Prevalence of New Delhi Metallo - Beta - Lactamase (NDM) and Other Carbapenemases in Commonly \Isolated Gram-negative Bacilli by Multiplex PCR Isolated from Cancer Patients Suffering with Infections

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Summary

Life threatening infections are caused by antibiotic resistant bacteria leading to a significant mishap in antimicrobial treatment. The boundless utilization of carbapenems has brought about the development of carbapenemase producing bacteria. Among carbapenemase, metallo-beta lactamases are of prime importance produced by gene clusters, carried on the mobile plasmids. This study was aimed to know the prevalence of metallo-beta-lactamase(MBL) by PCR in commonly isolated pathogenic Enterobacteriaceae and non-lactose fermenting bacilli like Acinetobacter spp. and pseudomonas spp. This study was for seven months (May to November 2018). The pathogenic Enterobacteriaceae, Acinetobacter and Pseudomonas were collected and stored. After preliminary identification by Vitek 2 (AES analysis) the isolates were subjected for DNA testing (multiplex PCR) using targeted primers for different MBL genes. Out of the 5062 clinical samples, we selected 160 random isolates of interest for further study. 66.25% of GNB were resistant to imipenem, 56.87% to meropenem and 81.25% to doripenem. The results of selected 104 different GNB carbapenemase positive showed that 24 had blaNDM genes, 1 each of blaVIM & blaIMP genes & VIM family, 2 had SIM family, 19 had IMP family & 22 had detectable GIM-1 genes. There were co-existing genes either two or three together in single isolate. Out of the six isolates of Klebsiella spp. five isolates had blaNDM-1 and blaGIM-1, three isolates had blaNDM and VIM family. There was also presence of blaNDM, blaIMP, IMP family and GIM in one isolate. Our study found that Klebsiella pneumonia is the principle supply of different plasmid borne beta lactamase qualities coding for carbapenemase hydrolyzing all beta lactam antibiotics. This is of concern because the mobile resistant plasmids are prevalent in the CRE in hospitals and also colonize the normal flora. This has a potential for outbreak infections due to these resistant bacteria.

In conclusion, these infecting bacteria having MBL, isolated from our patients causing morbidity and mortality are enthralling attention of the clinicians leading to vast awareness on the existence of multi drug resistant super bugs in day-to-day practice. It is a requirement of the day to have antibiotic policies for the hospitals and having strict infection prevention policies to prevent the spared of these super bugs in the hospitals from HCWs & patients to patients.

Keywords: Metallo-beta-lactamase (MBL), blaNDM, CRE (Carbapenem resistant Enterobactereceae), Multidrug resistance, Antibiotic policy

Introduction

NDM represents New Delhi metallo-beta-lactamases (MBL), which is an enzyme that makes gram negative bacteria (GNB) not sensitive to a wide range of beta lactam antibiotics. These incorporates antibiotics having a place with carbapenem family which are viewed as pillar for the treatment of diseases cause by antibiotic resistant bacteria. These includes antibiotics belonging to carbapenem family which are considered main stay for the treatment of antibiotic resistant bacterial infections. This enzyme has been reported most commonly from India and Pakistan. The concern to health industry in that it is spreading throughout the world as people travel from country to country.[1]

Carbapenem antibiotics (imipenem, ertapenem, meropenem, doripenem) are members of the beta lactam class of antibiotics like penicillin's & cephalosporins have broad spectrum of activity against the gram positive, gram negative and anaerobic bacteria. Carbapenem are unique because they relatively are resistant to hydrolysis by most of beta-lactamases or are inhibitors of beta lactamases.[1]

The beta-lactamases are classified based on their functional and molecular properties. Focusing on the molecular properties, they are classified into molecular classes A, C and D which includes serene beta lactamases, though atomic class B beta-lactamases are all metallo enzymes (NDM) with dynamic site zinc molecular class B beta lactamases are all metallo enzymes (NDM) with and active site zinc, and these enzymes are inhibited by ethylene diamine tetra acetic acid (EDTA) thereby called as metallo beta lactamases.[2]

It has been observed that over more than a decade the commonly isolated Enterobacteriaceae have become resistant to almost all generation of cephalosporin and carbapenems due to the wise actions taken by the superbugs fighting against the antibiotics used in the patient treatment be it in the ICU or treating the immunocompromised patients. They are called carbapenem resistant Enterobacteriaceae (CRE). This as a major challenge

being faced by the treating physicians and has become a major cause of morbidity and increased stay in the hospital also impacting the financial expenditure for the hospital stay and expensive antibiotics.

Therefore, this study was designed to know the prevalence of metallo-beta-lactamase (NDM) by molecular method called multiplex polymerase chain reaction (m-PCR) in commonly isolated pathogenic Enterobacteriaceae and non-lactose fermenting bacilli like Acinetobacter spp and Pseudomonas spp.

Materials and Method

This prospective study was conducted over a period of seven months from May to November 2018 in the laboratory of microbiology of The Gujarat Cancer & Research Institute, Ahmedabad, Gujarat, India. Pathogenic bacteria were isolated from different types of specimens received, like blood for blood cultures in patients suffering from bacteremia, pus material received on swab in complicating post-operative wound infections and urine culture from patients suffering from urinary tract infections.

The pathogenic isolates selected for further study belonged to commonly isolated Enterobacteriaceae family and non-lactose fermenters like Acinetobacter spp, Burkholderia Spingomonas spp and Pseudomonas spp, which were initially identified by automated identification and susceptibility testing instrument called Vitek-2 compact and advance expert system (AES) of the software of Vitek. Formal ethical clearance and approval was taken from the institutional ethics committee before starting the study.

Method:

Carbapenemase production was noted from the results produced by the advance expert system of Vitek-2 Software which flagged the carbapenemase producers.

Genotypic detection of metallo-beta lactamase genes was done by multiplex polymerase chain reaction as follows:

Focus was on the detection of blaNDM-1, blaVIM, blaIMP and IMP family, VIM family, GIM-1, SPM-1 genes, and documentation of amplicons was done by gel documentation system.

Extraction: DNA extraction was finished utilizing the spin column method (QIAGEN: GmbH, Hilden, Germany) according to the company guidelines.

Multiplex Polymerase chain reaction: Hi-Chrome PCR master mix, a ready to use mix of ChromTaq DNA Polymerase, Buffer, MgCl2 and dNTPs (HIMEDIA, MolBioTM) was used. All lysates were exposed to multiplex PCR utilizing preliminaries focusing on blaNDM-1. The conjunction of other MBL encoding genes specifically blaVIM and blaIMP were searched for by utilizing consensus primer. Other multiplex PCR primers introductions for the identification of other MBL qualities were for SIM, IMP, SPM, VIM, and GIM. The primers used in the multiplexing (MP-3 and MP-5) are listed in Table-1. MP-3 is where the forward and backward primers used were three, whereas in MP-5 they were five

Amplification for the PCR assay was carried out in a thermal cycler (Applied Biosystems Gene amp 9700 PCR system) with following protocol: Starting with DNA release and denaturation for 5 minutes at 94℃ temperature, trailed by 40 patterns of 94°C temperature for 30 second, 52°C temperature for 40 second and 72°C temperature for 50 second, trailed by a solitary, last extension step at 72°C temperature for 5 minutes.

Gel Electrophoresis: PCR product containing amplicons was analyzed in a 2.5% agarose gel in 1x TAE buffer at 130V and 180mA current for 1.5 hr and the band were visualized with ethidium bromide using a Gel Documentation UV illuminator system (Gel. Luminax, BioZen). A DNA size marker of 100bp was used for comparative analysis.

Table 1: Primers used in Multiplexing PCR (Shanthi et al. 2014)

Multiplex	Sr. No.	Oligonucleotide	Nucleotidesequence, 5'–3'	Ampliconsize (Basepair)
MP-3	1	BlaNDM-1–F BlaNDM-1–R	GGGCAGTCGCTTCCAACGGT GTAGTGCTCAGTGTCGGCAT	475
	2	BlaVIM–F BlaVIM–R	TTTGGTCGCATATCGCAACG CCATTCAGCCAGATCGGCAT	500
	3	BlaIMP–F BlaIMP–R	GTTTATGTTCATACWTCG GGTTTAAYAAAACAACCAC	432
MP-5	4	IMPfamily-F IMPfamily-R	GGAATAGAGTGGCTTAAYTCTC CCAAACYACTASGTTATCT	188
	5	VIMfamily-F VIMfamily-R	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390
	6	GIM-1-F GIM-1-R	TCGACACACCTTGGTCTGAA AACTTCCAACTTTGCCATGC	271
	7	SPM-1-F SPM-1-R	AAAATCTGGGTACGCAAACG ACATTATCCGCTGGAACAGG	477
	8	SIM-1-F SIM-1-R	TACAAGGGATTCGGCATCG TAATGGCCTGTTCCCATGTG	570

Table 2: Infecting Gram-Negative Bacilli and Carbapenem Resistance (n=160)

Total GNB	Imipenem		Meropenem		Doripenem	
n=160	R	%	R	%	R	%
	106	66.25%	91	56.87%	130	81.25%

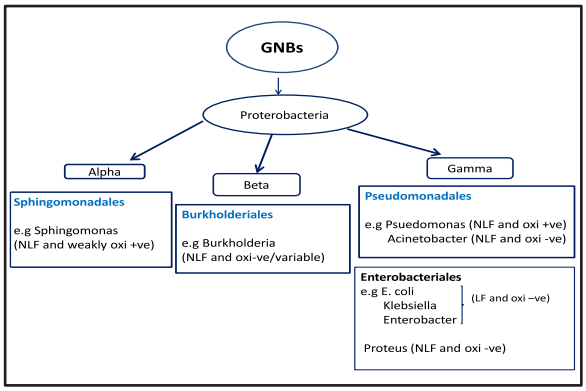

Figure 1: Classification of commonly isolated bacterial pathogens from cancer patients

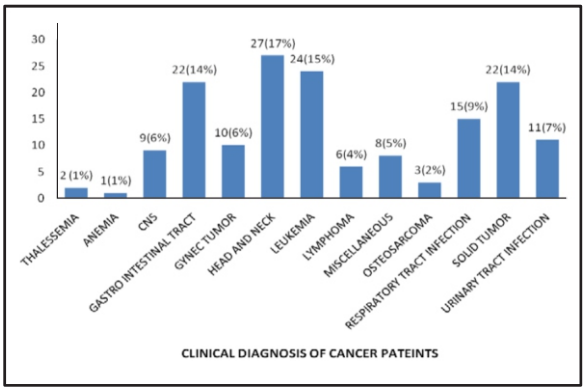

Figure 3: Gram negative bacilli infections in cancer patients enrolled in the study

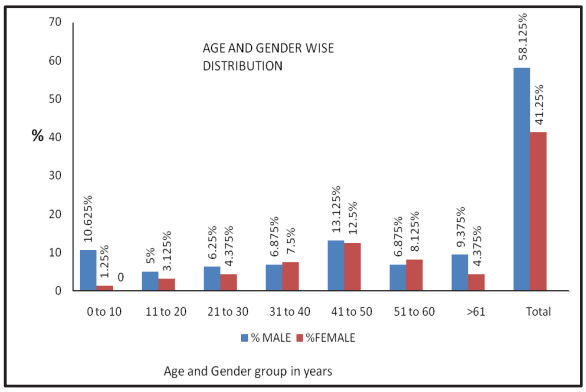

Figure 2: Age group and gender of patients enrolled in the study

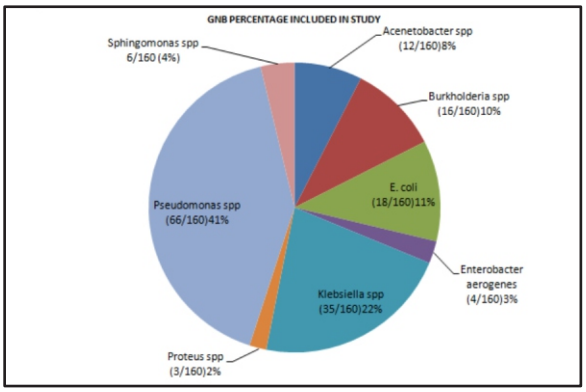

Figure 4: Types of organisms isolated in study group

Results

During the 6 months of study, a total number of 5062 clinical samples from various units were received for culture and sensitivity. From this randomly 160 isolates of different Gram-Negative bacilli (GNB) were included in the study. For the ease of identification in the laboratory commonly isolated bacterial pathogen is as classified below in the Figure 1.

Age and gender distribution of the patients who had infections is as per Figure 2. Highest percentage of infection was seen in 13.125% of male in the age group 41-50 yrs, followed by 10.625% in the age group of 0-10 years. 12.5% of the females having infections were seen in the age group of 41-50 years followed by 8.125% in age group of 51-60 years. Least percentage of infections (5%) in males was seen in the age group 11-20 years, while 1.25 % females having infections belonged to age group 0-10 years which is shown in the Figure 2.

Another finding of the study was patients with different cancers having infections showed that highest infections were seen in head and neck cancer 17% (27/160), followed by leukemia 15%(24/160). GI and solid tumor showed 14% (22/160), followed by respiratory cancer 9% (15/160) and urinary tract infections 7% (11/160), rest were less than 10% which is shown in Figure 3.

Out of 865 GNB were isolated, 160 isolates were randomly picked for future study. Out of the 160 isolates 41% (66/160) were Pseudomonas spp, 22% (35/160) were Pneumonia, 11% (18/160) were E. coli, 10% (16/160) were Burkholderia cepacia, 8% were Acinetobacter spp., Spingomonas paucimovilis 4%, Proteus spp 2% and Enterobacter aerogenes and Enterococcus spp showed 1 % each. (Figure 4)

According to Table 2, the carbapenem resistance of GNB shows 66.25% to imipenem, 56.87% to meropenem and 81.25% to doripenem, respectively. More than 57% of them were resistant to carbapenems.

It was an observation that 83% (10/160) of Acinetobacter spp. were carbapenemase producers. Similarly, Burkholderia spp. (6%), E. coli (94%), Enterobacter aerogenes (100%), Klebsiella spp. (97%), Proteus spp. (67%), Pseudomonas (52%) and Spingomonas spp. (33%) were carbapenemase

Table 3: Flagging of Carbapenamase activity shown by Advance Expert System (AES) of Vitek-2 (n=160)

Sr.No.	Name	Positive*	%	Negative	%
1	Acinetobacter spp (n=12)	10	83%	2	17%
2	Burkholderia spp (n=16)	1	6%	15	94%
3	E.coli (n=18)	17	94%	1	6%
4	Enterobacteraerogenes (n=4)	4	100%	0	0%
5	Klebsiella spp (n=35)	34	97%	1	3%
6	Proteus spp (n=3)	2	67%	1	33%
7	Pseudomonas spp (n=66)	34	52%	32	48%
8	Sphingomonas spp (n=6)	2	33%	4	67%
	Total	104	65%	56	35%

**Flagged by AES of Vitek-2 compact.*

Table 4: Carbapenamase by AES of Vitek system and Genotypic Comparison

	Carbapenamase detection by AES*1	Carbapenamase(MBL) detection by Multiplex PCR (Genotypic)							Total isolates	
		MP-3*2			MP-5*3					
NAME	Carbapenamase +ve	BlaNDM-1	BlaVIM	BlaIMP	VIM Family	SIM 1	IMP Family	SPM 1	GIM 1	
Acinetobacter spp (n=12)	10	-	-	-	-	-	-	-	-	0
Burkholderia spp (n=16)	1	-	-	-	-	-	-	-	-	0
E.coli (n=18)	17	7	-	-	-	-	8	1	8	14
Enterobacter aerogenes (n=4)	2	1	-	-	-	-	-	-	1	1
Klebsiella spp (n=35)	34	15	-	1	5	1	6	-	12	24
Proteus spp (n=3)	2	-	-	-	-	-	-	-	-	0
Pseudomonas spp (n=66)	34	1	1	-	3	1	5		1	5
Spingomonas spp (n=6)	2	-	-	-	-	-	-	-	-	0
Total=160	104	24	1	1	8	2	19	1	22	

*1AES-Advance expert system-Vitek-2 compact, *2 MP-3= multiplexing for 3 primers, *3MP-5= multiplexing for 5 primers

producers. On the whole there was 65% (104/160) of GNBs which were carbapenemase producers, flagged by AES of Vitek-2 compact.

As per Table 4, twenty-four of the GNB showed blaNDM-1 genes, twenty-two had GIM-1, nineteen had IMP family, eight had VIM family, two had SIM-1 and one each had blaVIM, blaIMP and SPM-1.

At the same time, it was also observed during the study that out of 35 Klebsiella spp., in 15 isolates blaNDM-1 was present and in 12 isolates GIM-1 was found. The carbapenamase genes were also detected in E.Coli, Enterobacter aeroginosa, Klebsiella spp. and Pseudomonas spp. These genes were not detected in Acinetobacter spp., Burkholderia cepecia, Proteus spp. and Sphingomonas spp.

The presence of single type of carbapenemase genes was found in E.coli, Klebsiella and Pseudomonas. Commonly found genes in E.coli were blaNDM-1 and IMP family, in Klebsiella there was blaNDM-1, IMP family and GIM-1 genes, and in Pseudomonas there was presence of only IMP family. Whereas there was also an observation that more than one gene was co-existing in single isolate. For example, in one species of E.coli there was presence of three genes i.e., blaNDM-1, IMP family and GIM-1 and in three isolates there was presence of two genes like IMP family and GIM-1, and blaNDM-1 and GIM-1. Likewise, there was also presence of four genes blaNDM-1, blaIMP, IMP family and GIM1 family in one strain of Klebsiella spp. In all there was presence of two to four genes in single isolate of gram-negative bacilli. (Table-5)

Metallo-beta-lactamase (MBL) resistant genes which were detected in this study using multiplex PCR are illustrated on agarose gel

Table 5: Shows presence of single Metallo-beta-lactamase (MBL) genes and Co-Existing genes found in GNB.

Strain	Single gene type								Co-existing										
									No.of genes										
									3	2	2	3	2	2	4	3	2	3	2
	BlaNDM-1	BlaVIM	BlaIMP	VIMFamily	SIM1	IMPFamily	SPM1	GIM1	BlaNDM-1,IMPFamily,GIM1	IMPFamily,GIM1	BlaNDM-1,GIM1	BlaNDM-1,GIM1,SPM1	BlaNDM-1,VIMFamily	VIMFamily,GIM1	BlaNDM-1,BlaIMP,IMPFamily,GIM1	IMPFamily,VIMFamily,GIM1	IMPFamily,SIM1	BlaNDM-1,VIMFamily,SIM1	BlaVIM,VIMFamily
E.coli	2					4			1	3	3	1							
Klebsiella spp	6					2		3		1	5		3	1	1	1	1		
Pseudomonas spp						2				1								1	1
Enterobacter aerogenes											1								

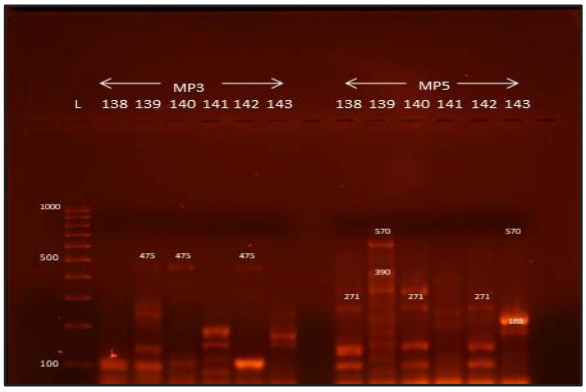

Figure 5: Gel documentation for the amplified products: 138,140,141 & 143 – klebsiella, 139-Pseudomonas, 142-E.Coli

electrophoresis.(Figure 5) Multiplex-3 had primers for blaNDM-1, blaVIM and blaIMP and MP-5 had primers for IMP, VIM, GIM, SPM and SIM genes. Three isolates of Klebsiella spp showed presence of either single MBL gene or double genes. Whereas Pseudomonas showed the presence of three genes namely blaNDM, SIM & VIM. E.coli showed blaNDM and GIM genes. There was presence of blaNDM genes in pseudomonas, Klebsiella and E.coli.

Discussion

Antimicrobial resistance is a major threat to patients admitted in the hospital. Carbapenems have been the last resort against the multidrug resistant gram-negative pathogens, cephalosporinase or extended spectrum beta-lactamase producing Enterobacteriaceae. In the line with the increased global burden of Carbapenemase Producing Enterobacteriaceae (CPE) over the period of recent years, and the production of various types of carbapenemase are increasingly reported in Klebsiella pneumonia and Escherichia coli.

The adaptable carbapenemase is the New-Delhi Metallo-beta-lactamase (NDM-1) is an adaptable molecular class B lactamase. The other normal metallo-beta-lactamase which has been a sensational expansion in the spread of obtained or adaptable families incorporates SIM, IMP, GIM and VIM proteins. The history of VIM (Verona Integron encoded MBL) is that it was isolated in France in 1997 and is most widespread MBL in Pseudomonas aeruginosa. SPM-1 (for Suo Paulo metallo beta lactamase) isolated from Sao Paolo, Brazil. This has caused multiple hospital outbreaks with high mortality in Brazil. The most recent group of procured metallo beta-lactamase from Korea is enzyme SIM-1 which means "Seoul imipenamases" has the nearest character to the IMP family was found when imipenem safe Pseudomonas spp. also, Acinetobacter spp were screened. Adaptable imipenem obstruction was first identified in Japan in 1990 in Pseudomonas aeruginosa and in B.fragilis. IMP-1(for dynamic on imipenem) found adaptable plasmid in P.aeroginosa clinical separates was found on S.marcescence and other Enterobacteriaceae. Its hydrolysis imipenem, penicillin's, extended spectrum cephalosporins but not aztreonam. Now IPM family has been found throughout the world. Identification of GIM-1 (for German imipenamases) has 30% homology to VIM, 43% to IMP & 20% to SPM. When this was isolated in 2007, it was not reported in elsewhere in the world. It is found in clones of Pseudomonas aeruginosa within class1 integrons in plasmid. Since their underlying

discovery (2007), SPM, SIM, GIM metallo-beta-lactamase have not spread past their nations of beginning. In any case, IMP and VIM keep on being distinguished around the world, the pattern moving past the metallo-beta-lactamases from P.aeruginosa and into the Enterobactereceae from P.aeruginosa.[2]

Our study showed out of 160 isolates of gram-negative bacilli , 41% were pseudomonas, 22% were Klebsiella pneumonia, 11% were E-Coli, 10% were Burkholderia cepeceae , 6% were Acinetobacter Bahmani, 4% were spingomonas paucimovilis etc. All these isolates were resistance to Imipenem, Meropenem and Doripenem and the percentage resistance was 66.25 %, 56.87 % & 81.25 % respectively. The study conducted by V Manchanda et al showed 90 % and 71 % to Imipenem and meropenem resistances respectively, in contrast to our study.[3]

The amplification product of polymerase chain reaction assay showed 475 bp amplicon product for NDM-1 gene. Out of the 104 GNB which were resistant to carbapenems, 24 isolates (23 %) were having the blaNDM-1 genes in our study. The isolates were from samples received from different infectious sites of patients.

Study conducted by Shyam sundar grover et al showed 50 % presence of blaNDM-1 gene in three of six isolates of E.coli.[4] Several other studies from India reported high incidence of blaNDH like enzyme production among carbapenem resistant E-coli from hospitals. Deshpande et al reported blaNDM-1 in nine E.coli isolates among 24 carbapenem resistant Enterobacteriaceae (37.5%).

In the study done by Mariappan shanthi et al in 2014 showed that among the Pseudomonas isolates blaNDM-1 coexisted with blaVIM in one isolate. They got blaVIM alone in 33 isolates and 2 isolates conveyed the blaIMP quality alone. Further blaGIM, blaSPM, blaSIM were not identified in any of their reviews isolates.[5] This study was only on the isolates of Pseudomonas spp, whereas our study included Gram negative bacilli belonging to Enterobacteriaceae and others, where in we found single genes of blaNDM-1 and blaIMP and blaGIM in E.coli, Klebsiella and Pseudomonas spp. coexistence of MBL genes was in E coli, Klebsiella, Pseudomonas and Enterobacter aerogenes. Among carbapenemase, metallo-β-lactamases (MBLs) are of prime significance for the locale under study in view of the development of new variations of MBL, for example, New Delhi metallo-β-lactamase (NDM) and different IMP variations from the subcontinent. MBLs have a place with class B carbapenemase as per the Ambler grouping framework. blaVIM, blaNDM, and blaIMP are significant MBL quality groups that are conveyed by versatile plasmids viable with a huge range of clinically significant microbes.[6]

The previously mentioned system of conjunction of genes has been accounted for in Klebsiella pneumoniae, Escherichia coli, Salmonella spp. and Enterobacter spp. Plasmids containing the blaNDM-1 gene have been seen to coexhibit gene for CTX-M, OXA-1, and TEM-1enzymes. Major ESBL and MBL qualities, including blaSHV, blaCTX-M, blaTEM, and blaOXA-51, and genes for the IMP-family and VIM family, have been accounted for to exist together in clinically safe Acinetobacter baumannii in Iran.[7] Notwithstanding, one review inferred that there was no critical connection among ESBL and MBL creation genes.[7] Ertapenem-resistant, ESBL-creating Klebsiella pneumoniae isolates have been accounted for in Italy and displayed to convey novel porin variations that added to the decreased vulnerability of disengages to meropenem and imipenem.Our study didn't include detection of ESBL thus there is no mention of these genes. Surprisingly, Acinetobacter baumanii and Burkholderia cepeceae did not demonstrate the presence of MBL genes.

In our study K. pneumonia is the fundamental supply of assorted plasmid-borne bla genes coding for carbapenemase, i.e., B-lactamases that hydrolyze practically all accessible b-lactams including carbapenems. The most significant being the KPCs and the metallo-b-lactamases (MBLs) NDM, VIM, IMP, GIM, and SIM happening separately or in existing together structures.

Treatment Agents: Polymyxins and tigecycline are the antimicrobials regularly utilized for the treatment of contaminations brought about via carbapenem-resistance Enterobacteriaceae. Polymyxin B has been utilized in clinical practice. This was found in excess of 50 yrs. back, has been re-found as an important helpful specialist with viability against multidrug-resistance GNB because of lack of new antimicrobials with exercises against these living beings. Exactly the same thing works out in a good way for the utilization of colistin provoking clinicians to rethink its utilization and is utilized in carbapenem-safe microscopic organisms. Colistin and tigecycline don't synergistically affect carbapenem-resistance Klebsiella in vitro, they are not hostile and may have an added substance impact when utilized together and forestall the rise of protection from these antibiotics. their mix or monotherapy can be utilized in multi-drug resistance P aeruginosa because of efflux. The constraint of tigecycline as a treatment choice in urinary tract contaminations and circulatory system diseases is notable.[8]

Clinical impact and Infection control: The issue of carbapenemase-intervened resistance escalated once genes for these compounds became related with obtained hereditary versatile components like plasmids and integrons. Natural organic entities might give hereditary material as a wellspring of these

enzymes and clinical strains might discard this data with the assistance of a portable piece of hereditary material (transposon) inside the medical clinic setting and into the general climate. Contamination with carbapenemase-creating Enterobacteriaceae (CRE) is arising as a significant test in the medical care setting. The rise and spread of carbapenem-resistance GNB forms are troubling general wellbeing advancement and highlight the prompt requirement for forceful location and control systems. Patients with unnoticed CRE colonization in the normal flora have filled in as repositories for transmission during medical care related episodes. Outbreaks with carbapenemase producing organisms have been due to lack of adherence of infection control measures. The flare-up of diseases with CRE recommends that early identification using designated observation and the presentation of severe contamination control measures including support of hand cleanliness and contact safeguards can assist with controlling the spread of these microbes.[8]

Conclusion

Taking everything into account, MBL-delivering bacterial isolates are arising quickly around the world. An incredible number of carbapenem-safe clinical bacterial species are impervious to a large portion of the usually utilized antibacterial agents, showing the ascent of super-microbes and their skillet protection from antimicrobial treatment. Deciding the resistance systems and the main driver for their end are vital. Carry out the normal screening of MBLs and ESBLs in lab methodology before anti-microbial treatment starts. Further investigations are needed to determine different sorts of quality variations pervasive among clinical isolates in our area for the ramifications of medicine in clinical settings. The present study indicated that the Pseudomonas and Enterobacteriaceae (E. Coli and Klebsiella) were carbapenemase producers in our group of cancer patient. The data presented showed that not only blaNDM-1 were responsible but also the other genes co-existed which contributed to the resistance.

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Conflicts of interest: There are no irreconcilable situations.

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