



OCCURRENCE AND RESISTANCE PROFILE OF NONFERMENTATIVE GRAM-NEGATIVE BACILLI IN A TERTIARY CARE HOSPITAL – A THERAPEUTIC CONCERN

Microbiology

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ABSTRACT

Aim: To isolate, identify and characterize the prevalence of non-fermenting gram negative bacteria (NFGNB) along with their antimicrobial resistance pattern among the patients attending a tertiary care Hospital in Meerut.

Materials and Methods: The isolates of NFGNB obtained from various samples were identified by standard bacteriological technique and VITEK® 2 system (Biomerieux, France). Antimicrobial susceptibility test was carried out to assess the resistance profile both by Kirby Baur disk diffusion method and VITEK® 2 system (Biomerieux, France).

Result: A total of 16,296 clinical samples from the indoor and outdoor patients were processed in the bacteriology laboratory. Isolation rate of non-fermenters was 7.82% (1274/16296). *Pseudomonas* spp. was the predominant isolate (66.56%) followed by *Acinetobacter* spp. (27.16%). Other isolated non-fermenters were *Burkholderia cepacia complex* (BCC) (1.57%), *Stenotrophomonas maltophilia* (0.86%), *Sphingomonas paucimobilis* (0.71%), *Achromobacter xylosoxidans* (0.16%), *Ochrobactrum anthropic* (0.16%).

Conclusion: NFGNB are emerging as important opportunistic pathogens and are mostly resistant to commonly used antimicrobials. Therefore early diagnosis and initiation of appropriate antibiotic therapy would help in efficient management of patients and result in reduction of morbidity and mortality caused by these multi drug resistant organisms.

KEYWORDS

Non Fermentative Gram Negative Bacilli, Antimicrobial Resistance, Hospital Acquired Infections

Introduction

The non-fermentative gram-negative bacilli (NFGNB) are a group of aerobic, non-spore forming, gram-negative bacilli that either do not utilize carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation.[1] This group includes various organisms but most common causative agents of nosocomial infections are *Pseudomonas aeruginosa*, *Burkholderia cepacia complex* (BCC), *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*. In the recent years, the liberal and empirical use of broad spectrum antimicrobials has led to emergence of NFGNB as important healthcare-associated pathogens.[2]

These organisms are ubiquitous in nature and are particularly present in soil and water. However their pathogenic potential has been established because of their frequent isolation from clinical specimens and their association with the disease. They can be recovered from hospital environment. These organisms commonly cause device related infections as they are present in ventilator machines and humidifiers. They are often resistant to disinfectants and have the potential to spread from patient to patient via fomites or the hands of the health care personnel. Also the antimicrobial resistance exhibited by the NFGNB creates an epidemiologic niche for these pathogens that facilitates colonization and super infection in antibiotic-treated patients. [2,3]

NFGNB are among the most clinically and epidemiologically relevant bacteria, however the phenotypic identification is not easy in clinical microbiology laboratories and requires more attention. NFGNBs are found at a high frequency in the bronchial tree, especially in immunosuppressed and cystic fibrosis patients, which is a big threat as these bacteria acquire resistance to a wide variety of antimicrobials including Penicillins, Cephalosporins, Aminoglycosides, Tetracycline, Fluoroquinolones, Trimethopim-Sulfamethoxazole, Carbapenems and even Polymyxins. Polymyxins are widely used in the treatment of infections caused by NFGNB. This causes a selection of resistant bacteria, making the treatment difficult.[4]

It has always been a tedious task to identify the NFGNBs from routine microbiological laboratory and thus automation plays a major role in their rapid and prompt identification and antimicrobial susceptibility.

Hence, the authors carried out this study to isolate, identify and determine the susceptibility of various non-fermenters isolated from patients either admitted or attending the OPD of a tertiary care hospital.

Aim

- To isolate and identify non-fermenting Gram-negative bacilli from various clinical samples received from indoor and outdoor patients.
- To assess the resistance pattern of these isolates.

Material and methods:

This prospective study was conducted in the Department of Microbiology, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut, for a period of 15 months (January 2017 to March 2018). The approval from the Institutional Ethical and Research Committee was obtained before conducting the study. A total of 16,296 non repeat clinical isolates of NFGNB were obtained from different clinical samples (pus, sputum, endotracheal aspirate, urine and blood) received in Clinical Microbiology laboratory. The samples were inoculated on Blood agar, Chocolate agar and Mac Conkey agar plates and incubated overnight at 37°C. The isolates were identified as per standard bacteriological techniques.[6]

Identification of isolates:

All the isolates producing non lactose fermenting (NLF) colonies on Mac Conkey agar and showing cytochrome oxidase activity were provisionally considered to be NFGNB. They were further subjected to Gram-stain, motility, Decarboxylase test, Hugh-Leifson oxidation fermentation test for glucose and maltose, Triple sugar iron fermentation with hydrogen sulphide production on lead acetate paper and gelatin liquefaction. The identification was confirmed by VITEK® 2 system (Biomerieux, France) using GN REF 21341 card.

Antimicrobial susceptibility testing:

The Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method using commercially available discs (Hi-media) as per CLSI recommendation [5] The susceptibility was further confirmed by VITEK® 2 system using AST-N281 REF 414532 card.

The different antimicrobials tested and their disc potency were ; Piperacillin (100mcg), Cefazidime (30mcg), Ceftriaxone (30mcg),

Cefepime (30mcg), Tetracycline (30mcg), Aztreonam (30mcg), Imipenem (10µg), Meropenem(10µg), Piperacillin-Tazobactam (100/10mcg), Ampicillin-Sulbactam (10/10mcg), Ciprofloxacin (5mcg), Amikacin (30mcg), Gentamicin (10mcg), Tobramycin (10mcg), Trimethoprim-Sulfamethoxazole (1.25/23.75mcg), and Colistin (10mcg). The interpretation of results was done as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.[5] ATCC 27853 *Pseudomonas aeruginosa* was used as Quality control strain.

RESULTS

During the study period, a total of 16296 clinical samples were received in Clinical Microbiology Laboratory from the indoor and outdoor patients. Isolation rate of Non-fermenters was 7.82% (1274/16296). The maximum isolation of NFGNB (31.08%) was observed in geriatric age group of (>60years). NFGNB were predominantly isolated from male patients (60.28%). *P. spp.* was the predominant isolate (66.56%) followed by *Acinetobacter* species (27.16%). Other isolated nonfermenters were *Burkholderia cepacia complex* (BCC)(1.57%), *Stenotrophomonas maltophilia* (0.86%), *Sphingomonas paucimobilis* (0.71%), *Achromobacter xylosoxidans* (0.16%) and *Ochrobactrum anthropi*(0.16%).(Table1)

Maximum isolation of NFGNB was from respiratory samples followed by pus, blood and urine (43.01%, 33.36%, 11.46% and 11.15%) respectively. Few cases were also found in central line tip, vitreous tap, ascitic fluid and corneal scraping. (Table 2)

Non fermenters were predominantly isolated from admitted (indoor) patients(62.08%) as compared to outdoor patients(37.92%) highlighting the fact that these pathogens are most commonly associated with hospital acquired infections.

A total of 3.16% *Pseudomonas spp.* showed resistance to Colistin. However, resistance to Imipenem and Meropenem was 36.53% and 35.74% in *Pseudomonas spp.* respectively, followed by Piperacillin-Tazobactam, Aminoglycosides and Cephalosporins. [Table 3]

Acinetobacter spp. showed maximum resistance to Cephalosporins, Piperacillin, Piperacillin-Tazobactam followed by Cotrimoxazole, Aminoglycosides and Carbapenems. Colistin resistance was observed only in 4 isolates (1.15%). Isolates of *Burkholderia cepacia complex* showed minimal resistance to Carbapenems (10-20%). Only 1 isolate each of *S. maltophilia* and *S. paucimobilis* was resistant to Colistin. All isolates of *A. xylosoxidans* and *O. anthropi* were sensitive to Colistin.

Discussion

A total of 7.82% NFGNB were isolated from clinical samples received from indoor and outdoor patients of our hospital. Studies carried out by different researchers have reported varied isolation rates; Mahajan *et al.* [2] reported isolation rate of 12.40%, Rit *et al.* [6] have reported 12.1% and Krishnan *et al.* [7] have reported an isolation rate of 10.2%. Few studies have reported a higher positivity rate as compared with our study; Rahbar *et al.* [8] 15%, Sidhu *et al.* [9] 45.91%. However, Malini *et al.* [10] have reported only 4.5% in their study. These variations might be due to varying degrees of the hospital infection control practices in different institutes.

Nonfermenters were more frequently isolated from indoor patients (62.08%) in our study. This highlights the fact that the NFGNB are most commonly associated with nosocomial infections. On clinical correlation there was history of presence of indwelling devices in majority of cases which could probably have been the source of infection.

In our study, *Pseudomonas* species (69.39%) along with the *Acinetobacter* species(27.16%) accounted for 96.55% of the total non-fermenters. Our observation correlated with work carried out by various other authors like Mahajan *et al.* [2] (95.62%), Rit *et al.* [6](84.06%) who have also reported these two organisms as predominant nosocomial pathogens. They have been associated with various clinical conditions like VAP, SSI, UTI and septicemia and most of them were found to be multidrug-resistant. *Pseudomonas aeruginosa* plays an important role in the pathogenesis of central venous catheter-related infection, urinary catheter cystitis, contact lens-associated corneal infection, lung infection in cystic fibrosis, and ventilator-associated pneumonia.

Majority of *Pseudomonas* species isolated were sensitive to

Colistin(96.84%), the last resort of drug. However, 36.53% and 35.74% isolates showed resistance to Imipenem and Meropenem respectively, followed by Piperacillin-Tazobactam (41.06%), Aminoglycosides (45.92%-49.32%) and Cephalosporins (66%-69%). Our findings correlated with the study of Mahajan *et al.* [2] who have reported high susceptibility with Colistin(96.79%). Different study have reported different rates, resistance with Piperacillin-Tazobactam (38%), 41% resistance with Carbapenems [11], in another study 42% resistance with Carbapenems [12] Similar to our study, Veenakumari *et al.* [13] has reported 60-70% resistance with Cephalosporins. These differences in rate of drug resistance might be because of the variations in type of antimicrobials being prescribed by the clinicians.

Acinetobacter species showed maximum resistance to Cephalosporins (89-92%) followed by Piperacillin(92.19%), Piperacillin Tazobactam (78.90%), Cotrimoxazole(74.85%), Aminoglycosides (67%-70%) and Carbapenems(61%-63%). Colistin was sensitive in 98.85% isolates. Our findings correlate with Rit *et al.* [6] where 94% susceptibility was reported for Colistin and 88% resistance to Cephalosporins. In another study, Mahajan *et al.* [2] reported 86.66% sensitivity to Colistin and 25% resistance to Carbapenems.

The antibiotic susceptibility patterns may change with time and may vary from hospital to hospital. Susceptibility patterns may be altered due to resistance transfer and mutant selection from indiscriminate and excessive use of antibiotics. Furthermore, our hospital being a tertiary care referral center and also most of our patients came from rural areas after visiting the local practitioners and being exposed to antibiotics. Differences in susceptibility could be attributed to these above mentioned factors.

In our study we could also isolate some rare pathogens like *Achromobacter xylosoxidans* and *Ochrobactrum anthropi* [Table 1]. Unusual biochemical results in conventional reporting alerted us regarding isolation of some unusual pathogen and this prompted us to perform identification by automated VITEK 2 system which helped us in identifying such rare pathogens.

Conclusion

To conclude, NFGNB are of significant importance, since they constitute an imminent risk to the hospitalized patients. Proper identification of NFGNB up to the species level along with monitoring of their susceptibility patterns are important for proper management of the infection caused by them. Studies like this, carried out periodically in the hospital environment, may help to control resistance rates, considering that microbiological data may contribute to the choice of the most appropriate therapy for each patient. Moreover, it is also important to establish their clinical relevance, before they can be considered as pathogens to avoid unnecessary usage of antibiotics and emergence of drug-resistant strains. Implementation of good healthcare practices in association with Hospital Infection Control Committee is the key measures for the management of hospital-acquired infections caused by this group of bacteria.

CONFLICT OF INTEREST – None

Table1: Frequency of isolation of NFGNB (n=1274)

Organisms isolated	Number	Percentage
<i>Pseudomonas spp.</i>	884	69.39%
<i>Acinetobacte</i> spp.	346	27.16%
<i>Burkholderiacepacia complex</i>	20	1.57%
<i>Stenotrophomonas maltophilia</i>	11	0.86%
<i>Sphingomonas paucimobilis</i>	9	0.71%
<i>Achromobacter xylosoxidans</i>	2	0.16%
<i>Ochrobactrum anthropi</i>	2	0.16%
Total	1274	100%

Table2: Distribution of NFGNB isolates in clinical samples

ORGANISM	RESPIRATORY	PUS	BLOOD	URINE	CENTRAL LINE	VITREOUS	ASCITIC FLUID	CORNEAL SCRAPING
ISM	ATATORY SAMPL ES		D	E	AL LINE TIP	REO US TAP	TIC FLUI D	CORN SCRA PING
<i>Pseudomonas spp.</i>	373 (42.19%)	330 (70.37%)	70 (9.7%)	103 (11.65%)	3 (0.33%)	1 (0.11%)	3 (0.33%)	1 (0.11%)

<i>Acinetobacter</i> spp.	155 (44.79%)	93 (26.87%)	55 (15.89%)	39 (11.27%)	4 (1.15%)	0	0	0	0	<i>Stenotrophomonas maltophilia</i>	6(54.54%)	0	5(45.45%)	0	0	0	0	0
<i>Burkholderia Cepacia complex</i>	8(40%)	2(10%)	9(45%)	0	1(5%)	0	0	0	0	<i>Ochrobactrum Anthropi</i>	1(50%)	0	1(50%)	0	0	0	0	0
<i>Achromobacter xylosoxidans</i>	1(50%)	0	1(50%)	0	0	0	0	0	0	<i>Sphingomonas paucimobilis</i>	4(44.44%)	0	5(55.55%)	0	0	0	0	0
										TOTAL	548(43.01%)	425(33.36%)	146(11.46%)	142(11.15%)	8(0.62%)	1	3	1

Table 3: Antimicrobial resistance pattern of NFGNB isolates (n=1274).

	<i>Pseudomonas</i> spp.	<i>Acinetobacter</i> spp.	<i>Burkholderia cepacia complex</i>	<i>Achromobacter xylosoxidans</i>	<i>Stenotrophomonas</i>	<i>Ochrobactrum anthropi</i>	<i>Sphingomonas maltophilia</i>
PI	574(64.93%)*	319(92.19%)	8(40.00%)	1(50.00%)	6(54.54%)	1(50%)	7(77.77%)
PiT	363(41.06%)	273(78.90%)	6(30.00%)	1(50.00%)	2(18.18%)	1(50%)	2(22.22%)
A/S	--	289(83.52%)	--	---	---	-	-
TE	--	267(77.16%)	--	---	---	-	-
COT	--	259(74.85%)	--	---	---	-	-
CIP	480(54.29%)	267(77.16%)	6(30.00%)	0	7(63.63%)	1(50%)	4(44.44%)
CAZ	588(66.51%)	319(92.19%)	10(50.00%)	2(100%)	9(81.81%)	1(50%)	8(88.88%)
CTR	--	321(92.77%)	--	---	-	-	-
CPM	610(69.00%)	310(89.59%)	6(30.00%)	2(100%)	10(90.90%)	1(50%)	8(88.88%)
AZ	556(62.89%)	--	8(40.00%)	2(100%)	9(81.81%)	2(100%)	8(88.88%)
GEN	436(49.32%)	244(70.52%)	IR	0	4(36.36%)	2(100%)	5(55.55%)
AK	413(46.71%)	236(68.20%)	IR	0	4(36.36%)	2(100%)	5(55.55%)
TOB	406(45.92%)	232(67.05%)	IR	0	4(36.36%)	2(100%)	5(55.55%)
MRP	316(35.74%)	212(61.27%)	4(20.00%)	0	2(18.18%)	1(50%)	2(22.22%)
IMP	323(36.53%)	219(63.29%)	2(10.00%)	0	2(18.18%)	1(50%)	2(22.22%)
CL	28(3.16%)	4(1.15%)	IR	0	1(9.09%)	0	1(11.11%)

PI:Piperacillin, PiT:Piperacillin-Tazobactam, A/S:Ampicillin-Sulbactam, TE:Tetracycline, COT:cotrimoxazole, CIP:Ciprofloxacin, CAZ:Ceftazidime, CTR:Ceftriaxone, CPM:Cefepime, AZ:Aztreonam, GEN:Gentamicin, AK:Amikacin, TOB:Tobramycin, MRP:Meropenem, IMP:Imipenem, CL:Colistin, IR:Intrinsic Resistance

*showing % resistance

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