Prevalence of Carbapenem Resistance in Nonfermenting Gram Negative Bacteria in Patients with Respiratory Tract Infection Admitted in Intensive Care Units in Tertiary Care Centre

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Introduction

Non-Fermenting Gram Negative Bacteria (NFGNB) are aerobic, non-lactose fermenting, catalase-positive coccobacilli which are developing as a major threat to critically ill patients (Agarwal S. et al., 2017). Most common bacterial agents of Lower Respiratory Tract Infection in the Intensive Care Units (ICUs) are Pseudomonas, Acinetobacter, Klebsiella, Citrobacter (Mukhopadhyay C et al., 2003; Gonugur U. et al., 2004). which are multi drug resistant, and with limiting the therapeutic options (Goossens H. et al., 2003). Carbapenem which were introduced first in 1980 are now frequently used as the last choice in treating serious infections caused by multidrug
resistant, gram negative bacilli which are stable to β-lactamases including the Extended Spectrum β-Lactamases (ESBLS) and Ampc (Brahmadathan K. et al., 2005; Quinn J.P. et al., 1998).

Nonfermenting Gram-negative bacilli are known to produce ESBLs and metallo β-lactamases (Gales A.C. et al., 2001). Unfortunately, resistance to these antibiotics started emerging from 1990 and has been reported in nonfermenting gram negative bacilli (NFGNB) worldwide over the years with varying frequencies (Tognim M.C.B. et al., 2004). *Pseudomonas aeruginosa* and *Acinetobacter spp.* in particular are most often associated with carbapenem resistance.

The combination of porin loss and class c β-lactamase expression is an important cause of imipenem resistance in *Pseudomonas aeruginosa* (Livermore DM. et al., 1992) and *Acinetobacter baumannii* (Devi P. et al., 2015). Here, we document the microbiological aspects of the prevalence of carbapenem resistance in NFGNB isolated from patients with respiratory tract infections in the ICU.

**Materials and Methods**

A total of 430 samples were processed from patients of all age groups with clinical evidence of lower respiratory tract infection admitted to medical, surgical, and paediatric ICUS from October 2017 to September 2019.

Samples were collected before starting antibiotics in sterile, wide mouthed, disposable, screw-capped container of about 100 ml capacity (J.G. Collee et al., 1996, p63). Sample is collected before starting antibiotics (J.G. Collee et al., 1996 p63). Samples collected were Endotracheal aspirates from suction tips of patients on ventilators (Devi P. et al., 2015). Specimens were delivered and processed within 2 hours (J.G. Collee et al., 1996, p63).

Homogenization of sputum done with dithiothreitol followed by gram staining (Duguid J.P. et al., 1996). If more than 10 polymorph per square, then Processed further. All sample were inoculated on blood agar, MacConkey agar, chocolate agar and fildes digest agar overnight at 37°C.

Sputum samples were processed in semiquantitative method (J.G. Collee et al., 1996, p64-66). Bacterial isolates were identified according standard procedure using gram stain (Duguid J.P. et al., 1996) and using various biochemical tests (J.G. Collee et al., 1996, p131-149).

The antimicrobial susceptibility testing of non-fermenting gram negative isolates was done by Kirby Bauer disc diffusion method according to CLSI 2019 (Clinical Laboratory Standards Institute) guidelines (CLSI guidelines 2019).

**Statistical analysis**

The data were recorded in the MS excel and analysed by using software -SPSS version 20.

**Results and Discussion**

Out of 430 samples processed, 306 (71.16%) were positive for pathogenic isolates, 73 (16.97%) were showing normal flora growth and 51 (11.87%) were showing no growth.

Among 430 sample processed, maximum samples were from age group 51-60 i.e. 106 (24.66%) as depicted in table 1. Out of total 306 positive sample for pathogenic isolates, 227 (74.18%) were positive for gram negative isolates and 79 (25.82%) were positive for gram positive isolates.
Table.1 Age wise distribution of total samples

| Age   | Number of samples | Percentage |
|-------|-------------------|------------|
| 0-10  | 14                | 3.25%      |
| 11-20 | 49                | 11.40%     |
| 21-30 | 30                | 6.97%      |
| 31-40 | 36                | 8.37%      |
| 41-50 | 84                | 19.55%     |
| 51-60 | 106               | 24.66%     |
| 61-70 | 79                | 18.37%     |
| 71-80 | 23                | 5.34%      |
| 81-90 | 09                | 2.09%      |
| total | 430               | 100%       |

Figure.1 Prevalence of NFGNB and other isolates in the total No. of positive samples

Among total 306(71.16%) isolated pathogenic bacteria, most common Gram-negative bacteria was *klebsiella pneumoniae* i.e. 93 (30.39%) followed by *Pseudomonas aeruginosa* 76(24.83%) followed by *Acinetobacter baumannii* 42(13.73%) followed by *Citrobacter freundii* 14(4.58%) followed by *Escherichia coli* 2(0.65%). Out of total 227-gram negative isolates, nonfermenting gram negative bacteria were 118.out of 118, 76 were *Pseudomonas aeruginosa* and 42 were *Acinetobacter baumannii* as depicted in table 2 and figure 1.
Table 2: Prevalence of NFGNB and other isolates in the total No. of positive samples

|总阳性分离菌 |
|----------------|
|总革兰氏阴性菌 227 (74.18%) | 总革兰氏阳性菌 79 (25.82%) |
| Klebsiella pneumoniae 93 (30.39%) | Streptococcus pneumoniae 54 (17.65%) |
| Pseudomonas aeruginosa 76 (24.83%) | Staphylococcus aureus 17 (5.56%) |
| Acinetobacter baumannii 42 (13.73%) | Coagulase negative staphylococcus (CONS) 8(2.61%) |
| Citrobacter freundii 14 (4.58%) |
| Escherichia coli 2(0.65%) |

Figure 2: carbapenem resistance in nonfermenting gram negative bacilli. (n=118)

Out of total 430 samples, 179 were sputum and 251 were endotracheal aspirates. Out of 306 samples positive for pathogenic bacteria, 124(40.52%) were sputum and 182(59.48%) were endotracheal aspirates.

Out of 124 positive sputum samples, 31(25%) were Pseudomonas aeruginosa and 15(12.09%) were Acinetobacter baumannii. Out of 182 positive endotracheal aspirate samples, 45(24.72%) were Pseudomonas aeruginosa and 27(14.83%) Acinetobacter baumannii as depicted in table 3. Out of total 76 isolated Pseudomonas aeruginosa, 33(43.42%) were resistant to imipenem and 22(28.94%) were resistant to meropenem as shown in table 4.

Out of total 42 isolated Acinetobacter baumannii, 16(38.10%) were resistant to imipenem and 11(26.19%) were resistant to meropenem as shown in table 5.

Out of 118 isolates of nonfermenting gram negative bacteria 49(41.52%) were resistance to imipenem and 33(27.96%) were resistance to meropenem as depicted in table 6 and figure 2.

Table 3: Prevalence of total isolates NFGNB in different samples.
Samples | Positive samples for pathogenic isolates | *Pseudomonas aeruginosa* | *Acinetobacter baumannii*
---|---|---|---
Sputum (179) | 124 (40.52%) | 31 (25%) | 15 (12.09%) |
Endotracheal aspirates (251) | 182 (59.48%) | 45 (24.72%) | 27 (14.83%) |
Total (430) | 306 (60.51%) | 76 | 42 |

**Table 4** antibiotic susceptibility testing of *Pseudomonas aeruginosa* (n=76)

| Antibiotics       | Resistance | Resistance percentage |
|-------------------|------------|-----------------------|
| ceftazidime       | 70         | 92.10%                |
| cefepime          | 34         | 44.73%                |
| amikacin          | 37         | 48.68%                |
| gentamicin        | 58         | 76.31%                |
| imipenem          | 33         | 43.42%                |
| meropenem         | 22         | 28.94%                |
| Piperacillin -tazobactam | 38 | 50%                   |

**Table 5** Antibiotic susceptibility testing of *Acinetobacter baumannii* (n=42)

| Antibiotics         | Resistance | Resistance percentage |
|---------------------|------------|-----------------------|
| ceftazidime         | 39         | 92.85%                |
| cefepime            | 31         | 73.80%                |
| amikacin            | 28         | 66.6%                 |
| gentamicin          | 39         | 92.85%                |
| imipenem            | 16         | 38.10%                |
| meropenem           | 11         | 26.19%                |
| Piperacillin -tazobactam | 18 | 42.85%                |

**Table 6** Carbapenem resistance in nonfermenting gram negative bacilli
Total no. of isolates 118

|                      | Imipem resistance | Meropenem resistance |
|----------------------|-------------------|---------------------|
| Pseudomonas aeruginosa (76) | 33 (43.42%)       | 22 (26.94%)        |
| Acinetobacter baumannii (42) | 16 (38.10%)       | 11 (26.19%)        |
| Total (118)          | 49 (41.52%)       | 33 (27.96%)        |

Non-Fermenting Gram Negative Bacilli (NFGNB); world-wide over the years with varying frequencies of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in particular are most often associated with Carbapenem resistance causing fatal lower respiratory tract infections in patient admitted in ICUs. In our study, 306 (74.16%) among 430 were showing growth of pathogenic isolates. This is consistent with study conducted by Isa H. et al., (Isa H., Mahmood and Tirmidi 2010) showing high prevalence rate of isolation i.e. (92.5%), and contradict with study conducted by Mishra et al., and V. Ramana et al., (Mishra et al., 2012; V. Ramana et al., 2013) showing isolation rate of 44% and 39.4% respectively. Higher isolation rate is may be due to proper sample collection with timely transportation and before starting antibiotics. In this study gram negative isolates 227(74.18%) were more frequently isolated than gram positive isolates 79(25.82%). Other study conducted by Regha et al., (Regha et al., 2018) Galatelatabaswanna et al., (Galatelatabaswanna et al., 2015) and Ravichitra et al., (Ravichitra et al., 2016) also showing higher isolation of gram negative than gram positive bacteria. Gram negative prevalence is may be due to unequal cases of community acquired infections and hospital acquired infections.

In our study, among total 227 isolated gram-negative bacteria, 118 were nonfermenting gram negative bacilli. Among 118 nonfermenting gram negative bacilli 76 were *Pseudomonas aeruginosa* and 42 were *Acinetobacter baumannii*. These nonfermenting gram negative isolates were tested for antibiotic susceptibility for carbapenems and other antibiotics. Our study shows higher carbapenem resistance in *Pseudomonas aeruginosa* than *Acinetobacter baumannii*. This is consistent with study conducted by Devi p et al., (Devi p et al., 2015) and contradict with Taneja N et al., and Agrawal S et al., (Taneja N et al., 2003; Agrawal S et al., 2017) where carbapenem resistance was found more frequently in *Acinetobacter baumannii* than *Pseudomonas aeruginosa*. In our study imipenem resistance to *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is found to be 43.42% and 38.10% respectively. our study is consistent with study conducted by Devi p et al., (Devi p et al., 2015) showing 42% and 28% imipenem resistance to *Pseudomonas aeruginosa* and *Acinetobacter baumannii* respectively.

Our study is contradicting with study conducted by Agrawal S et al., (Agrawal S et al., 2017) showing imipenem resistance to *Pseudomonas aeruginosa* and *Acinetobacter baumannii* 52% and 90.54% respectively. This may be due to Frequent use of imipenem might attribute to resistant against imipenem of its multidrug-resistant pattern and its ability to adapt to various environments (Jean SS et al., 2014.).

In our study meropenem resistance to *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is found to be 28.94% and 26.19% respectively. Our study is consistent with study conducted by Hashem H. et al., (Hashem H. et al., 2016) showing 24% of
meropenem resistance in *Pseudomonas aeruginosa* and Sharma D. *et al.*, (Sharma D. *et al.*, 2015) and Cai B. *et al.*, (Cai B. *et al.*, 2017) showing 19% and 26% of meropenem resistance to *Acinetobacter baumannii* respectively. Study conducted by Sahu *et al.*, (Sahu *et al.*, 2016) showing higher resistance of meropenem in *Pseudomonas aeruginosa* (84%) and in *Acinetobacter baumannii* (81.9%).

The relatively low prevalence in our study is no way a reason for satisfaction, since our study was done in a setup including rural population, in whom carbapenem often are not the first-choice drug. Matter of concern is selective multiplication and dissemination of multiple resistant NFGNB in near future.

Our study has put forward the carbapenem resistance NFGNB among the respiratory isolates of our ICUs. In view of carbapenem resistance amongst the isolates, antibiotic therapy should be advocated or modified following culture and sensitivity. This would not only help in the proper treatment of the patient but also would discourage the indiscriminate use of available antibiotics and stop the spread of drug resistance bacteria. Moreover, considering the prevalence of carbapenem resistant bacteria, it is necessary to carry out regular monitoring of drug resistance and molecular characteristics of carbapenem resistant isolates in this region.

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