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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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A B S T R A C T

Most common bacterial agents of Lower Respiratory Tract Infection in the Intensive Care Units are *Pseudomonas*, *Acinetobacter*, *Klebsiella*, *Citrobacter;* which are multi drug resistant, and with limiting the therapeutic options.Nonfermenting Gram-negative bacilli are known to produce Extended spectrum β-lactamases (ESBLs) and metallo β-lactamases. *Pseudomonas aeruginosa* and *Acinetobacter spp*. in particular are most often associated with carbapenem resistance. 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. Out of total 227gram negative isolates, nonfermenting gram negative bacteria were 118. out of 118, 76 were *Pseudomonas aeruginosa* and 42 were *Acinetobacter baumannii*. Out of total 76 isolated *Pseudomonas aeruginosa*, 33(43.42%) were resistant to imipenem and 22(28.94%) were resistant to meropenem. Out of total 42 isolated *Acinetobacter baumannii*,16(38.10%) were resistant to imipenem and 11(26.19%) were resistant to meropenem. 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.

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). Carbapenems 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 ware 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 37^0 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%

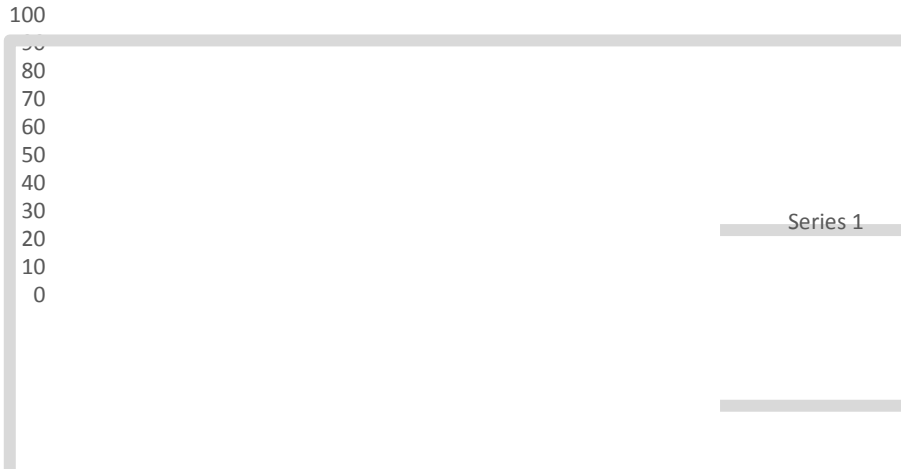

Figure.1 Prevalence of NFGNB and other isolates in the total No. of positive samples

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

Total positive isolates for pathogenic bacteria (306)	
Total gram-negative isolates 227 (74.18%)	Total gram-positive isolates 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%)**	

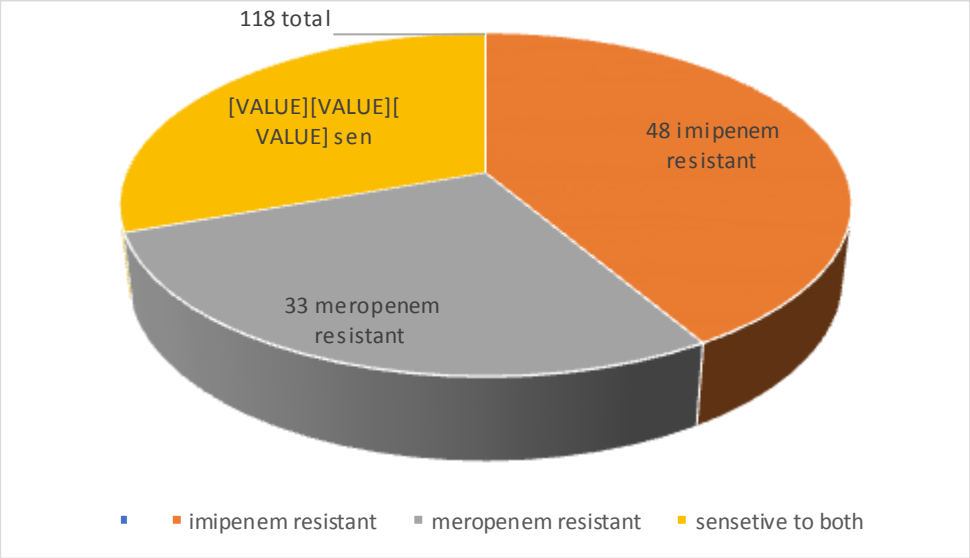

Figure.2 carbapenem resistance in nonfermenting gram negative bacilli. (n=118)

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 and182(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	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	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	**Imipenem 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 Tirmidhi2010) showing high prevalence rate of isolation i.e. (92.5%), and contradict with study conducted byMishra *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) Galatelata-baswanna *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 *Acinetobacterbaumannii* 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% and28% 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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