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Prevalence of Carbapenemase Producing Genes among Carbapenem Resistant Enterobacteriaceae Isolated from Blood in a Tertiary Care Hospital, Kashmir

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A B S T R A C T

Carbapenemase producing carbapenem resistant *Enterobacteriacea* were almost non-existent till 1990s but nowadays they are routinely encountered in hospitals. KPC producing *Klebsiella pneumoniae* were the first to develop and occur commonly. Lately NDM producing *Enterobacteriaceae* have emerged. IMP and VIM genes in Carbapenem resistant *Enterobacteriaecae* have started to emerge but the prevalence is low.

Introduction

Carbapenems are a class of β lactams which are highly effective antibiotic agents and are commonly used for the treatment of severe bacterial infections. They have greatest potency against gram positive and gram negative bacteria.[1] Both gram positive and gram negative bacteria are becoming resistant to carbapenems.[2] This distressing pattern possess a major public health threat. Resistance in gram positive cocci is typically the result of acquisition of altered PBP. In gram negative rods production of β lactamases, porin loss, efflux pumps, alterations in PBP are all associated.[3]Combination of these mechanisms can cause high levels of resistance to carbapenems in certain bacterial species such as *Klebsiella pneumonia.* The mechanism that has been studied in details is the production of β lactamases.

β lactamases are enzymes that hydrolyse β lactams and hence render them ineffective. They are classified into four classes (A, B, C and D).Class A, B and D are carbapenemases. In class A the most problematic are KPC enzymes encountered in US and are increasingly seen elsewhere. Class B enzymes are metallo β lactamases requiring zinc ions for activity. Most common being NDM, VIM and less commonly IMP types. Among class D enzymes few are carbapenemases OXA 23, 40, 51.

Detection of carbapenemases can be done by several phenotypic methods like modified hodge test (MHT) and combined disc test (CDT). Recently molecular methods like PCR have been introduced for the detection of genes responsible for carbapenemase production. The multiplex PCR assays being the fastest and most sensitive.[4]

Materials and Methods

Aim

To detect the prevalence of VIM, IMP, KPC and NDM-1 gene in CRE by multiplex PCR and its correlation with phenotypic test like modified hodge test and combined disc test. Blood received for blood culture was processed by automated blood culture system. Further identification and susceptibility testing were done on the Vitek 2 system. All CRE isolates were included in this study. Susceptibility criteria are according to CLSI guidelines[5] All screen test positive isolates were subjected to combined disc test and modified hodge test. CDT was performed using imipenem, meropenem and ceftazidime disc alone and in combination with EDTA. The CDT was performed as described by Yong *et al.*,[6] In modified hodge test, the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (*E. coli* ATCC 25922) towards a carbapenem disc.

Multiplex PCR

DNA was extracted by boiling at 95°C for 20 min, and discarding the cellular debris by centrifugation. (12000 xg; 2 min at 4°C).Extracted DNA was used for PCR. The resulting PCR products were analyzed in a 1% agarose gel with ethidium bromide staining and UV light. The design of the primers for detection of *bla*KPC, *bla*NDM-1, *bla*VIM, *bla*IMP genes are

NDM 1 FP	**GCATAAGTCGCAATCCCCG**	**237**
NDM 1 RP	CTTCCTATCTCGACATGCCG	
VIM FP	GTTTGGTCGCATATCGCAAC	382
VIM RP	AATGCGCAGCACCAGGATAG	
IMP FP	GAAGGCGTTTATGTTCATAC	587
IMP RP	GTAAGTTTCAAGAGTGATGC	
KPC FP	TCGAACAGGACTTTGGCG	201
KPC RP	GGAACCAGCGCATTTTTGC	

Observations

The blood samples received over a period of one year from in-patients and out-patients were processed for isolation and identification of bacterial pathogens according to the standard microbiological techniques.

A total of 120 non duplicate *Enterobacteriaceae* were isolated from patients admitted or attending the OPD. Out of these 52 were CRE.40 (76.9%) were *Klebsiella peumoniae*, 12 (21.4%) were *Escherichia coli*. Out of these 40 carbapenem resistant *Klebsiella pneuomiae* isolates, 33(82.5%) were recovered from males and 7(17.5%) were recovered from female patients. Out of 12 meropenem resistant *Escherichia coli* isolates, 7 (58.3%) were recovered from males and 5 (41.6%) from female patients (Figure 1) These 52 CRE were subjected to phenotypic tests MHT and CDST. Multiplex Polymerase chain reaction was done for *bla*KPC gene, *bla*NDM gene,

*bla*IMP gene and *bla*VIM gene detection in 52 CRE isolates, 42(80.7%) isolates were found harbouring one or more than one gene. *bla*KPC alone was present in 38(73.0%) isolates, while as *bla*KPC with *bla*NDM was present in 1(1.9%) isolate and *bla*KPC with *bla*IMP was seen in 1(1.9%) isolate *bla*NDM alone in 2(3.8%) isoates, *bla*IMP and *bla*VIM alone in none(0%) of the isolates. However in 10(19.2%) isolates, none of the gene was detected.

Out of 40 KPC gene detected from PCR, 8 were isolated from *E. coli* and 32 from *Klebsiella*. Of these 8 KPC genes isolated from *E. coli,* 3 were positive by MHT and 6 were positive by CDT. Similarly out of 32 KPC genes isolated from *Klebsiella*, 22 were MHT positive and 27 were CDT positive. Out of 3 NDM genes detected by PCR, 2 were isolated from *E. coli* and 1 from *Klebsiella.* Of these 2NDM genes isolated from *E. coli*, none was MHT positive and 1 CDT positive. Similarly out of 1 NDM gene isolated from *Klebsiella,* 1 was MHT positive and none CDT positive. Only1 IMP gene was isolated from *Klebsiella*, and it was given positive both by MHT and CDT (Table 1, Figure 2, 3). Out of 40 *Klebsiella* isolates, 33 were PCR positive which included 31 KPC, 1KPC+IMPand I NDM alone. Of these 31 KPC genes, 18 were positive both by MHT and CDT. 3 were CDT positive and MHT negative. Further 8 were MHT positive and CDT negative. And 2 were missed by both the phenotypic tests. KPC+IMP detected was positive by both MHT and CDT, and NDM detected was MHT positive and CDT negative (Table 2). Out of 3 were PCR negative *E. coli* isolates, 1 was given negative results by both MHT and CDT while 2 were falsely given positive by CDT. Similarly out of 7 PCR negative *Klebsiella* isolates, 4 were falsely given positive by MHT and by CDT (Table 3).

Results and Discussion

Carbapenem resistant *Enterobacteriaceae* (CRE) are worldwide a public health concern. These multidrug-resistant organisms cause infections associated with high mortality and limited treatment options, and are increasingly recognized as an important cause of health care-associated infections.[7,8] Genes encoding carbapenemases are mostly plasmid –located and associated with various mobile genetic structures, such as transposons or intergrons.[9]Such a characteristic certainly accelerates inter/intra- species dissemination of carbapenemase genes. Other factors, being travel, long term hospitalization and frequent use of invasive medical devices have also led to rapid rise in carbepenems resistance.[10]

Reliable detection methods with rapidity, high sensitivity and specificity are required for combating the spread as it allows physicians to start proper anti-microbials. The preliminary screening for Carbapenamase producers in clinical specimens is based first on phenotypic tests, whereas confirmation tests are mainly based on molecular assay.

Out of these 120 isolates only 52(43.3%) were found to be carbapenem resistant, which includes 40(76.9%) *Klebsiella pueumoniae* isolates and 12(21.44%) *E. coli* isolates. None *Enterobacter cloacae* isolate was found resistant to meropenem. In our study it was found that out of 52 resistant *Enterobacteriaceae* isolates 40(76.9%) were isolated from male patients and 12(23%) from female patients. According to a study conducted by Namratha *et al.*, out of 100 *Klebsiella* isolates, 63 were from males and 37 were females with a male female ratio of 1.7:1.[11] KPC production was seen more in carbapenem resistant *Klebsiella* isolates i.e. 80% and comparatively less in carbapenem resistant *E. coli* isolates (66%). Among the KPC producers *E. coli* (n=8), MHT was

positive in (n=3)37.5% of *E. coli* isolates and CDT was positive in (n=6)75% of the isolates. It has been seen that out of 12 carbapenem resistant *E. coli* isolates, 9 were PCR positive, and 7 out of these 9 PCR positive isolates were carrying KPC gene, 1 was carrying both KPC and NDM gene and one was harbouring NDM gene alone. Out of these 7 KPC genes which were detected alone in *E. coli*, 2 were picked up by both MHT and CDT, While 3 were picked by CDT and not by MHT. Also one among them were not picked up by either of the phenotypic tests.

Our results are in accordance with the study conducted by Maryam AlTamimi *et al.*, where out of 26 carbapenem resistant *Escherichia coli* isolates only 4(15.3%) were MHT positive and 22(84.6%) were MHT negative.[13] Contrary to our results, Rachana Solanki *et al.*, showed that of 4 KPC producer *E.coli* isolates (n=3)75% were MHT positive and (n=1)25% was MHT negative. While CDT was positive in (n= 1) 25% isolates and (n=3)75% were CDT negative.[12]In our study among KPC producing *Klebsiella* isolates (n=32), MHT was positive in (n=27)84.3% of isolates and CDT was positive in (n=22)68.7% of the isolates. Out of 40 carbapenem resistant *Klebsiella* isolates33 were PCR positive and 31 among them were harbouring KPC gene.1 KPC+IMP and I NDM alone was detected. Further out of 31 isolates, 18 were picked by both the phenotypic tests, 3 were missed by MHT and picked by CDT. However 8 were missed by CDT and picked by MHT. In 2 of such isolates both CDT and MHT were negative. Similarly study by Rachana Solanki *et al.*, showed that of 5 KPC producer *Klebsiella* isolates, all were picked by MHT and none by CDT.[12] As CDT is more sensitive for MBLs than for class a enzymes which explains less sensitivity of CDT then MHT for KPC in *Klebsiella.* One of the isolate was harbouring KPC and IMP which has been detected both by MHT and CDT, and in one of the isolate only NDM was present which has been detected by MHT and not by CDT. Contrary to our results, a study by Maryam AlTamimi *et al.*, showed MHT is less reliable to detect NDMs, VIMs, and IMPs producing bacteria.[13] This however can be explained because in our study IMP was isolated with KPC.88In our study NDM production was seen more in carbapenem resistant *E. coli* isolates (n=2) 66.6% than in carbapenem resistant *Klebsiella* isolates (n=1)33.3%. MHT could not detect this gene in *E. coli* isolates, however CDT could pick it in one of the isolate (50%).In one of the isolates KPC was co-existing with NDM which was picked by CDT and not by MHT. One NDM gene was also detected in *E. coli* which has been missed by both CDT and MHT. In this study, there was one NDM producer *Klebsiella* which was picked up by MHT and not by CDT. In a study by Maryam AlTamimi *et al.*, showed that MHT failed to detect one isolate of *K. pneumonia* which was PCR positive for NDM gene.[13]

Table.1 Correlation of multiplex PCR with MHT and CDT among carbapenemase producing isolates

	Total KPC(40)			**Total NDM(3)**			**Total IMP(1)**		
	KPC +ve	**MHT**	**CDT**	**NDM +ve**	**MHT**	**CDT**	**IMP +ve**	**MHT**	**CDT**
E. coli	8	3	6	2	0	1	0	0	0
Klebsiella	32	22	27	1	1	0	1	1	1

Table.2 Results of Modified Hodge test, combined disc test and genotypic test for KPC, NDM and IMP detection

Organism	Total	PCR +ve	MHT+ve, CDT+ve, PCR+ve				MHT-ve, CDT+ve, PCR+ve				MHT+ve, CDT-ve, PCR+ve				MHT-ve, CDT-ve, PCR +ve			
			KPC	NDM	KPC + NDM	KPC + IMP	KPC	NDM	KPC + NDM	KPC + IMP	KPC	NDM	KPC + NDM	KPC + IMP	KPC	NDM	KPC + NDM	KPC + IMP
E. coli	12	9	2	-	-	-	3	-	1	-	1	-	-	-	1	1	-	-
Klebsiella	40	33	18	-	-	1	3	-	-	-	8	1	-	-	2	-	-	-

Table.3 Comparison of Modified Hodge test and combined disc test for PCR negative isolates

Organism	Total	PCR - ve	MHT -ve CDT -ve PCR-ve	MHT +ve CDT –ve PCR -ve	MHT –ve CDT +ve PCR-ve	MHT +ve CDT +ve PCR-ve
E.coli	12	3	1	0	2	0
Klebsiella	40	7	0	4	3	0

Fig.1 Sex wise distribution of CRE isolates

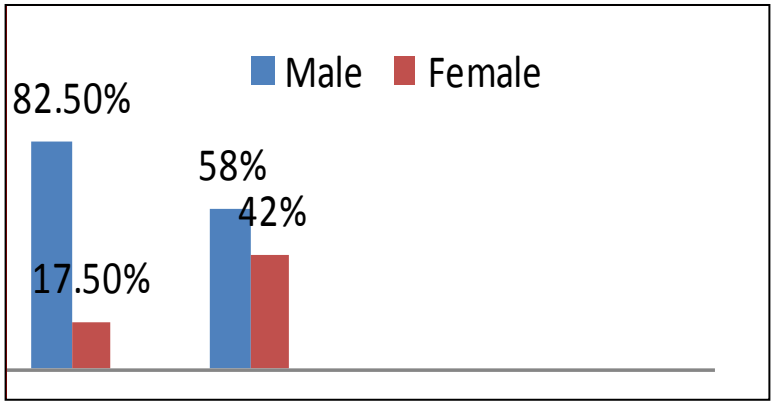

Fig.2 Distribution of *bla*KPC, *bla*NDM, *bla*IMP in *E. coli* and *Klebsiella* isolates

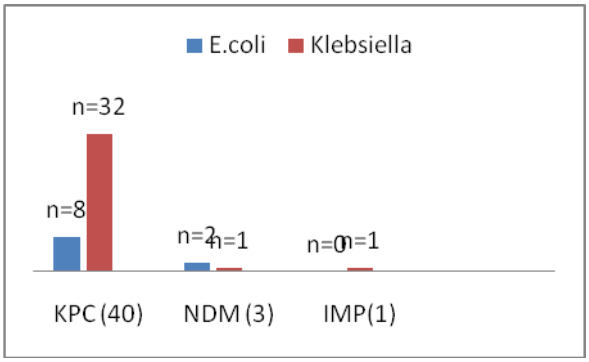

Fig.3 Comparison between MHT, CDT and PCR among *E. coli* and *Klebsiella* isolates

Our results are in accordance with the study done by DelphineGirlich *et al.*, who found that out of 14 NDM detected MHT could detect only 7(50%).54In this study out of 10 PCR negative isolates, 1 was negative by both MHT and CDT, while 4 were falsely given positive by MHT. Further 5 among them were falsely given positive by CDT.[14]

It has been concluded, while determining the prevalence of various carbapenemase producing genes, the most prevalent gene being *bla*KPC followed by *bla*NDM and *bla*IMP, while no *bla*VIM could be detected *bla*KPC were isolate more from *Klebsiella pneumonia* and combined disc test was more sensitive than modified hodge test *bla*NDM were isolated more from *Escherichia coli* and both the phenotypic test test were equally sensitive.

References

1. Bardley, J.S., *et al.*, 1999.Carbapenems in clinical practice: a guide to their use in serious infection. Int. J. Antimicrob. Agents 11:93-100.
2. Chouchani, C., Marrakchi R., EL., Salabi, A., 2011. Evolution of b-lactams resistance in gram-negative bacteria in Tunisia. Crit.Rev.Microbiol. 37:167-177.
3. Nordmann, P., *et al.*, 2011.Comparative activity of carbapenem testing: the compact study. J. Antimicrob. Chemother 66: 1070-1078.
4.Tripathi, KD., editor. In: Essentials of Medical Microbiology. 5th edition. Jaypee 2003; 627-40.
5. Wayne, MZ., PA., Performance Standards for antimicrobial susceptibility testing. Clinical and Lab standards Institute

[CLSI].2014.

6. Young, D., Lee, K., Yun JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo β lactamase producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. Journal of clinical Microbiology. 2002; 40(10): 3798-37801.
7. Nordmann, P., Cuzon .G, Naas T. The real threat of *Klebsiella pneumaniae* Carbapenemases [KPC] resistance. Lancet Infectious Disease 2009; 9: 228-236.
8. Gupta, N., Limbago, BM., Patel, JB., Carbapenem resistant Enterobacteriaceae: Epidemiology and prevention. Clin. Infect. Dis. 2011; 53: 60-67.
9. Cuzon, G., Nass, T., Nordmannn, P., Functional characterization of Tn4401, aTn3 based transposon involved in bla KPC gene mobilization. Antimicrob Agents Chemther. 2011; 55: 5370-73.
10. Castanheria, M., Deshpande, LM., Mathai D *et al.*, Early dissemination of NDM-1 and OXA-181 producing Enterobacteriaceae in Indian hospitals: a report from SENTRY antimicrobial surveillance Program. 2006-2007.
11. Namratha, KG., Sreshma, P., Subbannayya, K., Dinesh PV, Champa H. Characterization and Antibiogram of *Klebsiella* spp. Isolated from clinical specimens in a Rural teaching Hospital. Sch. J. App Med Sci. 2015; 3: 878-883.
12. Nicolas Kieffer, Patrice Nordmann, Marta Aires-de-Sousa, Laurent Poirel. High Prevalence of Carbapenemase-Producing Enterobacteriaceae among Hospitalized Children in Luanda, Angola. Antimicrobial Agents and Chemotherapy.2016; 60.
13. Maryam AlTamimi, *et al.*, Comparison of phenotypic and PCR methods for detection of carbapenemases production by Enterobacteriaceae. Saudi J. Biological Sciencs.2016; 24:155-161.
14. Girlich, D., Poirel L., Nordmann, P., Detection of Carbapenemase producers in Enterobacteriaceae by use of novel screening medium. Journal of Clinical Microbiology. 2012; 50:2761-2766.

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