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DETECTION OF METALLO BETA LACTAMASE PRODUCTION IN IMIPENEM RESISTANT GRAM NEGATIVE BACILLI NON FERMENTERS ISOLATED IN A TERTIARY CARE HOSPITAL.

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ABSTRACT: Objectives: Metallo-beta lactamase (MBL) producing non fermenter Gram negative bacilli is an emerging warning and cause of worry as they have established as one of the most feared resistance mechanisms and are the foremost cause of nosocomial infections worldwide. Carbapenem, including Imipenem, Meropenem and Doripenem are often used as a last remedy for treatment of infections caused by Pseudomonas aeruginosa, Acinetobacter and other Gram-negative. The present study was designed to explore the distribution of imipenem resistant non-fermenter Gram-negative bacilli isolates in different age groups. **Study Design:** Cross sectional Descriptive study. **Setting:** Microbiology laboratory, PGMI, Lahore. **Period:** January 2015 to December 2015. **Material & Methods:** 53 imipenem resistant NFGNB that were isolated from appropriate sampling of patients suffering from several infections were analyzed by using different standard microbiological techniques like microscopy, culture methods, biochemical reactions and antibiotic susceptibility using Kirby-Bauer method. MBL recognition was performed by imipenem-2MPA double disc synergy test (DDST). **Results:** This study shows the frequency of imipenem resistant non-fermenter Gram-negative bacilli isolated from various clinical wards. Maximum NFGNB were recovered from surgery/surgical allied 35.84% followed by ICU 28.3%, medicine /medicine allied 20.75%, pediatrics 9.4% and gynae/obs 5.6% respectively. MBL production was identified among different imipenem resistant non-fermenter Gram-negative bacilli isolates by DDST using 2-MPA. Out of total 53 imipenem resistant non-fermenter Gram Negative Bacilli 37 Pseudomonas aeruginosa 20(54.05%) were MBL positive. Out of 13 Acinetobacter baumannii and 2 Pseudomonas luteola, 11(84.61%) Acinetobacter baumannii and 1(50%) Pseudomonas luteola were positive for MBL production. None of the Acinetobacter junii indicated MBL production. **Conclusion:** Double disc synergy test is operational for detection of MBL producers among NFGNB. It can be established in our routine clinical microbiology laboratories, for the MBL recognition especially in imipenem resistant isolates as of its cost efficiency.

Key words: Carbapenems, Double Disc Synergy Test, Metallo-Beta-Lactamase, Mercaptopropionic Acid, Non-Fermenting Gram-Negative Bacilli.

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INTRODUCTION

Metallo beta lactamase (MBL) producing non-fermenter Gram negative bacilli is an evolving intimidation and cause of terror, as they have established as one of the most dreaded resistance mechanisms and are the prominent cause of nosocomial infections globally.[1] Carbapenem, including imipenem, meropenem and doripenem are often used as a last option for treatment of infections, which caused by Pseudomonas aeruginosa, Acinetobacter and other Gram-negative bacteria. However, carbapenem-resistant P. aeruginosa has become prevalent worldwide.[2] Carbapenem resistance is mainly due to up regulation of efflux pumps, reduced outer membrane permeability.[3] MBLs are reticent by metal chelators, such as an ethylene-diamine tetra acetic acid (EDTA) and thiol-based compounds. MBL-encoding genes have been testified worldwide in GNB, such as Pseudomonas spp. Acinetobacter spp, and members of the Enterobacteriaceae family.[4] The non-fermenter Pseudomonas aeruginosa is a well-known dreaded pathogen in hospital surroundings and

play a noteworthy role in causing nosocomial outbreaks among susceptible patients, especially mechanically ventilated patients VAP.[5] It is estimated that at least 2 million people gain bacterial infections that are resistant to standard therapy each year only in in the United States.[6]

Up to 25% of cutaneous colonization is caused by Acinetobacter and are the most common bacteria found on the skin of hospital personnel, which, often a times, cause a number of outbreaks of nosocomial infections such as septicemia, pneumonia, wound sepsis, endocarditis, meningitis, urinary tract infections.[7] Eventually, infection control specialists and clinical microbiologists need to work together to regulate the risk carried by Carbapenem resistant non fermenting Gram-negative bacilli in their institution and what methods should be measured for surveillance and revealing of these pathogens.

METHODOLOGY

This descriptive study has been conducted in the Microbiology department of Post Graduate Medical Institute from January 2015 to December 2015. All the labeled samples received in microbiology laboratory from Lahore General Hospital were inoculated on Blood agar and MacConkey agar and incubated at 37°C for 24 hours. Non-lactose fermentation was noted on Mac Conkey agar. Preliminary identification of NFGNB was done by Gram staining, Catalase and Oxidase test. The final confirmation of all the NFGNB was done upto specie level by Analytical profile index Non Enterobactericae Kit (API 20 NE Biomerieux). Phenotypic verification of provisional MBL producers was done by Double Disc Synergy Test. Isolates which were imipenem resistant were advance investigated for Metallo-beta lactamase production by double disc synergy method. The bacterial suspension with turbidity equivalent to 0.5 Mc Farland standards was arranged and inoculated on Mueller Hinton agar.

After inoculation of the experimental organism on Muller Hinton agar, 2 imipenem disc containing 10 micrograms were positioned on the surface of agar plate at the distance of 4-5 cm center to center. A disc comprising 3 microliters of diluted 1:8 2-Mercaptopropionic acid was placed near one of the IPM disc at a distance of 1-1.5 cm. The plates were incubated at 37˚C overnight. The expansion of the diameter of growth inhibitory zone around imipenem and 1:8 2-Mercaptopropionic containing disc by ≥ 7mm was reflected as positive for MBL production. Quality control of Pseudomonas aeruginosa (ATCC 27853) and Stenotrophomonas maltophilia (ATCC 13636) was run with each batch of the test. The results were interpreted as per Clinical and Laboratory Standard Institute guidelines.

RESULTS

Total 53 imipenem resistant non-fermenter Gram-negative bacilli were isolated for culture and sensitivity during the study period. Positive synergy test is shown in Figure-1. The imipenem resistant NFGNB isolates was distributed according to the age of the patients shown in Figure-2. Table-I. Shows frequency of imipenem resistant non-fermenter Gram-negative bacilli isolates recovered from several clinical wards. Among 53 imipenem resistant non-fermenter Gram-negative bacilli, most common non-fermenter bacteria isolated was Pseudomonas aeroginosa 37 (69.81%) rest of the results showed in Table-II. In our study, most common MBL producing bacteria was Pseudomonas aeruginosa 20 (54.05%), rest of MBL making bacteria are shown in Table-III.

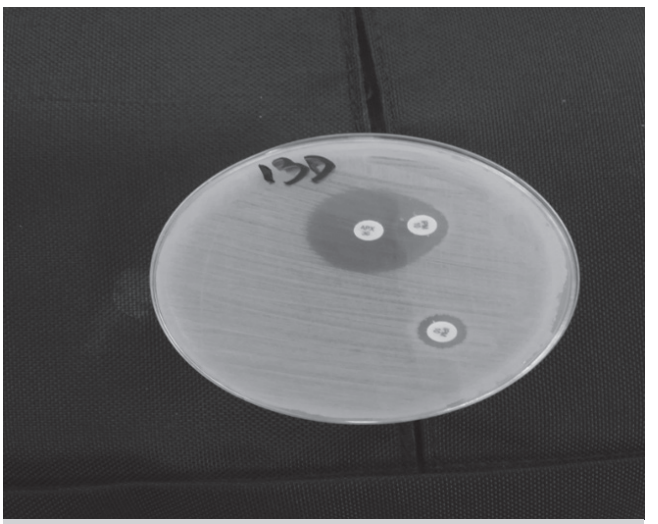

Figure-1. Double disc synergy test showing positive synergy test.

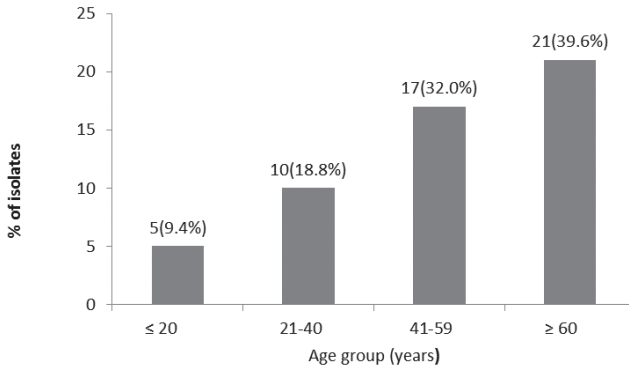

Figure-2. The distribution of imipenem resistant non-fermenter Gram negative bacilli isolates according to age (n=53)

Ward	No. of Isolates	Percentage (%)
Medicine/Medicine Allied	11	20.75%
Surgery/Surgical Allied	19	35.84%
Gynae/Obs	3	5.66%
ICU	15	28.3%
Pediatrics	5	9.4%

Table-I. Frequency of imipenem resistant non fermenter Gram negative bacilli isolated from several clinical wards (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%

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

NFGNB isolated (n=53)	MBL Positive on DDST	Per-centage (%)
Pseudomonas Aeruginosa (37)	20	54.05%
Acinetobacter Baumannii (13)	11	84.61%
Pseudomonas Luteola (2)	1	50%
Acinetobacter Junii (1)	0	0%

Table-III. Frequency of Metallo beta lactamase creating different non fermenter Gram negative bacilli isolates by double disc synergy test using 2-MPA disk (n=53)

DISCUSSION

Due to excessive and irrational use of carbapenems, a shocking increase in carbapenem resistance has been recounted in many Gram-negative bacteria including Acinetobacter spp.[8] The resistance produced by broad-spectrum cephalosporin is a tenacious problem in managing infections caused by Pseudomonas areuginosa, and Enterobacter species, as well as other Enterobacteriaceae.[9] There are limited treatment options for carbapenem - resistant Acinetobacter baumannii and Enterobacteriaceae, so clinicians must completely evaluate all offered therapeutic options for treating these multidrug-resistant organisms.[10] The present study showed the dispersal of imipenem resistant non-fermenter Gram-negative bacilli isolates according to age. The maximum age in which we stated distribution of imipenem resistance was 39.6% in 60 years. Similarly, a study was conducted in hospitals of Kermansha by Akya et al in 2015, which conveyed 36.4% imipenem resistant isolates in age group of 60 years and above.[11] Another study conducted by Lefevre et al in 2013 reported maximum isolation of NFGNB between 50-60 years of age.[12] In contrast, El-Mahallawy et al in 2015 in Egypt reported the high prevalence in age group of 48 years.[13]

Table-I of our study shows the frequency of imipenem resistant non-fermenter Gram-negative bacilli isolates recovered from several clinical wards. Maximum NFGNB were recovered from surgery/surgical allied 35.84%. The results were nearly similar to the study conducted by Prasanna et al in 2016. They reported maximum imipenem resistant NFGNB recovered from surgical wards 30% followed by surgical ICU 20.5%.[14] One more study conducted by Maniyan et al in 2016 indicated high prevalence of non-fermenters from surgical wards 40% followed by ICU 20%.[15] Contrary to this, some of the researches however stated maximum recovery of NFGNB from intensive care units. Dash et al in 2013 in India reported utmost number of isolates recovered from ICU 45.2%.[16] Table-II of our study shows the frequency of imipenem resistant non-fermenter Gram-negative bacilli isolates recovered from different clinical specimen. Amongst 53 imipenem resistant non-

fermenter Gram-negative bacilli 37 (69.81%) were Pseudomonas aeroginosa. Our results were alike with the number of studies such as study carried out chawla et al in 2013; they recounted that Pseudomonas aeruginosa was the most commonly isolated non-fermenter pathogen.[17] In difference to our study, Goel et al in 2013 in tertiary care hospital of India described that Acinetobacter baumannii 48.78% was the chief isolated pathogen among the non-fermenters trailed by Pseudomonas aeruginosa 31.71%.[18]

Table-III shows percentage of imipenem non-susceptible metallo-beta lactamase producing different non-fermenter Gram-negative bacilli isolates by Double disc synergy test. All 53 imipenem resistant NFGNB were tested for MBL production by DDST using imipenem disc only and in combination with 2-MPA. Out of 53 imipenem resistant, NFGNB isolates 20 (54.05%) Pseudomonas aeruginosa, 11(84.61%) Acinetobacter baumannii, 1(50%) Pseudomonas luteola were originate positive for MBL production by DDST test. Acinetobacter junii did not show MBL production on DDST. We establish high percentage of MBL production in Acinetobacter baumannii in this study, which is in harmony to the study conducted by Kabbaj et al in 2013 in Morocco, which revealed 74% of MBL producing Acinetobacter baumannii. Among imipenem resistant isolates which is parallel to our study.[19] In contrast to our study, Ahir et al in 2012 mentioned the prevalence of MBL in P.aeroginosa to be as high as 11.42% followed by 10.40% in Acinetobacter spp. Tellis et al in 2013 in India also stated the high percentage of MBL producing Pseudomonas aeruginosa.[20,21]

CONCLUSION

Non-fermenting Gram-negative bacilli isolated from diverse clinical specimens should not be overlooked and recognized by using standard methods, to institute appropriate and timely antibiotic reporting. There is an alarmingly high rate of resistance to many antimicrobials created by the non-fermenter Gram-negative bacilli including carbapenems. We suggest that further studies should be carried out to assess the usefulness of older and newer antimicrobial agents to prevent the emergence of multi drug resistant bacteria in clinical spacimens.

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2	Muna Malik	Literature review, Sample processing.	
3	Iffat Javed	Concept, help in write up.	
4	Sohaila Mushtaq	Literature review.	
5	Fareeha Imran	Literature review.	
6	Rabiya Jamil	Literature review.	