Isolation of Different Non-Lactose Fermenting Gram Negative Bacilli (NLFGNB) and their Antimicrobial Resistant Pattern: A Retrospective Analysis

Raval Pankaj V[1], Goswami Parijat N[2], Desai Nilima S[3], Patel Hiren A[4]
Fellow[1], Professor and Head[2], Junior Scientific Officer[3], Resident[4]
Department of Microbiology
The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad, Gujarat, India.
Corresponding Author: parijath.goswami@gcriindia.org
[1]https://orcid.org/0000-0003-1675-3827;
[2]https://orcid.org/0000-0002-7889-3676;
[3]https://orcid.org/0000-0002-1798-2472;
[4]https://orcid.org/0000-0001-9395-9840

Summary

The emergence of gram-negative bacilli causing infections has created untreatable problems due to increasing antibiotic resistance thus complicating cancer treatment, prolonging stay in the hospital and escalating the burden of cost on the patients. Our study of retrospective analysis was focused on the common and uncommon pathogenic isolates of non-lactose fermenter gram negative bacilli (NLFGNB) to know the prevalence of different isolates which were less (42%) in contrast to lactose fermenter gram negative bacilli (58%). All the standard microbiological methods were followed including the identification (ID) and antibiotic susceptibility testing (AST) by Vitek-2 and the analysis was for a period of two years (2019-2020). The isolates identified were Pseudomonas 59%, Acinetobacter 23%, Burkholderia 10%, and least isolation was of Sphingomonas 5% and proteus 3%. The most uncommon NLFGNB isolated were only two, namely Achromobacter xylosoxidans and Elizabethkingia. Antimicrobial resistance showed that more than 50% of them were MDR (Multiple Drug Resistance) (MDR is shown by at least one antimicrobial drug in three or more antimicrobial category). Even though there were two unusual bacilli isolated (Achromobacter, Elizabethkingia) they were sensitive to most of the antimicrobitics but 100% of Achromobacter spp. (single isolate) was resistant to gentamycin and aztreonam. Forty to fifty percent of pseudomonas spp were resistant to carbapenems, aminoglycosides, and quinolones. Thirty to forty percent of them were resistant to betalactam+betalactamase inhibitors (BL+BLI) and to also third and fourth generation cephalosporins. Acinetobacter species had 6.6% to 28% resistance to tigecycline, minocycline and colistin. In other words, around 93.4% to 72% were sensitive and can be drug of choice to treat infections caused by them. Ninety percent of Burkholderia spp were resistant to betalactam+betalactamase inhibitors (BL+BLI) and 25% - 28% of them were resistant to carbapenems. Ninety to hundred percent of Proteus spp. were resistant to minocycline and tigecycline. To carbapenems there was low and high resistance like 27.6% to meropenem and 77.4% to imipenem. Sphingomonas Paucimobilis showed 39.7% to 77.4% resistant to most of the panel of antibiotics used. In conclusion, there was isolation of NLFGNBs which are multidrug resistant and complicating the treatment of cancer patients. There in a need for development of clinico-microbiological meetings and discussions to prevent the spread of antibiotic resistant NLFGNB from patient to patient and form antibiotic policies through antibiotic stewardship program (ASP).

Keywords: NLF, GNB, Antibiotic Resistance, Cancer, MDR.

Introduction

Non-Lactose fermenting gram-negative bacilli (NLFGNB) emerged as important health care associated infections leading to morbidity in the patients. Risk factors associated with the surge of these infections are prolonged hospital stay, lack of antibiotic policies, lapses in asepsis and unhygienic conditions prevailing in most of the hospitals.

Most common NLFGNB isolated from the patients are Pseudomonas aeruginosa, Acinetobacter baumanii, Burkholderia cepacia, Morganella morganii, Proteus mirabilis and Salmonella typhi.[1] Pseudomonas aeruginosa is an important and common pathogen in hospitalized patients, causing treatment failure due to its multiple resistant mechanisms in critically ill patients specifically in intensive care units and in wards because of its ubiquitous nature and ability to survive in moist hospital environment. It has multifactorial resistance mechanism like mutations in genes encoding porins, efflux pumps, penicillin-binding proteins, and chromosomal β-lactamase production, ESBL (Extended Spectrum Beta- Lactamases), carbapenemase etc.[2] Strains of P. aeruginosa are the cause of several diseases predominantly pneumonia, bacteraemia, meningitis, urinary tract infections, as well as skin and soft-tissue infections Sphingomonas paucimobilis, also an opportunistic pathogen that take advantage of underlying conditions and causes infectious disease. Burkholderia cepacia is an aerobic gram-negative bacillus found in various aquatic environment and has low virulence and is a frequent colonizer of fluids used in the hospital (e.g., irrigation solutions, intravenous fluids).

Thus, the purpose of this study was to know the prevalence of non-lactose fermenting gram negative bacilli causing super or opportunistic infections and its antibiotic susceptibility pattern in

cancer patients, thereby helping the clinicians for treating the patients

Methods and Materials

This retrospective study was conducted at the microbiology laboratory of The Gujarat Cancer & Research Institute for a period of 2 years (2019 to 2020). Patient's infectious samples were received in the laboratory who were suffering with different types of infections in clinically diagnosed cancers. All standard Microbiological methods were followed for isolation, preliminary identification, and further identification (ID), and antibiotic susceptibility testing (AST) performed by automated system called Vitek-2 compact. The results were entered in software of WHO Net, and data was analysed for antibiotic resistance pattern of NLFGNB.

Results

A total of 3121 gram-negative bacilli were isolated during the retrospective analysis. Out of these 58 % (1820/3121) were lactose fermenters and 42 % (1301/1321) were non lactose fermenter. (Figure 1) The isolation of NLF gram negative bacilli were from different patients' diagnosis having post operate infection (34%), leukaemia patient (20%), head & neck cancers (18%), gynec & gastrointestinal cancer (8%).(Figure 2)

As per Figure 3, out of 1301 NLFGNB, Pseudomonas isolated were 59%, Acinetobacter spp. were 23%, Burkholderia were 10%, Sphingomonas was 5%, and Proteus were 3%. There were two rare bacilli which were isolated. One each of Achromobacter zylosoxidans and Elizabethkingia.

Analysis of antimicrobial resistance was done for all 1299 NLFGNB (in general) and individual isolates. Combined antimicrobial resistance of all NLF GNB showed that 77.4% were resistant to aztreonam, around 53-55% resistance was observed for co-trimoxazole and ciprofloxacin. Around 40-49% resistance was seen for antibiotics like cefaperazone/sulbactam, ticarcillin /clavulanic acid, piperacillin/tazobactam, ceftazidime, cefepime carbapenems. There was 14-27% resistance to tigecycline, minocycline and colistin respectively.(Figure 4) The two isolates of NLF GNB, showed sensitivity to all the antibiotics, except single isolate of Achromobacter species showed 100% resistant to gentamycin & aztreonam.

Now looking into the individual species, antimicrobials resistance of pseudomonas spp (n=763) showed that 54% resistance was there for co-trimoxazole, around 40-50% resistance to carbapenem, aminoglycosides, quinolone, and 30-40% resistant to BL (Beta Lactamases) +BLI (Beta

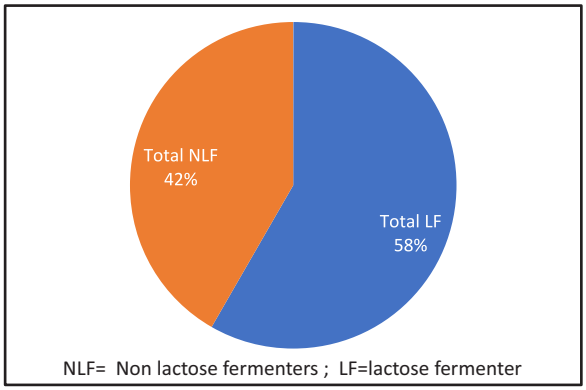

Figure 1: Differential growth of gram-negative bacilli on MacConkey agar (n=3121)

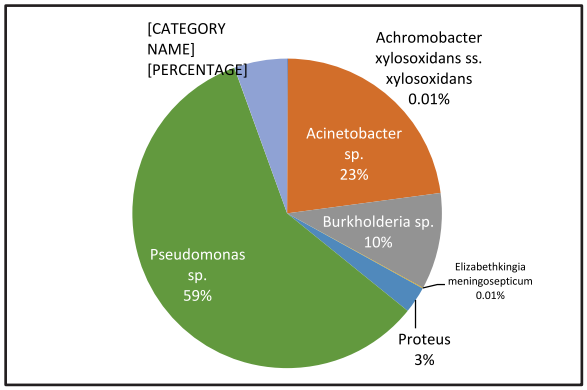

Figure 3: Different Isolates of Non-lactose fermenter Gram negative bacilli (n=1301)

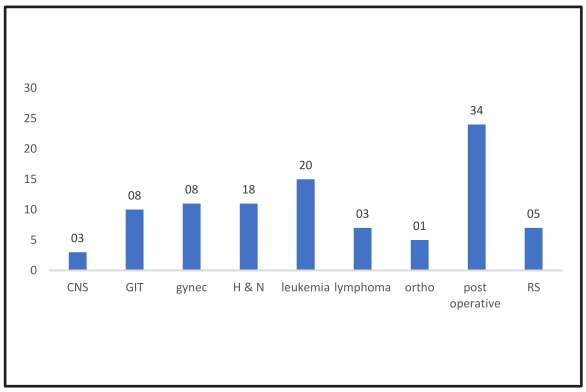

Figure 2: Percentage of Non-lactose fermenter GNBs in different cancers (n=1301)

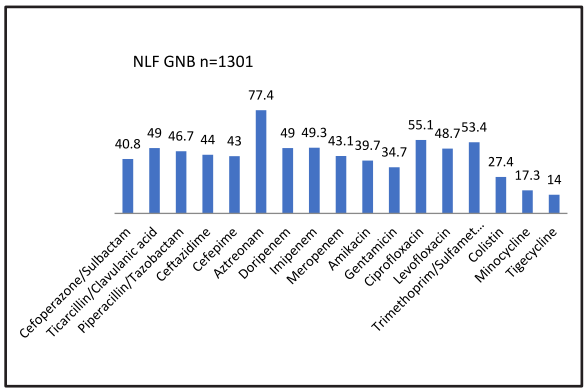

Figure 4: Antibiotic Resistant pattern of all NLF GNBs.

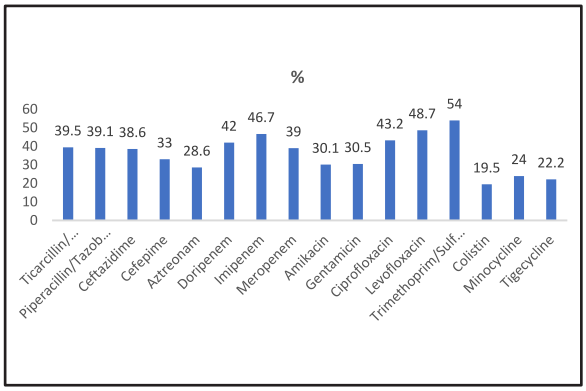

Figure 5: Antibiotic Resistant pattern of Pseudomonas Spp (n=763)

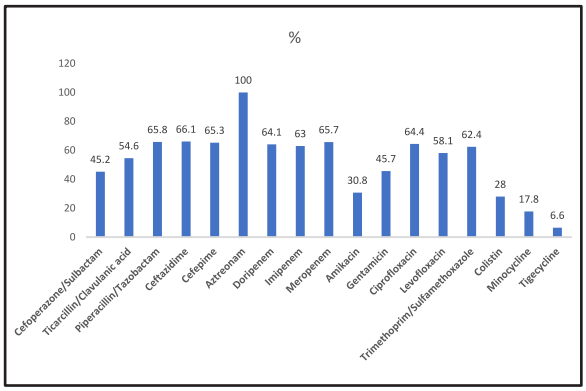

Figure 6: Antibiotic Resistant pattern of Acinetobacter spp (n =297)

Lactamases Inhibitor) like ticarcillin clavulanic acid, piperacillin / tazobactam, 3rd and 4th generation cephalosporins.(Figure 5)

In the case of Acinetobacter baumanii (n=297) isolates, they were 100% resistant to aztreonam, (50-66.1%) ticarcillin/clavulanic acid, piperacillin/tazobactam (65.8%), ceftazidime (66.1%), cefepime (65.3%), carbapenems (65.7%), ciprofloxacin (64.4%), levofloxacin (58.1%), co-trimoxazole (62.4%). Nonetheless, there was least resistance between 6.6% to 28% to tigecycline (6.6%) minocycline (17.8%) and colistin (28%).(Figure 6)

The NLF Burkholderia spp showed more than 90% resistance to BL+BLI. It was good that there was less resistance to carbapenems (25-28%), levofloxacin (32.2%) and tigecycline (21.3%) and minocycline (9.5%).(Figure7)

Non-lactose fermenting Proteus species have always been an issue and complicates admitted patients causing hospital acquired infections. The scenario is contrary to other NLFs showing that 90-100% resistance is seen to antibiotics like colistin, minocycline & tigecycline, to carbapenems there was low & high resistance to meropenem (27.6%) and imipenem (77.4%). To rest of the antibiotics like BL-BLI, Proteus was less resistant varying from 20 to 24.2%. (Figure 8)

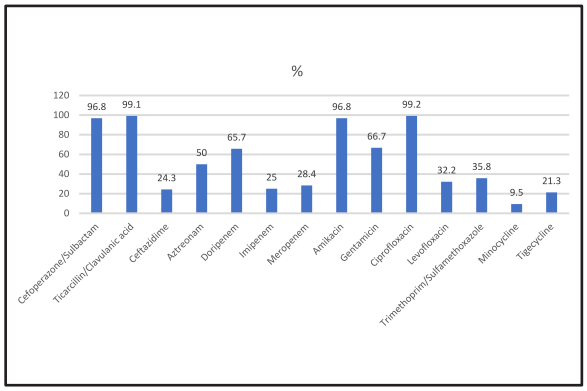

Figure 7: Antibiotic Resistant pattern in Burkholderia Sp.(n=131)

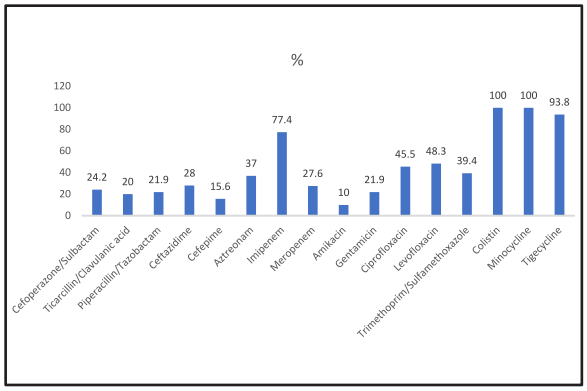

Figure 8: Antibiotic Resistant pattern of Proteus sp. (n=36)

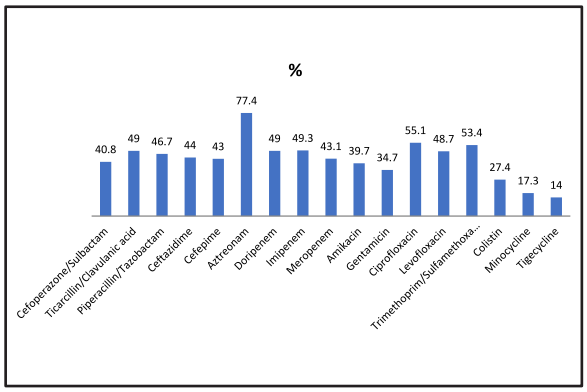

Figure 9: Antibiotic resistant pattern of Sphingmonas sp. (n=72)

There were (72) isolates of Sphingomonas paucimobilis and there was Sphingomonas showing 39.7% to 77.4 % resistance to most of the antibiotics.(Figure 9) But to tigecycline, minocycline and colistin the percentage resistance was 14%, 17.3% and 27.4% respectively.

As per Table 1 comparison was done between NLF GNB in total and singly isolated bacilli. Grouping of percentage resistance was done having above 50% and below 50% and were again grouped as per the Table 1. Fifty percent antibiotic resistance of other single NLFGNBs which ranged from 10-20%, for Pseudomonas was colistin and minocycline, for Acinetobacter and Sphingomonas was tigecycline, for

Table 1: Comparative antibiotic Resistance of all NLF GNBs in General and Individual Isolates

Resistant Range	AllNLF	Pseudomonas	Acinetobacter baumanii	Burkholderia	Proteus	Sphingomonas
Above 50%	Aztreonam-774% Ciprofloxacin-55.1 % Co-trimoxazole-53.4%	Co-trimoxazole–54%	Ticarcillin / Clavulanicacid Piperacillin/ Tazobactam Ceftazidime Cefepime	Cefoperazone/ Sulbactam Ticarcillin/ Clavulanicacid Aztreonam Doripenem Amikacin Ciprofloxacin Gentamicin Piperacillin/ Tazobactam Ceftazidime Cefepime Levofloxacin Trimethoprim/ Sulfamethoxazole	Imipenem Colistin Minocycline Tigecycline	Aztreonam Ciprofloxacin Co-trimoxazole
40–50%	Cefoperazone/ Sulbactam Ticarcillin/ Clavulanicacid Piperacillin/ Tazobactam Ceftazidime Cefepime Doripenem Imipenem Meropenem Levofloxacin	Doripenem Imipenem Ciprofloxacin Levofloxacin	Cefoperazone/ Sulbactam Gentamicin		Ciprofloxacin Levofloxacin	Cefoperazone/ Sulbactam Ticarcillin / Clavulanicacid Piperacillin / Tazobactam Ceftazidime Doripenem Imipenem Meropenem Levofloxacin Cefepime
30–40%	Amikacin Gentamicin	Ticarcillin / Clavulanicacid Piperacillin / Tazobactam Ceftazidime Cefepime Meropenem	Amikacin	Levofloxacin Co-trimoxazole	Aztreonam Co-trimoxazole	Amikacin Gentamicin
20–30%	Colistin	Cefoperazone/ Sulbactum Aztreonam Minocycline Tigecycline	Colistin	Cefepime Imipenem Meropenem Tigecycline Colistin	Cefoperazone / Sulbactam Ticarcillin / Clavulanicacid Piperacillin / Tazobactam Ceftazidime Meropenem Gentamicin	Colistin
10–20%	Minocycline Tigecycline	Colistin	Minocycline Tigecycline	Minocycline	Amikacin Cefepime	Minocycline Tigecycline

Burkholderia was minocycline and for proteus was amikacin and cefepime.

Discussion

Non-Lactose fermenting gram negative bacilli (NLFGNB) have now become multidrug resistant to most of the panels of antibiotics been used for invitro antibiotic susceptibility testing given as per CLSI guidelines. Once considered as contaminants are now gaining importance and emerging as health care associated infections.

In our study, the NLFGNB isolation was 42% in contrast to the study conducted by Grewal et al, where they found 11.6% (216) yield of NLF GNB out of 1854 culture-positive samples.

Two hundred and sixteen (11.6%) yielded NFGNB.[4] Since our institute is dedicated cancer centre and we have tumour surgeries done the isolation of NLFGNB were maximum (34%) from post-operative wounds.

Isolation of Pseudomonas sp. by Grewal et al was 87.96% whereas in our analysis we found much less (59%) when compared to them.[4] The other NLFGNB like Acinetobacter sp. isolation was more with us (23%) than when compared to study of Grewal et al, where the isolation of Acinetobacter was 7.87%.

The current study analysed antimicrobial sensitivity testing by focusing on the resistance pattern of all the NLF GNB in general and individual species and analysis showed that 40-50 % of them were resistant to BL + BLI, 3rd generation, 4th generation cephalosporin, carbapenems and

fluroquinolone, levofloxacin. Overall, the best drugs that can be used for treatment are minocycline, tigecycline (tetracycline group) and colistin (CLSI 2019-2020). But the latest guideline of CLSI 2021 does not interpret sensitivity to colistin. Only interpretation as intermediate and resistant in given. In such cases the recommendation for pseudomonas is to give loading dose and maximum renal adjusted dose. Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy even if intermediate result is obtained. Alternative agents were strongly preferred. Consultation with an infection diseases specialist is recommended in such cases.

Antimicrobial resistance of pseudomonas species was much less (<40%) to most of the antibiotics like ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, minocycline, tigecycline, aztreonam, cefoperazone/sulbactam etc, unlike the study conducted by Grewal et at, where they had MDR pseudomonas aeruginosa. Our analysis showed that Burkholderia spp were multidrug resistant where in the resistance was more than 50% to most of the antibiotics and only 25% resistant to imipenem (i.e.,75% sensitive) and the results of Grewal et at showed 10% sensitivity to imipenem.[4]

A.baumanii showed maximum susceptibility to the imipenem (88.2%) followed by cefoperazone/sulbactam in Grewal et at study.[4] Whereas our study showed maximum susceptibility 37% (as 63% were resistant) to imipenem and to cefoperazone/sulbactam 54.8% (as 45.2% were resistant) were sensitive. Since our study focused on resistant pattern, we could analyse that Acinetobacter showed maximum sensitivity to minocycline, tigecycline (10-20% resistance) and amikacin (30-40 % resistant). Therefore, these drugs can be considered to patients who are critically ill.

Antimicrobial stewardship: In the early era of antibiotics there were only fourteen new classes of antibiotics between 1935 and 2003. This led to overuse and misuse and the impact is the development of antimicrobial resistance. After the exhaustion of the development of newer drugs there is now a method of conserving the antibiotics. Therefore, antimicrobial stewardship (ASP) has come into being wherein there is optimal selection of doses and duration of antimicrobial treatment that results in the best clinical outcome for the treatment and prevention of infection with minimal toxicity to the patient or minimal impact on subsequent resistance. Thus, the goals of ASP are three:

1) To work with the health care practitioner to help each patient receive the most appropriate antibiotic with correct dose and treatment.
2) To prevent antimicrobial overuse, misuse, and abuse.
3) To have prescription audits to know the prevalent use of different antibiotics.
4) To minimise the development of resistance both at the individual patient level and at the community level.

Conclusion

Infections caused by gram negative bacilli (GNB) is gradually increasing the morbidity and mortality. Unresolved postoperative infections caused by opportunistic GNB are increasing hospital stay and expenditure on costly antibiotics. Immunocompromised leukemic patients are prone to opportunistic infections due to the multidrug resistant bacteria leading to mortality.

Our study focused on the infections caused in cancer patients by NLF GNB complicating the cancer treatment, which accounted for around 42% isolation amongst all the Gram-negative bacilli.

Though Sphingomonas, Proteus and Burkholderia isolation is less as compared to Pseudomonas and Acinetobacter spp they were multidrug resistant, and this raises concern of rapidly spreading of these bacteria in the hospital leading to emergence of outbreak of uncontrolled infection.

Thus, it is necessary to have frequent and ongoing screening of these bacteria, regular assessment of antibiotic susceptibility profiles and judicious use of antibiotics are recommended for effective management of infection caused by NLF GNB or any other bacteria and limiting emergence of multi drug resistance.

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