Section: Microbiology

Original Research Article

NON-FERMENTING GRAM NEGATIVE BACTERIA: A STUDY ON THEIR PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN AMONG PATIENTS ADMITTED IN A TERTIARY CARE HOSPITAL, PRAYAGRAJ, UTTAR PRADESH

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Abstract

Background: The present study aimed to isolate and identify NFGNB from clinical samples and to assess prevalence and antimicrobial susceptibility profiles in a tertiary care hospital in Eastern India. **Materials and Methods:** An observational study with a cross-sectional design was conducted between January 2022 to December 2022 (1 year) in the Department of Microbiology, United Institute of Medical Sciences, Prayagraj. All the clinical samples, including urine, pus, blood, wound swab and body fluids, were received in the laboratory, inoculated on blood and Mac-Conkey agar or CLED agar, and incubated aerobically at 37°C for 18 to 24 hours. The non-lactose fermenting isolates that showed alkaline change (K/NC) reaction in triple sugar iron agar media were provisionally considered as NFGNB. They were further identified using standard protocols for identification, like gram staining for morphology, hanging drop for motility, pigment production, oxidase test, catalase test etc. Kirby-Bauer disc diffusion method performed an antimicrobial susceptibility test using commercially available discs (Hi-Media).Statistical analysis was done by using Excel and SPSS V21. **Result:** Out of 1021 clinical samples, cultures were positive in 695 samples. Out of 695 culture positive samples, 131 (18.8%) yielded NFGNB. The mean of our study participants was found to be 42.22 ± 12.46 years, with a male: female ratio 2.6:1. P. aeruginosa was isolated in 74/131 (56%) samples, followed by A. baumannii (50/131, 38%), Burkholderia pseudomallei (4/131, 3%), A. lwoffii (2/131, S2%), and Stenotrophomonas maltophilia were rarely isolated, accounting together for 1% of the isolates. **Conclusion:** Since these organisms have great potential to survive in the hospital environment, improved antibiotic stewardship and infection control measures will be needed to prevent the emergence and spread of drug-resistant NFGNB in healthcare settings.

INTRODUCTION

Non-fermenting 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] They occur as saprophytes in the environment and some are also found as commensals in the human gut.[2,3] NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory.[4] In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important healthcare-associated pathogens. They have been incriminated in infections, such as, septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections (SSI).[3] NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum ß-lactamases and metallo β-lactamases. Non-fermenters are now resistant to many routinely used antibiotics and even to cephalosporins and carbapenems. Resistance compromises treatment, prolongs hospital stay, increases mortality and healthcare costs.[3-6]

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The aim of the present study was to isolate and identify NFGNB from clinical samples and to assess prevalence and antimicrobial susceptibility profiles in a tertiary care hospital of Eastern India.

MATERIALS AND METHODS

An observational study with cross-sectional design was conducted between January 2022 to December 2022 in the Department of Microbiology, United Institute of Medical Sciences, Prayagraj. All the clinical samples including urine, pus, blood, wound swab and body fluids were received in the laboratory and inoculated on blood and Mac-Conkey agar or CLED agar and incubated aerobically at 37°C for 18 to 24 hours. The isolates which were non-lactose fermenting and showed alkaline change (K/NC) reaction in triple sugar iron agar media were provisionally considered as NFGNB. They were further identified using standard protocols for identification, like gram staining for morphology, hanging drop for motility, pigment production, oxidase test, catalase test, Hugh-Leifson oxidative fermentative test for glucose, lactose, sucrose, maltose and mannitol, nitrate reduction test, indole test, citrate utilization test, urease test, utilization of 10% lactose, lysine and ornithine decarboxylation, arginine dehydrolation, growth at 42°C and 44°C.[1] The clinical significance of isolated NFGNB was assessed retrospectively by analyzing the case sheets for relevant laboratory and clinical criteria. Laboratory criteria included the presence of pus cells along with gram-negative bacilli in the stained smear from the sample, isolation of the same organism from a repeat sample, leukocytosis, and relevant radiological evidence. The clinical criteria included the presence of risk factors such as underlying diseases (diabetes mellitus, chronic renal failure, malignancy, cystic fibrosis, pneumonia and other immunosuppressive conditions), presence of intravenous or urinary catheters, duration of stay in intensive care unit (ICU), mechanical ventilation and recent surgery.[7,8] Antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion method using commercially available disc (Hi-Media). The different antimicrobials used were gentamicin (10µg), amikacin (30µg), ceftazidime (30µg), ceftriaxone (30µg), piperacillin/tazobactam (100µg/10µg), imipenem (10µg), meropenem (10µg), ciprofloxacin (5µg), and cotrimoxazole (25µg). The results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains.[9]

Statistical analysis was done by using Excel and SPSS V21. The result of this analysis was used for comparison of data and to finalize the study results. P-value was determined to evaluate the levels of significance using Excel and SPSS ver. 20.0, p-value of < 0.05 was considered to be significant.

RESULTS

Out of 1021 clinical samples, cultures were positive in 695 samples. Out of 695 culture positive samples, 131 (18.8%) yielded NFGNB. The mean of our study participants was found to be 42.22 ± 12.46 years, with a male: female ratio 2.6:1. P. aeruginosa was isolated in 74/131 (56%) samples, followed by A. baumannii (50/131, 38%), Burkholderia pseudomallei (4/131, 3%), A. lwoffii (2/131, 2%), and Stenotrophomonas maltophilia were rarely isolated, accounting together for 1% of the isolates.

Clinical sources of various NFGNB isolates are shown in Figure 1. Out of 131 clinical samples positive for NFGNB, pus swab accounted for 30 (22.9%) samples, followed by urine culture 34 (25.95%), blood culture 16 (12.21%), sputum culture 24 (18,32%), tracheal swab 8 (6.12%). and endotracheal tube, 19 (14.5%).

Clinico-microbiological correlation of NFGNB isolates in our study is shown in [Figure 1].

Overall, most of the NFGNB isolates were susceptible to polymyxin B (90.4%), imipenem (89.2%) and cefoperazone + sulbactam (52.7%). Percentage antibiotic susceptibility of the various isolates is shown in [Table 1].

Table 1: Sensitivity pattern of isolated NFGNB to various antimicrobial agents

Antimicrobial	Sensitivity pattern Isolated NFGNB (n=131)				
	P. aeruginosa (74)	A. baumannii (50)	B. pseudomallei (4)	A. lwoffii (2)	S. maltophilia (1)
Piperacillin/Tazobactam	50 (67.5%)	20 (40.0%)	0	2 (100%)	0
Ceftazidime	36 (48.6%)	18 (36.0%)	0	2 (100%)	0
Ceftriaxone	18 (24.3%)	15 (30.0%)	0	2 (100%)	0
Cefepime	36 (48.6%)	17 (34.0%)	0	2 (100%)	0
Amoxicillin + clavulanic acid	36 (48.6%)	17 (34.6%)	0	1 (50%)	1 (100%)
Amikacin	42 (56.7%)	22 (44.0%)	0	2 (100%)	0
Gentamycin	44 (59.4%)	28 (56.0%)	0	2 (100%)	0
Ciprofloxacin	48 (64.8%)	22 (44.0%)	0	1 (50%)	1(100)
Ofloxacin	27 (37.45)	12 (24.95)	2 (50%)	0	0
Norfloxacin	22 (29.8%)	8 (16.7%)	1 (25%)	0	0
Cotrimoxazole	20 (28.1%)	-	4 (100%)	2 (100%)	1 (100%)
Meropenem	42 (56.7%)	26 (52.0%)	1 (75%)	2 (100%)	0
Imipenem	64 (87.3%)	41 (82.8)	3 (70%)	2 (100%)	0
Polymyxin B	74(100%)	8 (16.7%)	0	0	0

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Cefoperazone + sulbactam	36 (49.8%)	37 (74.6%)	2 (50%)	100%	100%

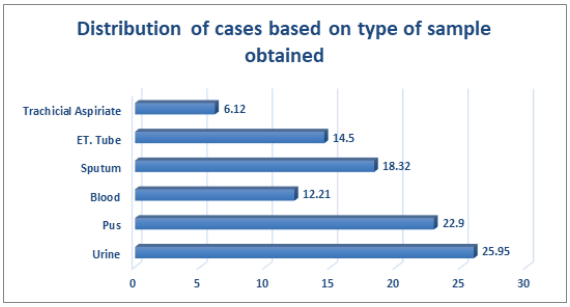

Figure 1: Distribution of cases based on type of sample obtained

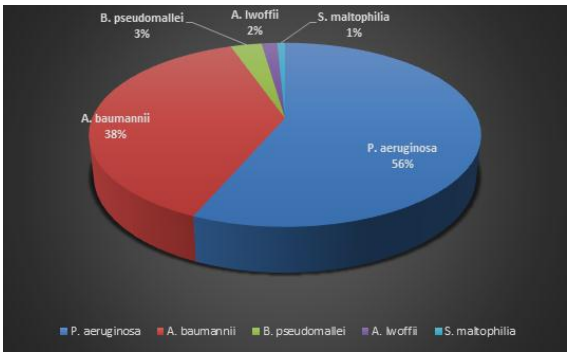

Figure 2: Distribution of cases based on isolated NFGNB

DISCUSSION

NFGNB, which were only considered to be contaminants in the past, have now emerged as important nosocomial pathogens.[2] In our study, isolation rate of NFGNB was 18.8%, which is in parallel to other studies by Rit et al,[5] and Benachinmardi et al,[10] that reported isolation rates of 12.8% and 10%, respectively. The most common NFGNB isolated in our study was P. aeruginosa (56%), followed by A. baumannii (38%) which is similar to the results obtained by Malini et al,[2] who reported P. aeruginosa as the most common isolate accounting for 104/189 (53.8%) isolates, followed by A. baumannii (43/189, 22.2%).[2] Similarly, the study done by Rit et al.[5] also found P. aeruginosa to be the predominant isolate (101/201, 50.24%), followed by A. baumannii (50/201, 24.8%). Other Gram negative. Non-fermenters such as S. maltophilia that were rarely isolated by us (1%) vary from study to study both in terms of their genera and prevalence. However, their role as opportunistic pathogens in immunocompromised and debilitated individuals has been invariably established.[11]

In our study, the highest number of isolates was isolated from urine (25.95%), which is in accordance with the observations made by Rit et al,[5] and Gokale and Metgud,[12] who also reported Urine and pus swabs as the source of maximum percentage of the isolates i.e., 27.86% and 58.4%, respectively. As evident from [Figure 1], NFGNB were majorly found to cause urinary tract infections (25.95%) and would infections (22.9%).

P. aeruginosa isolates in our study were found to be most susceptible to polymyxin B (100%), which is not routinely used to treat infections caused by P. aeruginosa and is only tried as a last resort in case of severe multidrug resistant Gram-negative bacterial infections.[13] Nearly 87.3% of the P. aeruginosa isolates were found to be sensitive to imipenem. Similarly, Malini et al,[2] and Rit et al,[5] documented 94.2% and 91.08% susceptibility to imipenem, respectively. In contrast with the studies done by Benachinmardi et al,[10] and Naqvi et al,[14] that showed higher susceptibility to quinolones, only 64.8%, 37.4% and 24.9% of P. aeruginosa isolates in the present study showed susceptibility to the quinolones such as ciprofloxacin, ofloxacin and norfloxacin, respectively. In our study, P. aeruginosa showed least susceptibility to cefepime (48.6%) and amoxicillin + clavulanic acid (48.6%).

Almost 23.2% of the isolates of P. aeruginosa in our study were labelled as multidrug resistant (MDRPA), comparable to the findings of Jayakumar and Appalaraju who reported 22% isolation rate of MDRPA in their study.[15] About 100% of the MDRPA isolates were found to be susceptible to polymyxin B, which is similar to the results obtained by Ramakrishnan et al. who also reported 100% susceptibility to imipenem.[16] Nearly 70.1% of the MDRPA isolates in our study showed resistance to imipenem, which is usually the preferred therapeutic choice for treating the infections caused by them. As carbapenems are a potent antimicrobial weapon against MDRPA, this bacterium has developed resistance even against this group of drugs by producing metallo-beta-lactamases (carbapenemase).[17] Goossens,[18] and Ramakrishnan et al,[16] showed 44.9% and 40% resistance of MDRPA isolates to imipenem in their studies, respectively [Figure 2]. Imipenem resistance in MDRPA may possibly be influenced by the amount and duration of utilisation of the antibiotic used to treat these infections.

Isolates of A. baumannii in our study showed maximum susceptibility to imipenem (82.8%), followed by cefoperazone + sulbactam (74.6%). Results obtained by other studies show variable results. Rit et al. documented 90% and 16% susceptibility of A. baumannii isolates to imipenem and cefoperazone + sulbactam, respectively.[5] Tunyapanit et al. have reported 100% susceptibility to imipenem and 47% susceptibility to cefoperazone + sulbactam in A. baumannii isolates.[19] Highest resistance amongst these isolates in our study was recorded against aztreonam (susceptibility = 17.1%). Similarly, Juyal et al,[20] reported least susceptibility of A. baumannii isolates to aztreonam (8.33%) in their study.

A total of 33 (67.41%) of A. baumannii isolates showed multidrug resistance (MDRAB) in the present study which is in accordance with Cai et al. who reported 72.23% prevalence of MDRAB

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isolates.[21] Fortunately, MDRAB isolates in our study showed good susceptibility to imipenem (87.5%), which is usually the most common therapeutic choice for MDRAB bacteraemia.[22] This is, however, in contrast with the findings of Tunyapanit et al.[19] and Cai et al. [Figure 2] who documented only 12% and 9.27% susceptibility to imipenem, respectively.[21] Nearly 55.45% of the MDRAB isolates in our study were found to be susceptible to cefoperazone + sulbactam, which is comparable to Tunyapanit et al. who reported 47% susceptibility to cefoperazone + sulbactam combination.[19]

B. pseudomallei was the third most commonly isolated NFGNB (3%) in our study. Sidhu et al. reported a prevalence of 2.31%.[23] The isolate of B. pseudomallei showed maximum susceptibility (70%) to imipenem and ciprofloxacin. Sidhu et al. reported 100%, and 75% susceptibility of B. pseudomallei isolates to imipenem and ciprofloxacin, respectively, in their study.[23] There is a lack of substantial data regarding the prevalence and antibiotic susceptibility profile of B. pseudomallei due to its limited pathogenic role and rare isolation.

S. maltophilia showed high resistance to almost most of the antibiotics tested for susceptibility.

In our study, A. lwoffii was isolated from urine culture and showed maximum (100%) susceptibility to imipenem, in accordance with Sidhu et al. who also reported 100% susceptibility to imipenem.[23] Similarly, in the study done by Rit et al., B. cepacia isolates showed excellent susceptibility to imipenem (92.85%).[5] Therefore, it can be inferred that imipenem offers excellent therapeutic effect in infections caused by A. lwoffii, which is known to be resistant to many first-line therapeutics of choice against serious pseudomonal infections, such as beta-lactam drugs, polymyxin B and aminoglycosides.[24]

S. maltophilia, isolated from a pus swab, showed 100% susceptibility to some of the antibiotics, notably ciprofloxacin. Similar were the results obtained by Malini et al,[2] and Chawla et al,[11] who reported 100% and 93.3% susceptibility of S. maltophilia to ciprofloxacin, respectively. S. maltophilia was found to be 100% resistant to majority of the antibiotics in our study, including imipenem, which could be attributed to the production of a zinc-dependent β-lactamase by this bacterium.[25]

CONCLUSION

Our study showed a significantly high prevalence of NFGNB, the most common being P. aeruginosa and A. baumannii. P. aeruginosa isolates showed good susceptibility to polymyxin B and imipenem whereas the isolates of A. baumannii showed good susceptibility to imipenem and cefoperazone + sulbactam. Isolation of MDRPA and MDRAB in the present study raises the concern of rapidly emerging antibiotic resistance in this group of bacteria in our region. Proper screening of non-fermenters in nosocomial settings, regular assessment of their antibiotic susceptibility profiles and judicious use of antibiotics are suggested for effective management of the infections caused by them and limiting the emergence of multidrug resistance.

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