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Original Research Article

Occurrence of Non Fermenting Gram Negative Bacilli and their Antibiotic Susceptibility patterns among clinical isolates: Special reference to Beta-lactamase production

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Abstract:

Introduction: Non-fermenting Gram Negative Bacilli (NFGNB) including Pseudomonas aeruginosa and Acinetobacter baumannii are one of the leading causes of Health Care Associated Infections & pose a serious challenge in treatment especially in Intensive care units. Resistance to antimicrobial agents in these bacteria has become an increasingly relevant problem in present scenario. With the increase in the incidence of Multidrug resistant organisms (MDROs) among NFGNB there are only limited options available.

Methods: 100 consecutive samples of NFGNB isolated from various clinical samples were included in the study. Identification & Antimicrobial Susceptibility patterns of NFGNB were performed according to Standard conventional methods. Phenotypic detection of various Beta-lacatamases like ESBL, AmpC & Carbapenemase were performed according to Clinical Laboratory Standards Institute (CLSI) guidelines.

Results: Out of 100 consecutive NFGNB isolates Pseudomonas aeruginosa (66%), Acinetobacter baumannii complex (12%) & Other NFGNB (22%) were isolated. Cefipime was found to be the most effective antibiotic with 74% of the isolates being sensitive while Ciprofloxacin was found to be least effective with only 56% of the isolates being sensitive. 34% of isolates showed Multidrug Resistance. Out of 100 NFGNB isolated 96% isolates were AmpC producers, 40% isolates produced ESBL & 4% were Carbapenemase producers.

Conclusion: Pseudomonas aeruginosa is the most common NFGNB isolated from Clinical samples causing Healthcare Associated Infections. Multidrug Resistant organisms of NFGNB producing significant amount of Beta-lactamases are an issue of concern for clinicians. Strict Infection Prevention & control measures along with effective Antimicrobial Stewardship Programme in a healthcare set up is the need of the moment.

Keywords: Non-fermenting Gram Negative bacilli(NFGNB), Antimicrobial Susceptibility Patterns, Beta-lactamases, Phenotypic tests.

Introduction

Non-fermenting Gram Negative bacilli (NFGNB) are one of the most crucial organisms to deal with as they are known to be common causative agents of Healthcare Associated infections often leading to increased morbidity & mortality. [1]

NFGNB exhibit various multidrug resistance mechanisms making them difficult to treat [2],[3]. These organisms are ubiquitous in nature & thus pose a greater risk in Infection Prevention & Control in a hospital setting. [2],[4],[5] NFGNB are known to cause infections in patients with certain predisposing factors like Reticuloendothelial system malignancies, instrumentation, surgery, Urinary & Intravascular catheterization, Lumbar Puncture, tracheostomy, dialysis, lavages, presence of shunts, prosthesis & prolonged use of antibiotics.[6],[7]

NFGNB constitute about 15% of Gram Negative isolates from the clinical samples. They are difficult to isolate & speciate by conventional culture methods making it a herculean task without automation. Further they also have to be differentiated as pathogens since they can be common contaminants in culture.[8]

NFGNB are innately resistant to many antibiotics & are known to produce Extended Spectrum Beta-lactamases (ESBL), Metallo-beta-lactamase's (MBL) & AmpC Beta-lactamases. Detection of Beta-lactamases among NFGNB is challenging due to lack of standard guidelines in the literature.[8]

Materials & Methods:

This prospective cross sectional study was conducted in Shridevi Institute of Medical Sciences & Research hospital, Tumkur from January 2022 to December 2022 after obtaining approval from Institutional Ethics Committee (ECR/831/Inst/KA/2016/RR-19). A total of 100 consecutive isolates of NFGNB isolated from various clinical samples were included in the study. Processing of samples & identification of isolates were performed by conventional methods[9].

The antibiotics (Himedia laboratories) tested against Pseudomonas species were Piperacillin (100µg), Piperacillin-tazobacatm (100/10µg), Ceftazidime (30µg), Cefipime (30 µg), Imipenem (10µg), Meropenem (10µg), Gentamicin (10µg), Amikacin (30µg), Netilmicin (30µg), Ciprofloxacin (5µg). In addition to the above antibiotics the other NFGNB were also tested against Cefotaxime (30µg), Tetracycline (30µg), Co-trimoxazole (1.25/23.75µg) & Tigecycline (15µg). All isolates showing intermediate susceptibility were considered as resistant for ease of analysis & interpretation. Pseudomonas aeruginosa ATCC 27853 & Escherichia coli ATCC 25922 were used for quality control. Testing for Beta-lactamases for NFGNB was performed as per Clinical Laboratory Standards Institute (CLSI) guidelines 2021[10] as stated for Enterobacteriaceae & Pseudomonas aeruginosa, for NFGNB other than Pseudomonas same tests were applied as there are no CLSI guidelines for the same. Organisms resistant to at least one agent in more than or equal to three different classes of antibiotics were considered as MDROs.

Extended Spectrum Beta-lactamase (ESBL) testing:

Isolates of NFGNB showing Zone diameter of ≤22mm for Ceftazidime were presumably identified as ESBL producers. Double Disk Synergy Test (DDST) & Phenotypic Combined Disk Diffusion Test (PCDDT) were performed as confirmatory tests for ESBL. An extention of Zone of Inhibition of Ceftazidime towards disc of Amoxycillin-Clavulanic acid disc in DDST was considered positive. An increase in Zone diameter of ≥5mm for Ceftazidime & Calavulanic acid in comparison to disc containing only Ceftazidime in PCDDT were considered positive. Himedia ESBL Identification kit 1 & 3 was used for PCDDT.

AmpC Beta-lacatamase testing:

Isolates that were resistant to 3rd generation Cephalosporins & Cefoxitin were subjected to AmpC disc test to detect plasmid mediated AmpC production. Lawn of Escherichia coli ATCC 25922 was made. Cefoxitin disc was placed close to filter paper disc containing 4-5 colonies. After overnight incubation an indentation of zone of inhibition for Cefoxitin was considered as Plasmid-mediated AmpC positive.

Isolates that were sensitive to 3rd generation Cephalosporin & Cefoxitin were subjected to Disc Antagonism test to detect Inducible AmpC production. Inducible AmpC was identified by blunting of Ceftazidime zone towards inducer (Imipenem) & Sensitivity to Cefipime.

Carbapenamase detection:

All isolates resistant to either Meropenem or Imipenem were further tested for Carbapenemase production using Modified Carbapenem Inactivation Method (mCIM). Isolates that tested positive by mCIM were considered positive for Carbapenemase production. Escherichia coli ATCC 25922 was used for quality control.

Statistical Analysis:

All the data collected was compiled and entered into a Microsoft excel worksheet. Descriptive statistics was used for describing continuous & categorical data. The qualitative variables have been presented in frequency, percentages & graphs.

Results:

Out of the 100 consecutive NFGNB obtained from all clinical isolates, Pseudomonas aeruginosa (66%) was the most common organism followed by Other NFGNB (22%) & Acinetobacter baumanii complex (12%).

The most common age group from which NFGNB was isolated include 31-40years (20%). Majority of the isolates were obtained from patients above 40 years of age (62%).

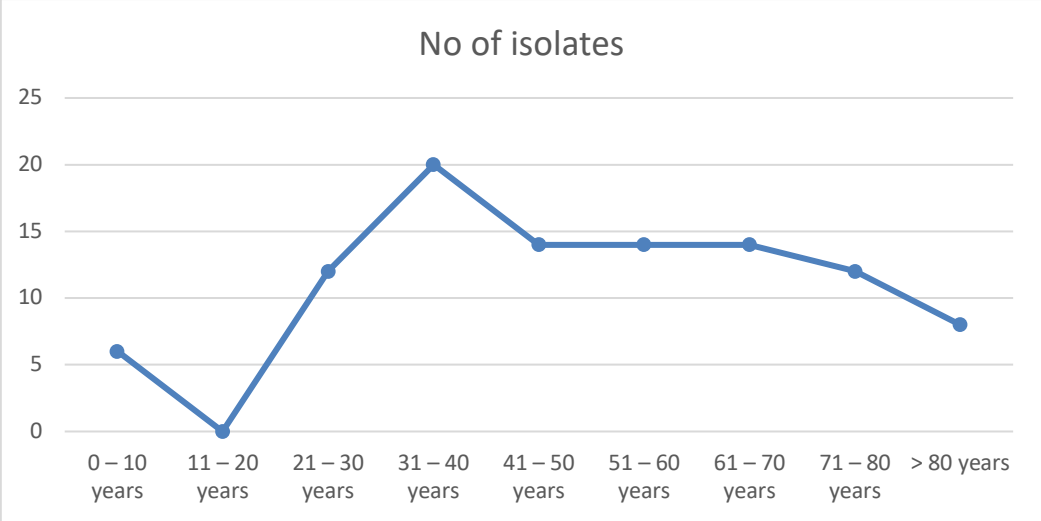

Figure 1: Age-wise distribution of isolates

Majority of NFGNB isolates were isolated from Males (64%) followed by females (36%).
48 out of 100 isolates were obtained from Respiratory samples (Sputum& Endotracheal Tube Aspiration) followed by Pus (28), Blood (14), Urine (10).

Table 1: Antimicrobial Susceptibility patterns of NFGNB

Sl. No	Antibiotic	No. of NFGNB Tested	N(%)
1	Ceftazidime	100	60(60)
2	Cefipime	100	74(74)
3	Piperacillin	100	66(66)
4	Piperacillin-tazobactam	100	64(64)
5	Amikacin	100	72(72)
6	Netilmicin	100	68(68)
7	Ciprofloxacin	100	56(56)
8	Imipenem	100	64(64)
9	Meropenem	100	66(66)
10	Tigecycline	34	16(47.05)
11	Cefotaxime	34	18(52.94)
12	Co-trimoxazole	34	21(61.76)
13	Tetracycline	34	19(55.88)

Table 1 shows the Antibiotic Susceptibility patterns of NFGNB in the study. For those antibiotics tested for all 100 isolates of NFGNB, Cefipime (74%) & Amikacin (72%) are most sensitive & Ciprofloxacin (56%) being least sensitive.

Table 2: Phenotypic tests for Detection of Beta-lactamases:

Beta-lactamase Enzyme detected	Screen Positive (%), N=100 isolates	Confirmatory test positive (%) N=100 isolates
ESBL	40	DDST only-6 PCDDT only-28 Both DDST & PCDDT-6 Total positive-40
AmpC	96	Inducible AmpC - 34 De-repressed AmpC - 62 AmpC Disk Test (Plasmid mediated AmpC) - 60 Total positive-96
Carbapenemase	36	mCIM -4 Total positive-4

As shown in the Table 2 majority of the NFGNB isolates were AmpC producers (96%) followed by ESBL production (40%) & Carbapenemase producers(4%).

Discussion

NFGNB is still one of the most common pathogen isolated from the clinical samples particularly causing Healthcare Associated Infections. In our study we had taken 100 consecutive NFGNB out of which Pseudomonas aeruginosa (66%) was the most common organism isolated similar to the findings of other studies like Grewal et al[1]& Rit et al[11]. Unlike other studies where the second most common NFGNB isolated is Acinetobacter, we isolated only 12% among the whole which could be due to lack of individual identification of other NFGNB (22%).

In our study majority of NFGNB were isolated from Respiratory samples (48%) followed by pus (28%) unlike in other studies like Grewal et al[1] & Rit et al[11] where pus was the most common sample. This is because the prevalence of NFGNB varies from place to place & also on the Infection control practices followed in the hospital. Higher percentage of NFGNB isolation from sputum causing Respiratory infections suggests a possible environmental source.

In our study Males (64%) were commonly affected compared to females (36%) similar to the findings in studies like Purimitla et al[8] & Varaiya et al[12].

In our study 34% of NFGNB were found to be Multidrug resistant organisms. Highest sensitivity to Cefipime (74%) & Amikacin (72%) was recorded compared to other studies like Grewal et al[1]& Mandira et al[2] which showed reduced sensitivity to Cefipime. This finding is promising for the use of Cefipime in combination with Amikacin for serious infections caused by NFGNB which also helps in curbing the injudicious use of Carbapenems as empirical therapy.

In our study 40% of NFGNB isolates where known to produce ESBL enzymes which is much higher compared to that found in other studies like Ahmed et al[5] & Faraj et al[7]. The Phenotypic Combined Disk Diffusion(PCDDT) was showing much higher sensitivity than Double Disk Synergy (DDST) in detecting ESBL production. However, these findings have to be compared with a molecular test for enzyme producing genes which forms the limitation of this study.

Prevalence of AmpC in our isolates was found to be 96% which is significantly high compared to other studies like Soma et al[13] & Shoba et al[14], nevertheless most of the NFGNB are known to intrinsically produce AmpC beta-lacatamases. Among the 96 AmpC producing isolates 34 were classified as Inducible AmpC producers, 62 as Derepressed AmpC producers & 60 showed AmpC production by Plasmid mediated mechanism.

In our study 4 isolates of NFGNB produced Carbapenemase enzyme as confirmed by mCIM (Modified Carbapenem Inactivation test). Though initial screening by Carbapenem resistance was high (36) subsequently only 4 isolates turned out to be phenotypic confirmatory test positive. This percentage is very low in comparison with the findings of other studies like Faraj et al[7], Purimitla et al[15] & Rohith et al[16], this could be because of other mechanisms of Carbapenemase production which was not be detected by mCIM. Use of mCIM as confirmatory test is very crucial to detect Carbapenemase production as it is one of the few phenotypic test recommended by CLSI currently,more approved phenotypic tests to detect all the type of Carbapenemases are required.

Conclusion

Managing NFGNB infections is still a nightmare for many physicians especially in ICU setup. Fourth generation Cephalosporins like Cefipime can still be a possible option for treating NFGNB infections in the era of emerging Multidrug Resistant Organisms(MDROs) & Pan Drug Resistant(PDROs) organisms. Effective & rigorous testing methods including both phenotypic & molecular detection of beta-lactamases is the need of the hour along with the implementation of strict Hospital Infection Control policies & Antimicrobial Stewardship Programme for cutting down on Antimicrobial Resistance (AMR) thus saving the patients from increased morbidity & mortality caused by these NFGNB.

References

1. Grewal US, Bakshi R, Walia.G, Shah PR.Antibiotic Susceptibility profiles of non-fermenting gram-negative bacilli at a Tertiary Care Hospital in Patiala, India. Niger Postgrad Med J. 2017; 24:121-5.
2. Sarkar M, Jena J, Pattnaik D, Mallick. Prevalence of Nonfermenting gram-negative bacilli and their antimicrobial susceptibility profiles in a tertiary care hospital of Eastern India. Int J Adv Med. 2018; 5:366-70.
3. Jesudasan MV, Kandathil AJ, Balaji V.Comparision of two methods to detect carbapenemases & metallo-betalactamase production in clinical isolates. Indian J Med Res. 2005;121(6):780-83.
4. Chitrabanu NA, Mallya S. Identification, Speciation and Antibiogram along with Detection of Metallo Beta-lactamase production in Acinetobacter Isolated from Clinical Samples in a Tertiary Care Hospital. J Pure Appl Microbiol. 2021;15(2): 839-44.
5. Ahmed AO, Abdellatif HH, Abdullah AE. Various phenotypic techniques for detection of beta-lactam resistance in Pseudomonas species and Acinetobacter species: a single centre

experience. J of Cur Med Res and Practice. 2022; 99(21):329-36
6. Solanki S, Saileela K, Dinesh, Vasantha, Kumar S, Trinain et al. Detection of Metallo Beta Lactamase Production in Imipenem Resistant Gram-negative non Fermenting Bacilli Isolated in a Tertiary Care Hospital in South India. International J of Cont Med Res. 2020;7(9):11-15.
7. Faraj DN, Mohammed OJ. Detection of Extended Spectrum β-lactamases and Metallo β-lactamases in Pseudomonas Aeruginosa isolated from Burns. Mal J Med Health Sci. 2022; 18:70-75.
8. Rani PU, Vijayalakshmi P. Epidemiological pattern of Non-fermenting Metallo Beta Lactamase Bacterial Pathogens Isolated from clinical specimens in a Tertiary care Hospital. MRJI. 2017;18(3):1-10.
9. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simsons A. Laboratory strategy in the Diagnosis of infective syndromes. In: JG C, AG F, BP M, A S, editors. Mackie & McCartney Practical Medical Microbiology; 1999; 84-90.
10. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI Supplement M100. Clinical & Laboratory Standards Institute, USA, 2021.
11. Rit K, Nag F, Raj HJ, Maity PK. Prevalence & Susceptibility profiles of nonfermentive gram-negative bacilli infection in a tertiary care hospital in India.Indian J Clin Pract. 2013; 24:451-55.
12. Varaiya A, Kulkarni M, Bhalekar, Dorga J.Incidence of Crabapenem Resistant Pseudomonas aeruginosa in diabetes & cancer patients. Ind J Med Microbiol. 2008;26(3):238-40.
13. Sarkar S, Dutta S, Namhata A, Banerjee C, Sengupta M, Sengupta M. Beta-lacatamse profile & Biofilm production of Pseudomonas aeruginosa isolated from a tertiary care Hospital in Kolkata, India. J of Clin & Diagnostic Res. 2020;14(10):22-27.
14. Nadigar, Shobha and Kumar M. Prevalence of AmpC β Lactamasesin Non-Fermenting Gram-Negative Isolates in Clinical samples. Semantic Scholar 2014.
15. Rani PU, Vijayalakshmi P.Detection of Metallo-Beta-lactamase Production in Rare Carbapenem-Resistant Non-fermentative Gram-Negative Bacilli Isolated in a Tertiary Care Hospital, Vishakapatnam, India. J Med Microbiol Infec Dis. 2016;4(12):31-36.
16. Sachdeva R, Sharma B, Sharma R.Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenem-resistant Pseudomonas aeruginosa. J of Lab Physicians. 2017;9:249-53.