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EMERGENCE OF MULTIDRUG-RESISTANT NON-FERMENTATIVE GRAM-NEGATIVE BACILLI IN A TERTIARY CARE HOSPITAL IN WESTERN UTTAR PRADESH: A THERAPEUTIC CONCERN

Clinical Microbiology

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ABSTRACT

Introduction- Non-fermentative Gram-negative bacilli (NFGNB) are ubiquitously distributed in the environment and have a high proclivity for antibiotic resistance. They have emerged as important healthcare-associated pathogens. Identification of these NFGNB is essential owing to the intrinsic multidrug resistance exhibited by them. **Aims & Objectives-** i) To isolate and identify NFGNB from various clinical samples ii) To determine the antibiotic susceptibility pattern of clinical isolates of NFGNB. **Method-** A total of 14203 clinical samples were subjected to culture i) by conventional methods as per standard bacteriological techniques, and ii) by an automated BACTEC System (BD) as per the manufacturer's instructions. Identification of isolates was done by conventional tests and by an automated VITEK 2 system (Biomerieux, France) using GN card. Antibiotic susceptibility testing of the isolates was done by Kirby Bauer disc diffusion method as per CLSI guidelines. The minimum inhibitory concentration (MIC) of certain antibiotics was determined by VITEK 2 system using AST-N281 card. **Results-** Isolation rate of NFGNB from various clinical samples was 9.4%. Non-fermenters were isolated more frequently from indoor patients. The frequency of isolation was maximum from endotracheal aspirate (43.3%), followed by pus (36.5%), blood (10.9%) and urine (5.6%). The most common isolates were of Pseudomonas aeruginosa (67.1%), followed by Acinetobacter calcoaceticus-Acinetobacter baumannii complex (25.3%), Burkholderia cepacia complex (5.3%), Stenotrophomonas maltophilia (0.7%), Ochrobactrum anthropi (0.5%), Sphingomonas paucimobilis (0.4%), Chryseobacterium indologenes (0.3%), Achromobacter xylosoxidans (0.2%) and Chryseobacterium gleum (0.1%). The antibiogram of these NFGNB revealed multidrug resistance profile, acquired and intrinsic. **Conclusion:** The upthrust in antimicrobial resistance in NFGNB continues to be a therapeutic catastrophe. Timely diagnosis and initiation of appropriate antibiotic therapy, coupled with adoption of infection prevention and control practices, becomes essential in management of patients, and curbing the burden of these multidrug resistant organisms.

KEYWORDS

Non-fermentative Gram-negative bacilli (NFGNB), Antibiotic Resistance, Healthcare Associated Infections

INTRODUCTION

The non-fermentative Gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, non-sporing bacilli that either do not utilize carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation.[1,2] Being ubiquitously distributed in nature, they are often disregarded as contaminants when isolated in clinical laboratory. However, their pathogenic potential has been established because of their frequent isolation from clinical samples and their association with the nosocomial infections.[3,4] In the hospital environment, they may be isolated from instruments such as ventilators, humidifiers, and other equipments, as well as from the skin of healthcare workers. All these organisms have the potential to spread horizontally on fomites or the hands of healthcare personnel.[4,5] This group of bacteria have been incriminated in infections such as septicemia, meningitis, pneumonia, urinary tract infection and surgical site infections.[6] NFGNB poses a therapeutic menace in healthcare settings because of their multiple, innate, or acquired antibiotic resistance. The burden of drug resistance is presumably due to injudicious and empirical use of antibiotics, that has enabled NFGNB emerge as important healthcare-associated pathogens.[5,7] They show acquired resistance to a wide range of antibiotics including penicillins, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracycline, cotrimoxazole and polymyxins, thus challenging the treatment options.[7,8] The correct identification of NFGNB becomes crucial due to the fact that most of them exhibit multidrug-resistant patterns and inherent resistance to many antibiotics. Owing to overlapping phenotypic characteristics, many non-fermenters are not adequately identified by the standard conventional methods, and thus automated techniques becomes important for their correct and rapid identification, and for antimicrobial susceptibility testing. To the best of our knowledge, limited data is available from India, wherein the various NFGNB have been identified and their clinical significance assessed. Hence, the present study was designed to identify the clinical isolates of NFGNB and to determine their antibiotic susceptibility in our healthcare setup.

MATERIALS AND METHOD

The prospective study was carried out in a tertiary care hospital in Western Uttar Pradesh, over a period of one year (March 2024 - February 2025). The study included all the clinical samples received in bacteriology laboratory from various in-patient and out-patient departments, irrespective of age and gender.

Ethics Approval: Approval from the institutional ethics committee was taken before conducting the study.

Sample Processing :[1,9,10]

Clinical samples such as blood, sputum, endotracheal aspirate, pus, urine, cerebrospinal fluid and other body fluids, received in the laboratory, were processed for culture and antibiotic susceptibility testing. Samples were subjected to culture i) by conventional methods as per standard bacteriological techniques,[1,9] and ii) by an automated BACTEC System (BD) as per the manufacturer's instructions.

Identification of Isolates: The isolates grown onto culture media (Blood agar, Chocolate agar, MacConkey's agar) were identified by conventional methods such as colony characteristics, culture smears, motility and the biochemical tests.[1,9] All the isolates producing non lactose fermenting colonies on Mac Conkey's agar and showing cytochrome oxidase activity were presumptively considered to be non-fermenters. NFGNB isolates were further subjected to biochemical tests such as nitrate reduction, citrate utilization, urea hydrolysis, amino acids decarboxylase tests, triple sugar iron test, Hugh and Leifson's oxidation fermentation tests for glucose and maltose, and gelatin liquefaction.[1,9] Identification was confirmed by an automated VITEK 2 system (Biomerieux, France) using GN REF 21341 card.

Antimicrobial Susceptibility Testing: Antibiotic susceptibility testing was performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines[10] by Kirby Bauer disc diffusion method on Mueller-Hinton agar. Antibiotics tested were piperacillin (100µg), ceftazidime (30µg), ceftriaxone (30µg), cefepime (30µg), aztreonam (30µg), piperacillin-tazobactam (100/10µg), ampicillin-sulbactam (10/10µg), imipenem (10µg), meropenem (10µg), ciprofloxacin (5µg), amikacin (30µg), gentamicin (10µg), tobramycin (10µg), tetracycline (30µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), colistin (10µg), polymyxin B (300µg) and tigecycline (15µg). For urinary isolates, norfloxacin (10µg), nitrofurantoin (300µg) and fosfomycin (200µg) were tested in addition. The interpretation of results was done as per CLSI guidelines.[10] Pseudomonas aeruginosa ATCC 27853 was used as quality control strain. Further, the minimum inhibitory concentration (MIC) of certain antibiotics was determined by VITEK 2 system using AST-N281 card.

RESULTS

A total of 14203 clinical samples were received in the laboratory. The number of isolates of NFGNB obtained from various clinical samples were 1338. Thus, the isolation rate of non-fermenters was found to be 9.4% (1338/14203). Non-fermenters were isolated more frequently from indoor patients (71.4%, 956/1338). The isolation of NFGNB (36.4%) was seen predominantly in geriatric age group (>60 years). NFGNB were recovered mainly from male patients (67.6%).

The frequency of isolation was maximum from endotracheal aspirate (43.3%), followed by pus (36.5%), blood (10.9%), urine (5.6%), central line tip (3.4%), ascitic fluid (0.2%) and cerebrospinal fluid (0.1%) (Table-1). Out of total of 1338 isolates of NFGNB, most frequent were of Pseudomonas aeruginosa (67.1%), followed by Acinetobacter calcoaceticus-Acinetobacter baumannii (ACB) complex (25.3%), Burkholderia cepacia complex (5.3%), Stenotrophomonas maltophilia (0.7%), Ochrobactrum anthropi (0.5%), Sphingomonas paucimobilis (0.4%), Chryseobacterium indologenes (0.3%), Achromobacter xylosoxidans (0.2%) and Chryseobacterium gleum (0.1%) (Table-2). Looking at the sample-wise distribution of various NFGNB isolates (Table-3), P. aeruginosa was isolated most frequently from pus (44.8%), followed by endotracheal aspirate (39.9%), blood (7.2%) and urine (4.8%). Isolation of ACB complex was maximum from endotracheal aspirate (53.4%), followed by pus (23.6%), blood (9.7%) and urine (8.8%). Burkholderia cepacia complex (BCC) isolates were recovered mainly from blood (47.9%), followed by endotracheal aspirate (39.4%) and pus (8.5%). Isolation of Stenotrophomonas maltophilia from endotracheal aspirate and blood was found to be 55.6% and 44.4%, respectively. Achromobacter xylosoxidans was also obtained from endotracheal aspirate (66.7%) and blood (33.4%). Chryseobacterium indologenes showed isolation from endotracheal aspirate and blood (50% each) (Table-3). Isolates of Ochrobactrum anthropi, Sphingomonas paucimobilis and Chryseobacterium indologenes were recovered mainly from blood. We could obtain one isolate of Chryseobacterium gleum from endotracheal aspirate (Table-3). Among the isolates of P. aeruginosa, 3.6% showed resistance to colistin as well as to polymyxin B. However, resistance to meropenem was noticed in 46.3% and resistance to imipenem in 44.3% of isolates of P. aeruginosa. Resistance to ceftazidime and cefepime was noticed in 65.4% and 64.7% of these isolates, respectively. Ciprofloxacin resistant isolates of P. aeruginosa were found to be 56.9%, followed by isolates resistant to piperacillin-tazobactam (47.4%), and resistant to aminoglycosides (gentamicin 47.3%, amikacin 45.2%, tobramycin 43.2%) (Table-4). Isolates of ACB complex showed maximum resistance to cephalosporins, piperacillin, ampicillin-sulbactam, piperacillin-tazobactam followed by ciprofloxacin, tetracycline, cotrimoxazole and aminoglycosides. Resistance to imipenem and meropenem was seen in 65.2% and 63.7% of these isolates, respectively. ACB complex isolates resistant to colistin and polymyxin B were 1.5% each (Table-4). Isolates of BCC resistant to imipenem and meropenem were 36.6% and 32.4%, respectively.

Resistance of BCC to piperacillin-tazobactam, cefepime, ceftazidime and cotrimoxazole was found to be 53.5%, 59.1%, 63.4% and 29.6%, respectively. Isolates of Stenotrophomonas maltophilia showed maximum resistance to cephalosporins. Only one isolate of S. maltophilia was resistant to both colistin and polymyxin B. All the isolates of Ochrobactrum anthropi were resistant to aminoglycosides. High rate of resistance of O. anthropi was also noticed for carbapenems, cephalosporins and piperacillin-tazobactam. Sphingomonas paucimobilis showed 66.7% resistance to colistin and polymyxin B. Chryseobacterium indologenes reflected 100% resistance to carbapenems, colistin and polymyxin B.

All the isolates of Achromobacter xylosoxidans were resistant to cephalosporins and aztreonam but susceptible to aminoglycosides, carbapenems, colistin and polymyxin B. Chryseobacterium gleum was found to be sensitive to only piperacillin-tazobactam, ciprofloxacin and cotrimoxazole (Table-4). Except for P. aeruginosa and Chryseobacterium gleum, all other isolates of NFGNB were found to be sensitive to tigecycline, P. aeruginosa being intrinsically resistant to tigecycline (Table-4). Looking at urinary isolates of P. aeruginosa (n=43), resistance to norfloxacin, nitofurantoin and fosfomycin was detected in 61.5%, 83.6% and 22.8%, respectively. Urinary isolates of ACB complex (n=30) showed 78.1% and 29.4% resistance to norfloxacin and nitofurantoin, respectively. Both the two urinary isolates of BCC were resistant to norfloxacin and nitofurantoin. Moreover, ACB complex and BCC are intrinsically resistant to fosfomycin.

Table-1: Frequency of isolation of NFGNB (n=1338) from various clinical samples:

Clinical samples	Number of isolates (n=1338)	Percentage (%)
Endotracheal aspirate	580	43.3%
Pus	488	36.5%
Blood	146	10.9%
Urine	75	5.6%
Central line tip	45	3.4%
Ascitic fluid	3	0.2%
Cerebrospinal fluid	1	0.1%

Table 2: Frequency of clinical isolates of NFGNB (n=1338):

Organism isolated	Number of isolates (n=1338)	Percentage (%)
Pseudomonas aeruginosa	898	67.1%
Acinetobacter calcoaceticus-Acinetobacter baumannii complex	339	25.3%
Burkholderia cepacia complex	71	5.3%
Stenotrophomonas maltophilia	9	0.7%
Ochrobactrum anthropi	7	0.5%
Sphingomonas paucimobilis	6	0.4%
Chryseobacterium indologenes	4	0.3%
Achromobacter xylosoxidans	3	0.2%
Chryseobacterium gleum	1	0.1%

Table 3: Sample-wise distribution of various NFGNB isolates (n=1338):

Organism isolated	Endotracheal aspirate	Pus	Blood	Urine	Central line tip	Ascitic fluid	Cerebrospinal fluid
Pseudomonas aeruginosa (n=898)	358 (39.9%)	402 (44.8%)	65 (7.2%)	43 (4.8%)	28 (3.1%)	2 (0.2%)	0
Acinetobacter calcoaceticus-Acinetobacter baumannii complex (n=339)	181(53.4%)	80 (23.6%)	33 (9.7%)	30 (8.8%)	13 (3.8%)	1 (0.3%)	1 (0.3%)
Burkholderia cepacia complex (n=71)	28 (39.4%)	6 (8.5%)	34 (47.9%)	2 (2.8%)	1 (1.4%)	0	0
Stenotrophomonas maltophilia (n=9)	5 (55.6%)	0	4 (44.4%)	0	0	0	0
Ochrobactrum anthropi (n=7)	1 (14.3%)	0	3 (42.9%)	0	3 (42.9%)	0	0
Sphingomonas paucimobilis (n=6)	2 (33.3%)	0	4 (66.7%)	0	0	0	0
Chryseobacterium indologenes (n=4)	2 (50.0%)	0	2 (50.0%)	0	0	0	0
Achromobacter xylosoxidans (n=3)	2 (66.7%)	0	1 (33.4%)	0	0	0	0
Chryseobacterium gleum (n=1)	1 (100%)	0	0	0	0	0	0
Total (n=1338)	**580 (43.3%)**	**488 (36.5%)**	**146 (10.9%)**	**75 (5.6%)**	**45 (3.4%)**	**3 (0.2%)**	**1 (0.1%)**

Table 4: Antibiotic resistance pattern of clinical isolates of NFGNB (n=1338):

Antibiotic	Antibiotic resistance profile of isolates								
	Pseudomonas aeruginosa (n=898)	Acinetobacter Calcoaceticus-Acinetobacter baumannii complex (n= 339)	Burkholderia cepacia complex (n=71)	Stenotrophomonas maltophila (n=9)	Ochrobactrum anthropi (n=7)	Sphingomonas paucimobilis (n=6)	Chryseobacterium indologenes (n=4)	Achromobacter xylosoxidans (n=3)	Chryseobacterium gleum (n=1)

PI	585 (65.1%)	299 (88.2%)	IR	IR	5 (71.4%)	6 (100%)	4 (100%)	2 (66.7%)	1 (100%)
PIT	426 (47.4%)	255 (75.2%)	38 (53.5%)	IR	5 (71.4%)	4 (66.7%)	2 (50%)	1 (33.3%)	0
A/S	*IR	276 (81.4%)	IR	IR	-	-	4 (100%)	-	1 (100%)
CAZ	587 (65.4%)	314 (92.6%)	45 (63.4%)	8 (88.9%)	5 (71.4%)	5 (83.3%)	4 (100%)	3 (100%)	1 (100%)
CTR	IR	316 (93.2%)	-	IR	-	-	4 (100%)	-	1 (100%)
CPM	581 (64.7%)	301 (88.8%)	42 (59.1%)	7 (77.8%)	5 (71.4%)	5 (83.3%)	3 (75%)	3 (100%)	1 (100%)
AZ	513 (57.1%)	IR	31 (43.7%)	IR	5 (71.4%)	5 (83.3%)	IR	3 (100%)	1 (100%)
TE	IR	258 (76.1%)	-	IR	-	-	-	-	-
COT	IR	246 (72.6%)	21 (29.6%)	-	-	-	0	-	0
CIP	511 (56.9%)	262 (77.3%)	39 (54.9%)	6 (66.7%)	4 (57.1%)	2 (33.3%)	2 (50%)	1 (33.3%)	0
GEN	425 (47.3%)	236 (69.6%)	-	IR	7 (100%)	3 (50.0%)	IR	0	IR
AK	406 (45.2%)	224 (66.1%)	-	IR	7 (100%)	3 (50.0%)	IR	0	IR
TOB	388 (43.2%)	211 (62.2%)	-	IR	7 (100%)	3 (50.0%)	IR	0	IR
MRP	416 (46.3%)	216 (63.7%)	23 (32.4%)	IR	5 (71.4%)	4 (66.7%)	4 (100%)	0	IR
IPM	398 (44.3%)	221 (65.2%)	26 (36.6%)	IR	5 (71.4%)	4 (66.7%)	4 (100%)	0	IR
CL	32 (3.6%)	5 (1.5%)	IR	1 (11.1%)	0	4 (66.7%)	4 (100%)	0	IR
PB	32 (3.6%)	5 (1.5%)	IR	1 (11.1%)	0	4 (66.7%)	4 (100%)	0	1 (100%)
TG	IR	0	0	0	0	0	0	0	1 (100%)

Note- PI:Piperacillin, PIT:Piperacillin-Tazobactam, A/S:Ampicillin-Sulbactum, CAZ:Ceftazidime, CTR:Ceftriaxone, CPM: Cefepime, AZ:Aztreonam, TE:Tetracycline, COT:Cotrimoxazole, CIP:Ciprofloxacin, GEN:Gentamicin, AK:Amikacin, TOB:Tobramycin, MRP:Meropenem, IMP:Imipenem, CL:Colistin, PB:Polymyxin B, TG:Tigecycline, *IR:Intrinsic Resistance

DISCUSSION

Non-fermentative Gram-negative bacilli have emerged as important healthcare-associated pathogens. NFGNB pose significant challenges in health care settings because of their widespread antibiotic resistance. The burden of resistance is presumably due to the liberal and empirical use of broad spectrum antibiotics.[5,7,11] It is important to correctly identify the clinically significant NFGNB, considering the innate and acquired multidrug resistance exhibited by these bacteria.[11]

Isolation rate of NFGNB from various clinical samples in our tertiary care hospital was 9.4%. Similar observation was made by the study carried out in Tamil Nadu by Krishnan S et al.[12] in 2018, where they have reported an isolation rate of 10.2%. Mahajan R et al.[5] and Rit K et al.[13] have reported frequency of isolation as 12.4%, 12.1%, respectively. As compared to our findings, much higher recovery rate of NFGNB has been was documented by Bhagava D et al. (29.6%) from Nepal[14] and Sharma D et al. (25.6%) from Jaipur,[15] while Rahbar M et al.[4] reported a positivity rate of only 15% from Iran. However, study done in Karnataka by Malini A et al.[6] have shown 4.5% isolation rate. These variations in the isolation rates could be attributed to varying degrees of infection prevention and control practices in different hospital settings.

In our study, NFGNB were recovered more frequently from indoor patients (71.4%), suggesting their ability to cause nosocomial infections. On clinical data analysis, severe immunosuppression and frequent use of invasive devices appeared to be the significant factors in most of our cases. Presence of longstanding intravenous catheters provides an important reservoir for non-fermenters. NFGNB are becoming a threat to health care systems because these bacteria are mainly associated with opportunistic infections in debilitated and immunocompromised patients.[16,17] Sidhu S et al.[16] observed that 45.91% of NFGNB isolates were obtained from critically ill hospitalized patients. Costerton JW et al.[18] documented that biofilm formation by P. aeruginosa pose an important factor in the pathogenesis of device-related infections such as central venous catheter-related infections, urinary catheter cystitis and ventilator-associated pneumonia.

In our set up, the frequency of isolation of NFGNB was maximum from endotracheal aspirate (43.3%), followed by pus (36.5%), blood (10.9%), urine (5.6%), central line tip (3.4%), ascitic fluid (0.2%) and cerebrospinal fluid (0.1%). Our findings are in alignment with the study done in Nepal by Yadav SK et al.[19], where the maximum number of NFGNB were recovered from lower respiratory tract samples (43.0%), followed by pus (24.6%) and urine (12.2%). However, Mahajan R et al.[5] has reported highest isolation of NFGNB from pus (59.96%), followed by blood (12.23%), CSF (8.39%), endotracheal secretions (7.34%) and urine (6.29%). In our present study, amongst the non-fermenter isolates, most frequent were of Pseudomonas aeruginosa (67.1%), followed by ACB complex (25.3%), BCC (5.3%), Stenotrophomonas maltophilia (0.7%), Ochrobactrum anthropi (0.5%), Sphingomonas paucimobilis (0.4%), Chryseobacterium indologenes (0.3%), Achromobacter xylosoxidans (0.2%) and Chryseobacterium gleum (0.1%). Our observation of high rate of isolation of P. aeruginosa and ACB complex matched with the study done by Mahajan R et al.[5] who had reported P. aeruginosa as the predominant non-fermenter (54.54%), followed by Acinetobacter baumanii (41.08%), Stenotrophomonas maltophilia (2.62%) and BCC (1.70%). Yadav SK et al.[19] observed that most common NFGNB was A. baumannii (44.0%), followed by P. aeruginosa (40.1%) and BCC (8.2%). The predominant isolation of Pseudomonas and Acinetobacter has also been reported by various other authors.[6,13,20]

In our study, sample-wise distribution of various NFGNB isolates (Table-3) suggests frequent isolation of P. aeruginosa from pus (44.8%), followed by endotracheal aspirate (39.9%), blood (7.2%) and urine (4.8%). Isolation of ACB complex was maximum from endotracheal aspirate (53.4%), followed by pus (23.6%), blood (9.7%) and urine (8.8%). BCC isolates were recovered mainly from blood (47.9%), followed by endotracheal aspirate (39.4%) and pus (8.5%) (Table-3). These observations reflects the potential of these non-fermenters to cause nosocomial infections such as blood-stream infections, respiratory tract infections, burn wound infections, postoperative wound infections and urinary tract infections.[6] In a study done by Yadav SK et al.[19], majority of A. baumannii and P. aeruginosa were recovered from lower respiratory tract specimens. The morbidity and mortality of patients with cystic fibrosis is influenced by chronic lung infections caused by bacterial pathogens. P. aeruginosa is by far the most common and dreadful pathogen that infects the patients with cystic fibrosis.[21] Malini A et al.[6] found that among NFGNB, 62.2% were obtained from pus samples, of which P. aeruginosa and A. baumannii were the common isolates. In our tertiary care hospital, we isolated Acinetobacter from CSF sample, which is in agreement with the study done by Siegman-Igra Y et al.[22] who had reported cases of nosocomial Acinetobacter meningitis secondary to invasive procedures. In 2023, a study done in western Uttar Pradesh observed that A. baumannii was most commonly isolated from endotracheal aspirate (28.9%), followed by blood (24.4%), pus (21.7%), sputum (14.3%), and urine (5.8%).[23]

B. cepacia complex is another NFGNB colonizing and infecting patients with chronic respiratory illness. It is known to cause disease in cystic fibrosis patients.[21] In our study, BCC isolates were recovered mainly from blood (47.9%), followed by endotracheal aspirate (39.4%) and pus (8.5%). As found in our set-up, BCC causing blood stream infections has been also reported by other workers.[19,24] BCC causing hospital-acquired pneumonia has been observed in a study

done in Manipal by Chawla K et al.[25]

Recovery of Stenotrophomonas maltophilia from endotracheal aspirate and blood in our study correlates with the findings by Yadav SK et al.[19], whereas Malini A et al.[6] had reported the isolation of S. maltophilia from pus and urine. S. maltophilia causing pneumonia has been noted by Chawla K et al.[25] In our present study, we could isolate rare pathogens like Ochrobactrum anthropi, Sphingomonas paucimobilis, Achromobacter xylosoxidans and Chryseobacterium indologenes and Chryseobacterium gleum (Table-2 and 3). The inert or unusual biochemical reactions by conventional techniques prompted us to identify these rare organisms by automated VITEK 2 system. In our set-up, isolates of Ochrobactrum anthropi found to be culprit for bloodstream infections were 42.9%, and isolates recovered from endotracheal aspirate were 14.3%. In 2022, authors from western Uttar Pradesh reported a case of Ochrobactrum anthropi causing septicemia and pneumonia in a diabetic patient with chronic kidney disease who was on regular haemodialysis, thus highlighting the incrimination of O. anthropi causing catheter-related bloodstream infections.[26] We isolated Sphingomonas paucimobilis from blood (66.7%) and endotracheal aspirate (33.3%). Chawla K et al.[25] had shown the isolation rate of Sphingomonas paucimobilis as 9.1% from the respiratory samples. Achromobacter xylosoxidans, generally considered as a nosocomial colonizer, has gained attention as an emerging opportunistic pathogen in cystic fibrosis.[27] In our study, we could recover Achromobacter xylosoxidans from endotracheal aspirate (66.7%) and blood (33.4%). A. xylosoxidans causing respiratory illness has been reported by Chawla K et al.[25] In our study, Chryseobacterium indologenes was isolated from endotracheal aspirate and blood (50% each). Izaguirre-Anariba DE et al.[28] had reported a case of central line-associated bloodstream infection due to C. indologenes in 2020. C. indologenes was first reported in 1993 by Bhagawati G et al.[29] in a patient presenting with ventilator-associated pneumonia. Nosocomial infections due to C. indologenes have been linked to the use of indwelling catheters. Additionally, the colonization of patients through contaminated medical devices involving fluids such as respirators, endotracheal tubes and humidifiers has been documented.[28-30] In our set-up, we could obtain one isolate of Chryseobacterium gleum from endotracheal aspirate from a patient with idiopathic interstitial lung disease.

Looking at the antibiotic susceptibility profile of NFGNB in our set-up, the rising drug resistance poses a therapeutic menace. In the present study, among the isolates of P. aeruginosa, 96.4% were sensitive to colistin as well as to polymyxin B. However, resistance to meropenem was noticed in 46.3% and resistance to imipenem in 44.3% of isolates of P. aeruginosa. Resistance to ceftazidime and cefepime was noticed in 65.4% and 64.7% of these isolates, respectively. Ciprofloxacin resistant isolates were found to be 56.9%, followed by isolates resistant to piperacillin-tazobactam (47.4%) and aminoglycosides (gentamicin 47.3%, amikacin 45.2%, tobramycin 43.2%) (Table-4). Our observation regarding sensitivity of P. aeruginosa to colistin was in agreement with the study done by Mahajan R et al.[5] who had also reported high susceptibility to colistin (96.79%), whereas sensitivity to other antibiotics, as reported by authors[5] was found to be amikacin 75%, tobramycin 75%, piperacillin-tazobactam 62.85% and imipenem 59.61%. A study done in Bangalore by Veenakumari HB et al.[31] made the observation of P. aeruginosa exhibiting 60-70% resistance to amikacin, ceftazidime, and ciprofloxacin. In a study conducted in Chandigarh by Taneja N et al.[32], 42% of P. aeruginosa isolates were found to be resistant to imipenem, which is somewhat in concordance with our present study where we have reported imipenem resistance in 44.3% of these isolates.

In the present study, isolates of ACB complex showed maximum resistance to cephalosporins, piperacillin, ampicillin-sulbactam, piperacillin-tazobactam followed by ciprofloxacin, tetracycline, cotrimoxazole and aminoglycosides. Resistance to imipenem and meropenem was seen in 65.2% and 63.7% of these isolates, respectively. Sensitivity of Acinetobacter to colistin was 98.5% (Table-4), which correlated with the findings by Rit K et al.[13] where 94% susceptibility had been reported for colistin. In contrast, as observed by Mahajan R et al.[5] sensitivity to colistin was found to be 85.10%. Mahajan et al.[5] reported the sensitivity of Acinetobacter to imipenem as 75.31%, which is much more than we noticed (34.8%). Our observations are in agreement with the study done in Meerut by Vashishtha A et al.[33] who had found that among the Acinetobacter isolates, 65.07% were resistant to meropenem and 60.02% were resistant to imipenem, however, all the isolates were sensitive to colistin. Another study carried out in Uttar Pradesh[23] reported higher meropenem resistance (72.1%) and 100% sensitivity to colistin and tigecycline in A. baumannii.

In our study, isolates of BCC resistant to imipenem and meropenem were 36.6% and 32.4%, respectively. Moreover, BCC is intrinsically resistant to colistin and polymyxin B.[10] Resistance of BCC to piperacillin-tazobactam, ceftazidime and cotrimoxazole was found to be 53.5%, 63.4% and 29.6%, respectively. Resistance pattern of BCC seen in our study is quite similar to that observed by Mahajan R et al.[5] It has been noticed that BCC strains are resistant to most antibiotics commonly used for treatment of Gram-negative bacterial infections, including the broad-spectrum beta-lactam antibiotics and aminoglycosides.[11] In the present study, Stenotrophomonas maltophilia showed maximum resistance to cephalosporins, however 88.9% of S. maltophilia were sensitive to both colistin and polymyxin B. Stenotrophomonas maltophilia is inherently resistant to ampicillin-sulbactam, piperacillin-tazobactam, imipenem and meropenem and aminoglycosides.[10] Multidrug resistance in S. maltophilia as observed in our study has been reported by various other authors.[5,6,19]

In our setup, all the isolates of Ochrobactrum anthropi showed resistance to aminoglycosides, followed by high resistance to piperacillin-tazobactam, imipenem and meropenem (71.4% each). In 2022, authors from western Uttar Pradesh had reported a case of catheter-related bloodstream infection caused by an AmpC beta lactamase producing Ochrobactrum anthropi.[26]

In the present study, all the isolates of Achromobacter xylosoxidans were resistant to cephalosporins, however, resistance to piperacillin-tazobactam was found to be 33.3%. Chawla K et al.[25] had reported 100% sensitivity of A. xylosoxidans to beta lactam-beta lactamase inhibitor combination.

The emergence of multi-drug resistance among NFGNB contrive a significant challenge to effective therapeutic options. Mechanisms of antibiotic resistance in non-fermenters includes enzyme production, porin deficiencies, overexpression of efflux pumps, and target-site alterations. Multiple drug resistance genes often coexist in the same organism.[7] Antibiotic resistance genes can spread via horizontal gene transfer through mobile genetic multi-drug resistance elements among bacteria.[7,11] The dissemination of antibiotic resistance genes in the hospital environment or clinical setting pose a serious threat to human health, making the treatment of infections caused by these multi-drug resistant bacteria more arduous and costly. Strict adherence to good healthcare and clinical practices becomes pivotal to scuffle against and to restrain these drug resistant bacteria.

CONCLUSIONS

The non-fermentative Gram-negative bacilli are of increasing concern in the hospital settings, considering the intrinsic and acquired multi-drug resistance exhibited by them. The correct identification and antibiotic susceptibility testing becomes incumbent for the appropriate management of infections caused by these nosocomial pathogens. Booming of drug resistance among NFGNB highlights the need for regular surveillance of antibiotic susceptibility profiles, improved antibiotic stewardship, and rigorous infection control measures.

Limitations

Due to resource constraints, molecular characterization of resistant phenotypes and genetic mechanism of antibiotic resistance could not be determined.

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