

Molecular Characterization Of Non-Fermenting Gram-Negative Bacilli And Its Virulence Markers From Various Clinical Samples In A Tertiary Care Center, South India

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Abstract

Introduction: Non-fermentative Gram-negative bacilli (NFGNB) are an important cause of healthcare-associated infections, and they are primarily opportunistic pathogens.

Aim and objectives: To isolate and characterize the non-fermenting Gram-negative bacilli (NFGNB) from various clinical samples in a tertiary care center. To detect the virulence markers and drug resistance using molecular methods.

Material and methods: Non-fermentative Gram-negative bacilli (NFGNB) from various clinical samples were identified using standard bacteriological identification methods. Conventional PCR for detection of MBLs genes of *P. aeruginosa* such as *bla*_{IMP}, *bla*_{VIM}, *bla*_{NDM} and a virulence marker, *Exo S* gene and also MBL genes of *A. baumannii* such as *bla*_{IMP}, *bla*_{VIM}, *bla*_{NDM}, *bla*_{OXA-23}, *bla*_{OXA-58}, and a virulence marker, *OmpA* gene.

Results: Out of 104 NFGNB isolates *Pseudomonas aeruginosa* was the most common bacteria comprising 62% (n=65), followed by *Acinetobacter baumannii* 25% (n=26), *Acinetobacter lwoffii* 4% (n=4), *Pseudomonas fluorescens* 3% (n=3), *Burkholderia cepacia* 3% (n=3), *Stenotrophomonas maltophilia* 1% (n=1), *Myroides odoratimimus* 1% (n=1), and *Rhizobium radiobacter* 1% (n=1). The result of *P. aeruginosa* showed that the *bla*_{VIM} gene was expressed in 25 % (n=2), the *bla*_{NDM} gene was expressed in 50 % (n=4) and *bla*_{IMP} was found in 25 % (n=2). For *A. baumannii*, the *bla*_{NDM} gene was expressed in 33% (n=6), *bla*_{OXA-23} gene was expressed in 78% (n=14), *bla*_{OXA-58} gene was expressed in 56% (n=10) with no expression of *bla*_{IMP} and *bla*_{VIM} genes.

Conclusion: In our study we report the increased prevalence of MBL genes in *P. aeruginosa* and *A. baumannii*. This highlights the importance of accurate identification of MBL producers and judicious use of carbapenems. Early identification and continued surveillance of these multidrug resistant organisms will help to prevent their spread in a hospital environment.

Key Words : NFGNB, *P. aeruginosa*, *A. baumannii*, MBL genes.

1. INTRODUCTION

Non-fermentative Gram-negative bacilli (NFGNB), a group of aerobic bacteria, which are non-spore formers. NFGNB utilize carbohydrates only through oxidative fermentation and does not use carbohydrates as main source of energy or either degrade them by other metabolic pathways [1]. The major factors contributing to NFGNB infections are prolonged hospital stay or frequent hospitalization, and longer course of antibiotic therapy. They have emerged as a threat to health care systems with more drug-resistant bacteria [2].

The common NFGNB-causing infections in humans are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Burkholderia spp*, *Stenotrophomonas maltophilia etc*. Among the NFGNB, nosocomial pathogens such as *P. aeruginosa* and *A. baumannii* are commonly associated with severely ill patients and also to patients admitted in intensive care unit [3].

The increased emergence of these organisms as multidrug-resistant organisms contributes to mortality and morbidity among hospitalized patients. In developing countries like India, it is considered a major public health concern [4]. The multidrug resistance is due to various underlying mechanisms. [5]. These resistance bugs interfere in the management of the infections due to it acquire resistance to a different class of antimicrobials [6].

World Health Organization (WHO) has categorized priority level 1 pathogens as carbapenem-resistant *P. aeruginosa* and carbapenem-resistant *A. baumannii* [7]. *Acinetobacter baumannii*, is categorised as red-alert human pathogen, and added to priority list of pathogens for novel antibiotics [8]. Emerging resistance of non fermenting Gram-negative bacilli, especially *P. aeruginosa* and *A. baumannii* may lead to an increase in morbidity & mortality, which further can be controlled by enforcement of the strict antibiotic policy [9]. Various international authorities emphasize, that each and every hospital should have its local antibiotic policy. Since the standard antibiotic sensitivity pattern, vary for every area as NFGNB are ubiquitous. This study will help in the characterization of NFGNB and provide the data on antimicrobial resistance of those isolates, which further helps in formulating local antimicrobial policy.

2. MATERIALS AND METHODS

2.1 Study design

Our study was a prospective study, done in the Dept of Microbiology, SRM Medical College hospital and Research center, Kattankulathur during the period from February 2021- July 2022. All NFGNB which were isolated and reported from various clinical samples during the study period were included and isolates other than NFGNB were excluded. A total of 104 isolates of NFGNB were received during the study period in the laboratory.

2.2 Ethics approval of research

This study was approved by the Institutional ethical committee with reference number 2361/IEC/2021. Informed written consent was obtained from all the participants of our study.

2.3 Phenotypic identification

The identification of NFGNB was done using standard microbiological methods. It includes Gram staining, motility testing, morphological appearance of the colonies, and a series of biochemical tests. Further, the NFGNB identification was confirmed by the Vitek-2 compact. The antimicrobial susceptibility testing of bacterial isolates was done by the Kirby–Bauer disk diffusion method to detect carbapenem-resistant isolates. Interpretations of antibiotics were made according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI 2022) [10]. Carbapenem-resistant bacterial isolates for tested for detection of MBLs production phenotypically using a combination disk diffusion test (CDDT) using imipenem disks (10mg) in combination with EDTA(ethylenediaminetetraacetic acid). An increase in diameter of >7mm is considered as positive [11,12].

2.4 Molecular detection of MBL genes

The conventional PCR was done for the detection of MBLs (metallo beta-lactamases) genes and virulence markers. For *P. aeruginosa*, the MBL genes were *bla_{IMP}*, *bla_{VIM}*, *bla_{NDM}*, and a virulence marker- *Exo S* gene. For *A. baumannii*, the MBL genes *bla_{IMP}*, *bla_{VIM}*, *bla_{OXA-23}*, *bla_{OXA-58}*, and a virulence marker- the *OmpA* gene.

3. RESULTS

All the NFGNB included in our study was isolated and characterized by standard bacteriological methods. Out of 104 NFGNB isolates *Pseudomonas aeruginosa* was the most common bacteria comprising 62% (n=65), followed by *Acinetobacter baumannii* 25% (n=26), *Acinetobacter lwoffii* 4% (n=4), *Pseudomonas fluorescens* 3% (n=3), *Burkholderia cepacia* 3% (n=3), *Stenotrophomonas maltophilia* 1% (n=1), *Myroides odoratimimus* 1% (n=1), and *Rhizobium radiobacter* 1% (n=1). The distribution of NFGNB from various clinical samples is illustrated in Figure.1

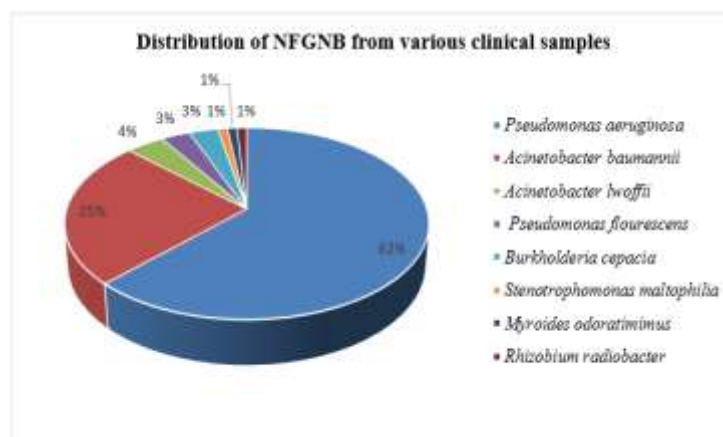


Figure 1: Distribution Of NFGNB From Various Clinical Samples

In our study, carbapenem resistance was found in 17% (n=11) and 77% (n=20), of *P. aeruginosa* and *A. baumannii* respectively. Of these 31 isolates, 26 isolates (84%) were found to be MBL producers by the phenotypic method. The production of MBL in *P. aeruginosa* and *A. baumannii* was found to be seen in 73% (8 out of 11 isolates) and 90% (18 out of 20 isolates) respectively.

The MBL producing NFGNB that expresses *bla*_{IMP}, *bla*_{VIM}, and *bla*_{NDM} gene for *P. aeruginosa* and *bla*_{IMP}, *bla*_{VIM}, *bla*_{NDM}, *bla*_{OXA-23}, and *bla*_{OXA-58} gene for *A. baumannii* identified by PCR. The result of *P. aeruginosa* showed that the *bla*_{VIM} gene was expressed in 25 % (n=2), the *bla*_{NDM} gene was expressed in 50 % (n=4) and *bla*_{IMP} was found in 25 % (n=2). For *A. baumannii*, the *bla*_{OXA-23} gene was expressed in 78% (n=14), *bla*_{OXA-58} gene was expressed in 56% (n=10) and *bla*_{NDM} gene was expressed in 33% (n=6), with no expression of *bla*_{IMP} and *bla*_{VIM} genes. The distribution of various MBL genes was illustrated in Figure 2.

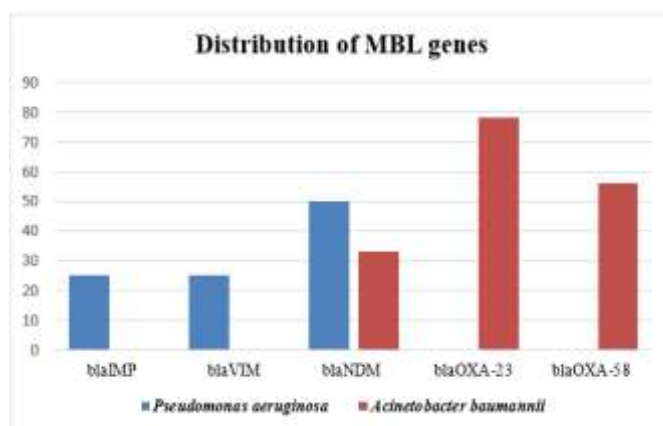


Figure 2: The Distribution Of Various MBL Genes Of *P. Aeruginosa* And *A. Baumannii*

Virulence markers, *ExoS* gene for *P. aeruginosa* and *OmpA* gene for *A. baumannii* were studied which showed the presence of *ExoS* gene 50% (n=2) and *OmpA* gene in 56% (n=10) respectively.

4. DISCUSSION

The multidrug resistance of *P. aeruginosa* and *A. baumannii* was due to various underlying mechanisms, among this production of β -lactam hydrolyzing enzymes such as ESBL (extended-spectrum β -lactamases), AmpC producers, and enzymes hydrolyzing carbapenems such as metallo- β -lactamases, oxacillinase plays a major role. The presence of acquired MBL genes such as IMP, VIM, SPM & GIM, indicates the association of multidrug resistance and these genes were present in the mobile genetic elements [13]. The plasmid-mediated resistance contributes to more for the carbapenem hydrolyzing enzyme, which further limits the therapeutic options for its treatment [14].

In our study, we report as increased prevalence of certain NFGNB among various clinical samples in descending order such as of *Pseudomonas aeruginosa* 62% (n=65), followed by *Acinetobacter baumannii* 25% (n=26), *Acinetobacter lwoffii* 4% (n=4), *Pseudomonas fluorescens* 3% (n=3), *Burkholderia cepacia* 3% (n=3), *Stenotrophomonas maltophilia* 1% (n=1), *Myroides odoratimimus* 1% (n=1), and *Rhizobium radiobacter* 1% (n=1). This was similar to the study by al charrakh et al., where *P. aeruginosa* was a predominant pathogen among various NFGNB [15].

In our study, *P. aeruginosa* showed increased prevalence the *bla*_{NDM} gene (50%) followed by *bla*_{VIM} gene (25%) and *bla*_{IMP} gene (25%), these findings were similar to the study by Namaei et al., *bla*_{NDM} gene (40%) followed by *bla*_{VIM} gene (29%) and *bla*_{IMP} gene (13%) [16,17]. In our study, for *A. baumannii* we observed an increased prevalence of *bla*_{OXA-23} gene (78%) followed by *bla*_{OXA-58} (56%) and *bla*_{NDM} gene (33%). This findings were similar to the study by Saranya et al., *bla*_{OXA-23} gene followed by *bla*_{NDM} gene [18].

5. CONCLUSION

To conclude, we report the increased prevalence of MBL genes in *P. aeruginosa* and *A. baumannii*. This study demonstrated a higher rate of resistance among these isolates especially *Acinetobacter baumannii* with an additional burden of MBL production. This highlights the importance of accurate identification of MBL producers and judicious use of carbapenems. This strongly reflects the need to rapidly promote the implementation of antimicrobial stewardship policies. Early identification and continued surveillance of these multidrug resistant organisms will help to prevent their spread in a hospital environment.

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Ramprabu.R : Sample collection, analysis, or interpretation of data for the work, drafting the work.

Leela.K.V. & Jaya Lakshmi.S.S.: design of the work, drafting the work or revising it critically for important intellectual content, final approval of the version to be published

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