Identification & Importance of Antimicrobial Susceptibility Of Non-Fermenting Gram-Negative Bacilli Among Various Clinical Specimens In A Tertiary Care Hospital

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ABSTRACT

Introduction: Non-fermenting gram-negative bacilli (NFGNB)have emerged as important healthcare-associated pathogens. NFGNBs are innately resistant to many antibiotics and are known to acquire resistance by producing extended-spectrum beta-lactamase. This makes treatment of infections caused by these pathogens both difficult and expensive. Therefore, this study is undertaken with the aim of identification of NFGNB and their antimicrobial susceptibility pattern in our hospital. **Material and Method:** A total of 3233 specimens were received in the bacteriology section of the microbiology department from September 2022 to May 2023. Clinical specimens were processed for culture according to standard operating procedures. Identification and antibiotic sensitivity testing were performed by an automated: VITEK-2 compact system.**Result:** A total of 486 (15.03%) NFGNB were isolated from 3233 clinical specimens. Out of 486 isolates, *Acinetobacter baumannii* complex was the most common non-fermenter, accounting for 238 (48.97%) isolates, followed by *Pseudomonas aeruginosa* 229 (47.12%). Other significant NFGNBs isolated were: *Stenotrophomonas maltophilia* 4 (0.82%), *Myroides* spp. 4 (0.82%) etc. Vitek 2 detected carbapenem resistance in 226 (95%) of *A. baumannii* complex and 135 (59%) of *P. aeruginosa* isolates. **Conclusion:** *A. baumannii* complex and *P. aeruginosa* were the most common NFGNB isolated from wound swabs, bronchoalveolar lavage (BAL), blood, sputum, tissue, and pus. They were found to be most resistant to quinolones, carbapenems, and aminoglycosides.

Keywords:– Non-Fermenting Gram-Negative Bacilli, Antimicrobial susceptibility, VITEK -2

INTRODUCTION

The non-fermentative gram-negative bacilli (NFGNB) are a group of aerobic, non-spore-forming bacilli, that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation[1]. These bacteria are common inhabitants of soil and water[2]. Although frequently considered contaminants, most of them have emerged as important nosocomial pathogens causing opportunistic infections in immunocompromised hosts. NFGNB are known to account for about 28.26% of all bacterial isolates from a clinical microbiology laboratory[3]. NFGNBs causes various infections including wound infections, urinary tract infections, meningitis, pneumonia, septicemia, osteomyelitis, etc.[4] They are usually associated with risk factors like immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostics, therapeutic procedures, prolonged hospital stay, and broad-spectrum antibiotics[5]. This group includes *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*, *Burkholderia*, *Alcaligenes*, *Weeksella* spp, etc. Among these *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most isolated non-fermenters which cause pathogenic infections in humans[3]. Members of nonfermenting gram-negative bacteria show innate resistance to many antibiotics and are known to produce extended-spectrum beta-lactamases (ESBL) and Metallo Beta Lactamases (MBL)[1]. Resistance to carbapenems in non-fermenters can be intrinsic or acquired. Intrinsic resistance is seen in *S. maltophilia* while acquired Class B metallo beta-lactamases (MBL) and Class D serine carbapenamases are frequently found in *P. aeruginosa* and *Acinetobacter* spp respectively[6]. Multidrug resistance (MDR) exhibited by non-

fermenters poses a major clinical problem in treating infections caused by them. Therefore, early identification and institution of appropriate treatment is necessary to reduce the morbidity and mortality due to these organisms in hospitalized patients[7]. In recent years, the number of diagnoses of infections caused by non-fermentative bacilli has increased, largely because of a heightened awareness of the possible roles of these bacteria as pathogens rather than contaminants. Hence this study has been undertaken to isolate and speciate the nonfermenting gram-negative bacilli in various clinical samples.

MATERIAL AND METHOD

A retrospective study was conducted in a tertiary care hospital in Ahmedabad over a period of 7 months from September 2022 to March 2023. The analysis of the reports was done in the Microbiology Department.

Patient Enrollment: Samples from patients visiting the Outpatient Department and admitted to the hospital were included in our study.

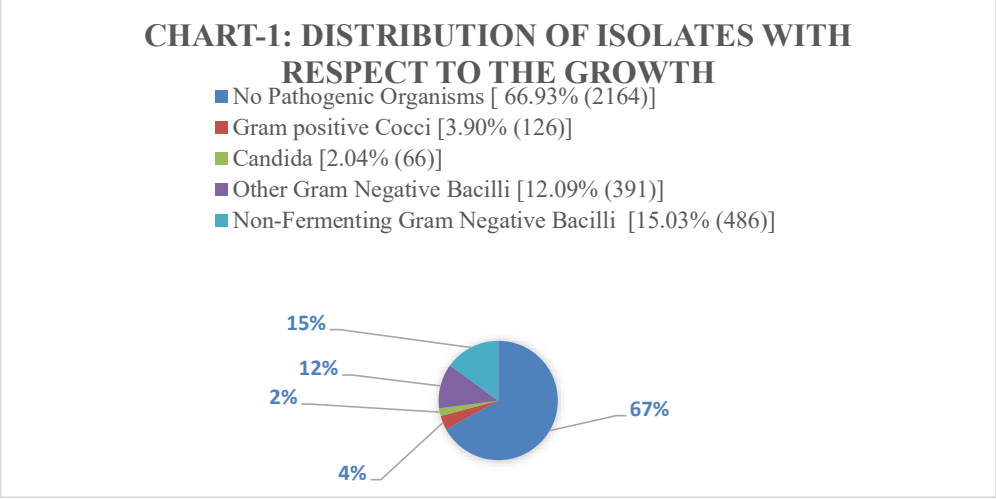
Sample Collection: All the clinical specimens received in the bacteriology lab of the microbiology department in a sterile leak-proof container for bacterial culture and sensitivity were included in the study.

Any specimens received in formalin were excluded from the study.

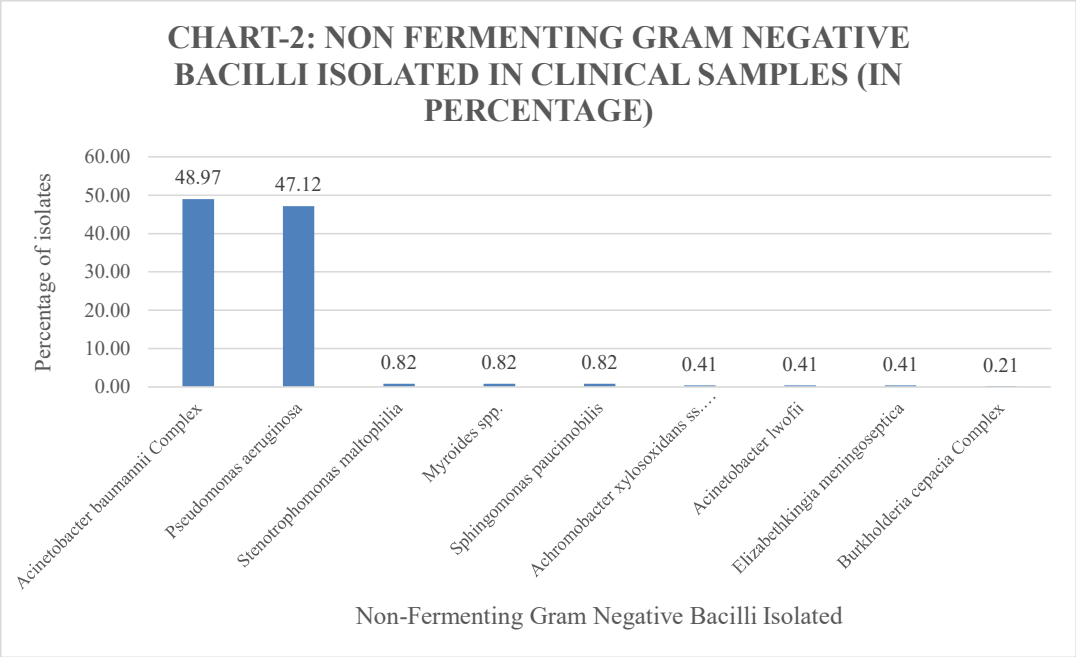
A total of 3233 specimens were enrolled in the study. The samples that were received were cultured on nutrient agar, blood agar, and MacConkey agar and incubated at 35 ± 2°C for 18-24 hrs. Gram staining was done of pale colonies that grew on the MacConkey agar and these gram-negative bacilli were provisionally considered as Non-Fermenting Gram Negative Bacilli (NFGNB). Thereafter, identification with the VITEK-2 compact system was performed using a Vitek 2 GN card according to the manufacturer's instructions. Antibiotic susceptibility testing with the VITEK-2 compact system was performed using an AST N406 susceptibility card. AST card 406 contains amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, colistin, gentamicin, imipenem, levofloxacin, meropenem, minocycline, piperacillin/tazobactam, trimethoprim/sulfamethoxazole. The reporting of the antimicrobial susceptibility was done using CLSI M100 guidelines[8,9].

RESULTS

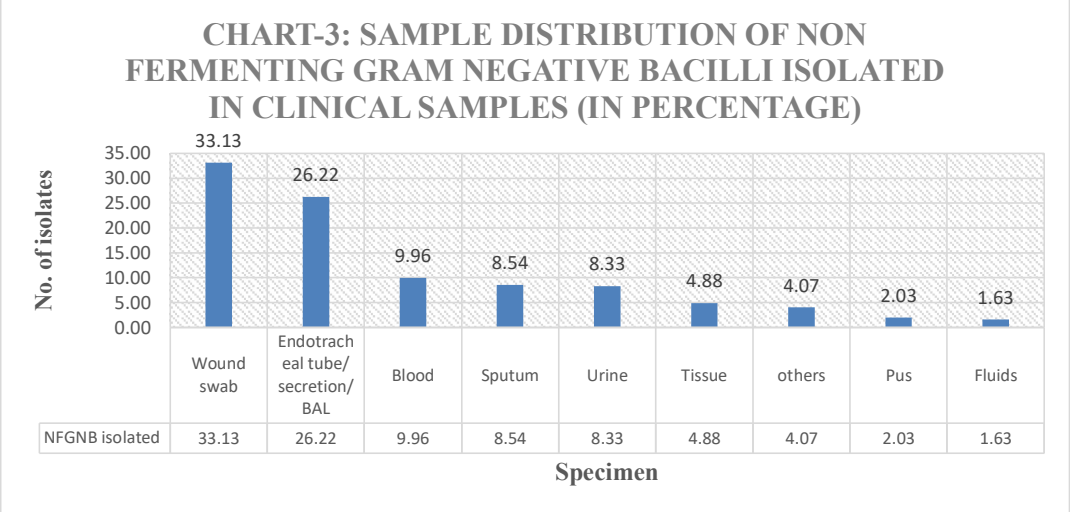
In total, 3233 culture specimens were received during the study period; 1069 (33.07%) cultures yielded significant pathogens and no pathogenic organisms were isolated in 2164 (66.93%) cultures. Among the 1069 (33.07%) of growth cultures, 486 (15.03%) were identified as non-fermenting gram negative bacilli, 391 (12.09%) as other gram-negative bacilli, 126 (3.90%) were identified as gram-positive cocci and 66 (2.04%) isolates were identified as *Candida* by the VITEK-2 compact system [Chart-1].



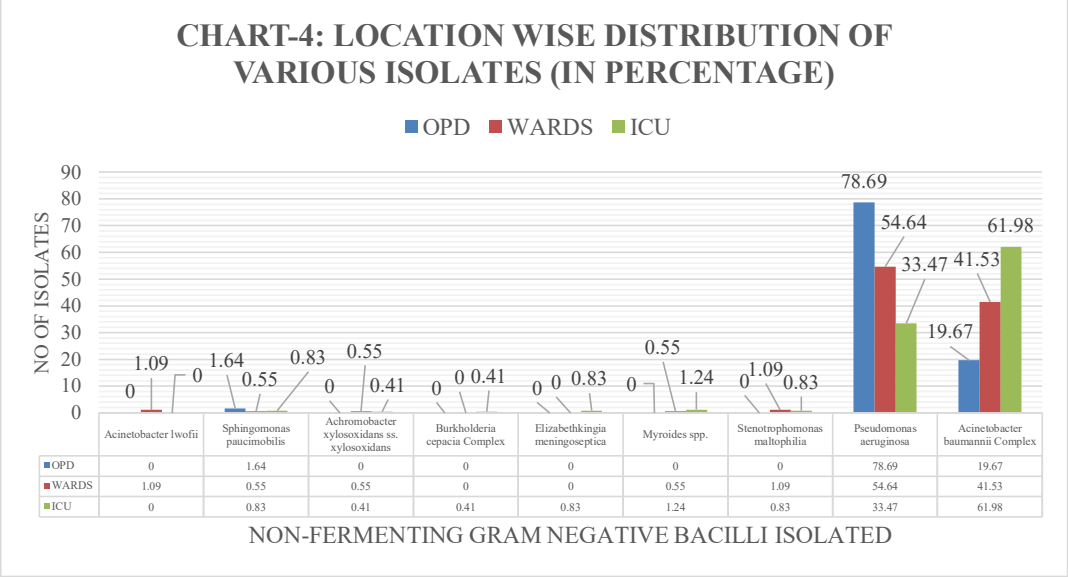
Out of 486 NFGNB, *Acinetobacter baumannii* complex 238 (48.97%) and *Pseudomonas aeruginosa* 229 (47.12%) contributed to the major number of isolates. The other non-fermenting gram-negative bacilli isolated were *Stenotrophomonas maltophilia* 4 (0.82%), *Myroides spp.* 4 (0.82%), Sphingomonas *paucimobilis* 4 (0.82%), *Achromobacter xylosoxidans ss. xylosoxidans* 2 (0.41%), *Acinetobacter lwofii* 2 (0.41%), *Elizabethkingia meningoseptica* 2 (0.41%), and *Burkholderia cepacia* Complex 1 (0.21%) [Chart-2].



Out of 486 NFGNB, 163 (33.13%) NFGNB were isolated from wound swab, 129 (26.22%) from Endotracheal tube/secretion/BAL, 49 (9.96%) from blood, 42 (8.54%) from sputum, 41 (8.33%) from urine, 24 (4.88%) from tissues, 10 (2.03%) from pus, 8 (1.63%) from fluids and rest 20 (4.07%) from other specimens [Chart 3].



Most non-fermenters were isolated from ICU [242 (49.79%)] followed by wards [183 (37.65%)] and OPD [61 (12.55%)] [Chart-4].



The antimicrobial resistance pattern of the isolated strains are shown in Table 1.

Table 1: Anti-microbial resistance pattern of the isolated strains in percentage

TOTAL ISOLATES	AZT	AMI	CEP	CTZ	LEV	CIP	COL	GEN	IMI	MER	MIN	PIT	TRS
A. baumannii Complex (n=238)	IR	84 (n=201)	89 (n=213)	86 (n=204)	79 (n=187)	94 (n=224)	0 (n=0)	92 (n=218)	95 (n=226)	94 (n=224)	35 (n=84)	95 (n=225)	92 (n=218)
P. aeruginosa (n=229)	9 (n=20)	59 (n=134)	55 (n=125)	64 (n=147)	72 (n=165)	71 (n=162)	3 (n=7)	62 (n=143)	55 (n=125)	59 (n=135)	IR	63 (n=144)	IR
S. maltophilia (n=4)	IR	IR	NT	NT	50 (n=2)	NT	NT	IR	IR	IR	25 (n=1)	IR	75 (n=3)
Myroides spp. (n=4)	100 (n=4)	100 (n=4)	100 (n=4)	100 (n=4)	100 (n=4)	100 (n=4)	NT	100 (n=4)	100 (n=4)	100 (n=4)	0 (n=0)	100 (n=4)	100 (n=4)
S. paucimobilis (n=4)	75 (n=3)	25 (n=1)	25 (n=1)	50 (n=2)	25 (n=1)	75 (n=3)	NT	50 (n=2)	50 (n=2)	50 (n=2)	0 (n=0)	25 (n=1)	25 (n=1)
A. xylosoxidans ss. xylosoxidans (n=2)	100 (n=2)	50 (n=1)	50 (n=1)	0 (n=0)	0 (n=0)	100 (n=2)	NT	50 (n=2)	0 (n=0)	0 (n=0)	0 (n=0)	0 (n=0)	100 (n=2)
A. lwofii (n=2)	IR	100 (n=2)	100 (n=2)	100 (n=2)	50 (n=1)	50 (n=1)	0 (n=0)	50 (n=1)	100 (n=2)	100 (n=2)	50 (n=1)	100 (n=2)	100 (n=2)
E. meningoseptica (n=2)	50 (n=1)	100 (n=2)	100 (n=2)	100 (n=2)	50 (n=1)	100 (n=2)	NT	100 (n=2)	100 (n=2)	100 (n=2)	50 (n=1)	100 (n=2)	100 (n=2)
B. cepacia Complex (n=1)	NT	NT	NT	100 (n=1)	100 (n=1)	NT	IR	NT	NT	0 (n=0)	0 (n=0)	NT	100 (n=1)

Abbreviation of antibiotic: EUCAST System for Antimicrobial Abbreviations[10]. AZT- Aztreonam, AMI- Amikacin, CEP- Cefepime, CTZ- Ceftazidime, LEV- Levofloxacin, CIP- Ciprofloxacin, COL- Colistin, GEN- Gentamicin, IMI- Imipenem, MER- Meropenem, MIN- Minocycline, PIT- Piperacillin-tazobactam, TRS- Trimethoprim-sulfamethoxazole. Abbreviations: IR:- Intrinsic resistance; NT:- Not tested

Vitek 2 data was entered into WHONET which detected Multi-Drug Resistant (MDR), Possible Pan-Drug Resistant (PDR), and Possible Extreme Drug Resistant (XDR) isolates. 403 (82.92%) of 486 NFGNB were found to be MDR, out of which 229 (47.12%) isolates were *A. baumannii* complex, 170 (34.98%) isolates were *P. aeruginosa* isolates, 2 (0.411%) were *A. lwofii*, 1 (0.21%) was *Elizabethkingia meningoseptica* and 1 (0.21%) was *Sphingomonas paucimobilis*. These 403 NFGNB were susceptible to colistin only. 178 (30.25%) of the total 486 isolates were found to be possible XDR with 135 (27.78%) isolates of *A. baumannii* complex, 42 (8.64%) isolates of *P. aeruginosa*, and 1 (0.21%) isolate of *Acinetobacter lwofii*. Possible PDR was found to be in 214 (44.03%) of total 486 isolates; 116 (23.86%) *A. baumannii* complex, 95 (19.54%) *P. aeruginosa*, and 1 (0.21%) isolate each of *Sphingomonas paucimobilis*, *Acinetobacter lwofii*, *Elizabethkingia meningoseptica*.
Table 2 shows the carbapenem resistant *A. baumannii* complex isolates and *P. aeruginosa* isolates.

Table 2: Carbapenem resistant *A. baumannii* complex and *P. aeruginosa* isolates.

A. baumannii **Isolates (%)**	*P. aeruginosa***Isolates (%)**
95 (n=225)	59 (n=135)

DISCUSSION

Non-fermenting Gram-Negative bacilli (NFGNB) are being isolated with increasing frequency from clinical specimens and treatment failure due to their multidrug resistance in recent years. 15.03% (486) clinical isolates of non-fermenting Gram-negative bacilli isolated from various clinical samples like pus, urine, endotracheal aspirates, blood, sputum, body fluids, and were evaluated for their role in infections in hospitalized patients including the characteristics of their drug resistance. A similar isolation rate (14.6%) was seen in a study done by Shweta Sharma et al[11]. Less isolation rate (7.59%) was reported in a study by Suhani Gondha et al[12] in 2022. In this study, the maximum number of isolates were from intensive care units (49.79%) followed by wards (37.65%) and OPDs (12.55%).

In the present study, the commonest isolates were *Acinetobacter baumanii* complex 48.97% (238) followed by *Pseudomonas aeruginosa*47.12% (229), *Stenotrophomonas maltophilia* 0.82% (4), *Myroides spp.* 0.82% (4), Sphingomonas *paucimobilis* 0.82% (4), *Achromobacter xylosoxidans ss. xylosoxidans* 0.41% (2), *Acinetobacter lwoffii*0.41% (2), *Elizabethkingia meningoseptica* 0.41% (2), and *Burkholderia cepacia* Complex 0.21% (1).

On comparison with the similar study done by Anu Sharma et al.[13], it was found that the highest number of non-fermentative gram-negative bacilli isolated was *Pseudomonas aeruginosa* with the rate of isolation (56%) different than this study (47.12%) . Study done by Yadav et al[14] showed 44% isolation rate of *Acinetobacter baumanii* complex.

In a study done by Ndzabandzaba S et al[15], *Acinetobacter baumanii* complex (20.4%) was the third most commonly isolated organism from ICUs after *Burkholderia cepacia* (24%) and *S.maltophilia* (23.3%). In the present study, *Acinetobacter baumanii* complex (61.98%) was the most commonly isolated NFGNB followed by *Pseudomonas aeruginosa*(33.47%).

Because of the high intrinsic resistance of different NFGNBs to different antimicrobial agents, the value of proper identification and resistance testing is foremost important in each setup to guide the appropriate selection of empiric therapy. The antimicrobial susceptibility pattern of *A. baumanii* complex showed resistance to imipenem(95%), meropenem(95%), and piperacillin/ tazobactam(95%) followed by ciprofloxacin(94%) gentamicin (92%), trimethoprim-sulfamethoxazole (92%), cefepime (89%), ceftazidime (86%), and amikacin (84%). *P. aeruginosa* showed resistance to ciprofloxacin (72%), levofloxacin(72%) followed by ceftazidime (64%), gentamicin(63%), piperacillin/tazobactam (63%), meropenem(59%), amikacin (59%), imipenem(55%) and cefepime (55%).

NFGNB, especially *P. aeruginosa* and *A. baumannii*, are remarkably adept at adapting to environmental pressures, upregulating their intrinsic resistance mechanisms, and acquiring and transferring drug-resistance genes through mobile genetic elements such as plasmids and transposons[16]. As a result, these bacteria exhibit an alarmingly high overall drug resistance prevalence rate against diverse categories of drugs. The acquired drug resistance genes enable bacteria to produce beta-lactamase enzymes, particularly ESBLs, which confer resistance to most beta-lactam antibiotics. Furthermore, ESBL-producing Gram-negative bacteria have been identified to possess additional

resistance mechanisms to other categories of antimicrobials, including phenicol's, sulfonamides, fluoroquinolones, tetracyclines, and aminoglycosides.
Multidrug resistance is a major problem with non-fermenting gram-negative bacilli and so the infections caused by them are very difficult to treat. Polymyxins are the last resort antimicrobial drug class with consistent activity against multidrug-resistant strains of non-fermenters.

CONCLUSION

Non-fermenting gram-negative bacteria are a group of microorganisms that pose significant challenges in clinical microbiology. These bacteria, which include members of the *Acinetobacter* group, are commonly found in various environments such as human skin, soil, water, and sewage. They are opportunistic pathogens and have the potential to cause nosocomial infections, making them a concern for healthcare providers. The clinical importance of these non-fermenting gram-negative bacteria is still unknown but these studies should raise awareness among physicians. In recent years, there has been a global increase in infections caused by gram-negative bacteria, with gram-negative organisms often surpassing gram-positive infections in prevalence in various healthcare settings. This trend highlights the need for effective strategies to detect and manage infections caused by non-fermenting gram-negative bacteria. Given their resistance to antibiotics and ability to cause infections in different body sites, including the lungs, abdomen, and incision sites, healthcare providers must be vigilant and proactive in identifying and treating these infections. The Infectious Diseases Society of America recognizes non-fermenting gram-negative bacteria as some of the most problematic pathogens in the field of infectious diseases. These bacteria present unique challenges due to their ability to resist multiple antibiotics, including some of the most commonly used drugs in clinical settings. Therefore, healthcare providers must stay updated on the latest research and clinical guidelines surrounding non-fermenting gram-negative bacteria.Efforts must be made to improve detection methods and develop effective strategies for managing infections caused by these bacteria.

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