RESEARCH PAPER

Comparative Evaluation of Conventional phenotypic Methods and VITEK 2 automated system for Identification and Antimicrobial Susceptibility testing of Non fermenting gram negative bacilli

Nalina C [1], Mathavi Sureshkumar [2]

[1]*Final Year Postgraduate Department of Microbiology Vinayaka Mission's Kirupananda Variyar Medical College and Hospital Vinayaka Mission's Research Foundation (Deemed to be University) Salem, Tamil Nadu, India*
Email: nalinachitrarasu@gmail.com
[2]*Professor and Head Department of Microbiology Vinayaka Mission's Kirupananda Variyar Medical College and Hospital Vinayaka Mission's Research Foundation (Deemed to be University) Salem, Tamil Nadu, India*
Email: drmathavimicro@gmail.com.

ABSTRACT
Background: Non-fermenting gram-negative bacilli (NFGNB) once considered as contaminants have emerged as major cause of life threatening nosocomial infections and are frequently associated with multidrug resistance. Pseudomonas aeruginosa and Acinetobacter baumannii are among the most clinically important NFGNB implicated in healthcare-associated infections. Objectives: To isolate and identify NFGNB from clinical samples and to compare conventional phenotypic method with automated VITEK-2 system for their identification and antimicrobial susceptibility testing. Methodology: A cross-sectional study was conducted over six months (May–October 2025) in the Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College, Salem. A total of 1138 clinical samples were processed using microscopy, culture and standard biochemical tests. Presumptive NFGNB isolates were further identified using conventional techniques and confirmed by VITEK-2 automated system. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion and compared with VITEK 2 AST results. Results: Out of 1138 samples, 147 (12.9%) isolates were identified as NFGNB. Pseudomonas aeruginosa was the predominant isolate 108 (73.5%) followed by 13 isolates of Acinetobacter baumannii and 26 Stenotrophomonas maltophilia isolates. Multidrug resistance was predominantly observed in Acinetobacter baumannii followed by Stenotrophomonas maltophilia and Pseudomonas aeruginosa. The turnaround time for identification and AST was significantly shorter with VITEK-2 (8-18 hours) compared to conventional methods (48-72 hours). Conclusion: VITEK-2 provides rapid and accurate identification of NFGNB, enabling timely antimicrobial therapy and strengthening antimicrobial stewardship. The predominance of Pseudomonas aeruginosa and high MDR rates among Acinetobacter baumannii highlight the need for continuous surveillance and strict infection control measures.

Keywords: Non fermenting gram negative bacilli (NFGNB), VITEK-2, Multidrug resistance (MDR), Antimicrobial susceptibility testing.
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INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) such as Pseudomonas aeruginosa, Acinetobacter baumannii, and Stenotrophomonas maltophilia are increasingly recognized as significant pathogens in hospital environments, particularly among postoperative and critically ill patients [1,2]. These organisms are commonly associated with bloodstream infections, ventilator-associated pneumonia, urinary tract infections and surgical site infections contributing to high morbidity, mortality and extended hospital stays [3,4].

The persistence of NFGNB on moist hospital surfaces and medical devices combined with their intrinsic and acquired resistance mechanisms makes eradication difficult [5]. Acinetobacter baumannii, in particular, has emerged as a major concern due to its multidrug-resistant (MDR) profile which limits therapeutic options and complicates clinical management[6]. Pseudomonas species and Stenotrophomonas maltophilia also exhibit variable resistance patterns posing challenges for empirical therapy [7].

Accurate and timely identification of NFGNB is essential for effective patient management and infection control. Conventional phenotypic methods based on colony morphology, Gram staining, and biochemical reactions are labor-intensive, time-consuming, and may fail to differentiate closely related species [8]. Automated systems such as VITEK 2 provide rapid, reproducible species-level identification along with simultaneous antimicrobial susceptibility testing, significantly reducing turnaround time and facilitating early initiation of targeted therapy [9].

**Author for Correspondence: drmathavimicro@gmail.com*

Considering the rising prevalence of MDR NFGNB in hospital settings, it is imperative to evaluate the performance of conventional methods in comparison with automated identification systems. Such comparative studies are valuable for optimizing antimicrobial stewardship programs, guiding empirical therapy, and strengthening infection control strategies.

This study aimed to isolate and identify non-fermenting Gram-negative bacilli (NFGNB) from various clinical samples using conventional methods, to compare the performance of conventional methods with the automated VITEK 2 system for accurate identification of NFGNB and to assess antimicrobial susceptibility patterns of NFGNB isolates and correlate conventional and automated AST results.

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 six months from May 2025 to October 2025. 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

A total of 1138 clinical specimens including pus, urine, sputum, endotracheal aspirates, blood, wound swabs, catheter tips and other body fluids were collected under strict aseptic precautions after obtaining informed consent. All specimens were subjected to direct gram staining and cultured onto Blood agar and MacConkey agar plates followed by aerobic incubation at 37°C for 18–24 hours.

Non lactose fermenting isolates on MacConkey agar were subjected to further analysis.

Conventional Identification

NFGNB isolates were identified using standard biochemical tests including catalase test, oxidase test, citrate utilization, urease test, nitrate reduction test, Triple Sugar Iron (TSI) agar, Hugh–Leifson oxidative-fermentative test, mannitol fermentation, lysine and ornithine decarboxylation, arginine dehydrolation, growth at 42°C and 44°C and utilization of 10% lactose. NFGNB were presumptively identified using oxidase test, pigment production and characteristic colony morphology with special emphasis on oxidase positive isolates.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. Inoculum was standardised to 0.5 McFarland turbidity. Plates were incubated at 37°C for 16–18 hours and zone diameters were measured and interpreted according to CLSI guidelines [10].

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).

Quality control was ensured using standard reference strains Pseudomonas aeruginosa ATCC 27853 as per CLSI guidelines.

Automated Identification using VITEK 2

All presumptive NFGNB isolates were subjected to identification using the VITEK 2 Compact system with GN identification cards. Antimicrobial susceptibility testing was carried out simultaneously using AST cards as per CLSI guidelines. AST results obtained by conventional disk diffusion were compared with VITEK-2 findings. Concordance and turnaround time between both methods were analyzed.

Results

Out of 1138 clinical samples processed, 634 showed growth of which 147 isolates were identified as NFGNB. *Pseudomonas aeruginosa* were predominant with 108 isolates and 13 *Acinetobacter baumannii* identified by conventional methods and remaining 26 isolates were reported only as NFGNB by conventional methods. VITEK-2 system accurately identified all 26 unclassified NFGNB isolates as *Stenotrophomonas maltophilia,* highlighting the limitations of conventional methods in species level identification.

Figure 1: Distribution of organisms among the isolated NFGNB (N = 147)

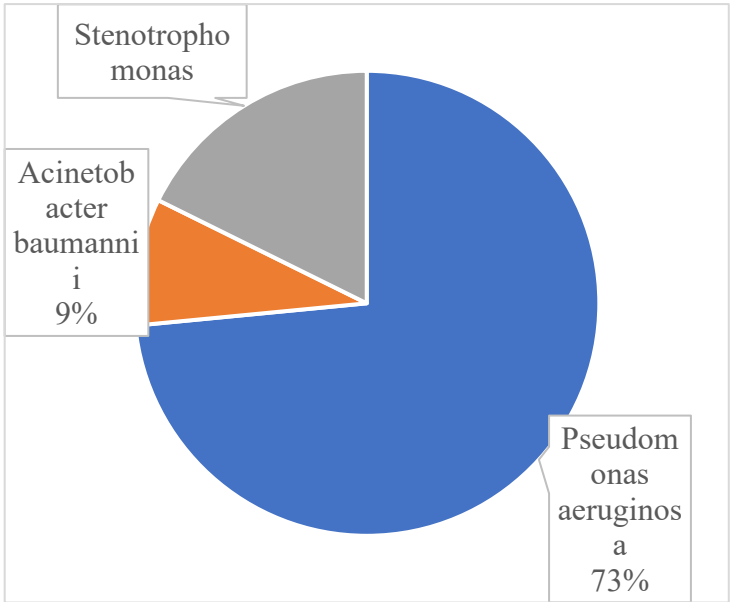
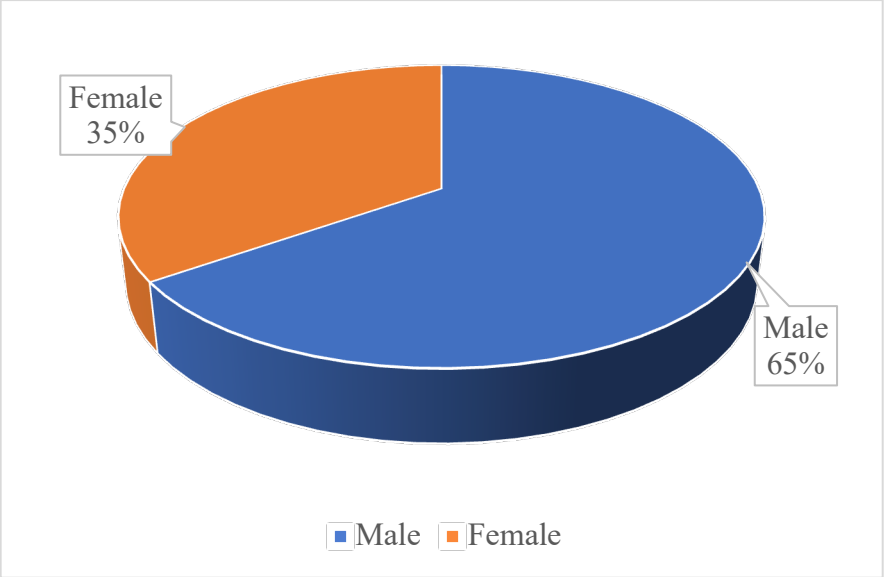


Table 1: Age distribution among the isolated NFGNB (N = 147)

Age	Total
< 18 years	11 (7.4%)
18 - 59 years	87 (59%)
≥ 60 years	49 (33%)

Majority of isolates were obtained from patients aged 18 to 59 years followed by ≥ 60 years and only 11 (7.4%) were paediatric age group (<18 years). This distribution indicates a predominance of NFGNB infections among adult and elderly populations, attributable to prolonged healthcare exposure and underlying comorbidities.

Figure 2 : Distribution of sex among isolated NFGNB (N = 147)



From this diagram it was observed that more than half n=96 (65.3%) were males
and n= 51 (35 %) were females. Similar observations have been noted in hospital based studies on non-fermenting gram negative bacilli likely reflecting increased healthcare exposure and associated risk factors among male patients[11].

Table 2: Distribution of type of samples among the isolated NFGNB (N = 147)

S.No	Type of sample	Total samples	NFGNB isolates	Percentage
1	Urine	497	8	5.4%
2	Sputum	306	27	18%
3	Pus	202	91	62%
4	ET aspirate	76	16	11%
5	Blood	44	3	2%
6	Central line tip	13	2	2%

Out of 147 NFGNB, 91 (62 %) were isolated from pus followed by 27 (18.3 %) from sputum, 16 (11%) from Endotracheal aspirate followed by urine (5.4 %), blood (2 %) and central line tip (2 %).

Table 3 : Department wise distribution of isolated NFGNB (N = 147)

Departments	Total
General Medicine	20 (14%)
Emergency Medicine	11 (7%)
General Surgery	57 (39%)
Pediatrics	7 (5%)
Orthopedics	36 (24%)
ICU	10 (7%)
ENT	2 (1%)
Chest & TB	4 (3%)

Most isolates of NFGNB were from the Department of Surgery (MSW>FSW) 57 (39%) followed by 36 (24%) Orthopaedics, 20 (14%) General Medicine and Emergency Medicine 11 (7%).

Table 4: Concordance analysis between Conventional methods and VITEK-2

Organism	Total isolates	Identified by conventional methods	Identified by VITEK-2	Concordance (%)
Pseudomonas aeruginosa	108	108	108	100
Acinetobacter baumannii	13	13	13	100
Stenotrophomonas maltophilia	26	Identified as NFGNB	26	0
Total	147	121	147	82.3

The overall concordance between conventional methods and VITEK 2 was 82.3%. Conventional methods accurately identified Pseudomonas and Acinetobacter but failed to detect Stenotrophomonas. A statistically significant difference ($p < 0.001$) was observed only for Stenotrophomonas maltophilia highlighting the superior performance of VITEK-2 in species level identification of NFGNB.

Table 5: Comparison of turnaround time between Conventional methods and VITEK-2

Method	ID (hrs)	AST (hrs)	Total TAT (hrs)
Conventional	24-48	24	48-72
VITEK-2	4-8	6-18	8-18

VITEK-2 system provided organism identification and AST results within 8-18 hours, whereas conventional methods required 48-72 hours. Independent t-test analysis confirmed that the difference in TAT was statistically significant ($p<0.001$), confirming the efficiency of automated systems for rapid diagnosis.

Table 6: Antimicrobial susceptibility pattern of Pseudomonas aeruginosa (N=108)- Conventional and automated method

Drugs	Conventional AST(N=108)			VITEK-2 AST(N=108)		
	Sensitive n (%)	Moderately sensitive n (%)	Resistant n (%)	Sensitive n (%)	Moderately sensitive n (%)	Resistant n (%)
Amikacin (30µg) (U)	78 (72.2)	10 (9.3)	20 (18.5)	82 (75.9)	8 (7.4)	18 (16.7)
Cefepime (30µg)	72 (66.7)	9 (8.3)	27 (25.0)	76 (70.4)	SDD 7 (6.5)	25 (23.1)
Ceftazidime (30µg)	70 (64.8)	8 (7.4)	30 (27.8)	74 (68.5)	6 (5.6)	28 (25.9)
Ciprofloxacin (5µg)	66 (61.1)	12 (11.1)	30 (27.8)	70 (64.8)	10 (9.3)	28 (25.9)
Levofloxacin (5µg)	68 (63.0)	10 (9.3)	30 (27.8)	72 (66.7)	8 (7.4)	28 (25.9)
Imipenem (10µg)	60 (55.6)	8 (7.4)	40 (37.0)	62 (57.4)	8 (7.4)	38 (35.2)
Meropenem (10µg)	58 (53.7)	10 (9.3)	40 (37.0)	60 (55.6)	10 (9.3)	38 (35.2)
Netilmicin (30µg) (U)	75 (69.4)	8 (7.4)	25 (23.2)	78 (72.2)	6 (5.6)	24 (22.2)
Norfloxacin (10µg) (U)	72 (66.7)	10 (9.3)	26 (24.0)	75 (69.4)	8 (7.4)	25 (23.2)
Piperacillin-Tazobactam (100 + 10µg)	74 (68.5)	9 (8.3)	25 (23.2)	78 (72.2)	7 (6.5)	23 (21.3)
Colistin	-			-	33 (30.5)	75 (69.4)
Polymyxin-B	-			-	31 (28.7)	77 (71.2)

^SDD- Susceptible Dose Dependent interpretation was applied only for MIC based testing using VITEK 2 as per 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 7: Antimicrobial susceptibility pattern of Acinetobacter baumannii (N=13) Conventional and automated method

Drugs	Conventional AST(N=13)			VITEK-2 AST(N=13)		
	Sensitive n (%)	Moderately sensitive n(%)	Resistant n (%)	Sensitive n (%)	Moderately sensitive n(%)	Resistant n (%)
Ampicillin-Sulbactam (10µg+10µg)	7 (53.8)	2(15.4)	4 (30.8)	8 (61.5)	2(15.4)	3 (23.1)
Amikacin (30µg)	3 (23.0)	1 (7.7)	9 (69.3)	3 (23.0)	1 (7.7)	9 (69.3)
Gentamicin (10µg)	2 (15.4)	1 (7.7)	10 (76.9)	2 (15.4)	1 (7.7)	10 (76.9)
Cefepime (30µg)	1 (7.7)	1 (7.7)	11 (84.6)	1 (7.7)	1 (7.7)	11 (84.6)

Ceftazidime (30µg)	1 (7.7)	0	12 (92.3)	1 (7.7)	0	12 (92.3)
Cefotaxime (30µg)	0	0	13 (100)	0	0	13 (100)
Ceftriaxone (30µg)	0	0	13 (100)	0	0	13 (100)
Ciprofloxacin (5µg)	2 (15.4)	1 (7.7)	10 (76.9)	2 (15.4)	1 (7.7)	10 (76.9)
Levofloxacin (5µg)	2 (15.4)	1 (7.7)	10 (76.9)	2 (15.4)	1 (7.7)	10 (76.9)
Cotrimoxazole (25µg)	4 (30.8)	1 (7.7)	8 (61.5)	4 (30.8)	1 (7.7)	8 (61.5)
Minocycline (30µg)	5 (38.5)	2 (15.4)	6 (46.1)	6 (46.1)	2 (15.4)	5 (38.5)
Imipenem (10µg)	2 (15.4)	0	11 (84.6)	2 (15.4)	0	11 (84.6)
Meropenem (10µg)	2 (15.4)	0	11 (84.6)	2 (15.4)	0	11 (84.6)
Piperacillin-Tazobactam (100 + 10µg)	3 (23.0)	1 (7.7)	9 (69.3)	3 (23.0)	1 (7.7)	9 (69.3)
Colistin	-			-	2 (15.4)	11 (84.6)
Polymyxin-B	-			-	2 (15.4)	11 (84.6)

The antimicrobial susceptibility patterns obtained by conventional and VITEK 2 methods showed good concordance with most antibiotics. Both techniques showed high resistance to carbapenems, indicating a predominance of carbapenem resistant Acinetobacter baumannii. Ampicillin-sulbactam showed comparatively better susceptibility by both methods suggesting its therapeutic relevance. VITEK 2 additionally enabled MIC based detection of colistin and polymyxin B, which cannot be assessed by disc diffusion. This underscores the importance of automated systems in guiding treatment of multidrug resistant infections.

Table 8: Antimicrobial susceptibility pattern of Stenotrophomonas maltophilia (N=26) Conventional and automated method

Drugs	**Conventional AST(N=26)**			**VITEK-2 AST(N=26)**		
	Sensitive n (%)	**Moderately sensitive n(%)**	**Resistant n(%)**	**Sensitive n (%)**	**Moderately sensitive n(%)**	**Resistant n (%)**
Levofloxacin (5µg)	17 (65.4)	3 (11.5)	6 (23.1)	18 (69.2)	3 (11.5)	5 (19.3)
Cotrimoxazole (25µg)	19 (73.1)	2 (7.7)	5 (19.2)	21 (80.8)	2 (7.7)	3 (11.5)
Minocycline (30µg)	21 (80.8)	3 (11.5)	2 (7.7)	23 (88.5)	3 (11.5)	1 (3.8)

While conventional methods identified isolates only as NFGNB, species level confirmation and optimized AST were achieved using VITEK 2. Minocycline and cotrimoxazole emerged as the most effective agents. These results emphasize the importance of automated systems for accurate management.

DISCUSSION:

Non-fermenting Gram-negative bacilli (NFGNB) have emerged as notable nosocomial pathogens due to their multidrug resistance, ability to survive in hospital environments and potential to cause severe infections in hospitalized patients. In this study, NFGNB were isolated from 147 of 1,138 clinical specimens (12.9%), which is comparable to isolation rates reported in other tertiary care hospitals. Sahu et al., observed a 13% prevalence, Singh et al., reported 12.5%, and Gupta et al., found 11.8%, demonstrating that NFGNB are consistently encountered in clinical specimens [12,13,14].

Among the isolates identified by conventional methods, Pseudomonas aeruginosa was the most frequent comprising 73.5% of cases. This predominance is similar to the findings of Gopalakrishnan et al., who reported Pseudomonas as the leading NFGNB (70%) [15]. Pseudomonas is widely recognized for colonizing moist hospital environments and for its involvement in postoperative wound infections, catheter-associated infections and ventilator-associated pneumonia. The high incidence in surgical and ICU wards in our study aligns with the findings of Borkar et al., which indicated these areas are particularly susceptible to NFGNB infections [16].

Conventional phenotypic identification methods had limited sensitivity for certain species. Stenotrophomonas maltophilia isolates were categorized only as non-fermenters without species-level confirmation. In contrast, the VITEK 2 system successfully identified all S. maltophilia isolates. Similar results were reported by Yildirim et al., highlighting the superior species-level accuracy of automated systems, while Ryan et al., emphasized the challenges of identifying S. maltophilia using conventional methods and the benefits of automation [17,18].

Acinetobacter baumannii constituted 8.8% of isolates, with a high level of multidrug resistance. Carbapenem resistance was observed in 84.6% of isolates, which is comparable to findings by Jain et al., and Peleg et al., who reported MDR rates exceeding 70% in hospital-acquired A. baumannii [2,19]. The organism's ability to acquire multiple resistance mechanisms, including OXA-type carbapenemases, aminoglycoside-modifying enzymes and efflux pumps, contributes to its persistence and makes infections challenging to treat. Such MDR strains are often associated with prolonged hospitalization, increased morbidity and higher healthcare costs.

Pseudomonas aeruginosa displayed moderate resistance to cephalosporins and fluoroquinolones but remained largely susceptible to carbapenems, consistent with the observations of Sharma et al., who noted preserved carbapenem efficacy despite rising resistance trends [20]. S. maltophilia isolates were resistant to most β-lactams and carbapenems but remained susceptible to co-trimoxazole, confirming Brooke et al., findings that co-trimoxazole continues to be the treatment of choice for this organism. These susceptibility patterns emphasize the necessity of local surveillance data to guide empiric therapy. Colistin susceptibility was assessed only by VITEK 2 using MIC methodology as per CLSI guidelines. Among Acinetobacter baumannii isolates, 30.8% showed intermediate susceptibility while 69.2% were resistant, highlighting the limited therapeutic options.

Pus and wound swabs accounted for more than 65% of NFGNB isolates, reinforcing the role of these pathogens in surgical site infections studies which is similar to studies by Borkar et al., and Gopalakrishnan et al. Department-wise analysis revealed that most isolates originated from general surgery and ICU wards highlighting their relevance in postoperative and critically ill patients, as similarly reported by Biswajit et al [21].

The VITEK 2 system offers the advantage of a significantly reduced turnaround time (8–18 hours) for identification and AST compared to conventional methods, which require 48–72 hours. Funke et al., reported similar improvements in laboratory efficiency with automated systems allowing for more timely initiation of appropriate antimicrobial therapy. Humphries et al., also emphasized that rapid TAT is critical in managing nosocomial infections caused by MDR pathogens to reduce morbidity and prevent transmission [22]. Patel et al., reported that automated AST systems are generally reliable but require careful validation in settings with high prevalence of multidrug-resistant organisms[23]. The combined use of conventional and automated methods thus provides a comprehensive approach for detecting resistance patterns accurately and supporting informed clinical decision-making.

Overall, our findings are in concordance with previous studies and highlight the growing burden of MDR-NFGNB. The rapid and accurate performance of VITEK 2 supports its routine use in clinical laboratories. Continuous monitoring and rational antibiotic use remain essential to combat antimicrobial resistance.

Future studies incorporating molecular characterization of resistance genes such as OXA carbapenemases and NDM would further enhance understanding of resistance mechanisms and support targeted therapy.

CONCLUSION:

Non-fermenting Gram-negative bacilli are important nosocomial pathogens, with Pseudomonas aeruginosa being the most prevalent isolate. The VITEK 2 system demonstrated superior accuracy and faster turnaround time compared to conventional methods, enabling timely and appropriate antimicrobial therapy. The high multidrug resistance observed, particularly in Acinetobacter baumannii, highlights the need for strict infection control and rational antibiotic use. Continuous surveillance is essential to monitor resistance patterns and guide effective treatment strategies..

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