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NON LACTOSE FERMENTER GRAM NEGATIVE BACILLI ISOLATED FROM CANCER PATIENTS

Microbiology

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ABSTRACT

AIM AND OBJECTIVE: To know the different types of NLFs and their Antimicrobial Resistant Pattern.

MATERIALS AND METHODS: This retrospective study was done from January 2012 to December 2016, in the Microbiology Laboratory of the GCRI, Ahmedabad. Different sampes like Pus discharges from post operative surgeries, absesses, flap infections, septicaemia and bacteremia, respiratory and urinary tract infections were received in the laboratory for culture and sensitivity. Standard laboratory methods were used for growth (Bactec), and automated ID AST (Vitek 2 Compact) was used for diagnosis of organism.

Results: Out of 25154 samples, 48.12% (12105) bacterial growth was present including gram positive and gram negative bacteria. Out of 12105 bacterial isolation, 42.09% (5096) gram positive and 57.90% (7009) gram negative bacteria were detected. It was observed that the NLFs were quite less when compared to lactose fermenter and they accounted to 36.05% (2527/7009). Most of the NLFs, were Pseudomonas spp. 56.19% (1420) followed by next common NLF, which was Acinetobacter Spp. 34.82% (880) and others like Burkholderia spp., Stenotrophomonas spp., Achromobacter Spp. and Alcaligenes Spp. 1.22% (31) NLFs were unidentified.

CONCLUSIONS: NLFs were resistant to most of the commonly used antibiotics like Beta Lactam, Beta Lactam inhibitors, Carbapenems, Aminoglycosides, Flouroquinolones. It was surprising to note that the uncommonly isolated NLF like stenotrophomonas was resistant (50-100%) to most of the antibiotics. So, the frequency of antibiotic resistant NLFs is increasing and complicating the treatment of cancer patients.

KEYWORDS

Non Lactose Fermenter, Antibiotic Resistant, Cancer.

INTRODUCTION

Non-fermenters are gram-negative bacteria that cannot ferment sugars to produced energy for cell physiology. Gram negative non-fermenting bacteria (NFGNB) were isolated from different clinical samples. Because of extreme multidrug resistance problems, species of this group offer a serious challenge for healthcare management [1]. As mostly, non-fermenting (gram-negative) bacteria are niche pathogens that cause infections in critically ill or immune-compromised patients. As they are primarily healthcare associated pathogens, they rarely cause infection in healthy individuals [2].There are different mechanisms for resistance in non-fermenting gram-negative bacteria, including (i) production of enzymes (ii) enzymatic inactivation of antimicrobial agents (iii) specific targeted enzyme that is inhibited by antimicrobial agents (iv) alterations in target sites, (v) production of efflux pumps (vi) loss of outer membrane proteins or porins (vii) reduced uptake of the antimicrobial agent. That is because of these different resistance mechanisms that the therapeutic options are severely limited to treat infections caused by them [3,4]. Non-fermenters include many species belonging to several genera. Some studies suggest four species rarely found in hospitals, significant problems in hospital practice, including *Pseudomonas aeroginosa*, *Morganella morganii*, *Proteus mirabilis* and *Salmonella typhi* [5]. The emergence of multidrug resistance *P. aeruginosa* has emerged as a severe health problem [6], which is of low permeability of the cell wall, mutation in chromosomal genes regulating resistance genes and also acquiring resistance genes from other organisms via plasmids, transposons and bacteriophages [7] [8]. As the infection caused by multiple antibiotics resistant *P. aeroginosa* may result in worse clinical outcomes, this bacterium has gained particular importance [9]. Multi-antibiotic resistant of *M. morganii* strain is due to change in outer membrane permeability and mutation of the major porin or by a change in the number of porins in the outer membrane [10]. Increasing resistance to β-Lactam antibiotics in *P.mirabillis*, is mediated by the production of acquired β-lactamases. Plasmid-mediated ESBLs, including TEM type derivatives active against expanded spectrum cephalosporin are also spreading in *P. mirabilis* [11]. Since 1991, the cases of infections caused by *Salmonella* were increasingly resistant to extended-spectrum *cephalosporins* and *fluoroquinolones* [13]. *Salmonella typhi* resistance to *Fluoroquinolone* is associated with point mutations occurring mostly within a domain of gyrA. *Cephalosporins* resistance is usually mediated by extended spectrum β-lactamases derived from TEM- and SHV-type enzymes [14].

Gram-negative bacilli *Pseudomonas aeruginosa* is an important pathogen in hospitalized patients, contributing to their morbidity and mortality due to its multiple resistance mechanisms. Therefore, as therapeutic options become restricted, the search for new agents is a priority. Latterly an accelerated increase in frequency of multidrug-resistant clinical strains has severely limited the availability of therapeutic options. Several *in vitro* and *in vivo* studies evaluating the efficacy of different antimicrobials agents and development of vaccines against *P. aeruginosa* have been reported as novel approaches, such as inhibition of virulence factor expression or inhibition of their metabolic pathways.

Pseudomonas aeruginosa is an opportunistic pathogen that may cause severe invasive diseases in critically ill patients. The frequency of infections caused by them is increasing and multidrug-resistant (MDR) strains, resis- tant to almost all available antimicrobials, are emerging in hospitalized patients. Because of its ubiquitous nature, ability to survive in moist environments, and innate resistance to many antibiotics and antiseptics, *P. aeruginosa* is a common pathogen in hospitals and particularly in intensive care units. It has become increasingly clear that resistance development in *P. aeruginosa* is multifactorial, with mu- tations in genes encoding porins, efflux pumps, penicillin-binding proteins, and chromosomal β-lactamase, all contributing to resistance to β-lactams, carbapenems, aminoglycosides, and fluoro quinolones [16]. Strains of *P. aeruginosa* are the cause of several diseases in nosoco- mial environments, predominantly pneumonia, bacteraemia, meningitis, urinary tract infections, as well as skin and soft-tissue infections [17].Due to the emergence of MDR pathogens, it is of ultimate importance to develop new antimicrobial drugs. *P. aeruginosa* has been characterized as one of the most versatile microbial organisms, with a wide span of habitats including soil, disinfectant solution and jet plane fuel [18].

METHODS AND MATERIALS

Study Design

This study is conducted at Gujarat cancer research Institute Clinical Microbiology lab. which provides medical care. Participants of this study comprised patients who came to the laboratory department of Microbiology Dept.in GCRI hospital with signs and symptoms of Respiratory, urinary tract infections, Hospital infections during year 2016 to 2017.This is a retrospective study conducted as an observational cross-sectional study design.

Sample is collected in microbiology lab then it is inoculated in blood agar, Mac conkey Agar, Sabrauds Agar, Keep at 37. C in Incubator. Next day observe the colonies. Differentiate Non Lactose Fermenter (NLF) and Lactose Fermenter (LF) by Mac conkey Agar. If NLF

colony do Oxidase test, observe it and then do Gram Stain. After GS 2 card selected for Vitek for Identification and Sensitivity.

Sample Collection

A total clinical samples were collected from suspected patients in M P Shah Cancer hospital *Samples were collected from urine*, *pus/wound*, *blood*, *ascetic/plural fluids* and were analyzed for colonial morphology *and routine biochemical identification.* The isolation of clinical samples was carried out according to standard protocol. The collected samples from urine, pus/wound, blood and ascetic/plural fluids were spread on blood, MacConkey agar plates and incubated at 37˚C for 24-48 hours. Gram staining was carried out as early described to identify the NFGNB bacteria.

Biochemical Characterizations

Biochemical characterizations were performed through biochemical tests of clinical isolates. The protocol for clinical sample's identification was according to Cheesbrough *et al)*. Indole, Methyl red, Citrate utilization, Triple sugar iron, Oxidase, Urease and Nitrate tests were also carried out.

Antibiotic Sensitivity Test

The Kirby-Bauer Disc Diffusion Method was used to test the *in vitro* susceptibility of the identified isolates to Ceftazidime (30 µg), Cefoperazone (75 µg), Ceftriaxone (30 µg), *Cefepime* (30 µg), Imipenem (10 µg). *Pseudomonas aeroginosa* colonies were picked up from the culture plate with the help of a sterile platinum wire loop and emulsified in 4 ml of sterile peptone water to match with 0.5 McFarland turbidity standards (1.5 × 108cfu/ml). The surface of Mueller Hinton Agar (Oxoid, Basingstoke, UK) in a Petri dish was inoculated evenly through a sterile swab and for 10 minutes was allowed the agar to dry. A multichannel disc dispenser (Oxoid, Basingstoke, UK) was used to deposit the antibiotics discs onto the surface of the inoculated medium. The plate was then incubated at 37˚C for 24 hours. With measuring scale the diameters of zone of inhibition were measured in millimeters after 24 hours of period of incubation.

Data Collection and Processing

Data under this study were retrospectively collected with existing records of the laboratory. Briefly, bacteriological raw results that were recorded by the laboratory of Microbiology, GCRI were collected following the authorization of the Head of Laboratory and subjected to analyses. These data were thereafter entered into Microsoft Excel software for Window version 2013 whereby various computations and statistical analyses were carried out. Notably the different species of bacteria isolated during this period were sorted out. The proportion of isolation of each bacterium species was calculated per year in order to assess the most predominant species of each year. Comparisons were performed in order to find out the most prevalent bacterium isolated from patients with UTIs at Microbiology lab. GCRI,

RESULTS

TABLE 1: INCIDENCE OF THE PATHOGENIC BACTERIA

Parameter	Outcome	Percentage
Study Duration	January 2012 to December 2016	--
Total No. of Samples Received for Culture & Sensitivity	25154	--
Bacterial Growth Present	12105	48.12 %
Gram Positive Bacteria	5096	42.09 %
Gram Negative Bacteria	7009	57.90 %
NLF Bacteria	2527	36.05 %

Out of 25154 samples, 48.12% (12105) bacterial growth was present including gram positive and gram negative bacteria. Out of 12105 bacterial isolation, 42.09% (5096) gram positive and 57.90% (7009) gram negative bacteria were detected. It was observed that the NLFs were quite less when compared to lactose fermenter and they accounted to 36.05% (2527/7009).

TABLE 2: Incidence of Non Lactose Fermenter Gram Negative Isolated bacilli

NLF Isolates	No. of Isolates	Percentage
Achromobacter Species	10	0.40
Acinetobacter Species	880	34.82
Burkholderia Species	95	3.76
Pseudomonas Species	1420	56.19
Sphingomonas Species	55	2.18
Stenotrophomonas Species	26	1.03
Other Speceis of NLF	10	0.40
Unidentified NLF	31	1.23
Total	2527	

Pseudomonas spp. 56.19% (1420) followed by next common NLF, which was Acinetobacter Spp. 34.82% (880) and others like Burkholderia spp., Stenotrophomonas spp., Achromobacter Spp. and Alcaligenes Spp. 1.22% (31) NLFs were unidentified.

CHART 2: Non Lactose Fermenter Gram Negative Isolated bacilli

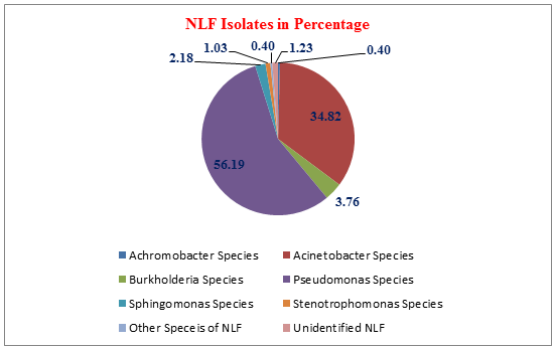
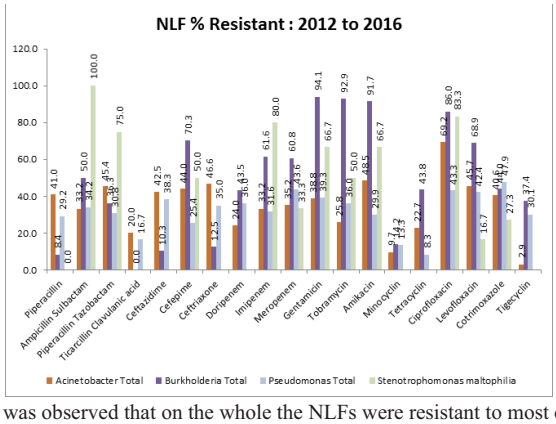


Chart 3: Percentage Of NLF Resistance



It was observed that on the whole the NLFs were resistant to most of the commonly used antibiotics like Beta Lactam, Beta Lactam inhibitors, Carbapenems, Aminoglycosides, Flouroquinolones. It was surprising to note that the uncommonly isolated NLF like stenotrophomonas was resistant (50-100%) to most of the antibiotics. So, the frequency of antibiotic resistant NLFs is increasing and complicating the treatment of cancer patients.

DISCUSSION

Longer duration of hospital stay and surgical intervention are important risk factors for infection with NFGNB High levels of resistance among NFGNB isolates were detected to carbapenems and also to the different antibiotics tested. In general, high rates of MDR are increasingly reported worldwide limiting the options of treatment in high risk patients.

In the present study, NFGNB constituted 51% of gram negative pathogens isolated from different clinical specimens of adult cancer patients in one and a half year duration. Similar figures were recorded, whilst higher figures (50%) were also reported. The wide spread use of antibiotics and other chemotherapeutic agents has a major role in the increased frequency of infection by these organisms due to disruption of the normal flora .*Pseudomonas* and *Acinetobacter* are the most commonly isolated NFGNB human pathogens In the present study, NFGNB isolates were *A. baumannii*, *Pseudomonas species*, *S. maltophilia*, *B. cepacia* and *A* In a similar study, *Pseudomonas species*

was the most commonly isolated NFGNB (70%), followed by *Acinetobacter species* (30%) .

A significant problem in managing BCC infected patients is the antimicrobial resistance and lack of newer effective antibiotics.BCC is intrinsically resistant to antimicrobial agents such as aminoglycosides, first and second generation cephalosporins, antipseudomonal penicillins and polymyxins. These various groups are commonly used in Pseudomonas infections,and the value of proper identification of BCC comes to the forefront.

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