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Original Article

Microbiology Section

Molecular Profile of Emerging Multidrug Resistant *Klebsiella pneumoniae* Clinical Isolates from Southern India

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ABSTRACT

Introduction: Multidrug Resistance (MDR) in *Klebsiella pneumoniae* isolates is an increasingly recognised threat to hospital infection control. It is known to produce a wide array of cephalosporinase and carbapenemase enzymes.

Aim: This study was done to determine the prevalence of MDR in *K. pneumoniae* with phenotypic and genotypic characterisation of Extended Spectrum Beta-Lactamase (ESBL), AmpC and carbapenemase mediated resistance mechanisms.

Materials and Methods: Out of 562 *K. pneumoniae* isolates recovered during November 2014 to June 2015 in our tertiary care hospital in Pondicherry, 117 MDR strains were phenotypically analysed for presence of various types of beta lactamases and carbapenemases by ceftazidime-clavulanic acid combined disc test, AmpC disc test, Modified Hodge's test and meropenem-EDTA combined disc test. These isolates were further screened for ESBL (*bla*CTX-M, *bla*SHV-1, *bla*TEM) and Carbapenemase genes (*bla*NDM-1, *bla*IMP-1, *bla*VIM-2, *bla*SIM-1 and *bla*KPC) by multiplex PCR.

Results: Prevalence of MDR strains of *K. pneumoniae* was 20.8%. Out of 117 MDR *K. pneumoniae*, ESBL, AmpC and MBL mediated resistance was identified by phenotypic method in 91, 27 and 16 isolates respectively. Among the ESBL and MBL genes, *bla*CTX-M (60.6%), *bla*SHV-1 (69%), *bla*NDM-1 (33%) and *bla*IMP-1 (9%) genes were detected. Co-production of multiple enzymes was observed in 32% isolates.

Conclusion: Beta-lactam hydrolysing enzymes are prevalent among MDR *K. pneumoniae* stains in our region. Co-expression of ESBL and MBL genes are found in a large proportion of clinical isolates of *K. pneumoniae*.

Keywords: Extended spectrum beta-lactamase gene, Metallo beta-lactamase gene, Molecular characterisation

INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae*) is one of the commonest isolate in both hospital and community acquired infections. This non-motile member of the Enterobacteriaceae family is Voges-Proskauer test positive, urease positive and produces enormous amounts of capsular polysaccharides besides other virulence factors viz., cell wall lipopolysaccharide, fimbriae, siderophores. It is conventionally known to clinicians as the aetiological agent for Friedlander's pneumonia, a fulminant form of community-acquired pneumonia which is more common among chronic alcoholic and diabetic persons [1]. It is also associated with a number of diverse pyogenic infections, such as, urinary tract infection, septicaemia, endocarditis, wound infection and nosocomial infections. Hypermucoviscous strains of *K. pneumoniae* have been described which were reported to cause complications like liver abscess and metastatic infections [1]. These infections are increasingly becoming intractable and resistant to antimicrobial therapy due to emergence of resistance to commonly used antibiotics. While penicillins and cephalosporins are generally used for regular treatment, carbapenems, polymyxin B and colistin are reserved for multi-resistant gram negative infections [2]. Multidrug resistant (MDR) and carbapenem resistant *K. pneumoniae* has become a major therapeutic challenging scenario in several countries due to the lack of alternative existing antibiotics [2]. Furthermore, there are lack of new antibiotics in the process of development [3]. MDR *K. pneumoniae* is predominantly due to production of beta-lactam hydrolysing enzymes like ESBL, AmpC, Metallo Beta-Lactamase (MBL) and *K. Pneumoniae* Carbapenemase (KPC). Mobile genetic elements like plasmids are the usual location of ESBLs and several MBL genes. As a result these genes are increasingly becoming widespread due to extensive horizontal dissemination among gram negative bacterial population. In addition, significant air travel and human traffic in recent times have been a key factor for the global spread of these resistant strains [4]. As per current estimates, 70-90% Enterobacteriaceae in India are ESBL producer [2]. A lesser prevalence is noted in United States of America and Middle East countries [5,6]. At the same time, there are increasing occurrence of carbapenems and colistin resistance [7]. This leads to prolonged hospital stay and increased expenditure. Currently, there are both phenotypic tests and genotypic tests for detection of ESBL and MBL. Since hospital infection control cannot be effective without a close monitoring of these resistant strains, it is obligatory to detect these resistant genes and characterise MDR *K. pneumoniae* isolates by molecular methods. The objective of this study was to estimate the prevalence of MDR in *K. pneumoniae* and to characterise the ESBL, AmpC and carbapenemase mediated resistance mechanisms by both phenotypic and genotypic methods.

MATERIALS AND METHODS

A prospective analytical study was carried out over a period of 8 months (November 2014 to June 2015) in our tertiary care hospital in Pondicherry after obtaining the Institutional Ethical Committee (IEC) clearance (Registration number: Faculty/2014/19). All non-duplicate isolates of *K. pneumoniae* recovered from various clinical samples received in microbiology department for bacterial culture during the study period were included in this study. In case of recovery of more than one isolates of *K. pneumoniae* from the same patients having identical antibiogram, the first isolate was included and further isolates were considered as duplicate and were excluded from our study. Samples were collected and processed as per standard operative procedures. These isolates were subjected to antibiotic susceptibility test against a Gram negative antibiotic panel comprising of cotrimoxazole (1.25/23.75 µg), cefoxitin disk (30 µg), cefotaxime (30 µg), cefepime-tazobactam (30/10 µg),

ciprofloxacin (5 µg), gentamycin (10 µg), amikacin (30 µg), imipenem (10 µg), meropenem (10 µg), piperacillin-tazobactam (100/10 µg), cefoperazone-sulbactam (75/30 µg). Antibiotic susceptibility was determined by Kirby-Bauer disc diffusion method. *E. coli* ATCC 25922 was used for quality control as per CLSI 2015 guidelines [8].

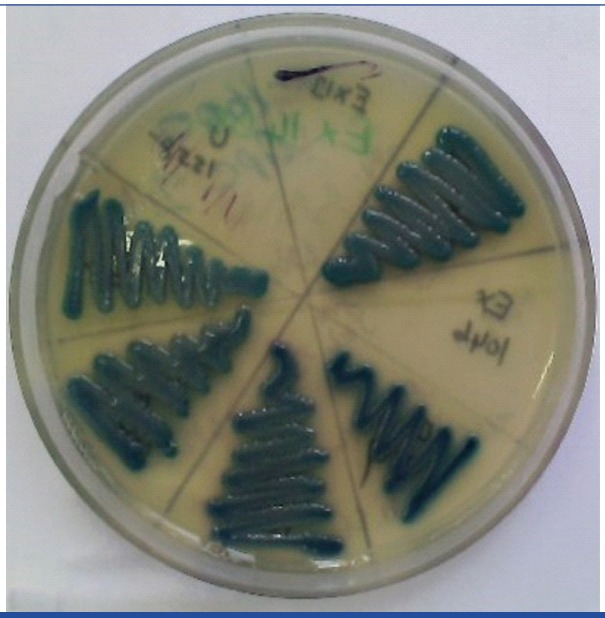
ESBL Detection by Phenotypic Method

K. pneumoniae strains showing cefotaxime zone ≤27 mm were considered as ESBL producers and were confirmed by combined disc test using ceftazidime (30 µg) and ceftazidime with clavulanic acid (30/10 µg) discs [8].

Lawn culture of the test organism with a bacterial suspension of 0.5 McFarland was made on a Mueller Hinton agar plate. The Ceftazidime (CAZ) disc and ceftazidime with clavulanic acid (CAC) discs were placed 25 mm apart on the Mueller Hinton agar plate. The plates were aerobically incubated at 37°C. Isolates showing an increase ≥5 mm in a zone diameter in CAC versus CAZ alone were considered to be ESBL producers. *K. pneumoniae* ATCC 700603 (positive control) and *E. coli* ATCC 25922 (negative control) were used for quality control [Table/Fig-1].

[Table/Fig-1]: ESBL combined disc test using ceftazidime and ceftazidime with clavulanic acid.

ESBL production was further compared phenotypically by HiCrome ESBL Agar (Himedia, Mumbai, India). ESBL producing *K. pneumoniae* strains had luxuriant growth with bluish green colonies [Table/Fig-2].

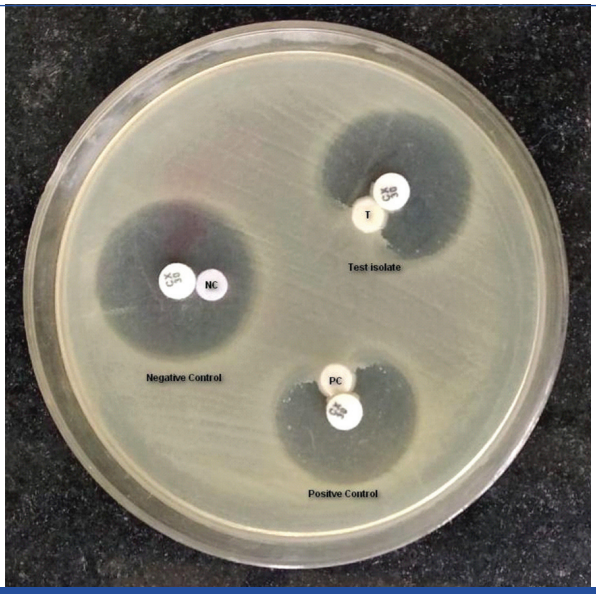


[Table/Fig-2]: HiCrome ESBL agar showing bluish green colonies.

AmpC Detection by Phenotypic Method

An isolate was considered as AmpC beta lactamase producer if it was cefoxitin resistant (zone ≤14 mm) and showed positive AmpC disc test [9,10].

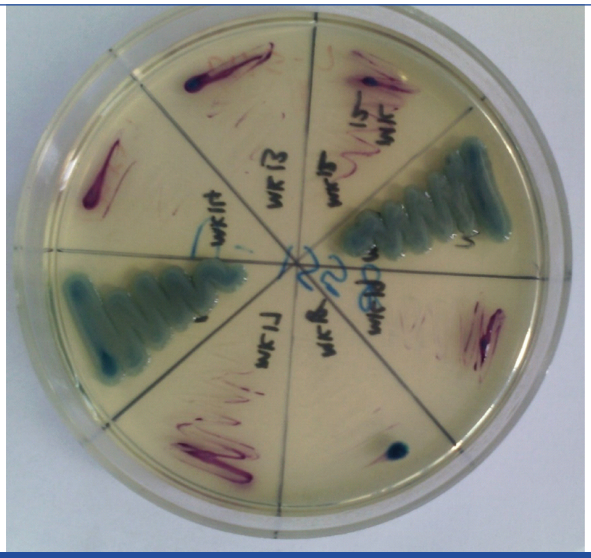
For AmpC disc test, filter paper discs containing Tris-EDTA (20 µL of a 1:1 mixture of saline and 100× Tris-EDTA) were prepared and stored. Lawn of *E. coli* ATCC 25922 on Mueller-Hinton agar plate was made. A cefoxitin disk (30 µg) was placed on Mueller-Hinton agar. An AmpC disk is moistened with 20 µL of saline, smeared with test isolate colonies and kept close to the cefoxitin disk on Mueller-Hinton agar. We also included AmpC discs with known AmpC positive and negative laboratory isolates as Positive Control (PC) and Negative Control (NC) respectively. An indented inhibition zone after overnight incubation is indicative of AmpC beta lactamase production [Table/Fig-3].



[Table/Fig-3]: AmpC disc test showing indented zone of inhibition of cefoxitin disc (CX) near the Test isolate (T) and Positive Control (PC) discs. Inhibition zone near Negative Control (NC) disc shows no indentation.

Phenotypic Tests for Carbapenemase Detection

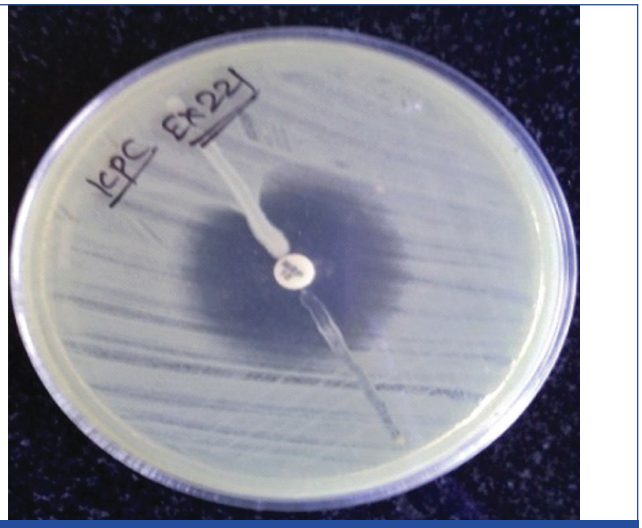
Isolates showing resistance to one or more carbapenems i.e., imipenem and/or meropenem were subjected to Modified Hodge test (MHT), meropenem-EDTA combined disc test and culture on HiCrome KPC Agar (Himedia, Mumbai, India) to differentiate various carbapenemase producers phenotypically. Isolates that grew were considered as carbapenemase producers. The KPC producing *K. pneumoniae* had luxuriant growth with bluish green colonies [Table/Fig-4].



[Table/Fig-4]: HiCrome KPC agar showing bluish green colonies.

Modified Hodge Test

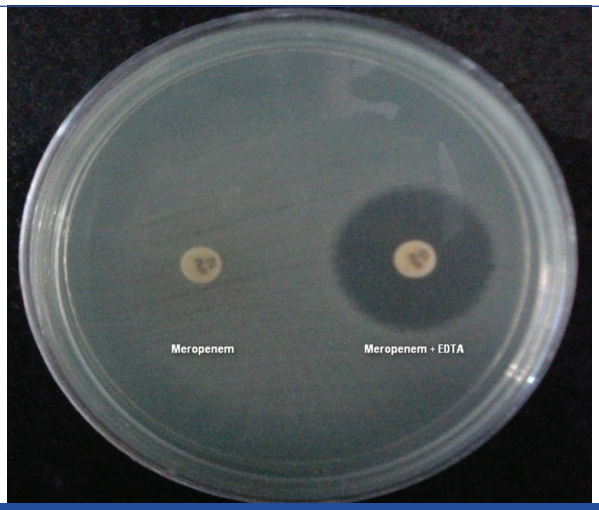
K. pneumoniae strains considered to produce carbapenemase if they were positive in MHT. The test was carried out as per CLSI 2015 guidelines [8]. A lawn culture of imipenem sensitive *E. coli* ATCC 25922 was made on a Mueller Hinton agar plate. An imipenem disk (10 µg) was placed in the centre. *K. pneumoniae* isolates were streaked as straight lines from the centre to the periphery of the plate. After overnight incubation, a clover leaf-like distorted zone of inhibition of the imipenem disc produced by a test isolate was interpreted as a positive result [Table/Fig-5].



[Table/Fig-5]: Modified hodge test.

Meropenem-EDTA Combined Disc Test

A lawn culture of the test organism with a bacterial suspension of 0.5 McFarland was made on a Mueller Hinton agar plate [11,12]. A meropenem disc (10 µg) and another meropenem disc with 10 µL of 0.1 M EDTA were placed 25 mm apart on the Mueller Hinton agar plate. The plates were aerobically incubated at 37°C. Isolates showing an increase ≥5 mm in a zone diameter in meropenem-EDTA versus meropenem alone were considered to be MBL producers [Table/Fig-6].



[Table/Fig-6]: Combined disc test using meropenem and meropenem-EDTA.

Molecular Methods

All cefotaxime resistant 117 *K. pneumoniae* isolates were screened for *bla*CTX-M, *bla*SHV-1, *bla*TEM genes by multiplex PCR with specific primers as described by Kiratisin P et al., and Abujnah AA et al., [13,14]. Carbapenem resistant 33 strains were tested for *bla*NDM-1, *bla*IMP-1, *bla*VIM-2, *bla*SIM, and *bla*KPC genes using specific primers as described by Kazi M et al, and Saranathan R et al., [15,16]. The forward and reverse primers used for PCR are given in [Table/Fig-7]. PCR condition followed were 95°C for 5 minutes, 30 cycles with 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 90 seconds and final extension at 72°C for 7 minutes. Amplicons were identified by electrophoresis of PCR products on 1% agarose gel.

Beta lactamase genes	Forward and reverse primers	Sequence (5' to 3')	Base pair (bp) size	Reference
*bla*CTX-M	F	TCTTCCAGAATAAGGAATCCC	909 bp	Kiratisin P et al., [13]
	R	CCGTTTCCGCTATTACAAAC		
*bla*SHV-1	F	TGGTTATGCGTTATATTCGCC	868 bp	Kiratisin P et al., [13]
	R	GGTTAGCGTTGCCAGTGCT		
*bla*TEM	F	CAGCGGTAAGATCCTTGAGA	643 bp	Abujnah AA et al., [14]
	R	ACTCCCCGTCGTGTAGATAA		
*bla*NDM-1	F	GTAGTGCTCAGTGTCGGCA	475 bp	Kazi M et al., [15]
	R	GGGCAGTCGCTTCCAACGGT		
*bla*IMP-1	F	CTACCGCAGCAGAGTCTTTGC	640 bp	Saranathan R et al., [16]
	R	GAACAACCAGTTTTGCCTTACC		
*bla*VIM-2	F	ATGTTCAAACTTTTTGAGTAGTAAG	801 bp	Saranathan R et al., [16]
	R	CTACTAACGACTGAGCG		
*bla*SIM-1	F	TTGCGGAAGAAGCCCAGC	681 bp	Saranathan R et al., [16]
	R	GCGGCGGTTTTGATTTGC		
*bla*KPC	F	SACCRCSTCGCRGSACCSRT	275 bp	Kazi M et al., [15]
	R	SCCRSCASGCCSGRTRTCS		

[Table/Fig-7]: Primers used for PCR [13-16].

RESULTS

In our study, the prevalence of MDR *K. pneumoniae* was 20.8%. Out of a total of 562 non-duplicate *K. pneumoniae* strains isolated during study period, 117 isolates were found to be MDR having resistance to three or more classes of antibiotics and were included for further characterisation. Most of these MDR isolates were recovered from respiratory (n=45) and pus/exudate samples (n=40), followed by urine (n=19) and blood (n=13) samples. Gender-wise distribution of our samples showed, the majority of our MDR isolates were from male patients with age group of 26 to 45 (76%) followed by females.

We performed antibiotic susceptibility test against a panel of Gram negative antibiotics by disc diffusion test. Among the non-beta-lactam agents, highest resistance was found against co-trimoxazole (82%, n=97) and ciprofloxacin (75%, n=88), followed by gentamycin (56%, n=66) and amikacin (46%, n=54) [Table/Fig-8].

Antibiotics	Sensitive	Resistant	Intermediate
Cotrimoxazole	9% (10)	82% (97)	9% (10)
Gentamycin	23% (27)	56% (66)	21% (24)
Amikacin	32% (37)	46% (54)	22% (26)
Ciprofloxacin	21% (24)	75% (88)	4% (5)
Imipenem	72% (84)	28% (33)	0% (0)
Meropenem	77% (90)	23% (27)	0%
Cefoxitin	31% (36)	63% (74)	6% (7)
Cefotaxime	0%	100% (117)	0%
Cefoperazone-sulbactam	69% (81)	25% (29)	6% (7)
Cefepime-tazobactam	78% (91)	20% (23)	2% (3)
Piperacillin-tazobactam	35% (41)	45% (53)	20% (23)

[Table/Fig-8]: Antibiotic susceptibility pattern of MDR *K. pneumoniae*.

All 117 MDR *K. pneumoniae* isolates were cefotaxime resistant. Out of these isolates, 91 isolates were ESBL positive by ceftazidime-clavulanic acid combined disc method and 95 isolates by HiCrome ESBL agar. Amp C screening was positive in 74 isolates which showed cefoxitin resistance. Of them, 27 isolates (23%) were identified as AmpC beta lactamase producer Amp C by disc test.

A total of 33 isolates resistant to one or more carbapenems were tested for MBL and KPC phenotypically and genotypically. Out of the 33 isolates, 16 were positive in Meropenem-EDTA combined disc test, 17 were Modified Hodge test positive and 18 isolates were identified as carbapenemase producer by HiCrome KPC agar [Table/Fig-9].

Resistance mechanism	Phenotypic methods	Number of Isolates (%)
ESBL	ESBL combined disc test	91 (77.7%)
	HiCrome ESBL agar	95 (81%)
AmpC beta-lactamase	AmpC disc test	27 (23%)
Carbapenemase	MBL combined disc test	16 (48%)
	Modified Hodge test	17 (51.5%)
	HiCrome KPC agar	18 (54.5%)

[Table/Fig-9]: Resistance detected by phenotypic methods.

The results of molecular tests are outlined in [Table/Fig-10]. Two ESBL genes i.e., *bla*SHV-1 and *bla*CTX-M were identified in 81 and 71 isolates respectively. Whereas, *bla*NDM-1 (n=11) and *bla*IMP-1 (n=3) were the carbapenemase genes found among our 33 carbapenem resistant isolates.

Resistance mechanism	Genes detected	Number of Isolates (%)
ESBL	*bla*CTX-M	71 (60.6%)
	*bla*SHV-1	81 (69%)
	*bla*TEM	0 (0%)
Carbapenemase	*bla*NDM-1	11 (33%)
	*bla*IMP-1	3 (9%)
	*bla*VIM-2	0 (0%)
	*bla*SIM-1	0 (0%)
	*bla*KPC	0 (0%)

[Table/Fig-10]: Resistance genes detected by PCR.

DISCUSSION

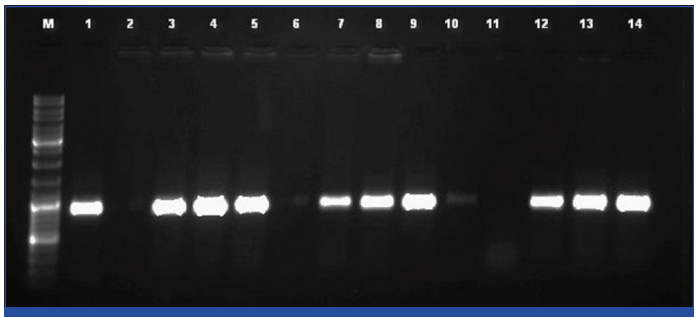
K. pneumoniae is notorious for its drug resistance. While MDR is a more common scenario, extensively drug-resistant and pan-drug-resistant clones of *K. pneumoniae* have also been reported [17,18]. Isolates displaying resistance to three or more categories of antibiotics are considered as MDR [19]. In this study, all 117 MDR *K. pneumoniae* isolates were cefotaxime resistant. Among these isolates, positive results obtained in HiCrome ESBL agar (81%) was higher than that of ceftazidime-clavulanic acid combined disc test (77.7%) [Table/Fig-9]. This higher positivity of ESBL in chromogenic media in comparison to combined disc test was also noted in previous studies. Kałużna E et al., found 92.9% and 95.2% positive results using CHROM agar ESBL (GRASO) and ChromID ESBL (bioMérieux) respectively which was higher than double-disc synergy test (47.6%), combined disc test (40.5%), E-test ESBL (26.2%) and VITEK 2 (35.7%) [20]. Overdevest ITMA et al., also reported lower specificity of chromogenic ESBL detection media indicating the chance of obtaining false-positive results [21].

In the present study, AmpC production was seen in 27 (23%) *K. pneumoniae* by AmpC disc test [Table/Fig-9]. The prevalence of AmpC enzyme has been variable in studies from different parts of India. While Singhal S et al., from Delhi and Hemalatha V et al., from Chennai reported 36.06% and 47.3% AmpC prevalence in gram-negative bacilli respectively [22,23], it is less in a study from Karnataka [24]. The mechanism of cefoxitin resistance in gram negative bacteria could be either AmpC beta-lactamase or porin mutations [10]. Since 47 of our isolates were cefoxitin resistant and AmpC disc test negative, these may indicate presence of other mechanism of cefoxitin resistance such as porin mutations. However, this finding was not substantiated by molecular methods.

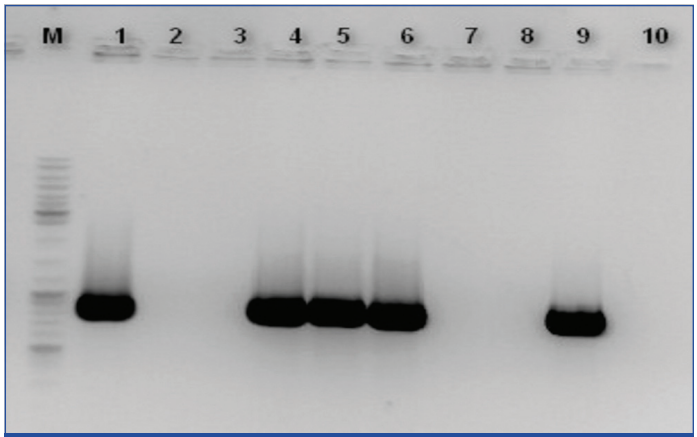
Carbapenem resistance in gram negative bacteria is an emerging problem and a serious threat to infection control in hospital. Expression of carbapenem hydrolysing enzymes like MBLs, KPC, OXA and overproduction of ESBLs or AmpC along with porin loss can confer resistance to this class of antibiotics [25,26]. In this study, 33 carbapenem resistant isolates were phenotypically tested by HiCrome KPC agar, Modified Hodge test and combined disc test. Several chromogenic media, namely CHROM agar KPC, chromID CARBA, Brilliance CRE and HiCrome KPC agar are available commercially and have been recommended for screening gram negative isolates with reduced carbapenem susceptibility.

Modified Hodge test has been effectively used for detection of carbapenemase production in Enterobacteriaceae. A positive result in MHT may indicate carbapenemase of diverse class viz. MBLs including New Delhi metallo betalactamase (NDM), KPC and SME-1 [27]. In our study, HiCrome KPC agar was used and 18 out of 33 carbapenems resistant isolates were found positive for carbapenemase. Among this 18 HiCrome KPC agar positive isolates, 17 were MHT positive and 16 were MBL producer by meropenem-EDTA combined disc test [Table/Fig-9]. This may suggest presence of other carbapenemases in at least one isolate. The remaining 15 isolates were non-carbapenemase producing and are assumed to have other mechanisms of carbapenem resistance.

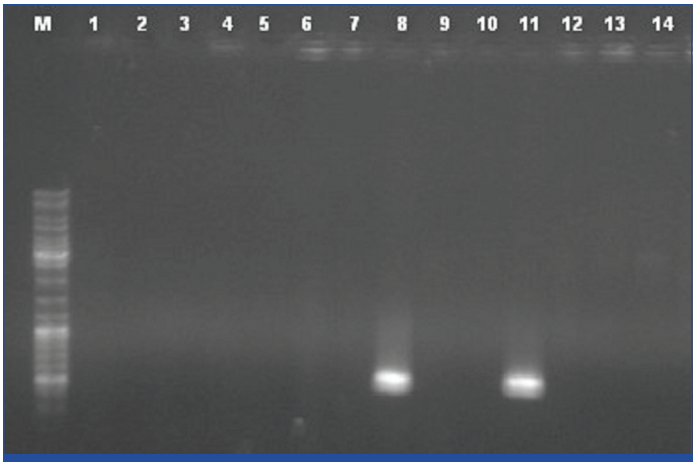
We have done molecular identification of ESBL and carbapenemase genes [Table/Fig-11-14]. Among the ESBL genes, *bla*SHV-1 was most common (n=81, 69%), followed by *bla*CTX-M (n=71, 60.6%) in 117 MDR *K. pneumoniae* isolates [Table/Fig-10]. No isolates were positive for *bla*TEM gene. This is in accordance with other studies. High prevalence of *bla*CTX-M, and *bla*SHV have been reported in *K. pneumoniae* in Indian subcontinent [28,29]. Goyal A et al., from India found *bla*CTX-M in 85.4% ESBL producing isolates, followed by *bla*TEM (54.9%) and *bla*SHV (32.9%) [28]. A study from Bangladesh identified *bla*CTX-M gene in 51.4% *K. pneumoniae* isolates, followed by *bla*SHV (27%) [29]. *bla*CTX-M have been the most clinically significant ESBL gene due to its worldwide dissemination. It has replaced *bla*TEM in bacterial population in several countries including India [3]. Although *bla*TEM is not a rarity in Enterobacteriaceae, it was not detected in our isolates.



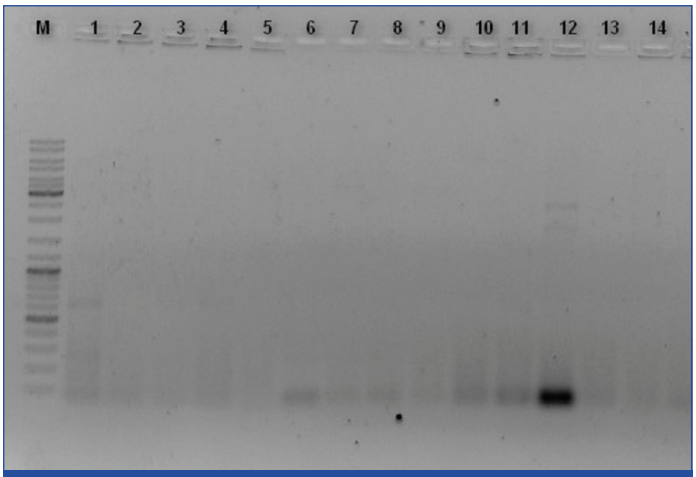
[Table/Fig-11]: PCR amplification of bla_{CTX-M} among the phenotypic ESBL positive *Klebsiella pneumoniae* isolates. Lane M-10 Kb Gene O' Ruler (Thermo Scientific), Lane 1,3,4,5,7,8,9,12,13 and 14-Positive for bla_{CTX-M}, Lane 2,6,10 and 11-Negative for $bla_{CTX-M.}$



[Table/Fig-12]: PCR amplification of bla_{SHV} among the phenotypic ESBL positive *Klebsiella pneumoniae* isolates. Lane M-10 Kb Gene O' Ruler (Thermo Scientific), Lane 1,4,5,6 and 9-Positive for bla_{SHV}, Lane 2,3,7,8 and 10-Negative for $bla_{SHV.}$



[Table/Fig-13]: PCR amplification of bla_{NDM-1} among the phenotypic MBL positive *Klebsiella pneumoniae* isolates. Lane M-10 Kb Gene O' Ruler (Thermo Scientific), Lane 8 and11 -Positive for bla_{NDM-1}, Lane 1-7,9,10,12-14 - Negative for bla_{NDM-1}.



[Table/Fig-14]: PCR amplification of bla_{IMP} among the phenotypic MBL positive Klebsiella pneumoniae isolates. Lane M-10 Kb Gene O' Ruler (Thermo Scientific), Lane 12 -Positive for bla_{IMP}, Lane 1-11, 13 and 14 -Negative for bla_{IMP}.

The molecular analysis of our 33 carbapenem resistant isolates showed preponderance of *bla*NDM-1 gene (n=11, 33%) followed by *bla*IMP-1 in 3 (9%) isolates [Table/Fig-10]. *bla*VIM-2, *bla*SIM-1, *bla*KPC genes were not detected in our isolates. Similar findings were noted in other studies. Various authors have reported prevalence of various genes for carbapenem resistance. *bla*NDM, *bla*KPC, *bla*IMP, *bla*VIM and *bla*OXA-48 were commonly implicated [26,30,31]. In a study from Taiwan, Tseng IL et al., found 18.4% carbapenem non-susceptible *K. pneumoniae* had carbapenemase genes and *bla*KPC (15.8%), *bla*IMP-8 (1.6%), *bla*VIM-1 (0.9%) and *bla*NDM-1 (0.1%) were the prevalent genotypes [31]. However, in Saudi Arabia *bla*OXA-48 was commonest (81.5%) followed by *bla*NDM-1 and *bla*VIM [32]. NDM-1 is a novel MBL enzyme, first reported in New Delhi in a Swedish patient colonised with *K. pneumoniae*. The initial outbreaks were from India and Pakistan rapidly followed by its worldwide spread [2,30].

The co-production of two or more of these enzymes has become frequently now-a-days. This not only confers wider spectrum of antibiotic resistance, but also leads to greater chance of survival and dissemination of the resistant bacterial strains. Furthermore, presence of high-level of AmpC enzymes may preclude the detection of the ESBLs [33]. In the present study, 32% isolates were co-producers [Table/Fig-15]. Several of these isolates were found to be positive for multiple ESBL and MBL genes. Out of the three *bla*IMP-1 positive isolates, two were co-expresser of *bla*CTX-M, *bla*SHV-1, *bla*IMP-1, and *bla*NDM-1 genes and one isolate co-expressed *bla*IMP-1 and *bla*SHV-1. Likewise, out of 11 *bla*NDM-1 positive isolates, *bla*CTX-M and *bla*SHV-1 genes were co-expressed along with *bla*NDM-1 in 3 and 5 isolates respectively. This is in accordance to a study from North India, which found co-production of ESBL and AmpC (13.5%), ESBL and MBL (10%), AmpC and MBL (1%) and ESBL, AmpC and MBL (2.5%) among E. coli isolates [9]. A higher prevalence of these co-producer strains were reported in other studies [33,34].

Mechanism	Number of Co-producer isolates detected by phenotypic method (%)
ESBL+AmpC	9 (8%)
Carbapenemase+AmpC	5 (4%)
ESBL+Carbapenemase	14 (12%)
ESBL+Carbapenemase+AmpC	9 (8%)

[Table/Fig-15]: *K. pneumoniae* isolates showing multiple resistance mechanisms.

LIMITATION

The limitations of our study were inability to confirm OXA, AmpC and porin mediated resistance mechanisms by molecular methods due to cost constrains. This is especially important for OXA enzymes as there are no phenotypic tests for its detection due to absence of specific inhibitor. Recently high level Temocillin resistance on MIC or disc diffusion test has been described to be an effective indicator of OXA-48 [26]. However, it needs further confirmation.

CONCLUSION

MDR strains of *K. pneumoniae* is prevalent in our hospital. While, *bla*SHV and *bla*CTX-M were the major ESBL genes, *bla*NDM-1 and *bla*IMP-1 were prevalent MBL genes. Co-production of ESBL, AmpC and MBL enzymes was found in our *K. pneumoniae* isolates. It indicates the need for close surveillance of antimicrobial use and resistance profile of gram negative bacteria for effective infection control.

ACKNOWLEDGEMENTS

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