IDENTIFICATION OF NONFERMENTATIVE GRAM–NEGATIVE BACILLI ISOLATED FROM CLINICAL SPECIMENS

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ABSTRACT

BACKGROUND
Nonfermentative Gram-negative bacilli (NFGNB), once regarded as contaminants, have now gained high relevance as opportunistic pathogens which are usually multidrug resistant.

AIM
Identification of nonfermentative Gram-negative bacilli isolated from clinical samples during the study period and to determine the antibiotic susceptibility pattern of Pseudomonas aeruginosa.

MATERIALS AND METHODS
The study was conducted in the Department of Microbiology at a Tertiary Care Teaching Hospital for a period of 6 months. NFGNB isolated from clinical samples were identified by standard procedures and antibiotic susceptibility test was performed for the most frequently isolated nonfermenter, P. aeruginosa.

RESULTS
Majority of the NFGNB isolates were recovered from pus samples (36.36%), miscellaneous specimens ranking second (33.52%). P. aeruginosa was the most common isolate (76.70%) followed by Acinetobacter baumannii (15.90%). P. aeruginosa isolates were most susceptible to piperacillin/tazobactam (71.85%), followed by meropenem (71.11%), and least susceptible to tobramycin (31.85%).

CONCLUSION
As NFGNB have emerged as an important group of organisms responsible for causing multidrug resistant infections particularly in hospitals, their identification to the species level is necessary. Maintenance of a high quality of infection control practices and judicious use of antibiotics are the cornerstone in the control of these microbes.

KEYWORDS
Nonfermentative Gram-negative bacilli, Identification, Multidrug resistant infections.

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INTRODUCTION: Nonfermentative Gram-negative bacilli (NFGNB) are physiologically versatile group of bacteria that flourish as saprophytes in warm moist situations in the human environment. They were earlier thought to be of little clinical significance.(1,2) But, they are now recognised as opportunistic pathogens that cause infections mainly in debilitated and immune compromised individuals. They have been incriminated in infections such as bacteraemia, meningitis, pneumonia, urinary tract infections, surgical site infections, wound infections, osteomyelitis, etc.(3) Many potential reservoirs of infection have been identified including respiratory equipment, cleaning solutions, disinfectants, sinks, endoscopes etc.(3,4,5) An important source of colonization of NFGNB is the hospital water supply.(6) This widespread nature is mainly due to their ability to thrive in environment with minimal nutrients and also due to their innate resistance to several disinfectants and antibiotics. Many studies report Pseudomonas aeruginosa (P. aeruginosa) as the most frequently isolated nonfermenter, followed by Acinetobacter baumannii (A. baumannii).(7,8,9) The potential of P. aeruginosa to cause infection at anybody site is because of the wide array of virulence factors it produces and also its ability to counteract host defences.(10)

Multidrug resistant (MDR) nonfermenters have already got established in the hospital environment. The crisis of MDR infections is especially problematic in ICUs which have the highest burden of MDR Gram-negative bacteria.(11) MDR Acinetobacter spp. are often responsible for causing hospital outbreaks.(12) The digestive tracts of ICU patients are important reservoirs of MDR A. baumannii.(13) Another important nonfermenter is Burkholderia cepacia (B. cepacia) which is one of the most antibiotic-resistant organisms encountered in the laboratory.(14) The major impact of B. cepacia infection is in patients with cystic fibrosis.(15)

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Multiple drug resistance, with special reference to carbapenem resistance among P. aeruginosa and Acinetobacter spp. has complicated the management of infections caused by these organisms. P. aeruginosa produces multiple β-lactamases including AmpC, metallo β-lactamases (MBL), KPCs, and OXA enzymes and also express resistance by efflux pumps and changes in outer membrane permeability.[16] Acinetobacter spp. exhibit antibiotic resistance by means of β-lactamases, aminoglycoside-modifying enzymes and efflux pumps.[12] MDR infections make treatment options limited. This has led to the revival of polymyxins, an old class of polypeptide antibiotics.[17] Considering all these issues, identification of NFGNB to species level has become important. Hence, this particular study was aimed at identifying and characterizing NFGNB isolated from clinical specimens.

MATERIALS & METHODS: The study was a hospital based one. During the study period of 6 months, NFGNB isolated from various clinical specimens in the Microbiology Department were further identified. A total of 176 NFGNB isolates were studied. Of these, 64 isolates were from pus, 59 isolates from miscellaneous specimens such as tissue, drain tip, catheter tip, and bronchial wash, 36 from sputum, 11 from urine, 5 from body fluids including CSF and 1 from blood. The NFGNB isolated were either as pure growth in culture or as the predominant isolate in a mixed culture. An organism was considered a nonfermenter if it failed to produce acid in routine glucose medium or utilised glucose oxidatively.[18] Growth on triple sugar iron agar slant with no growth extending into the butt and no acidification of the slant was taken as that of a nonfermenter.

Identification of the NFGNB were done by studying various characteristics such as odour, colony morphology, pigment production, Gram staining, motility, biochemical reactions, and antibiotic susceptibility test results.

The culture media used to study the colony characteristics were blood agar, MacConkey agar, and nutrient agar. The biochemical tests done were oxidase test, Indole production, TSI agar, mannitol motility medium, urea hydrolysis, citrate utilization, nitrate reduction, Hugh-Leifson's OF medium, decarboxylases, phenyl alanine deaminase, acetamide utilisation, aesculin hydrolysis, DNAse, MacConkey agar with 10% lactose, and Nutrient broth with 6.5% NaCl.

Antibiotic susceptibility testing was done by Kirby-Bauer method on Mueller-Hinton agar. Antibiotic susceptibility test was performed only for P. aeruginosa since it was found to be the most frequently isolated NFGNB. The drugs used for testing P. aeruginosa included piperacillin, ceftazidime, cefoperazone, amikacin, gentamicin, netilmicin, tobramycin, ciprofloxacin, piperacillin/ tazobactam, cefoperazone/ sulbactam, and meropenem.

RESULTS: Of the 176 nonfermenters isolated from various clinical samples during the study period, 64(36.36%) were obtained from pus samples, 59(33.52%) from miscellaneous specimens, 36(20.45%) from sputum, 11(6.25%) from urine, 5(2.84%) from body fluids, and 1(0.57%) from blood (Table 1). The NFGNB isolated during the study were Pseudomonas aeruginosa (135 isolates, 76.70%), Acinetobacter baumannii (28 isolates, 15.90%), Pseudomonas stutzeri (5 isolates, 2.84%), Burkholderia cepacia (4 isolates, 2.27%), Ralstonia pickettii (1 isolate, 0.56%), Achromobacter piechaudii (1 isolate, 0.56%), Elizabethkingia meningoseptica (1 isolate, 0.56%), and Acinetobacter lwoffii (1 isolate, 0.56%) (Table 2). P. aeruginosa was the most frequently isolated nonfermenter in the study.

Being the most common nonfermenter, antibiotic susceptibility test was performed only for P. aeruginosa. The isolates were most susceptible to piperacillin/tazobactam (71.85%), followed by meropenem (71.11%) and cefoperazone/sulbactam (63.70%). The isolates were least susceptible to tobramycin (31.85%) (Tables 3 & 4). Amikacin was the most effective aminoglycoside in vitro (49.63%). This was followed by gentamicin (37.78%), netilmicin (34.81%), and tobramycin (31.85%) (Table 5).

Specimen	No. of non-fermenter isolates	Percentage (%)
Pus	64	36.36%
Miscellaneous	59	33.52%
Sputum	36	20.45%
Urine	11	6.25%
Body fluids	5	2.84%
Blood	1	0.57%

Table 1: Distribution of non-fermenters in various clinical specimens

Organism	Number	Percentage (%)
Pseudomonas aeruginosa	135	76.70%
Acinetobacter baumannii	28	15.90%
Pseudomonas stutzeri	5	2.84%
Burkholderia cepacia	4	2.27%
Ralstonia pickettii	1	0.56%
Achromobacter piechaudii	1	0.56%
Elizabethkingia meningoseptica	1	0.56%
Acinetobacter lwoffii	1	0.56%

Table 2: List of non-fermenters isolated from clinical specimens

Antibiotic	No. susceptible	% susceptible
Piperacillin	75	55.56%
Ceftazidime	68	50.37%
Amikacin	67	49.63%
Cefoperazone	64	47.41%
Ciprofloxacin	55	40.74%
Gentamicin	51	37.78%
Netilmicin	47	34.81%
Tobramycin	43	31.85%

Table 3: Antibiotic susceptibility pattern of pseudomonas aeruginosa

Antibiotic	No. susceptible	% susceptible
Piperacillin/Tazobactam	97	71.85%
Meropenem	96	71.11%
Cefoperazone/Sulbactam	86	63.70%

Table 4: Antibiotic susceptibility pattern of pseudomonas aeruginosa to higher-level antibiotics

No. of isolates	GENTA		AMIK		NETIL		TOBRA	
	No.	%	No.	%	No.	%	No.	%
135	51	37.78	67	49.63	47	34.81	43	31.85

Table 5: Susceptibility pattern of pseudomonas aeruginosa to aminoglycosides

Genta=Gentamicin, Amik=Amikacin, Netil=Netilmicin, Tobra=Tobramycin.

DISCUSSION: 176 nonfermenters were isolated during the study period of which the majority (64 isolates, 36.36%) were obtained from pus samples followed by 59(33.52%) from miscellaneous specimens. This correlates with the data published by many authors. Malini A. et al[19] isolated 62.2% of nonfermenters from pus and 11.92% from urine samples. Gokale SK et al[20] also reports higher isolation rate of NFGNB from pus (58.4%). In an article by Bhatnagar R et al,[21] majority of nonfermenters were obtained from pus samples (49.20%), the next common being sputum (19.84%). But in the study by Nautiyal et al,[8] nonfermenters were most frequently isolated from respiratory specimens (42.33%) followed by pus samples (28.6%).

P. aeruginosa was the most frequently isolated nonfermenter (135 isolates, 76.70%) followed by A. baumannii (28 isolates, 15.90%). This agrees with the findings of Patel et al[22] (P. aeruginosa 76.97%, A. baumannii 21.36%), Malini A. et al[19] (P. aeruginosa 53.8%, A. baumannii 22.2%), Nautiyal S et al[8] (P. aeruginosa 62.92%, A. baumannii 21.05%), Benachimardi et al[7] (P. aeruginosa 60%, A. baumannii 22%), and Gokale et al[20] (P. aeruginosa 82.3%, A. baumannii 15.4%). In a study by El-Mahallawy et al,[23] A. baumannii was the most frequently isolated nonfermenter (51.9%) followed by Pseudomonas spp. (45.6%).

Antibiotic susceptibility test was performed for P. aeruginosa. Majority of the strains were susceptible to piperacillin/tazobactam (71.85%) which tallies with a study by Benachinmardi et al[7] (73.3%). The P. aeruginosa strains were least susceptible to tobramycin (31.85%).

Carbapenems, particularly imipenem and meropenem have been widely used for the management of infections caused by multidrug-resistant P. aeruginosa infections. Several mechanisms of resistance to antibiotics exist in P. aeruginosa strains. Reduced porin expression due to loss of OprD, overexpression of efflux pumps, and production of metallo β-lactamases (MBL) are the important mechanisms.[23,24,25] Resistance in P. aeruginosa to imipenem is primarily due to OprD loss, whereas resistance to meropenem was mainly due to the presence of MexAB-OprM efflux pump. The percentage susceptibility of P. aeruginosa isolates to meropenem in our study was 71.11%. Many studies have used imipenem to check carbapenem susceptibility in P. aeruginosa and were found to be highly effective in vitro.[7,19,22] Gokale etal[20] reported that the P. aeruginosa strains in their study were highly susceptible to meropenem (96.2%), which was followed by ciprofloxacin (50.4%). Only 40.74% of P. aeruginosa isolates in our study were susceptible to ciprofloxacin. Increased use of fluoroquinolones can lead to carbapenem resistance in P. aeruginosa. Amikacin exhibited the highest susceptibility among the aminoglycosides (49.63%). This result tallies with that of several other studies.[7,19,20]

CONCLUSION: A total of 176 nonfermenters isolated from clinical samples were identified. Majority of the NFGNB (64 isolates, 36.36%) were obtained from pus samples. This was followed by 59 nonfermenters (33.52%) from miscellaneous specimens. P. aeruginosa was the most common isolate (76.70%) followed by A. baumannii (15.90%). The P. aeruginosa isolates were most susceptible to piperacillin/tazobactam (71.85%) and least susceptible to tobramycin (31.85%). The nonfermenters continue to pose a significant challenge to effective therapeutic strategies because of their intrinsic and acquired resistance to many antibiotics. The NFGNB, in particular P. aeruginosa, are responsible for numerous hospital-acquired infections in immune compromised and noncompromised patients, continually challenging infection control practices. Improved infection control measures and judicious antibiotic usage are necessary to contain the emergence and spread of multiply drug resistant nonfermenters in health care setting. Moreover, timely dissemination of the local antibiogram will aid the clinician in choosing the appropriate antibiotic.

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