International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 13, No. 3, March 2024 Online ISSN: 2250-3137
Print ISSN: 2977-0122

ORIGINAL RESEARCH

Comparison of various phenotypic methods for detection of metallo β lactamases in imipenem resistant (carbapenem resistant) clinical isolates of *Enterobacterales*, *Acinetobacter* spp and *Pseudomonas* spp

[1]Dr. Shipra Galhotra, [2]Dr. Shaveta Kataria, [3]Dr. Ashish Chhabra, [4]Dr. Neerja Jindal

[1]Associate Professor, [4]Ex Professor& Head, Department of Microbiology, Guru Gobind Singh Medical College, Faridkot, Punjab, India
[2]Assistant Professor, Department of Microbiology, Mahatma Gandhi Medical College, Jaipur, Rajasthan, India
[3]Professor, Department of Pediatric Surgery, Guru Gobind Singh Medical College, Faridkot, Punjab, India

Corresponding Author
Dr. Shipra Galhotra
Associate Professor, Department of Microbiology, Guru Gobind Singh Medical College, Faridkot, Punjab, India
Email: shipragalhotra@gmail.com

Received Date: 19 February, 2024 Acceptance Date: 1 March, 2024

ABSTRACT
Background: Antibiotic resistance specially in the form of metallobetalactamase production (MBL) in gram negative bacilli is major health concern and present a major therapeutic challenge nowadays. In this study, phenotypic confirmation of various methods for detection of MBL is done by various methods. **Material& Methods:** The isolates were screened for metallo-beta-lactamase production by using imipenem. All the imipenem resistant isolates were further subjected to a phenotypic confirmation by the combined disk synergy test, modified Hodge test and Vitek 2 system. The results were analyzed and tabulated. **Results:** 63% of our isolates were MDR organisms. Of 260 isolates, 68 were found to be carbapenem resistant. A total of 34 of 37 (91.8%) *A. baumannii* isolates were carbapenem resistant.. In case of *Enterobacterales* only 29 of 119 (24.3%) and 5 of 8 (62.5%) *P. aeruginosa* were carbapenem resistant. Many of these isolates were resistant to multiple antimicrobial drugs with the highest resistance shown by carbapenem resistant isolates of *A. baumannii*. The majority of the strains were sensitive to colistin and polymyxin B. In our study, MHT for detection of carbapenemases was positive in 76% (52/68) of carbapenem resistant isolates. This study also depicts that Class B carbapenemases i.e. MBL can be detected by Vitek as compared to CDST and VItek. **Conclusions:** Carbapenem resistance has been found to be widespread. This emergence might outcome from the absence of public health surveillance programs in most countries. So the public health surveillance programs must be set up in all countries to discover these problems as early as possible.
Keywords: Gram Negative Organisms, MBLs, Combined disk synergy test, Modified Hodge test.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution- Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

INTRODUCTION

Antimicrobial resistance is a major clinical and public health problem. In 2014, World Health Organization (WHO) reported alarmingly high rates of bacterial resistance across all WHO regions and declared ‘combat drug resistance: no action today, no cure tomorrow’ a theme to fight drug resistance.[1] It has been estimated that at-least 2 million people every year acquire bacterial infections which are resistant to standard therapy in the United States alone. GNB have a propensity to develop and acquire resistance to multiple antibiotics and in the recent years, many reports of increased prevalence of MDR GNB (resistant to three or more antibiotics of different classes or groups) have been published globally which presents a major challenge to modern medical practice.[4,5]

GNB have a number of mechanisms by which they become resistant to antibiotics. However, the primary mechanism is the production of beta-lactamase (β-lactamase) enzymes and there are more than one thousand of them in GNB.[6] Production of β-lactamases was first recognized mechanism of bacterial resistance to β-lactam antibiotics and still

remains the major cause of drug resistance in gram negative pathogens.[6]β-lactam enzymes which confer resistance to third generation cephalosporins (ceftriaxone, cefotaxime, cefoperazone and ceftazidime) are called Extended Spectrum Beta Lactamases (ESBLs). These enzymes are sensitive to carbapenems and β-lactamase inhibitors (sulbactam, clavulanic acid and tazobactam).[7]

But nowadays, carbapenem resistant GNB has emerged as an issue of major concern.[8]Carbapenems are the broad spectrum antimicrobials and often viewed as the last line of antimicrobial agents for the treatment of MDR GNB.[12]Carbapenemases are the enzymes which confer resistance to carbapenems. The carbapenemases are diverse enzymes that vary in their abilities in hydrolyzing carbapenems and other beta lactams. Hence, their detection is a crucial issue because they often show an extensive and sometimes a total antibiotic resistance. The more resistant organisms like the strains of *Pseudomonas* and *Acinetobacter* spp. have acquired the resistance from the Enterobacterales[2]. The carbapenemases belong to the molecular classes A, B and D. The class B enzymes (Bush group 3) are metallobetalactamases (MBLs). The MBLs hydrolyze almost all the beta lactam antibiotics. The MBLs typically hydrolyze carbapenem efficiently, but they are inhibited by chelating agents such as EDTA.

New Delhi Metallo-beta-lactamase (NDM-1) is a newer type of metallo-beta-lactamase (MBLs) which was first described in 2009. NDM is a type of carbapenemase produced by certain strains of bacteria and is able to inactivate all β-lactams except aztreonam. Thus, it provides resistance against all compounds that contain a beta-lactam ring such as penicillins, cephalosporins and carbapenems. NDM-1 has been identified mostly in Enterobacterales, but nowadays it has been found in non-enterobacterales members like *Pseudomonas aeruginosa* and *Acinetobacter*.[9]

Organisms which carry plasmid for the blaNDM-1 gene also carry a number of other genes conferring resistance to all aminoglycosides, macrolides and sulphamethoxazole, thus making these isolates multidrug resistant. [10] Various phenotypic and genotypic tests are employed for detection of MBL production in bacteria. The phenotypic methods are based on the ability of metal chelators, such as EDTA and thiol-based compound to inhibit the activity of MBLs. These phenotypic methods are Modified Hodge Test (MHT) and Combined Disk Synergy Test (CDST). [11, 12] These tests have proved to be rapid and convenient tests for their detection in the clinical laboratory. Various studies have shown that although phenotypic tests bear significant role in the detection of MBLs and help in the preliminary screening of the NDM-1 but these tests do not differentiate between the chromosomal and plasmid encoded genes. So, genetic confirmation by PCR and analysis of the genetic context and relatedness of the MBL producers mandatory for isolates screened positive by phenotypic tests.[13]

Present study aimed to detect metallo-beta-lactamase production in GNB by two phenotypic methods-Modified Hodge Test (MHT), Combined Disk Synergy Test (CDST). It also aimed to compare MHT and CDST in the diagnosis for MBL production

MATERIAL & METHODS

This descriptive study was conducted over the period of 6 months in the Department of Microbiology of Guru Gobind Singh Medical College and Hospital, Faridkot among patients presenting to various departments of Guru Gobind Singh Medical College and Hospital, Faridkot. A sample size of 260 was taken for the purpose of the above study.

Inclusion criteria

- All the clinical specimens from the indoor patients which were received in the Microbiology department of Guru Gobind Singh Medical College and Hospital, Faridkot for culture sensitivity.
- Multidrug resistant gram negative bacteria (resistant to 3 or more antibiotics of different classes or groups)

Exclusion criteria

- Samples showing organisms other than gram negative bacteria.
- Samples showing no growth of organisms on culture.
- GNB resistant to one or two drugs only.

Sample collection and transport

Various clinical samples were collected using the standard techniques. Samples received from patients was inoculated on Blood agar, MacConkey agar or CLED and incubated at 37*C for 24-48 hrs. Identification of bacteria was done by colony morphology, gram staining and biochemical tests. The antibiotic susceptibility test of GNB isolates was performed by Kirby Bauer Disc Diffusion method as per CLSI guidelines and VITEK 2 compact automatic system. Phenotypic assay was done by double disk synergy test and Modified hodge test according to standard clinical and laboratory standards institute (CLSI) guidelines for detection of carbapenemases.

RESULTS

The present study was conducted on 260 GNB isolated from 5307 clinical specimens processed in the department of microbiology. The following observations were made.

©2024Int. J. LifeSci.Biotechnol.Pharma.Res.

Table 1- Distribution of various GNB isolated (n=260)

ORGANISMS	GNB	Percentage (%) of GNB
Enterobacterales group	185	71.2
P. aeruginosa	35	13.5
A.baumannii complex	40	15.4
	260	100

Table 2- Distribution of various MDR GNB (n=164)

ORGANISMS	MDR GNB	Percentage (%) of GNB
Enterobacterales group	119	72.7
P. aeruginosa	8	4.8
A. baumannii complex	37	22.5
	164	100

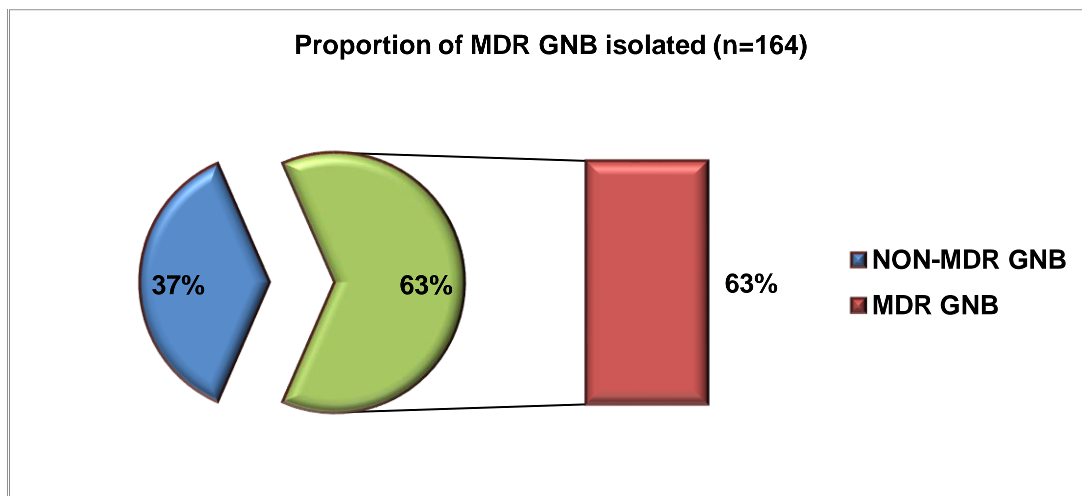

Figure 1: Proportion of MDR GNB isolated (n=164)

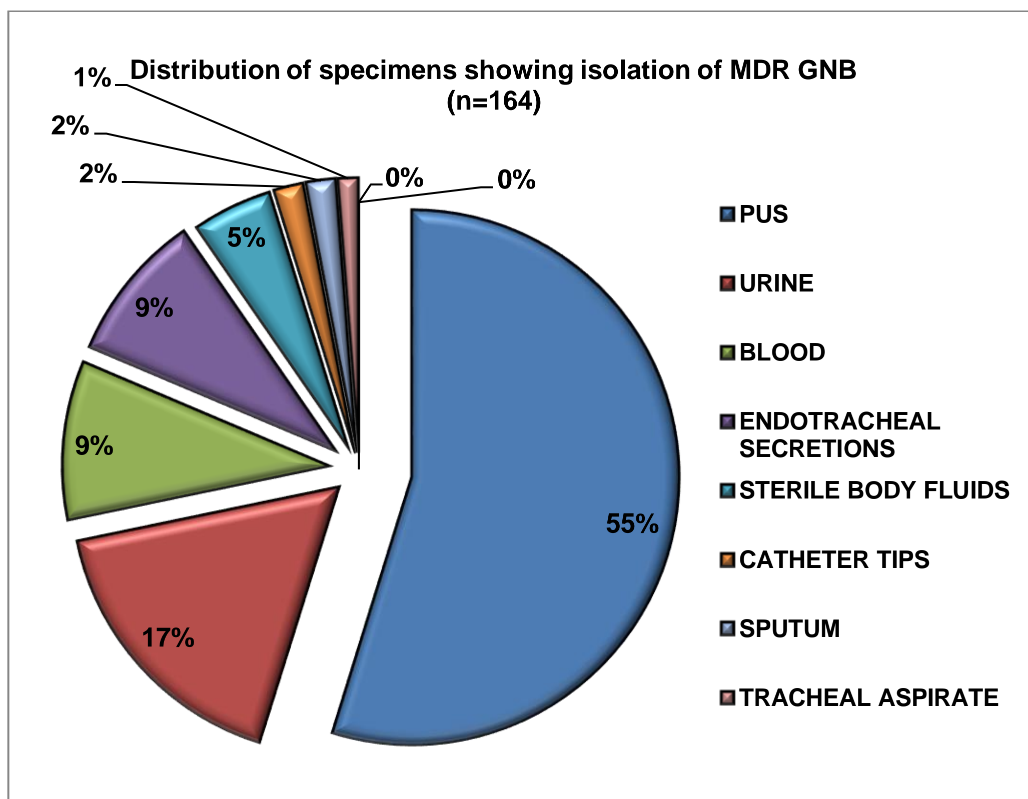

Figure 2: Distribution of specimens showing isolation of MDR GNB (n=164)

©2024Int. J. LifeSci.Biotechnol.Pharma.Res.

Online ISSN: 2250-3137
Print ISSN: 2977-0122

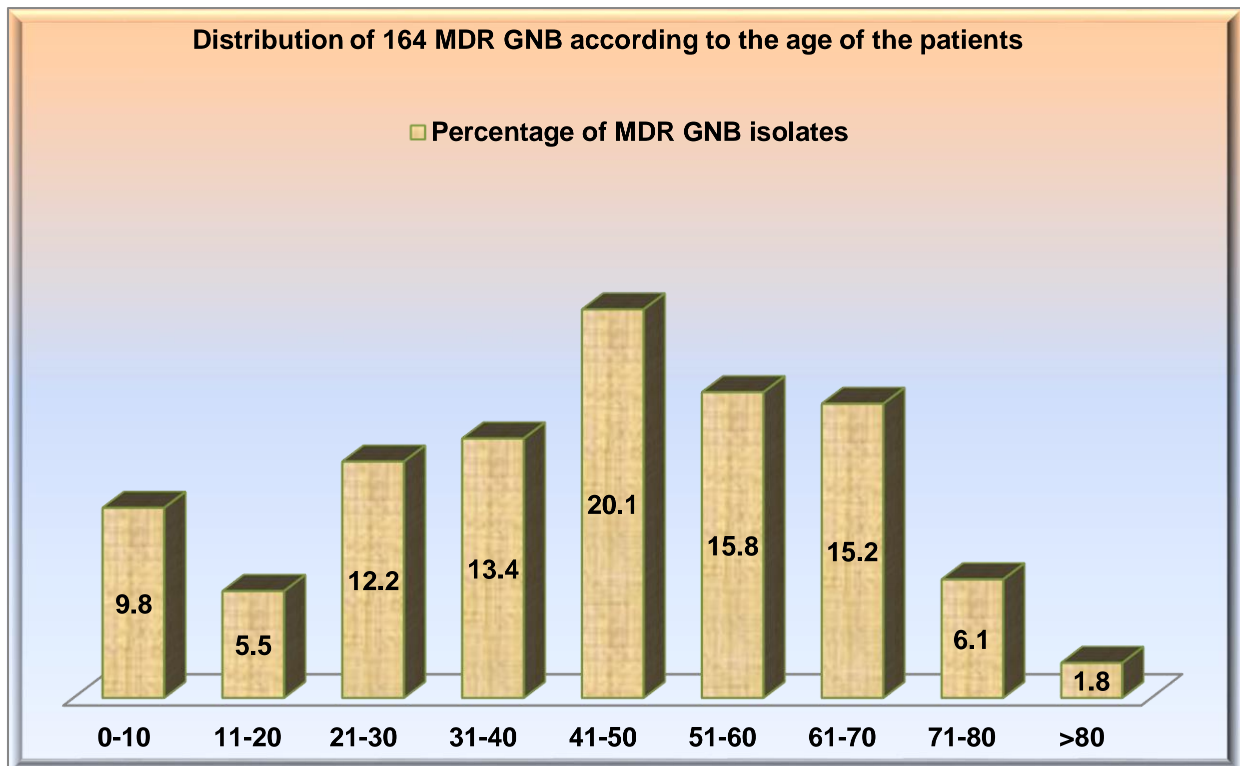

Figure 3: Distribution of 164 MDR GNB according to the age of the patients

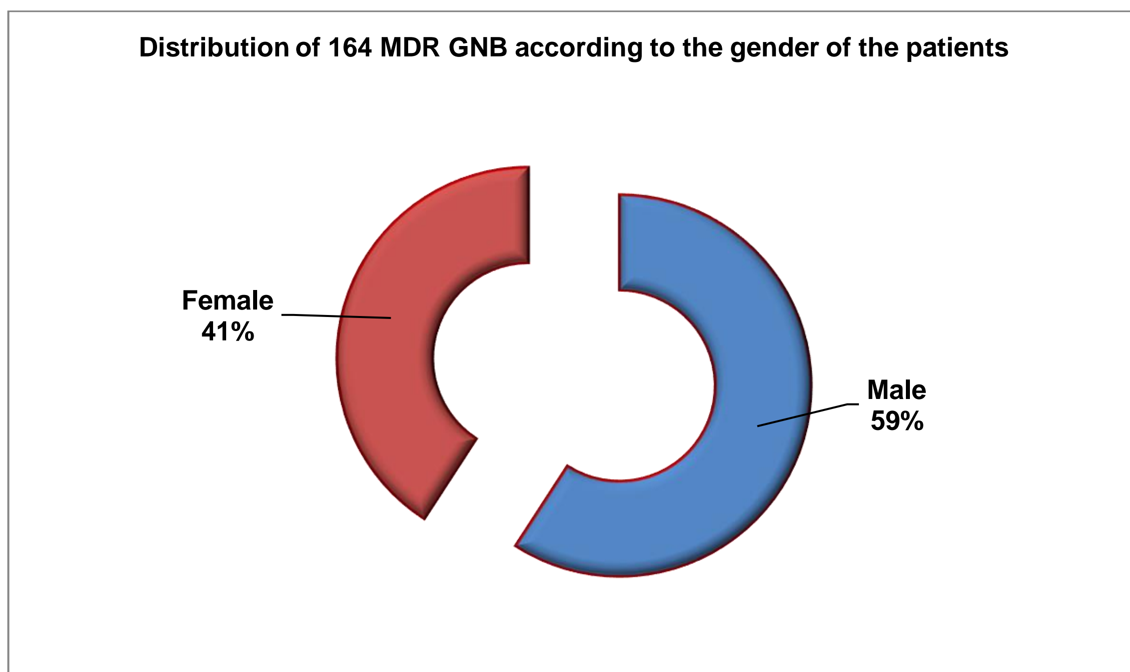

Figure 4- Distribution of 164 MDR GNB according to the gender of the patients

Table 3- Distribution of Imipenem Resistant GNB (n=68)

ORGANISMS	Imipenem Resistant	Imipenem Sensitive
Enterobacterale group	29	90
P. aeruginosa	5	3
***A. baumannii* complex**	34	3
	68	96

©2024Int. J. LifeSci.Biotechnol.Pharma.Res.

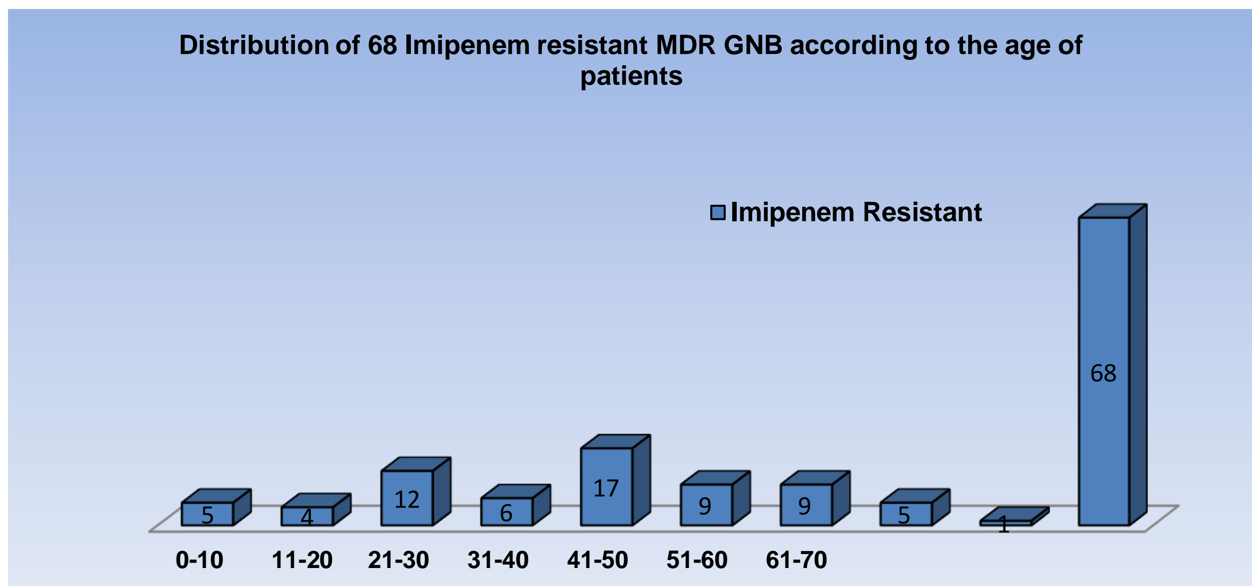

Figure 5- Distribution of 68 Imipenem resistant MDR GNB according to the age of patients

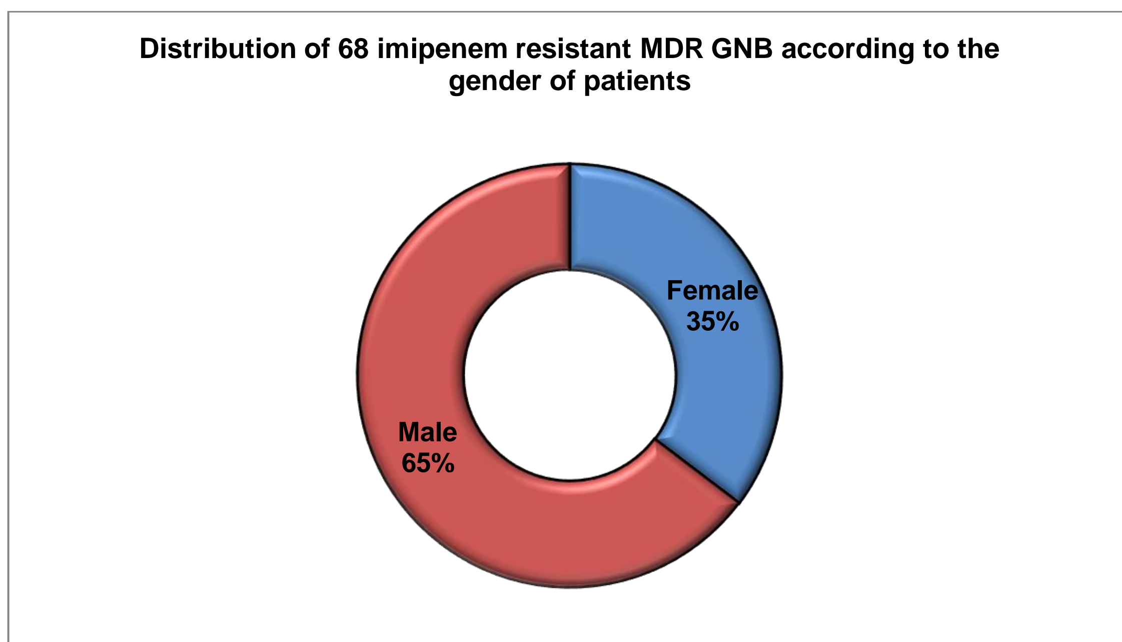

Figure 6-Distribution of 68 imipenem resistant MDR GNB accordingto the gender of patients

Table 4: Sensitivity and Specificity of tests

S.No	Name of the test	Positives	Negatives	Sensitivity	Specificity
1	**Modified Hodge Test**	52	16		
2	**Combine disc synergy test**	49	19		
3	**Vitek**	44	24		

Table 3: Number of positive and negative results by using different phenotypic methods in different organisms (n=68)

Result	MHT		CDST		VITEK	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Enterobacteriales (29)	24	5	19	10	20	9
Pseudomonas aeruginosa (5)	3	2	3	2	3	2
Acinetobacte rbaumannii complex (34)	25	9	26	8	21	13
P value <.001 Not significant						

©2024Int. J. LifeSci.Biotechnol.Pharma.Res.

Table 4: Comparison of different phenotypic tests for metallo-beta-lactamase production in Enterobacterales (*n*=29)

	MBL positive by Vitek	MBL negative by Vitek
MHT (Positive) 24	18	6
MHT (Negative)5	2	3
CDST (Positive) 19	15	4
CDST (Negative) 10	5	5

Table 5: Comparison of different phenotypic tests for metallo-beta-lactamase production in *Acinetobacter baumannii complex* (*n*=34)

	MBL positive by Vitek	MBL negative by Vitek
MHT (Positive) 25	21	4
MHT (Negative) 9	1	8
CDST (Positive) 26	18	8
CDST (Negative) 8	3	5

Table 6: Comparison of different phenotypic tests for metallo-beta-lactamase production in *Pseudomonas aeruginosa* (*n*=5)

	MBL positive by Vitek	MBL negative by Vitek
MHT (Positive)	3	0
MHT (Negative)	0	2
CDST (Positive)	3	0
CDST (Negative)	0	2

DISCUSSION

Gram negative bacteria producing Metallo-β-lactamase (MBL) enzyme are associated with severe bacterial infections. Antibacterial drug resistant microorganisms limit and complicate treatment due to limited treatment options. Carbapenems are one of the antibiotics that offer broad spectrum activity and are used as a last resort drug.

In many healthcare facilities around the world, bacterial pathogens that express multiple resistance mechanisms are becoming rampant, complicating treatment and increasing both human morbidity and financial costs. This necessitates the need for detecting the resistant bacteria so that unnecessary use of broad spectrum antimicrobials can be avoided. The significant finding of our study was the fact thatquite large percentage i.e 63% (Fig 1) of our isolates were MDR organisms.

Of 260 isolates, 68 were found to be carbapenem resistant. A total of 34 of 37 (91.8%) *A. baumannii*isolates were carbapenem resistant. In case of *Enterobacteriales* only 29 of 119 (24.3%) and 5 of 8 (62.5%) *P. aeruginosa*were carbapenem resistant. Many of these isolates were resistant to multiple antimicrobial drugs with the highest resistance shown by carbapenem resistant isolates of *A. baumannii*. The majority of the strains were sensitive to colistin and polymyxin B.

The MHT is used as the earliest developed phenotypic method to detect carbapenemase; however, the poor sensitivity in detection of NDM carriers has hindered its wide clinical application. In our study, MHT for detection of carbapenemases was positive in 76% (52/68) of carbapenem resistant isolates.Amjad et al and Kalra et al have detected carbapenemase in 69%and 85% of isolates by MHT method. Other methods such as loss of porin channel or increased efflux mechanism may be responsible for IPM resistance in the rest of MHT-negative isolates. CDST test was used for detection of MBL production incarbapenem resistant isolates and it was positive in 70%(48/68) of isolates. Vitek was used for detection of Carbapenemases which was positive in 44 isolates .Fifty-eight percent of total carbapenem-resistant isolates were MBL positive by the DET, whereas 56% were MBL positive bythe CDST.Hence majority of such patients would be prescribed carbapenems which would be disastrous on two accounts, firstly the patient would end up in treatment failure and secondly unnecessary usage of carbapenems would further expose this antimicrobial with potential for more resistance. The treatment of infections caused by drugs resistant bacteria is sometimes impracticable. In another study on comparison of various phenotypic tests (DDST, CDT, MHT). Modified Hodge test was comparatively more specific and sensitive than Double disc synergy test and combination disc test4.

Though the definite diagnosis of NDM-1 rests on PCR but Modified Hodge test can be a very useful screening test for suspecting such cases for epidemiological purpose.This study also depicts that Class B carbapenemases i.e. MBL can be detected by Vitek as compared to CDST and VItek

CONCLUSIONS

We found carbapenem resistance is widespread. This emergence might outcome from the absence of public health surveillance programs in most countries. So the public health surveillance programs must be set up in all countries to discover these problems as early as

©2024Int. J. LifeSci.Biotechnol.Pharma.Res.

possible. Also new substitute therapies are required to be developed to face bacteria with this kind of resistance; and the hospitals require excellent infection treatment and control to prevent the morbidity.

REFERENCES

1. World Health Organization. Antimicrobial resistance: global report on surveillance [Internet]. Geneva: WHO; 2014. [cited 2016 Dec 20]. Available from:www.who.int/dg/speeches/2011/WHD_20110407/en.
2. Centers for disease control and prevention. Antibiotic resistance threats in the United States, 2013 [Internet]. Atlanta: Conference on decision and control; 2013. [cited 2016 Dec 24]. Available from:http://www.cdc.gov/drugresistance/threat-report.2013/pdf/ar-threats-2013-50 8.pdf
3. Weber MA, Linseman JV, Lindholm LH.Urgently needed: new antibiotics. Lancet. 2009;374:1868.
4. D'Agata EMC. Rapidly rising prevalence of nosocomial multi drug resistant gram negative bacilli: a 9 year surveillance study. Infect Control HospEpidemiol. 2004;25:842-6.
5. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug resistant, extensively drug resistant and pandrug resistant bacteria: an International expert proposal for interim standard definitions for acquired resistance. ClinMicrobiol Infect. 2012;18:268-81.
6. Jacoby GA, Munoz-Price LS. The new beta-lactamases. N Engl J Med. 2005;352:380-91.
7. Bradford PA. Extended spectrum β-lactamases in the 21st Century: characterization, epidemiology and detection of this important resistance threat. ClinMicrobiol Rev. 2001;14:933-51.
8. Tzouvelekis LS, Markogiannakis A, Psichogiou M. Carbapenemases in Klebsiellapneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. ClinMicrobiol Rev. 2012;25:682-707.
9. Fomda BA, Khan A, Zahoor D. NDM-1 (New Delhi metallo-beta-lactamase-1) producing gram negative bacilli: emergence and clinical implications. Indian J Med Res. 2014;140(5):672-8.
10. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan and the UK: a molecular, biological and epidemiological study. Lancet Infect Dis. 2010;10(9):597-602.
11. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 27th informational supplement. Wayne, PA: CLSI;2017.
12. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem susceptible metallo-beta-lactamase producing gram negative bacilli in the clinical laboratory. J Clin Microbiol.2006;44:3139-44.
13. Shenoy KA, Jyothi EK, Ravikumar R. Phenotypic identification and molecular detection of blaNDM-1 gene in multidrug resistant gram negative bacilli in a tertiary care centre. Indian J Med Res.2014;139:625-31.

©2024Int. J. LifeSci.Biotechnol.Pharma.Res.