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ANTIMICROBIAL SUSCEPTIBILITY;

ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND PERCENTAGE OF NON-FERMENTER GRAM NEGATIVE BACILLI (NFGNB) IN OUR SETUP.

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Abstract… Background: The emergence and spread of antibiotic resistant bacteria causing infection is a great health issue for clinicians. The problem of multidrug resistant (i.e. resistant to at least three groups of antimicrobial) is becoming more and more threatening. The rate of resistance among Gram negative bacteria especially non fermenters are increasing to all available antibiotic groups. **Objectives:** The aim of study was to detect the pattern of antimicrobial susceptibility in non-fermenter Gram negative bacilli (NFGNB) in a tertiary care hospital (Postgraduate Medical Institute, Lahore). **Study Design:** Descriptive study. **Settings:** The study was conducted in Pathology Department, Postgraduate medical institute (PGMI), Lahore. **Study Period:** Six months from August 2014 to December 2014. **Material and Methods:** Non-fermenter Gram negative bacteria were collected from various clinical specimen including blood, pus, urine, fluid aspirates and respiratory tract in a period of six months in pathology department, PGMI. All non-fermenter Gram negative isolates were identified up to species level by standard laboratory procedures using API (Analytical profile index) 20 NE (non-enterobacteriaceae). Antimicrobial susceptibility testing of non-fermenter Gram negative isolates were performed by modified Kirby Bauer disk diffusion method as recommended in CLSI. **Results:** Out of the total 51 samples the most commonly isolated NFGNB were Acinetobacter baumanni and Burkholderia cepacia (16 each) followed by Pseudomonas aeruginosa (7), Pseudomonas luteola (5), Stenotrophomonas maltophilia (4), Pseudomonas fluorescens (2) and Pseudomonas stutzeri (1). **Conclusion:** The study showed that rate of multidrug resistance increased in non-fermenter Gram negative organisms.

Key words: Antimicrobial Susceptibility Pattern, Multidrug Resistance, Non-Fermenter Gram Negative Bacilli, .

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INTRODUCTION

Humans are struggling against infectious diseases for a long period of time.[1] The antimicrobial therapy has played an important role in the treatment of human infectious diseases in the 20th century.[2] Re-emergence of diseases once controlled and more importantly the development of antimicrobial resistance are the main reasons for these casualties.[3]

Antibiotic resistance occurs when an antibiotic has lost its ability to efficiently control or kill bacterial growth, in other words, the bacteria are "resistant" and keep on multiply in the presence of therapeutic levels of an antibiotic".[4,5] The emergence and spread of antibiotic resistant bacteria is one of the pre-eminent public health concerns of the 21st century.[3,6] Mostly these organisms are multi-resistant to more than one or two classes of antibiotics.[7,8]

According to CDC, multi drug resistance organisms are defined as" acquired non susceptibility to at least one agent in three or more antimicrobial categories".[6] The primary reasons of concerned of these multi drug resistant pathogens are limited treatment option, prolong hospital stay, increase mortality& morbidity and increase financial burden on patient and provider.[9] The world is facing two dramatic challenges. Firstly the organisms are becoming resistant to all available drugs.[10] Secondly the antimicrobial pipeline is becoming dry.[11]

The antibiotics resistant are increasing due to multiple factors. These include exposure of drugs to sub optimal level, exposure to expanded spectrum drugs and exposure of resistant genes carrying by microbes. Other causes are lack of cleanliness in hospital environment, and use of antibiotics in agricultural & food industries.[12,13]

The greatest challenge today for the clinicians is emergence of anti-microbial resistance to commonly used antibiotics.[14] The rate of resistance among non-fermenter Gram negative bacteria is increasing against all available antibiotic groups.[15,16] These bacteria accounts for one fifth of all Gram negative bacteria.[9] They produce energy for their cell function without fermentation of sugars and do not utilize the carbohydrates.[17] They are aerobic and non-spore forming Gram negative bacilli.[19]

Non-fermenter Gram negative bacilli are widely distributed in nature and mainly isolated from water and soil. They are common in hospital environment, on the skin of hospital peoples and in clinical specimen.[18] They are also found on surfaces of medical devices such as humidifiers, dialysis machines and ventilator.[19]

The main pathogens isolated from non-fermenter Gram negative bacilli are Pseudomonas species, Acinetobacter species, Burkholderia species, Aeromonas species and Stenotrophomonas species.[20]

An important purpose of this study was based on the knowledge of antibiotics resistance and its pattern among major non-fermenter Gram negative bacilli causing infections. Therefore it is necessary for the physicians to be updated with the current data concerning efficacy of commonly prescribed drugs.[21,22]

MATERIALS AND METHODS

The current study was descriptive in nature and done in Postgraduate Medical Institute. Non-probability and purposive sampling technique was used. A total of 51 clinical specimens were isolated includes blood, pus, urine, fluid aspirates, central venous pressure tip secretions, Foley's catheter secretions and respiratory tract specimens (bronchial lavage, tracheal secretions and endotracheal tube secretions) from the patients admitted in Lahore General Hospital. Majority of clinical samples taken were pus specimens. Non-fermenter Gram negative bacteria was identified by preliminary tests i.e. colony morphology, Gram staining, catalase test and oxidase test. All non-fermenter Gram negative isolates were identified up to species level by standard laboratory procedures using API 20 NE. Antimicrobial susceptibility testing for all non-fermenters was performed by modified Kirby Bauer disk diffusion method as recommended by CLSI guidelines. Antibiotics (14) used were Imipenem (10μg), Ceftazidime (30μg), Cefepime (30μg), Cefoperozone (30μg), Ceftriaxone (30μg), Amikacin (30μg), Levofloxacin (5μg), Ciprofloxacin (5μg), Aztreonam (30μg), Tetracycline (30μg), Piperacillin (100μg), Piperacillin-tazobactam (100/10μg), Chloramphenicol and Trimethoprin-sulfamethoxale (25μg).

RESULTS

Among 51 clinical samples of non-fermenter Gram negative bacilli, 16 (31.4%) were Acinetobacter baumanii, 16(31.4%) were Burkholderia cepacia, 7(13.7%) were Pseudomonas aeruginosa, 5(9.8%) were Pseudomonas luteola, 4(7.8%) were Stenotrophomanas maltophilia, 2(3.9%) were Pseudomonas fluorescens and 1(1.96%) was Pseudomonas stutzeri shown in Table-I.

Antimicrobial susceptibility pattern among non-fermenter Gram negative bacilli showed in Figure-1. The plotted graph shows that in case of Piperacillin (100ug), 48 were resistant and 3 were sensitive. Regarding Piperacillin/tazobactam (100ug/10ug), 47 were resistant and 4 were sensitive. In case of Ceftazidime (30ug) 45 were resistant and 6 were sensitive. In case of Cefepime (30ug) all 51 species were resistant to it. In case of Cefoperozone (30ug) 50 strains were resistant and 1 was sensitive. In case of Ceftriaxone (30ug) 50 were resistant and 1 was sensitive. In case of Amikacin (30ug) 45 were resistant and 6 were sensitive. Regarding Levofloxacin (5ug) and Ciprofloxacin (5ug), 49 strains were resistant and 2 were sensitive. In case of Imipenem (10ug) all 51

strains were resistant to it. In case of Aztreonam (30ug) 48 were resistant and 3 were sensitive. In case of Tetracycline (30ug) 49 were resistant and 2 were sensitive. In case of Chloramphenicol 50 strains were resistant and 1 was sensitive. In case of Trimethoprin-sulfamethoxale (25ug) 46 were resistant and 5 were sensitive.

Table-II shows the percentage of resistant pattern to different drugs in non-fermenter Gram negative bacilli. Most of the organisms were resistant to almost all 14 antimicrobial drugs which were applied. Pseudomonas stutzeri was the organism which showed 100% resistance to all 14 drugs followed by Pseudomonas fluorescens which showed resistance to 12 drugs. All of the isolates were resistant to cefepime.

DISCUSSION

Multiple drug resistance is common in NFGNB and is increasing day by day. The treatment options for these pathogens have become difficult and limited. The most common NFGNB are Pseudomonas aeruginosa, Acinetobacter baumanii, Stenotrophomonas maltophilia, Burkholderia cepacia.[19]

Over the past few years, serious hospital acquired infections are caused by NFGNB as,shown in 2013 by Hodiwala et al.[23] Table-I of our study shows distribution of frequency of species of NFGNB.

Species	Frequency	Percentage
Acinetobacter Baumanii	16	31.37%
Burkholderia Cepacia	16	31.37%
Psedomonas Aeruginosa	7	13.73%
Psedomonas Stutzeri	1	1.96%
Pseudomonas Fluorescens	2	3.92%
Pseudomonas Luteola	5	9.80%
Stenotrophomonas Maltophilia	4	7.84%
Total	51	100.00%

Table-I. Frequency and percentage of non-fermenter Gram negative bacilli isolates from Respiratory system, Urinary tract, Central nervous system and miscellaneous samples (n=51).

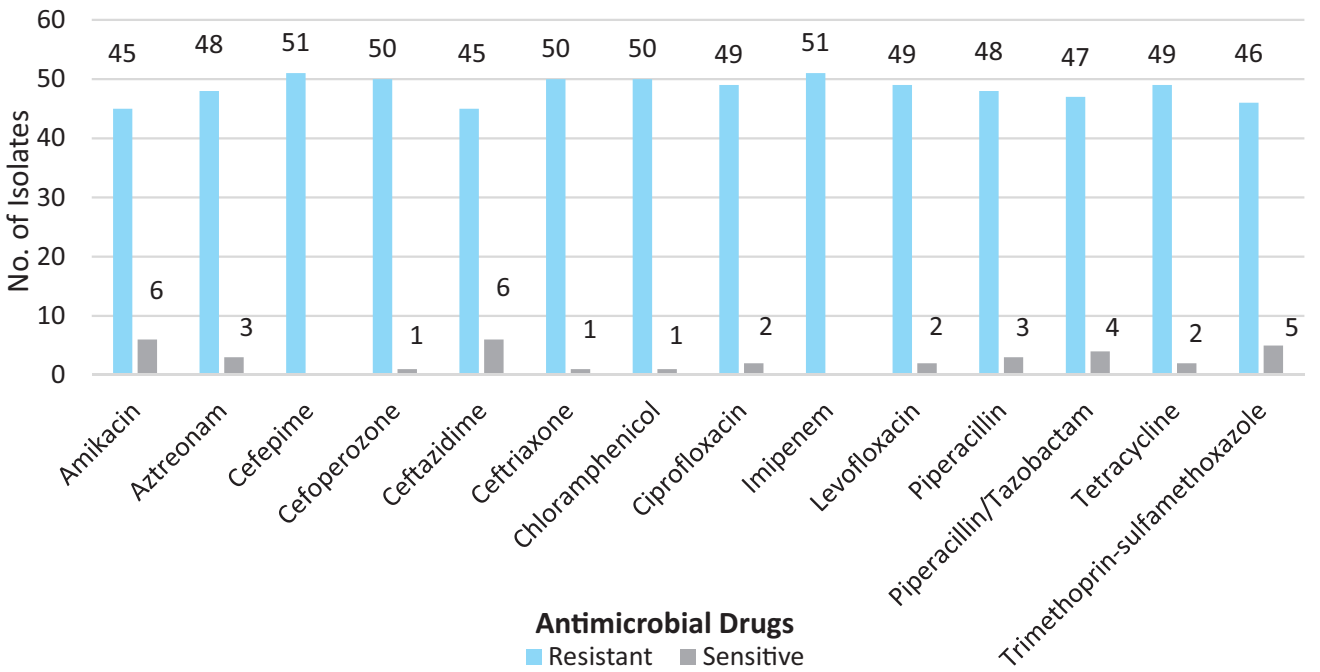

Figure-1. Antimicrobial susceptibility pattern among non-fermenter Gram negative Bacilli isolates (n= 51).

Drugs	Acinetobacter Baumanii n=16	Burkholderia Cepacia n=16	Pseudomonas Aeroginosa n=7	Pseudomonas Stutzeri n=1	Pseudomonas Fluorescence n=2	Pseudomonas Luteola n=5	Stenotro-phomonas Maltophilia n=4
Amikacin	87.5%	100%	71.4%	100%	100%	80%	100%
Aztreonam	100%	100%	71.4%	100%	100%	100%	75%
Cefepime	100%	100%	100%	100%	100%	100%	100%
Cefoperozone	100%	93.8%	100%	100%	100%	100%	100%
Ceftazidime	100%	87.5%	85.7%	100%	100%	80%	50%
Ceftriaxone	100%	93.8%	100%	100%	100%	100%	100%
Chloramphenicol	93.8%	100%	100%	100%	100%	100%	100%
Ciprofloxacin	100%	93.8%	100%	100%	50%	100%	100%
Imipenem	100%	100%	100%	100%	100%	100%	100%
Levofloxacin	100%	100%	85.7%	100%	100%	100%	100%
Piperacillin	100%	93.8%	100%	100%	100%	80%	75%
Piperacillin/ Tazobactam	100%	93.8%	85.7%	100%	50%	80%	100%
Tetracycline	93.8%	93.8%	100%	100%	100%	100%	100%
Trimethoprin-sulfamethoxazole	81.3%	100%	100%	100%	100%	60%	100%

Table-II. Percentage of resistant pattern to different antimicrobial drugs among non-fermenter Gram negative bacilli isolates (n=51)

Out of a total number of 51 isolates, the most frequently isolated non fermenters were Acinetobacter baumanii and Burkholderia cepacia (31.7%) each followed by Pseudomonas aeroginosa (13.7%), Pseudomonas luteola (9.8%), Stenotrophomonas maltophilia (7.8%), Pseudomonas flouresence (3.9%) and Pseudomonas stutzeri (1.96%). Our results are in line with a number of studies, like a study carried out by Veenu Gupta also showed that Acinetobacter baumanii was mainly isolated organism from NFGNB followed by Pseudomonas aeruginosa, Pseudomonas stutzeri, Burkholderia cepacia, Stenotrphomonas maltophilia & Pseudomoas flouresence. Goel et al (2013) from India and El-Mahallawy et al (2015) from Egypt have also reported that Acinetobacter baumanii was the most commonly isolated non fermenter pathogen.[24,25,26]

Among the NFGNB, multidrug resistance has been observed with a high frequency.[24] This finding was also observed in our study as shown in Figure-1, representing a high frequency of resistance to multiple antibiotics, cefepime (100%), cefoperazone (98%), ceftriaxone (96%), chloramphenicol (94%), ciproxacin and levofloxacin (96%) each, tetracycline (96%), aztreonam and piperacillin also showed 94% each, tazobactam (92%), septran (90%), amikacin (88%), and ceftazidime (88%). Similar results were seen in different studies conducted by Irfan et al. (2007), Anuradha et al. (2010) and Mohite et al. (2015). Gupta et al. (2013) conducted a study in India showing significant resistance to aminoglycosides, ciprofloxacin, cephalosporins, piperacillin and tazobactam among Acinetobacter baumanii and Pseudomonas aeruginosa which is in accordance with our study.[27,28,29,30]

Table-I of our result shows the percentage of resistance pattern to different antimicrobial drugs among non-fermenter Gram negative bacilli isolates. In our study Pseudomonas stutzeri showed 100% resistance to all 14 drugs. In other NFGNB the resistance pattern showed by Pseudomonas fluorescens is 100% resistance to 12 drugs followed by Stenotrophomonas maltophilia to 11 drugs, Acinetobacter baumanii to 10 drugs, Pseudomonas aeruginosa and Pseudomonas luteola to 9 drugs each and Burkholderia cepacia to 7 drugs.Gautam et al. in 2009 also reported that Burkholderia cepacia has high intrinsic resistance to multiple antibiotics which is concordant to our study.[31] A study conducted in India by Grewal et al. in 2017, also

showed that Stenotrophomonas maltophilia and Pseudomonas stutzeri were resistant to majority of drugs including imipenem which is similar to our result.[32] Prudhivi et al, also reported in their study that majority of NFGNB especially Acinetobacter species, Psedomonas species, Burkholderia cepacia and Stenotrophomonas maltophilia were resistant to ciprofloxacin, gentamycin and carbapanem which is in concordance with our study.[33]

These pathogens (NFGNB) are highly resistant to most antibiotics especially the carbapenem group, which is used for treating these MDR organisms. In the last few years, due to excessive use of antibiotics, non-fermenter Gram negative bacilli have become an important health care associated organisms.[34] An increasing microbial resistance to antibiotics need to be controlled rapidly by a multidisciplinary approach. This involves the active participation of community in term of controlling self-medications and educating health care professionals to follow the protocols of cultures rather than empirical treatment.[35] Also implementation of infection control measures to prevent the spread of resistance pathogens from hospital to community.[36]

For proper treatment, identification of these organisms should be done up to species level along with monitoring their susceptibility pattern. Antimicrobial susceptibility pattern mostly changes with time and also vary from one hospital to another.[37]

The problem of emergence of MDR among NFGNB should be detected in a cost effective and timely manner both in hospital and community setup. That's why it is urgent to carry out studies on monitoring the antimicrobial susceptibility pattern in non-fermenter Gram negative bacilli. It will help the clinicians for proper management of infection caused by non-fermenter Gram negative bacteria. Thus it would reduce the unnecessary usage of drug and prevent the existence of multidrug resistance pathogen.

CONCLUSION

The study showed that rate of multidrug resistance increased in non-fermenter Gram negative organisms.

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