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Analysis of Multidrug Resistant NDM Producing Bacteria in ICU

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Abstract

➢ ***Background:***

β-Lactam antibiotics are commonly used to treat bacterial infections. The groups of antibiotics in this catagory include penicillins, cephalosporins, carbapenems & monobactams. Increased use of antibiotics, particularly the third generation of cephalosporins, has been associated with the emergence of β-Lactamases mediated bacterial resistance, which subsequently led to the development of ESBL producing bacteria. ESBL screening as a routine test has not yet been practiced in Bangladesh. So, main objective is to determine the extended spectrum beta lactamase (ESBL) among *Escherichia coli, Klebsiella* spp. and other strains and to detect main ESBL and NDM producing genes by PCR.

➢ ***Materials/Methods:***

Patients with Bacterial infection. UTI reporting, PUS, RTI and Wound infection in RMCH. These sample was cultured in a MacConkey agar. Disk diffusion and Double disk diffusion test was done.

➢ ***Results:***

Among 1423 meropenem resistant isolates, the breakup was, *Klebsiella sp* - 381, *Proteus sp* - 176, *Esch. coli* - 189, *Pseudomonas sp* - 119, *Others* - 147. In the present study, test strains showed potentiality against higher classes of antibiotics such as carbapenem group. After PCR we found NDM-1 positive isolate.

➢ ***Conclusions:***

The results shows that bla_{NDM-1} is the most frequent gene identified in most of the isolates. bla_{NDM-1} appear to be an emerging cause of carbapenem resistance in enterobacteriaceae.

Keywords:- *Enterobacteriaceae, NDM-1, ESBLs.*

I. INTRODUCTION

Antibiotic resistance is a growing concern all over the world especially in the third world where malnutrition is present. (Prabakar & Weinstein, 2011). Carbapenems (imipenem, ertapenem, meropenem, and doripenem) are β-Lactam antibiotics that are classified according to their chemical structures (Mohamed *et al.*, 2013). Carbapenems were the medications of choice to treat severe infections caused by extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae*.

β-Lactam antibiotics were normally used to treat bacterial infections. The groups of antibiotics in this catagory include penicillins, cephalosporins, carbapenems & monobactams. Increased use of antibiotics, particularly the third generation of cephalosporins, had been related with the occurrence of β-Lactamases mediated bacterial resistance, which subsequently led to the development of ESBL producing bacteria. ESBLs were enzymes that mediate resistance to extended spectrum e.g., Carbapenem, third generation cephalosporins as well as monobactams such as aztreonam (CLSI 2010). These enzymes catalyze the hydrolysis of the β-lactam ring of antibiotic, thereby destroying the antimicrobial activity. ESBLs had reported globally in several different genera of *enterobactericeae* and *Pseudomonas aeroginosa* (Friedman *et al*. 2005). However, these were most common in *Klebsiella pneumoniae and E. coli* (Agarwal *et al*. 2008). ESBL producing organisms were often resistant to numerous other classes of antibiotics, as the plasmids with the gene encoding ESBLs often carry other resistance determinants. In the beginning ESBL producing organisms were isolated from nosocomial infections but these organisms were now also being isolated from community (Pitout and Laupland 2008).The colonization rate for *K. pneumoniae* was little in strong persons in the common inhabitants. But it was increased in hospitalized patients especially with long care facilities, health care manipulations. e.g., use of catheters (Yusha'u *et al*. 2010).

In this study, an effort had made to research the features and analysis of the current spreading bla_{NDM-1} producing enterobacteriaceae in several organisms and to determine its occurrence in organisms isolated from patients.

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II. MATERIAL AND METHODS

A. Materials

HiCrome ESBL agar, HiCrome UTI Selective agar, Nutrient agar were purchased from HiMedia Laboratories and Difco ™ Mueller Hinton agar was provided by Becton, Dickinson and Company. The preparations of both media were registered and secret. And other media were acquired from Thermofisher (Basingstoke, UK).

B. Specimen Collection and Culture

Between April 2013 to November 2013 and July 2014 to June 2015, samples were collected from Rajshahi Medical College and Hospital, Rajshahi, Bangladesh. The hospital has 1000 beds, respectively. The Hospital had a collection of specified surgical units, including orthopaedics and plastic surgery units. The samples were randomly selected from hospitalized and non-hospitalized individuals attending different outpatient departments of the Rajshahi Medical College and Hospital. Samples were taken randomly and were not selected on the basis of suspected enteric infection or diarrhoea. Study was conducted in accordance with the Declaration of Helsinki and national and institutional standards. Consent for screening for carbapenemase-producing Enterobacteriaceae was taken from all subjects and their attending doctors. The samples were cultured on Mac-Conkey agar at the Rajshahi Medical College and Hospital, and Molecular Pathology Laboratory, IBSc, University of Rajshahi and incubated at 37^0C for 24 h. The mixed growth from MacConkey plates was then harvested using a swab and inoculated into glycerol broth for storage at -80^0C for up to further analysis. Immediately prior to shipment, the glycerol broths were thawed and 0.5 mL amounts were added to tubes containing 1.5 mL of Mueller–Hinton agar at 50^0C. Once the agar had solidified, the tubes were securely sealed

C. Bacterial Isolates and Susceptibility Tests

Among 1423 sample 1012 sample were confirmed as ESBL. (71.1%), resistant enterobacteriaceae organisms were collected between the periods of 2013 through 2015 from scientific specimens and tested for reduced susceptibility to carbapenems by agar dilution method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2011). According to CLSI references, the production of carbapenemase among the isolates was confirmed by meropenem/ethylene diamine tetra-acetic acid (EDTA) double-disc diffusion test as per CLSI recommendation 2011. ATCC *Escherichia coli* 25922 were used as a negative control.

D. Susceptibility Testing

Entire Gram-negative organisms were used for susceptibility test. Minimum inhibitory concentration (MICs) of meropenem, doripenem, fosfomycin and methicillin were ascertained using standard agar dilution techniques (Kumarasamy *et al.* 2010). For fosfomycin, 25 mg/L glucose-6-phosphate was assimilated into the Mueller–Hinton agar as recommended (Livermore *et al.* 2007). Enterobacteriaceae were tested for the existence of extended spectrum β-lactamases (ESBLs) and New Delhi metallo β-lactamases with the HiCrome ESBL agar and meropenem with EDTA. Bacterial lawns were organized as for susceptibility tests and were separated using discs comprising meropenem alone and with various β-lactamase inhibitors (such as clavulanate, cloxacillin and clavulanate plus cloxacillin). Analysis was in accordance with the manufacturer's guidelines.

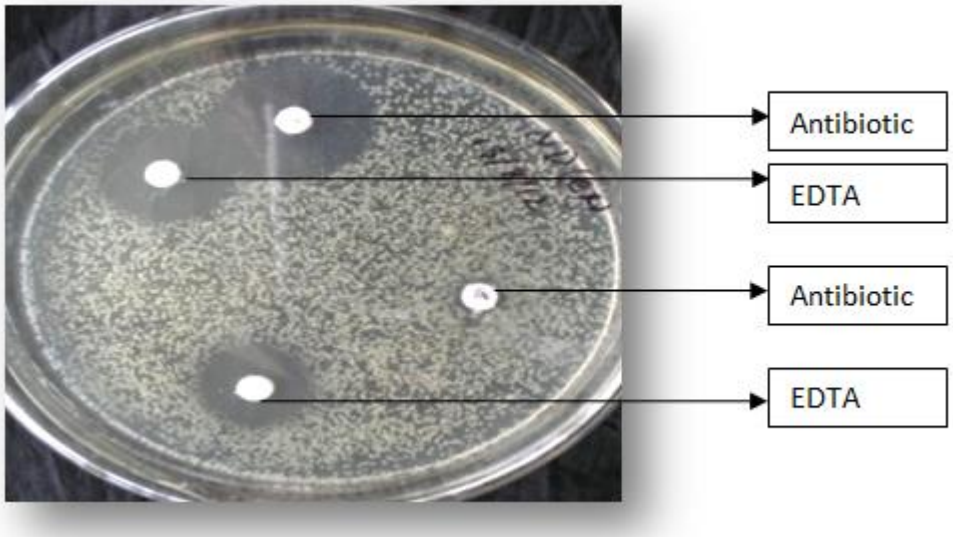

Fig 1:- Antibiotic with EDTA (Mueller Hinton Agar) PCR Amplification

Total DNA was extracted from all strains by boiling lysis method. A multiplex polymerase chain reaction (PCR) was used to amplify genes encoding $bla_{NDM-1,}$ (table 1). A total of 40 cycles were performed with a thermal cycler (Master cycler gradient, eppendorf) consisting initial denaturation step for 3 minutes at 95°C, denaturation step for 30 second at 95°C, an annealing step for 30 second at 55°C and extension step for 30 sec at 72 °C. After the final cycle there was a step of final extension of 10 min at 72°C. The PCR products generated were visualized by ethidium bromide staining after electrophoresis in a gel containing 1.5% agarose.

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E. Statistical Analysis

Variances among the efficiencies of the two chromogenic media for isolation of carbapenemase-producing Enterobacteriaceae were compared using McNemar's test with the continuity correction applied. A χ^2 test was used for comparison of occurrence in hospitalized and non-hospitalized persons. Statistical significance was taken as $P<0.05$.

III. RESULTS

A. Prevalence of CRS and CIR Isolates in Rajshahi Medical College and Hospitals from 2012 to 2015.

A total of 1423 clinically important organisms were collected from April 2013 to June 2015, inclusive, as part of my PhD Study. During this period of my research, a total of 1423 Gram negative organisms from numerous experimental samples were comprised in the research. The isolated strains were characterized for their antibiogram, MIC, production of ESBLs and presence of NDM-1 genes and different clusters of CTX-M β-lactamases. The distribution of various specimens were given in Table -I. The samples were urine 925 (65%), wound swab 302 (21.2 %), pus 196 (13.8%).

Types of specimen	Total no.
Urine	925 (65 %)
Wound swab	302 (21.2 %)
Pus	196 (13.8 %)
Total	1423 (100 %)

Table 1:- Distribution of Sample from Various Specimens

K. pneumoniae and *Pseudomonas spp* ranked first and fifth in terms of numbers of organisms submitted and made up 381 (83.6%) and 119 (68%) of all isolates, respectively. After collecting those sample, At first, I am going to see ESBL positive and their percentage of several organisms in the study population. For the isolation of ESBL positive isolates, we have selective or specific media.

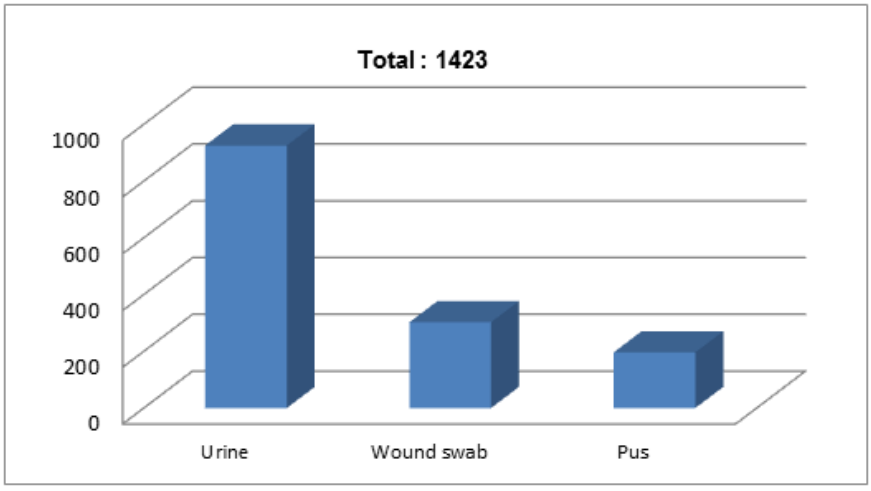

Fig 2:- Bar Diagram Shows the Distribution of Various Samples.

We use these ESBL agar base media and inoculate different strains of bacteria and saw the percentage of ESBL positive isolates. Following table shows the detection rate of several organisms in the study population.

Name of organism	**Total**	**ESBL positive (%)**
Klebsiella spp	456	381 (83.6)
E.coli	317	189 (59.6)
Proteus spp	257	176 (68.5)
Pseudomonas spp	175	119 (68)
*others	218	147 (67.4)
Total	1423	1012(71.1)

Table 2:- Detection Rates of Several Organisms in the Study Population
**Others- Enterobacter spp., Citrobacter*

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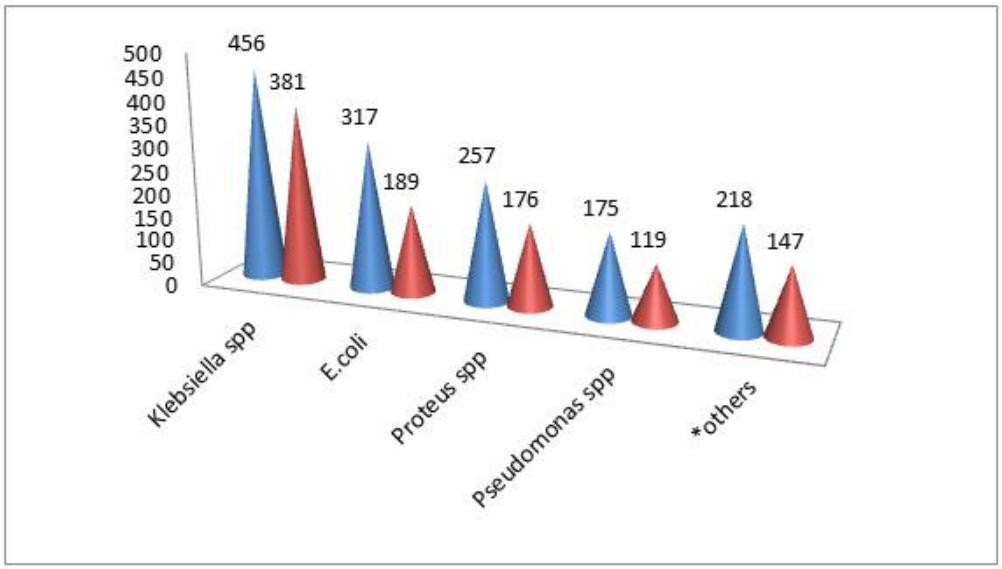

Fig 3:- Bar Diagram Shows the Detection Rate of ESBL Positive Isolates in the Study Population
*Series 1: Total strains, Series 2: ESBL positive.

Out of the 456 *Klebsiella spp*, 2.4% (11/456) had an imipenem MIC of 0.12 or 0.25 µg/ml and were categorized as carbapenem reduced susceptible (CRS); 2.2% (10/456) had an imipenem MIC ≥0.5 µg/ml and were categorized carbapenem intermediate/resistance (CIR). Out of the 317 *E.coli*, 2.8% (9/317) were categorized as CRS; 2.5% (8/317) were categorized as CIR. When looking at the increase from 2012 to 2014 of CRS and CIR isolates, no observable trend was present when the two cohorts were separated. However there was a doubling in prevalence for *E. coli* (2012: 1%, 2013: 1.2%, 2014: 0.5%, and 2015: 0.9%; P value=NS) when looking at all isolates with MICs ≥0.12 µg/ml. When looking at all *K. pneumoniae* isolates with MICs ≥0.12 µg/ml, the prevalence from year to year was relatively stable (2012: 1.5%, 2013: 1.2%, 2014: 1%, and 2015: 0.9%; P value=NS). This is summarized in Table 3.

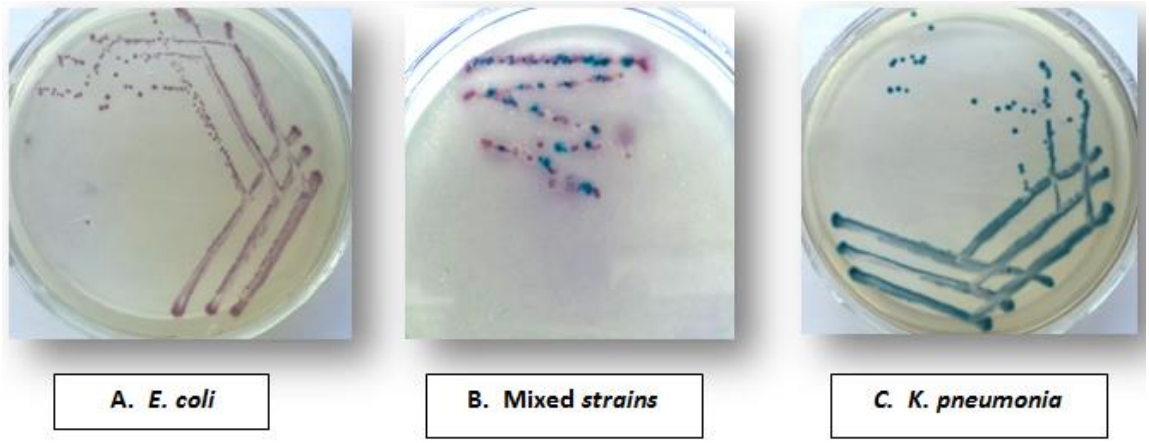

Fig 4:- Isolation of ESBL Producing Strains

	#/total (%)			
Study year	**CRS *K. pneumoniae***	**CIR *K. pneumoniae***	**CRS *E. coli***	**CIR *E. coli***
2012	3/200 (1.5)	3/200 (1.5)	3/200 (1.5)	2/200 (1)
2013	3/250 (1.2)	3/250 (1.2)	2/250 (0.8)	3/250 (1.2)
2014	2/200 (1)	2/200 (1)	1/200 (0.5)	1/200 (0.5)
2015	3/220 (1.4)	2/220 (0.9)	3/220 (0.9)	2/220 (0.9)

Table 3:- The national prevalence rates of CRS/CIR *E. coli* and *K. pneumoniae* from Rajshahi medical college and hospital 2013 to 2015.

B. Patient Demographics

➢ *CRS-E. coli and CRS-K. pneumonia*

Patient demographics for all CRS and CIR isolates are summarized in Table 4 and Table 5. The prevalence of CRS-*bacteria* (CRS) was even among males (1.97%) and females (2.1%) although it was slightly higher in females (P value=NS).CRS-Bacterial infection was seen more commonly in patients with ≥18 years of age (P value=NS). Infections with CRS-Bacteria was most likely seen in urban (1.98%) than rural (2.1%) (P value=NS).CRS-bacteria were most commonly isolated from ICUs (2.1%) followed by surgical wards (1.75%), medical wards (2.35%), outpatient clinics (1.62%), and ERs (2.13%)(P value=NS). The most common source of isolation of CRS-bacteria was the respiratory tract (1.72%) followed by wound (2.08%), urine (2.39%), and blood (1.79%) (P value=NS).

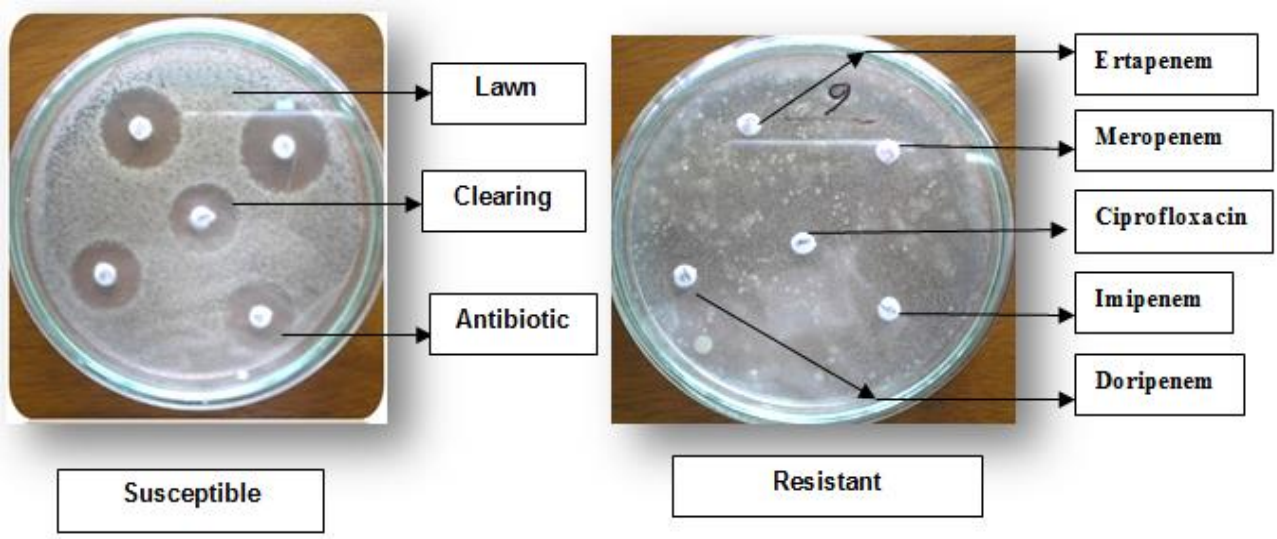

Fig 5:- Antimicrobial Susceptibility Testing

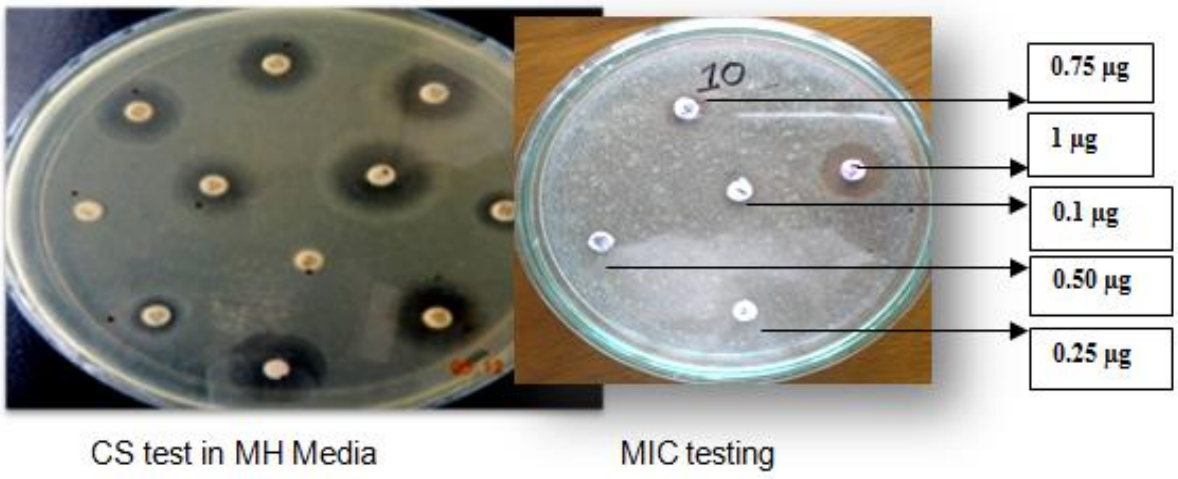

Fig 6:- Mueller Hinton Agar with Different Concentration of Carbapenem Antibiotics

Parameter	Value	#CRS/#Total (%)	#CIR/#Total (%)	# (%) of patients	
		CRS bacteria *n*=29/*n*=1423 (2.04)	CIR bacteria *n*=23/*n*=1423 (1.62)	CRS bacteria *n*=29	CIR bacteria *n*=23
Sex	**M** **F**	11/559 (1.97) 18/864 (2.1)	8/559 (1.4) 15/864 (1.7)	11 (37.93) 18 (62.1)	8 (34.78) 15 (65.22)
Age	**≤17** **18-65** **≥66**	3/234 (1.3) 16/768 (2.1) 10/421 (2.4)	2/234 (0.85) 13/768 (1.69) 8/421 (1.9)	3 (10.34) 16 (55.17) 10 (34.48)	2 (8.7) 13 (56.52) 8 (34.78)
Patient region[a]	**Urban** **Rural**	17/857 (1.98) 12/566 (2.1)	13/857 (1.52) 10/566 (1.77)	17 (58.62) 12 (41.38)	13 (56.5) 10 (43.5)

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Hospital ward[b]	**Medical**	9/383 (2.35)	11/578 (1.9)	9 (31.03)	11 (47.83)
	Surgical	5/285 (1.75)	0/55	5 (17.24)	0 (0)
	ICU	7/335 (2.1)	5/346 (1.45)	7 (24.14)	5 (21.74)
	ER	5/235 (2.13)	4/297 (1.35)	5 (17.24)	4 (17.4)
	Clinic	3/185 (1.62)	3/147 (2.04)	3 (10.34)	3 (13.04)
Specimen source[c]	**Urine**	12/503 (2.39)	7/430 (1.6)	12 (41.38)	7 (30.43)
	Blood	7/390 (1.79)	9/560 (1.6)	7 (24.14)	9 (39.13)
	Wound	5/240 (2.08)	4/240 (1.7)	5 (17.24)	4 (17.39)
	Resp	5/290 (1.72)	3/193 (1.6)	5 (17.24)	3 (13.04)

Table 4:- Demographics, Hospital Ward and Specimen Source types from Patients with CRS-Bacteria and CIR-Bacteria Infections in RMCH (2013 to 2015).

ICU, intensive care unit; ER, Emergency room; clinic, Outpatient clinic. Resp., respiratory.

C. Antimicrobial Susceptibilities

➢ *CRS-E. coli and CRS-K. pneumonia*

Antimicrobial susceptibilities among CRS isolates are summarized in Table 5. Among CRS isolates, resistance rates were relatively high for cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole. MDR was seen in 21/40 (52.5%) and 5/12 (41.7%) of CRS-EC and CRS-KP isolates, respectively. The most active agents against CRS- EC were tigecycline (100% susceptible), amikacin (95% susceptible), and colistin (97.5% susceptible), and the carbapenems. Piperacillin-tazobactam remained relatively active (77.5% susceptible). The most active agents against CRS-KP were amikacin (91.7% susceptible), gentamicin (83.3% susceptible), and colistin (100% susceptible). Tigecycline was less active against CRS-KP with only 66.7% remaining susceptible. Carbapenems were still highly active.

	CRS-Bacteria (*n* = 29)				
Drug*	**MIC_{50}**	**MIC_{90}**	**% Susceptible**	**% Intermediate**	**% Resistant**
Cefazolin	64	>128	0	0	100
Ceftazidime	16	>32	20.7	3.4	75.9
Ceftriaxone	32	>64	13.8	0	86.2
Cefepime[a]	4	>32	51.7	6.9	41.4
Cefoxitin	32	>32	24.1	13.8	62.1
Doripenem	≤0.12	≤0.12	100	0	0
Ertapenem	0.12	0.25	100	0	0
Meropenem	≤0.12	≤0.12	100	0	0
Imipenem	≤0.12	≤0.12	100	0	0
AMC	16	>32	44.9	31	24.1
TZP	8	16	79.3	17.2	3.4
Ciprofloxacin	8	>16	31.03	10.3	58.6
Levofloxacin[b]	16	>32	24.1	0	75.9
SXT	0.5	>8	55.2	0	44.8
Tigecycline	0.5	1	100	0	0
Amikacin	2	8	93.1	3.4	3.4
Gentamicin	1	>32	58.6	0	41.4
Colistin	0.5	1	96.6	0	3.4

Table 5:- Antimicrobial Susceptibilities of CRS-Bacteria from Hospitals (2013-2015).

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	CIR-Bacteria (*n* = 23)				
Drug*	**MIC_{50}**	**MIC_{90}**	**% Susceptible**	**% Intermediate**	**% Resistant**
Cefazolin	>128	>128	0	0	100
Ceftazidime	>32	>32	0	8.7	91.3
Ceftriaxone	>64	>64	13.04	0	87
Cefepime[a]	32	>32	34.8	8.7	56.5
Cefoxitin	32	>32	0	30.4	69.6
Doripenem	≤0.12	0.5	91.3	0	8.7
Ertapenem	1	2	0	39.1	60.9
Meropenem	≤0.12	1	100	0	0
Imipenem	≤0.12	1	91.3	0	8.7
AMC	16	32	21.7	21.7	56.5
TZP	32	64	47.8	34.8	17.4
Ciprofloxacin	>16	>16	17.4	8.7	73.9
Levofloxacin[b]	16	>32	26.1	0	73.9
SXT	2	>8	56.5	0	43.5
Tigecycline	1	4	95.7	4.3	0
Amikacin	8	16	82.6	13.04	4.3
Gentamicin	16	>32	34.8	8.7	56.5
Colistin	0.5	0.5	91.3	0	8.7

Table 6:- Antimicrobial susceptibilities of CIR-Bacteria from hospitals (2012-2015).

	CRS-EC (*n* = 9)					CRS-KP (*n* = 3)				
Drug*	**MIC_{50}**	**MIC_{90}**	**% Susceptible**	**% Intermediate**	**% Resistant**	**MIC_{50}**	**MIC_{90}**	**% Susceptible**	**% Intermediate**	**% Resistant**
Cefazolin	>128	>128	0	0	100	64	>128	0	33.3	66.7
Ceftazidime	32	>32	22.2	0	77.8	16	>32	33.3	0	66.7
Ceftriaxone	32	>64	11.1	0	88.9	16	>64	66.7	0	33.3
Cefepime[a]	4	>32	33.3	11.1	55.6	8	32	66.7	0	33.3
Cefoxitin	32	>32	33.3	11.1	55.6	8	>32	66.7	0	33.3
Doripenem	≤0.12	≤0.12	100	0	0	≤0.12	≤0.12	100	0	0
Ertapenem	0.12	0.25	100	0	0	0.25	0.25	100	0	0
Meropenem	≤0.12	≤0.12	100	0	0	≤0.12	0.25	100	0	0
Imipenem	≤0.12	≤0.12	100	0	0	≤0.12	0.25	100	0	0
AMC	16	>32	100	0	0	8	32	66.7	0	33.3
TZP	8	64	66.7	22.2	11.1	16	32	66.7	0	33.3
Ciprofloxacin	>16	>16	22.2	0	77.8	8	>16	33.3	0	66.7
Levofloxacin[b]	16	>32	33.3	0	66.7	8	>32	33.3	0	66.7
SXT	0.5	>8	77.8	0	22.2	4	>8	33.3	0	66.7
Tigecycline	0.5	1	100	0	0	1	8	33.3	33.3	33.3
Amikacin	2	8	77.8	11.1	11.1	1	16	100	0	0
Gentamicin	1	>32	55.6	0	44.4	0.5	>32	66.7	0	33.3
Colistin	0.5	1	88.9	0	11.1	0.5	1	100	0	0

Table 7:- Antimicrobial susceptibilities of CRS-EC and CRS-KP from hospitals (2013-2015).

*Drug concentrations in µg/ml; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; SXT, trimethoprim-sulfamethoxazole. [a]Cefepime *n*=34 for CRS-EC; *n*=7 for CRS-KP [b]Levofloxacin *n*=34 for CRS-EC; *n*=7 for CRS-KP

➢ *CIR-E. coli and CIR-K. pneumonia*

Antimicrobial susceptibilities among CIR isolates are summarized in Table 14. CIR isolates are those that are intermediate or resistant to ertapenem. MDR was seen in 11/17 (64.7%) and 9/11 (81.8%) of CIR-EC and CIR-KP, respectively. Doripenem and meropenem still remained active with 100% and 90.9% of CIR-EC and CIR-KP isolates still susceptible. Resistance to third generation cephalosporins was very high at >90% of isolates being resistant. The most active agents against CIR-EC were tigecycline (94.1% susceptible), amikacin (82.4%), and colistin (100% susceptible). The most active agents against CIR-KP were amikacin and colistin, both with 90.9% of isolates still remaining susceptible.

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	CIR-EC (*n* = 4)					CIR-KP (*n* = 5)				
Drug*	**MIC_{50}**	**MIC_{90}**	**% Susceptible**	**% Intermediate**	**% Resistant**	**MIC_{50}**	**MIC_{90}**	**% Susceptible**	**% Intermediate**	**% Resistant**
Cefazolin	>128	>128	0	0	100	>128	>128	0	0	100
Ceftazidime	>32	>32	0	25	75	>32	>32	20	0	80
Ceftriaxone	>64	>64	0	0	100	>64	>64	20	0	80
Cefepime[a]	32	>32	25	25	50	32	>32	20	20	60
Cefoxitin	32	>32	0	25	75	8	>32	60	20	20
Doripenem	≤0.12	0.5	100	0	0	≤0.12	≤0.12	80	0	20
Ertapenem	1	2	0	25	75	0.5	1	0	60	40
Meropenem	≤0.12	1	100	0	0	≤0.12	0.25	80	0	20
Imipenem	≤0.12	1	100	0	0	≤0.12	0.25	80	0	20
AMC	32	>32	25	25	50	16	32	40	20	40
TZP	32	64	25	0	75	16	64	20	60	20
Ciprofloxacin	>16	>16	25	0	75	>16	>16	20	0	80
Levofloxacin[b]	16	>32	25	0	75	16	>32	20	0	80
SXT	2	>8	50	0	50	4	>8	40	0	60
Tigecycline	0.5	1	75	25	0	1	4	80	20	0
Amikacin	8	32	75	0	25	2	16	80	20	0
Gentamicin	2	>32	75	0	25	16	>32	20	20	60
Colistin	0.5	0.5	100	0	0	0.5	0.5	80	0	20

Table 8:- Antimicrobial susceptibilities of CIR-EC and CIR-KP from hospitals (2013-2015).

*Drug concentrations in µg/ml; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; SXT, trimethoprim-sulfamethoxazole. [a]Cefepime *n*=11 for CIR-EC; *n*=8 for CIR-KP [b]Levofloxacin *n*=11 for CIR-EC; *n*=8 for CIR-KP

D. PCR for Detecting NDM-1 Genes:

DNA isolated from the colony was completed by alkaline lysis technique. DNA amplification in thermal cycler. Detection of *bla* $_{NDM-1}$ gene : by the orthodox PCR analyze. NDM-1 precise primers F (5'-GGG CAG TCG CTT CCA ACG GT-3') and R (5'-GTA GTG CTC AGT GTC GGC AT-3') (synthesised at NCDC, Delhi, India). A 478 bp section of NDM -1 gene was amplified. The PCR product was investigated in 2 % agarose gel in 1x TAE buffer at 120 V for 30 min and was visualized with ethidium bromide under a gel documentation method. (Biometra GmbH, Goettingen, Germany).

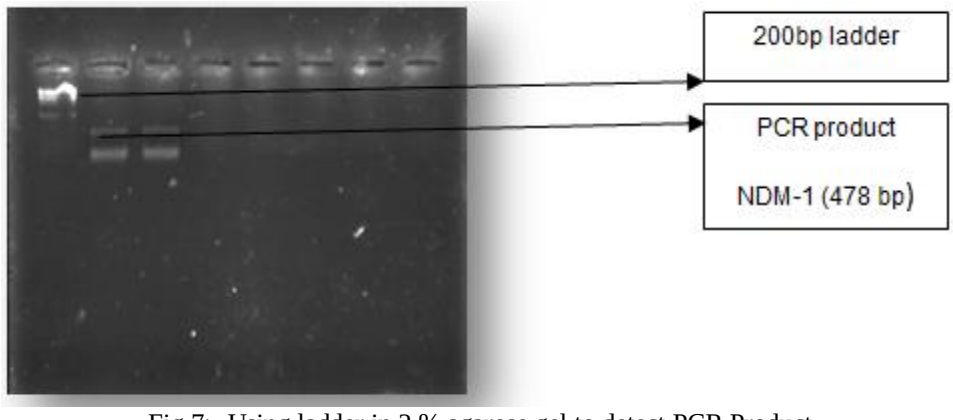

Fig 7:- Using ladder in 2 % agarose gel to detect PCR Product

IV. DISCUSSION

The emersion of carbapenemase-conducting enterobacteriaceae had been stated in numerous researches globally (Mohamed *et al.,* 2013; Duin *et al.,* 2013; 2011; Poirel *et al.,* 2011). The current research revealed the emergence of bla_{NDM-1} co-producer between enterobacteriaceae organisms. It was similarly witnessed that there was loss of porin OMPK36 in enterobacteriaceae organisms developing to the carbapenem resistance. PCR analysis demonstrated the existence of Metallo-β-Lactamase-encoding genes for example bla_{NDM-1} and bla_{OXA-48} in both the OMPK36 deficient and non-deficient enterobacteriaceae organisms. Out of the 456 *Klebsiella spp*, 2.4% (11/456) had an imipenem MIC of 0.12 or 0.25 µg/ml and were categorized as carbapenem reduced

susceptible (CRS); 2.2% (10/456) had an imipenem MIC ≥0.5 µg/ml and were categorized carbapenem intermediate/resistance (CIR). Out of the 317 *E.coli*, 2.8% (9/317) were categorized as CRS; 2.5% (8/317) were categorized as CIR. When looking at the increase from 2012 to 2014 of CRS and CIR isolates, no observable trend was present when the two cohorts were separated. However there was a doubling in prevalence for *E. coli* (2012: 1%, 2013: 1.2%, 2014: 0.5%, and 2015: 0.9%; P value=NS) when looking at all organisms with MICs ≥0.12 µg/ml. When looking at all *K. pneumoniae* organisms with MICs ≥0.12 µg/ml, the prevalence from year to year was relatively stable (2012: 1.5%, 2013: 1.2%, 2014: 1%, and 2015: 0.9%; P value=NS). Numerous researches globally had described the occurrence of transconjugants bearing bla_{NDM-1} plasmids (Balm *et al.*, 2013).

The recent studies focus the occurrence of bla_{NDM-1} in the clinical organisms of enterobacteriaceae that was isolated in this research. It was also notable that bla_{NDM-1} was initiate to be the most dominant in this research, *K. pneumoniae* and *Pseudomonas spp* ranked first and fifth in terms of numbers of organisms submitted and made up 381 (83.6%) and 119 (68%) of all isolates, respectively. Moreover, conjugation researches verified that *Klebsiella spp*, *Enterobacter spp.* and *Esch. coli* harboured bla_{NDM-1} plasmids. The results of this study bring home the point that there was growing incidence of transmissible genes such as bla_{NDM-1} and bla_{OXA-48} between the enterobacteriaceae strains isolated from the experimental samples.

V. CONCLUSION

bla_{NDM-1} and bla_{OXA-48} seem to be a developing reason of carbapenem resistance in enterobacteriaceae. The results shows that bla_{NDM-1} is the most recurrent gene recognized in most of the organisms and for few organisms, resistance to carbapenems was also associated to the existence of bla_{OXA-48}.

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