Occurrence of Non-Fermenting Gram-Negative Bacilli and Their In Vitro Susceptibility Pattern by Vitek 2 at a Tertiary Care Teaching Hospital – An Observational Study

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ABSTRACT

BACKGROUND

Non fermenting gram-negative bacilli (NFGNB) are a group of heterogenous, aerobic and non-sporing saprophytic bacteria, found as commensals in humans and other animals primarily causing opportunistic healthcare-associated infections**.** They are innately resistant to many antibiotics and are known to acquire resistance by various mechanisms. They pose a particular difficulty for the healthcare community because multidrug resistance is common and increasing among them and a number of strains have now been identified that exhibit pan drug resistance. This study was conducted to isolate and identify various non-fermenter gram negative bacilli (NFGNB), to study their antibiotic sensitivity pattern and their clinical significance from various clinical samples.

METHODS

A study was undertaken from March 2019 to February 2020 to isolate NFGNB from various clinical samples received for culture and sensitivity in the department of microbiology in a tertiary care hospital, Ahmedabad. Non lactose fermenting colonies on MacConkey agar plates were further processed by Vitek 2 to identify them and to study their antimicrobial susceptibility testing (AST).

RESULTS

A total of 2010 NFGNB were isolated from various clinical samples and their AST was evaluated by Vitek 2. *Pseudomonas aeruginosa* (52.7 %) and *Acinetobacter baumannii* (36.5 %) were the most common NFGNB isolated. Carbapenem resistance was 93 % for Acinetobacter species and 61 % for Pseudomonas species.

CONCLUSIONS

Accurate and rapid identification and antimicrobial susceptibility testing of NFGNB help in early initiation of appropriate antimicrobial therapy and proper management of patients thereby help in reducing emergence of MDR strains of NFGNB, mortality and overall hospital stay.

KEYWORDS

NFGNB – Non-Fermenting Gram-Negative Bacilli, Multidrug Resistance, Pan Drug Resistance, Carbapenem Resistance

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BACKGROUND

Non-fermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, non-spore forming bacilli that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation.[1] They are ubiquitous in nature, found as saprophytes inhabiting soil or water and some are also found as commensals in human and animal gut.[2]

In the hospital environment, they have been isolated from instruments such as ventilator machine, humidifiers, suction tubes, mattresses, other equipment and even from the skin of healthcare workers.[3] They can cause device associated nosocomial infections and have the potential to spread from patient-to-patient via fomites or the hands of health care workers.[4,5] These organisms are niche pathogens that primarily cause opportunistic healthcare-associated infections in patients who are critically ill or immunocompromised.[5]

NFGNB are innately resistant to many antibiotics and are known to acquire resistance by producing extended spectrum beta lactamase and metallobetalactamase.[2] They pose a particular difficulty for the healthcare community because multidrug resistance is common and increasing among them and a number of strains have now been identified that exhibit resistance to all commonly used antibiotics. A variety of multidrug resistance makes treatment of infections caused by these pathogens both difficult and expensive.

NFGNB accounts for nearly 15 % of all gram-negative bacilli cultured from clinical specimens in a clinical microbiology laboratory.[2,3] The important members of the group include *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Acinetobacter lwoffii*, *Stenotrophomonas maltophilia*, Myroides spp. and *Burkholderia cepacia*. They cause various infections such as septicaemia, meningitis, pneumonia, urinary tract infections and surgical site infections (SSI).[2]

Identifying them in a routine microbiological laboratory was a diagnostic challenge as many of them are slow growers requiring battery of biochemical tests for their identification. This is not only time consuming and cumbersome, but many of them were also either misidentified or remained unidentified. Many of them were intrinsically resistant to various antibiotics. So, an automated system which identified them rapidly and accurately up to species level was indeed a need of an hour. Several automated systems were then developed and evaluated. Vitek-2 compact system is one such system developed by Biomerieux detects metabolic changes by fluorescence-based methods which facilitate the identification of gram-negative bacteria (GN cards) within 6 hours reducing turnaround time for identification**.** The instrument also processes the antimicrobial susceptibility cards (AST cards) until MIC's are obtained. The system monitors the kinetics of bacterial growth and calculates minimum inhibitory concentrations (MIC) using a unique algorithm.[6] So the current study was undertaken to isolate and identify NFGNB and to know their antibiotic susceptibility pattern by Vitek 2 method with their clinical significance.

Objectives

1. To isolate and identify non-fermenting gram negative bacilli from various clinical samples
2. To study antimicrobial susceptibility pattern (AST) of NFGNB
3. To get recent trend of antibiotic resistance in NFGNB

METHODS

The present observational study was conducted in department of Microbiology of a 1500 bed tertiary care teaching hospital, Ahmedabad during the period from March 2019 to February 2020. A total of 21053 samples were received for bacterial culture from OPD as well as admitted patients in different wards. Samples were plated on blood agar and MacConkey agar and incubated at 37^0 c. for 18 - 24 hours. The isolates that showed non lactose fermenting colonies on MacConkey agar or that do not grow on MacConkey agar were provisionally considered as non-fermenting gram-negative bacilli. Further identification was done on the basis of TSI changes and their identification as well as antimicrobial susceptibility testing was performed by Vitek 2 compact system using gram-negative identification card and antimicrobial susceptibility testing cards (GN ID and AST) (280 or 281). The cultures were incubated for 48 hours before declaring them as negative. To rule out chances of contamination, for specimens from non-sterile sites like urine and respiratory specimen like bronchoalveolar lavage (BAL) or endotracheal aspirate (ET), quantitative cultures were done and only those showing > 10^5 cfu / ml were considered for follow up. Whenever in doubt, a repeat sample was requested and only those showing growth of same organism in repeat culture were included in study.

Data was collected in a record form having primary details regarding specimen number, date of sample collection, date of reporting, specimen collection site and specimen type. The specimen record form has additional information whether specimen was positive or negative in culture, name of organisms isolated, colony count and their minimum inhibitory concentration (MIC) value in µg / ml and their final interpretation as sensitive (S), resistant (R) or intermediate (I) as per Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines. Data so obtained as per case record form was presented in tabulated form as site wise distribution of clinical samples (Table 1 – whether OPD or indoor), specimen wise distribution of samples (Table 2), species distribution of NFGNB (Table 3) and their antimicrobial susceptibility pattern (Table 4). Data was further analysed using MS-Excel software.

Identification of NFGNB

The identification card (GN ID) contains substrate for various biochemical tests including tests for sugar assimilation, sugar fermentation, decarboxylase tests and other miscellaneous tests (urease, utilisation of malonate and tryptophan deaminase). Identification cards were inoculated with microorganism suspensions of 0.5 McFarland standards

from a plate of pure culture using an integrated vacuum apparatus.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing with Vitek 2 compact system was performed using N281 card according to the manufacturer's instructions. Antibiotics tested in AST N281 card included levofloxacin, gentamicin, cefepime, meropenem, imipenem, ticarcillin / clavulanic acid, doripenem, ceftazidime, cefoperazone / sulbactam, amikacin, ciprofloxacin, minocycline, tigecycline, colistin, trimethoprim / sulfamethoxazole (cotrimoxazole), cefotaxime, piperacillin / tazobactam, cefuroxime, ceftriaxone, tobramycin.

The Vitek-2 System automatically processes the antimicrobial susceptibility cards until MIC's are obtained. The Vitek-2 compact system subsequently corrects MIC where necessary as per clinical category in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations.[4]

Quality Control

The Vitek 2 compact machine was validated using the standard strain as per manufacturer's instructions. *Pseudomonas aeruginosa* American Type Culture Collection (ATCC) 27853 strain was used. During the study period, the control strain was checked at every 15 days.

RESULTS

A total of 21053 clinical samples received from in-patient and Out Patient Departments of SVP hospital, NHL Medical College, Ahmedabad during period from March 2019 to February 2020 were included in study. Majority of samples were received from indoor patients (95.01 %) including wards, emergency room (ER), intensive care unit (ICU) & critical care unit (CCU) and only 5.05 % (1065) samples received from OPD.

Majority of samples received were blood (34.5 %), urine (29.5 %), wound swabs (11.5 %), respiratory secretions (11.6 %) and fluids (5.6 %). A total of 6354 (30 %) of samples were positive in culture growing 6932 different isolates. Culture positivity was found maximum in endotracheal specimens (94 %) followed by wound swab (66 %) while lowest culture positivity was found in blood specimen (15 %). Some samples have grown more than one organism. Samples showing 2 or more isolates were repeated and only those isolates confirmed by repeated isolations were considered for study. Out of total of 6932 isolates, NFGNB were 2010 in number. (Isolation rate of 29 %). NFGNB were maximally isolated from respiratory specimen, endotracheal specimen, wound swabs and catheter tips. *P. aeruginosa* (52.7 %) and *A. baumannii* (36.5 %) were the commonest isolates followed by *Stenotrophomonas maltophilia* (2 %). *Myroides* spp. and *A. lwoffii* were isolated from 36 samples each (1.8 %). Other significant isolates were *Burkholderia cepacia* (N = 20, 1 %), *Pseudomonas putida* (N = 18, 0.9 %) and *Sphingomonas paucimobilis* (N = 11, 0.54 %). *Acinetobacter junii* was isolated from 7 samples while *Elizabethkingia meningosepticum*, *P. fluorescens* and *Chryseobacterium indologenes* were isolated in 5 samples. Other isolates were Achromobacter, *Ochrobactrum anthropic*, *Ralstonia mannitolilytica*, *Delftia acidovorans*, *B. pseudomallei*, & *Oligella ureolytica*.

Pseudomonas aeruginosa was the commonest isolate and was most susceptible to colistin (90.89 %) and least susceptible to ticarcillin / clavulanic acid (16.43 %). Carbapenem resistance was found in almost around 60 %. Amongst carbapenem, highest resistance was noted against meropenem (Sens - 38.68 %) followed by doripenem (Sens - 42.51 %). Imipenem was found to be most effective amongst carbapenem with sensitivity of 44.4 %. Piperacillin-tazobactam (Sens - 34.4 %) and aminoglycosides (Sens - 44 %) once used very frequently for pseudomonas infection were also found to be less sensitive. Resistance to ceftazidime (Sens - 41.74 %), cefepime (Sens - 46.29 %), ciprofloxacin (Sens - 38.75 %) and levofloxacin (Sens - 31.54 %) was also very high. Amongst fluoroquinolones, ciprofloxacin was found better than levofloxacin.

Acinetobacter spp. were most susceptible to colistin (Sens - 97.78 %) and tigecycline (Sens - 82.57 %). Minocycline is another drug showing good sensitivity against *Acinetobacter baumannii* (Sens - 53.4 %). Majority of other antibiotics showed high resistance of nearly 90 %. These were ceftazidime (Sens - 5.71 %), fluoroquinolones and aminoglycosides. Amongst fluoroquinolones, there was not much difference in susceptibility to ciprofloxacin (Sens - 6.36 %) and levofloxacin (Sens - 7.53 %). Carbapenem resistance was as high as 93 % for imipenem, meropenem and doripenem. Cefoperazone + sulbactam once thought to be active drug also showed poor susceptibility (Sens - 11 %) against *A. baumannii*.

	OPD	ER	Indoor Wards	ICU	Total
Total number	1065 (5.05 %)	250 (1.18 %)	18956 (90.03 %)	782 (3.71 %)	21053 (100 %)

Table 1. Site Wise Distribution of Clinical Samples

	OPD	Indoor
Sample received	1065	19988
Culture positive	276 (25.9 %)	6070 (30.3 %)
Number of isolates	294	6638
Number of NFGNB	69 (23.4 % of isolates)	1941 (29.2 % of isolates)

Table 1. B. Site Wise Distribution of NFGNB

Sl. No.	Type of Sample	Total Number Received		Culture Positive		Number of Isolates		
		Number	%	Number	%	Total	NFGNB	% NFGNB
1	Blood	7277	34.57	1082	14.87	1107	106	9.57
2	Urine	6208	29.49	1484	23.90	1548	249	16.08
3	Wound swab	2428	11.53	1615	66.52	1831	680	37.13
4	Sputum	1324	6.29	467	35.27	503	174	34.59
5	Fluids	1172	5.57	142	12.12	154	37	24.02
6	Endotracheal tube / secretion / BAL	1126	5.35	1062	94.32	1232	645	52.35
7	Pus	459	2.18	167	36.38	186	22	11.82
8	Stool	435	2.07	33	7.59	34	0	0
9	Catheter tip	118	0.56	44	37.29	47	17	36.17
10	Tissue	506	2.40	258	50.99	290	80	27.58
	Total	**21053**	**100.00**	**6354**	**30.18**	**6932**	**2010**	**28.99**

Table 2. Specimen Wise Distribution of NFGNB

In *S. maltophilia*, 34 strains out of 41 were susceptible to trimethoprim-sulfamethoxazole (Sens - 84 %) and 33 strains were susceptible to levofloxacin (Sens - 80.7 %). *B. cepacia* showed resistance to almost all drugs except minocycline (Sens - 64.3 %) and tigecycline (Sens - 57.1 %).

On observing the antimicrobial resistance pattern many isolates were seen to be resistant to 3 or more drugs. Multidrug resistance was very high amongst NFGNB. Pan drug resistance was also found in *P. aeruginosa*, *A. baumannii* and *B. cepacia*.

Sl. No.	Bacterial Species	Number	%
1	*Pseudomonas aeruginosa*	1061	52.79
2	*Acinetobacter baumannii*	735	36.57
3	*S. maltophilia*	41	2.04
4	Myroids species	36	1.79
5	*Acinetobacter lwoffii*	36	1.79
6	*Burkholderia cepacia*	20	1
7	*Pseudomonas putida*	18	0.90
8	*Sphingomonas paucimobilis*	11	0.55
9	*P. stutzeri*	8	0.40
10	*Acinetobacter junii*	7	0.35
11	Achromobacter species	6	0.30
12	*Chryseobacterium indologenes*	5	0.25
13	*P. fluorescens*	5	0.25
14	*Elizabethkingia meningoseptica*	5	0.25
15	*Ochrobactrum anthropi*	3	0.15
16	*Ralstonia mannitolilytica*	3	0.15
17	Acinetobacter spp	2	0.10
18	*Acinetobacter ursingii*	2	0.10
19	*Delftia acidovorans*	2	0.10
20	*Burkholderia pseudomallei*	2	0.10
21	*Chryseobacterium gleum*	1	0.05
22	*Oligella ureolytica*	1	0.05
	Total	**2010**	**100**

Table 3. Species Distribution of NFGNB

	AST distribution (281)	*P. aeruginosa*	*A. baumannii*	*B. cepacia*	*S. maltophilia*	Myroids spp.	Pseudomonas other than *P. aeruginosa*	Acinetobacter other than baumannii complex	Others
		%	%	%	%	%	%	%	%
1	Amikacin	44.39	-	-	-	-	57.69	69.44	7.41
2	Cefepime	46.29	6.93	-	-	-	51.85	61.11	25.93
3	Cefoperazone + sulbactam	38.59	10.94	-	-	-	37.04	94.12	33.33
4	Ceftazidime	41.74	5.71	20.00	-	-	44.44	46.67	29.17
5	Ciprofloxacin	38.75	6.36	5.88	-	3.13	44.44	55.88	22.22
6	Colistin	90.89	97.78	-	-	-	-	88.24	14.81
7	Doripenem	42.51	6.19	-	-	-	-	46.67	0.00
8	Gentamycin	43.79	9.28	-	-	-	62.96	75.68	11.11
9	Imipenem	44.44	6.65	-	-	3.13	42.31	55.88	37.04
10	Levofloxacin	31.55	7.54	-	80.77	6.25	40.74	48.28	20.83
11	Meropenem	38.68	6.23	12.50	-	6.25	44.44	48.48	33.33
12	Minocycline	0.00	53.49	64.29	-	96.88	70.37	93.55	75.00
13	Piperacillin + tazobactam	34.43	5.57	-	-	3.13	40.74	45.45	25.93
14	Ticarcillin+ clavulanic acid	16.43	6.52	7.14	-	-	19.23	75.00	29.17
15	Tigecycline	-	82.57	57.14	-	12.50	50.00	94.12	40.74
16	Cotrimoxazole	-	13.02	41.18	84.00	-	26.92	58.82	51.85

Table 4. Antibiotic Sensitivity Pattern of Non-Fermenters by Vitek-2 AST Cards 281

DISCUSSION

Isolation rate of around 15 % were found in most of studies while in our study it was 29 %. High isolation rate in our study was due to predominance of in-patient samples and a smaller number of OPD samples. Similar isolation rate of 31.62 % was found in study by Chang & Huang.[7] Prevalence of NFGNB varies greatly from time to time and place to place. High isolation rate of NFGNB was found in similar studies carried out by Rao & Shivananda (66.8 %) and S. Sidhu & Pushpa Devi (45.9 %).[8] Culture positivity was found maximum in endotracheal specimen (94 %) with highest isolation of NFGNB (52 %) from them followed by wound swab (37 %). NFGNB were predominantly isolated from pus or wound swab in many studies previously conducted in India.[9,10] In our study, *Pseudomonas aeruginosa* (52.7 %) was commonest isolate followed by acinetobacter spp. (36.5 %), *Stenotrophomonas maltophilia* (2 %), *Acinetobacter lwoffii* (1.79 %), Myroids species (1.79 %) and *Burkholderia cepacia* (1 %). This is in concordance with many other studies by Benachinmardi KK et al., Martino R et al. & Gales AC et al.[9,11,12]

The rates of carbapenem resistance in non-glucose-fermenting gram-negative bacilli have been gradually increasing worldwide over the last 10 years and vary geographically.[13,14] Recent studies from South Korea have reported the proportion of carbapenem resistance in acinetobacter to be as high as 32 to 56 % in hospitalized patients.[15,16] In the United States, resistance rates have been reported from approximately 34 % to as high as 62.6 % (8 – 10). Reports from the National Healthcare Safety Network (NHSN) in the United States have demonstrated an increase from 33 % carbapenem resistance in 2006 to 2007 to 60 % among acinetobacter species isolates in 2009 to 2010. Carbapenem resistance in our study was as high as 93 % for acinetobacter species and 61 % for pseudomonas species. A study from Iran by Saderi H et al. also showed imipenem resistance of 68 % in *Pseudomonas aeruginosa* from hospitalised burn patients.[17] A study from India by Agarwal S et al. also showed imipenem resistance of 90.54 % in *Acinetobacter baumannii* and 52 % in *Pseudomonas aeruginosa*.[18] Similar results of high carbapenem resistance was also observed in a study conducted by Sharma D et al. in Jaipur, India.[19]

P. aeruginosa also showed poor sensitivity to cephalosporins-ceftazidime (41.7 %) and cefepime (46.3 %), fluoroquinolones-ciprofloxacin (38.8 %) and levofloxacin (31.5 %) and aminoglycosides–gentamycin (43.8 %) and amikacin (44.4 %), while for *A. baumannii* resistance in these groups were very high compared to *P. aeruginosa.* For *A. baumannii*, sensitivity to ceftazidime was (5.7 %), cefepime (6.9 %), ciprofloxacin (6.4 %), levofloxacin (7.5 %) and gentamycin (9.3 %). Resistance in these pathogens may arise due to intrinsic mechanisms or may be acquired through mutations or plasmids. Sometimes resistance may develop during prolonged therapy, which was initially effective. A variety of resistance mechanisms have been identified including enzyme production (e.g., β-lactamases, AmpC, carbapenemase), overexpression of efflux pumps (fluoroquinolones), porin deficiencies (Beta-lactam antibiotics) and target-site alterations (aminoglycosides and fluoroquinolones). In some cases, these resistance mechanisms affect the susceptibility of individual antibiotics differently (even in the same group);

this is the reason why some isolates may be resistant to meropenem, but not imipenem, or resistant to amikacin, but not tobramycin. Results similar to our study were also observed by studies carried out in same hospital previously by Parimal Patel et al.[20] in 2013.

S. maltophilia infections are also a serious concern, as this microorganism is intrinsically resistant to a wide range of antimicrobial drugs and data on clinical effectiveness is only available for sulfamethoxazole / trimethoprim and fluoroquinolones. In our study we have found good susceptibility to both sulfamethoxazole / trimethoprim (84 %) and fluoroquinolones–levofloxacin (80.8 %). Results similar to our study were obtained by Sun E et al.[21] from China.

Colistin resistance was observed 9 % in *Pseudomonas aeruginosa* and 2.2 % in *A. baumannii*.

CONCLUSIONS

Pseudomonas aeruginosa and acinetobacter species were commonest NFGNB isolated in our study. *P. aeruginosa* showed high degree of resistance to beta-lactam, beta-lactam-beta-lactamase inhibitor combinations, fluoroquinolones, aminoglycosides and carbapenems. Colistin remained drug of choice for both isolates. *A. baumannii* had shown good susceptibility to minocycline and tigecycline too. For other NFGNB like *B. cepacia* and myroides species, minocycline is the drug of choice, showing good susceptibility. Resistant pattern among nosocomial bacterial pathogens may vary from country to country and from region to region. Different species of NFGNB have shown a varied sensitivity pattern in our study. Therefore, identification of NFGNB and monitoring their susceptibility pattern are important for proper management of these infections. Treatment of infections by NFGNB presents a therapeutic challenge to clinicians due to their increasing levels of resistance to several classes of antibiotics. Early diagnosis and institution of empirical antibiotic therapy based on recent antibiogram of institute would reduce mortality and improve patient management. The Vitek 2 compact system identifies NFGNB along with their antibiotic susceptibility pattern within a time period of 8 to 16 hours helping in early institution of therapy, thereby reducing mortality and providing better care and management of patients. Implementation of stringent antibiotic stewardship and strict infection control practices will be required to prevent the emergence or slow down the spread of multi drug and pan drug resistance strains of NFGNB.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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