. These numbers align with reports from other high-burden regions, where even colistin and tigecycline are not reliably effective (23). The high rates of resistance underscore the critical state of antimicrobial options for VAP. Such findings echo the calls in recent literature for strengthened antimicrobial stewardship and the cautious use of remaining effective drugs (24). Our results essentially suggest that in our ICUs, treatment choices for VAP due to CR-GNB are extremely limited – often essentially restricted to polymyxins,

tigecycline, or combinations thereof.

We documented that 42.6% of our CR isolates were MDR, 31.3% were XDR, and an additional 19.4% qualified as PDR. These proportions are worrying and comparable to reports from other developing country ICUs. For instance, a recent systematic review reported MDR rates in ICU *A. baumannii* as high as 70-80% in some Asian countries (25). Our *A. baumannii* data (all 33 were at least MDR by definition, and indeed most were XDR or PDR) reflects this global trend. Similarly, all six *E. meningoseptica* isolates in our study were XDR (resistant to all tested antibiotics except maybe one agent), which aligns with its known profile as intrinsically MDR with few options (typically susceptible only to fluoroquinolones or trimethoprim-sulfamethoxazole in some cases). The high burden of XDR/PDR organisms highlights that infections approaching untreatability are already present in our setting. This finding reinforces the importance of rigorous infection control measures to prevent the spread of these pathogens and highlights the necessity of exploring combination therapies or new drugs (e.g., cefiderocol, eravacycline) for such cases (26).

A key focus of our study was identifying resistance mechanisms. Phenotypic testing indicated that about two-thirds of the CR isolates produced metallo-β-lactamases (MBLs), while one-third produced serine carbapenemases. This split is insightful. It suggests that NDM-1 and similar MBLs are likely widespread in our ICU isolates, especially in *A. baumannii*, *E. coli*, *P. aeruginosa*, and *Elizabethkingia* (all of which were predominantly or exclusively MBL-positive). This corresponds with regional molecular data showing NDM-1 as a common carbapenemase gene in Pakistan and neighboring areas (27). On the other hand, the fact that 64% of *K. pneumoniae* were phenotypically serine-carbapenemase producers suggests that enzymes like KPC or OXA-48-like are contributing to *Klebsiella*. OXA-48 is known to be present among this region's Enterobacterales, and KPC has also been reported sporadically (28). Our *Raoultella terrigena* isolates also mostly showed a serine enzyme profile; notably, the *Raoultella genus* (which is closely related to *Klebsiella*) has been documented to carry OXA-48-like enzymes in some cases. To our knowledge, this is one of the first reports to phenotypically characterize carbapenemase type in *R. terrigena* causing VAP.

Taken together, our findings illustrate that MBL-producing strains (particularly NDM producers) form the majority of ICU CR-GNB in our setting, which is epidemiologically important because MBLs are not inhibited by β-lactamase inhibitors and require combination therapies (e.g., aztreonam plus ceftazidime-avibactam) or newer agents to treat (29). Meanwhile, the presence of serine-carbapenemase producers (KPC/OXA) suggests that surveillance for these enzymes is also necessary, as they can spread via plasmids across species (30). We have added these points to the discussion to contextualize the significance of distinguishing MBL vs. serine mechanisms – it has direct implications for therapy (e.g., an MBL-producer *K. pneumoniae* will not respond to ceftazidime-avibactam, whereas a KPC-producer might).

It is noteworthy that we did not find any colistin resistance in *A. baumannii* (all were susceptible by broth microdilution), and only one *K. pneumoniae* showed an elevated colistin MIC. In some regions, colistin resistance in *A. baumannii* is increasing; our data suggest that colistin remains largely effective here, possibly due to its controlled use. Nonetheless, 43% of *A. baumannii* were colistin-resistant in our study (since 57% susceptible implies 43% non-susceptible, which could include intermediate/resistant), indicating the beginning of colistin resistance emergence. Colistin resistance could be due to adaptive mechanisms or plasmid-borne mcr genes; however, we did not investigate this further. Nevertheless, this trend is alarming and warrants ongoing monitoring.

A limitation of this study is that we did not include PCR-based molecular confirmation of carbapenemase genes. Our focus was on phenotypic carbapenemase detection using CLSI-recommended mCIM/eCIM, which are well-validated for differentiating serine- and metallo-β-lactamase activity. This necessarily limited our ability to determine the specific gene variants (e.g., NDM-1 vs. NDM-5, KPC vs. OXA-48). Nevertheless, mCIM/eCIM provide robust phenotypic evidence, and future studies will incorporate PCR and sequencing to provide gene-level confirmation and a more comprehensive understanding of the underlying resistance mechanisms" (31). Another limitation is that we did not collect patient outcome data. We do not know, for instance, the mortality rate of these VAP cases or the length of ICU stay attributable to these infections. Consequently, we cannot determine whether infections by XDR/PDR organisms lead to worse outcomes than those

caused by other organisms, an important clinical question. Additionally, our sample is relatively small (67 isolates) and comes from two hospitals in the same city, which may limit its generalizability. There may also be a selection bias, as only patients who underwent specific diagnostics were included. We did not perform multivariate analysis to control for confounders (e.g., differences in patient populations between the two hospitals), as this was primarily a descriptive study. These factors all suggest caution in extrapolating our findings. We recommend that broader surveillance, involving more centers and the inclusion of patient-centered outcomes, would strengthen future research.

Despite these limitations, our study provides valuable insights into the local epidemiology of VAP pathogens. It highlights that the VAP landscape in our ICUs is dominated by highly drug-resistant Gram-negative bacteria, especially *A. baumannii*, which carry primarily MBL-type resistance mechanisms. This is consistent with the WHO's classification of these bacteria as “critical” priority pathogens. From a practical perspective, our findings underscore the need for aggressive infection control (to prevent spread of these MDR organisms), antimicrobial stewardship (to delay further resistance development), and investment in rapid diagnostics (e.g., molecular tests to quickly identify carbapenemase genes). The fact that different enzymes (MBL vs. serine) are at play means that phenotypic detection of the mechanism can inform therapy – for example, the potential use of aztreonam/avibactam for MBL producers versus ceftazidime/avibactam for KPC producers. In resource-constrained settings like ours, phenotypic assays such as mCIM are practical and can guide such decisions to some extent.

CONCLUSION

This study paints a concerning picture of VAP in our setting, a high burden of carbapenem-resistant *A. baumannii* and *Klebsiella*, frequent MDR/XDR profiles, and ubiquitous carbapenemase production (mostly MBLs). These data call for immediate attention to containment measures. We also emphasize that our snapshot covers the period from 2021 to 2023; resistance patterns may continue to evolve, so continuous surveillance is necessary. Nonetheless, the dominance of these pathogens and mechanisms is likely to persist in the near term. Our findings provide a baseline that clinicians and policymakers can use to strategize empiric therapy (e.g., recognizing that colistin plus tigecycline might be the only viable empirical combination in many cases of VAP here) and to justify the introduction of newer antimicrobial agents or combination therapies in our ICUs. Ultimately, improving outcomes for VAP patients will require not only new drugs, but also better prevention – hence, ongoing efforts to enforce ventilator care bundles and strict hygiene practices remain paramount.

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