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Original Article

NON-FERMENTING GRAM-NEGATIVE BACILLI ISOLATES FROM VARIOUS CLINICAL SAMPLES AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: Non-fermenting Gram-negative bacilli (NFGNB) were once considered as a common laboratory contaminant (15-20%), but in recent years they have emerged as a major concern for nosocomial infections as the frequency of their isolation and resistance towards antimicrobial agents is increasing rapidly. Infections caused by these bacteria are almost always secondary to some predisposing factors in patients such as burns, prolonged antimicrobial therapy, immunosuppression etc. The major problem leading to high mortality lies in the appearance of drug-resistant strains. The Objectives of the study was to identify and isolate NFGNB and study their antibiogram profile so that empirical therapy could be selected accordingly.

Methods: This is a cross-sectional study conducted in the Department of Microbiology, Sri Venkateswara Medical College, Tirupati for a period of 6 mo. A total of 7875 various clinical samples were received and processed. Isolates were tested against 8 different antibiotics by a Kirby-Bauer disk diffusion method.

Results: Out of 7875 clinical samples processed, 2666 samples showed culture positive, among which 539 samples (20%) were NFGNB isolates. Male to female ratio was 1.5:1. Most common age group affected was 41-60 y. Predominant NFGNB isolates were *Pseudomonas species* (73.47%) followed by *Acinetobacter species* (26.53%). Pus (42%) was the commonest sample from which NFGNB was isolated; followed by Urine (16.88%), Sputum (16.69%), Blood (15.39%), ET tube (6.30%), Body fluids (1.66%) and cervical/vaginal swabs (1.11%). Both isolates showed higher resistance towards cephalosporins followed by ciprofloxacin, whereas least resistance towards Meropenem followed by Imipenem. Most of the isolates showed multidrug resistance (MDR).

Conclusion: Antimicrobial resistance (AMR) should be monitored on a regular basis in hospital settings. This study suggests that due care must be taken to adequately diagnose NFGNB infections and prescribe the antibiotic treatment most effective in preventing the increase in multidrug-resistant strains.

Keywords: Non-fermenting Gram-negative bacilli, Multidrug resistance, Antimicrobial resistance

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INTRODUCTION

Non-fermenters are a group of aerobic Gram-negative bacilli that do not form spores and have no ability to use carbohydrates as energy sources or to degrade through oxidation rather than fermentation [1]. Non-fermenters were previously considered as commensals or contaminants but now in recent era, they have been emerged as an important nosocomial pathogen with frequent epidemics and even lead to life-threatening condition [2]. The innate resistance of these bacteria to routinely used disinfectants and their tendency to colonize different surfaces are decisive for their emergence as important nosocomial pathogens [3]. About 15% of all Gram-negative bacteria cultured from clinical specimens are NFGNB [4]. These NFGNB infections are nearly always a result of other risk factors in patients, such as burns, neutropenia, indwelling catheters, prolonged hospital stay, long-term use antimicrobial therapy, use of immunosuppressive drugs, and the ability of advanced surgical and medical care to prolong a patient's life with serious illnesses [5].

Pseudomonas aeruginosa and *Acinetobacter baumannii*, the two most prevalent NFGNB are well-known nosocomial pathogens with a wide range of illness; other organisms included in this group are *Burkholderia, Stenotrophomonas, Sphingomonas, Flavobacter* etc [5]. Due to their predominant association with opportunistic infections in seriously ill and immunocompromised individuals, NFGNB are increasingly posing a hazard to healthcare systems [2].

Non-fermenters are now resistant to several commonly used antibiotics. This resistance can be attributed to a mutation in the genes encoding the protein, efflux pump mechanisms, chromosomal beta-lactamases or due to alteration of penicillin-binding proteins [6]. The surfacing of NFGNB infections along with rising drug resistance, calls for close monitoring of antibiogram of these organisms [7]. The aim and objective of this study was to isolate and identify NFGNB and determine their antimicrobial susceptibility pattern.

MATERIALS AND METHODS

This study was conducted for a period of 6 mo in a tertiary care hospital, Tirupati after obtaining approval from the Institutional Ethics Committee. A total of 7875 clinical specimens were received in laboratory from various departments and processed as soon as possible. These included urine samples, blood, pus, sputum, CSF, pleural fluid, peritoneal fluid, ascitic fluid, endotracheal secretions, tracheal aspirate, catheter tips, ear swabs and cervical/vaginal swabs.

Samples were inoculated on Blood agar and MacConkey agar, Cystine-lactose-electrolyte deficient agar (CLED agar urine samples) under strict aseptic condition and incubated at 37 °C for 18-24 h under aerobic conditions before being reported as sterile. Non-lactose fermenting colonies obtained on MacConkey agar were subjected to Gram staining and all Gram-negative bacilli/cocci/coccobacilli obtained were further identified by using a standard protocol for identification.

The characters assessed for isolation of NFGNB were Motility (By Hanging drop method), Catalase test, Oxidase test, Indole test, Methyl red test, Vokes-Proskauer test, Citrate test, Urease test, Triple Sugar Iron test, Oxidative Fermentative test (O/F), Nitrate reduction test, Sugar fermentation tests (Glucose, Lactose, Sucrose, Mannose, Maltose, Mannitol, Arabinose, Xylose, growth at 25 °C, 37 °C, 42 °C, Lysine and Ornithine decarboxylase and Arginine dehydrolase activity tests.

Antimicrobial susceptibility testing was determined by the Kirby Bauer Disc Diffusion Method on Muller Hinton agar as per CLSI

guidelines. Following Antibiotic discs were used: Ceftazidime (30µg), Piperacillin-Tazobactam (100µg/10 µg), Aztreonam (30µg), Imipenem (10µg), Meropenem (10 µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg). Turbidity compared to 0.5 McFarland units were inoculated on MHA plates by Lawn culture method and were incubated at 37 °C for 18-24 h. Results were interpreted according to the measurement of zone sizes mentioned in the CLSI guidelines [8].

Control strains used were *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. Medias and antibiotic discs were obtained from HiMedia Labs, Mumbai, India.

RESULTS

A total of 7875 samples were processed; among them 2666 samples showed culture positive, out of which NFGNB accounted for about 20% (539 samples) [fig. 1].

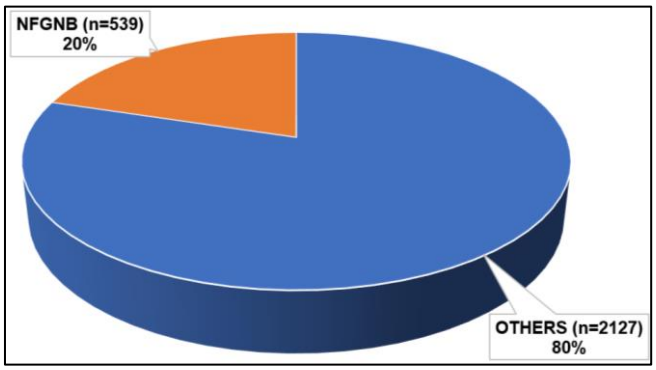

Fig. 1: Distribution of various NFGNB isolates obtained in the study

The isolation rate of NFGNB was highest among males (59.55%) compared to females (40.44%) fig. 2. Most common age group affected was 41-60 y (45%), 21-40 y (30%), 60-80 y (15%), 0-20 y (9%) and above 80 y (less than 1%).

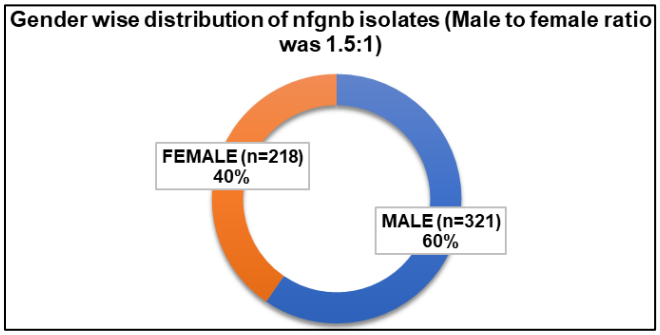

Fig. 2: Gender distribution of NFGNB

Pseudomonas aeruginosa was the predominant isolate accounting for about 65% followed by *Acinetobacter baumannii* (22.54%), *Pseudomonas species* (8.57%) and *Acinetobacter lwoffii* (7.45%) [fig. 3]. Majority of NFGNB were isolated from Pus (42%) and least from cervical/vaginal swabs (1.11%) [fig. 4]. Most of the NFGNB were isolated from ICUs followed by surgery, Orthopaedics, Medicine, Obstetrics and Gynaecology and Paediatric wards.

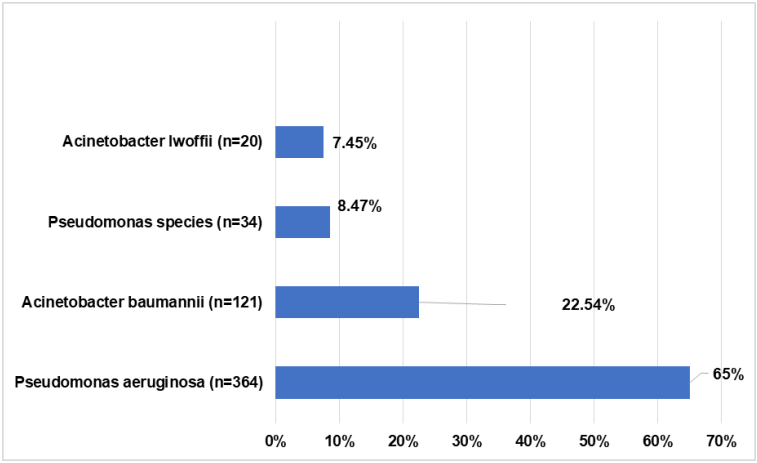

Fig. 3: Distribution of isolated NFGNB

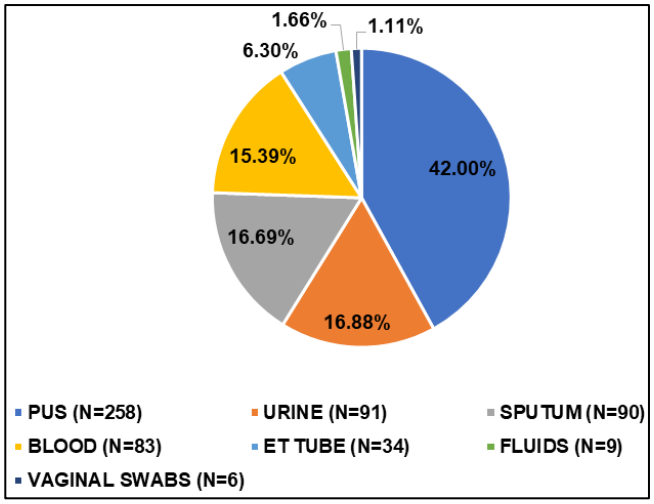

Fig. 4: Sample-wise distribution of NFGNB isolates

The antibiotic susceptibility results are given in table 1. Isolates showed higher sensitivity to Meropenem and Imipenem; Least sensitivity to Ceftazidime and Ciprofloxacin. Multidrug resistance was noted among most of the isolates.

Table 1: Antibiotic susceptibility test showing sensitive and resistant pattern of NFGNB isolates

Antibiotic susceptibility pattern of NFGNB isolates				
Antibiotics tested	**Pseudomonas species**		**Acinetobacter species**	
	Sensitivity	**Resistance**	**Sensitivity**	**Resistance**
Meropenem (MRP)	382 (96%)	16 (4%)	126 (89.7%)	15 (10.3%)
Amikacin (AK)	368 (92.5%)	30 (7.5%)	119 (84.7%)	22 (15.3%)
Gentamicin (GEN)	353 (88.6%)	45 (11.4%)	104 (73.5%)	37 (26.5%)
Imipenem (IMP)	328 (82.5%)	70 (17.5%)	100 (71.4%)	41 (28.6%)
Piperacillin-tazobactam (PIT)	295 (71.8%)	116 (28.2%)	95 (67.3%)	46 (32.7%)
Aztreonam (AT)	286 (66%)	112 (34%)	-	-
Ciprofloxacin (CIP)	227 (57.2%)	171 (42.8%)	94 (66.7%)	47 (33.3%)
Ceftazidime (CAZ)	159 (40%)	239 (60%)	71 (50.50%)	70 (49.50%)

DISCUSSION

The importance of NFGNB infections have been increased not only because of the seriousness of the infections but also due to its resistance to various antibiotics. Therefore, the present study was undertaken to identify commonly encountered clinically significant NFGNB from clinical specimen along with their antibiogram. Different researchers have reported varied isolation rates. Prevalence varies from place to place and from time to time. In our study, prevalence rate of NFGNB was about 20%. Similar results were reported in a study conducted by Jeyaraman *et al.* (2023) [9], Anshu Shastri *et al.* (2019) [4], where their prevalence rate was about 18.10%, 18.5 %, respectively. Study by Amandeepkaur *et al.* (2018) [10], Sharma *et al.* (2020) [11], Soni *et al.* (2023)[12], reported less prevalence rate of about 16.1%, 14.6%, 10.01%, respectively compared to our study; whereas a study by Yadav *et al.* (2020)[13] reported highest prevalence rate of about 27.1%.

Innate immunity and susceptibility to all types of infections are mostly determined by host characteristics such as age and sex. In present study, males (59.55%) were most commonly affected compared to females (40.45%). Male to female ratio was about 1.5:1. These results were in concordance with the study conducted by Sharma *et al.* (2020) [11], Anshu Shastri *et al.* (2019) [4], Yadav *et al.* (2020) [13], Ami Patel *et al.* (2021) [14], where they have reported predominance for male gender.

The most common age group affected was 41-60 y (45%), which was in accordance with the results reported by Soni *et al.* (2023) [12], Ridhima *et al.* (2016) [15], Kaur *et al.* (2014) [16], Kalidas *et al.* (2013) [17], whereas in a study conducted by Anu Sharma *et al.* (2018) [18], Ami Patel *et al.* (2021) [14], the maximum age group affected was between 31-46 y and 21-40 y respectively.

In present study, predominant isolate was *Pseudomonas aeruginosa* (65%) followed by *Acinetobacter baumannii* (22.54%). These results were corroborated with the study conducted by Soni *et al.* (2023) [12], where *Pseudomonas aeruginosa* (51.7%) was the most common NFGNB isolate followed by *Acinetobacter baumannii* (23.4%). Studies by Ami Patel *et al.* (2021) [14], Anshu shastri *et al.* (2019) [4], Anu Sharma *et al.* (2018) [18], Grewal *et al.* (2017)[19], Juyal *et al.* (2013) [20] also reported *Pseudomonas aeruginosa* as the most predominant isolate followed by *Acinetobacter baumannii*. In contrast to the present study, studies by Sharma *et al.* (2020) [11], Yadav *et al.* (2020) [13] reported *Acinetobacter baumannii* as the most common isolate followed by Pseudomonas aeruginosa.

Majority of NFGNB isolates were obtained from pus samples (42%). These results are in concordance with the findings obtained by Soni *et al.*, Benachinmardi *et al.* (2014) [21], where highest NFGNB isolates were from pus samples compared to other clinical samples.

In our study, both *Pseudomonas species* and *Acinetobacter species* showed higher sensitivity to Meropenem, Amikacin, Gentamicin, Imipenem, Piperacillin-Tazobactam and least sensitivity to Ciprofloxacin and Ceftazidime. Study conducted by Soni *et al.* (2023) [12], Anshu Shastri *et al.* (2019) [4] also showed similar findings that NFGNB isolates showed more sensitivity to Meropenem, Amikacin, Imipenem and least sensitivity to Ceftazidime.

CONCLUSION

Globally, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* was one among the most common NFGNB isolates that poses a threat by possessing numerous virulence factors and accumulating numerous intrinsic antimicrobial resistance mechanisms. Henceforth, Antimicrobial resistance should be monitored on a regular basis in

hospital settings. Antibiotic policies should be updated on regular basis to combat the emergence of resistant strains. This study suggests that due care must be taken to adequately diagnose NFGNB infections and prescribe the antibiotic treatment most effective in preventing the increase in multidrug-resistant strains.

ETHICAL APPROVAL

Lr. No. 99/2024 (Institutional Ethics Committee, S. V. Medical College, Tirupati).

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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