Prevalence and Molecular characterisation of Carbapenem Resistant Acinetobacter baumannii (CRAB) from clinically significant Non fermenters isolated in a tertiary care hospital

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ABSTRACT

Introduction

Non-fermenting Gram-negative bacilli (NFGNB) are increasingly implicated in healthcare-associated infections because of their ability to persist in hospital environments and their propensity to develop multidrug resistance. Among these organisms, Acinetobacter baumannii has become a prominent pathogen, particularly in intensive care units, owing to its capacity to acquire diverse resistance mechanisms. The emergence of carbapenem-resistant A. baumannii (CRAB) has further limited therapeutic options and is associated with adverse clinical outcomes. Understanding the local prevalence and molecular basis of carbapenem resistance is essential for guiding antimicrobial stewardship and infection control strategies.

Aim and Objectives

To determine the prevalence of carbapenem resistant Acinetobacter baumannii among clinically significant non-fermenting Gram-negative bacilli isolated in a tertiary care hospital and to characterize their antimicrobial susceptibility profiles and underlying carbapenem resistance genes.

Methodology

A cross-sectional study was conducted on clinical specimens received in the microbiology laboratory over a period of 2 years in a tertiary care hospital. Non-fermenting Gram-negative bacilli were isolated using standard culture techniques and identified by conventional biochemical methods and the VITEK-2 automated system. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method and interpreted according to CLSI guidelines. A. baumannii isolates showing resistance to carbapenems were subjected to polymerase chain reaction (PCR) for detection of carbapenemase-encoding genes, including blaOXA-23, blaOXA-51, blaOXA-58, blaNDM, and blaVIM.

Results

A total of 200 clinically relevant non-fermenter isolates were examined, with a mean patient age of 53.94 ± 15.79 years and male prevalence (61.5%). The most prevalent isolate was Pseudomonas aeruginosa (49.5%), followed by Stenotrophomonas maltophilia (33.5%) and Acinetobacter baumannii (16.5%). 16.5% of A. baumannii were carbapenem-resistant (CRAB). The isolates exhibited substantial multidrug resistance, with A. baumannii having very strong carbapenem resistance (imipenem 97.1%, meropenem 100%). Respiratory samples were significantly associated with carbapenem resistance ($p < 0.05$). RT-PCR revealed carbapenemase genes in 16.5% of isolates, primarily OXA-23/NDM, confirming carbapenemase-mediated resistance as the prevalent mechanism.

Conclusion

The study provides insight into the burden of CRAB and the molecular mechanisms contributing to carbapenem resistance in a tertiary care setting. These findings emphasize the necessity for continuous surveillance, rational antimicrobial use and stringent infection control measures to limit the spread of resistant strains.

Keywords: Non fermenting gram negative bacilli, Acinetobacter baumannii, Carbapenem resistance, OXA carbapenemases, Molecular characterization.

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INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) have emerged as important opportunistic pathogens in modern healthcare settings. These aerobic organisms are widely distributed in the environment and possess the ability to survive on hospital surfaces, medical devices and moist reservoirs, facilitating persistent transmission. [1,2] Over the past two decades, infections caused by NFGNB have increased substantially, particularly among critically ill patients, individuals with prolonged hospitalization and those receiving broad-spectrum antimicrobial therapy. [3,4]

Among NFGNB, *Acinetobacter baumannii* has gained considerable attention because of its association with severe healthcare-associated infections such as ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound infections and device-related infections. [5,6] The organism possesses a highly adaptable genome that facilitates the acquisition, maintenance and horizontal gene transfer of resistance determinants contributing to the rapid emergence and spread of multidrug resistant strains. [7]

Carbapenems were traditionally regarded as reliable agents for the treatment of multidrug-resistant A. baumannii infections. However, the global emergence of carbapenem-resistant A. baumannii (CRAB) has significantly compromised their effectiveness. [8,9] Resistance is primarily mediated by the production of carbapenem-hydrolyzing enzymes, particularly OXA-type carbapenemases and metallo-β-lactamases such as NDM and VIM, often carried on mobile genetic elements. [10–12] The spread of these resistance mechanisms has resulted in limited therapeutic options and increased morbidity and mortality. [13]

Local epidemiological data on the prevalence of CRAB and the distribution of resistance genes are essential for guiding empirical therapy, strengthening antimicrobial stewardship programs and implementing targeted infection control measures. [14,15] Therefore, the present study was undertaken to determine the prevalence and molecular characteristics of CRAB isolated from clinically significant NFGNB in a tertiary care hospital.

OBJECTIVES

1. To isolate the Non fermenting Gram negative bacilli (NFGNB) from clinical samples.
2. To know the prevalence and speciate the clinically significant Acinetobacter.
3. To determine the antimicrobial susceptibility pattern of Acinetobacter species by Kirby Bauer disc diffusion and their phenotypic confirmation by VITEK-2.
4. Genotypic detection of Carbapenem resistant genes by molecular assay.

METHODOLOGY

This prospective cross-sectional study was conducted in the Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College and Hospitals, Salem, over a period of two years from July 2024 to February 2026. The study was approved by the Institutional Ethics Committee (IEC) [Ethical clearance No: IEC / 24 /149]. The study included patients of all age groups presenting with clinically suspected infections.

Inclusion and Exclusion Criteria

Patients with clinical evidence of infection whose samples yielded non-fermenting Gram-negative bacilli were included in the study. Patients without signs or symptoms of infection and isolates other than NFGNB were excluded. Contaminated samples, leaky containers and specimens with improper labeling were rejected.

Sample Collection and Processing

Clinical specimens were collected using standard aseptic techniques and processed according to routine microbiological procedures. Samples were inoculated onto Blood agar and MacConkey agar and incubated aerobically at 37°C for 18–24 hours.

Isolation and Identification of NFGNB

Non-lactose fermenting colonies on MacConkey agar were subjected to Gram staining and preliminary biochemical tests. Identification and speciation were performed using conventional biochemical methods and confirmed using the VITEK-2 automated identification system.

Identification of Acinetobacter baumannii

Presumptive Acinetobacter isolates were identified based on characteristic colony morphology, Gram-negative coccobacillary appearance, oxidase negativity and further confirmed by VITEK-2.

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. AST was also performed using the VITEK-2 automated system for confirmation.

Following antibiotic discs (HiMedia) were used : Amikacin (30μg), Ampicillin-sulbactam (10μg+10μg), Ceftazidime (30μg), Cefepime (30μg), Cefotaxime (30μg), Ceftriaxone (30μg), Ciprofloxacin (5μg), Co-trimoxazole (25μg), Gentamicin (10μg), Imipenem (10μg), Levofloxacin (5μg), Meropenem (10μg), Minocycline (30μg) and Piperacillin-Tazobactam (100μg + 10μg). In addition to that, for urine isolates following discs were used: Nalidixic acid (30μg), Norfloxacin (10μg), Nitrofurantoin (300μg) and Netilmicin sulphate (30μg).

Detection of Carbapenem Resistance

Isolates showing resistance to imipenem and/or meropenem were categorized as carbapenem resistant and subjected to molecular analysis.

Molecular Detection of Carbapenemase Genes

Genomic DNA was extracted from carbapenem-resistant A. baumannii isolates using a commercially available DNA extraction kit according to the manufacturer's instructions. PCR was performed for detection of carbapenemase genes including blaOXA-23, blaOXA51, blaOXA58, blaNDM and blaVIM.

RESULTS

Figure 1: Distribution of clinically significant non fermenters (N=200)

Out of 200 NFGNB isolates, *Pseudomonas aeruginosa* was the most frequently isolated organism accounting for 99 (49.5%) isolates, followed by *Stenotrophomonas maltophilia* with 67 isolates (33.5%) and *Acinetobacter baumannii* with 34 (17%) isolates.

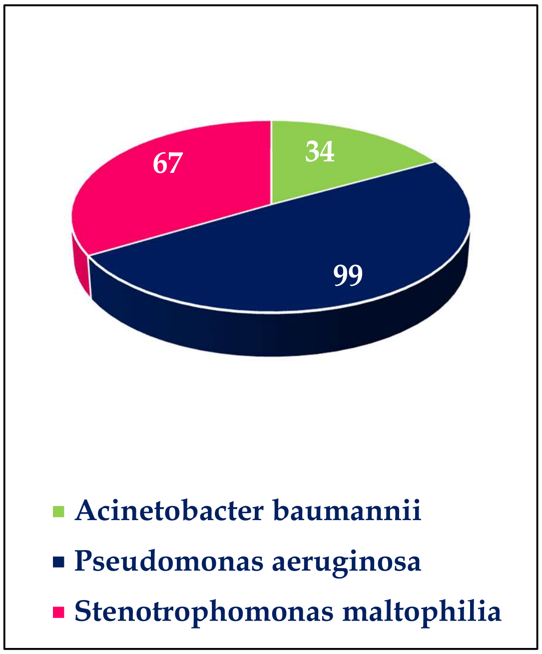
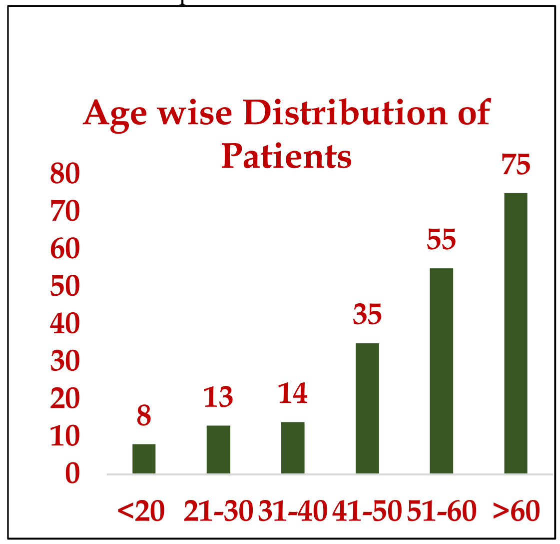


Figure 2 : Distribution of Patients based on Age

Majority of isolates were recovered from patients aged >60 years, followed by 51-60 years age group, indicating a higher prevalence of NFGNB infections among elderly patients.

Figure 3: Type of Samples

Pus samples constituted the predominant clinical specimen followed by Endotracheal aspirate (ETA), wound swab and sputum.



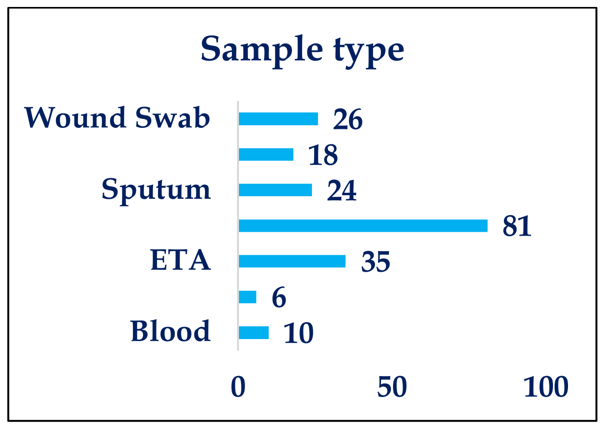
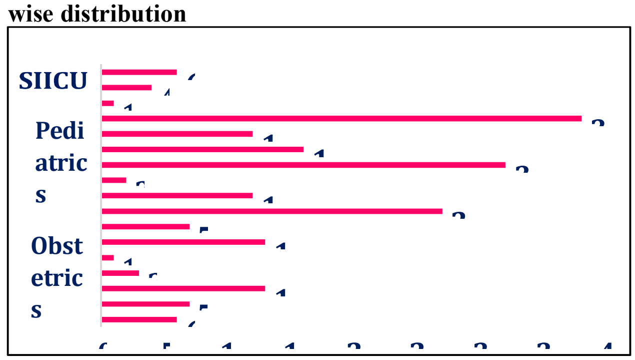


Figure 4: Department wise distribution



The Male Surgical Ward (MSW) recorded the most cases with 38 patients (19.0%), followed by the Medical Intensive Care Unit (MICU) with 32 patients (16.0%) and the Female Surgical Ward (FSW) with 27 patients (13.5%).

Table 1: Antibiogram of Acinetobacter baumannii (N=34)

Drugs	Conventional AST(N=34)			VITEK-2 AST(N=34)		
	Sensitive n (%)	Moderately sensitive n(%)	Resistant n (%)	Sensitive n (%)	Moderately sensitive n(%)	Resistant n (%)
Ampicillin-Sulbactam (10µg+10µg)	10(29.4)	4 (11.8)	20 (58.8)	11 (32.4)	5 (14.7)	18 (52.9)
Amikacin (30µg)	8 (23.5)	3 (8.8)	23 (67.6)	9 (26.5)	2 (5.9)	23 (67.6)
Gentamicin (10µg)	6 (17.6)	2 (5.9)	26 (76.5)	7 (20.6)	3 (8.8)	24 (70.6)
Cefepime (30µg)	4 (11.8)	3 (8.8)	27 (79.4)	5 (14.7)	4 (11.8)	25 (73.5)
Ceftazidime (30µg)	3 (8.8)	2 (5.9)	29 (85.3)	4 (11.8)	2 (5.9)	28 (82.4)
Cefotaxime (30µg)	2 (5.9)	1(2.9)	31 (91.2)	3 (8.8)	1(2.9)	30 (88.2)
Ceftriaxone (30µg)	2 (5.9)	1(2.9)	31 (91.2)	3 (8.8)	1(2.9)	30 (88.2)
Ciprofloxacin (5µg)	5 (14.7)	3 (8.8)	26 (76.5)	6 (17.6)	2 (5.9)	26 (76.5)
Levofloxacin (5µg)	6 (17.6)	2 (5.9)	25 (73.5)	6 (17.6)	3 (8.8)	25 (73.5)
Cotrimoxazole (25µg)	7 (20.6)	2 (5.9)	16 (47.1)	8 (23.5)	2 (5.9)	24 (70.6)
Minocycline (30µg)	15 (44.1)	3 (8.8)	28 (82.4)	16 (47.1)	2 (5.9)	16 (47.1)
Imipenem (10µg)	1(2.9)	0	33(97.0)	0	0	34 (100)
Meropenem (10µg)	2 (5.9)	0	32 (94.1)	0	0	34 (100)
Piperacillin-Tazobactam (100 + 10µg)	5 (14.7)	2 (5.9)	27 (79.4)	6 (17.6)	2 (5.9)	26 (76.5)
Colistin	-			-	5 (14.7)	29 (85.2)
Polymyxin-B	-			-	5 (14.7)	29 (85.2)

Among 34 Acinetobacter baumannii isolates, higher susceptibility was observed to minocycline and ampicillin-sulbactam, whereas a high level of resistance was noted against carbapenems.

Table 2: Antibiogram of Pseudomonas aeruginosa (N=99)

Drugs	Conventional AST(N=99)			VITEK-2 AST(N=99)		
	Sensitive n (%)	Moderately sensitive n (%)	Resistant n (%)	Sensitive n (%)	Moderately sensitive n (%)	Resistant n (%)
Amikacin (30µg) (U)	60 (60.6)	10 (10.1)	29 (29.3)	62 (62.6)	8 (8.1)	29 (29.3)
Cefepime (30µg)	45 (45.5)	12 (12.1)	42 (42.4)	48 (48.5)	SDD 10 (10.1)	41 (41.4)
Ceftazidime (30µg)	40 (40.4)	15 (15.2)	44 (44.4)	43 (43.4)	14 (14.1)	42 (42.4)
Ciprofloxacin (5µg)	50 (50.5)	9 (9.1)	40 (40.4)	52 (52.5)	8 (8.1)	39 (39.4)
Levofloxacin (5µg)	48 (48.5)	11 (11.1)	40 (40.4)	50 (50.5)	9 (9.1)	40 (40.4)
Imipenem (10µg)	38 (38.4)	10 (10.1)	51 (51.5)	40 (40.4)	9 (9.1)	50 (50.5)
Meropenem (10µg)	35 (35.4)	12 (12.1)	52 (52.5)	37 (37.4)	10 (10.1)	52 (52.5)
Netilmicin (30µg) (U)	55 (55.6)	8 (8.1)	36 (36.3)	57 (57.6)	7 (7.1)	35 (35.4)
Norfloxacin (10µg) (U)	46 (46.5)	10 (10.1)	43 (43.4)	48 (48.5)	9 (9.1)	42 (42.4)
Piperacillin-Tazobactam (100 + 10µg)	42 (42.4)	13 (13.1)	44 (44.4)	45 (45.5)	11 (11.1)	43 (43.4)
Colistin	-			-	87 (87.9)	12 (12.1)
Polymyxin-B	-			-	84 (84.8)	15 (15.2)

^SDD- Susceptible Dose Dependent interpretation was applied to conventional disk diffusion and MIC-based susceptibility testing using the VITEK 2 system, in accordance with CLSI guidelines.

Both methods showed comparable susceptibility patterns for most of antibiotics, including cefepime. However, VITEK 2 proved superior by providing rapid, MIC based results and enabling reliable testing of colistin and polymyxin B which cannot be assessed by disc diffusion. This highlights the clinical advantage of automated systems in guiding antimicrobial therapy.

Table 3: Antibiogram of Stenotrophomonas maltophilia (N=67)

Drugs	Conventional AST(N=67)			VITEK-2 AST(N=67)		
	Sensitive n (%)	Moderately sensitive n(%)	Resistant n(%)	Sensitive n (%)	Moderately sensitive n(%)	Resistant n (%)
Levofloxacin (5µg)	40 (59.7)	10 (14.9)	17(25.4)	42 (62.7)	8 (11.9)	17(25.4)
Cotrimoxazole (25µg)	45(67.2)	5(7.5)	17(25.4)	46 (68.7)	4 (6.0)	17(25.4)
Minocycline (30µg)	50 (74.6)	8(11.9)	9 (13.5)	51 (76.1)	7 (10.4)	9 (13.5)

S. maltophilia isolates exhibited high sensitivity to cotrimoxazole (67.2%) and minocycline (74.6%).

Table 4: Prevalence of Acinetobacter baumannii Isolates Based on Carbapenem Resistance Detected by VITEK-2 System

Total isolates tested	CRAB identified	Prevalence (%)
200	34	16.5

Out of total 200 non-fermenting gram negative bacilli isolates tested by the VITEK-2 system, 34 (16.5%) were identified as carbapenem resistant Acinetobacter baumannii (CRAB). Remaining 166 (83.5%) isolates were Pseudomonas aeruginosa and Stenotrophomonas maltophilia.

Table 5: Distribution of Carbapenemase Genes among Isolates Detected by RT-PCR

RT-PCR	Frequency	Percent	Cumulative Percent
OXA-23	3	8.8	8.8
OXA-23 + OXA-51	8	20.6	29.4
OXA-23 + NDM	21	64.7	94.1
OXA-58 + NDM	2	5.9	100.0
Total	34	100.0	

Molecular analysis of carbapenem resistant isolates revealed that the most common gene detection was OXA-23 + NDM detected in 21 isolates followed by OXA 23 + OXA-51.

DISCUSSION

In the present study, *Pseudomonas aeruginosa* was the most predominant isolate, followed by *Stenotrophomonas maltophilia* and *Acinetobacter baumannii*. Similar findings were reported by Kaur et al., [16] who observed P. aeruginosa as the leading non-fermenting Gram-negative bacillus (NFGNB) in clinical specimens. The predominance of P. aeruginosa may be attributed to its intrinsic resistance mechanisms, biofilm-forming ability and persistence in hospital environments.

The majority of isolates in this study were obtained from patients aged >60 years, followed by the 51–60 years age group. Comparable age distribution patterns have been described by Falagas et al., [17] who reported increased susceptibility to NFGNB infections among elderly individuals due to comorbidities, prolonged hospitalization, and frequent invasive procedures.

Pus samples constituted the major clinical specimen, followed by endotracheal aspirates (ETA), indicating the significant role of wound infections and ventilator-associated infections in the transmission of NFGNB. Similar observations were documented by Munoz-Price et al., [18] highlighting the association of NFGNB with surgical site infections and ventilator-associated pneumonia.

With regard to antimicrobial susceptibility, A. baumannii isolates in the present study demonstrated higher sensitivity to minocycline and ampicillin–sulbactam. Similar susceptibility patterns have been reported by Queenan et al., [19] who noted better activity of sulbactam-based combinations against multidrug-resistant A. baumannii. However, a high level of resistance to carbapenems (meropenem and imipenem) was observed, which is consistent with findings by Magiorakos et al., [20] who described rising carbapenem resistance among A. baumannii globally.

Polymyxin resistance observed in this study is particularly concerning, as colistin and polymyxin B are often considered last-resort drugs for carbapenem-resistant isolates. Falagas et al. have reported emerging polymyxin resistance in A. baumannii, emphasizing the therapeutic challenges posed by extensively drug-resistant strains.

Among P. aeruginosa isolates, amikacin retained good activity, whereas high resistance to carbapenems was noted. Similar trends were reported by Kaur et al., indicating that aminoglycosides remain relatively effective compared to beta-lactams in certain settings. The considerable proportion of intermediate susceptibility to colistin and polymyxin B may suggest evolving reduced susceptibility patterns, warranting careful antimicrobial stewardship.

Stenotrophomonas maltophilia isolates in the present study showed high sensitivity to cotrimoxazole and minocycline. This finding is in agreement with previous studies by Brooke et al.,[(21)] who identified cotrimoxazole as the drug of choice for S. maltophilia infections.

Molecular analysis revealed that OXA-23 combined with NDM was the most frequently detected carbapenemase gene pattern, followed by OXA-23 with OXA-51. Similar gene combinations have been reported by Poirel et al., [(22)] who highlighted OXA-type carbapenemases as predominant mechanisms of resistance in A. baumannii. The coexistence of OXA and NDM genes indicates the dissemination of highly resistant clones and limits therapeutic options.

Overall, the study highlights the increasing burden of multidrug-resistant NFGNB, particularly carbapenem-resistant and polymyxin-resistant strains, underscoring the urgent need for stringent infection control practices, antimicrobial stewardship, and continuous molecular surveillance.

CONCLUSION

This study demonstrates a notable prevalence of *Acinetobacter baumannii* among clinically significant non-fermenting Gram-negative bacilli in a tertiary care hospital. Marked carbapenem resistance and the presence of OXA-23 in combination with NDM and OXA-51 indicate the spread of important carbapenemase genes. Emerging reduced susceptibility to polymyxins further narrows treatment options. These findings highlight the need for ongoing molecular monitoring and strengthened infection control and antimicrobial stewardship measures.

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