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Prevalence and Susceptibility Profiles of Non-Fermentative Gram-Negative Bacilli Infection in Tertiary Care Hospital

Siddiqui Heena Kausar*, V.P. Bansal and M. Bhalchandra

Department of Microbiology, MGM Hospital and college, Aurangabad, India

**Corresponding author*

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A B S T R A C T

Non-fermenting gram negative bacilli (NFGNB) have emerged as a major cause of nosocomial infection. They can be recovered from the hospital environment, commonly cause device related infections, are often resistant to disinfectant. These NFGNB are becoming increasingly resistant to antibiotics which are used in critically ill patients. Aims: To know the prevalence and antimicrobial susceptibility profile of NFGNB from various clinical specimens. The main objectives of this study is to know the various NFGNB and the prevalence in hospital. And to study the antimicrobial susceptibility test (AST) pattern of NFGNB the isolates identified by standard bacteriological methods Vitek-2 system. AST pattern was generated by using Vitek-2 system except *Burkholderia cepaciae* complex (BCC). BCC AST was done by standard disc diffusion method. A total of 121 NFGNB for 1850 clinical specimens were isolated and identified by its bacteriological methods and Vitek-2 system. AST was performed by Vitek-2 system because method for the AST pattern of their isolated revealed variation to this sensitivity pattern. Thus correct identification of these isolates is of utmost importance for the correct antibiotic treatment. The organisms were identified by standard bacteriological and Vitek-2 system. Out of 1850 clinical specimens NFGNB we found 121 specimens with a prevalence rate. The various isolated in the present study were *Pseudomonas* species-79, *Acinetobacter*-27, *Burkholderia* species-7, *Stenotrophomonas maltophilia* (*S. maltophilia*)-4, *Sphingomonas* species-4. The AST pattern of these isolates is being studied.

Introduction

Non-fermenting gram-negative bacilli (NFGNB) including *Pseudomonas* species, *Acinetobacter* species, *Burkholderia* species, *Stenotrophomonas maltophilia* species, are emerging as an important causes of blood stream infections (BSI) world-wide particularly immune compromised patients with haematological malignancies and patients admitted in intensive care unit (ICUs) (Ramphal *et al.*, 2008). The organisms are ubiquitous in nature particularly in soil and water in the hospital environment, they may

be isolated from instruments such as ventilator machine, humidifiers, mattresses and other equipment as well as from the skin of health care workers (Kiran Chawla *et al.,* 2013). Non-fermenting bacteria associated with nosocomial infections are becoming increasingly resistant to commonly used antimicrobial agents (Kalidas Rit *et al.,* 2013). The high intrinsic resistance of these bacteria to different antimicrobial agents, thus there is a value of proper identification that comes to the forefront. *Burkholderia cepacia* complex (BCC) is intrinsically resistant to many β-lactam drugs, aminoglycosides, colistin and polymyxin B, the first line therapeutics of choice against Pseudomonal infections. Limited sensitivity of BCC isolates to meropenem is significant as it is one of the first line drugs against *Pseudomonas* species. There are few studies that provide the antibiogram data of NFGNB especially, and this may vary in every hospital set up knowing the antibiogram data of NFGNB in our set up will be very useful in deciding appropriate treatment strategies for these bacteria. With above facts in mind we undertook this study to identify various NFGNB from various clinical specimens in our hospital settings and to know their antibiotic sensitivity pattern in our set up.

Materials and Methods

Study material and place

121 isolates of NFGNB from 1850 clinical specimens received in microbiology department of MGM hospital Aurangabad were included in this study. The specimens were blood, pus, urine, BAL, ET secretion and various body fluids. The study was carried out after the approval of Ethical committee of institution.

Study period

May 2015- December 2015.

Materials and Methods

All specimens except blood were inoculated on Blood Agar and MacConkey's Agar.

Blood specimens were inoculated in Bac T Alert blood culture system. Subcultures from these were made on Blood Agar and MacConkey's Agar when the system fledged the bottles positive.

Presumptive identification of isolates were made by a combination of conventional bacteriological methods like colony morphology, gram staining, biochemical tests and Vitek-2 system was used for confirmation and identification of these bacteria was important. Organisms that failed to acidify the butt of TSI media were considered as non-fermenting organisms.

AST was done of these isolates excluding *Burkholderia* species on the Vitek-2 system.

Antibiotic tested usingVitek-2 system are Ticarcillin/Clavulanic acid (TI), Piperacillin/ Tazobactam (PI/TAZ), Ceftazidime (CAZ), Cefoperazone/ Sulbactam (CFS), Cefepime (CPM), Doripenem (DOR), Imipenem (IM), Meropenem (MRP), Amikacin(AK), Gentamycin (GEN), Ciprofloxacin (CIP), Levofloxacin (LE), Minocyclin (MI), Tigecycline (TG), Colistin (CL) and Trimethoprim/ Sulfamethoxzole (COT).

The Vitek system for some unknown reason was not giving AST result of *Burkholderia* species so for *Burkholderia* species AST was done manually on Muller Hilton Agar (MHA) using Kirby-Baeur disc diffusion method, using for the following antibiotics as per CLSI guidelines. Ceftazidime (CAZ), Trimethorpim/Sulfamethoxzole (COT), Levofloxacin (LE), Minocyclin (MI), Chloramphenicol (C) and Meropenem (MRP).

Results and Discussion

Results will be discussed in tabular form under following heading.

To study the antimicrobial susceptibility test (AST) pattern of NFGNB as to prepare guidelines for treatment of infection caused by these organisms.

Organisms isolated from specimens.

AST pattern of the organisms isolated from specimens.

A total of 121 (11.62%) NFGNB were isolated from 1850 clinical specimens. The various clinical specimens from which NFGNB were isolated are pus (38), urine (21), blood (16), sputum (6), BAL (6), ET secretions (8) and other body fluids (26). The predominant isolate was *Pseudomonas* species (79), followed by *Acinetobacter* (27), *Burkholderia cepacia* complex (7), *Stenotrophomonas maltophilia* (4), and *Sphingomonas paucimobilis* (4) (Table 1).

In this study the two major isolates *Pseudomonas* (79) and *Acinetobacter* (27) accounted for 87.6% of all isolated NFGNB. Both showed 100% sensitivity towards colistin. For other antibiotics *Pseudomonas* gave a decent sensitivity for Meropenem (75.9%), Amikacin (75.9%), Doripenem (74.6%). Whereas *Acinetobacter* had a good sensitivity of 92.5% for Tigecyclin. For *Burkholderia cepacia* complex 100% sensitivity was for Trimethoprim/ Sulfamethoxzole and a sensitivity around 85% for Ceftazidime and Chloramphenicol. It gave a decent sensitivity of 71.4% for Meropenem.

For *Stenotrophomonas maltophilia* the 100% sensitivity was for colistin, cefipime and ciprofloxacin. It had a decent sesnsitivity of 75% for Cefaperazone/Sulbactam, Levofloxacin and co-trimaxzole. For *Sphingomonas paucimobilis* 100% sensitivity was for Amikacin, Cefaperazone/Sulbactam, Minocyclin, Tigecyclin and Trimethoprim/ Sulfamethaxazole and 75% sensitivity for Ceftazidime, Gentamycin and ticarcillin (Table 2).

Table.1 Sample source of NFGNB

Organism	Pus	Blood	Urine	Sputum	BAL	ET Secretions	Body Fluids	Total
Pseudomonas species	29	7	12	4	5	5	17	79
Acinetobacter species	5	6	4	1	1	2	8	27
Burkholderia species	2	1	1	1	-	1	1	7
St.maltophilia species	1	2	1	-	-	-	-	4
S.paucimobilis species	1	-	3	-	-	-	-	4
total	38	16	21	6	6	8	26	121

Table.2 Antibiotic susceptibility pattern for various isolates

Sr.no	Antibiotics	AST Pattern of Bacteria				
		Pseudomonas (n=7)	*Acinetobacter* (n=27)	BCC (n=7)	*S. maltophilia* (n=4)	*S. paucimobilis* (n=4)
1.	Colistin	79(100%)	27(100%)	-	4(100%)	2(50%)
2.	Meropenem	60(75.9%)	10(37.0%)	5(71.4%)	1(25%)	3(75%)
3.	Amikacin	60(75.9%)	79(25.9%)	-	1(25%)	4(100%)
4.	Doripenem	59(74.6%)	9(33.3%)	-	1(25%)	2(50%)
5.	Cefaperazone/ Sulbactam	56(70.8%)	9(33.3%)	-	3(75%)	4(100%)
6.	Cefipime	55(69.6%)	9(33.3%)	-	4(100%)	2(50%)
7.	Ceftazidime	52(65.8%)	8(29.6%)	6(85.7%)	3(75%)	3(75%)
8.	Peperacillin/ Tazobactam	51(64.5%)	8(29.6%)	-	1(25%)	2(50%)
9.	Gentamycin	51(64.5%)	11(40.7%)	-	1(25%)	3(75%)
10.	Imipenem	50(63.2%)	10(37.0%)	-	1(25%)	2(50%)
11.	Ciprofloxacin	45(56.9%)	9(33.3%)	-	4(100%)	2(50%)
12.	Levofloxacin	45(56.9%)	7(25.9%)	4(57.1%)	3(75%)	2(50%)
13.	Ticarcillin	44(55.6%)	8(29.6%)	-	2(50%)	3(75%)
14.	Minocyclin	21(26.5%)	18(66.67%)	4(57.1%)	-	4(100%)
15.	Tigecyclin	04(5.6%)	25(92.5%)	-	3(75%)	4(100%)
16.	COT	03(3.79%)	9(33.3%)	7(100%)	3(75%)	4(100%)
17.	Chloramphenicol	-	-	6(85%)	-	-

In recent years isolation of NFGNB has gained importance with increasing reports of these bacteria relating them to hospital outbreaks or health care associated infections (Malini *et al.,* 2009). The most common of these NFGNB are *Pseudomonas* and *Acinetobacter* (Ramphal *et al.,* 2008). So is also true in the present study. In the present study majority of isolates were from pus specimen which is similar to the study done by Kalidas Rit *et al.,* (2013) in the present study 100% sensitivity was shown by *Pseudomonas* and *Acinetobacter* which are similar to the findings of Kalidas Rit *et al.,* (2013) who has reported a 100% sensitivity for *Pseudomonas* and 94% for *Acinetobacter*. *Buekholderia cepacia* complex in the present study had the highest sensitivity for Trimethoprim Sulphamethaxazole. These findings are in accordance to the study by Fehlberg *et al.,*

(2016) who have mentioned a high sensitivity of 97.6% of this antibiotic. Similar a sensitivity of 85% for Ceftazidime matches the sensitivity of 93.9% in the study of Fehlberg *et al.,* (2016). But Fehlberg *et al.,* (2016) had reported a low sensitivity of 30.5% to Chloramphenicol as compared to 85% in our study. In our study a lower sensitivity of 71.4% was achieved for Meropenem which matches the study of Loren *et al.,* but higher a sensitivity of 100% was reported by Kiran *et al.,* (2013).

Stenotrophomonas maltophilia had 100% sensitivity for Colistin, Cefipime, Ciprofloxacin. Similar higher sensitivity to aminoglycosides was reported by Malini *et al.,* (2009) (100%) and Kiran *et al.,* (2013) (93.3%). A sensitivity of 75% to Trimethoprime/ sulphamethaxazole in this study is less as compared a sensitivity of 100% reported by Malini *et al.,* (2009), Kiran *et al.,* (2013) has reported a sensitivity of 86.7% for trimethoprime/ sulphamethaxazole, colistin was 100% sensitive in the present study which is much higher than 56.1%

reported by Nicodemo *et al.*, (2004). As for as sensitivity of *Sphingomonas paucimobilis* 100% to trimethoprime/ sulphamethaxazole, tigecycline, minocycline and meropenem and cefoperazone/ sulbactam is concerned it is difficult to comment on there therapeutic usefulness as studies for *Sphingomonas paucimobilis* could not be found. It will require more studies in future to assist the clinical response of these drugs to infections by this particular bacteria. From their findings it is evident that variable antibiotic sensitivity pattern of the bacteria and intrinsic resistance of some of these to various antibiotics is making proper identification and antibiotic sensitivity for these isolates is important in reducing the mortality and morbidity because of these infections. The antibiotic sensitivity pattern will vary among hospitals and geographic areas, so findings and establishing antibiotics policies for these NFGNB in setups will go a long way with infections because of these bacteria.

NFGNB are nowadays important pathogens causing life threatening hospital associated infections. These bacteria differ in antimicrobial treatment as bacteria are intrinsically resistant to a variety of antibiotics. This brings into focus the importance of their correct identification and antibiotic sensitivity testing. For this automated ID and AST systems like Vitek-2 could be handful addition in conventional bacteriological methods. One should go forward and find the in vivo response of these antibiotics so as to decide the best treatment options in their own hospital set ups.

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