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Original Article

Microbiology Section

Antibiotic Sensitivity Pattern of Non Fermentative Gram Negative Bacilli in a Tertiary Care Hospital, Kakinada, Andhra Pradesh, India

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ABSTRACT

Introduction: Non Fermentative Gram Negative Bacilli (NFGNB), which are saprophytic in nature, have emerged as important pathogens. They have been associated with infections such as septicaemia, meningitis, pneumonia, Urinary Tract Infection (UTI) and surgical site infections. They exhibit resistance to beta lactams and also to other groups of antibiotics and carbapenems.

Aim: To identify and isolate NFGNB from various clinical samples and to know their antimicrobial susceptibility pattern.

Materials and Methods: The present study was a cross-sectional study done in the Department of Microbiology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India, from July 2018 to August 2019. The study was done on 204 clinical samples that were inoculated on different growth media. The non fermenters were identified by conventional methods and isolates that were not identified, were subjected to automated system (MicroScan autoSCAN-4 system). The susceptibility testing was done by Kirby-Bauer disc diffusion method. Data was entered in Microsoft Excel sheet 10 and all analysis was performed by Statistical Package for the Social Sciences (SPSS) 24.0.

Results: A total of 100 NFGNB were isolated from 204 clinical samples. *Pseudomonas aeruginosa (P. aeruginosa)* was the most common non-fermenter (49%), followed by *Acinetobacter baumannii (A. baumannii)* (19%), *P. fluorescens* (17%), *Acinetobacter lwoffii (A. lwoffii)* (4%), *Alcaligenes faecalis (A. faecalis)* (4%), *Burkholderia cepacia (B. cepacia)* (3%), *Pseudomonas putida (P. putida)* (2%) and *Stenotrophomonas maltophilia (S. maltophilia)* (2%). The NFGNB showed good sensitivity to imipenem (97%), amikacin (53%) , and 92% resistance to cefipime, 75%, 73%, 49%, and 48%, resistance to ceftazidime, ticarcillin, ciprofloxacin, piperacillin, respectively.

Conclusion: *P. aeruginosa* and *A. baumannii* were the common NFGNB isolated in the present study from surgical site infections, urinary tract infections, bacteraemia, and ventilator associated pneumonia. The different species of NFGNB have shown a varied sensitivity pattern. All NFGNB showed higher rate of resistance to cefipime and ceftazidime and good sensitivity to imipenem, and amikacin.

Keywords: *Acinetobacter baumannii*, Imipenem, Nosocomial pathogens, *Pseudomonas aeruginosa*, Resistance pattern

INTRODUCTION

The NFGNB are a diverse group of non spore forming aerobic bacilli that either do not utilise glucose or utilise it oxidatively and occur as saprophytes in the environment and some also found as commensals in the human gut. About 15% of NFGNB are known to be isolated among bacterial isolates from a clinical microbiology laboratory [1]. They can tolerate harsh environmental conditions and shows marked drug resistance to antimicrobial agents and so frequently described as hospital acquired pathogens [2]. Commercial systems like MicroScan, Rapid NF Plus, API Rapid NFT, Vitek system, Remel systems are used to identify non fermenting bacilli for faster and accurate diagnosis of NFGNB infections [3]. The NFGNB are intrinsically resistant to commonly using antibiotics and are known to produce Extended Spectrum β-lactamases (ESBL) and Metallo β-lactamases (MBL) [4]. Risk factors are immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostic and therapeutic procedures [5]. In recent years, due to the liberal and empirical use of antibiotics, they have emerged as important healthcare-associated pathogens. They have been associated with infections like septicaemia, meningitis, pneumonia, urinary tract infections and surgical site infections [6]. They are highly prevalent in moist environmental conditions [7]. This group of bacteria was earlier considered to be colonisers but are now frequently isolated from different clinical specimens and are responsible for a wide range of human infections especially in immunocompromised individuals [8].

Infections produced by NFGNB may be endogenous and exogenous in origin [9].This heterogenous group includes *Pseudomonas* spp., *Acinetobacter* spp. *Alkaligens* spp. *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex [10]. They are often resistant to disinfectants and can spread from patient to patient [11]. The NFGNB are emerging as an important cause of blood stream infections [12]. They have been commonly found on the skin of healthcare workers, ventilator machines, humidifiers and mattresses [13].

Multidrug resistance in NFGNB is progressively increasing, so there is a need to identify NFGNB and monitoring their susceptibility pattern for the proper management of infections caused by them to avoid unnecessary usage of antibiotics and emergence of drug resistant strains. Hence, the aim of the present study was to identify and isolate NFGNB from various clinical samples and to determine their antimicrobial susceptibility pattern with Kirby-Bauer disc diffusion method from patients admitted to Government General Hospital, a tertiary care hospital, Kakinada, Andhra Pradesh, India.

MATERIALS AND METHODS:

The present study was a cross-sectional study done in the Department of Microbiology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India from July 2018 to August 2019. Informed consent was obtained from the participants of the present study. Institutional Ethical Committee (IEC) approval was obtained approval number- IEC /RMC/2018 1355A.

Inclusive criteria: Patients of age ≥14 years was included. Urine from UTI patients, blood from septicaemia, pus, sputum, bronchial washings and pleural fluid were included.

Exclusive criteria: Cerebrospinal Fluid (CSF) samples and samples from paediatric age group (<14 years) were excluded. Mixed growth isolates were also excluded.

Study Procedure

A total of 204 clinical samples were received in the laboratory during July 2018 and August 2019 which included 101 pus, 42 urine, 31 blood cultures, 18 sputum, seven pleural fluid, five bronchial washings. These samples were inoculated on Nutrient agar, Blood agar, MacConkey agar, and incubated at 37°C for 18-24 hours. All non lacgtose fermenting colonies from MacConkey agar were inoculated into Triple sugar iron agar. All the organisms that grew on triple sugar iron agar and gave an alkaline reaction (no acid production) were provisionally considered to be NFGNB and subjected to a battery of tests including Gram stain, Hanging drop for motility, Pigment production, Oxidase production, Nitrate reduction, Utilisation of 10% lactose, Utilisation of carbohydrates of Oxidative Fermentative (OF) media (Hugh-Leifsons medium) [14]. Isolates not identified by conventional methods, were subjected to automated system– by using Micro Scan autoSCAN-4 system.

The sensitivity test was done with the help of Kirby-Bauer disc diffusion [15], method using commercially available discs (Hi-media). The different antimicrobial agents tested were cefipime (30 µg), ceftazidime (30 µg), imipenem (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), ticarcillin (75 µg) and piperacillin (100 µg) .The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) [16] guidelines. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as the control strains.

STATISTICAL ANALYSIS

Data was tabulated in Microsoft Excel sheet 10 and all analysis performed by SPSS 24.0. Data was presented in tables, graph and charts.

RESULTS

A total of 100 NFGNB were isolated from 204 clinical samples with an isolation rate of 49%. Out of 204 different clinical samples 156 (76.5%) gram negative bacilli were isolated. In remaining 48 (23.5%) samples, 11 (22.9%) samples showed no growth, and 37 (77.1%) samples showed polymicrobial infection. Out of 156 (76.5%) gram negative bacilli, 126 (80.8%) were non lactose fermenters and 30 (19.2%) were lactose fermenters. Out of 100, 40 isolates were found from surgical wards. Higher number of NFGNB were isolated in age group of 41-60 years 41 (41%). 32 (47.1%) *Pseudomonas* spp and 2 (75%) *A. faecalis* were more common in 41-60 yrs age group. A total of 9 (39.1%) *Acinetobacter* spp were isolated in 14-20 yrs age group. NFGNB isolates were higher in males 74 (74%) as compared to females 26 (26%) [Table/Fig-1,2].

The most common isolate was *P. aeruginosa* (49%), followed by *A. baumannii* and *P. fluorescens* (19%, 17%) [Table/Fig-3]. Majority of NFGNB were isolated from pus (71%) and urine (11%) samples. Out of 71 (71%) isolates from pus, majority were *P. aeruginosa* (35), only two isolates are *B. cepacia* and *S. maltophilia*. Out of 11 (11%) isolates from urine, majority were *P. aeruginosa* (3), and only one isolate is *A. lwoffii*.

Out of 10 (10%) isolates from sputum, majority were *P. aeruginosa* (6) and one isolate is *B. cepacia*. Out of three (3%) isolates from pleural fluid, *A. baumannii* (2), and *P. aeruginosa* (1) were isolated.

Out of 49 isolates of *P. aeruginosa*, all 49 (100%) were resistant to cefipime, 33 (67.34%) isolates were resistant to ceftazidime and only 1 (2.04%) isolate was resistant to imipenem. Out of 17 isolates of *P. fluorescens*, all 17 (100%) were resistant to both ceftazidime, cefipime [Table/Fig-4]. Overall, out of 100 NFGNB, 92

Variables	Number
Age (in years)	
14-20	23
21-40	29
41-60	41
≥61	7
Gender	
Male	74
Female	26
Wards	
Surgical wards	40
Chest and communicable diseases	17
Orthopaedics	14
Medicine	12
Obstetrics and Gynaecology	11
Nephrology	6

[Table/Fig-1]: Demographic details of the 100 NFGNB.

Age (years)	*Pseudomonas* spp. (n=68)	*Acinetobacter* spp. (n=23)	*A. faecalis* (n=4)	*B. cepacia* (n=3)	*S. maltophilia* (n=2)
14-20	13 (19.1%)	9 (39.1%)	1(25%)	0	0
21-40	18 (26.4%)	8 (34.8%)	1 (25%)	1 (33.3%)	1 (50%)
41-60	32 (47.1%)	6 (26.1%)	2 (75%)	0	1 (50%)
61 and above	5 (7.4%)	0	0	2 (66.7%)	0
Total	68	23	4	3	2

[Table/Fig-2]: Distribution of NFGNB in relation to age (N=100).

Organism	Urine	Blood	Pus	Pleural fluid	Sputum	Bronchial washings	Total
P. aeruginosa	3	2	35	1	6	2	49
P. fluorescens	2	0	13	0	2	0	17
P. putida	0	0	2	0	0	0	2
A. baumannii	3	0	13	2	0	1	19
A. lwoffii	1	0	2	0	1	0	4
Alkaligenes faecalis	2	0	2	0	0	0	4
Burkholderia cepacia	0	0	2	0	1	0	3
S. maltophilia	0	0	2	0	0	0	2
Total	11	2	71	3	10	3	100

[Table/Fig-3]: NFGNB isolated from various clinical samples (n=100).

Organism \ Antibiotics	Ceftazidime	Cefipime	Ciprofloxacin	Imipenem	Amikacin	Ticarcillin	Piperacillin
P. aeruginosa (n=49)	33 (67.34%)	49 (100%)	20 (40.81%)	1 (2.04%)	20 (40.81%)	37 (75.51%)	17 (34.69%)
P. fluorescens (n=17)	17 (100%)	17 (100%)	12 (70.58%)	2 (11.76%)	9 (52.94%)	16 (94.11%)	14 (82.35%)
P. putida (n=2)	1 (50%)	1 (50%)	1 (50%)	0	2 (100%)	2 (100%)	0
A. baumannii (n=19)	14 (73.68%)	15 (78.94%)	11 (57.8%)	0	9 (47.36%)	10 (52.63%)	9 (47.36%)
A. lwoffii (n=4)	3 (75%)	3 (75%)	2 (50%)	0	2 (50%)	3 (75%)	3 (75%)
A. faecalis (n=4)	2 (50%)	2 (50%)	1 (25%)	0	2 (50%)	2 (50%)	2 (50%)
B. cepacia (n=3)	3 (100%)	3 (100%)	1 (33.33%)	0	1 (33.33%)	2 (66.66%)	1 (33.33%)
S. maltophilia (n=2)	2 (100%)	2 (100%)	1 (50%)	0	2 (100%)	1 (50%)	2 (100%)
Total	75%	92%	49%	3%	47%	73%	48%

[Table/Fig-4]: Resistance pattern of NFGNB isolates to different antibacterial agents (n=100).

(92%) isolates are resistant to cefipime, 75 (75%) to ceftazidime, 73 (73%) to ticarcillin, respectively. Out of 100, 75% of NFGNB resistance to ceftazidime, indicated positive for ESBL production. And, only 3% of NFGNB resistance to imipenem indicated MBL producers.

DISCUSSION

Earlier NFGNB that were considered to be contaminants have now emerged as important health care associated pathogens. *P. aeruginosa* and *Acinetobacter* species are common nosocomial pathogens [1,2,4-8]. Similar observations was also made in the present study as *P. aeruginosa* and *P. fluorescens*, along with the *Acinetobacter* species accounted for 91% of the isolates.

In all the studies *P. aeruginosa* was the major isolate. In some studies it was followed by *P. fluorescens* and *P. putida*. In all the studies *A. baumannii* followed by *A. lwoffii* [2, 5]. Alkaligenes faecalis and Stenotrophomonas maltophilia and Burkholderia cepacia were rarley isolated NFGNB [1,10]. In the present study, majority of non fermenters were isolated from pus samples, similar to observations made in previous literature [9,10].

P. aeruginosa in the present study was highly resistant to ceftazidime (67.34%) similar with studies by Peerzada BY et al., (74%) [4] Juyal D et al., (72%) [5], Pappu RK et al., (89.5%) [7], Bhatnagar R et al., (68.6%) [10], and its resistance to cefipime (100%) was more than that of Pappu RK et al., (66.3%) [7] and Singamsetty S et al., (38.1%) [11]. *P. aeruginosa* resistance to amikacin (40.81%) in the present study was more than the studies of Peerzada BY et al., (30%) [4], and Singamsetty S et al., (33.4%) [11]. Resistance to ticarcillin (75.51%) less than the study of Pappu RK et al (35.72%) [7], and its resistance to piperacillin (34.69%) correlating with Juyal D et al., (47.87%) [5]. *P. aeruginosa* in the present study showed less resistance (2.04%) to imipenem in similar to Pappu RK et al., (2.05%) [7], and was less than the studies done by Singamsetty S et al., (29.8%) [11], and Grewal US et al., (17%) [13], Shrihari N and Ishwarya M, (20.4%) [17]. While its resistance to ciprofloxacin (40.81%) in the present study was correlating to Bansal R et al., (<50%) [9].

In current study, majority isolates of *Acinetobacter* species showed higher resistance to ceftazidime and cefipime and lower resistance to imipenem which is corresponding to studies of Peerzada BY et al., [4], and Pappu RK et al., [7]. In the present study *P. aeruginosa* and *A. baumannii* were commonly isolated non fermenters and they showed maximum resistance. The prevalence , phenotypic nature and antibiotic sensitivity pattern of nonfermenters may show regional variation, therefore antimicrobial susceptibility profile is needed to establish appropriate therapeutic management to prevent infections caused by them.

Limitations

The CSF samples and samples from paediatric age group and mixed growth isolates were not included in the present study as NFGNB were rarely isolated from those samples and they might be contaminants.

CONCLUSION(S)

The P.aeruginosa and *A. baumannii* were the most common NFGNB isolated in the present study and showed good sensitivity to imipenem, amikacin and piperacillin. NFGNB have shown a varied sensitivity pattern and they showed higher rate of resistance to cefipime and ceftazidime. So, the present study highlights that there is a need to identification and monitoring sensitivity patterns of NFGNB for proper management of infections cause by nonfermenters and also to avoid unnecessary usage of antibiotics and emergence of drug resistance.

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