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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF NON-FERMENTING GRAM-NEGATIVE BACILLI (NFGNB) ISOLATED FROM CLINICAL SAMPLES AT A TERTIARY CARE HOSPITAL

Medical Microbiology

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ABSTRACT

Background: Non-fermenting gram-negative bacilli (NFGNB) or non-fermenters have emerged as major causes of nosocomial infections. They can cause minor infections, such as urinary tract infections, and severe infections such as pneumonia. They can be recovered from the hospital environment, devices such as ventilators and are often resistant to commonly used antibiotics. These NFGNBs are becoming increasingly resistant to antibiotics which are used in critically ill patients. **Materials & Methods:** This prospective study was carried out in the Department of Microbiology, 540 bedded tertiary care hospitals, for a period of Two year from January 2018 to December 2019. Various clinical samples were collected aseptically and processed according to standard laboratory protocols. **Results:** A total of 1446 NFGNBs (11%) were isolated from 13129 clinical specimens. Of the 1446NFGNB isolates, 1344(93%) were from IPD and 102 (7%) were from OPD samples. The predominant isolates were Pseudomonas aeruginosa 840(58%), Acinetobacter baumanii 419 (29%), Burkholderia cepecia 115 (8 %), and Stenotrophomonas maltophila 72 (5%). Among clinical samples, non-fermenters were found in blood 366 (25.3%), urine 288(19.9%), , sputum 266 (15%), pus 118 (8.1%), BAL 66 (4.4%), ET secretions 352(24.4%), central line tip 22(1.5%), tissue 12(0.8%), and swabs 8(0.6%). Most of the isolates were susceptible to Colistin (95-98%) **Conclusion:** Identification of non-fermenters in clinical settings is very important to prevent antibiotic resistance outbreaks and improved antibiotic stewardship, and infection control measures should be implemented to prevent nosocomial infections and spread of drug resistant nonfermenters.

KEYWORDS

NFGNBs, Pseudomonas aeruginosa, Acinetobacter baumanii, Burkholderia cepecia, Stenotrophomonas maltophila, nosocomial infections

INTRODUCTION:

Health care associated infections (HCAIs) are the infections that first appear 48 hours or more after hospitalization or within 30 days after having received health care [1]. The US Centre for Disease Control and Prevention identifies that nearly 1.7 million hospitalized patients annually acquire HCAIs while being treated for other health issues and that more than 98,000 of these patients (one in 17) die due to HCAIs (15). Developing countries like India found that 5-15% of the hospitalized patients acquire HCAIs which can affect from 9% to 37% of those admitted to intensive care units (ICUs) [1]. Europe hospital-wide prevalence rates of HCAIs range from 4.6% to 9.3%. Around 12–17 microorganisms cause 80%–87% of HCAIs i.e. Staphylococcus aureus, Enterococcus species (eg, faecalis, faecium), E. coli, coagulase-negative Staphylococci, Candida species (e.g. C.albicans, C.glabrata), K. pneumoniae and Klebsiella oxytoca, P. aeruginosa, A. baumannii, Enterobacter species, Proteus species, Burkholderia species, Stenotrophomonas maltophila, Yeast, Bacteroides species, and other pathogens . All these organisms are isolated from Hospital environment, Hospital supplied water, and medical devices like ventilators about 76% of nosocomial infection in the intensive care unit (ICU) were Ventilator- associated pneumonia (VAP). Ventilator-associated pneumonia (VAP) is associated with increased mortality, morbidity, and medical costs.

Non-fermentative gram-negative bacilli (NFGNB)or non-fermenters are a group of aerobic, non-spore forming bacilli; some of them are present as normal commensal flora of the gut and skin, most of which are non-pathogenic but cause opportunistic infections if the immunity is compromised [2]. These organisms play a major role in the development of HCAIs.

There are at least 15 families of non-fermenting gram-negative bacilli (NFGNB), but few of them are routinely isolated in clinical microbiology laboratories, including Pseudomonas, Acinetobacter, Burkholderia and Stenotrophomonas maltophila species. These are emerging as important causes of bloodstream infections (BSI) worldwide, particularly in immunocompromised patients with haematological malignancies and patients admitted to intensive care units (ICUs) [3].

In recent times, nonfermenters have recovered with increasing frequency from clinical specimens in a higher proportion of hospitalised patients suffering from illnesses such as urinary tract infection, ventilator-associated pneumonia, surgical site infection, and septicaemia. These organisms are highly resistant to commonly used antibiotics, leading to high morbidity and mortality rates [2,3,4].

The identification of these non-fermenters is important because most of them are resistant to many antibiotics and spread their genetic material to other organisms [5]. The prevalence and antimicrobial susceptibility profiles of NFGNB strains may show regional variations. Therefore, epidemiological studies are needed to establish appropriate therapeutic management strategies to prevent NFGNB induced infections. Hence, the present study was carried out to determine the prevalence and antibiotic susceptibility pattern of NFGNBs at the Department of Microbiology, between January 2018

and December 2019.

Aim Of The Study:
The aim of this study was to identify the NFGNBs and their prevalence and antimicrobial susceptibility profiles in various clinical specimens.

METHODOLOGY:
This prospective study was carried out in the Department of Microbiology, ESI Medical College Hospital, for a period of Two year from January 2018 to December 2019. Ethical clearance was obtained from the Institute. Inpatient and outpatient samples were included in the study. Non-fermenting bacteria isolated from various clinical specimens such as pus, urine, blood, bronchoalveolar lavage, endotracheal aspirations, drain tip, and cerebrospinal fluid were collected from both OPD and IPD of the 540 bedded tertiary care Hospital.

Sample processing: The samples were processed according to standard procedures. The collected samples were subjected to direct Gram staining, and all specimens were inoculated onto 5% sheep blood agar and MacConkey agar medium. Urine samples were inoculated onto Cysteine lactose electrolyte deficient agar (CLED). Blood culture was performed by collecting 5-10 mL of blood in BacTAlert culture bottles and subsequently incubated in BacTAlert, a fully automated blood culture system for detection of growth in blood culture. To Obtain a positive alarm, Gram staining was carried out in positive bottles, followed by subculturing on 5% sheep blood agar and MacConkey agar plates which were incubated aerobically at37°Covernight for bacterial isolation and were isolated and identified by standard protocol [6].

Antibiotic susceptibility patterns were determined using Mueller Hinton Agar by the Kirby- Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI-2018,2019). The following antibiotic discs were used for disc diffusion testing: ceftazidime(30µg), cefepime(30µg), ciprofloxacin (30µg), levofloxacin (30µg), norfloxacin (30µg), amikacin (30µg), gentamycin (30µg), tobramycin (30µg), piperacillin-tazobactam, imipenem (30µg), meropenem (30µg), colistin (Etest strip) (Hi-Media). The control strains used were E. coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 overnight broth culture compared to 0.5 McFarland's was used as inoculum. After incubation at 37oC for 16-18 hrs., the zone of inhibition was noted. The results were interpreted according to the CLSI standard [7].

Results
A total of 1446 (11%) NFGNBs were isolated from 13129 clinical specimen. Of the 1446NFGNB isolates, 1344(93%) were from IPD and 102 (7%) were from OPD samples. The predominant isolates from OPD and IPD samples were Pseudomonas aeruginosa 840(58%), Acinetobacter baumanii 419 (29%), Burkholderia cepecia 115 (8 %), and Stenotrophomonasmaltophila72 (5%) (fig 1) (Table1)

The various clinical specimens from which NFGNB were isolated where blood 366 (25.3%), urine 288(19.9%), , sputum 266 (15%), pus 118 (8.1%), BAL 66 (4.4%), ET secretions 352(24.4%), central line tip 22(1.5%), tissue 12(0.8%), and swabs 8(0.6%) (Pic 2) (Table1). The highest NFGNBs were from blood cultures, and the lowest was isolated from swabs.

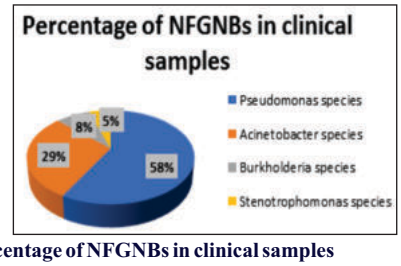

Fig1: Percentage of NFGNBs in clinical samples

Table 1: NFGNBs Isolated in Various samples

		Pseudomonas species		Acinetobacter species		Burkholderia species		Stenotrophomonas species		Total	
S. NO	Samples	No. of Strains	%	No. of Strains	%	No. of Strains	%	No. of Strains	%	No.GNB	%
1	Urine	252	30%	-	-	10	4%	26	38%	288	19.9
2	Blood	202	24%	56	13%	87	41%	21	27%	366	25.3
3	Sputum	151	18%	65	15%	-	- -	-	-	216	15.0
4	Pus	118	14%	-	-	-	-	-	-	118	8.1
5	BAL	17	2%	47	11%					64	4.4
6	Tracheal secretion	101	12%	251	60%	-	-	-	-	352	24.4
7	Central Line tip	-	-	-	-	18	16%	4	5%	22	1.5
8	Tissue	-	-	-	-	-	-	12	16%	12	0.8
9	Pus swab	-	-	-	-	-	-	8	11%	8	0.6
	Total	840	-	419	-	115	-	72	-	1446	100.0
	% NFGNB	58%	-	29%	-	8%	-	5%	-	-	100.0

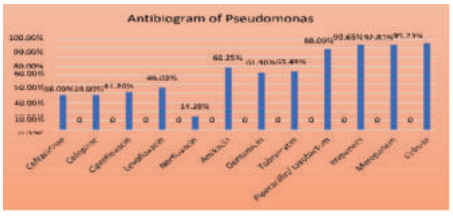

Fig 2: Antibiogram of Pseudomonas aeruginosa

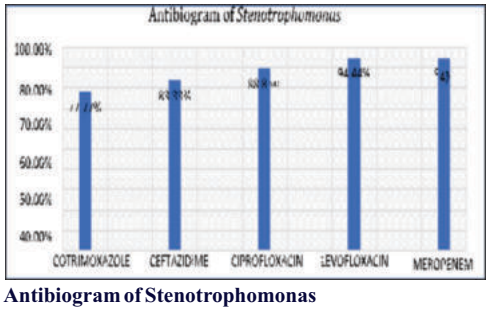

Fig 3: Antibiogram of Stenotrophomonas

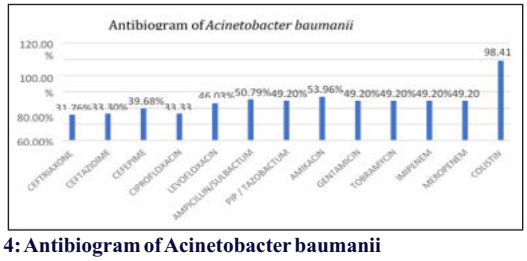

Fig 4: Antibiogram of Acinetobacter baumanii

DISCUSSION:
In this present study, the prevalence rate of NFGNBs was 11%, which is similar to the study conducted by Siddiq Heena kausar et al. (2018). The prevalence rate is dependent on place and hospital infection control measures; currently there is no official review data available on the prevalence rate of non-fermenters. Previous studies mentioned that the most prevalent organism in NFGNBs was Pseudomonas aeruginosa, followed by Acinetobacter baumanii [2, 3, 4, 8, 9,10,11]. In the present study, more than half of the isolates were Pseudomonas aeruginosa (58%), followed by Acinetobacter baumanii (29%), Burkholderia cepecia 115 (8 %), and Stenotrophomonas maltophila72 (5%).

Among clinical samples, non-fermenters were predominantly observed in blood cultures (25%), followed by tracheal aspiration (24.3%) and urine (19%). In earlier studies, a high percentage of NFGNBs were isolated from Pus samples followed by urine [2,3], which was in contrast to other previous studies, but this was correlated with the study conducted by (Frincy K Baruah et al.(2015).

In this study,95% of Pseudomonas aeruginosa strains were sensitive to colistin which is similar to the results reported by Fehlberg et al. and Siddiqui et al. and Lakshmi et al.[2,11,14], who reported 100% sensitivity. Compared to other studies, 95% of Pseudomonas aeruginosa strains were sensitive to carbapenems, and this was the highest sensitivity Siddique et al. reported75.9% sensitivity, Malina et al. reported 82% [2,4]. Unsceptibility to piperacillin-tazobactam in the present study was 88 %, Siddique et al et al. 64.5%, quinolones showed the highest resistance, 55% higher than other studies [2]. (fig 3).

A. baumanii is an organism which exhibits high resistance to antibiotics in hospital environment. In the present study,98.5% of isolates were sensitive to colistin, carbapenems showed 51% resistance which is like Sarkar et al[12]. 44% higher than the study conducted by Siddique et al [2], WHO reported 67% piperacillin-tazobactam, 53% to amikacin, and 43% to ceftazidime, while ceftazidime and cefepime showed maximum resistance at 60-70% (fig 4) S. maltophila is an emerging nosocomial pathogen in hospital settings. The significance of this organism lies in its intrinsic multidrug resistance. S. maltophila is highly resistant to many antibiotics commonly used in hospital settings. In contrast, in the present study, these isolates were very sensitive to quinolones 80-90%, cotrimoxazole (77%), and ceftazidime (77%) [11,13].

Burkholderia cepecia has emerged as a significant respiratory pathogen. A study conducted in Chandigarh showed that majority of these isolates were from blood. In the present study, organisms were isolated from blood. These organisms were sensitive to carbapenems (98%), quinolones (62%), and ceftazidime (73%). Another study found a similar susceptibility profile with resistance to aminoglycosides, as observed in the current study [12,13].

CONCLUSION:

In this study most of the NFGNBs were isolated from IP samples; It may be due to contamination of the IPD departments, needs to be rule out, most of the isolates have shown a very good sensitivity to Carbapenems and Colistin (those are the last resort of drugs) still 5-7% of the isolates are resistant to carbapenems, these resistance genes may transfer their genetic material to other susceptible organisms. Therefore, microbiologists and clinicians must be updated with the prevalence and antimicrobial susceptibility patterns of circulating pathogens in their healthcare settings.

Appropriate antimicrobials should be used for empirical therapy. Since, these organisms have great potential to survive in hospital environment, improved antibiotic stewardship and infection control measures are needed to prevent the emergence and spread of drug resistant NFGNBs in the healthcare settings.

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