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

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Abstract

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.

Keywords
Non-fermenting gram-negative bacilli, Prevalence, Pseudomonas aeruginosa, Acinetobacter species, Nosocomial infection.

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Introduction

Non-fermenting gram-negative bacilli (NFGNB) including Pseudomonas species, Acinetobacter species, Burkholderia species, Stenotrophomonas maltophilia species, are emerging as an important causes of bloodstream 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 using Vitek-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/ Sulfamethoxazole (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-Baer disc diffusion method, using for the following antibiotics as per CLSI guidelines. Ceftazidime (CAZ), Trimethorprim/Sulfamethoxazole (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/Sulfamethoxazole 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 sensitivity of 75% for Cefaperazone/Sulbactam, Levofloxacin and co-trimazoxazole. 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.   | Levofoxacin       | 45(56.9%)               | 7(25.9%)   | 4(57.1%)             | 3(75%)    | 2(50%)   |
| 13.   | Ticarclin         | 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 is 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 trimethoprim/ 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 finding 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 helpful 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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