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Detection of ESBL and MBL in non fermenting gram negative bacilli isolated from a tertiary care hospital

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Abstract: *Background:* NFGNB either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. They are innately resistant to many antibiotics and are known to produce ESBL and MBL that limits therapeutic options. Our objectives were to identify the NFGNB and to determine their Antibiogram, including ESBL and MBL detection. *Methods:* The study included 91 NFGNBs isolated from clinical samples, identified by conventional method. AST was done by Kirby Bauer disk diffusion method. ESBL detection was performed by DDST method and MBL detection by Imipenem-EDTA combined disc test, as per CLSI Guidelines. *Results:* NFGNB were predominantly isolated from swabs (41%), pus (20.87%) and sputum (20.87%). *P.aeruginosa* (69.23%) was the predominant isolate followed by *A.baumanii* (27.49%), *S.maltophilia* (2.19%), and *B.cepacia* (1.09%). 46% of the isolates were ESBL producers (60 % *P.aeruginosa,* 28% *A.baumannii*, all isolates of *B.cepacia* and *S.maltophilia*). Approximately 20% NFGNB were MBL producers (17.46% P.aeruginosa and 16% A.baumanii, 4.34% S.maltophilia and all isolates of B.cepacia). *Conclusion:* Early detection of ESBL and MBL, evaluation of effective antibiotic option, prudent use of antibiotics by formulating antibiotic policy and infection control measures would assist in the effective management of patients.

Keywords: NFGNB, ESBL, MBL, Tertiary care.

Introduction

Non-fermenting gram negative bacteria (NFGNB) are taxonomically heterogeneous group of bacteria of the division Proteobacteria that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation [1].

The major genera of non-fermenting Gram negative bacilli have been classified into at least 15 families, with several clinically essential non-fermenters with uncertain taxonomic positions. These organisms have emerged as important healthcare associated pathogens. They have been incriminated in infections such as septicaemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections [2].

These bacteria are innately resistant to several antibiotics and are known to produce Extended Spectrum Beta Lactamases (ESBLs) and Metallo-Beta Lactamases (MBLs), which make them more notorious pathogens, and have emerged as a public health threat by limiting therapeutic options [3].

This study aimed to evaluate the frequency and Antibiogram of NFGNB in a tertiary care hospital. The objectives of our study were to identify the NFGNB, determine their Antibiogram along with detection of ESBL & MBL production.

Material and Methods

Study Design: This was a cross sectional study conducted for a period of one year from March 2019 to February 2020 after obtaining Institutional Ethical Clearance (protocol no. 2019/096). The study included 91 non repetitive NFGNB isolated from various clinical samples.

Identification & Antibiogram of NFGNB: The NFGNB isolates were identified by conventional methods using relevant biochemical tests. The Antibiogram was performed by Kirby Bauer disk diffusion method and interpreted as per CLSI guidelines.

Detection of ESBL production: Double disc synergy test (DDST) as per CLSI guidelines was used to detect ESBL production. The Ceftazidime (30 µg) discs alone and in combination with Clavulanic acid (10 µg) were used. An increase of 5mm in zone of inhibition of the combination discs in comparison to the Ceftazidime disc alone was considered to be ESBL producer.

Detection of MBL production: MBL was detected by Imipenem EDTA combined disc test. Two (10 µg). Imipenem discs were placed on MHA inoculated with the test organism, and 10 µl of 0.5 M EDTA solution was added to one disc. A zone diameter difference of 7 mm between the Imipenem and Imipenem + EDTA was interpreted as a positive result for MBL production.

Statistical analysis: Data obtained was recorded in MS Excel work sheets and statistical analysis was done using IBM SPSS22 programme running on windows operating system. Categorical data was expressed in counts and percentages. Visualization of data was done using relevant charts/diagrams.

Results

Among the 91 NFGNB, 37 (41%) were from swab, 19 (20.87%) each from pus and sputum, 11 (12.08%) from urine, 3 (3.29%) from blood, 2 (2.15%) from tissue. *Pseudomonas aeruginosa 63* (69.23%) was the predominant isolate followed by *Acinetobacter baumanii 25* (27.49%), *S. maltophilia 2* (2.19%), and single isolate (1.09%) of *Burkholderia cepacia.*

Antibiogram: Pseudomonas aeruginosa (n=63) showed higher resistance to Cephalosporins as compared to other group of drugs (as depicted in Figure 1). More than 85% of *Acinetobacter baumanni* isolates exhibited resistance to all the tested antibiotics, as shown in Figure 2. Antibiogram of *S. maltophilia* and *B.cepacia* are depicted in Figure 3.

Fig-1: Antibiotic resistance pattern of Pseudomonas aeruginosa (n=63)

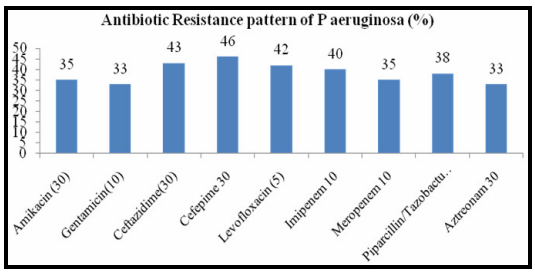


Fig-2: Antibiotic resistance pattern of *A.baumannii* (n=25)

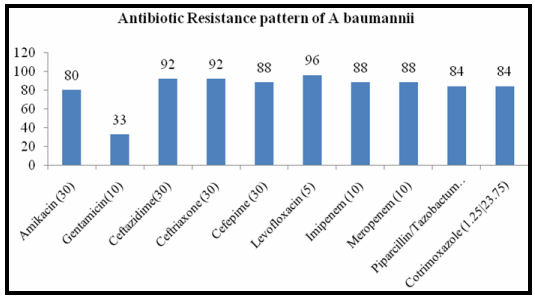
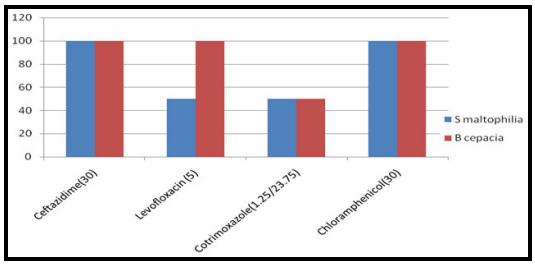


Fig-3: Antibiotic resistance pattern of *S.maltophilia* (n=1) and *B cepacia* (n=1)



ESBL & MBL production: A total of 42(46%) isolates were ESBL producers and 18(20%) isolates were MBL producers, *P. aeruginosa* being the predominant ESBL& MBL producer as shown in Table 1.

Table-1: Frequency of ESBL production among the NFGNB (N=91)

Organism	ESBL Producers (n, %)	MBL Producers (n, %)
P.aeruginosa (n=63)	32 (50%)	11 (17%)
A.baumanii (n=25)	7 (28%)	4 (16 %)
S.maltophilia (n=2)	2 (100%)	2 (100%)
B.cepacia (n=1)	1 (100%)	1 (100%)

Discussion

Non fermenting Gram Negative bacilli (NFGNB) are frequently isolated from clinical specimens and have emerged as important nosocomial pathogen. Their multi drug resistance poses clinical problem in treating the infections caused by NFGNBs. Out of 91 non fermenters isolated in this study, majority were from swab (41.1%) followed by pus (20.87%) and sputum samples (20.87%). Varying isolation rates of NFGNB from different samples have been reported by various authors. Kamalraj et al [4] from India has reported isolation rates of 48 % & 33% in pus and sputum samples respectively.

The studies by Anshu sastri et al., [5] (27%, 21%) and A J M Al-saadi et al.,[6] (24%, 36%) have reported highest isolation rates from sputum and ear swab. Isolation of NFGNB from various clinical samples proved their existence in all the sites leading to a wide range of diseases. *Pseudomonas aeruginosa* (69%) was the predominant isolate in our study followed by *Acinetobacter baumanii* (27%), *S. maltophilia* (2%) and *Burkholderia cepacia* (1%).The results are similar to those reported by kamalraj et al.,[4] Juyal D et al.,[7] and Nazir A et al.,[8] However in the studies done by Kumar R et al., [9] and Yadav S K et al.,[10] in contrary to our findings have reported *Acinetobacter baumanni* as predominant isolate 53% and 44% respectively.

In this study, the antimicrobial susceptibility pattern of *P. aeruginosa* showed highest sensitivity (65%) to amikacin, meropenem, piperacillin and lowest (46%) to ciprofloxacin. The second predominant isolate *Acinetobacter species* showed highest sensitivity (16%) to piperacillin /tazobactam, amikacin (16%) and least to (4%) gentamicin and levofloxacin as depicited in figure no 1 and 2 respectively. similar results reported by Nautiyal et al.,[11] S.maltophilia and *Burkholderia cepacia* isolates in the present study were sensitive to levofloxacin (50)%) and trimethoprim/ sulfamethoxazole (100%) respectively. There were 63 (67%) multi drug resistant isolates in our study.

A.baumani 18(72%) followed by *P.aeruginosa* 40 (63%) were the most common multi drug resistant strains which is comparable to the study by Patwardhan et al.,[12] which showed > 68% of *Pseudomonas* and >90% *Acinetobacter* isolates as multi drug resistant. *S.maltophilia* is an emerging nosocomial pathogen in a hospital setting its significance lies in its intrinsic multidrug resistance. In this study, there were 2 (2%) isolates which is similar to the studies done by Vijaya et al,.[13] but less as compared to the study from China (9.2%) by Wang H et al.,[14] *Burkholderia cepacia* has emerged as a significant respiratory pathogen being intrinsically resistant to amino glycosides and polymyxins and often develops resistance to þ-lactams due to the presence of inducible chromosomal þ-lactamases and altered penicillin-binding proteins.

Antibiotic efflux pumps in *Burkholderia* mediate resistance to chloramphenicol, trimethoprim and fluoroquinolones [15]. The only isolate in the present study was sensitive to Levofloxacin and Trimethoprim/ Sulfamethoxazole and resistant to Ceftazidime and Chloramphenicol. We observed 46% ESBL and 20% MBL production among the total NFGNBs as described in table 1.

Several studies by Goel et al,. [2], Juyal D et al.,[7] Kimura et al.,[16] Anil VK et al,. [17] and Kamalraj et al.,[4] have reported similar isolation frequencies of ESBL and MBL production among NFGNBs. The intrinsic and acquired antimicrobial resistance including ESBL and MBL production exhibited by NFGNBs pose great problems in treatment leading to increased length of hospital stay. It is therefore important to institute a system for the surveillance of antimicrobial resistance that will involve the collection and collation of both clinical and microbiological data. Antibiotic therapy either empiric or documented should be based upon antibiotic combination supplemented by knowledge of local epidemiology of resistance and susceptibilities in choosing a suitable antibiotics.

Conclusion

NF GNBs which were earlier considered as containments have now emerged as an important health care pathogen causing a wide range of infections. The variation in their prevalence and sensitivity pattern pose a challenge for healthcare professionals. Identification of NFGNB and detection of

ESBL and MBL along with their routine drug susceptibility patterns will help in proper management of the infection caused by them and will help in preventing spread of drug resistant NFGNB in healthcare settings.

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