ORIGINAL ARTICLE

FREQUENCY AND SUSCEPTIBILTY PATTERN OF NON FERMENTER GRAM NEGATIVE BACILLI IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background and Objective: There has been steady rise in imipenem resistant Gram negative bacilli non fermenters resulting from metallo beta lactamases that has been reported to be an important cause of hospital acquired infections and is serious therapeutic problem worldwide. The resistance produced by these pathogens has been mostly increased in Pseudomonas aeruginosa and Acinetobacter spp. during the last decade. These isolates pose not only a therapeutic problem but are also a serious concern for infection control management. This study was designed for the isolation and identification of non fermenter Gram negative bacilli in all the clinical samples and to determine their frequency and antibiotic susceptibility pattern. It was a Descriptive study and was performed in the Microbiology laboratory, Post Graduate Medical Institute (PGMI) Lahore from January 2015 to December 2015.

Methods: All specimens obtained from Lahore General Hospital were processed in microbiology lab of Post Graduate Medical Institute, Lahore. Non fermenter Gram negative bacilli NFGNB were isolated by routine microbiological and biochemical procedures. Antibiotic susceptibility pattern was determined by Modified Kirby-bauer disc diffusion method. For this purpose all commercially available antimicrobial disks were used.

Results: There were a total of 53 NFGNB that were isolated by convenient sampling. Respiratory samples were the most common specimens (39.6%) followed by urine (26.4%). Pseudomonas aeruginosa was the most common isolate (69.81%) followed by Acinetobacter baumannii (24.52%) and Pseudomonas luteola (3.77%). The maximum number of NFGNB were isolated in patients of age group above 60 years. Among NFGNB the maximum susceptibility was shown by Ciprofloxacin and Aztreonam whereas Acinetobacter junii showed 100% resistance.

Conclusion: It is necessary to identify NFGNB in tertiary care hospitals to take strict infection control measures to limit the spread of the underlying resistance mechanisms caused by these pathogens and to monitor their susceptibility pattern to guide the clinician for better care and management of patients. They are now emerging as a cause of hospital and community acquired infections so their antibiotic sensitivity testing and infection control measures are needed to prevent the emergence and spread in health care settings.

Keywords: Non fermenter Gram negative bacilli (NFGNB), Imipenem resistant, Metallo beta lactamase, Antimicrobial susceptibility.

INTRODUCTION

Multidrug resistance produced by *non fermenter Gram negative bacilli* is widely considered as a major global healthcare problem of 21st century and there are no good estimates of net global health burden of this antibiotic resistance.[34] The recent literature review shows that these organisms are now associated with life-threatening infections such as septicemia, pneumonia, urinary tract infection, meningitis, surgical site infections, wound infection, osteomyelitis etc.[23] Antimicrobial resistance greatly limits the treatment options for patients and the global disaster of multi drug resistant bacterias in the last several decades called the reliance on various antimicrobial agents into question.[10] *Gram negative non fermenter bacilli* are resistant to different antibiotics from different structural classes intrinsically.[3] *Pseudomonas aeroginosa* is of great clinical and epidemiological importance in hospital acquired infections.[30] Intrinsic and acquired antibiotic resistance makes them most difficult pathogen to treat.[3] The non fermenter *Pseudomonas aeruginosa* is a well known feared pathogen in hospital settings and play an important role in causing nosocomial outbreaks among susceptible patients especially mechani-

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cally ventilated patients.[9] Due to frequent use of antibiotics most of these organisms are now resistant to many routinely used antibiotics causing prescription failure. Hence, this study was undertaken to isolate and identify NFGNB and also to characterize the antibiotic susceptibility pattern at a tertiary care teaching hospital.

METHODOLOGY

This descriptive study has been conducted in the Microbiology department of Post Graduate Medical Institute from January 2015 to December 2015. All the labelled samples received in microbiology laboratory from Lahore General Hospital were inoculated on Blood agar and MacConkey agar and incubated at 35°C for 24 hours. Non–lactose fermentation was noted on MacConkey agar. Preliminary identification of NFGNB was done by Gram staining, Catalase and Oxidase test. The final confirmation of all the NFGNB was done up to specie level by Analytical profile index Non Enterobactericae Kit (API 20 NE Biomerieux). Antimicrobial sensitivity was determined by Kirby-Bauer disc diffusion method on Mueller Hinton agar using commercially available antimicrobial discs. The different antimicrobials tested were piperacillin (100 µg), ceftazidime (30 µg), amikacin (30 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), ciprofloxacin (5 µg), cefepime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg) and cefoperazone (30 µg). The results were interpreted as per Clinical and Laboratory Standard Institute guidelines. Controls used were *Pseudomonas aeruginosa (*ATCC 27853) and *stenotrophomonas maltophilia (*ATCC 13636).

RESULTS

A total of 53 imipenem resistant NFGNB were isolated for culture and sensitivity during the study period. Respiratory samples was the most common specimen accounting for 39.6% followed by urine (26.4%), pus (16.9%), blood (11.3%) and genital samples (5.6%) shown in Figure 1. Age of our cases ranged from ≤ 20 years to ≥ 60 years. Maximum number of cases were observed in the age group above 60 years (39.6%) and 41 – 59 years (32.0%). The age distribution is shown in Figure 2. Among the NFGNB isolated *P. aeruginosa* (69.81%) was the most common followed by *Acinetobacter baumannii* (24.52%), *Pueudomonas luteola (*3.77%) and *Acinetobacter junii* (1.88%) shown in Table 1. The antibiotic sensitivity pattern of NFGNB is shown in Table 2. NFGNB demonstrated high resistance to many groups of antimicrobial drugs. The antimicrobial resistance was 100% to imipenem, 98% to cefepime, chloramphenicol 96.2% to amikacin, ceftazidime, ceftriaxone, 94% to cefoperazone, and tetracycline, 88.6% to piperacillin, 86.7% to aztreonam and 84.9% to ciprofloxacin. Highest sensitivity showed to ciprofloxacin (15%) and aztreonam (13.2%). The least sensitivity to cefepime (1%), chloramphenicol (1%) and imipenem (0%) was observed.

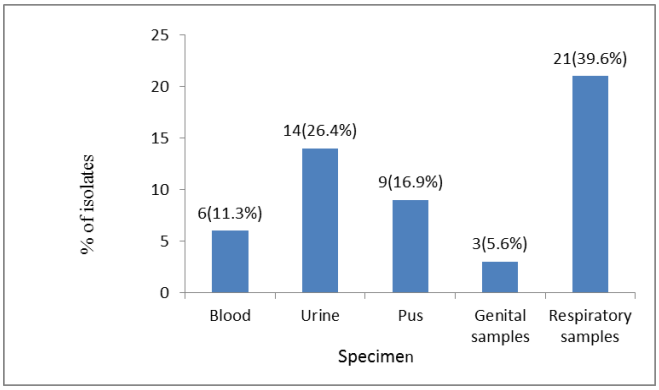

Fig. 1: *Distribution of imipenem resistant non fermenter Gram negative bacilli isolates according to clinical specimens (n = 53).*

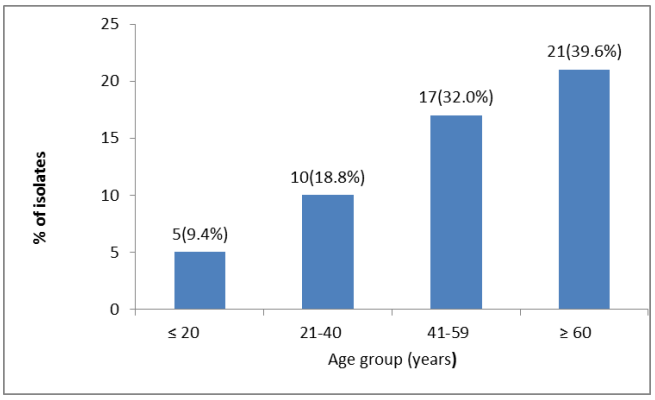

Fig. 2: *The distribution of imipenem resistant non fermenter Gram negative bacilli isolates according to age (n = 53).*

Table 1: *Frequency of different Imipenem resistant non-fermenter Gram negative bacilli isolates in various clinical specimens (n = 53).*

Species	*No of Isolates*	*Percentage (%)*
Pseudomonas aeruginosa	37	69.81%
Acinetobacter bauminii	13	24.52%
Pseudomonas luteola	2	3.77%
Acinetobacter junii	1	1.88%

DISCUSSION

An increased prevalence of antimicrobial drug resistant pathogens is emerged as a cause of global health concern at a terrifying rate.[21,32] The resistance produced by broad-spectrum cephalosporins is a persistent problem in managing infections caused by *Pseudo-*

monas areuginosa, and *Enterobacter* species, as well as other *Enterobacteriaceae*.[20] The pan-resistant bacterial strains and unavailability of new antimicrobials foretells a grave future for the treatment of infections acquired in hospitals.[24] There are limited treatment options for carbapenem-resistant *Acinetobacter baumannii* and *Enterobacteriaceae*, so it is necessary that clinicians fully assess all available therapeutic options for treating these multidrug-resistant organisms.[28]

Our study shows the distribution of *non fermenter Gram negative bacilli* recovered from various clinical samples. It was observed that majority of non-fermenters were recovered from respiratory samples 39.6% followed by urine 26.4%, pus 16.9, blood 11.3% and genital specimens 5.6%. Similar findings were observed by many researchers. Harris et al in 2002 reported that the maximum number of imipenem resistant non fermenters were isolated from respiratory secretions 50%, followed by urine samples 15%, wound swabs 11% and blood samples 7% respectively.[15] Moghaadam et al from Iran (2016) and Kaur et al in 2014 in tertiary care hospital of Iran conducted the study and reported maximum number of isolates recovered from respiratory samples.[18,25] Similar results were reported in Italy by Cornaglia et al in 2000.[8]

In contrast, Malini et al in 2009 showed highest incidence of *non fermenter Gram negative bacilli* recovered from pus 62.2% followed by respiratory samples 12.9% and urine 11.9%.[23] A study conducted by Pitout et al in 2005 and Benachinmardi et al in year 2014 observed that most of the MBL producers were recovered from pus.[6,29]

The present study showed the distribution of imipenem resistant *non fermenter Gram negative bacilli* isolates according to age. The maximum age in which we reported distribution of imipenem resistance was 39.6% in 60 years and above followed by 32% in 41 to 59 years of age, 18.8% in 21 – 40 years and 9.4% in age group ≤ 20 years. Similarly a study was conducted in hospitals of Kermansha by Akya et al in 2015 which reported 36.4% imipenem resistant isolates in age group of 60 years and above.[1] Another study conducted in Kashmir in 2011 by Bashir et al and Lefevre et al in 2013 reported maximum isolation of NFGNB between 50 – 60 years of age.[5,22] Similar results were shown by Kalam et al 2014 in SIUT (Sindh Institute of Urology and Transplantation) in Karachi and Varaiya et al in 2008 in India with higher prevalence in age group above 60 years.[19,33]

Table 2: *Frequency of resistance to different antimicrobial drugs among imipenem resistant non fermenters Gram negative bacilli isolates (n = 53).*

Drugs	*Non susceptible Isolates No (%)*	*Susceptible Isolates No (%)*
Amikacin	51 (96.2%)	2 (3.77%)
Aztreonam	46 (86.7%)	7 (13.2%)
Cefepime	52 (98.1%)	1 (1.88%)
Cefoperazone	50 (94.3%)	3 (5.66%)
Ceftazidime	51 (96.2%)	2 (3.77%)
Ceftriaxone	51 (96.2%)	2 (3.77%)
Chloramphenicol	52 (98.1%)	1 (1.88%)
Ciprofloxacin	45 (84.9%)	8 (15%)
Imipenem	53 (100%)	0 (0%)
Levofloxacin	48 (90.5%)	5 (9.43%)
Piperacillin	47 (88.6%)	6 (11.3%)
Piperacillin/Tazobactam	49 (92.4%)	4 (7.54%)
Tetracycline	50 (94.3%)	3 (5.66%)
Trimethoprin-sulfamethoxazole	50 (94.3%)	3 (5.6%)

In contrast, Benachinmardi et al in India (2014) showed highest prevalence of imipenem resistant NFGNB reported in age groups of 21 – 30 years and 41 to 50 years respectively.[6] El-Mahallawy et al in 2015 in Egypt reported the high prevalence in age group of 48 years that is in contrast with this study.[11]

Our study shows the frequency of imipenem resistant *non-fermenter Gram negative bacilli* isolates recovered from various clinical samples. Among 53 imipenem resistant *non fermenter Gram negative bacilli* 37 (69.81%) were *Pseudomonas aeroginosa,* 13 (24.52%) were *Acinetobacter baumannii spp*, 2 (3.77%) *Pseudomonas luteola* and 1 (1.88%) was *Acinetobacter Junii*. Our results were similar with the number of studies such as study carried out by Patzer et al in (2008) in Paedriatric ICU in Warsaw also showed that *Pseudomonas aeruginosa* was main organism 55% isolated among NFGNB followed by *Acinetobacter baumannii* 30% and other non fermenters 15% including *Acinetobacter junii* 5%.[27] Similarly a study done by Chawla et al in 2013 and Malini et al in 2009 in India also reported that *Pseudomonas aeruginosa* was the most commonly isolated non-fermenter pathogen.[7,23] Franklin et al in Australia (2006) reported *Pseudomonas aeruginosa* followed by *Acinetobacter baumannii* and *Acinetobacter junii*.[12]

In contrast to our study Goel et al in 2013 in tertiary care hospital of India reported that *Acinetobacter baumannii* 48.78% was the major isolated pathogen among the non fermenters followed by *Pseudomonas aeruginosa* 31.71%.[14] Souto et al in 2014 in Brazil and Najla et al in 2015 also reported the high prevalence of *Acinetobacter baumannii* followed by *Pseudomonas aeruginosa* among NFGNB.[26,31]

This study shows the percentage of resistance pattern to different antimicrobial drugs among *non fermenter Gram negative bacilli* isolates. Our study represents a high frequency of resistance to multiple antibiotics: imipenem (100%), cefepime (98.1%), cefoperazone (94.3%), ceftriaxone (96.2%), chloramphenicol (98.1%), ciprofloxacin (84.9%), levofloxacin (90.5%), tetracycline (94.3%), aztreonam (86.7%) piperacillin showed (88.6%), tazobactam (92.4%), trimethoprim (94.3%), amikacin (96.2%) and ceftazidime (96.2%).

In this study the maximum sensitivity was showed by ciprofloxacin and aztreonam.

Out of 53, forty five (84.9%) isolates were resistant and eight (15%) isolates were susceptible to ciprofloxacin followed by aztreonam which shows 46 (86.7%) isolates were resistant and 7 (13.2%) were sensitive to this drug. Gencer et al in 2002 in Turkey reported high susceptibility to ciprofloxacin 75% followed by amikacin 73%, ceftazidime 65% meropenem 63%.[13] Similarly, Hariharan et al in 2015 reported high sensitivity (77.6%) to ciprofloxacin in *Pseudomonas aeruginosa*.[16] Ionescu et al in 2014 showed high sensitivity of NFGNB against aztreonam which is similar to our study.[17]

In contrast a study conducted by Anwar et al in 2016 in Pakistan who reported 83.3% resistance to amikacin, 100% to imipenem, 100% to cefepime, 98% to ceftriaxone, 86% to ciprofloxacin and 97% to ceftazidime.[2]

It is ***concluded*** that *non-fermenting Gram-negative bacilli* isolated from different clinical specimens should not be ignored and identified by using standard methods so as to institute appropriate and timely antibiotic coverage. There is an alarmingly high rate of resistance to many antimicrobials produced by the *non fermenter Gram negative bacilli* including carbapenems. We suggest that further studies should be carried out to evaluate the usefulness of older and newer antimicrobial agents to prevent the emergence of multi drug resistant bacteria.

Authors' Contribution

AS: Designing of research work, sample collection, analysis, write-up. IJ: Supervision, planning of research, literature review, help in write-up. SM: Literature review, sample analysis. SA: Concept, overall supervision.

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