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Current Trend of Nonfermenting Gram Negative Bacilli in a Tertiary Care Hospital in Trivandrum

Ashish Jitendranath, Radhika R, L. Bhargavi, Geetha Bhai and Ramani Bai

Department of Microbiology,Sree Gokulam Medical College and Research Foundation, Trivandrum - 695 607, India.

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Non fermenting gram negative bacilli initially thought to be contaminants and commensal are now an important pathogens. They frequently exhibit multi drug resistance and harbour multidrug resistant gene of the present study was to characterize the prevalence of NFGNB along with their antimicrobial sensitivity pattern among the patients coming to our hospital a tertiary care center. This study has been conducted in the Department of Microbiology at a tertiary care teaching hospital over a period of 6 months from June to November 2015. NFGNB were isolated and identified from clinical specimens by standard procedure and antibiotic sensitivity test was performed. Prevalance rate of NFGNB was found to be 4.9%. Pseudomonas aeruginosa was the most common isolate at 57.7% followed by Acinetobacter baumanii at 26.8%. Imipenem and Meropenem were found to be most sensitive followed by Piperacillin tazobactum. First and second generation drugs showed high level of resistance

Keywords: NFGNB, Pseudomonas aeruginosa, Acinetobacter baumanii, Imipenem, Multidrug resistance.

Non-fermenting Gram-negative bacilli (NFGNB) are a group of organisms that either do not utilize glucose as a source of energy or utilize it oxidatively. NFGNB can cause a significant number of infections, ranging from superficial to deep-seated and disseminated infections in immunocompromised hosts, neutropenic patients, and patients with cystic fibrosis, patients on mechanical ventilation and indwelling catheters, and patients undergoing invasive diagnostic and therapeutic procedures[1].

Inherent resistance of these bacterial agents to commonly used disinfectants and there tendency to colonize various surfaces have been pivotal in their emergence as important nosocomial pathogens[2]. NFGNB are known to account for 15% of all bacterial isolates from clinical microbiological laboratory. Basically, they are saprophytes and previously they were considered as contaminants or commensals of little significance[3]. However, recent literature review shows that these organisms are now associated with life-threatening infections such as septicemia, pneumonia, urinary tract infection, meningitis, surgical site infection, ventilator associated pneumonia (VAP), wound infection, osteomyelitis etc.[2,4] Prolonged hospital stay broad spectrum antibiotic use and underlying host factors arethe best predictors of outcome[5]. Due to rampant use of antibiotics most of these organisms are now resistant to many routinely used antibiotics causing prescription failure[3]. Hence, this study was undertaken to isolate and identify NFGNB and also to characterize the antibiotic susceptibility at a tertiary care teaching hospital

MATERIALS AND METHODS

Present study was conducted at the Department of Microbiology, Sree Gokulam Medical College and Research Foundation,

* To whom all correspondence should be addressed.
Mob.: +91-8129823337;
E-mail: ashishjit11@gmail.com

Venjaramoodu, Trivandrum over a six month period from May 2015 to Oct 2015.

All the samples received in bacteriology section of laboratory were inoculated on blood agar, MacConkey agar and incubated at 37°C for 48 h before being reported as sterile. The isolates which were non-lactose fermenting were further processed according to standard protocol. The test conducted included Gram stain for morphology, hanging drop for motility, oxidase test, catalase test, indole, methyl red, Voges–Proskauer, citrate utilization, urease production oxidative-fermentative test (Hugh-Leifson medium) for glucose, utilization of 10% lactose, gelatin liquefaction, lysine and ornithine decarboxylation, arginine dihydrolase test, growth at 42°C and 44°C, esculin hydrolysis and ONPG test. [6,7]

All the strains were confirmed by Vitek-2 test

The sensitivity test was performed by Kirby-bauer disc diffusion method using commercially available discs (Himedia).

The results were interpreted as per the CLSI guideline. [8]

RESULTS

Among 5132 clinical samples, NFGNB were isolated from 253 samples accounting for an isolation rate of 4.9%. Monomicrobial growth was

Table 1. NFGNB from various clinical samples

Sample	Nos	%
Pus	135	53.3
Sputum	89	35.3
Urine	24	9.4
Blood	2	0.7
Endotracheal aspirate	2	0.7
Pleural fluid	1	0.3

Table 2. Various NFGNB isolated

Isolate	Nos	%
Pseudomonas aeruginosa	146	57.7
Acinetobacter baumanii	68	26.8
Pseudomonas fluorescens	30	11.8
Acinetobacter lwoffi	4	1.4
Stenotrophomonas maltophilia	2	0.7
Burkholderia cepacia	2	0.7
Burkholderia pseudomallei	1	0.3

Table 3. Antibiotic sensitivity

Antibiotics	Organisms						
	Pseudomonas aeruginosa (146) Sensitivity (%)	Acinetobacter baumanii (68) Sensitivity (%)	*Pseudomonas fluorescens* (30) Sensitivity (%)	Acinetobacter lwoffi (4) Sensitivity (%)	*Stenotrophomonas maltophilia* (2) Sensitivity (%)	Burkholderia cepacia (2) Sensitivity (%)	*Burkholderia pseudomallei* (1) Sensitivity (%)
Amikacin	65.1	60.5	77.1	75	100	0	0
Ciprofloxacin	68.5	60.4	60.2	75	50	0	0
Piperacillin	20.44	16	16.5	25	50	0	0
Cotrimoxazole	40.14	30.9	44.6	25	100	0	100
Ceftazidime	62.2	52.1	58.8	50	100	50	100
Cefipime	68.8	60.7	62.5	50	100	50	100
Cefaperazone sulbactum	85	89.4	85.4	75	100	100	100
Piperacillin tazobactum	90.8	85.1	90.6	75	100	100	100
Imipenem	95	90.1	95	75	100	100	100
Meropenem	95	90.1	95	75	100	100	100

N= 253

seen in 148 (57.26%) specimens whereas 105 (42.74%) specimens showed polymicrobial growth where nonfermenters were isolated with other organisms, of which *S.aureus*, *E. coli*, *K.*pneumoniae and Citrobacter species were common.

NFGNB were isolated from 135 pus/wound site swab, 89 sputum, 24 urine, 2 from blood and endotracheal aspirate and 1 from pleural fluid.

A major chunk of the isolates were from Pus/wound site, sputum and urine sample accounting for 98% of the total isolates.

Pseudomonas aeruginosa was the commonest isolate accounting for 146 (57.7%), followed by Acinetobacter baumanii 68 (26.8%) and *Pseudomonas fluorescens* 30 (11.8%). Acinetobacter lwoffi, Stenotrophomonas maltophilia, Burkholderia cepacia and Burkholdera pseudomallei were rarely isolated and together accounted for 11 (2%) isolates.

The antibacterial sensitivity pattern of isolated NFGNB as a pathogen showed that 95% of *P. aeruginosa* were sensitive to Imipenem & Meropenem, 90% were sensitive to Piperacillin tazobactum , 85% were sensitive to Ceftazidime-Sulbactam,65 % were sensitive to Amikacin, 20% for Piperacillin and 40% for Co-trimoxazole. While the second most isolated NFGNB, A. baumanii showed highest sensitivity to Imipenem & Meropenem(90%). Similarly Pseudomonas sp. showed maximum sensitivity towards Imipenems and the least towards Piperacillin(Table 3).

Resistance pattern of all the isolates when analysed as a group against various classes of antibiotics showed that the isolates exhibited high resistance to Piperacillin and Cotrimoxazole while, the resistance against ceftazidime and cefipime was >40%. On the other hand, isolates showed a low level of resistance against piperacillintazobactam, cefoperazone-sulbactam and ceftazidime. Extremely low level of resistance was observed against imipenem and meropenem

Table 4. Isolation rate of *Pseudomonas* species and Acinetobacter species in various studies

Authors	Year	Place	Pseudomonas (%)	Acinetobacter (%)
Vijaya et al[4]	2000	Bangalore	78.1	6.1
Eltahawy and Khalaf[12]	2001	Saudi Arabia	56	34
Malini et al[10]	2009	Kolar	64.6	25.3
Sidhu et al[14]	2010	Amritsar	32.88	23.28
Juyal et al[2]	2012	Uttarakhand	49.58	43.09
Gokhale andMetgud[15]	2012	Belgaum	82.3	16
Nautiyal S et al[16]	2013	Deharadun	62.9	25.1
Benachinmardi, et al[3]	2013	Bangalore	61	34
Our study	2015	Trivandrum	57.7	26.8

DISCUSSION

Initially NFGNB were considered contaminants and commensal but their continuous isolation and association with clinical disease shows their pathogenic potential[3]. NFGNBs have emerged as important opportunistic pathogens in the increasing population of patients who are immunocompromised by their disease or medical/ surgical treatments[9]. Resistance to antimicrobials has increased over the years among NFGNB and number of strains are now resistant nearly too all commonly used antibiotics. Multi drug resistance among these organisms makes the treatment of infections caused by them difficult and expensive.[2]. NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum beta-lactamase and metallo-beta-lactamase.[10]

There are various risk factors for NFGNB to emerge as an important pathogen, they are immunosuppression, neutropenia, indwelling catheter, mechanical ventilation and invasive diagnostic and operative procedures.[3]

Most of the isolates were from pus samples. This is in concordance with most studies.[2,10,11]

In our study prevalence of NFGNB was 4.9% which is in parallel to studies by Malini et

al[10]and Benachinmardi et al[3] whose isolation rate was 4.5% and 3.9% respectively. On the other hand, Juyal et al[2]Eltahawy and Khalaf[12] V3aya et al[12]and Bhargava[11]and Sharma D [13] have reported higher rate of isolation i.e., 10%, 16%, 21.80% , 29% and 29.1%respectively. These differences in NFGNB prevalence in various isolates is most likely due to various local variables.

The commonest isolated strains in our study were *P. aeruginosa* (57.7%), and Acinetobacter species (26.8%), correlates with the data published by various workers.

There is variation in the resistance patter of NFGNB from country to country and within the same country over a period of time. Due to presence of high level of intrinsic resistance among various NFGNB we need to identify and detect antibiotic sensitivity accurately. Therefore, various international authorities emphasize that every hospital should have antibiotic policy of its own.[2]. Most of our patients were from rural background without much exposure to antibiotics. From the present study it is clear that the first and second line antibiotics showed a high level of resistance thus confirming the multi drug attributes of NFGNB.

In our study, it was observed that P. aeruginosa isolates were sensitive to most of the antibiotics as seen in other studies. Imipenem and Meropenem at 95%were found to be the most sensitive antibiotic which is concordance with Malini et al[10] and Benachinmardi et al[3] Among the Pseudomonas species high levels of resistance were recorded for piperacillin (79.66) cotrimoxazole (60%), ciprofloxacin (60%), and ceftazidime (38.85%), cefipime (38.48%).

Resistance to Amikacin at 35% was found to be similar to study done by Juyal et al [2] but is in contrast to study done by Benachinmardi et al[3].

Over 70% of strains of Acinetobacter spp. Isolated showed resistance to various antibiotics similar to other studies.[2,3,4] However, imipenem was sensitive (90%) as observed in other studies

Acinetobacter baumannii is getting more attention now a days due to its ability to form biofilms which confers on it, antibiotic resistance, and survival properties and increased virulence.[17] Higher resistance pattern was observed in A. baumannii when compared with A. lwoffi i.

CONCLUSION

Isolation of non-fermenters and their antibiotic susceptibility pattern should be regarded with all seriousness in clinical practice and clinical epidemiology because by being resistant to multiple antibiotics, their prevalence not only limits the treatment options but also act as a reservoir of drug resistance genes. They are an emerging pathogen and every effort needs to be undertaken to prevent its spread and control its infection. In our present study the NFGNB showed good sensitivity to Imipenem and Meropenem but development of resistance needs to be closely monitored.

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