Section: Microbiology

Original Research Article

BACTERIOLOGICAL PROFILE OF NONFERMENTING GRAM-NEGATIVE BACILLI CAUSING INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

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Abstract

Background: The aim is to determine the proportion and the antibiotic susceptibility pattern of Nonfermenting Gram-negative bacilli(NFGNB) from clinical samples of immunocompromised patients. To compare the antibiogram of Pseudomonas aeruginosa and Acinetobacter baumannii with the state antibiogram by Kerala Antimicrobial Resistance Surveillance Network(KARS-NET). **Materials and Methods:** The study was conducted in the Department of Microbiology at a Tertiary Care Teaching Hospital for a period of 5 months. NFGNB isolated from clinical samples were identified by standard procedures and antibiotic susceptibility test was performed. **Result:** Out of this 1231 samples, isolation rate of significant NFGNBs was7.15%. Risk factors were chronic kidney disease (10%) followed by diabetes mellites (8.89%) and patients on immunosuppressive drugs (4.5%). In our study 60.2%of the NFGNBs were from pus aspirates,25% from the blood samples followed by others. Among the isolates 60.2 % of the NFGNBs were from ICU patients. NFGNBs isolated in our study was Pseudomonas aeruginosa (47.7%) followed by Acinetobacter baumannii (40.9%) Burkholderia cepacia complex (5.6%), Burkholderia pseudomallei(2.2%) Achromobacter xyloxidans(1.1%), Elizabethkingia meningoseptica (1.1%), and Stenotrophomonas maltophilia (1.1%) . P.aeruginosa was the predominant isolate from pus aspirate and urine samples . Among the blood and tracheal aspirate Acinetobacter baumannii was the predominant NFGNB. The only one isolate from CSF was Elizabethkingia meningoseptica. Burkholderia pseudomallei were isolated from aspirated pus from liver and spleen absscess. Achromobacter xyloxidans was from pulmonary abscess. Pseudomonas aeruginosa were most sensitive to Meropenem (95%), Tobramycin (88%), Piperacillin tazobactam(81%) & Ceftazidime(74%). Acinetobacter baumannii were sensitive to Minocycline (67%) Cotrimoxazole (56%),Amikacin(47%) and Meropenem (42%).Most of the antibiotics showed similar sensitivity when it was compared to state antibiogram. **Conclusion:** As NFGNBs have emerged as an important group of organisms responsible for health care associated infections(HAIs), their identification to the species level is necessary. Maintenance of a high quality of infection control practices is very important in the control of these microbes.

INTRODUCTION

Non-fermenting, gram-negative bacilli (NFGNB) are described as non-sporulating group of microbes that rely on oxidative pathways because they are unable to get energy from carbohydrates by fermentation. They are organisms with a low level of virulence that seldom cause disease in healthy individuals. However, they may cause severe infections in hospitalized and immunocompromised patients. NFGNB are known to account for 15% of all bacterial isolates from clinical specimens.[1] They can tolerate harsh environmental conditions, show remarkable resistance to antimicrobials, and are frequently described as hospital-acquired opportunistic pathogens.[2–4] Multidrug resistance of these organisms stems from different factors, such as up-regulated production of enzymes metabolizing the drugs, target site changes and over expression of efflux pumps.[5,6] Inherent resistance of these

bacterial agents to commonly used disinfectants and tendency to colonize various surfaces helps in their emergence as important nosocomial pathogens.[7]

Pseudomonas aeruginosa and Acinetobacter baumannii are the most commonly isolated non-fermenters. They emerged as important nosocomial pathogens that mainly infects critically ill patients. However, cases of community-acquired infections which are usually associated with preexisting conditions such as old age, diabetes, cancer, obstructive pulmonary disorders etc. have also been reported.[8] The Centres for Disease Control and Prevention (CDC) has included carbapenem-resistant Acinetobacter and Multidrug-resistant Pseudomonas aeruginosa in the category of 'urgent threat', thus calling for increased surveillance and prevention activities to manage these pathogens.[9]

Area-specific prevalence studies aimed at gaining knowledge about different non-fermenters and their resistance pattern may help clinicians to choose the correct empirical treatment. In view of this, the present study aims to identify the different non-fermenters causing significant infections in immunocompromised patients and their antibacterial susceptibility and alsoto compare the antibiogram of Pseudomonas aeruginosa and Acinetobacter baumannii with the state antibiogram by Kerala Antimicrobial Resistance Surveillance Network (KARS-NET) at a tertiary care centre in south India.

MATERIALS AND METHODS

This prospective study was conducted in the Department of microbiology at a tertiary care centre from May 2021 to September 2021 after Institutional Review board approval IRB No 3/2021. A total of 1231 clinical samples including exudate, blood, csf, urine ad tracheal aspirate from the immunocompromised patients admitted in Intensive care units and various wards of the hospital were included in this study. [Table 1 &2] Samples were received and processed as per standard laboratory techniques at the department of microbiology. They were plated on blood agar and MacConkey agar and incubated at 37°C for 18–24 h under aerobic conditions. Appropriate biochemical tests were done to identify the organisms. Organisms showed growth on triple sugar iron agar and producing an alkaline reaction were provisionally considered as NFGNB, and further identified using a standard laboratory protocol. Characters assessed include morphology, motility, oxidase, catalase, indole, urease, nitrate, citrate tests and oxidation-fermentation reactions of glucose, lactose, DNase, 10% Lactose lysine and ornithine decarboxylase, arginine dihydrolase tests.

Clinical significance of the NFGNBs were assessed by using presence of pus cells along with gram negative bacilli in the direct microscopy, repeated isolation of the organism, leucocytosis, relevant radiological, blood investigations, clinical features.

Antibiotic susceptibility testing (ABST) were performed by the Kirby–Bauer disc diffusion method using commercially available discs on Mueller–Hinton agar. Vitek 2 compact was also used for identification and susceptibility testing. The different antibiotics tested were ceftazidime 30 µg, piperacillin tazobactam100/10 µg, ciprofloxacin 5 µg, amikacin 30 µg, tobramycin, levofloxacin, cotrimoxazole 1.25/23.75 µg, meropenem 10 µg, doxycycline & minocycline. ABST results were interpreted according to the Clinical and Laboratory Standards institute (CLSI) guidelines except for Burkholderia pseudomallei. For Burkholderia psedomallei EUCAST guidelines were used. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains.

RESULTS

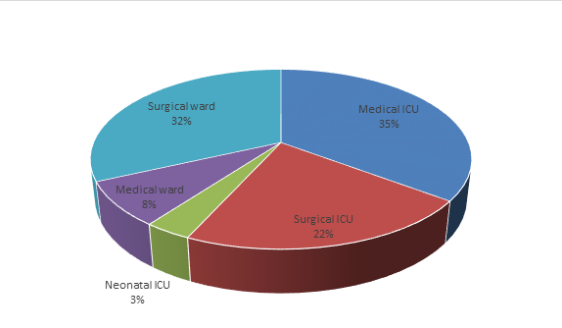

Figure 1: NFGNBs in Different ICUs/Wards)

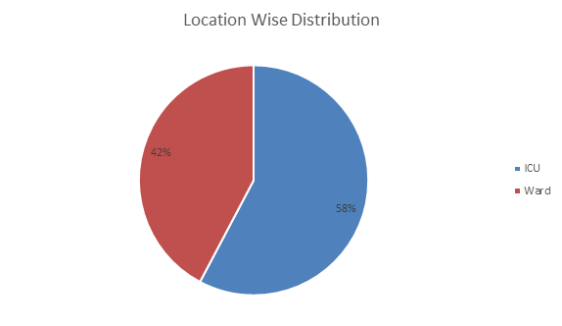

Figure 2: Location wise distribution of NFGNBs

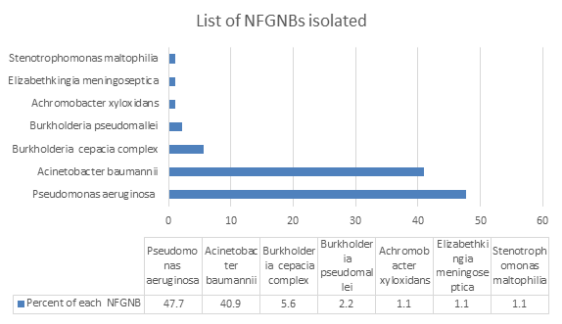

Figure 3: Species wise distribution of NFGNBs

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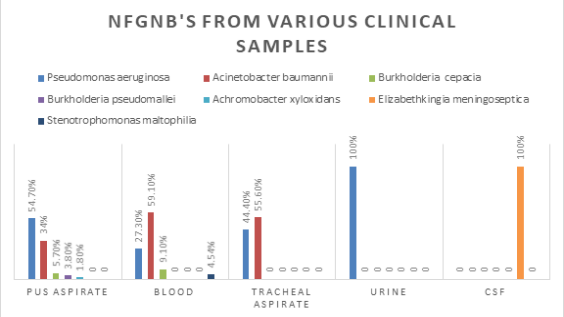

Figure 4: Isolation rate of NFGNBs from various clinical samples

Table 1: Sample wise distribution of NFGNB.

Sample	Number (percent) of NFGNB isolated
Blood(N=450)	22(25%)
Pus aspirate(N=401)	53(60.2%)
Urine(N=298)	3(3.4%)
CSF(N=52)	1(1.2%)
Endotracheal aspirate(N=30)	9(10.2%)
Total (N=1231)	88(100%)

Table 2: Distribution of NFGNBs among the study population

Risk groups included in the study	NFGNB isolated	NFGNB isolated
	Number	Percent
Diabetes mellites (N= 686)	61	8.89%
On immunosuppressive drugs (N= 200)	9	4.5%
New born (N=200)	3	1.5%
Chronic kidney disease (N= 50)	5	10%
Chronic liver disease (N= 46)	1	2.2%
Coronary artery disease(N=24)	1	4.16%
Multiple risk factors(N=25)	8	32%
Total	1231	

Table 3: Antibiotic sensitivity of NFGNBs Isolated

Antibiotic sensitivity of isolates number(percent)											
Isolate	**Ceftazidime N(%)**	**Ciprofloxacin N (%)**	**Gentamicin N(%)**	**Amikacin N(%)**	**Tobramycin N(%)**	**Piperacillin + Tazobactam N(%)**	**Meropenem N(%)**	**Minocycline N(%)**	**Levofloxacin N(%)**	**Doxycycline N(%)**	**Cotrimoxazole N(%)**
P.aeruginosa (N=42)	31(74)	22(52)	NA	36(86)	37(88)	34(81)	40(95)	NA	NA	NA	NA
A.baumannii(N=36)	13(36)	6(17)	16(44)	17(47)	NA	14(39)	15(42)	24(67)	13(36)	NA	20(56)
B.cepacia (N=5)	3(60%)	NA	NA	NA	NA	NA	5(100)	4(80)	NA	NA	59100)
B.pseudomallei(N=2)	2(100)	NA	NA	NA	NA	NA	2(100)	NA	NA	2(100)	2(100)
A.xyloxidans(N=1)	1(100)	1(100)	NA	NA	NA	1(100)	1(100)	NA	NA	NA	1(100)
E.meningoseptica (N=1)	NA	1(100)	NA	NA	NA	1(100)	NA	1(100%)	NA	NA	1(100)
S.maltophilia(N=1)	NA	NA	NA	NA	NA	NA	NA	NA	1(100)	NA	1(100)

Table 4: Comparison of the current Study with Indian Studies.

Author	Region	Year	Isolation rate of NFGNB	Most common NFGNBs isolated	carbapenem resistant A. baumanni	Carbapenem resistant P.aeruginosa
Rajesh Bansal et al,[10]	Rajasthan	1st July 2018 to June 2019.	7.84%	P.aeruginosa (47.88%) A.baumannii (38.09%)	19.4%	15%
Abhishekmehta et al,[11]	Madhya Pradesh, India	October 1 2018, to April 30, 2020	8.2%	P.aeruginosa (50%) A.baumannii (23%)	17.4%	16%

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Rajeev Kumar et al,[12]	Gujarat, India	6 month from January 2015 to may 2015.	6.9%	P.aeruginosa(56.77%), A. baumanii (36.97 %)	26.7%	6.4%
Mandira Sarkar et al,[14]	Odisha	January 2015 to October 2016	13.18%	A. baumannii (51.34%) P.aeruginosa (42.09%),	43.6%	34.6%
Ranjan Kumar et al [15]	Bihar	January 2022 to December 2022 (1 year)	15.4%	A.baumannii(48.78%) P.aeruginosa(37.71%)	3.8%	3.8%
Mitisha Soni et al,[16]	bhopal	January 2021 to July 2022	10%	P. aeruginosa (51.7%), A. baumannii (23.4%)	60%	43.4%
Veena Manjunath et al,[17]	Karnataka	December 2020–May 2022.	28.3%	P. aeruginosa (63.6%) A. baumannii. (36.3%)	19.4%	15%
Kalidas Rit et al,[13]	Kolkata	July 2011 to June 2012.	12.18%	Pseudomonas aeruginosa (50.24%) Acinetobacter baumannii (24.87%	10%	8.92%
Amandeep Kaur et al,[18]	Punjab	2018	16.1%	P. aeruginosa(52.6%) A. baumannii(31.7%).	68.1%	35.5%
Kirtilaxmi K. Benachinmardi et al,[19]	Karnataka,	2013	3.58%	P. aeruginosa(60%) A. baumannii(22%)	42.86%	20%
Malini et al,[20]	Karnataka	2009	4.5%	Pseudomonas aeruginosa (53.8%), Acinetobacter baumannii (22.2%)	0%	5.8%
Present study	Kerala	2021	7.15%	P. aeruginosa (47.7%) A. baumannii(40.9%).	58%	5%

The clinical specimen included in our study were blood 450(36.5%), Pus aspirate 401 (32.5%), Urine 298 (24.2%), CSF 52(4.2%), and Endotracheal aspirate 30 (2.4%) from immunocompromised patients. [Table 1] Out of this 1231 samples, the significant NFGNBs isolated were 88, accounting for an isolation rate of 7.15%. We have observed that rate of isolation of NFGNBs were more from patients diagnosed with chronic kidney disease (10%) followed by diabetes mellites (8.89%) and patients on immunosuppressive drugs (4.5%) [Table 2]. In our study 60.2%of the NFGNBs were isolated from pus aspirates,25% from the blood samples followed by others [Table 1]. In our study 31(35.2%) of NFGNBs were isolated from medical ICU followed by surgical ward 28(31.8%) and Surgical ICU 19(21.5%).[Figure 1] Among the isolates 60.2 % of the NFGNBs were from ICU patients compared to ward (39.8%). [Figure 2] The most common NFGNB isolated in our study was Pseudomonas aeruginosa (47.7%) followed by Acinetobacter baumannii (40.9%) Burkholderia cepacia complex (5.6%), Burkholderia pseudomallei (2.2%) Achromobacter xyloxidans (1.1%), Elizabethkingia meningoseptica (1.1%), and Stenotrophomonas maltophilia (1.1%). [Figure 3] Pseudomonas aeruginosa was the predominant isolate from pus aspirate (54.7%) and urine samples (100%). [Figure 4]

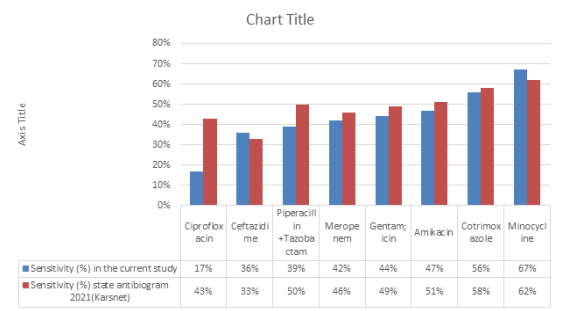

Figure 5: Comparison of antibiogram of Acinetobacter with Kerala state antibiogram (KARSNET)

Among the blood and tracheal aspirate Acinetobacter baumannii was the predominant NFGNB. The only one isolate from CSF was Elizabethkingia meningoseptica. Burkholderia pseudomallei (N=2) were isolated from pus aspirated from liver and spleen absscess. Achromobacter xyloxidans was from pulmonary abscess. Pseudomonas aeruginosa were most sensitive to Meropenem (95%), Tobramycin (88%),Piperacillin tazobactam(81%) and Ceftazidime(74%).[Table 4] Acinetobacter baumannii were sensitive to Minocycline (67%) Cotrimoxazole (56%), Amikacin (47%) and Meropenem (42%).[Table 3]

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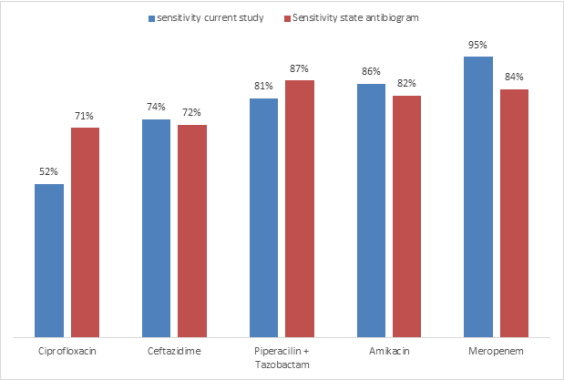

Figure 6: Comparison of antibiogram of Pseudomonas with Kerala state antibiogram (Karsnet)

DISCUSSION

Nonfermenting gram-negative bacilli are ubiquitous in the environment and were considered as contaminants or commensals. They have now emerged as important pathogens of healthcare-associated infections.

In the present study, the isolation rate of NFGNB from immunocompromised patients was 7.14 %. This was parallel to the results of the studies conducted by Rajesh Bansal et al, Abhishekmehta et al and Rajeev Kumar et al where isolation rates were 7.84 % ,8.2% and 6.9% respectively.[10-12] Most of the Indian studies showed high isolation rates compared to the current study.[13-18] In the studies conducted by Benanchinmardi et al. and Malini et al, the positivity of NFGNBs was 3.5% and 4.5%, respectively, which were very low compared to the present study.[19,20] The isolation rates in the present study is compared to few studies in [Table 4]. In a study from Nepal NFGNB isolation rate was 16%.[21] Regarding the risk factors chronic kidney disease, diabetes and immunosuppressive drugs were the most common , similar to the most of the studies.[10,14]

In our study 35.2% of NFGNBs were isolated from the medical ICU followed by the surgical ward (31.8%) and Surgical ICU (21.5%). However in other studies conducted by Rajesh et al and Rajeeev kumar et al we have observed the maximum percentage of NFGNBs was from surgical wards.[10,12]

Majority of the nonfermenter isolates in our study were from pus samples similar to other studies [10-13,16-20] But in some other Indian studies conducted by Mandira Sarkar et al ,Ranjan Kumar et al maximum isolation rate was from urine samples.[14,15] In a study conducted at Nepal, lower respiratory samples yielded maximum isolation.[21] we have also noted that positivity from blood samples (25%)s in our study was high compared to other studies.[10-20]

P. aeruginosa (47.7%) was the commonest non-fermenter in this study, followed by Acinetobacter baumanni (40.9%). This is in concordance with other studies and is depicted in [Table 4].[10-13,16-20] A.baumannii and Pseudomonas aeruginosa are the two most common ESKAPE organisms that pose a global threat to human health due to emerging and constantly increasing antibiotic resistance.[10-20] we have also observed that in the studies conducted by Mandira Sarkar et al Ranjan Kumar et al, Acinetobacter spp. was the most common isolate followed by P. aeruginosa.[14,15] Acinetobacter was the commonest isolate in a study conducted at NEPAL also.[21]

In the present study, Burkholderia cepacia complex, Burkholderia pseudomallei, Achromobacter xyloxidans, Elizabethkingia meningoseptica and Stenotrophomonas maltophilia were the rare but relevant nonfermenters which were isolated from the clinical samples. Few other studies conducted in India also reported similar findings.[10-20] It is evident that even though they were less common ,cause significant infections in the immunocompromised patients. Identification and monitoring of their susceptibility profiles are essential in proper management of these infections due to their variable sensitivity patterns.

Pseudomonas aeruginosa, the most common isolate in our study was more sensitive to Piperacillin Tazobactam (81%), Amikacin (86%) Tobramycin (88%) and Meropenem (95%). P.aeruginosa was found to be most susceptible to Meropenem(95%), which is similar to the findings of studies done by Rajeev Kumar et al , Ranjan Kumar et al, Kalidas Rit et al and Malini et al.[12,15,20,23] We have also noted that carbapenem resistance of Pseudomonas aeruginosa are very high in other Indian studies by Mandira Sarkar et al, Mitisha Soni et al and Amandeep Kaur et al.[14,16,18] Acinetobacter was more sensitive to PiperacillinTazobactam (39%), Gentamicin (44%), Amikacin (47%) and Meropenem (42%), Cotrimoxazole (56%) and Minocycline (67%). Studies conducted by Mandira Sarkar et al, Mitisha Soni et al, Amandeep Kaur et al and Kirtilaxmi K. Benachinmardi et al also demonstrated high carbapenem resistance similar to the present study.[14,16,18,19] Kerala was the first Indian state to implement an AMR containment plan called the Kerala Antimicrobial Resistance Strategic Action Plan (KARSAP) in 2018, and the state's first antibiogram report has been published in 2022.[22] Antibiogram of above two isolates are compared with Kerala state antibiogram 2021 by Kerala Antimicrobial Resistance Surveillance Network (KARS-NET). Most of the antibiotics showed similar sensitivity when it was compared to state antibiogram. [Figure 5 & 6]

CONCLUSION

These organisms have great potential to survive in hospital environment, so they are now emerged as main pathogens of Health Care Associated Infections.. Resistance pattern of these nosocomial pathogens shows wide variation not only from country to country but also within the same country over a period of time. Thus, it is very important that

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each hospital should have its own antibiotic policy based on the antibiogram. This will definitely helps the clinicians to start appropriate empirical therapy. Continuous surveillance and stringent infection control measures are key to control the spread of these pathogens in nosocomial settings.

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