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Original Research Article

BACTERIOLOGICAL PROFILE OF NON-FERMENTING GRAM NEGATIVE BACILLI FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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Abstract

Background: Nonfermenting gram negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, non-sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively. Nonfermenting gram negative bacilli (NFGNB) are ubiquitous in the environment and occur as saprophytes. Besides that, some NFGNB are found in the human gut as commensals. Despite being classified as commensals or contaminants, NFGNB have a well-established pathogenic potential due to their frequent isolation from clinical samples and their association with clinical disease. **Aim and Objectives:** 1. To isolate and identify non fermenting gram negative bacilli from a given sample.2. To study antimicrobial susceptibility pattern of isolated bacteria. **Materials and Methods:** Study design: A hospital based observational, descriptive, cross-sectional study. Study setting: Department of Microbiology, Government medical college and Hospital, Chhatrapati Sambhaji Nagar. Source of Data: Various clinical samples like blood, sputum, pus, stool, tracheal aspirate, urine, CSF and other body fluids received for culture and sensitivity from various wards, ICUs and OPDs in a Tertiary Care Hospital were included in study. Study period: One and half year (January 2021 to June 2022. Sample size = 1227. **Results:** Out of 19316 clinical specimens, 1227 NFGNB were isolated, yielding a 6.35% isolation rate. The age group 31- 40 accounted for 28.52 % (n=350) which was the highest percentage followed by age group 21-30 with 19.23 % (n=236). out of 1227 samples isolated, 736 (59.98 %) were males and 491 (40.02 %) were females. Male to female ratio was 3:2. location wise distribution of NFGNB. 47 NFGNB were isolated from OPD patient. From IPD, highest NFGNB were isolated from surgery ward accounting for 528 and from ICU, 189 were isolated from MICU. Pus was the commonest sample from which NFGNB were isolated accounting for 56.23 % followed by tracheal aspirate (13.62 %), blood (13.45 %), urine (6.60 %). Pseudomonas aeruginosa was the commonest isolate 720 (58.68 %) followed by Acinetobacter baumannii 477 (38.86 %) and Stenotrophomonas maltophilia 22 (1.79 %). the number of distinct isolates from various clinical samples. P. aeruginosa was most commonly isolated from pus sample (n=481) followed by tracheal aspirate (n=80) and blood (n=65). A. baumannii was isolated most frequently from pus (n=196), blood (n=90) and tracheal aspirate (n=87). Most of the S. maltophilia were isolated from pus (n=10) followed by blood (n=6). 59.44% isolates of P. aeruginosa were sensitive of Meropenem followed by Piperacillin-Tazobactam (56.39%), Amikacin (51.81%) and Tobramycin (50.69%) while it was significantly resistant to ciprofloxacin (54.44%) and levofloxacin (65.28%). P. aeruginosa was isolated from 38 urine samples. Nitrofurantoin was tested in these samples which showed only 2.63% sensitivity to nitrofurantoin. **Conclusions:** It may be concluded that P. aeruginosa and A. baumannii were the commonest NFGNB isolated in our study from various clinical samples. They were associated with number of infections like wound infection, surgical site infection, respiratory tract infections, ventilator associated pneumonia, septicaemia and urinary tract infection. Meropenem sensitivity was high in P. aeruginosa, while Levofloxacin sensitivity was low. A. baumannii was multidrug resistant. It showed 50.52% sensitivity to Minocycline and least susceptible to Ceftazidime. This study also gives an alarming signal of high prevalence of multidrug resistant NFGNB.

INTRODUCTION

Nonfermenting gram negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, non-sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively.[1] Nonfermenting gram negative bacilli (NFGNB) are ubiquitous in the environment and occur as saprophytes. Besides that, some NFGNB are found in the human gut as commensals.[2] Despite being classified as commensals or contaminants, NFGNB have a well-established pathogenic potential due to their frequent isolation from clinical samples and their association with clinical disease.[3]

About 15% of all bacterial isolates from a clinical microbiology laboratory are known to be NFGNB.[4,5,6,7] Infections due to NFGNB constitute about one fifth of all gram-negative bacilli infection.8 Some of the most important members of this group are Pseudomonas followed by Acinetobacter, Burkholderia, Stenotrophomonas, Moraxella, Alcaligenes, Flavobacterium, Achrobacterium, Oligella, Flavimonas etc.[7,9] Currently, the most frequently isolated microbial species is Pseudomonas aeruginosa, which is followed by Acinetobacter baumannii and Stenotrophomonas maltophilia.[10,11] NFGNB can lead to a number of infections, such as a wound infection, urinary tract infection, septicaemia, pneumonia, meningitis, osteomyelitis etc.[1,4] Immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, presence of indwelling catheters, and invasive diagnostic and therapeutic techniques are all risk factors.[4]

NFGNB can be recovered from hospital environment which commonly cause device related infections. They can spread from patient to patient through fomites or the hands of medical personnel because they are frequently resistant to disinfectants.[6]

Because of slow growing property of non-fermenters, they require the use of special culture media and biochemical tests for their identification. Very few laboratories in India routinely identify these organisms.[12] Their isolation from patient's samples should be clinically correlated to avoid unnecessary administration of antibiotics and thereby preventing the development of MDR strains.[8] There will be increased mortality, increased hospital stays and costs because of the resistance pattern produced by NFGNB which compromises the treatment.[13]

NFGNB multidrug resistance is a serious issue in hospital settings because they are a major source of infections. It is found that carbapenems resistant organisms are also resistant to other classes of drugs, like fluoroquinolones and aminoglycosides, making them very challenging to treat and increasing patient morbidity and mortality.[14] As Pseudomonas and Acinetobacter are the most frequently isolated species, carbapenem resistance among them is of major concern.[15] Carbapenem-Hydrolysing Class D β-lactamases (CHDLs) enzyme is mainly responsible for carbapenemase activity in A. baumannii. Loss of the carbapenem-specific porin, OprD2, is the main cause of carbapenem resistance in P. aeruginosa.[15]

Since there has been increased incidence of infection and drug resistance by NFGNB, it becomes necessary to identify them up to species level and also to perform their antibiotic sensitivity test in anticipation with increasing drug resistance among NFGNB. In order to lessen the threat of multidrug resistance, this study was conducted to isolate, identify, and study the antimicrobial susceptibility pattern of the various non fermenters from patients admitted to our tertiary care Center.

Aim and Objectives

1. To isolate and identify non fermenting gram-negative bacilli from a given sample.
2. To study antimicrobial susceptibility pattern of isolated bacteria.

MATERIALS AND METHODS

Study design: A hospital based observational, descriptive, cross-sectional study. **Study setting:** Department of Microbiology, Government medical college and Hospital, Chhatrapati Sambhaji Nagar. **Source of Data:** Various clinical samples like blood, sputum, pus, stool, tracheal aspirate, urine, CSF and other body fluids received for culture and sensitivity from various wards, ICUs and OPDs in a Tertiary Care Hospital were included in study. **Study period:** One and half year (January 2021 to June 2022. **Sample size** = 1227

Inclusion Criteria

1. Samples from any patients with undergoing treatment in ICU or various wards.
2. Samples from outdoor patients.
3. Non repetitive samples.

Exclusion Criteria

1. Isolates of repeated samples from same patient.
2. Patients who did not give consent.
3. Patients who left hospital against medical advice.

Ethical Committee Approval: Institutional ethical committee approval was obtained before the start of the dissertation.

Processing of Samples: Various samples like blood, pus, wound swab, CSF, urine and other body fluids, sputum, endotracheal aspirates were collected aseptically and transported to the microbiology laboratory. Processing was done as soon as possible. Blood samples received in laboratory were incubated for 48 hours before processing.

Culture: Samples were inoculated on Blood agar, MacConkey agar and Nutrient agar. Urine sample was inoculated on Cystine Lactose Electrolyte Deficient (CLED) agar. Before inoculating a culture medium plate, the surface of the medium was dried by removing the plate's lid and positioning the plate facing upward on the shelf of an incubator or hot air oven at 50^0 C-55^0 C. The base containing the medium was inverted and kept at an angle on the lid for drying for 30-40 minutes.

For inoculation, sample was applied to small area of plate with the help of a sterile Nichrome wire loop. This was called as well. The loop was re- sterilised and 4-5 parallel lines were drawn from well known as primary streak. This process was repeated, secondary streak was made from primary streak. From the secondary streak, tertiary streak was made using back and forth movements in the remaining quadrant. Urine samples was cultured with calibrated loop of 1.26 mm internal diameter holding one micro litre of urine.

A loopful of urine was streaked as straight line in middle of the area of media to be inoculated. Without sterilizing the loop zig-zag streaks were drawn at right angle to the original streak. Plates were incubated overnight at 37^0C. Same samples were used for smears preparation. Clean, grease free glass slides were used for smear preparation. Smears were stained with Gram staining and observed for the presence of gram-negative bacilli. After incubation, inoculated plates were observed for growth.

Organism Identification: Identification of an isolate in present study was done by using following methods

Table 1: Identification of an Isolate

Identification from culture plate	Biochemical tests for identification
1. Colony morphology	1. Catalase test
2. Haemolysis	2. Oxidase test
3. Pigment production	3. IMViC Tests a. Indole test b. Methyl red test c. Voges - Proskauer test d. Citrate utilisation test
4. Odour from colony	4. Urea hydrolysis
5. Gram stain	5. Triple sugar iron test
6. Motility	6. Oxidative – Fermentative test
	7. Decarboxylation tests a. Lysine decarboxylase test b. Ornithine decarboxylase test c. Arginine dehydrolase test

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out by using Modified Kirby-Bauer Disc Diffusion methods using commercially available discs (Himedia). Results were interpreted according to zone sizes as per Clinical & Laboratory Standards Institute (CLSI) 2022- M100 guidelines.

Saline suspension of the isolate was prepared from an overnight incubated agar plate (a nonselective medium blood agar was used) with turbidity matching to 0.5 McFarland turbidity. Muller Hinton Agar (MHA) plate was inoculated within 15 minutes after the inoculum had been adjusted. A sterile cotton swab was dipped into the suspension, rotated several times and gently pressed onto the inside wall of the tube above the fluid level to remove excess inoculum from the swab.

The swab was streaked across the entire surface of the agar plate three times, rotating the plate approximately 60° each time to ensure even distribution of the inoculum, and a final sweep around the agar rim was made. With the help of a sterile forceps, selected antimicrobial discs were applied evenly onto the agar plate. Each disc was pressed down to ensure complete contact with the agar surface. Plate was incubated at 35°C-37°C for overnight incubation. On the next day, zone of inhibition was calculated for each drug and interpreted as sensitive, intermediate or resistant according to CLSI 2022 guidelines. Commercially available disc (Himedia) with known potency were used. The antibiotics used for susceptibility testing were-

Table 2: Drugs Used for Antimicrobial Susceptibility Testing

Drug Class	Drug
Aminoglycosides	Amikacin
	Gentamicin
	Tobramycin
Beta Lactams	Ampicillin - Sulbactam
	Aztreonam

	Cefepime
	Ceftazidime
	Imipenem
	Meropenem
	Piperacillin - Tazobactam
Fluoroquinolones	Ciprofloxacin
	Levofloxacin
Sulphonamides	Cotrimoxazole
Tetracyclines	Minocycline
	Tetracycline
Nitrofurans	Nitrofurantoin

Sensitivity testing was done with control strains using standard American Type Culture Collection (ATCC) bacteria Staphylococcus aureus ATCC 25923, E. coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumoniae ATCC 700603 and Methicillin resistant Staphylococcus aureus (MRSA) ATCC 43300. Quality control for MHA was tested by E. faecalis ATCC 29212 as a general quality control laboratory procedure.

DATA ANALYSIS

All the data collected was entered in excel spreadsheet and analyzed using SPSS version 21 software. Chi square test was used to study associations. P<0.05 was considered as significant.

RESULTS

The present study entitled 'BACTERIOLOGICAL PROFILE OF NON- FERMENTING GRAM NEGATIVE BACILLI FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL' was performed on various clinical samples received from various wards and OPDs in a tertiary care hospital from January 2021 to June 2022. Out of 19316 clinical specimens, 1227 NFGNB were isolated, yielding a 6.35% isolation rate.

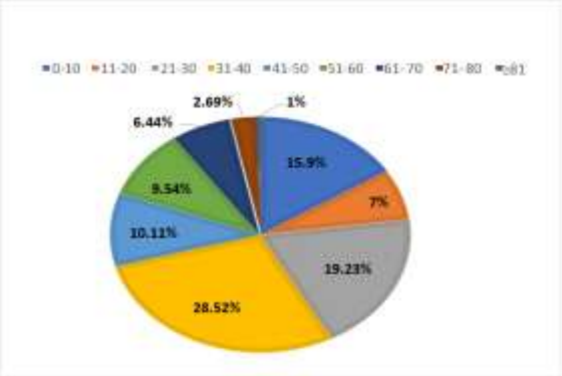

Chart 1: Age-wise Distribution of NFGNB

The age group 31- 40 accounted for 28.52 % (n=350) which was the highest percentage followed by age group 21-30 with 19.23 % (n=236).

Table 1: Gender-wise Distribution

Gender	No. of Isolates (n)	Percentage
Male	736	59.98
Female	491	40.02
Total	1227	100

Above table is showing, out of 1227 samples isolated, 736 (59.98 %) were males and 491 (40.02 %) were females. Male to female ratio was 3:2. Following Chart 5 depicts Gender-wise distribution of NFGNB.

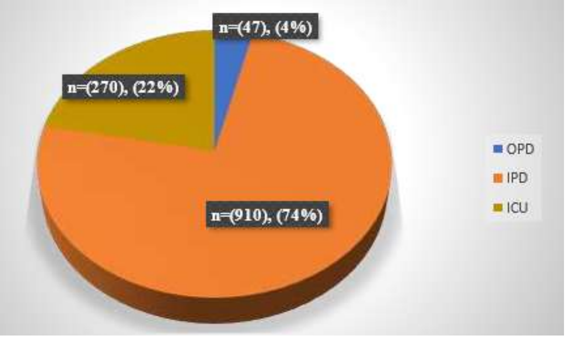

Chart No: 2 Patient Location-wise Distribution

Above figure shows 47 NFGNB were isolated from OPD patient. From IPD, highest NFGNB were isolated from surgery ward accounting for 528 and from ICU, 189 were isolated from MICU.

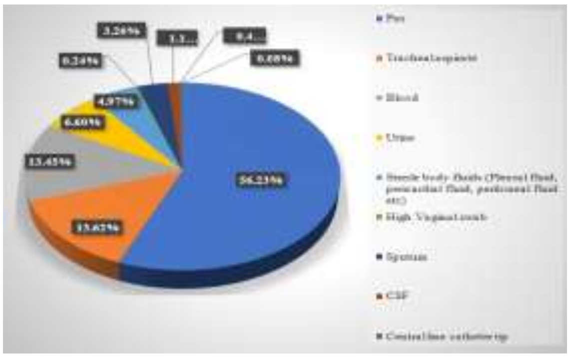

Chart no. 3: Sample wise Distribution

The above figure shows Pus was the commonest sample from which NFGNB were isolated accounting for 56.23 % followed by tracheal aspirate (13.62 %), blood (13.45 %), urine (6.60%).

Table 2: Speciation of NFGNB

Species	No. of Isolates (n)	Percentage
Pseudomonas aeruginosa	720	58.68
Acinetobacter baumannii	477	38.86
Stenotrophomonas maltophilia	22	1.79
Acinetobacter lwoffii	5	0.41
Burkholderia cepacia	2	0.16
Sphingomonas paucimobilis	1	0.08
Total	1227	100

Above table shows Pseudomonas aeruginosa was the commonest isolate 720 (58.68 %) followed by Acinetobacter baumannii 477 (38.86 %) and Stenotrophomonas maltophilia 22 (1.79 %)

The above figure shows P. aeruginosa was most commonly isolated from pus sample (n=481) followed by tracheal aspirate (n=80) and blood (n=65). A. baumannii was isolated most frequently from pus (n=196), blood (n=90) and tracheal aspirate (n=87). Most of the S. maltophilia were isolated from pus (n=10) followed by blood (n=6). [Chart 4]

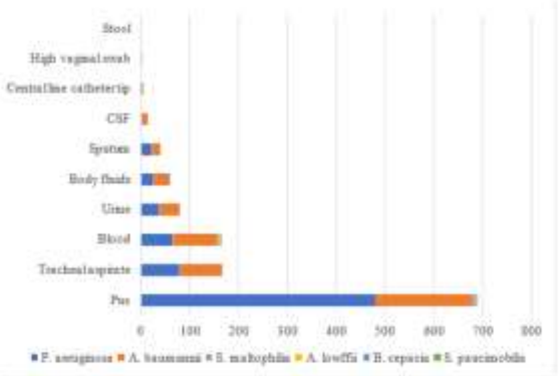

Chart no: 4 Specimen-wise Distribution of Each NFGNB

Table 3: Organism-Wise Antimicrobial Susceptibility Pattern
Pseudomonas aeruginosa (n = 720)

Test / Report Group	Drug	S	%S	I	%I	R	%R
Group A	Ceftazidime	326	45.28%	17	2.36%	368	51.11%
	Gentamicin	347	48.19%	20	2.78%	353	49.03%
	Tobramycin	365	50.69%	14	1.94%	341	47.36%
	Piperacillin–Tazobactam	406	56.39%	53	7.36%	233	32.36%
Group B	Amikacin	373	51.81%	36	5.00%	311	43.19%
	Aztreonam	328	45.56%	165	22.92%	227	31.53%
	Cefepime	332	46.11%	38	5.28%	350	48.61%
	Ciprofloxacin	297	41.25%	31	4.31%	392	54.44%
	Levofloxacin	196	27.22%	54	7.50%	470	65.28%
	Imipenem	346	48.06%	73	10.14%	301	41.81%
	Meropenem	428	59.44%	32	4.44%	260	36.11%
Group U	Nitrofurantoin (n = 38)	1	2.63%	0	0.00%	37	97.37%

Acinetobacter baumannii (n = 477)

Test / Report Group	Drug	S	%S	I	%I	R	%R
Group A	Ampicillin–Sulbactam	188	39.41%	64	13.42%	225	47.17%
	Ceftazidime	88	18.45%	12	2.52%	377	79.04%
	Ciprofloxacin	117	24.53%	12	2.52%	348	72.96%
	Imipenem	93	19.50%	16	3.35%	368	77.15%
	Meropenem	142	29.77%	31	6.50%	304	63.73%
	Gentamicin	139	29.14%	8	1.68%	330	69.18%
	Tobramycin	160	33.54%	15	3.14%	302	63.31%
Group B	Amikacin	146	30.61%	16	3.35%	315	66.04%
	Piperacillin–Tazobactam	144	30.19%	24	5.03%	309	64.78%
	Cefepime	105	22.01%	24	5.03%	348	72.96%
	Minocycline	241	50.52%	49	10.27%	187	39.20%
	Cotrimoxazole	127	26.62%	12	2.52%	338	70.86%
Group U	Tetracycline (n = 41)	10	24.39%	1	2.44%	30	73.17%

Stenotrophomonas maltophilia (n = 22)

Report Group	Drug	S	%S	I	%I	R	%R
Group A	Minocycline	8	36.36%	10	45.45%	4	18.18%
	Cotrimoxazole	7	31.82%	0	0.00%	15	68.18%
Group B	Ceftazidime	7	31.82%	3	13.64%	12	54.55%

Acinetobacter lwoffii (n = 05)

Report Group	Drug	S	%S	I	%I	R	%R
Group A	Ampicillin–Sulbactam	5	100.00%	0	0.00%	0	0.00%
	Ceftazidime	2	40.00%	0	0.00%	3	60.00%
	Ciprofloxacin	3	60.00%	0	0.00%	2	40.00%
	Imipenem	4	80.00%	1	20.00%	0	0.00%

	Meropenem	5	100.00%	0	0.00%	0	0.00%
	Gentamicin	4	80.00%	0	0.00%	1	20.00%
	Tobramycin	5	100.00%	0	0.00%	0	0.00%
Group B	Amikacin	3	60.00%	0	0.00%	2	40.00%
	Piperacillin–Tazobactam	5	100.00%	0	0.00%	0	0.00%
	Cefepime	4	80.00%	0	0.00%	1	20.00%
	Minocycline	5	100.00%	0	0.00%	0	0.00%
	Cotrimoxazole	2	40.00%	0	0.00%	3	60.00%
Group U	Tetracycline (n = 1)	0	0.00%	0	0.00%	1	100.00%

Burkholderia cepacia (n = 2)

Report Group	Drug	S	%S	I	%I	R	%R
Group A	Meropenem	2	100.00%	0	0.00%	0	0.00%
	Imipenem	2	100.00%	0	0.00%	0	0.00%
	Cotrimoxazole	1	50.00%	0	0.00%	1	50.00%
Group B	Ceftazidime	1	50.00%	1	50.00%	0	0.00%
	Minocycline	1	50.00%	0	0.00%	1	50.00%

Sphingomonas paucimobilis (n = 1)

Report Group	Drug	S	%S	I	%I	R	%R
Group A	Ceftazidime	1	100.00%	0	0.00%	0	0.00%
	Gentamicin	1	100.00%	0	0.00%	0	0.00%
	Tobramycin	1	100.00%	0	0.00%	0	0.00%
	Amikacin	1	100.00%	0	0.00%	0	0.00%
Group B	Cefepime	1	100.00%	0	0.00%	0	0.00%
	Ciprofloxacin	1	100.00%	0	0.00%	0	0.00%
	Imipenem	1	100.00%	0	0.00%	0	0.00%
	Meropenem	1	100.00%	0	0.00%	0	0.00%
	Piperacillin-Tazobactam	1	100.00%	0	0.00%	0	0.00%
	Cotrimoxazole	1	100.00%	0	0.00%	0	0.00%
Group U	Tetracycline (n=0)	0	0.00%	0	0.00%	0	0.00%

59.44% isolates of P. aeruginosa were sensitive of Meropenem followed by Piperacillin-Tazobactam (56.39%), Amikacin (51.81%) and Tobramycin (50.69%) while it was significantly resistant to ciprofloxacin (54.44%) and levofloxacin (65.28%). P. aeruginosa was isolated from 38 samples. Nitrofurantoin was tested in these samples which showed only 2.63% sensitivity to nitrofurantoin.

A. baumannii was resistant to most of the drugs during the study. A. baumannii showed 50.52% sensitivity to minocycline followed by Ampicillin – Sulbactam (39.41%) and significant resistance was seen to Cefepime (72.96%), Imipenem (77.15%), Ceftazidime (79.04%). A total 41 urine sample showed growth of A. baumannii. Tetracycline was tested for urine samples. A. baumannii showed very high resistance to tetracycline also which was 73.17%.

S. maltophilia also showed very high-level resistance to of the drugs tested. In group A, Minocycline and Cotrimoxazole were tested which showed sensitivity 36.36% and 31.82% respectively only. In group B, Ceftazidime was tested with sensitivity 31.82%.

Isolates of A. lowffii were sensitive to most of the drugs. In A. lowffii, sensitivity to meropenem was more than imipenem. It was 100% sensitive to Ampicillin- Sulbactam, Meropenem, Tobramycin, Piperacillin-Tazobactam and Minocycline. Resistance was seen more in Cotrimoxazole (60%). One isolate of A. lowffii was also resistant to Tetracycline.

B. cepacia was 100% sensitive to group A drugs like Meropenem and Imipenem while 50% sensitive to group A drug, Cotrimoxazole and group B drugs, Ceftazidime and Minocycline. S. paucimobilis showed 100% sensitivity to all the drugs tested like Ceftazidime, Gentamycin, Tobramycin, Amikacin, Cefepime, Ciprofloxacin, Imipenem, Meropenem, Piperacillin- Tazobactam, Cotrimoxazole. For A. lowffii (n=5), B. cepacia (n=2), S. maltophilia (n=1), the isolates isolated in our study were very less so it is too preliminary to comment on their resistance pattern.

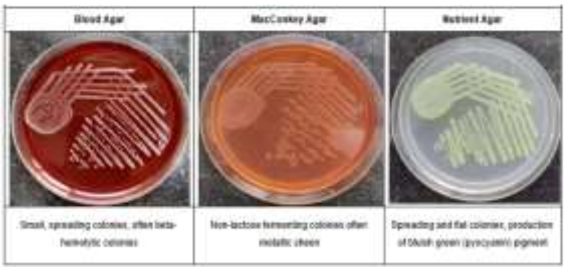

Figure 3: Pseudomonas aeruginosa

Figure 5: Pseudomonas aeruginosa

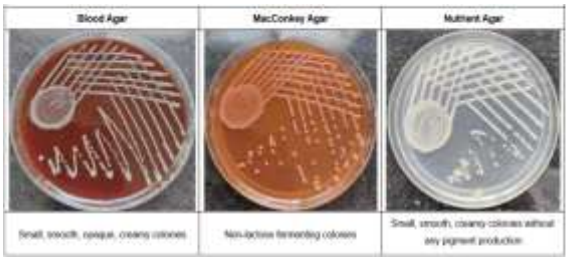

Figure 7: Acinetobacter baumannil

Figure 8: Acinetobacter baumannil

DISCUSSION

Over the past few years, clinical specimens showed increased isolation of Nonfermenting gram negative bacilli (NFGNB). NFGNB are also showing treatment failure due to emergence of multidrug resistance strains. This increases the economic burden, length of hospital stays, mortality and morbidity. As a result, we aimed to isolate commonly encountered, clinically significant NFGNB from clinical specimens, as well as their antimicrobial susceptibility pattern.

ISOLATION RATE

During a study of one and half year (January 2021 to June 2022), 1227 clinical isolates of NFGNB were isolated from various clinical samples. Previous studies showed varied isolation rate of NFGNB. Isolation rate of NFGNB in our study was found to be 6.35% which is in agreement with the study conducted by Madkey and Gajbhiye, et al,[16] where isolation rate was 5.19% and in study conducted by Malini, et al. isolation rate was 4.5%.[2]

Our study is not in agreement with study conducted by Nautiyal S et al. (16.18%),[11] where high isolation rate was reported. These variations in isolation rate might be because of poor hospital infection control practices and difference in patient population.

AGE WISE DISTRIBUTION

In our study, the age group 31-40 years showed the highest percentage of isolation which was 28.52% (n=350) followed by age group 21-30 years with 19.23% (n=236) isolation rate. Only 7 patients were ≥81 years of age. This finding was similar to finding of study conducted by Dr. Thipperudraswamy. T, et al,[17] where 42.92% patients were belonging to 31-40 years of age. But it is contradictory with the study carried out by Shah Vaghela, et al,[7] which showed most of the NFGNBs were isolated from age group 15-29 years. The study conducted by Gomathi Maniyan et al,[4] was showing maximum isolation was from <10 years of age.

GENDER WISE DISTRIBUTION

In our study males (59.98%) were more commonly affected than females (40.02%). Male to female ratio was 3:2. Similar observation was seen in study conducted by Madkey and Gajbhiye, et al,[16] where 56.36% males were affected and 43.64% females were affected. Study conducted by Benachinmardi, et al,[4] also showed males (n=68) were more affected than females (n=32). While study of Prasanna, et al,[13] was showing the opposite findings where females (56%) are more commonly affected than males (44%).

LOCATION WISE DISTRIBUTION:

In this present study, majority of isolates were from surgical ward (n=528) followed by Medicine Intensive Care Unit (n=189) and medicine ward (n=122). This finding was similar to study done by Gomathi Maniyan et al,[4] where 29.09% isolates were from surgery ward, 20.01% from ICU and 14.55% from medicine ward. Study of Prasanna, et al,[13] was also showing most of the samples were received from surgery ward (30%). Study conducted by Benachinmardi, et al,[3] showed NFGNB were isolated more from IPD patients than OPD patients.

Maximum isolation from the surgery ward was due to admission of patients with road traffic accidents, wound infection, burn patients. Surgery ward also showed more admission of patients suffering from diabetic foot. Diabetes made patients more to NFGNB infections. Isolation of NFGNB from ICU patients was greater due to immunosuppression of patients, greater invasive procedures, breach in normal defence mechanisms and more importantly use of contaminated fluids or life support equipment. These bacteria have tendency to grow and survive in the commonly used disinfectants and antiseptics resulting in infection of ICU patients.

SAMPLE WISE DISTRIBUTION

Non fermenters isolated from pus were 609 followed by tracheal aspirate (167), blood (165), wound swab (81) and urine (81), body fluids (81), sputum (40), CSF (14), central line tip (5), vaginal swab (3) and a single sample of stool. In our study, pus was the most common sample followed by tracheal aspirate.

This finding was parallel to study conducted by Benachinmardi, et al,[3] showed the most common specimen was pus (21) followed by tracheal aspirate (16) while this finding was not consistent with study of Madkey and Gajbhiye, et al,[16] which showed majority of NFGNB were isolated from tracheal aspirate (28.57%) followed by pus (6.37%). A study conducted in Belgaum in 2007 by Gokale and Metgud, et al,[1] and another study conducted by Malini, et al,[2] in 2009 in Kolar, Karnataka, also showed maximum number NFGNB were isolated from pus samples 58.4% and 62.2% respectively. Study of Mahajan, et al,[5] showed 59.96% isolates were from pus. Some studies showed urine was the common sample for NFGNB isolation.

Study conducted by Prasanna, et al,[13] showed sputum (30%) was predominant sample followed by urine (23.3%) while study of Susmitha Simgamsetty, et al,[6] showed blood (28.4%) was predominant sample followed by tracheal aspirate (23.7%). Study of Bose, et al,[8] showed maximum number of isolates were from blood (28.12%), pus (26.25%) and urine (18.12%).

Table 4: Isolation rate of P. aeruginosa and A. baumannii

Authors	Year	P. aeruginosa (%)	A. baumannii (%)
Present study	2021-2022	58.68	38.86
Gokhale and Metgud, et al.[1]	2012	82.3	16
Malini, et al.[2]	2005-2006	53.8	22.2
Benachinmardi, et at.[3] 3	2013	60	22
Ruchita Mahajan, et al.[5]	2016	54.54	41.08
Juyal, et al.[15]	2012	38.21	29.27

In some studies A. baumannii was the predominant isolate followed by P. aeruginosa. Studies conducted by Shah and Vaghela, et al,[7] (A. baumannii 54%, P. aeruginosa 42%), Madkey and Gajbhiye, et al,[16] (A. baumannii 56.82%, P. aeruginosa 40.92%) were showing predominance of A. baumannii.

In our study, P. aeruginosa (58.68%) and A. baumannii (38.86%) were the most common followed by S. maltophilia (1.79%) and A. lwoffii (0.41%). This finding is consistent with the study of Sonia Deb, et al,[18] where P. aeruginosa (52.8%) was followed by A. baumannii (39.3%), S. maltophilia (3.37%) and A. lwoffii (2.24%). Prevalence of particular bacteria may vary from one region to another and from time to time.

ANTIMICROBIAL SUSCEPTIBILITY PATTERN:

P. aeruginosa- 59.44% isolates of P. aeruginosa were sensitive of Meropenem in our study. Similar finding was seen in study of Jitendra, et al,[9] and Susmitha Simgamsetty, et al,[6] where sensitivity to Meropenem was 53% and 68.1% respectively. Study of Gomathi Maniyan et al,[4] showed 79.6% sensitivity.

In our study, the sensitivity rate for Piperacillin – Tazobactam was 56.39%. This finding was concordant with findings of Jitendra, et al,[9] (57%), Ruchita Mahajan, et al,[5] (62.85%), Juyal, et al,[15] (69.15%).

The sensitivity rate of Amikacin in our study was 51.81%. Similar results are seen in Gomathi Maniyan et al,[4] (59.3%). The sensitivity rate in Bose, et al,[8] was very low, 21.87% while highest sensitivity rate was seen in Sarkar M, et al,[19] 83.24%. Ceftazidime showed resistance almost in all studies. In our study sensitivity rate was 45.28%. Other studies with lower sensitivity rate are Bose, et al,[8] (5.72%), Juyal, et al,[15] (28.72%).

A. baumannii- A. baumannii isolated in our study were found to be multidrug resistant. They were extremely resistant to Ceftazidime (79.03%), Imipenem (77.14%), Cefepime (72.95%), Ciprofloxacin (72.95%) and Cotrimoxazole (70.85%) and Gentamicin (69.18%). This resistance pattern was similar to study of Nautiyal S, et al.[11] where resistance pattern was Gentamicin (85%), Ceftazidime (74%), Cotrimoxazole (83%).

S. maltophilia- S. maltophilia in our study showed very low sensitivity rate to antibiotics. It is 36.36% sensitive to Minocycline, 31.82% sensitive to Cotrimoxazole and 31.82% to Ceftazidime. Study of Madkey and Gajbhiye, et al,[16] showed 50% sensitivity to Minocycline and 83.33% to Cotrimoxazole. Study of Ruchita Mahajan, et al,[5] showed 26.6% sensitivity to Ceftazidime and 80% to Cotrimoxazole.

A. lowffii- Present study was showing that A. lowffii was 100% sensitive to Ampicillin–Sulbactam, Meropenem, Tobramycin, Piperacillin-Tazobactam and Minocycline while 80% sensitive to Imipenem, Gentamicin, Cefepime followed by Ciprofloxacin and Amikacin each 60%. The similar finding was seen in study of Gomathi Maniyan et al,[4] which showed 100% sensitivity to Imipenem, Meropenem, Piperacillin-Tazobactam while 80% sensitive to Ceftazidime and Amikacin and 50% sensitive to Ciprofloxacin, Gentamicin and Cotrimoxazole. Also study of Jitendra, et al,[9] showed similar sensitivity pattern. Susmitha Simgamsetty, et al,[6] study showed A. lowffii was 75% sensitive to Amikacin, Imipenem and Ciprofloxacin.

B. cepacia- Two isolates of B. cepacia were found in study. It was almost 100% sensitive Meropenem and Imipenem while 50% sensitive to Cotrimoxazole, Ceftazidime and Minocycline. This finding was similar to study of Susmitha Simgamsetty, et al,[6] where B. cepacia was 100% sensitive to Meropenem, Doripenem and Tigecycline. Study of Madkey and Gajbhiye, et al,[16] also showed B. cepacia was 100% sensitive to Ceftazidime and Meropenem, 75% sensitive to Cotrimoxazole and 50% sensitive to Minocycline. Study of Gokhale and Metgud, et al,[1] showed B. cepacian was 100% sensitive to all drugs.

S. paucimobilis- One isolate of S. paucimobilis was isolated in our study which showed sensitivity to all the drugs tested. Study of J.N. Lin, et al,[20] also showed 81.3% sensitive to Ciprofloxacin, Imipenem, Piperacillin – Tazobactam while 75% sensitive and Gentamicin, Amikacin. Study of Nuri Bayram, et al,[21] showed 79.1% sensitive to

Ceftazidime and 91.7% sensitive to Amikacin.

CONCLUSION

P. aeruginosa and A. baumannii were the commonest NFGNB isolated in our study from various clinical samples. Meropenem sensitivity was high in P. aeruginosa, while Levofloxacin sensitivity was low. A. baumannii was multidrug resistant. It showed 50.52% sensitivity to Minocycline and least susceptible to Ceftazidime. This study also gives an alarming signal of high prevalence of multidrug resistant NFGNB.

NFGNB are significant bacteria that cause a variety of nosocomial infections despite being considered contaminants. They are emerging as important opportunistic pathogens. It is important to practice infection control, manage biomedical waste, and perform routine environmental cleaning, sterilisation, and disinfection of various instruments and devices used in patient care. Hand hygiene should be performed when one comes in contact with patients, their secretions and the environment.

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