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Non-fermenting gram negative bacilli isolated from blood and their in vitro sensitivity pattern in a tertiary care hospital

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Abstract--Background: Bacteremia caused by non-fermenting gram negative bacilli (NFGNB) is a serious infection which is associated with high morbidity and mortality. Identification of NFGNB and monitoring their susceptibility pattern are important for proper management of these infections. Aim: Scope of the present study is to characterize the NFGNB along with their antimicrobial sensitivity pattern among the patients coming to our hospital, a tertiary care center. Materials & Methods: A retrospective study was conducted to determine the NFGNB isolated from blood during the one-year study period. A total of 8223 Blood cultures were received in the laboratory which was processed by semi-automated technique in Bac-T Alert. Antibiotic susceptibility was performed by VITEK and Kirby Bauer methods and interpreted by CLSI guidelines. 102 isolates of NFGNB were obtained from the Blood culture. Results: Most commonly isolated organism is *Acinetobacter* species (47%) followed by *Pseudomonas* spp (27%) and *Burkholderia* spp (15.6%). Maximum isolates of *Acinetobacter* spp were sensitive to colistin (91.9%), cefoperazone-sulbactam (88.4%), Tigecycline (62.5%). *Pseudomonas* spp was sensitive to colistin (100%), ciprofloxacin (59%). Conclusion: Identification of NFGNB and monitoring their susceptibility patterns will help in proper management of infections caused by them. Judicial use of antibiotics and infection-control measures will be needed to prevent or slow the emergence and spread of multidrug-resistant NFGNB in the healthcare setting.

Keywords---Acinetobacter, pseudomonas, non-fermenters, NFGNB, bacteremia.

Introduction

Non fermenting gram negative bacilli (NFGNB) are a taxonomically diverse group of organisms that either do not utilize glucose as a source of energy or utilize it oxidatively. They are saprophytic in nature but can cause a significant number of infections particularly in hospitalized patients, immunocompromised hosts and patients with hematological malignancies.¹

Bacteremia is a serious infection which is associated with high morbidity and mortality. Infections caused by non-fermentative gram-negative bacteria (NFGNB) constitute an emerging problem in nosocomial setting, especially in an immunocompromised host. Data from the Surveillance and Control of Pathogens of Epidemiological importance (SCOPE) study revealed that approximately one-fourth of gram-negative bacteremia attributed to NFGNB.²

Resistance pattern among nosocomial bacterial pathogens may vary widely from country to country at any given time and within the same country over time. Because of these variations a surveillance of the nosocomial pathogens and their susceptibility patterns in a given set up is needed in order to guide appropriate selection of empiric therapy.

Aims and objective

- 1) To determine the proportion of NFGNB isolated from blood.
- 2) To determine the *in vitro* sensitivity pattern of NFGNB isolated from blood.

Material and Methods

The retrospective study was conducted for a period of 12 months in a tertiary care hospital of Mangalore, India. A total of 8223 blood specimens were received in laboratory. All samples were processed and incubated in Bac-T Alert and positive samples of NFGNB were identified by Vitek-2 compact system. The antibiotic susceptibility test was also done by Vitek-2 compact System and modified Kirby Bauer method wherever required. The results were interpreted by using the CLSI guideline.³

Results

NFGNB were isolated from 102 out of 8223 clinical specimens accounting for an isolation rate of 1.24%. Our study showed that NFGNB can cause bacteremia in all age group (table1) though geriatric population is most commonly affected (36.2%).

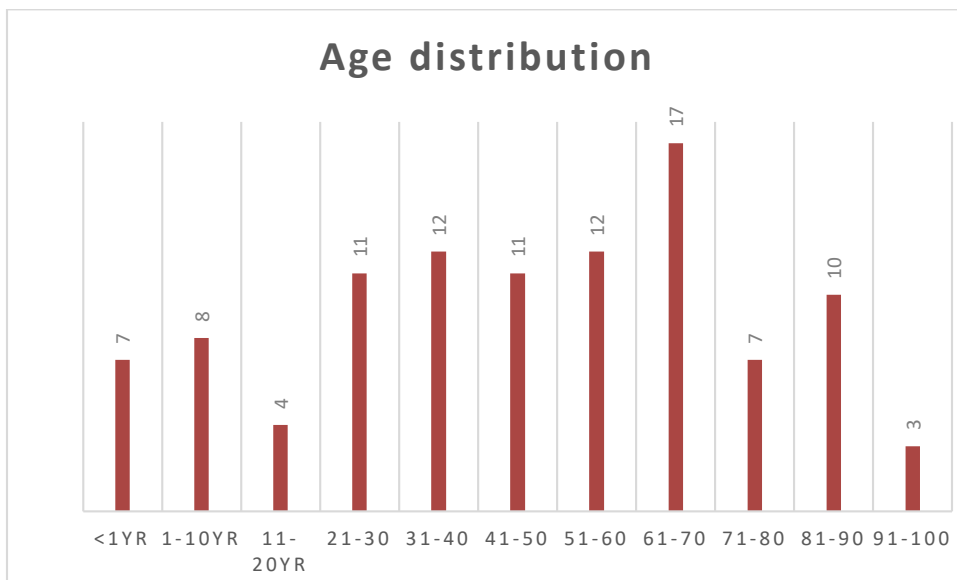


Figure 1: Age distribution

Male to Female ratio was 4:1, implying that it was more commonly isolated from Males.

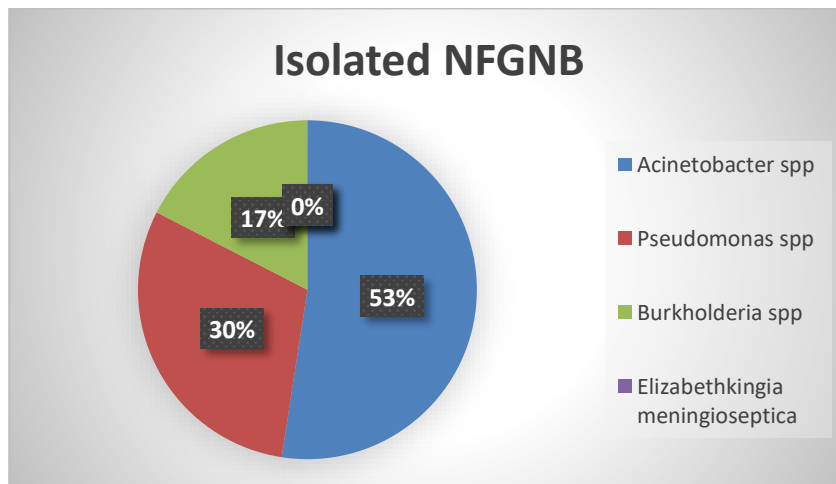


Figure 2: Isolated NFGNB

Among the 102 positive specimens 48 was identified as *Acinetobacter spp* (47%), 28 as *Pseudomonas spp* (27%), 16 as *Burkholderia spp* (15.6%), 4 as *Elizabethkingia meningoseptica* (0.03%), 2 as *Sphingomonas spp* (0.01%) and 1 as *Orchrobacterium anthropi* (0.009%). (fig 2). 3 NFGNB isolates could not be identified. Among *Acinetobacter spp*, *A. baumannii* ($n=32$) was most commonly isolated species (66.6%) followed by *A. lwoffii* ($n=6$, 0.13%), and *A. junni* ($n=2$, 0.04%). (Fig3) Among *Pseudomonas spp*, *P. aeruginosa* ($n=20$, 71.4%) and among *Burkholderia spp* *Burkholderia cepacia* ($n=14$, 87.5%) were most commonly isolated. Only seven specimens (0.06%) showed polymicrobial infection where

non-fermenters were isolated along with other organisms of which *Klebsiella pneumoniae* (n=4), *Enterococcus spp* (n=3), *Citrobacter spp* (n=1) and *Escherechia coli* (n=1) were commonly associated.

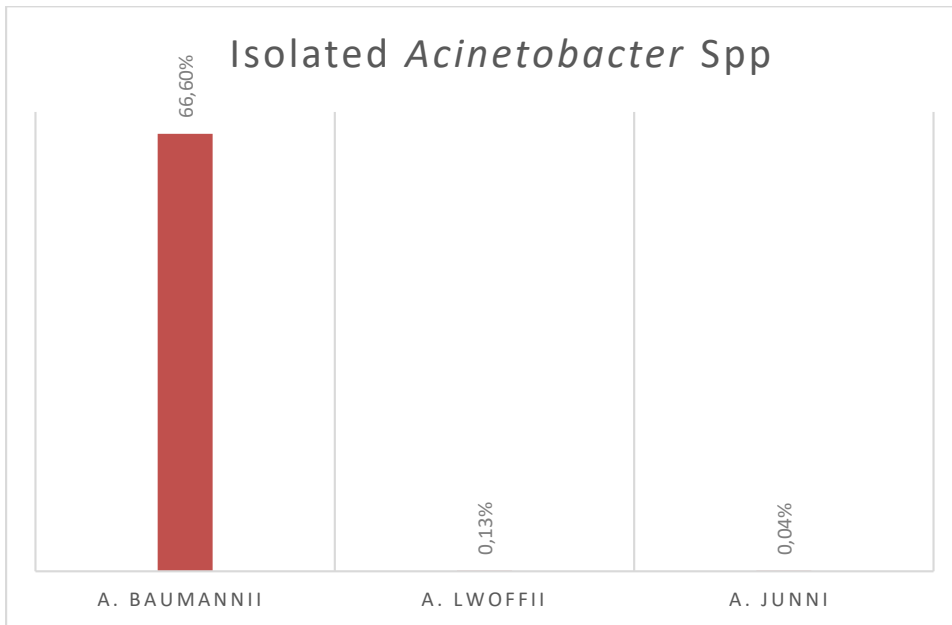


Figure: 3 Isolated *Acinetobacter* spp

Most isolates of *Acinetobacter spp* were sensitive to Tigecycline (92.3%), cefoperazone-sulbactam (92.6), colistin (91%) (figure 4).

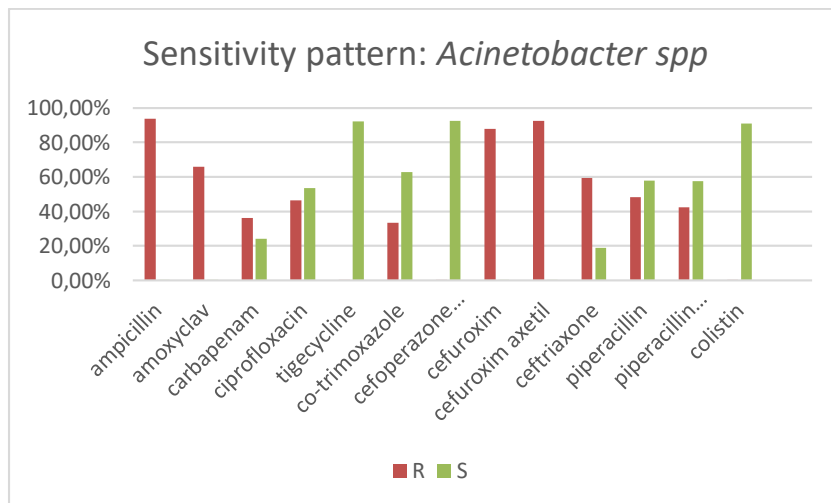


Figure: 4 Sensitivity pattern: *Acinetobacter* spp.

Pseudomonas spp was most susceptible to colistin (100%), piperacillin (65%), cefoperazone-sulbactam (65%), piperacillin-tazobactam (64%), ciprofloxacin (59%) (fig5).

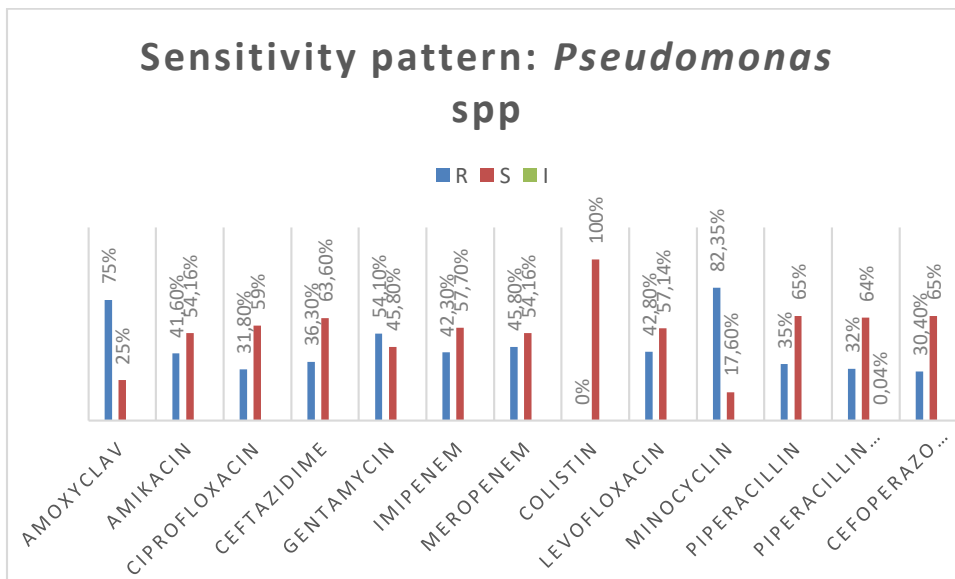


Figure: 5 sensitivity patterns: *Pseudomonas spp*

Burkholderia spp was found to be sensitive to ceftazidime (100%), cefoperazone (100%), meropenem (100%), piperacillin (100%) and ofloxacin (100%) and resistant to gentamycin (100%), amikacin (92.80%) and imipenem (85.70%). Figure 6.

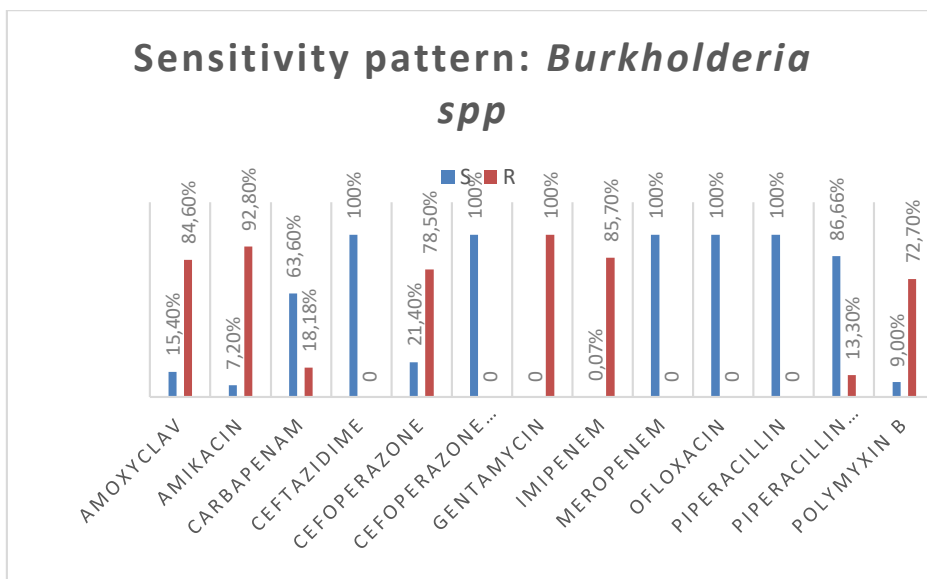


Figure: 6 sensitivity patterns: *Burkholderia spp*.

Discussion

A study by Hilmar Wisplinghoff et al showed that *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were the most commonly isolated NFGNB whereas some other organisms (e.g. *Burkholderia pseudomallei*, *Acinetobacter lwoffii*, *Burkholderia cepacia*, *Elizabethkingia meningioseptica* etc.) were isolated rarely.⁴ In our study, the most common pathogens isolated were *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Burkholderia cepacia*. These pathogens are usually considered as opportunistic pathogens.

The isolation rates in India differ in the various geographical regions. In Bangalore, the prevalence of *Pseudomonas* spp was 78.94% and *Acinetobacter* spp was 6.1% whereas in Tamilnadu it was 43% and 21% respectively and at Uttarakhand the prevalence of *Acinetobacter* spp was 49.59% and *Pseudomonas* spp was 43.03% in 2012.¹ In our study incidence of *Acinetobacter* spp, *Pseudomonas* spp and *Burkholderia* spp were found to be 47.05%, 27.45% and 15.68% respectively.

Resistance patterns among nosocomial bacterial pathogens may vary from country to country and also within the same country, over time. *Pseudomonas* spp showed high levels of resistance for cotrimoxazole, ciprofloxacin, ceftazidime, cefepime, gentamycin, cefoperazone-sulbactam, piperacillin-tazobactam. Unlike a study conducted at Kolar, Karnataka, we found that *Acinetobacter* spp is more sensitive to cotrimoxazole (66.90%) and piperacillin (58.00%) but less sensitive to imipenem (42.80%).⁵ *Pseudomonas aeruginosa* is found to be less sensitive to amikacin (41.60%) and gentamycin (45.80%) as compared to another study conducted at Taiwan in which resistance for amikacin and gentamycin was 98% and 94% respectively.⁶

There are few different mechanisms involved in drug resistance of *Pseudomonas*. The presence of over expressed efflux pump and low permeability of outer membrane is responsible for innate resistance, whereas the acquisition of resistance gene or mutation in genes encoding porins, penicillin-binding proteins, efflux pumps and chromosomal β -lactamase which can be termed as acquired mechanism of resistance. Mutation in chromosomal β -lactamase contributes to resistance to β -lactams, carbapenems, aminoglycosides and fluoroquinolones. The treatment option for drug resistant *Pseudomonas*, becomes very limited as the above-mentioned mechanisms often exist simultaneously resulting into combined resistance to many antibiotics.⁷

For *Pseudomonas* spp fluoroquinolone resistance is linked only to chromosomal genes with mutational changes in the fluoroquinolone targets DNA gyrase (*gyrA* and *gyrB*) and/or topoisomerase IV (*parC* and *parE*) and/or overexpression of multidrug efflux pumps, whereas imported resistance is seen against β lactams and aminoglycosides by the production of inactivating β -lactamases and enzymatic inactivation of the drug molecule through chemical modification respectively.⁸

A. baumannii shows various antimicrobial resistance mechanisms, may be constitutive or acquired via plasmids, integrons and transposons. Mechanisms

include enzymatic modification of antibiotic molecules, modification of antibiotic target sites, expression of efflux pumps, and downregulation of cell membrane porin channel expression. *A.baumannii* can become resistant due to modification of existing genes, their regulation mechanisms, or acquisition of exogenous DNA by horizontal gene transfer.⁹ Resistance to carbapenem is mediated by production of carbapenem – hydrolyzing enzymes encoded by horizontally transferrable genes e.g. class A (*bla_{GES-14}* and *bla_{KPC}*), class B (*bla_{IMP}*, *bla_{VIM}*, *bla_{SIM-1}*, and *bla_{NDM}*), and class D (*bla_{OXA-23-like}*, *bla_{OXA-24/40-like}*), so susceptible organisms can acquire resistance rapidly¹⁰. On the other hand, the main cause of aminoglycoside resistance is acquisition of genes encoding aminoglycoside-modifying enzymes (e.g. *aphA1*, *aphA6*, *aphA15*, *aacC1*, *aacC2*, *aacA4*, *aadB*, *aadA1*, and *aadA4*)¹⁰. Resistance to β -lactams is caused by β -lactamase production, most commonly oxacillinases (*bla_{OXA}*), metallo- β -lactamases (*bla_{IMP}*, *bla_{VIM}*, *bla_{SIM}*) and extended spectrum β -lactamases (*bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}*, *bla_{KPC}*).¹⁰

Resistance to fluoroquinolones (*gyrA*, *parC*) and aminoglycosides (*arm*, *rmt*), and rarely β -lactams is sometimes attributed by antibiotic target site alteration. Mutation of genes encoding efflux pumps (*tet*, *ade*, *abe*) can also contribute to resistance against β -lactams, fluoroquinolones, tetracyclines and aminoglycosides. Finally, porin channel deletion (*carO*, *oprD*) leads to β -lactam resistance and may contribute to rarely seen polymyxin resistance.¹¹ Documenting resistance among NFGNB is very important especially the carbapenem resistance, as they are often the causative agents for outbreaks in the ICU setting and therapeutic option is limited due to the high degree of multi - drug resistance.

Conclusion

To conclude, *A. baumannii* and *P. aeruginosa* were the most common NFGNB isolated in our study. *A. baumannii* showed good susceptibility to cefoperazone sulbactam and colistin, whereas *P. aeruginosa* was sensitive to cefoperazone sulbactam and piperacillin. Due to the difference of susceptibility pattern of the organisms the identification and monitoring of their antibiotic susceptibility is important for prior management of the infections caused by NFGNB. This would avoid unnecessary use of antibiotics and emergence of drug resistant strains.

Declarations:

Acknowledgements: None

Conflict of Interest: No conflict of interest

Authors' Contribution

1. Dr Maumita Deb, Tutor, Department of microbiology, K S Hegde Medical Academy Mangalore has contributed for data collection and analysis of the study undertaken
2. Dr Asha Rani S, Assistant Professor, Department of Microbiology, GIMS Kalaburagi has contributed in analysis of data in the study undertaken.
3. Dr Anitha T K, Associate professor, Department of Microbiology, East Point College of Medical Sciences and Research Centre, Bangalore, has contributed in writing the manuscript of the study undertaken.

Funding: none

Ethics Statement

none (as the study is retrospective in nature and does not involve any human or animal-based experiments)

Informed Consent

Retrospective study was conducted and this study does not involve an experiment on humans and animals

Data Availability

all datasets generated or analysed during this study are included in the manuscript.

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