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Research Article

Emergence of Non-Fermenting Gram Negative Bacilli as Multi-Drug Resistant Septicaemic Pathogen in A Tertiary Hospital

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Abstract

The Non Fermenting Gram Negative Bacilli were previously thought to be saprophytes existing solely as commensals or contaminants. But recent findings have established a clinical significance subject to their frequent isolation from clinical samples and pathological association with blood stream infections, immunocompromised hosts and patients with hematological malignancies. This study aims at providing a comprehensive view of this scenario through a span of one year in a tertiary hospital. *Acinetobacter species* was found to be the most common isolate comprising of 54.4% of all NFGNB, followed by Pseudomonas species (29.67%), *Burkholderia species* (2.75%) and *Sphingomonas paucimobilis* (0.55%). Analysis of antimicrobial susceptibility testing showed multidrug resistant pattern with majority of the isolates being resistant to three or more drugs

Keywords: NFGNB; Antimicrobial Susceptibility; Drug Resistance; Commensal; Septicaemia

Introduction

Non Fermenting Gram Negative Bacilli (NFGNB) are a taxonomically diverse group of ubiquitous organism that either do not utilize glucose as a source of energy or utilize it oxidatively [1]. These organisms were previously thought to be saprophytes existing solely as commensals or contaminants. However, recent findings have established a clinical significance subject to their frequent isolation from clinical samples and pathological association with blood stream infections, immunocompromised hosts and patients with hematological malignancies [2]. NFGNB have been recorded to account for 15% of all bacterial isolates from clinical microbiological laboratory [3]. These organisms normally cause device-related nosocomial infections in hospital environment owing to their potential to spread by hand-to-hand transmission between healthcare workers and patients through fomites [4]. Above all, their high resistance to disinfectants and the indiscriminate use of antimicrobials has facilitated colonization, creating an epidemiological niche for these pathogens [5-8]. Hence, our study aims at providing a comprehensive view of this scenario through a span of one year in a tertiary hospital. This study was conducted with the following objectives:

Primary: Identification and prevalence study of NFGNB from blood culture isolates of indoor patients and analysis of sensitivity patterns for a period of one year.

Secondary: Nomenclature atleast upto genus level and *Stenotrophomonas maltophilia* up to species level by recA gene detection.

Materials and Methods

This prospective study was conducted in the Department of Microbiology, Assam Medical College and Hospital, Dibrugarh which is a tertiary care facility, for a period of one year. A total of 4385 blood samples were received in blood culture bottles for

culture and sensitivity from in-patient wards of all departments. These were incubated in the BD BACTEC™ Automated Blood Culture System for detection of growth of organisms by fluorescent technology. The blood samples indicating growth were plated on blood agar (BA), chocolate agar (CA), and Mac Conkey's agar (MA), and incubated at 37°C for 18 - 24 hours before being reported as sterile or otherwise. The organisms isolated were identified using the appropriate biochemical tests [9]. After plating on MacConkey agar medium, all the organisms giving non-lactose fermenting (NLF) colonies and growing on Triple Sugar Iron Agar slant producing an alkaline reaction, denoted by K/K, were provisionally considered to be NFGNB.

Identification of isolate

Definative identification of NFGNB was primarily carried out by conventional biochemical testing based on phenotypic character involving an array of infrequently used media, reagents and a considerable amount of technical expertise. Vitek-2-compact was also used for the purpose of confirmation and detection of contaminants. The characters assessed included morphology on Gram-stain, motility by hanging-drop method; cytochrome oxidase production; reduction of nitrate and nitrite; production of DNase, onitrophenyl-3-D-galactopyranoside, and urease; liquefaction of gelatin; presence of lysine decarboxylase and arginine dihydrolase; indole production; activity in oxidation fermentation basal medium (Hugh-Leifson medium) to detect anaerobic breakdown of glucose [10,11].

Isolates of *Sphingomonas paucimobilis* was confirmed through molecular detection for RecA gene by Polymerase Chain Reaction, carried out in the Regional Medical Research Centre, Dibrugarh.

Antimicrobial susceptibility testing

The testing for antimicrobial susceptibility and detection of resistance was performed by the Kirby-Bauer disc diffusion method using commercially available discs (Hi-media). The organisms were tested against Carbapenem, Cephalosporin, Fluoroquinolone, Aminoglycoside groups and β-lactum combination agents.

The different antibiotics tested were Ceftriaxone (30mcg), Cefuroxime (30mcg), Cefepime (30mcg), Imipenem (10µg), Meropenem (10µg), Piperacillin-Tazobactam (100/10mcg), Ampicillin-Sulbactum (200/10mcg), Ciprofloxacin (5mcg), Amikacin (30mcg), Levofloxacin (5µg), Chloramphenicol (30µg), Polymixin-B (30µg) and Colistin (10mcg. The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines 2015.

Results and Discussion

Among 4385 total blood samples processed 1083 yielded pathogens and rest were reported as sterile or contaminated by poly-microbial growth. Out of this, 1056 pathogens were bacterial, of which non-fermenting gram negative bacilli were 182 in number, accounting for an isolation rate of 16.8% of all blood borne infections.

Phenotypic and genotypic detection

Acinetobacter species was found to be the most common isolate comprising of 54.4% of all NFGNB, followed by *Pseudomonas species* (29.67%), *Burkholderia species* (2.75%) and *Sphingomonas paucimobilis* (0.55%). Three isolates identified phenotypically as *Stenotrophomonas maltophilia* were outsourced to the Regional Medical Research Centre (RMRC) of Dibrugarh for confirmation by DNA extraction and recA gene polymerization reaction. 20 (10.9%) isolates could not be identified with limited available conventional tests and await genotypic detection. The recorded non-fermenters as enumerized in table 1 was isolated after processing the culture samples.

	Number (n)	Percentage (%)
Acinetobacter species	99	54.4
Pseudomonas species	54	29.67
Burkholderia species	5	2.75
Sphingomonas paucimobilis	1	0.55
Stenotrophomonas maltophilia	3	1.65
Unconfirmed non-fermenters	20	10.98
Total	182	100

Table 1

Antimicrobial sensitivity testing

Acinetobacter group showed 95.24% sensitivity towards Meropenem, 84% towards Imipenem and 68.48% towards Piperacillin-tazobactam with high resistance towards rest. Pseudomonas species also showed a similar trend with 91.30% sensitivity towards Meropenem, 82.93% towards Imipenem and 74% towards Piperacillin-tazobactam. A high multi-drug resistant trend is observed in this study with increasing resistance toeards the higher groups of antimicrobials. The resistance patterns of the two predominant organisms, Acinatobacter species and Pseudomonas species, isolated from the blood culture samples is given in table 2.

	Acinetobacter species	*Pseudomonas species*
Piperacillin-TZ	23.26	31.58
Cefuroxime	73.68	71.43
Ceftriaxone	48	50
Cefepime	100	42.86
Polymixin-B	50	0
Levofloxacin	9.09	20
Meropenem	14.29	0
Imipenem	19.05	26.32
Ciprofloxacin	52.27	14.29

Table 2

Following ATCC strains were used as Quality control strains:

- ATCC-25922 *Eschericia coli* – Gram-negative bacilli.
- ATCC-25923 *Staphylococcus aureus* – Gram-positive cocci.
- ATCC 27853 *Pseudomonas aeruginosa*- Non-Lactose Fermenters.

Discussion

Of total 4385 blood samples of indoor admitted patients sent for clinical detection, 182 Non fermenting Gram Negative bacilli were isolated from a total 1083 blood samples diagnosed with septicaemia, accounting for 16.8%. The isolation rate of NFGNB is 4.15%. This is in concordance with the finding of Malini A., *et al.* from Karnataka [12], with 4.1% isolation rate and less that of Samanta P., *et al*. from Chandigarh [13] with 10%. However a variation is expected depending on the technical expertise and infection control practices of different institutes. Since all the cases were seriously ill septicaemic subjects admitted to hospital, it can be safely commented that non-fermentative bacterimia is associated with immunocompromised state in this study. Similar findings from the studies of other workers have also shown that nosocomial nonfermentative bacteraemia has prepondency to patients with severe underlying diseases [14,15]. Moreover, since the patients were admitted, intravenous equipment such as intravenous lines might have played a major role in predisposing them to the pathogen as a source of infection. On that line, it has been emphasized by Vidal., *et al*. that non-fermenting bacteraemia is often associated with invasive devices and their study propagated that 60% of patients had central line [14].

Our study was conducted in a tertiary level public hospital catering as a reference centre to the entire upper Assam and bordering states as well. It offers a panoramic view of the microbial spectrum upto the genus level of pathogenic NFGNBs. *Acinetobacter species* was found to be the most common NFGNB (54.4%), followed by *Pseudomonas species* (29.67%). This finding is similar to other conducted works wherein also these two organisms were concluded as the predominant pathogens [15,16]. Non-fermenters other than *Acinetobacter spp*. and *Pseudomonas spp*. have also been isolated (Table-1). These organisms which were considered as saprophytic commensals in the past have recently emerged as important causes of morbidity and mortality especially in immuno-compromised patients. Recently plural studies have reported cases of bacteraemia caused by these unusual pathogens which can be proven by their frequent isolation from clinical materials and their association with disease [17-19].

High intrinsic resistance towards all antimicrobials is exhibited by all non-fermenters, which brings the importance of their proper identification to the forefront. They have been assigned as a major upcoming cause of multi-drug resistance in the world. For epidemiologic purposes, CDC/HICPAC has defined MDROs as microorganisms, predominantly bacteria, that are resistant to one or more classes of antimicrobial agents. Out study shows the emergence of MDR strains in seriously ill and debilitated patients admitted to the hospital. There was a high resistance towards cephalosporin group of drugs up to the fourth generation. Acinetobacter spp. showed 100% resistance towards cefepime with Pseudomonas species showing 43% resistance. Almost 50% of both pathogens were resistant to Ceftriaxone and more than 70% resistant to Cefoperazone. This strengthens the findings of other scientists lately where increasing resistance towards cephalosporins has been observed in non-fermenters [20].

Imipenem resistance was found to be 19% for *Acinetobacter baumannii* and 26.32% for Pseudomonas species. This is in contrast to findings of Juyal D., *et al*. [21] from Uttarakhand, who reported the resistance to be 31.13% and 27.05% respectively and in concordance with Gladstone P., *et al*. [22] (Tamil Nadu) who reported 12.2% and 50% respectively, although the difference is significant. This is important as studies have shown that several outbreaks due to carbapenem resistant NFGNB have resulted with considerable morbidity and mortality [23,24].

Resistance to the carbapenem group is due to reduced outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes, i.e. carbapenemases. These enzymes may be class B-metallo β -lactamases (IMP, VIM) or class D-oxacillinases (OXA-23 to OXA-27) or class A-clavulanic acid inhibitory enzymes (SME, NMC, IMI, KPC) [25]. The resistance hereby transmitted can be chromosomally or plasmid mediated and therefore pose a threat of spread of gene transfer among gram negative bacteria.

Conclusion

We can safely conclude on the basis of this study that non-fermenters, which were once considered commensals, have arrived as a potential pathological septicaemic agent in critically ill patients steadily gaining ground as multi-drug resistant strains. Although our study found Acinetobacter species to be comparatively more susceptible to regular antimicrobials than Pseudomonas group, the former has demographic significance here. Its rising resistance towards second and third generation Cephalosporins and Fluoroquinolones signify the emergence of multidrug resistance. Pseudomonas group also showed resistance towards second generation cephalosporins. Thus, antimicrobial agents which have been used and abused in the last century are now being rendered inactive and will consecutively turn useless in the long haul if rigorous practices of ethical antimicrobial usage and stewardship are not imposed globally.

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Conflict of Interest

None.

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