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Original Research Article

Phenotypic Detection and Antibiotic Resistance Profiling of Carbapenem Resistant Non-Fermenting Gram-Negative Rods

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Abstract:

Introduction: Carbapenem resistance in non-fermenting gram-negative bacilli (NF GNB), notably Pseudomonas and Acinetobacter, poses serious clinical challenges. Phenotypic detection methods like mCIM, Carba NP, and EDTA-based tests are crucial in resource-limited settings. This study evaluates their effectiveness for identifying carbapenemase producers in clinical isolates from a tertiary care hospital.

Methods: This four-month prospective study at CAIMS, Karimnagar, focused on identifying and characterizing non-fermenting Gram-negative bacilli from clinical specimens. Standard CLSI methods and biochemical tests were used. Carbapenem resistance was screened using meropenem disc diffusion, MHT, CDT, DDST, and CHROMagar KPC for phenotypic confirmation of carbapenemase production.

Results: Of 327 isolates, 102 were meropenem-resistant—64 P. aeruginosa, 38 A. baumannii. CHROM agar KPC showed highest carbapenemase detection (97.05%), followed by MHT, CDT, and DDST. Resistance to multiple antibiotics was 100%. Tigecycline and colistin showed limited activity (≤14%). Detection rates were significantly higher in A. baumannii.

Conclusion: Carbapenem resistance was significant in P. aeruginosa and A. baumannii, with CHROM agar KPC showing highest detection sensitivity. Multidrug resistance was common, and only colistin and tigecycline were partially effective. Continuous surveillance using phenotypic methods and rational antibiotic use are essential to manage rising resistance in clinical settings.

Keywords: Carbapenem resistance, Non-fermenting Gram-negative bacilli, Pseudomonas aeruginosa, Acinetobacter baumannii.

Introduction

Carbapenem resistance among non-fermenting gram-negative bacilli (NF-GNB), particularly Pseudomonas aeruginosa and Acinetobacter spp., poses a critical challenge in nosocomial settings due to limited therapeutic options and high morbidity. Phenotypic detection methods remain essential in many clinical microbiology laboratories, especially where molecular assays are not readily available.

Recent advances underscore the performance of standardized assays: "a method comparison study of 11 phenotypic carbapenemase detection assays for the detection of carbapenemase-producing non-fermenters (CPNFs) demonstrated variable accuracy across tests in non-fermenters, highlighting the need for optimized local protocols [1]. A comprehensive 2023 review emphasized that several techniques are available for the detection of resistance to Carbapenems in NF GNB, including modified carbapenem inactivation method (mCIM), Carba NP, and EDTA double-disk synergy tests [2]. In resource-limited settings, Kamel et al. (2022) found that the combined disk test (CDT) by EDTA and blue-carba test yielded acceptable sensitivity and specificity compared to genotypic detection among NF-GNB isolates [3]. More recently, Perumal et al. reported on a rapid detection platform (RAISUS S4) enabling detection of carbapenemase-producing Gram-negative rods within 18 hours—suggesting translational potential for NF-GNB diagnostics [4]. Given these findings, your study aims to evaluate and validate phenotypic approaches for carbapenem resistance detection in NF-GNB from clinical specimens, benchmarking methods such as disc diffusion screening, mCIM, Carba NP, and synergy tests against established standards in a tertiary hospital setting.

Methods

This prospective cross-sectional study was conducted over four months, from December 1, 2022, to March 30, 2023, at the Department of

Microbiology, Chalmeda Anand Rao Institute of Medical Sciences (CAIMS), Karimnagar. The study focused on isolating and characterizing NFGNB from a variety of clinical specimens. Repetitive samples from the same patient were excluded to avoid duplication. Institutional Human Ethical Committee approval was obtained prior to initiation. The data from the study were recorded in a structured proforma and analyzed using JAMOVI software version 2.3.18.

All collected clinical samples, including urine, blood, CSF, respiratory secretions, pus, and other sterile body fluids, were processed following standard microbiological procedures as per the Clinical and Laboratory Standards Institute (CLSI) guidelines [5]. Initial processing involved inoculation onto selective and differential media, followed by identification of non-lactose fermenting colonies using a combination of biochemical tests. The identification of NFGNB, particularly Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia, and Shewanella algae, was done using conventional phenotypic characteristics, including oxidative-fermentative (OF) tests, growth at elevated temperatures, pigment production, and decarboxylase reactions, as outlined by Schreckenberger et al. [6]. Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar and interpreted in accordance with CLSI standards. Control strains used included Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC BAA 1705, and BAA 1706 to ensure quality and accuracy in results.

Phenotypic detection of carbapenem resistance in NFGNB was performed using meropenem (10 µg) disc screening followed by confirmatory tests in isolates with reduced zone diameters (≤15 mm for Pseudomonas, ≤13 mm for Acinetobacter), as per CLSI recommendations [5]. To confirm the production of carbapenemases, modified hodge test (MHT), combined disc test (CDT) with EDTA, and double disc synergy test (DDST) were employed, which are well established phenotypic techniques validated in previous studies for detecting metallo-β-lactamase (MBL) producers [7]. Additionally, CHROM agar KPC medium was used as a selective differential medium for identifying carbapenemase-producing strains based on colony morphology and chromogenic properties [8]. These phenotypic assays allowed comprehensive screening and confirmation of carbapenem resistance mechanisms in NF GNB.

Results

Out of 327 isolates, 102 showed meropenem resistance, 64 Pseudomonas aeruginosa and 38 Acinetobacter baumannii. Carbapenemase producers were more common in males. Samples were mostly from pus, endotracheal secretions, and urine. All resistant isolates were tested using Modified Hodge Test (MHT), EDTA-disk synergy, CDT, and CHROM agar m Super CARBA agar.

Among the 102 meropenem-resistant isolates, CHROM agar KPC detected the highest number of carbapenemase producers (97.05%), followed by MHT (87.25%), EDTA-CDT (80.39%), and EDTA-Double Disk Synergy Test (DDST) (66.66%). Detection rates were higher in Acinetobacter baumannii than in Pseudomonas aeruginosa. Chi-square analysis revealed a statistically significant difference in detection rates across tests (χ^2=13.47, df=3, p=0.0038), indicating that CHROM agar KPC is significantly more sensitive for phenotypic detection of CPNFs.

Both Pseudomonas aeruginosa and Acinetobacter baumannii isolates showed 100% resistance to cefepime, piperacillin-tazobactam, ceftazidime, ceftriaxone, imipenem, and meropenem. High resistance was also noted to ciprofloxacin, amikacin, and gentamicin. Tigecycline and colistin showed the highest sensitivity, but with limited efficacy (≤14%) in both organisms.

Discussion

Out of the 327 NFGNB isolates obtained from diverse clinical specimens, 102 (31.19%) exhibited resistance to meropenem by the disc diffusion method. Of these, 64 (62.74%) were Pseudomonas aeruginosa and 38 (37.25%) were Acinetobacter baumannii, indicating that P. aeruginosa remains the predominant carbapenem-resistant NFGNB in clinical settings. Carbapenemase production was more frequently observed in male patients, suggesting possible gender-related exposure patterns or comorbidity profiles. The most common sources of carbapenem-resistant isolates included pus/wound swabs, endotracheal secretions, and urine, highlighting their prevalence in respiratory, urinary, and soft tissue infections.

Phenotypic detection methods employed in the study included MHT, EDTA-disk synergy test, EDTA-CDT, and growth on CHROM agar m Super CARBA. Among these, CHROM agar was found to have the highest sensitivity and ease of interpretation, corroborating findings by Funashima et al. that highlight its utility for rapid detection of carbapenemase producers in clinical microbiology laboratories [9]. According to Yong et al., the EDTA-based tests are effective in identifying metallo-β-lactamase producers, particularly in non-fermenters like Pseudomonas and Acinetobacter [10]. CLSI guidelines remain the gold standard for interpreting antimicrobial resistance and ensuring diagnostic accuracy [5].

The increasing prevalence of carbapenem-resistant NFGNB, particularly Pseudomonas aeruginosa and

Acinetobacter baumannii, has become a global healthcare concern due to the limited therapeutic options and high associated morbidity and mortality. In the present study, phenotypic detection methods were applied to 102 meropenem-resistant isolates (64 P. aeruginosa and 38 A. baumannii), revealing CHROM agar KPC as the most effective method, identifying 97.05% of carbapenemase producers. This was followed by Modified Hodge Test (87.25%), EDTA-CDT (80.39%), and EDTA DDST (66.66%). The higher positivity rate seen with CHROM agar KPC reflects its superior sensitivity and specificity in detecting carbapenemase-producing organisms. This aligns with studies by Funashima et al., who emphasized that chromogenic media enable rapid and accurate screening for carbapenemase production in Gram-negative organisms within 24 hours of incubation [9].

The MHT, though widely used, has variable sensitivity, especially in non-fermenters, and may yield false-positive results due to hyperproduction of AmpC β-lactamase or extended-spectrum β-lactamases (ESBLs) [11]. Despite its limitations, MHT detected 87.25% of carbapenemase producers in this study, indicating its continued relevance in routine microbiological testing when used alongside confirmatory tests. The EDTA-based methods, including the DDST and CDT, specifically target metallo-β-lactamase (MBL) producers and have been validated in previous research [12, 13]. Yong et al. demonstrated that the EDTA-impregnated disc significantly improves the sensitivity of detecting MBLs in Pseudomonas and Acinetobacter spp., which supports their continued application in diagnostic workflows [10]. However, false negatives in EDTA-based tests can occur in strains producing serine carbapenemases such as KPC or OXA-type enzymes [14], possibly explaining the relatively lower sensitivity (66.66%) of DDST observed in this study.

The statistical analysis using the chi-square test revealed a significant variation in detection rates across the four phenotypic tests ($\chi^2 = 13.47$, df = 3, $p = 0.0038$), confirming that not all phenotypic methods perform equally. CHROM agar KPC emerged as significantly more effective in detecting carbapenemase-producing isolates among both P. aeruginosa and A. baumannii. A similar pattern was noted in the work by Gaillot et al., where chromogenic media facilitated the differentiation of KPC-producing strains based on colony color, aiding rapid decision-making in infection control [8]. The higher detection rates in A. baumannii than P. aeruginosa observed in this study may reflect differing resistance mechanisms, as Acinetobacter more commonly harbors OXA-type carbapenemases that are more readily expressed phenotypically [12]. These findings underscore the need for multimodal phenotypic approaches complemented by molecular methods to accurately identify the resistance profile and inform antimicrobial stewardship strategies. Ultimately, while CHROM agar KPC appears highly effective for rapid phenotypic screening, laboratories should adopt a panel of tests to cover the wide enzymatic diversity of carbapenemases in NFGNB.

The alarming rise in antimicrobial resistance among NFGNB, particularly Pseudomonas aeruginosa and Acinetobacter baumannii, poses a major challenge in clinical settings. In this study, both organisms demonstrated 100% resistance to key β-lactam antibiotics including cefepime, piperacillin-tazobactam, ceftazidime, and ceftriaxone, as well as carbapenems such as imipenem and meropenem. This high-level resistance highlights the extensive dissemination of carbapenemases and β-lactamases among these species, limiting the utility of conventional broad-spectrum antibiotics. Furthermore, significant resistance was also observed to other classes of antibiotics, including ciprofloxacin, amikacin, and gentamicin, which were traditionally reserved for resistant Gram-negative infections. These findings are consistent with previous reports indicating increasing resistance to aminoglycosides and fluoroquinolones among Pseudomonas and Acinetobacter isolates due to efflux pumps and target site mutations [15]. Among the limited therapeutic options, tigecycline and colistin exhibited the highest sensitivity; however, their efficacy was low (≤14%), which aligns with recent concerns over emerging resistance to last-resort antibiotics [16]. The dwindling effectiveness of these agents necessitates urgent implementation of robust antimicrobial stewardship programs and accelerated research into novel therapeutic strategies to combat multidrug-resistant NFGNB infections.

Conclusion

This study highlights the high prevalence of carbapenem resistance among NF GNB, particularly Pseudomonas aeruginosa and Acinetobacter baumannii. CHROM agar KPC was the most sensitive phenotypic method for detecting carbapenemase production, followed by Modified Hodge Test, EDTA-CDT, and EDTA-DDST. Alarmingly, both organisms exhibited 100% resistance to several antibiotics, including carbapenems and β-lactams. Only colistin and tigecycline retained limited activity, emphasizing the urgent need for antimicrobial stewardship, strict infection control, and newer therapeutic options. Routine phenotypic screening remains vital in resource-limited settings to track resistance and guide appropriate therapy.

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