



## EVALUATION OF DIFFERENT PHENOTYPIC METHODS TO DETECT ESBL AND MBL PRODUCTION IN *Escherichia coli* AND *Klebsiella* SPECIES WITH DETECTION OF NDM-1 BY MOLECULAR METHOD

KUMAR M.<sup>1\*</sup>, DUTTA R.<sup>1</sup>, SAXENA S.<sup>1</sup> AND SINGHAL S.<sup>2</sup>

<sup>1</sup>Department of Microbiology, Lady Hardinge Medical College, New Delhi - 110 001, India.

<sup>2</sup>Group Leader, Infectious Diseases, Biology Department, Daiichi Sankyo India Pharma Private Limited, Gurgaon - 122 015, Haryana, India.

\*Corresponding Author: Email- [drmanu\\_05@rediffmail.com](mailto:drmanu_05@rediffmail.com)

Received: February 20, 2015; Revised: March 24, 2015; Accepted: March 30, 2015

**Abstract-** *Introduction:* Gram negative bacteria are becoming increasingly resistant to antibiotics due to the production of ESBL and MBL. Microbiology laboratories must be prepared to screen for ESBL and MBL producing isolates by a low cost, convenient and sensitive procedure. *Objectives:* To detect ESBL and MBL production in *Escherichia coli* and *Klebsiella* species by using three different phenotypic methods and NDM-1 detection by PCR. *Materials and Methods:* ESBL production was detected using double disc synergy test (DDST), ESBL Etest and ESBL detection kits. For detecting MBL production, combined disk test, Modified Hodge test (MHT) and MBL Etest were used. MIC was determined by agar dilution, microbroth dilution and NDM-1 production by PCR. *Results:* Among *Esch. coli*, ESBL production was observed in 81.2%, 80.0%, 76.8% and 75.2% of 250 isolates by ESBL detection kit, DDST, Etest for ceftazidime and cefotaxime respectively. MBL production was observed in 12.5% of 40 isolates by MHT and 10% by both combined disk test and Etest. Among *Klebsiella*, ESBL production was observed in 63.3%, 62.2% and 61.0% of 267 isolates by Etest, ESBL detection kit and DDST. MBL production was observed in 35.1% of 97 isolates by MHT and 21.6% by both the combined disk test and Etest. 1 *Esch. coli* and 8 *Klebsiella* spp. were strongly positive, 1 each of *Esch. coli* and *Klebsiella* spp. was weakly positive, and 1 *Esch. coli* and 6 *Klebsiella* spp. were negative for NDM-1 production. *Conclusion:* Etest was best for detecting ESBL and MBL production among Gram negative bacteria.

**Keywords-** Extended-spectrum  $\beta$ -lactamases, metallo- $\beta$ -lactamases, New Delhi metallo- $\beta$ -lactamase-1

**Citation:** Kumar M., et al. (2015) Evaluation of Different Phenotypic Methods to Detect ESBL and MBL Production in *Escherichia coli* and *Klebsiella* species with Detection of NDM-1 by Molecular Method. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 7, Issue 1, pp.-598-603.

**Copyright:** Copyright©2015 Kumar M., et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

### Introduction

$\beta$ -lactamases are the primary mechanism of conferring bacterial resistance to  $\beta$ -lactam antibiotics, such as penicillins and cephalosporins [1]. Extended-spectrum  $\beta$ -lactamases (ESBLs) are primarily produced by the Enterobacteriaceae family of Gram-negative organisms, in particular *Klebsiella pneumoniae* and *Escherichia coli*, and also by nonfermentative Gram-negative organisms, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. ESBLs are Class A plasmid-mediated  $\beta$ -lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by  $\beta$ -lactamase inhibitors such as clavulanic acid [2].

The growing prevalence of ESBL producers is sufficient to drive a greater reliance on carbapenems. But extensive and sometimes unnecessary use of carbapenems has facilitated the emergence of carbapenem resistant bacteria. Two types of carbapenem hydrolyzing enzymes exist, first is the serine  $\beta$ -lactamase (having serine at their active site) and second is the metallo- $\beta$ -lactamase (MBL) which requires divalent cations of  $Zn^{2+}$  as cofactors for enzyme activity. MBLs are class B  $\beta$ -lactamase belonging to Group 3 and

can hydrolyze all metallo- $\beta$ -lactams except monobactams and are not inactivated by  $\beta$ -lactamase inhibitors like clavulanic acid, sulbactam and tazobactam but are inactivated by metal ion chelators like ethylene diamine tetracetic acid (EDTA) and 1,10-o-phenanthroline [3]. With the global increase in the occurrence and types of MBLs, early detection is crucial; the benefits of which include timely implementation of strict infection control practices and treatment with alternative antimicrobials.

Phenotypic methods of detecting ESBL and MBL are important in resource poor settings where molecular methods are not available easily. The present study was designed to evaluate standard phenotypic methods for detection of ESBL and MBL production among *Escherichia coli* and *Klebsiella* spp. and detection of New Delhi metallo- $\beta$ -lactamase (NDM-1) production among them by PCR.

### Materials and Methods

A prospective observational descriptive study was carried out in the Department of Microbiology, Lady Hardinge Medical College (LHMC), New Delhi and associated hospitals - Kalawati Saran Children's Hospital (KSCH) and Smt. Sucheta Kriplani Hospital (SSKH) from November 2010 to March 2012.

Three hundred and fifty strains each of *Escherichia coli* and *Klebsiella* spp. isolated from various clinical samples of inpatients were collected consecutively. Repeat isolates of *Escherichia coli* and *Klebsiella* spp. with the same antibiogram from the same patient were excluded from the study.

### Processing of Specimens

Collection, transport and processing of specimens were done as per standard protocol [4,5]. The plates were incubated aerobically overnight at 37°C and examined for colony morphology and lactose fermenting colonies on MacConkey's agar and further identified by Gram stain and biochemical reactions [4,5].

### Antimicrobial Susceptibility Tests

#### Disk Diffusion Tests

All identified strains were tested for antimicrobial susceptibility by Kirby-Bauer method on Mueller-Hinton Agar (MHA) medium according to criteria recommended by CLSI [6].

Following antimicrobial agents (Hi-Media) were used for antibiotic susceptibility testing: Amikacin (30 µg), Ampicillin (10 µg), Cotrimoxazole (1.25/23.75 µg), Ertapenem (10 µg), Aztreonam (30 µg), Gatifloxacin (5 µg), Cefazolin (30 µg), Imipenem (10 µg), Cefotaxime (30 µg), Norfloxacin (10 µg), Ceftazidime (30 µg), Tetracycline (30 µg), and Ciprofloxacin (5 µg).

#### Minimum Inhibitory Concentration (MIC) Determination

MIC determination was done according to CLSI guidelines by agar dilution method [6]. MIC of Imipenem was determined for the strain found resistant by disk diffusion method, while MIC of Ceftazidime and Cefotaxime was determined for strains found resistant to Ceftazidime/Cefotaxime/Aztreonam. MIC of ESBL producing imipenem resistant and intermediate susceptible isolates of *Esch. coli* and *Klebsiella* spp. was determined using microbroth dilution and these were tested for NDM-1 production [7].

### Detection of ESBL Production

The strains resistant to Ceftazidime and/or Cefotaxime were further

tested for ESBL production by double disc synergy test (DDST), ESBL Etest and ESBL detection kits using standard methods [8].

### Detection of MBL Production

The strains which showed resistance to imipenem were further tested and interpreted for MBL production by combined disk test, Modified Hodge test (MHT) and using MBL Etest strips [9-12].

### Detection of NDM-1 Production

DNA was extracted from the strains by heat boil method and this DNA was subjected to single target PCR. Amplified products (250 bp) were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide. *Esch. coli* NDM-1 positive controls were included. (Thermo Scientific GeneRuler 1 kb DNA Ladder 250 to 10,000 bp).

### Results

Three hundred and fifty clinical isolates of *Esch. coli* and 350 of *Klebsiella* spp. (347 of *Klebsiella pneumoniae* and 3 of *Klebsiella oxytoca*) were obtained from 338 and 343 patients respectively.

### ESBL and MBL Production

#### *Escherichia coli* ESBL Production

Out of the 350 isolates, 247 isolates showed resistance to both cefotaxime and ceftazidime. Out of 3 isolates showing intermediate susceptibility to cefotaxime, 2 were susceptible to ceftazidime and 1 was resistant. The 2 isolates which were intermediate susceptible to cefotaxime and susceptible to ceftazidime did not show multidrug resistance. The remaining 248 isolates were multidrug resistant. Thus, a total of 250 isolates were further tested for ESBL production.

Of these 250 isolates, 203 (81.2%) tested positive for ESBL production by ESBL detection kit and out of these 203, 200 (80.0%) were also positive by the DDST method. ESBL Etest for ceftazidime and cefotaxime showed 192 (76.8%) and 188 (75.2%) isolates to be ESBL producers respectively out of the 200 isolates which were positive by both ESBL detection kit and DDST [Fig-1].

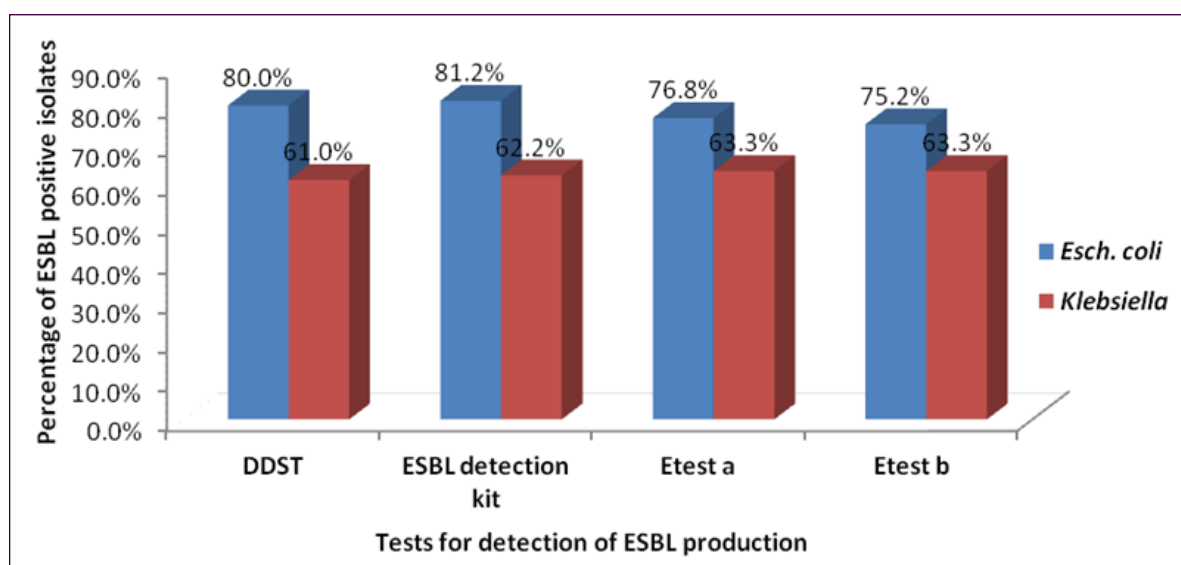


Fig. 1- Results of different methods for ESBL production in *Esch. coli* and *Klebsiella* spp. isolates.

(DDST – cefotaxime/amoxicillin+clavulanic acid, ESBL detection kit - ceftazidime/ceftazidime+clavulanic acid, Etest a – Etest using ceftazidime/ceftazidime+clavulanic acid, Etest b – Etest using cefotaxime/cefotaxime+clavulanic acid).

99.5 - 100% of ESBL producers were resistant to cephalosporins, 96.9% to monobactam, 78.7 - 89.1% to fluoroquinolones, 88.5% to penicillin, 85.4% to tetracycline and 76.6% to cotrimoxazole whereas lesser number of ESBL producers were resistant to carbapenems (2.6 - 14.1%) and aminoglycosides (25.5%) and this difference was statistically significant ( $p < 0.0001$ ).

6.4%, 6.8% and 86.8% of the 250 isolates were sensitive, intermediate susceptible and resistant by MIC test to cefotaxime respectively. 13.2%, 15.2% and 71.6% isolates were sensitive, intermediate susceptible and resistant by MIC test to ceftazidime respectively. 86%, 7.2% and 6.8% isolates were sensitive, intermediate susceptible and resistant by MIC test to imipenem respectively. Result of MIC<sub>50</sub> and MIC<sub>90</sub> values is depicted in [Table-1].

#### *Escherichia coli* MBL Production

40 isolates were tested for MBL production. Of these 40 isolates, 17 isolates were resistant to imipenem, 5 were intermediate susceptible and 18 were sensitive to imipenem but positive for ESBL production by DDST only. ESBL production (Etest positive) was seen in 5 of the 17 isolates resistant to imipenem and 3 of the 5 isolates which were intermediate susceptible to imipenem. MBL production was observed in 5 isolates (12.5%) by MHT. Of these 5 isolates, 4 isolates (10%) were also positive by both the combined disk test and MBL Etest [Fig-2].

#### *Klebsiella* Species ESBL Production

Of the 350 isolates, 262 isolates showed resistance to both cefotaxime and ceftazidime, 5 were intermediate susceptible to cefotaxime. Out of these 5, 4 were resistant to ceftazidime and 1 was susceptible to ceftazidime. One isolate which was intermediate susceptible to cefotaxime and susceptible to ceftazidime did not show multidrug resistance. The remaining 266 isolates were multidrug resistant. Thus, a total of 267 isolates were further tested for ESBL production.

Of these 267 isolates, 169 isolates (63.3%) were positive for ESBL

production by ESBL Etest, 166 isolates (62.2%) were positive by ESBL detection kit and 163 (61%) by DDST method [Fig-1].

99.4% of ESBL producers were resistant to cephalosporins, 98.2% to monobactam, 97% to penicillin, 88.2% to cotrimoxazole, 81.1% to tetracycline and 58 - 69.2% to fluoroquinolones whereas lesser number of ESBL producers were resistant to carbapenems (8.9 - 17.2%) and aminoglycosides (31.4%) and this difference was statistically significant ( $p < 0.0001$ ).

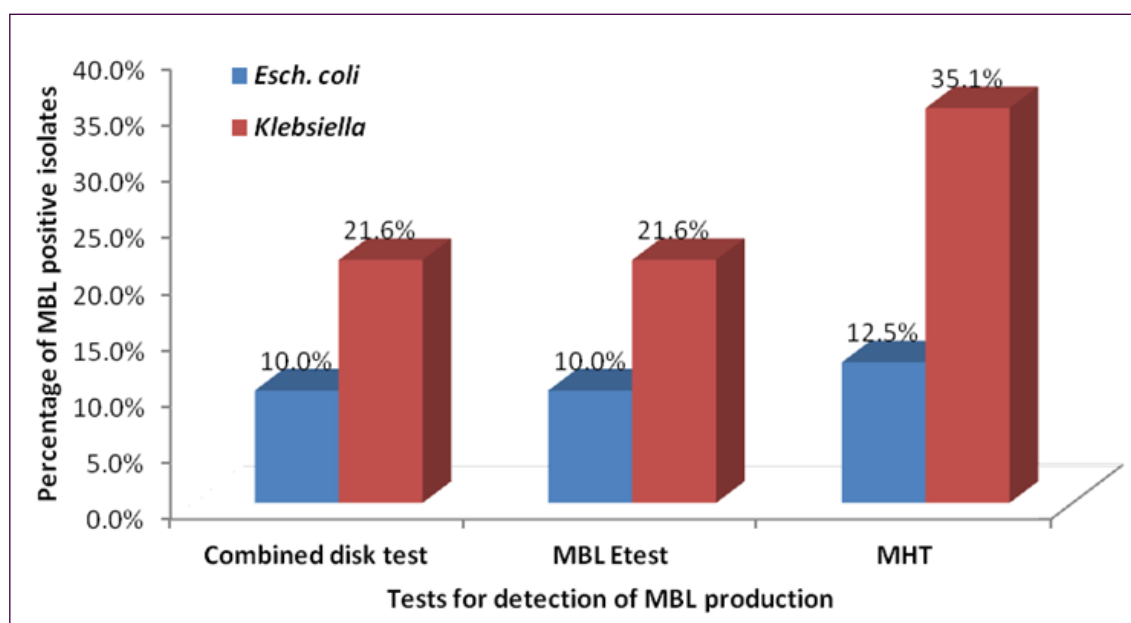
0.8%, 7.1% and 92.1% of the 267 isolates were sensitive, intermediate susceptible and resistant by MIC test to cefotaxime respectively. 8.6%, 13.5% and 77.9% isolates were sensitive, intermediate susceptible and resistant by MIC test to ceftazidime respectively. 84.3%, 3.7% and 12% isolates were sensitive, intermediate susceptible and resistant by MIC test to imipenem respectively. Result of MIC<sub>50</sub> and MIC<sub>90</sub> values is depicted in [Table-1].

**Table 1-** MIC<sub>50</sub> and MIC<sub>90</sub> values of cefotaxime, ceftazidime and imipenem for *Esch. coli* and *Klebsiella* spp.

Antibiotic	Range tested (µg/ml)	<i>Esch. coli</i>		<i>Klebsiella</i>	
		MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>
Cefotaxime	0.5-8	4	8	4	8
Ceftazidime	2-32	16	32	16	32
Imipenem	0.5-8	1	4	1	2

#### *Klebsiella* species MBL Production

A total of 97 isolates were tested for MBL production. Of these 97 isolates, 57 isolates were resistant to imipenem, 12 were intermediate susceptible and 28 were sensitive to imipenem but positive for ESBL production by DDST only. ESBL production (Etest positive) was seen in 14 of the isolates resistant to imipenem and 3 isolates which were intermediate susceptible to imipenem. MBL production was observed in 34 isolates (35.1%) by MHT. Of these 34, 21 isolates (21.6%) were also positive by both the combined disk test and MBL Etest [Fig-2].



**Fig. 2-** Results of different methods for MBL production in *Esch. coli* and *Klebsiella* spp. isolates.  
(Combined disk test and MBL Etest – imipenem/imipenem – EDTA, MHT – ertapenem)

## NDM-1 Production

Of the total 40 isolates of *Esch. coli* tested for MBL production, 8 isolates were ESBL producers. Of these 8, 4 isolates were MBL producers by MHT, combined disk test and MBL Etest and 1 isolate by MHT. These 8 isolates were tested for MIC determination by microbroth dilution. 3 isolates which showed multidrug resistance with this method were further evaluated for NDM-1 production by polymerase chain reaction. 1 isolate tested came out to be strongly positive for NDM-1 production, 1 isolate weakly positive and 1 neg-

ative for NDM-1 production [Table-2], [Fig-3].

Of the total 97 isolates of *Klebsiella* spp. tested for MBL production, 17 isolates were ESBL producers. All these isolates were MBL producers by the three tests. They were tested for MIC determination by microbroth dilution. 15 isolates showed multidrug resistance with this method. Of these 15, 8 isolates were strongly positive for NDM-1 production, 1 was weakly positive and 6 were negative for NDM-1 production [Table-2], [Fig-3].

**Table 2-** Minimum inhibitory concentration of antimicrobials for *Esch. coli* and *Klebsiella* spp. by microbroth dilution.

S.no	Strains	Imipenem	Meropenem	Ceftazidime	Aztreonam	Levofloxacin	Colistin	Tigecycline	Amikacin	NDM-1
1	<i>Esch. coli</i>	32	32	>32	>32	8	0.5	2	>64	Strongly positive
2	<i>K. pneumoniae</i>	8	16	>32	>32	>32	0.5	2	>64	Strongly positive
3	<i>K. pneumoniae</i>	>32	>32	>32	>32	>32	2	4	>64	Strongly positive
4	<i>K. pneumoniae</i>	16	32	>32	>32	16	4	1	>64	Strongly positive
5	<i>K. pneumoniae</i>	16	32	>32	>32	>32	>16	1	>64	Strongly positive
6	<i>Esch. coli</i>	16	>32	>32	>32	16	0.5	1	>64	Negative
7	<i>Esch. coli</i>	32	32	>32	>32	4	1	2	>64	Weakly positive
8	<i>K. pneumoniae</i>	16	32	>32	>32	16	1	4	>64	Negative
9	<i>K. pneumoniae</i>	>32	>32	>32	>32	>32	1	4	>64	Strongly positive
10	<i>K. pneumoniae</i>	>32	>32	>32	>32	>32	1	>16	>64	Strongly positive
11	<i>K. pneumoniae</i>	16	32	>32	>32	32	2	4	>64	Negative
12	<i>K. pneumoniae</i>	>32	>32	>32	>32	>32	1	8	>64	Strongly positive
13	<i>K. pneumoniae</i>	16	16	>32	>32	4	2	4	>64	Negative
14	<i>K. pneumoniae</i>	32	32	>32	>32	4	2	8	>64	Strongly positive
15	<i>K. pneumoniae</i>	16	32	>32	>32	16	4	16	>64	Negative
16	<i>K. pneumoniae</i>	16	16	>32	>32	>32	2	16	>64	Negative
17	<i>K. pneumoniae</i>	16	32	>32	>32	32	1	8	>64	Weakly positive
18	<i>K. pneumoniae</i>	<0.06	<0.06	0.125	<0.06	<0.06	1	0.25	4	Negative



**Fig. 3-** Gel picture of NDM-1 PCR results.

bp ng is a molecular weight marker. Last lane (19) is NDM-1 plasmid (positive control). Lane nos. 1,2,3,4,5,9,10,12 and 14 are strongly positive; 7 and 17 are weakly positive; 6,8,11,13,15,16 and 18 are negative.

## Discussion

Bacteria from clinical and non-clinical settings are becoming increasingly resistant to conventional antibiotics, especially the Gram-negative bacteria. Nowadays multidrug resistant Gram negative bacteria pose the greatest risk to public health because there is an increased risk of resistance in them [13,14] and drug development programmes seem insufficient to provide therapeutic cover in the

near future [15,16].

PCR analysis is the gold standard method for the detection of ESBL and MBL producers, but it is not suitable for routine testing in clinical laboratories due to the high cost and inconvenience. In this study, we have used three different phenotypic methods each to detect ESBL and MBL production in *Escherichia coli* and *Klebsiella* species.



In the present study, out of 250 isolates of *Esch. coli*, 80.0% tested positive for ESBL production by the DDST method while 81.2% by ESBL detection kit. ESBL Etest for ceftazidime and cefotaxime showed 76.8% isolates and 75.2% isolates to be ESBL producers respectively which were also positive by both DDST and ESBL detection kit. ESBL Etest was found to be very sensitive for detection of ESBL production. The present study showed a higher percentage of ESBL producers as compared to Shobha et al [17] who detected 32% of *Esch. coli* isolates to be ESBL producers with the screening test and 35% with the phenotypic confirmatory test using modified double disk method; Hassan et al [18] detected 54% of the 100 isolates as ESBL producers with the combination disk method; Jitsurong et al [19] detected 5.1% of *Esch. coli* isolates as ESBL producers with screening disk diffusion test, combination disk test and Etest. Lautenbach et al [20] detected 24.2% infections due to ESBL producing *Esch. coli*.

In the present study, out of 40 isolates of *Esch. coli*, 10% were detected as MBL positive by both the combined disk test and MBL Etest and 12.5% by the MHT. Both combined disk test and MBL Etest were found to be equally effective for MBL detection. In contrast, Chakraborty et al [21] reported a total of 41.2% of MBL producing bacteria out of which 28.57% were *Esch. coli* by combined disk test and Etest.

In the present study, of the 267 isolates of *Klebsiella* spp., 61.0% were positive for ESBL production by the DDST method while 62.2% were positive by ESBL detection kit. ESBL Etest showed 63.3% to be positive for ESBL production with Etest. All the 163 isolates found to be ESBL producers by DDST were also positive both with the Etest and ESBL detection kit and 3 isolates which were positive by ESBL detection kit but not by DDST were positive by the Etest. The present study showed a higher percentage of ESBL producers as compared to that reported by Paterson et al [22] who detected 18.7% of ESBL producing *K. pneumoniae* bacteraemia and Shobha et al [17] who detected 37% of ESBL producing *Klebsiella* isolates with the screening test and 41% with the phenotypic confirmatory test. Jitsurong et al [19] detected 44.4% of *K. pneumoniae* isolates as ESBL producers with screening disk diffusion test, combination disk test and Etest. However, higher percentage of ESBL producing *Klebsiella* infections (75.8%) was reported by Lautenbach et al [20].

In the present study, out of 97 isolates of *Klebsiella* spp., 21.6% isolates were detected as MBL producers by both the combined disk test and MBL Etest and 35.1% by MHT. Both combined disk test and MBL Etest were found to be equally effective for MBL detection. Cagnacci et al [23] identified nine isolates using combined disk test. Deshpande et al [24] detected 12 carbapenem resistant species by MHT, of which 10 were detected as NDM producers by PCR. Chakraborty et al [21] identified a total of 41.2% of MBL producing bacteria out of which 36.6% were *Klebsiella* by combined disk test and MBL Etest. Kumarasamy et al [25] detected 21.3% of isolates as MBL producing *K. pneumoniae* using any of the three methods.

In the present study, NDM-1 production was tested in only 25 isolates of *Esch. coli* (8) and *Klebsiella* (17) which showed resistance or intermediate susceptibility to imipenem and were ESBL producers by disc diffusion test. Amongst these, 1 *Esch. coli* and 8 *Klebsiella* spp. were strongly positive for NDM-1 production, and 1 each of *Esch. coli* and *Klebsiella* spp. was weakly positive. Deshpande et al [24] had identified 22 NDM producing organisms out of

24 carbapenem resistant Enterobacteriaceae of which 9 were *Esch. coli* and 10 *Klebsiella* spp. Kumarasamy et al [25] identified 19 *Esch. coli* and 14 *K. pneumoniae* from Chennai and 26 *K. pneumoniae* from Haryana as NDM-1 producers from carbapenem resistant Enterobacteriaceae.

## Conclusion

In the present study, incidence of ESBL production was high in both *Esch. coli* and *Klebsiella* spp. by the three methods though incidence of MBL production was not very high. Among all the methods evaluated, Etest was found to be the best test for detecting ESBL and MBL production among Gram negative bacilli.

**Conflicts of Interest:** None declared.

## References

- [1] Dhillon R.H.P. & Clark J. (2011) *Critical Care Research and Practice*, 625170.
- [2] Bradford P.A. (2001) *Clinical Microbiology Reviews*, 14(4), 933-951.
- [3] Bush K. (2001) *Clinical Infectious Diseases*, 32(7), 1085-1089.
- [4] Collee J.G. & Marr W. (1996) *Culture of bacteria*, Mackie and McCartney Practical Medical Microbiology, 14, 113-129.
- [5] Collee J.G., Miles R.S. & Watt B. (1996) *Tests for the identification of bacteria*, Mackie and McCartney practical medical microbiology, 14, 131-149.
- [6] Wikler M.A. (2006) *Performance standards for antimicrobial susceptibility testing: Sixteenth informational supplement*, Clinical and Laboratory Standards Institute, 26.
- [7] Andrews J.M. (2001) *Journal of Antimicrobial Chemotherapy*, 48(1), 5-16.
- [8] Livermore D.M. & Brown D. F. (2001) *Journal of Antimicrobial Chemotherapy*, 48(1), 59-64.
- [9] Behera B., Mathur P., Das A., Kapil A. & Sharma V. (2008) *Indian Journal of Medical Microbiology*, 26(3), 233-237.
- [10] Yong D., Lee K., Yum J.H., Shin H.B., Rossolini G.M. & Chong Y. (2002) *Journal of Clinical Microbiology*, 40(10), 3798-3801.
- [11] Hodge W., Ciak J. & Tramont E.C. (1978) *Journal of Clinical Microbiology*, 7(1), 102-103.
- [12] Walsh T.R., Bolmström A., Qwärnström A. & Gales A. (2002) *Journal of Clinical Microbiology*, 40(8), 2755-2759.
- [13] Cornaglia G. (2009) *Clinical Microbiology and Infection*, 15(3), 209-211.
- [14] Tan T.T. (2008) *Ann. Acad. Med. Singapore*, 37(10), 884-890.
- [15] Boucher H.W., Talbot G.H., Bradley J.S., Edwards J.E., Gilbert D., Rice L.B., Scheld M., Spellberg B. & Bartlett J. (2009) *Clinical Infectious Diseases*, 48(1), 1-12.
- [16] Rice L.B. (2009) *Current Opinion in Microbiology*, 12(5), 476-481.
- [17] Shobha K.L., Gowrish Rao S., Rao S., & Sreeja C.K. (2007) *Indian J. for the Practicing Doctor*, 3(6).
- [18] Hassan S.A., Jamal S.A. & Kamal M. (2011) *J. Basic Appl. Sci.*, 7(1), 39-43.
- [19] Jitsurong S. & Yodsawat J. (2006) *Southeast Asian Journal of Tropical Medicine and Public Health*, 37(1), 131-135.

- [20]Lautenbach E., Patel J.B., Bilker W.B., Edelstein P.H. & Fishman N.O. (2001) *Clinical Infectious Diseases*, 32(8), 1162-1171.
- [21]Chakraborty D., Basu S. & Das S. (2010) *American Journal of Infectious Diseases*, 6(2), 34-39.
- [22]Paterson D.L., Ko W.C., Von Gottberg A., Casellas J.M., Mulazimoglu L., Klugman K.P., Bonomo R.A., Rice L.B., McCormack J.G. & Yu V.L. (2001) *Journal of Clinical Microbiology*, 39(6), 2206-2212.
- [23]Cagnacci S., Gualco L., Roveta S., Mannelli S., Borgianni L., Docquier J.D., Dodi F., Centanaro M., Debbia E., Marchese A., & Rossolini G.M. (2008) *Journal of Antimicrobial Chemotherapy*, 61(2), 296-300.
- [24]Deshpande P., Rodrigues C., Shetty A., Kapadia F., Hedge A., & Soman R. (2010) *Journal of Association of Physicians of India*, 58(3), 147-149.
- [25]Kumarasamy K.K., Toleman M.A., Walsh T.R., Bagaria J., Butt F., Balakrishnan R., Chaudhary U., Doumith M., Giske C.G., Irfan S., Krishnan P., Kumar A.V., Maharjan S., Mushtaq S., Noorie T., Paterson D.L., Pearson A., Perry C., Pike R., Rao B., Ray U., Sarma J.B., Sharma M., Sheridan E., Thirunarayan M.A., Turton J., Upadhyay S., Warner M., Welfare W., Livermore D.M., & Woodford N. (2010) *The Lancet Infectious Diseases*, 10(9), 597-602.