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Assessment of Prevalence and Antibiogram of NFGNB isolates in a Tertiary care Hospital, Dehradun

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Abstract: Non-fermenting Gram Negative Bacilli (NFGNB) is emerging as a major cause of nosocomial infections as they exhibit great multidrug resistance thereby posing difficulty in combating the infections. Studies on assessing the prevalence rate and antibiogram of NFGNB is necessary for proper management of infections caused by them as there are high chances of regional variation in predominance and antimicrobial susceptibility pattern of NFGNB. Aim of the present study was to assess the prevalence rate of NFGNB along with antimicrobial sensitivity pattern revealing their drug sensitivity and resistance among the patients attending tertiary care hospital of varied age groups. A total of 1000 various clinical specimens were received in laboratory during the period of 4 months (August 2020-Novemebr 2020) and were subjected to processing using Vitek-2 compact system. Among 1000 clinical samples 328 yielded NFGNB i.e., *Pseudomonas aeruginosa, Acinetobacter baumannii, Achromobacter Xylosoxidans, Stenotrophomonas maltophilia* and *Burkholderia cepcia. Pseudomonas aeruginosa* (51.82%) and *Acinetobacter baumannii* (39.63%) were most prominent NFGNB isolates. Results showed that although NFGNB were found to be resistant against most of the subjected antibiotics but considerable intensity of effectiveness was also recorded against Colistin. Accurate, rapid identification and antimicrobial susceptibility testing of NFGNB by Vitek 2, is required in early diagnosis, treatment and proper management of patients will reduce emergence of MDR strains of NFGNB.

Key words: Antimicrobial susceptibility testing • MDR • NFGNB • Vitek2

Introduction

The diversity in the disease causing ability of bacteria has always presented a challenge in the treatment of their infections (Quiroga et al., 2001). NFGNB are found to produce ESBLs and Metallo-lactamases, as well as being inherently resistant to several antibiotics. *Pseudomonas spp., Acinetobacter spp., Alkaligenes spp., Stenotrophomonas maltophilia,* and *Burkholderia cepcia* are among the hetrogenous organisms found in the NFGNB. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most often isolated NFGNBs and they are both harmful to humans. Infections caused by other species are relatively infrequent (Prudhivi Sumana et al., 2017).

Antibiotic resistance in *P. aeruginosa, A. baumannii,* and *S. maltophilia* strains is becoming a major public health concern around the world. The selection of effective treatment choices for individuals with multidrug resistant NFGNB infection is still a difficult (GuptaV 2008; Yadav SK et al., 2020). All thanks to excessive use of antibiotics in the previous couple of years, most of the organism are immune to commonly used antibiotics due to resistance to beta-lactam antibiotics such as penicillin and cephalosporinsn is becoming more common

among NFGNB, posing a serious clinical dilemma in terms of treatment efficacy and good patient managrment. Despite this, carbapenems are frequently regarded as last choice for treatment infections caused by MDR-NFGNB isolates (Farhan SM et al., 2019;Tarashi S et al., 2016).NFGNB's multidrug resistance is caused by a number of causes, including increased production of drug-metabolizing enzymes, target site alterations, efflux pump overexpression, and porin insufficiency (Ruppé É et al., 2015; Kim UJ et al., 2014).Metallo-beta-lactamase (MBL) production, mainly by *Pseudomonas aeruginosa*, stands out as a frequent cause of severe nosocomial infections(Gonçalves DCPS et al., 2009; Hong DJ et al., 2015). An advance diagnostic procedure is required for the right and timely identification of those organism for correct patient management. The aim of our study is to assess the frequency rate of non-fermenting gram negative bacterial infectionsin a tertiary care hospital and to monitor their antimicrobial susceptibility pattern so as to improve the empirical therapy.

Materials and methods

Collection of Clinical Samples: The present study was conducted in the tertiary care hospital, Dehradun, during the time period from August 2020 to November 2020. Various clinical samples received were Pus, Urine, BAL Sputum, CSF, Endotracheal tubes tip, Blood and other specimen from patient of varied age groups.

Isolation and Detection of NFGNB Pathogens: Clinical samples collected from patients of varied age groups were subjected to isolation for procurement of pure colonies using MacConkey agar, Blood agar, Chocolate agar and incubated for 16-24hrs at 37°C. Isolated Colonies were further Characterized phenotypically.

Identification and Antimicrobial Susceptibility Testing of NFGNB Pathogens: Identification and antimicrobial susceptibility testing was performed by Vitek2 compact system (Prudhivi Sumana et al., 2017).

Suspension preparation

The colonies were grown on the culture plates were used to make a bacterial mixture in 3ml sterile saline ((0.45%NaCl) in a clean polystyrene test tube. With the help of Vitek 2 DensiCheck equipment, the suspension's turbidity was corrected to McFarland standard of 0.5.The time difference in inoculums preparations and card filling should not take longer than 30 minutes. The 64 well plastic Gram Negative (GN) card was used to identify the samples using the Vitek 2 compact system according to the manufacturer's instructions. With the use of a suction device inside the filling chamber, the culture suspension was injected into the GN card. The cards were then sealed and incubated in a spinning carousel at 37 degrees Celsius in the loading chamber. Each filled card was removed from carousel and delivered to the optical system, where response reading were taken and data was gathered (Prudhivi Sumana et al., 2017).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed with the Vitek 2 compact system utilising an AST N281 card, as directed by the manufacture. A total of seventeen antibiotics were tried in the AST N281 card. In the filling chamber, inoculum was prepared by transferring 200µl of culture suspension from the 0.5 McFarland culture suspension used for filling the identification cards into a fresh 3ml sterile solution, resulting in a final turbidity of $8x10^6$cfu/ml. The antimicrobial susceptibility cards are processed automatically by the Vitek 2 compact system corrects for MIC as needed using an internal database of potential phenotypes for microbe antimicrobial agent combinations **(**Prudhivi Sumana et al., 2017).

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Observation and Result

Isolation and Detection of NFGNB Pathogens:-

Detection of NFGNB on MacConkey agar, Blood agar and Chocolate agar:-Colonies were examined as non-Lactose fermenters on MacConkey agar as grayish colonies and hemolytic activity was observed (beta hemolysis) on Blood agar.

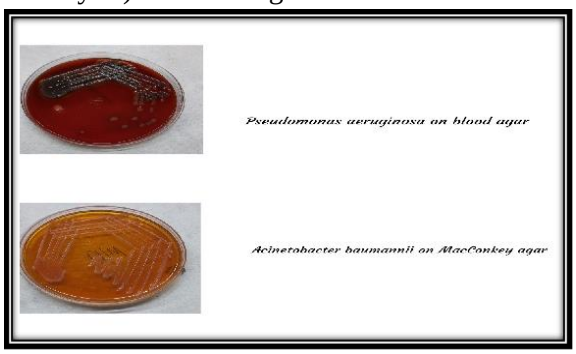

Fig:- 1 Colonies of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* on blood agar and MacConkey agar media.

Total no. of 1000 Clinical samples were collected from patients of varied age groups visiting tertiary care hospital, suffering from difference illness. Clinical samples were subjected to processing using Vitek 2 compact system and number of 328 isolates was found to be NFGNB. Results obtained showed that *Pseudomonas aeruginosa* are 51.82%, *Acinetobacter baumannii 39.63%*, *Achromobacter Xylosoxidans*4.26%, *Stenotrophomonas maltophilia*2.74%, *Burkholderia cepcia*1.52%.

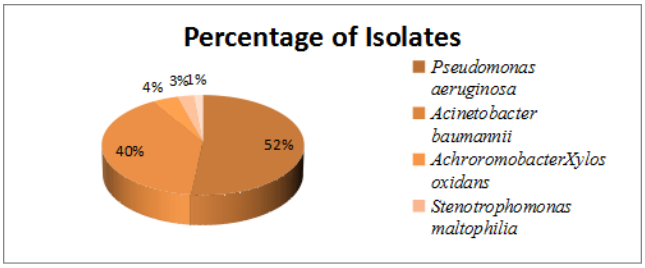

Fig:- 3 Percentage of NFGNB Isolates in present study (n=328)

The highest number of NFGNB were isolated from pus/wound swab (n=88),followed by Endotracheal tubes tip (n=77),blood (n=55),urine (n=44),sputum (20),body fluids (n=9), bronchioalveolar lavage fluid (n=5),CSF (n=5),and other specimen (33). A tabular detail of the distribution is as follows:

Table 1:- Specimen Wise Distribution of NFGNB isolates

Samples	*Pseudomonas* species (170)	*Acinetobacter* species (130)	*Achromobacter Xylosoxidans* (14)	*Stenotrophomonas maltophilia* (9)	*Burkholderiacepacia* (5)
Urine	31	10	01	01	01
Blood	15	33	00	05	02
Pus	50	26	02	02	00
Tip	33	34	10	00	00
CSF	02	03	00	00	00
Sputum	12	06	00	00	02
Body fluid	02	07	00	00	00
BAL	04	01	00	00	00
Others	21	10	01	01	00

Susceptibility of patients towards NFGNB infections were higher in the age group of 40-60 (n=105) followed by the age group 20-40 (n=100), then >60 age group (n=76) and the (n=47)from the age group below 20 years. Data collected indicated that highest number ofpatients were among the age group of 40-60 years. Age wise Graphical distributions of

clinical isolates of NFGNBs are shown as follows:

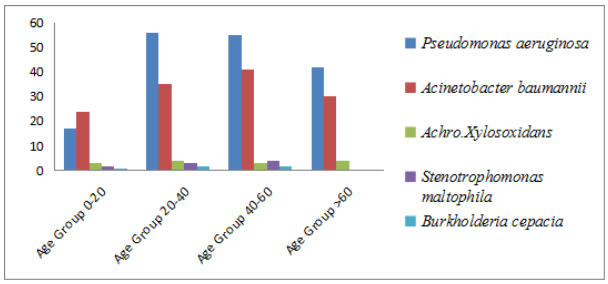

Fig:- 4 Age wise distributions of clinical isolate of NFGNB

Antibiotic susceptibility pattern reveals that colistin (77.07%) was the most effective drug against most of the isolated NFGNB pathogen followed by Tigecycline (31.34%), Minocycline (55.55%) and Amikacin (24.33%) whereas moderate to least susceptibility was recorded against Imepenem (10.50%), clavulanic acid (7.00%), Piperacillin (5.09%), Cefepime (5.09%).

Pseudomonas aeruginosa was highly susceptible against colistin (73.52%), amikacin (32.94%), moderately susceptible against Sulbactaum (16.47%), Doripenem (14.70%) and mild susceptibility was observed against Imepenem (11.76%) and Meropenem (11.76%).

Acinetobacter baumannii showed highest susceptibility against colistin (79.23%), tigecycline (68.46%) and minocycline (53.84%), moderate sensitivity was shown against Trimethprime(25.38%), Sulbactum (18.46%) and Livofloxacin (16.15%) followed by mild suppression against amikacin (13.07%) and ciprofloxacin (13.07%).

Achromobacterxylosoxidans showed highest suppression by colistin (100%), trimethoprime (100%), sulbactum(100%), moderate sensitivity byceftazidime (78.57%), impenem(64.28%) followed by Ticarcillin (42.85%), tigecycline (42.85%), meropenem (42.85%), cefepime (21.42%).

Burkholderiacepacia showed resistance against most of the antibiotics. Ceftazidime (100%), Livofloxacin (100%) and Minocycline (100%) were some of the antibiotics of higheffectiveness whereas Tigecycline (60%), trimethoprime (60%) and Meropenem (60%) were found to exhibit moderate effect on *Burkholderia cepacia.* *Stenotrophomonas maltophilia* due to intrinsic resistance mechanism have showed highest degree of resistance against all the antibiotics.

Table: 2 Antibiotic susceptibility profile of commonly isolated NFGNB tested by Vitek2.

ANTIBIOTICS	***Pseudomons aeruginosa* 170 (n)**	***Acinetobacter baumanii* 130 (n)**	***Achromobacter Xylosoxidans* 14 (n)**	***Stenotrophomonas maltophilia* 9 (n)**	***Burkholderia cepacia* 5 (n)**
Amikacin	32.94	13.07	00	NA*	00
Aztreonam	2.94	00	00	NA	00
Cefepime	5.88	2.30	21.42	NA *	00
Cefoperazone/ Sulbactum	16.47	18.46	100	NA *	00
Ceftazidime	7.05	2.30	78.57	00	100
Ciprofloxacin	2.94	13.07	21.42	NA *	00
Colistin	73.52	79.23	100	NA *	00

Doripenem	14.70	3.07	00	NA *	00
Gentamicin	7.05	3.07	00	NA *	00
Imipenem	11.76	3.07	64.28	NA *	00
Levofloxacin	6.47	16.15	21.42	55.55	100
Meropenem	11.76	3.84	42.85	NA *	60
Minocyclin	00	53.84	00	00	100
Piperacillin/Tazobactam	7.05	2.30	7.14	NA *	00
Ticarcillin/Clavulanic acid	7.05	3.07	42.85	00	00
Tigecycline	1.17	68.46	42.85	NA *	60
Timethoprime/Sulfomethoxazole	00	25.38	100	100	60

NA*: Due to the intrinsic resistance organism are not tested against these antibiotics according to the CLSI guidelines.

Discussion

The significant increase in the number of infections caused by NFGNB pathogens and their multiple patterns of resistance make them a notable and important pathogen. They are resistant to carbapenem as well as beta-lactams and other groups of antibiotics. In recent years, the indiscriminate and irrational use of antibiotics has made NFGNB an important nosocomial pathogen. Studies have confirmed a link between exposure to antibiotics and the emergence of antibiotic-resistant strains (J P Quinn 1998). Carbapenems are commonly used as a first-line therapy for serious bacterial infections. The development of carbapenem resistant bacteria in recent decades has increased theprobability of treatment failure (Buzilă ER et al., 2021). The difficulty in selecting a suitable empirical antibiotic treatment is one of the main effects of multidrug resistance. Patients with MDR/XDR infections are more likely to get insufficient initial antimicrobial therapy (Horcajada JP et al., 2019). In patients with P. Aeruginosa bloodstream infections, delayd in receiving effective antibiotic medication are linked to worse outcomes and higher fatality rates, and MDR/XDR patterns are linked to a higher risk of ineffective empirical treatment (Horcajada JP et al., 2019).

In the current study, *Pseudomonas aeruginosa* (51.82%) is the most prominent of all NFGNB isolates which is in accordance to study conducted by various researcher (Malini A et al., 2009; Susmitha Simgamsetty et al., 2016; Srividya Yeruva et al., 2018; Patel PH et al., 2013; Prudhivi Sumana et al., 2017). Malini et al., 2009andPatel et al., 2013 conducted Study which showed isolation rates of 6.7% and 7%. This is similar to our result that is (6.09%). In contrast, the segregation rate of NFGNB from sputum is Savita Singh et al., 2017 study was 22.5%. The maximum number of NFGNBs was isolated from pus / wound swabs at 24.61%, followed by the endotracheal tip, blood, urine, sputum which is similar to the study by Srividya Yeruva et al., 2018.Studies conducted by different researchers have found that different clinical samples have different NFGNB isolation rates. In our study, the isolation rate of NFGNB was 32.8%, and the isolation rate of NFGNB pathogen was reported to be 45.9% by Sidhu et al. , 2010 is

almost the same as the our study. In contrast to previous reports, A study by Juyal et al. 2012reported a very low NFGNB isolation rate of 9.32%. The segregation rates of *Pseudomonas* and *Acinetobacter* in different studies are compared with the results of this study. Various international organisations emphasizes that each hospital should have its own unique antibiotic sensitivity pattern, as the typical antibiotic sensitivity pattern may not apply to all areas (Vijaya D et al., 2000). The NFGNB pathogens are also known to cause nosocomial infections and have gained multiple antibiotic resistance pattern. Therefore the present study focusses on the necessity to monitor and study the antibiotic susceptibility pattern of Non Fermenting Gram Negative Bacilli isolates in order to identify the selection of empiric therapy for their treatment and also to keep a check on adoption of their multi drug resistance.

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Conflict of Interest

Authors have declared no conflicts of interest.

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