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Molecular Detection of Carbapenemase Genes among Carbapenem Resistant Gram-Negative Bacilli in Tertiary Care Hospital, Bangladesh

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Abstract: ***Background: Multi-drug resistance among gram negative bacteria with special interest to carbapenem resistance has been increasingly noticed worldwide leaving very few treatment options and associated with high morbidity and mortality. The rapid and accurate detection of several carbapenemase genes in these bacteria is important for both clinicians and infection control practitioners. In this work, we used a multiplex PCR assay for simultaneous detection of 5 carbapenemase genes among GNBs in a single run within a short time. Objectives: Use of Multiplex PCR for early detection of plasmid-borne carbapenemase genes among carbapenem resistant gram-negative bacteria isolated from various clinical samples of patients in community and hospital. Methods: This observational study was conducted at microbiology and molecular laboratory of a Tertiary care hospital in Dhaka, Bangladesh from June to July, 2023. Fresh culture colonies of 30 Carbapenem resistant and 04 Carbapenem sensitive gram-negative bacteria from different clinical samples were tested for carbapenem resistance by both Kirby-bauer disc diffusion and automated MIC detection as per CLSI guidelines. A multiplex PCR assay was done with Unimedica Multiplex Real time PCR Kit for identification of KPC, NDM, VIM, IMP and OXA-48 Carbapenem Resistance Genes and results were analyzed by software. Results: Among total tested 34 clinical isolates, 16 were Klebsiella pneumoniae, 04 E. coli, 08 Pseudomonas aeruginosa and 06 Acinetobacter baumannii. Of them, 24(71%) MDR-GNBs showed the presence of NDM and OXA-48 gene on Multiplex PCR. Both NDM and OXA-48 were co expressed predominantly in 50% isolates, while 33.3% NDM and 16.7% OXA-48 were detected solitarily. No KPC, VIM, IMP were determined. Minocycline (50%), tigecycline, fosfomycin and gentamicin (30%) & cotrimoxazole (25%) were sensitive for NDM encoded carbapenem resistant GNBs, however no sensitivity found to ceftazidime-avibactam. OXA-48 harbouring CR-GNBs showed 25% Fosfomycin & Ceftazidime-avibactam sensitivity and 100% resistance to all other tested antibiotics. Combined NDM & OXA 48 genes were positive in 60% K. pneumoniae, 50% E coli and 28.60% Pseudomonas aeruginosa. They were 33% sensitive to tigecycline, minocycline & fosfomycin, 17% to gentamicin and 8% to ceftazidime-avibactam & cotrimoxazole. All isolates were 100% sensitive to colistin and polymyxin B. Though having high MIC, no resistance genes were present in 6 carbapenem resistant Acinatobacter baumannii. Conclusion: Multiplex PCR overcome the limitations of the phenotypic methods and automated systems in identification of carbapenemase genes that enable physicians to select the most appropriate antibiotics. Our study has shown the co-existence of multiple genes in a single bacteria pointing out that different carbapenmases enzymes are utilized by the bacteria to inactive the carbapenem drugs. We recommend routine testing for carbapenem resistance genes among the MDR-GNB infections which will contribute in preserving carbapenems, the last resort antibiotics.***

Keywords: Carbapenem-resistant gram-negative bacteria; Multidrug resistance; Carbapenemases; NDM, KPC, OXA-48, VIM, IMP genes; Co-expression of genes

1. Introduction

Carbapenems are broad spectrum, highly efficient last resort antibiotics used for difficult to treat infections caused by multidrug resistant gram-negative bacteria (MDR-GNB). The emergence of carbapenem resistance (CR) among gram-negative bacteria particularly in *Enterobacteriaceae* has become a worldwide problem because of their high prevalence, wide range of clinical infections, associated multidrug resistance and rapid spread of plasmid-mediated resistance genes to other organisms. The overall mortality in such infections has been reported to be up to 50%.[1],[2],[3],[5] The 2017 World Health Organization (WHO) has recognized carbapenem resistant *Enterobacteriaceae* (CRE), *Pseudomonas aeruginosa* (CRPsA) and *Acinetobacter baumannii* (CRAB) on the priority ranking among top 10 global public health threats facing humanity.[4]

This carbapenem resistance is mediated mainly by production of carbapenemase enzymes which can hydrolyze carbapenems along with other β-lactam antibiotics that challenges empirical and targeted antibiotic treatment in healthcare settings and leads to use of expensive, complicated alternative anti-infective strategies with polymyxin-B, colistin, tigecycline, and fosfomycin.[6] Thus, early and rapid detection of carbapenemases in CR-GNBs is of paramount importance for optimizing antibiotic therapy, antimicrobial stewardship programs, hospital infection control, and improving patient outcomes by decreasing morbidity-mortality, reducing length of hospitalization and minimizing the cost of health care. [7]

Non-enzymatic carbapenem resistance mechanisms include hyper production of ESBL or AmpC enzymes combined with porin loss or upregulated efflux pumps, particularly in *P. aeruginosa* and *A. baumannii*. [8], [9] Carbapenemases are categorized in Ambler classification system from A-D classes. Enzymes in classes A (KPCs, GES), C, and D (oxacillinases) have serine in the active catalytic site, whereas metallo-β- lactamases (MBLs; VIM, IMP, NDMs) are class B enzymes with zinc in the active site. [10] Enzyme mediated resistance is more important clinically due to their ability to transfer horizontally through plasmids or transposons.[3]

Knowledge about specific carbapenemase enzymes in CR-GNBs will be the crucial step in effective treatment of CR infections and strengthen the necessity for developing new drugs to treat NDM and OXA positive GNB infections.[18] *Klebsiella pneumoniae* carbapenemase (KPC) was first identified in USA and now found globally. KPC and Verona integron-encoded metallo-β-lactamase (VIM) were most common carbapenemases in North America and Europe. In contrast, the New Delhi metallo-β-lactamase (NDM), other MBLs (IMP, VIM), and Oxacillinase (OXA) were the predominant carbapenemases among CR-GNBs of Southeast Asia. [11] Surveillance reports of India and surrounding countries revealed NDM and OXA-48 as the most frequent carbapenemase enzymes in *Enterobacteriaceae*. [12] Bacteria possessing NDM gene are resistant to almost all β-lactam drugs like Fluoroquinolones and Aminoglycosides except Aztreonam, Tigecycline and Colistin. [13], [14] OXA-48 producers are mostly found among *K. pneumoniae* and *E. coli* in India. Carbapenems and broad-spectrum Cephalosporins like Ceftazidime and Aztreonam are weakly hydrolyzed by OXA-48. [12], [16] Specific carbapenemases like OXA-23, OXA-40 and OXA-58 are mainly associated CR mechanism In Acinetobacter species. [15] Among the newer agents, avibactam inhibits KPC, AmpC and OXA 48 but does not significantly inhibit the activity of class B MBLs (NDM, VIM, IMP). [16]

Though MIC value detection is considered as an excellent screening test in new CLSI breakpoints but they have described low carbapenem MICs for CR-GNBs and cannot identify the mechanism of resistance. [30] Several phenotypic methods are available for detection of carbapenemases but they are growth dependent, turnaround time is 18 - 24 h, not clinically useful and results are also subjective. Therefore, molecular assays particularly Real time Multiplex Polymerase Chain Reaction (RT-PCR) is preferred as gold standard method for identification of resistance conferring genes by amplification of specific nucleic acid simultaneously within short time and with high sensitivity and specificity. [17] Despite their high cost, commercially available PCR-based tests have been used to screen clinically important carbapenemases, known as the "Big 5" genes: metallo-β-lactamases (MBLs) (ie, NDM, IMP, and VIM), KPC, and the OXA- 48 family. [18]

We found high carbapenem resistance in our laboratory. Therefore, the present study was conducted with an aim to determine carbapenemase genes through multiplex PCR in different CR gram negative bacteria isolated from clinical samples of patients in community and hospital settings.

2. Materials and Methods

Study place and period: This observational study was carried out in Microbiology and Molecular pathology Laboratory of a Tertiary care hospital in Dhaka, Bangladesh from June to July, 2023.

Study samples and microbial isolates: Fresh culture colonies of 30 Carbapenem resistant (15 *K. pneumoniae*, 02 *E. coli*, 07 *P. aeruginosa* and 06 *A. baumanii*) and 04 randomly selected Carbapenem sensitive (01 *K. pneumoniae*, 02 *E. coli* and 01 *P. aeruginosa*), non-repeat gram negative isolates from different clinical specimens (blood, urine, wound swab, sputum, tracheal aspirate and broncho-alveolar lavage) were included in this study. Total 34 strains were obtained from patients of mostly critical care along with Internal (IPD) and Out-patient department (OPD) of the hospital. All samples were processed for microbial culture, isolation, identification and antimicrobial susceptibility testing following standard method. [19]

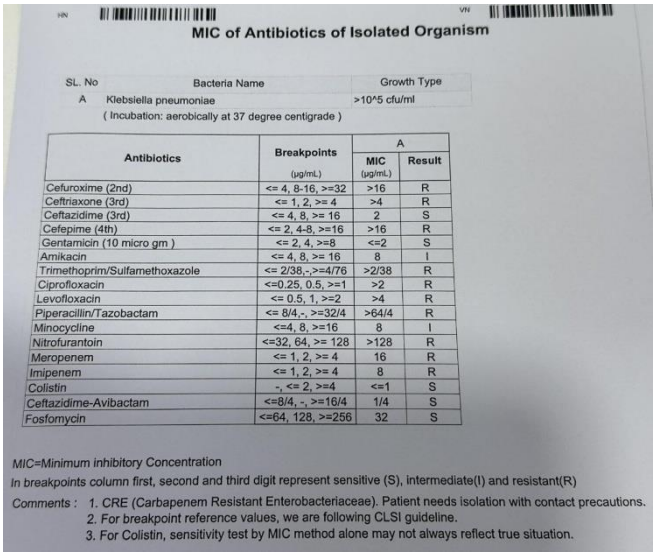
Antimicrobial susceptibility testing (AST): The AST was performed using Kirby-Bauer disc diffusion technique against a selected panel of antibiotics discs for gram negative organisms (Table-1). Based on the zone of inhibition, the results were interpreted as susceptible, intermediate or resistant as per CLSI 2022 guidelines. [20] Isolates were screened for possible Carbapenemase production using Meropenem (10_g) disc. All the isolates showing meropenem disc diffusion zone diameter less than 21mm were considered to be screen positive.

Table 1: AST panel used in Kirby-Bauer Disc diffusion technique

Sl	*Name of Antibiotics*	*Sl*	*Name of Antibiotics*
1	Amoxycillin-Clavulunic acid (AMC)	11	Pipercillin-tazobactam (TZP)
2	Cefuroxime (CXM)	12	Meropenem (MEM)
3	Cefixime (CFM)	13	Collistin (CT)
4	Ceftriaxone (CRO)	14	Polymyxin B (PB)
5	Cefepime (FEP)	15	Minocycline (MH)
6	Gentamicin (CN)	16	Tigecycline (Tig)
7	Amikacin (AK)	17	Fosfomycin (FOS)
8	Cotrimoxazole (SXT)	18	Aztreonam (ATM)
9	Ciprofloxacin (Cip)	19	Ceftazidime-avibactam (Cef-avi)
10	Levofloxacin (Levo)		

Phenotypic confirmatory methods: Minimum inhibitory concentration (MIC) breakpoints of all the meropenem screen

positive isolates were determined by automated BD Phoenix and Vitek 2 system. Isolates were considered as carbapenem resistant (CR) if they were found resistant or intermediate to one or more of the carbapenems (Ertapenem, Imipenem and Meropenem). Further to look for treatment options of these carbapenem-resistant isolates, MIC of other antibiotics like fosfomycin, minocycline, ceftazidime-avibactam, aztreonam and tigecycline were also determined and results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines [20] (Figure-1).



MIC of Antibiotics of Isolated Organism

SL. No	Bacteria Name	Growth Type
A	Klebsiella pneumoniae	>10^5 cfu/ml

(Incubation: aerobically at 37 degree centigrade)

Antibiotics	Breakpoints (µg/mL)	A MIC (µg/mL)	A Result
Cefuroxime (2nd)	<= 4, 8-16, >=32	>16	R
Ceftriaxone (3rd)	<= 1, 2, >= 4	>4	R
Ceftazidime (3rd)	<= 4, 8, >= 16	2	S
Cefepime (4th)	<= 2, 4-8, >=16	>16	R
Gentamicin (10 micro gm)	<= 2, 4, >=8	<=2	S
Amikacin	<= 4, 8, >= 16	8	I
Trimethoprim/Sulfamethoxazole	<= 2/38,-,>=4/76	>2/38	R
Ciprofloxacin	<=0.25, 0.5, >=1	>2	R
Levofloxacin	<= 0.5, 1, >=2	>4	R
Piperacillin/Tazobactam	<= 8/4,-, >=32/4	>64/4	R
Minocycline	<=4, 8, >=16	8	I
Nitrofurantoin	<=32, 64, >= 128	>128	R
Meropenem	<= 1, 2, >= 4	16	R
Imipenem	<= 1, 2, >= 4	8	R
Colistin	-, <= 2, >=4	<=1	S
Ceftazidime-Avibactam	<=8/4, -, >=16/4	1/4	S
Fosfomycin	<=64, 128, >=256	32	S

MIC=Minimum inhibitory Concentration
In breakpoints column first, second and third digit represent sensitive (S), intermediate(I) and resistant(R)
Comments : 1. CRE (Carbapenem Resistant Enterobacteriaceae). Patient needs isolation with contact precautions.
2. For breakpoint reference values, we are following CLSI guideline.
3. For Colistin, sensitivity test by MIC method alone may not always reflect true situation.

Figure 1: MIC breakpoints of different antibiotics for Meropenem resistant *K. pneumoniae*

PCR Amplification for Carbapenemase Genes: All Meropenem resistant 30 isolates and 4 Meropenem sensitive isolates were subjected to DNA extraction using bacterial DNA extraction kit (QuiagenQIAmp kit) as per the instructions of the manufacturer.

In this study, the multiplex PCR assay was done by Unimedica Multiplex Real time PCR Kit for detection of 5 Carbapenem Resistance Genes including KPC, NDM, VIM, IMP and OXA-48. [21] The kit contains two tubes. Primer sets and FAM labeled probe are designed for specific detection of NDM gene, VIC labeled probe for KPC gene, ROX labeled probe for OXA-48 gene. The other Primer sets and FAM labeled probe are designed for specific detection of VIM gene, VIC labeled probe for IMP gene. Human RNase P gene extracted concurrently with the test sample provides an internal control to validate nucleic acid extraction procedure and reagent integrity. Probe targeting human RNase P gene is labeled with CY5. (Table-2).

Table 2: Kit contents

Name	Components
PCR reaction buffer A	KPC, NDM, OXA-48, Internal reference gene, Primers probes, Buffer
PCR reaction buffer B	VIM, IMP, Internal reference gene, Primers, probes, Buffer
Positive control	Mixture of target bacterial liquid nucleic acid
Negative control	TE Buffer

PCR reaction buffer A and B, each 10 ul per test were prepared. Two PCR tube for each sample and Negative & Positive control were taken, to which 15 ul DNA sample, Negative control and Positive control were added respectively. PCR tubes were tightly caped, centrifuged at 6,000 rpm for 10s and placed into real-time PCR machine for amplification. The thermal cycling machine (RotorGeneQ 6000) was programmed for 38 cycles for DNA amplification (Table-3). Master mix and PCR Cycling conditions were prepared as per kit standard protocol. [21]

Table 3: RotorGeneQ 6000 Program for thermal cycling

Steps	Temperature	Duration	Cycle
1	50^0 C	2 min	1
2	95^0 C	2s	1
3	95^0 C	1s	45
	60^0 C	13s/35s	

Finally, the amplified DNA was determined using fluorescent signals of the samples. Results should be

Table 4: Result Interpretation

Channels (Ct value)				Curve shape	Interpretation
FAM	ROX	VIC	Cy5 (Internal control)		
<35	-	-	<35	S	NDM detected
<38	-	-	<35	No curve	NDM not detected
-	-	<35	<35	S	OXA 48 detected
-	-	<38	<35	No curve	OXA 48 not detected
<35	-	<35	<35	S (both channel)	NDM+ OXA48 detected
-	<38	-	<35	No curve	KPC not detected
<38	-	-	<35	No curve	VIM not detected
-	<38	-	<35	No curve	IMP not detected

interpreted according to Cycle threshold (Ct) value found in different channels and shape of amplification curve showed in software system, mentioned in Unimedica kit protocol. Result is read and analyzed using table-4 and Figure-2.

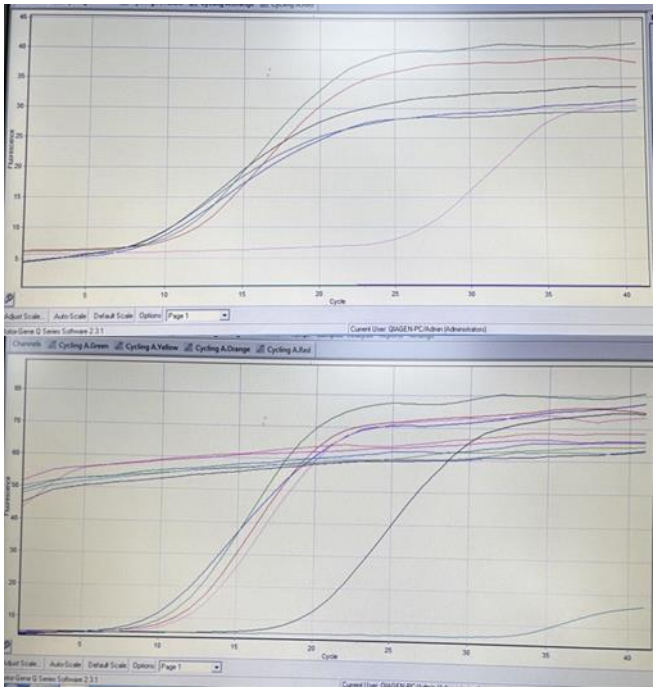


Figure 2: Amplification curve showing different gene in different channel

3. Results

Fresh culture colony of 34 Gram negative bacteria (GNBs) from various clinical samples were collected from Microbiology laboratory. Of them, 30 isolates were Carbapenem resistant (CR) and 04 were sensitive to Carbapenem (CS); Figure-3.

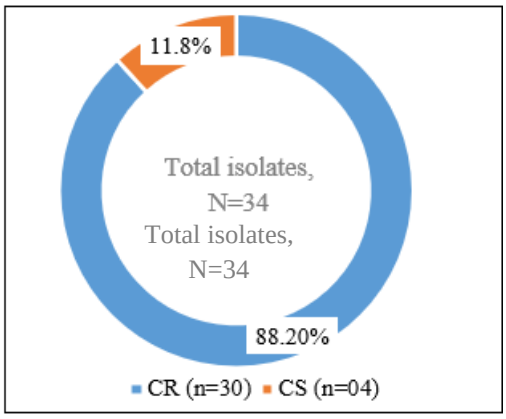


Figure 3: Distribution of Carbapenem Resistant (CR) and Carbapenem Sensitive (CS) Isolates *(Values represent the percentage of isolates studied)*

The majority samples were Urine followed by Respiratory samples [Sputum, Tracheal aspirate (T/A) and Broncho-alveolar lavage (BAL)], Blood and Wound swab respectively. Of total 34 samples, *Klebsiella pneumoniae* (16) was the most prevalent species identified followed by *Pseudomonas aeruginosa* (08), *Acinetobacter baumannii* (06) and *E. coli* (04). Distribution of samples and isolates in this study are shown below in Figure-4.

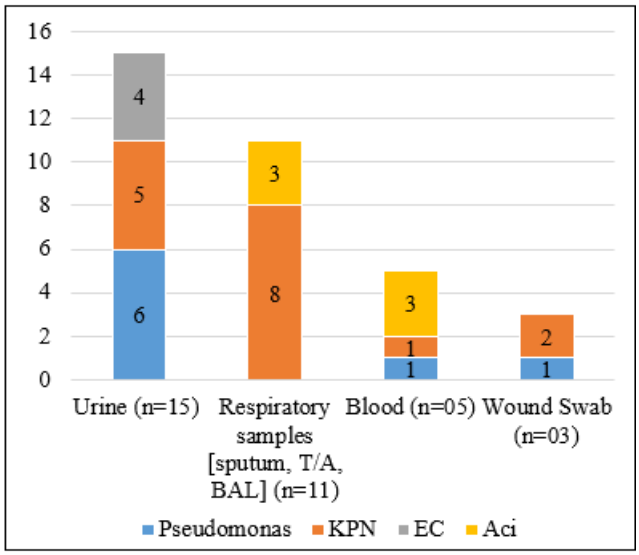


Figure 4: Sample wise distribution of Isolates studied *(Values represent the number of isolates studied in the samples). KPN-klebsiella pneumoniae, EC-Escherichia coli, Aci-Acinatobacter, T/A-tracheal aspirate, BAL-Bronchoalveolar lavage)*

Based on the Multiplex PCR assay targeted 5 genes done in our molecular laboratory, 71% (24/34) of MDR-GNB isolates were found positive for one or more of the carbapenemase genes. Overall, combined NDM+OXA-48 types were predominantly detected in 12 (50%) isolates, followed by 08 NDM (33.3%) and 04 OXA-48 (16.7%). Carbapenemase genes were more prevalent in culture of both Broncho-alveolar lavage (1/1; 100%) and wound swab (3/3; 100%) followed by urine (12/15;80%), sputum (4;7%), Tracheal aspirate (2/4;50%) and blood (2/5;40%) specimens studied. No KPC, VIM or IMP were identified in present study. Total 10 (29%) study isolates had no resistance genes, of which 4 were carbapenem sensitive *Klebsiella, E. coli* and *Pseudomonas* and 6 were carbapenem resistant *Acinatobacter baumannii*. The results of Multiplex PCR for different carbapenemase genes among organisms cultured from various clinical specimens are shown in Table-5.

All the clinical isolates were tested for resistance to Carbapenem class of drugs, mainly meropenem along with other relevant antibiotics by Kirby-Bauer disc diffusion method. Among carbapenem resistant GNBs, *Klebsiella pneumoniae* was found resistant to Gentamycin (69%), Ceftazidime-avibactam (56%), Tigecycline and Minocycline (25%) and Fosfomycin (13%). *E. coli* possessed 50% resistance to Cotrimoxazole, Pipercillin-tazobactam and Ceftazidime-avibactam; 25% to Amikacin, Levofloxacin, Aztreonam, Tigecycline and Minocycline and no resistance to Fosfomycin. Only Tigecycline and Minocycline showed 25% resistance to Pseudomonas. All antibiotics showed high resistance to Acinatobacter. This resistance pattern of GNBs used in this study are shown in Table-6.

Table 5: Frequency distribution of Carbapenemase genes by Multiplex PCR in different samples

Sample	Isolates	Frequency of Carbapenemase genes			No Resistance Genes Positive
		NDM	OXA-48	NDM + OXA 48	
Urine	Klebsiella pneumoniae	2	1	2	-
	Escherichia coli	1	-	1	2
	Pseudomonas aeruginosa	3	1	1	1
Sputum	Klebsiella pneumoniae	1	-	3	1
	Acinatobacter baumannii	-	-	-	1
Blood	Klebsiella pneumoniae	1	-	-	-
	Pseudomonas aeruginosa	-	1	-	-
	Acinatobacter baumannii	-	-	-	3
Tracheal aspirate (T/A)	Klebsiella pneumoniae	-	1	1	-
	Acinatobacter baumannii	-	-	-	2
Wound Swab (W/S)	Klebsiella pneumoniae	-	-	2	-
	Pseudomonas aeruginosa	-	-	1	-
Broncho-alveolar lavage (BAL)	Klebsiella pneumoniae	-	-	1	-
Sub Total		**8**	**4**	**12**	**10**
TOTAL		**24**			**10**
GRAND TOTAL		**34**			

Table 6: Resistance pattern of gram-negative Bacteria (GNBs) used in the study

Antibiotics	*K. pneumoniae* (N=16)	*E.coli* (N=04)	*P. aeruginosa* (N=08)	*Aci. Baumannii* (N=06)	TOTAL (N=34)
AMC	88%	75%			82%
CXM	88%	75%			82%
CFM	88%	75%			82%
CRO	88%	75%			82%
FEP	88%	75%	75%	100%	85%
CN	69%	25%	75%	100%	68%
AK	81%	25%	75%	100%	70%
SXT	75%	50%		0%	42%
LEVO	94%	25%	88%	100%	77%
TZP	88%	50%	88%	100%	82%
MEM	94%	50%	88%	100%	83%
CAZ	88%	75%	88%	100%	88%
AZT	75%	25%	75%	100%	69%
FOS	13%	0%		67%	27%
CZA	56%	50%	88%	100%	74%
TIG	18%	25%	25%	67%	34%
MH	25%	25%	25%	83%	40%

AMC, CXM, CFM, CRO, FEP, CN, AK, SXT, LEVO, TZP, MEM, CAZ, AZT, FOS, CZA, TIG and MH stand for amoxicillin/clavulanic acid, Cefuroxime, Cefixime, ceftriaxone, Cefepime, gentamicin, Amikacin, trimethoprim-sulfamethoxazole, levofloxacin, Pipercillin-tazobactam,meropenem, Ceftazidime, Aztreonam, Fosfomycin, Ceftazidime-Avibactam, Tigecycline and Minocycline respectively)

MIC of meropenem and imipenem for Carbapenemase producing isolates in this study may vary within a broad range of values, from ≥8 to ≥64 µg/mL but MICs of imipenem were found lower than Meropenem. Hundred percent isolates that co-carrying NDM+OXA-48 genes and 13% of only NDM producers have higher carbapenem MICs, about ≥64. However, OXA 48 producing GNBs has lower MICs. Though all our studied Though Acinatobacter showed higher MIC value to both imipenem and meropenem, but no resistance genes were found among them. Correlation between MIC of Imipenem and Meropenem and carbapenemase production among *K. pneumoniae*, *E. coli*, *P. aeruginosa* and*A. baumannii* is shown in Table-7.

Table 7: Correlation between MIC of Imipenem & Meropenem and Carbapenemase production among GNB

Organism and Carbapenemase	Imipenem MIC (µg/mL)						Meropenem MIC (µg/mL)					
	2	4	8	16	32	64	2	4	8	16	32	64
NDM (8)												
K.pneumoniae (n=15)	-	-	1	3	-	-	-	-	-	1	1	2
E.coli (n=2)	-	-	1	-	-	-	-	-	-	-	1	-
Pseudomonas aeruginosa (n=7)	-	-	2	1	-	-	-	-	1	-	1	1
OXA 48 (4)												
K.pneumoniae (n=15)	-	-	1	1	-	-	-	-	-	1	1	-
E.coli (n=2)	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa (n=7)	-	-	1	1	-	-	-	-	-	-	1	1
NDM+OXA 48 (12)												
K.pneumoniae (n=15)	-	-	5	4	-	-	-	-	-	2	5	2
E.coli (n=2)	-	-	-	1	-	-	-	-	-	-	-	2
Pseudomonas aeruginosa (n=7)	-	-	1	1	-	-	-	-	-	-	1	1
No Genes												
Acinatobacter sp (n=6)	-	1	3	2	-	-	-	-	1	1	2	2

Table-8 summarizes the antibiotic susceptibility pattern of carbapenem resistant isolates determining MIC of colistin, polymyxin B, tigecycline, minocycline, fosfomycin, ceftazidime-avibactam, cotrimoxazole, ceftazidime and gentamicin. In our study, all CR organisms with single or both genes were found 100% sensitive to colistin and polymyxin B. NDM encoded CR GNBs showed 50% sensitivity to Minocycline, 30% to tigecycline, fosfomycin and gentamicin, 25% to cotrimoxazole and no sensitivity to ceftazidime-avibactam. OXA 48 gene positive CR GNBs were found only 25% sensitive to Fosfomycin and Ceftazidime-avibactam and 100% resistant to all other tested antibiotics. Carbapenem resistant *Klebsiella*, *E. coli* and *Pseudomonas* which harboured both NDM and OXA 48 genes were found 33% sensitive to tigecycline, minocycline and fosfomycin followed by 17% to gentamicin and only 8% to both ceftazidime-avibactam and cotrimoxazole.

Table 8: Association between Antimicrobial Susceptibility Profile of Isolates and Carbapenemase Resistance Genes

No	Isolate	CT	TIG	MH	FOS	CZA	PB	SXT	CAZ	CN	Carbapenemase genes
1	K.pneumoniae	S	S	R	R	R	S	R	R	R	NDM+OXA 48
2	K.pneumoniae	S	R	R	R	R	S	R	R	R	OXA 48
3	E.coli	S	R	S	S	R	S	S	R	S	NDM
4	K.pneumoniae	S	R	R	S	S	S	R	R	S	NDM+OXA 48
5	Pseudomonas aeruginosa	S	R	R	R	R	S	R	R	R	NDM
6	K.pneumoniae	S	R	R	S	S	S	R	R	R	OXA 48
7	E.coli	S	R	R	R	R	R	R	R	S	NDM+OXA 48
8	K.pneumoniae	S	S	S	R	R	S	R	R	R	NDM+OXA 48
9	Pseudomonas aeruginosa	S	R	R	R	R	S	R	S	R	OXA 48
10	K.pneumoniae	S	R	S	R	R	S	R	R	R	NDM+OXA 48
11	Pseudomonas aeruginosa	S	R	R	R	R	S	R	R	R	NDM
12	K.pneumoniae	S	R	R	S	R	S	R	R	R	NDM+OXA 48
13	Pseudomonas aeruginosa	S	R	R	R	R	S	R	R	R	NDM
14	K.pneumoniae	S	S	S	S	R	S	S	R	R	NDM+OXA 48
15	Pseudomonas aeruginosa	S	R	R	R	R	S	R	R	R	NDM+OXA 48
16	K.pneumoniae	S	R	R	R	R	S	R	R	R	NDM+OXA 48
17	Pseudomonas aeruginosa	S	R	R	R	R	S	R	S	R	OXA 48
18	K.pneumoniae	S	S	S	S	R	S	R	R	R	NDM+OXA 48
19	Pseudomonas aeruginosa	S	R	R	R	R	S	R	R	R	NDM+OXA 48
20	K.pneumoniae	S	R	R	R	R	S	R	R	R	NDM+OXA 48
21	K.pneumoniae	S	S	S	R	R	S	S	R	S	NDM
22	K.pneumoniae	S	R	R	R	R	S	R	R	S	NDM
23	K.pneumoniae	S	S	S	S	R	S	R	R	R	NDM
24	K.pneumoniae	S	S	S	S	R	S	R	R	R	NDM

(K. pneumonia and E. coli stands for Klebsiella pneumonia and Escherichia coli. CT, TIG, MH, FOS, CZA, PB, SXT, CAZ and CN stands for Collistin, Tigecycline, Minocycline, fosfomycin, Ceftazidime-avibactam, Polymyxin b, Cotrimoxazole, Ceftazidime and Gentamycin respectively. NDM and OXA 48 stands for New-Delhi- Metallo Beta lactamase and Oxacillinase Enzymes.)

Table 9: Frequency of Single/Combined/No Carbapenemase genes included in PCR kit among different organisms

	K. pneumoniae (N=15)	*E.coli* (N=02)	*Pseudomonas sp (7)*	*Acinatobacter sp (6)*
Single Carbapenemase				
NDM	4	1	3	ND
OXA 48	2	ND	2	ND
KPC	ND	ND	ND	ND
VIM	ND	ND	ND	ND
IMP	ND	ND	ND	ND
Double Carbapenemase				
NDM/OXA-48	9	1	2	
KPC/NDM	ND	ND	ND	
NDM/VIM	ND	ND	ND	
No Carbapenemase				6

Note: ND, not detected by polymerase chain reaction assay. No KPC, VIM, IMP are detected

Table 9 showed presence of targeted five genes either solitarily in one bacterial isolate or more than one gene in one bacterial isolate. Out of 30 carbapenem resistant GNBs, 24 (80%) strains harbored single or double genes, while none of the gene was detected among 6 (20%) cases. The most common resistance gene found was NDM (33.3%) and less frequent was OXA 48 (16.7%). NDM and OXA 48 were co expressed in 50% of isolates. KPC, VIM, IMP were not isolated single or in combination in this study.

Among the 15 carbapenem resistant *K. pneumoniae* strains, 60% were mediated by both NDM & OXA 48 genes, 26.70% by NDM and 13.30% by OXA 48 only. The *E coli* has shown presence of NDM gene in 50%, while both NDM & OXA-48 genes in other 50% cases.

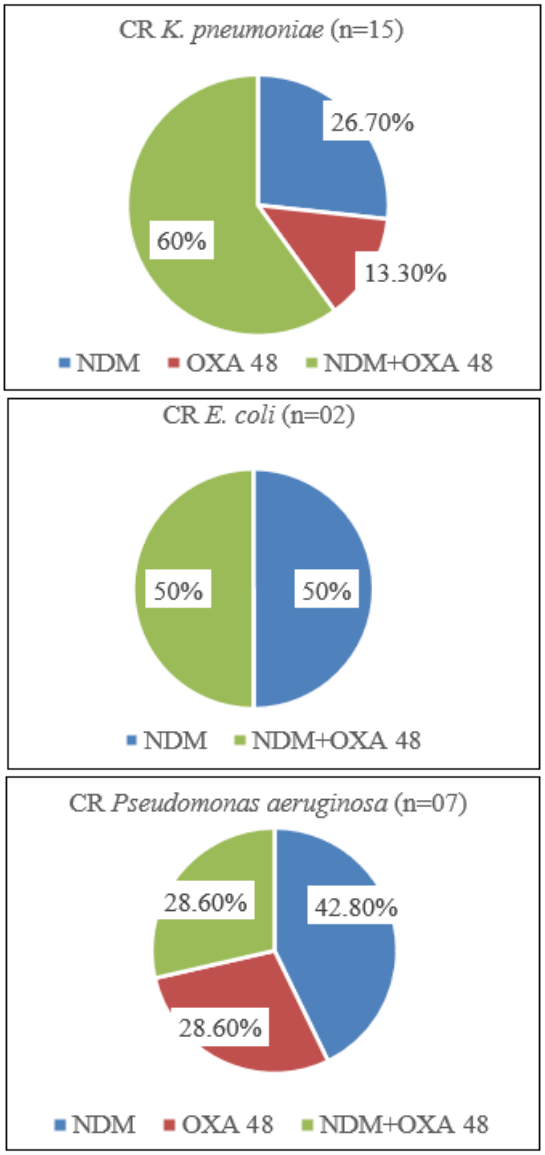


Figure 5: Distribution of carbapenem resistance mechanisms detected by Multiplex RT-PCR. *CR-Carbapenem resistant; NDM-New Delhi metallo-β-lactamase; OXA-oxacillin carbapenemase/oxacillinase*

Our studied *Pseudomonas aeruginosa* become carbapenem resistant by NDM in 42.80%; OXA 48 in 28.60% and both NDM+ OXA48 in 28.60% isolates (Figure-5)

4. Discussion

Carbapenem resistance (CR) occur due to presence of Carbapenemase enzymes, excessive production of ESBL, porin loss, presence of efflux pumps or combination of more than one mechanism. This resistance gradually increases among gram negative isolates including both *Enterobactericeae* and non-*Enterobactericeae* which are

responsible for community-acquired and nosocomial infections. Carbapenem resistance can spread clonally from person to person or through carbapenemase enzymes encoded genes that can easily transferable horizontally by extrachromosomal plasmids between isolates. [23] The most important carbapenemases are KPC, VIM, NDM and OXA-48. [22]

Urine was the most frequent sample received during our study period and in most of other studies analyzed. This may be attributed to urinary tract infection (UTI), being the most common hospital-acquired infection. Respiratory samples including Sputum, Tracheal aspirate (T/A) and Broncho-alveolar lavage (BAL) were the next in frequency followed by blood and wound swab which is in contrast to a comparable study.[38] The most common CRE species isolated in the US and European countries are *K. pneumoniae* followed by *Enterobacter aerogenes* and *E. coli*.[22], [28] Our study is partially similar to this study where highest isolation was *K. pneumoniae*, but second most was *Pseudomonas aeruginosa* followed by *Acinetobacter baumannii* and *E.coli*. Our study has also reported carbapenem resistant organisms with different carbapenemase genes more prevalently from both Broncho-alveolar lavage and wound swab. This is in contrast to other studies from various places of India that detected highest cases of carbapenem resistant gram-negative isolates from Urinary tract infections.[26], [29], [31]

Carbapenemase producing isolates are mostly multidrug resistant (MDR) while carbapenems are considered as the last-resort antibiotics for these difficult to treat infections. This leads CR-infections become highly detrimental with narrow treatment options and increase importance of detection of carbapenem resistance along with its encoded gene for healthcare worldwide.[27], [28] In present study, total 34 gram-negative bacteria were isolated from various clinical samples and tested for antimicrobial resistance with special target to carbapenem by both Kirby-Bauer disc diffusion technique and automated Minimum inhibitory concentration (MIC) detection. An Indian study found a high meropenem resistance of 42, 47 and 62 per cent among members of carbapenem resistant *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii* respectively. [29] Much high meropenem resistance was reported in our study, indicated 94%, 50%, 88% and 100% for CR *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinatobacter baumanii* respectively.

Accurate susceptibility data is required to provide effective therapy. Thus, for appropriate treatment of carbapenem-resistant isolates, MIC were determined for colistin, polymyxin B, tigecycline, minocycline, fosfomycin, ceftazidime-avibactam, cotrimoxazole, ceftazidime and gentamicin. We have detected sensitivity of Gentamycin (31%), Ceftazidime-avibactam (44%), Tigecycline and Minocycline (75%), Fosfomycin (87%) and Collistin (100%) against carbapenem resistant *Klebsiella pneumoniae* which corresponds to the previous studies. [29] [30], [31] Our study also reported sensitivity of CR- E. coli to Cotrimoxazole, Pipercillin-tazobactam and Ceftazidime-avibactam (50%); to Amikacin, Levofloxacin, Aztreonam, Tigecycline and Minocycline (75%) and to Fosfomycin (100%), while CR-Pseudomonas possessed 75% sensitivity to only Tigecycline and Minocycline and CR- Acinatobacter had no antibiotics with high sensitivity. Fosfomycin, though previously used mainly as oral treatment for uncomplicated urinary tract infections, but currently attracts clinicians' interest worldwide, particularly for the reported activity against pathogens with advanced resistance. [29]

Plasmids can harbor multiple carbapenemase genes in one single bacteria. Also, genes on different plasmids may jump from bacteria to bacteria, easily causing the rapid emergence of multidrug-resistance. [24] This study also observed co-carrying of NDM and OXA 48 gene in CR-GNBs isolates which were resistant to most tested antibiotics and found consistent with previous studies.[15], [28], [32] Co-expression of NDM and OXA 48 were identified 60% in *K. Pneumonia*, 50% in *E coli* and 28.60% among *Pseudomonas aeruginosa* in this study which corroborated with the study performed by Veeraraghavan et al. [29] But we did not found any other combination like NDM & VIM, VIM & IMP as reported in other previous studies. [33], [40]

Automated antibiotic susceptibility systems were found to be unreliable for detection of carbapenem resistance, as either over or under reported. A review of several automated systems showed that they incorrectly labeled up to 87% of carbapenemase-producing *K. pneumoniae* isolates as susceptible to imipenem.[22], [34] We found that meropenem and imipenem MICs for Carbapenemase producing isolates may vary within a broad range of values, from ≥8 to ≥64 µg/mL and both 13% of only NDM & 100% of combined NDM & OXA-48 genes producers possessed high MIC, ≥64 which is in accordance with a study reported MICs from 0.12 to >256 mg/L. [34]

In recent years, molecular diagnostic techniques have become a game changer for clinical laboratories of all sizes. Poirel et al. concluded with multiplex-PCR as a rapid, reliable and convenient tool for better evaluation of real prevalence of carbapenemase genes in different clinical isolates.[35] On multiplex PCR using Unimedica Multiplex Real time PCR Kit for detection of 5 Carbapenem Resistance Genes in our molecular laboratory [21], 24 (71%) of 34 multidrug resistant gram-negative bacteria were positive for one or more of the carbapenemase genes. Highest expression of combined NDM & OXA 48 type was detected among our 50% isolates which was much higher than a study showed 20 per cent co-observation of them. [33] This study reported 33.3% NDM and 16.7% OXA-48 positive isolates which were lower than a study where 65.6% NDM and 24.7% OXA 48 were identified. [38]

Studies of the South East Asian countries, most importantly India detected a high prevalence of NDM gene correlating to the findings of the present study. Contrary to this, most of the European countries, USA & Canada reported a higher incidence of KPC gene responsible for carbapenem resistance.[22], [32], [37] The KPC, VIM, IMP were not detected in any isolate of our study. No resistant gene was present among our 06 carbapenem resistant *Acinatobacter baumanii* isolates. This is not consistent to some previous studies done in India where carbapenem resistant Acinetobacter possessed OXA-23 and OXA-51.[37], [38] This might be due to absence of our 5 target genes and/or having other mechanism of

carbapenem resistance. The rest 04 carbapenem sensitive organisms found negative for Carbapenemase genes, validate our test results.

New Delhi metallo-β-lactamase (NDM) producing *Enterobacteriaceae* are now widespread in India, Pakistan, and Bangladesh. [29], [24], [39] Our study found highest NDM producers in *E coli* (50%) followed by 42.80% in *Pseudomonas aeruginosa* and 26.70% in *K. pneumoniae* strains. Similar observations were reported from other studies.[22], [23], [39] OXA-48 gene, though initially identified from a *K. pneumonia* strain in 2001 in Turkey, but currently, many other species of *Enterobacteriaceae* such as *E. coli* and other GNBs are known to possess them. Today OXA-48 producers have spread to Middle East, North Africa, Mediterranean countries, Europe, North America, South America and Asia and become increasingly important causes of nosocomial outbreaks in our country as well.[24], [25], [32], [33] In present study, majority of OXA-48 producing isolates were *Pseudomonas aeruginosa* (28.60%) followed by *K. Pneumoniae* (13.30%) which corresponds with a study conducted by Amaya et al. in Nicaragua [40], but not consistent to a study conducted in the USA by Hoelle et al., where 55% of E. coli isolates possessed VIM gene and 1% had IMP gene [29].

Carbapenem resistant GNBs in the present study having NDM, OXA 48 and combination of both genes showed maximum antimicrobial susceptibility to colistin and polymyxin B (100%) which correlates with a study where colistin & tigecycline were found 100% & 98% sensitive. However, some Indian studies are in concurrence with our study CR-GNBs regarding tigecycline.[26], [37], [38] Depending on presence of OXA 48, NDM and both NDM & OXA 48 genes, minocycline sensitivity was found from 0-50% among our tested isolates which was in accordance to other Indian and western literature.[24], [34] The OXA 48 enzyme have weak carbapenemase activity and poor or no action against extended spectrum cephalosporins and aztreonam.[25], [33], [35] Our findings of all OXA-48 positive isolates having only 25% sensitive to Fosfomycin and Ceftazidime-avibactam and 100% resistance to all other tested antibiotics suggested presence of other resistance mechanisms among them. Though NDM can yield high levels of resistance to carbapenems, but cannot hydrolyze aztreonam. [29] Our NDM encoded CR-GNBs showed 30% sensitivity to fosfomycin and gentamicin, 25% to cotrimoxazole and no sensitivity to ceftazidime-avibactam.

We acknowledge several limitations of our study. First, the present study includes a few random isolates that did not represent a large population number, second, the current research did not use primers to target all known carbapenemases genes and third, it did not investigate the clonality of the isolates and the sequence of the genes. Thus, some carbapenemase-producing isolates could not be identified and adequately characterized. Despite these limitations, the study has provided the distribution of the common carbapenemase genes and the magnitude of the problem.

5. Conclusions

The past few years have seen an unprecedented, rapidly increasing number of carbapenemase producing gram negative bacteria worldwide. In this study we used an efficient multiplex PCR assay for fast, accurate and simultaneous detection of five carbapenemase genes in a single reaction which could help in preventing emergence and spread of these pathogens through strict infection control practices, judicious use of antibiotics, and timely identification. Although further study is required to determine the prevalence of carbapenemase genes in a large sample size and to do sequencing of the genes for characterization of the isolates properly, our PCR technique provides a satisfied and reliable result. Therefore, this Multiplex PCR can be recommended as a routine molecular test for efficient and optimal detection of carbapenemase genes among the MDR-GNB in our hospital and other health facilities in developing countries.

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References

[1] Zhang Y, Wang Q, Yin Y, et al. Epidemiology of carbapenem-resistant Enterobacteriaceae infections: report from the China CRE network. Antimicrob Agents Chemother. 2018;62(2): e01882. doi: 10.1128/ AAC.01882-17

[2] Kang J, Yi J, Ko M, Lee S, Lee J, Kim K. Prevalence and risk factors of Carbapenem-resistant Enterobacteriaceae Acquisition in an Emergency Intensive Care Unit in a Tertiary Hospital in Korea: a Case-Control Study. J Korean Med Sci. 2019;34(18): e140. doi: 10.3346/ jkms.2019.34. e140

[3] J. Brink, J. Coetzee, C. G. Clay et al., "Emergence of New Delhi metallo-beta-lactamase (NDM-1) and Klebsiella pneumoniae carbapenemase (KPC-2) in South Africa," Journal of Clinical Microbiology, vol. 50, no. 2, pp. 525–527, 2012.

[4] World Health Organization Global Action Plan on Antibiotic Resistance. WHO Press: Geneva, Switzerland, 2015.

[5] Marr CM, Russo TA. Hyper virulent Klebsiella pneumoniae: a new public health threat. Expert Rev Anti Infect Ther. 2019;17(2):71-73. doi: 10.1080/14787210.2019.1555470

[6] M. Queenan and K. Bush, "Carbapenemases: the versatile β-lactamases," Clinical Microbiology Reviews, vol. 20, no. 3, pp. 440–458, 2007.
[7] Surojit Das, Subhanita Roy, Samadrita Roy, Gaurav Goel, KaminiWalia, Sudipta Mukherjee, Sanjay Bhattacharya and Mammen Chandy. Rapid and economical detection of eight carbapenem-resistance genes in Enterobacteriaceae, Pseudomonas spp, and Acinetobacter spp directly from positive blood cultures using an internally controlled multiplex-PCR assay. The Society for Healthcare Epidemiology of America; https://doi.org/10.1017/ice.2019.79; 2019.
[8] Quale J, Bratu S, Gupta J, Landman D. Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of Pseudomonas aeruginosa clinical isolates. Antimicrob Agents Chemother 2006; 50:1633–41.
[9] Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003; 67:593–656
[10] Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 2010; 54:969–76.
[11] P. Nordmann, T. Naas, and L. Poirel, "Global spread of carbapenemase-producing Enterobacteriaceae," Emerging Infectious Diseases, vol. 17, no. 10, pp. 1791–1798, 2011.
[12] Hsu LY, Apisarnthanarak A, Khan E, Suwantarat N, Ghafur A, Tambyah PA. Carbapenem-resistant Acinetobacter baumannii and Enterobacteriaceae in South and Southeast Asia. ClinMicrobiol Rev 2017; 30:1–22
[13] Jean SS, Hsueh PR. High burden of antimicrobial resistance in Asia. Int J Antimicrob Agents. 2011;37(4):291-5.
[14] Nordmann P, Poirel L, Toleman MA, Walsh TR. Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J Antimicrob Chemother. 2011;66(4):689-92.
[15] Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in Pseudomonas aeruginosa and Acinetobacter baumannii: mechanisms and epidemiology. Int J Antimicrob Agents 2015: 45:568–85.
[16] Abboud MI, Damblon C, Brem J, et al. Interaction of avibactam with class B metallo-β-lactamases. Antimicrob Agents Chemother 2016; 60:5655–62.
[17] Ong DC, Koh TH, Syahidah N, Krishnan P, Tan TY. Rapid detection of the blaNDM-1 gene by real-time PCR. J Antimicrob Chemother.2011;66(7):1647-9.
[18] Praful S. Patil, Harshada Shah, BrijNandan Singh, Dhruba Hari Chandi, Mrinangka Deb and Roshan Jha. Molecular Detection of Carbapenem Resistance in Clinical Isolates of Klebsiella pneumoniae in Tertiary Care Hospital Journal of Pure and Applied Microbiology. 2023;17(2):1109-1117. https://doi.org/10.22207/JPAM.17.2.41
[19] Mirza Nazim Uddin, NurunNahar Mawla, Zahidul Hasan, Faridul Islam, Anowar Hossain, "AntibioticResistance Pattern of Isolates in Intensive Care Unit and Source of Nosocomial Infection in a Tertiary CareHospital, Dhaka, Bangladesh", International Journal of Science and Research (IJSR), https://www.ijsr.net/archive/v6i9/ART20176855.pdf. Vol. 6 (9), 1117-1124; 2017 (Sept).
[20] Performance Standards for antimicrobial susceptibility testing, 23rd informational supplement. CLSI document M100-S23. Clinical and Laboratory Standards Institute. 2013.
[21] Multiplex Real time PCR Kit for Carbapenem Resistance Genes Shenzhen Uni-medica Technology Co. Ltd CMC MEDICAL DEVICES & DRUGS S.L. Málaga, Spain.
[22] Pollett S, Miller S, Hindler J, Uslan D, Carvalho M, Humphries RM. Phenotypic and molecular characteristics of carbapenem-resistant Enterobacteriaceae in a health care system in Los Angeles, California, from 2011 to 2013. J ClinMicrobiol. 2014; 52:4003–9.
[23] Demir Y, Zer Y, Karaoglan I. Investigation of VIM, IMP, NDM-1, KPC AND OXA-48 enzymes in Enterobacteriaceae strains. Pak J Pharm Sci. 2015; 28:1127–33.
[24] Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. ClinMicrobiol Infect. 2014; 20:821–30.
[25] Glasner C, Albiger B, Buist G, Tambić Andrasević A, Canton R, Carmeli Y, et al. Carbapenemase producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. Euro Surveill. 2013;18(28). Doi: 10.2807/1560-7917.
[26] Mustafeed Uddin Mohammed, Manisha D R, Nagamani K. Clinical, phenotypic and genotypic profile of carbapenem resistant gram-negative infections in intensive care units. Indian Journal of Microbiology Research 2021;8(1):28–34
[27] Sahin K, Tekin A, Ozdas S, Akin D, Yapislar H, Dilek AR, et al. Evaluation of carbapenem resistance using phenotypic and genotypic techniques in Enterobacteriaceae isolates. Ann Clin Microbiol Antimicrob. 2015; 14:44.
[28] Djahmi N, Dunyach-Remy C, Pantel A, Dekhil M, Sotto A, Lavigne JP. Epidemiology of carbapenemase-producing Enterobacteriaceae and Acinetobacter baumannii in Mediterranean countries. Biomed Res Int. 2014; 2014:305784.
[29] Atul Garg, Jaya Garg, Sachin Kumar, Amitabh Bhattachary6, Saurabh Agarwal & G.C. Upadhyay. Molecular epidemiology & therapeutic options of carbapenem-resistant Gram-negative bacteria. Indian J Med Res 149, February 2019, pp 285-289.
[30] Wang JT, Wu UI, Lauderdale TL, Chen MC, Li SY, Hsu LY, et al. Carbapenem- nonsusceptible Enterobacteriaceae in Taiwan. PLoS One. 2015;10: e0121668
[31] Hamzan NI, Yean CY, Rahman RA, Hasan H, Rahman ZA. Detection of blaIMP4 and blaNDM1 harboring Klebsiella pneumoniae isolates in a university hospital in Malaysia. Emerg Health Threats J. 2015; 8:26011.
[32] Irmak Baran and NerimanAksu. Phenotypic and genotypic characteristics of carbapenem‐resistant Enterobacteriaceae in a tertiary‐level reference

hospital in Turkey Baran and Aksu Ann Clin Microbiol Antimicrob (2016) 15:20

[33] Ellappan K, Belgode Narasimha H, Kumar S. Coexistence of multidrug resistance mechanisms and virulence genes in carbapenem-resistant Pseudomonas aeruginosa strains from a tertiary care hospital in South India. J Glob Antimicrob Resist.2018 Mar;12:37-43

[34] Solanki R, Vanjari L, Subramanian S, B A, E N, Lakshmi V. Comparative evaluation of multiplex PCR and routine laboratory phenotypic methods for detection of carbapenemases among gram negative bacilli. J ClinDiagn Res. 2014; 8: DC23–6.

[35] Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. DiagnMicrobiol Infect Dis 2011; 70:119-123.

[36] Mohanty S, Gaind R. In vitro susceptibility of carbapenem-resistant Enterobacteriaceae to colistin: A hope at present. Indian J Med Microbiol 2016; 34:558-60

[37] Parijat Das, Kumar AnandShrutiraaj, Manish Ranja1 and Sourav Sen. Prevalence of Carbapenem Resistance and their Genotypic Profile among Gram-Negative Bacteria in a Tertiary Care Hospital in Western India. Annals of Pathology and Laboratory Medicine, Vol. 8, Issue 5, May, 2021.

[38] La MV, Jureen R, Lin RT et al. Unusual detection of an Acinetobacter class D carbapenemase gene, bla OXA-23, in a clinical Escherichia coli isolate. J ClinMicrobiol 2014; 52: 3822–3.

[39] Moore NM, Cantón R, Carretto E, Peterson LR, Sautter RL, TraczewskiMM; Carba-R Study Team. Rapid Identification of Five Classes of Carbapenem Resistance Genes Directly from Rectal Swabs by Use of the XpertCarba-R Assay. J ClinMicrobiol. 2017 Jul;55(7):2268-2275

[40] E. Amaya, D. Reyes, M. Paniagua et al., “Antibiotic resistance patterns of Escherichia coli isolates from different aquatic environmental sources in Leon, Nicaragua,” Clinical Microbiology and Infection, vol. 18, no. 9, pp. E347–E354, 2012.