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Microbiology

# DETECTION OF NEW DELHI METALLO-BETA-LACTAMASES-1 GENE IN CLINICAL ISOLATES OF *E.COLI* IN A MULTISPECIALITY HOSPITAL.

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**ABSTRACT** Carbapenemases are diverse enzymes that vary in their ability to hydrolyze carbapenems and other beta-lactams. Detection of carbapenemase is a crucial infection control issue because they are often associated with extensive antibiotic resistance, treatment failures and infection-associated mortality. Among the beta-lactamases, the carbapenemases, especially transferrable metallo-beta-lactamases (MBLs) are the most feared because of their ability to hydrolyze virtually all drugs in that class, including the carbapenems. Currently, the bacteria receiving the most attention is New Delhi metallo-beta-lactamase-1 (NDM-1) producing superbug that confers resistance to most antibiotics including carbapenems. *NDM-1* has been increasingly isolated *from K.pneumoniae, E.coli, C.freundii, Morganella morgagnii, Providentia spp, Enterobacter cloacae.*

**Objective:** The present study was undertaken to detect *bla*NDM-1 gene in clinical isolates of *E.coli* in Multispecialty hospital.

**Materials & Methods**: All clinical isolates of *E.coli* from patients attending out-patient and in-patient department at JSS hospital from December 2013 to November 2014. Samples received in the laboratory were subjected to routine processing. Antimicrobial susceptibility testing was according to CLSI guidelines. MBL production was detected both by Modified Hodge Test and EDTA-Disc Diffusion Synergy Test. Genotypically PCR was carried out for the detection of *bla*NDM-1 genes.

**Result**: Total of 1,579 *E.coli* were isolated from various clinical samples. Out of these, 100 non-repetitive isolates, which were also ESBL producers by phenotypic tests, were subjected for blaNDM-1 gene detection. In these 100 isolates, 27(27%) NDM-1 gene were detected by conventional PCR. Among 27 positive NDM-1 gene isolates, 16(59.25%) Metallo-β lactamase (MBL) production was detected both by Modified Hodge test and EDTA-Disc Diffusion Synergy tests for ESBL *E.coli* isolates. Out of 27(27%) *NDM-1* positive isolates, 17(62.96%) isolates were also positive for *CTX-M* gene.

**Conclusion:** *NDM-1* producing strains appear to be an emerging worldwide problem. Based on our findings, we conclude that genotypic assay could be considered in the diagnostic workflow as confirmatory method for carbapenemase production and/or as an identification tool for the most important different carbapenemase genes. When the presence of a carbapenemase is suspected, PCR is the fastest way to determine which family of β-lactamase is present. Hence timely detection of *NDM-1* would help in timely institution of appropriate antibiotic therapy.

**KEYWORDS :** .Carbapenems, CTX-M gene, Metallo-beta-lactamases(MBL), Modified Hogde Test(MHT), Polymerase Chain Reaction (PCR).

## Introduction

*E. coli* is responsible for a wide variety of hospital and community-onset infections, affecting patients with normal immune systems as well as those with pre-existing conditions. They often comprise the most common gram negative bacteria found in clinical laboratories including the vast majority of urinary, blood culture and peritoneal isolates. They may also be isolates from other sites including the respiratory tract, cerebrospinal fluid and various types of abscesses.

*E. coli* is the most common cause of urinary tract infections (UTIs) in humans and is a leading cause of enteric infections and systemic infections. The systemic infections include bacteraemia, nosocomial pneumonia, cholecystitis, cholangitis, peritonitis, cellulitis, osteomyelitis, and infectious arthritis. *E. coli* is also leading cause of neonatal meningitis.

Beta lactamases seen in E.coli are SHV, VEB-1, OXA, CTX-M, NDM-1. Carbapenem antibiotics are considered the drugs of choice for the treatment of extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and other multidrug resistant bacteria. The emergence of bacterial strains that produce carbapenemases further limits the therapeutic options available to clinicians.

Bacteria receiving the most attention are New Delhi metallo-beta-lactamase-1(NDM-1) producing superbug that confers resistance to most antibiotics including carbapenems. The NDM-1 enzyme was named after New Delhi, the capital city of India, as it was first described by Yong *et al*.[1] in December 2009 in a Swedish national who fell ill with an antibiotic-resistant bacterial infection that he acquired in India.

This study was undertaken to know the prevalence of MBL *E.coli* and to detect *bla*NDM-1 gene in Multispecialty hospital; so that extent of drug resistance in *E.coli* can be extrapolated and necessary infection control measures can be taken to prevent spread of drug resistance.

## Materials & Methods:

This was a hospital based prospective study, undertaken in the department of Microbiology. All clinical isolates of *E.coli* from patients attending out-patient and in-patient department at JSS hospital from December 2013 to November 2014 was included in the study. Fecal *E. coli* was excluded from this study and inclusion criteria were Phenotypically confirmed ESBL *E.coli* isolates for detection of NDM-1 gene. The samples collected were processed as per standard methods. The study protocol was approved by the Institutional ethical committee.

Urine, Pus, ET secretions, Bile, Blood and other Body fluids were received to the laboratory and were subjected to routine processing as per standard (CLSI guidelines,2013)[2] operating procedures. Phenotypic Identification of *E.coli* was carried out on Blood Agar and Mac-Conkey Agar, followed by Gram Stain was carried indicating Gram negative rod, 1-3 x 0.4 - 0.7 µm arranged singly or in pairs. Colonies were later subjected to biochemical tests such as catalase, oxidase, nitrate reduction, Indole production, urease, utilization of citrate, Triple sugar Iron agar test, Methyl red test, Voges-Proskauer test as per standard CLSI guidelines,2013.[2]

Antimicrobial susceptibility testing was performed as per the CLSI guidelines (2013)[2] by modified Kirby Bauer method. All the strains were tested for their sensitivity to antimicrobial drugs using recommended CLSI guidelines combined with hospital formulary practices for the purpose of reporting to the clinician. The isolates were tested for Ampicillin (10 µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime(30µg), Cefepime(30µg), Gentamicin(10µg), Amikacin (30µg), Ciprofloxacin(5µg), Co-trimoxazole

(Trimethoprim/Sulphamethoxazole)(1.25/23.75µg), Imipenem (10µg), Meropenem (10µg), Piperacillin / Tazobactam(100/10 µg), Norfloxacin(10µg), Nitrofurantoin (100µg) (Hi-media, Mumbai).

**Phenotypic Detection of MBL - Detection methods :**

I. **Modified Hodge test[3]:** Prepare a 0.5 McFarland dilution of the *E.coli* ATCC 25922 in 5 ml of nutrient broth. Dilute 1:10 by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of saline. Streak a lawn of the 1:10 dilution of *E.coli* ATCC 25922 on Muller Hinton Agar (MHA) plates and allow to dry 3–5 minutes. Place a 10µg Meropenem susceptibility disk the center of the test area. In a straight line, streak test organism from the edge of the disk to the edge of the plate. Up to four organisms can be tested on the same plate with one drug. Incubate overnight at 35°C ± 2°C in ambient air for 16–24 hours. After 16–24 hrs. of incubation, examine plate for a clover leaf-type indentation at intersection of test organism & E. coli 25922, within zone of inhibition of carbapenem susceptibility disk. **MHT Positive test:** Clover leaf-like indentation of *E.coli* 25922 growing along test organism growth streak within disk diffusion zone. **MHT Negative test**: No growth of *E.coli* 25922 along test organism growth streak within disc diffusion zone.

ii. **EDTA-Disk Diffusion Synergy Test:** Overnight broth culture of the test strain, (opacity adjusted to 0.5 McFarland opacity standards) is used to inoculate a plate of MHA. After drying, 10 µg Imipenem disc and a blank filter paper disk (6 mm in diameter, Whatmann filter paper no. 2 placed 10mm apart from edge to edge. 10 µl of 0.5 M EDTA solution is applied to the blank disc, which results in approximately 1.5 mg/disc. After overnight incubation, presence of an enlarged zone of inhibition is interpreted as EDTA synergy positive.

**II. NDM-1 Gene detection by Conventional PCR.**

DNA was extracted from overnight broth culture of *E.coli,* using HiPurA Bacterial Genomic DNA Purification Kit MB505 as per the manufacture's protocol. The *bla*NDM-1 gene was identified by PCR using primers. *bla*NDM -1 : 5´ ---> 3´621bp

GGTTTGGCGATCTGGTTTTC CGG AAT GGC TCA TCA CGA TC

PCR tube consisted of 29 µl of Master Mix + 1 µL of DNA. The conditions included an initial denaturation step of 5 min at 95.8C, followed by 30 cycles of Holding Temperature:91°C, Annealing Temperature:55°C and then a final extension step of 5 min at 72°C. Isolates were screened for acquired *bla*CTX-M and *bla*NDM-1 gene by PCR using primers and conditions described previously. Post PCR Analysis by Gel Electrophoresis was carried out using 1% Agarose (DNA grade, low melting, Himedia) is prepared with 10X TAE buffer, heated till it becomes a clear solution. Allow the agarose to cool and then add 10µl tracking dye i.e, 1% ethidium bromide. Set electrophoresis tray with comb in position on levelling platform. Mix well and pour into the casting gel to set for 30 minutes. Fill the gel chamber with 10x TAE buffer(250 ml) till the gel is completely covered with buffer. Load the wells using micro pipettes. Add the tracking dye Bromophenol blue (5µl in 25 µl amplicon) before loading into the well. A DNA ladder (molecular weight marker) is run alongside in the 1st well. Electrophoresis is run using 150V for 25 minutes or till the tracking dye has travelled atleast 3/4th of the gel in longitudinal direction. The gel is immediately viewed in UV transilluminator.. The separated DNA fragments give orange red fluorescence. The results were recorded on gel documentation system.

**Sequencing:** Amplified DNA fragments were purified (Scigenom Lab, Kochi). Nucleotide sequence was analysed by Basic Local Alignment search tool available at the National Centre of Biotechnology Information website (www.ncbi.nlm.nih.gov/).

**Result**:

Total of 1,579 *E.coli* was isolated from various clinical samples over a period of one year from November 2013 – December 2014. Out of these 190 randomly selected isolates was tested for phenotypic identification of ESBL. Out of which 142 (74%) was positive for ESBL production. In these, 100 non repetitive isolates were randomly selected as sample size for detection of blaNDM-1 gene by genotypic methods.

**Figure 1: *bla*NDM-1 positive Isolates from Genotypic method:**

In total of 100 non-repetitive isolates, 27(27%) isolates were positive for *bla*NDM-1 gene by Convensional PCR and 73(73%) were negative respectively.

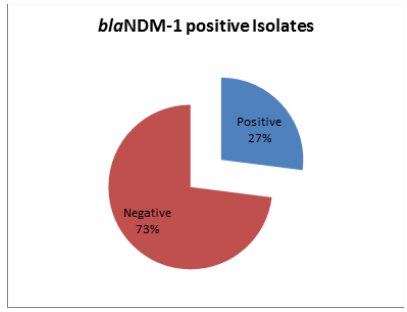



**Table 1: Age wise Distribution of blaNDM-1 positive ESBL *E.coli* isolates:**

| Age | Number | Percentage |
|---|---|---|
| 0-1mo | 00 | 00% |
| 1mo-18yrs | 02 | 7.40% |
| 18-30yrs | 03 | 11.11% |
| 31-45yrs | 06 | 22.22% |
| 46-60yrs | 10 | 37.03% |
| >60yrs | 06 | 22.22% |
| Total | 22 | 100% |

Out of 27(100%) blaNDM-1 positive ESBL *E.coli* clinical isolates, maximum number were from patients in the age group of 46-60 years, accounting for 37.03%, which was followed by the age groups 31-45 years and >60 years [06(22.22%)] and 18-30 years accounting for 3 (11.11%) and no Ndm-1 gene ESBL E.coli was isolated from 0-1Month respectively.

**Table 2: Department wise Distribution of blaNDM-1 positive ESBL *E. coli* clinical isolates:**

| Serial Number | WARDS/ Department | Number Number | Percentage Percentage |
|---|---|---|---|
| 1 | OPD | 04 | 14.81% |
| 2 | Medicine Ward | 02 | 7.40% |
| 3 | ICU's | 08 | 29.62% |
| 4 | Nephrology Ward | 01 | 3.70% |
| 5 | Orthopaedics ward | 02 | 7.40% |
| 6 | Surgery ward | 03 | 11.11% |
| 7 | Urology | 04 | 14.81% |
| 8 | Paediatric ward | 01 | 3.70% |
| 9 | OBG | 02 | 7.40% |
| Total | | 27 | 100% |

Maximum number of blaNDM-1 ESBL E.coli were isolated from various ICU's- 08(29.62%), followed by OPD and Urology – 04 (14.81%), Surgery ward- 03(11.11%), Medicine ward, Orthopaedics ward and OBG accounting for 02(7.40%) each respectively.

**Table 3: Distribution of blaNDM-1 positive *ESBL E.coli* clinical isolates from various ICU's:**

| Serial Number | WARDS/ Department | Number Number | Percentage Percentage |
|---|---|---|---|
| 1 | MICU | 02 | 7.40% |
| 2 | ICCU | 04 | 14.81% |
| 3 | RICU | 01 | 3.70% |
| 4 | SICU | 01 | 3.70% |
| 5 | PICU | 00 | 0.0% |
| 6 | NICU | 00 | 0.0% |
| Total | | 08 | 29.62% |

Maximum number of blaNDM-1 ESBL E.coli were isolated clinical from ICCU-07(14.81%), followed by MICU-02(7.40%), RICU and SICU each accounting for 01(3.70%) and no NDM-1 ESBL E.coli were isolated from both PICU and NICU respectively.

**Table 4: Sample wise Distribution of bla NDM-1 positive ESBL E.coli isolates:**

| Sl No. | Sample | Number | Percentage |
|---|---|---|---|
| 1 | Bile | 01 | 3.70% |
| 2 | Blood | 01 | 3.70% |
| 3 | ET secretions | 02 | 7.40% |
| 4 | Pus | 04 | 14.81% |
| 5 | Urine | 19 | 70.37% |
| | Total | 27 | 100% |

Among the *blaNDM-1* positive *ESBL E.coli* isolates, maximum were from urine-19(70.37%), Pus-04(14.81%), followed by Endotracheal secretions-02(7.40%), Blood and Bile each accounting for 01(3.70%).

**Figure 2 : Phenotypic Detection of MBL in *bla*NDM-1 positive ESBL *E.coli* isolates:**

In present study, among 27 blaNDM-1 ESBL *E.coli* positive isolates, 16(59%) were MBL producers and 11(41%) were MBL non-producers by phenotypic tests.

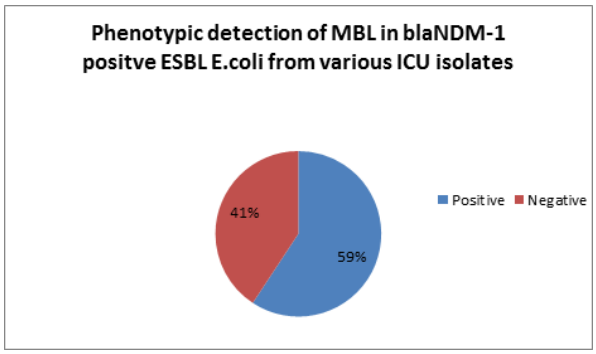
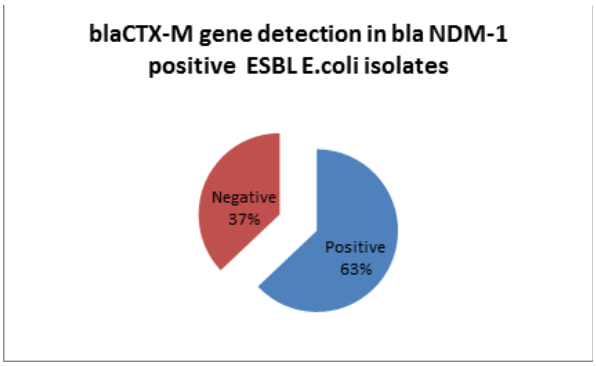



**Figure 3 : Genotypic Detection of *bla*CTX-M in *bla*NDM-1 positve ESBL *E.coli* isolates:**

In this current study, genotypic detection of blaCTX-M gene in bla NDM-1 positve ESBL *E.coli* isolates was detected by Polymerase Chain Reaction, in which 17(62.96%) were Positive and 10(37.03%) were Negative respectively.




**Discussion:**

*Escherichia coli* are emerging as an important cause of extra-intestinal infections in our hospitals. The growing increase in the rate of antibiotic resistance of these isolates is a major cause of concern. β-lactams have been the mainstay of treatment for serious infections, the most active of these being carbapenems, which are advocated for use in treatment of infections caused by ESBL producing Enterobacteriaceae, particularly *Escherichia coli* and *Klebsiella pneumoniae*. Pathogens that produce ESBL or AmpC β lactamases along with carbapenamases are particularly challenging for clinicians and are a major threat worldwide.

Extensive use of β- lactam antibiotics in hospital and community has created a major problem leading to increased morbidity, mortality and health care costs. ESBL production confers resistance to all the beta-lactam antibiotics, except Carbapenems and Cephamycins. In addition, ESBL encoding plasmids also carry genes which encode resistance to other class of antibiotics such as fluoroquinolones, aminoglycosides and sulfonamides. Thus, limited antibiotic choices are available for the treatment of infections caused by these strains. In these circumstances, it is imperative to quantify the problem and reinforce guidelines promoting appropriate antibiotic use.

Carbapenemases are diverse enzymes that vary in their ability to hydrolyze carbapenems and other beta-lactams. Detection of carbapenemase is a crucial infection control issue because they are often associated with extensive antibiotic resistance, treatment failures and infection-associated mortality. Among the beta-lactamases, the carbapenemases, especially transferrable metallo-beta-lactamases (MBLs) are the most feared because of their ability to hydrolyze virtually all drugs in that class, including the carbapenems.

In present study, 16(59%) of ESBL *E.coli* were positive for phenotypic tests for Metallo-β-Lactamase(MBL) production by Modified Hodge Test and Disc Diffusion Synergy test for 27(100%) blaNDM-1 positive isolates. Our study is comparable with Arjit Bora *et al*,.[3] (2012) ascertained that 18.98% were positive for carbapenemase production ; Vijaya Doddiah *et al*,.[4] (June 2012 - March 2013) concluded that 22.85% of *E.coli* were positive for phenotypic detection of metallo beta lactamase enzyme ; Arindam Chakraborty *et al*,.[5] (2013-14) observed that 9.5% of Extraintestinal *E.coli* isolates were carbapenemase producers; Mita D Wadekar, K. Anuradha *et al*,.[6] (2013) - 18% of Enterobacteriacae were MBL producers, 13.4% of E.coli were MBL producers; Kalpana Chauhan *et al,*.[7] (2014) 15% of *E.coli* were carbapenemase producers. Discordant with our study, Pandya *et al,*.[8] (2011) carbapenemase *E.coli* 2.87%, Rai et al,.[9] (2011) carbapenemase *E.coli* 50%.

Like other metallo-β-lactamases, NDM-1 inactivates all β-lactams (including carbapenems) except monobactams. It is the most recently discovered carbapenemase that is spreading rapidly worldwide. NDM-1 producers have been identified mainly in the United Kingdom, India, and Pakistan, but numerous studies within the last year reported NDM-1 producers from many countries in Europe, Asia, Africa, Australia, and North America.

In our study, prevalence of *bla*NDM-1was found to be 27% of ESBL *E.coli.* We observed that the carbapenem resistant ESBL *E.coli* was mainly from 19(70.37%) Urine isolates, 04(14.81%) from Pus, 02(7.40%) from endotracheal secretions, 01(3.70%) each from Blood and Bile. Identical to our study Karthikeyan Kumaraswamy *et al*,.[10] investigated and reported that 25.3% of *E.coli* were NDM-1producers; Martha Fidelis Mushi *et al*,.[11] (2012) concluded that 22% of *E.coli* isolates were positive for blaNDM-1 and A.Bora *et al*,.[12] (August 2009- July 2010) and told that all the screen positive 14 isolates were found to be positive for plasmid mediated *bla*NDM-1 gene by PCR. In another study Shenoy *et al*,.[13] (2013) reported that 14.7 per cent of E. coli isolates were NDM-1 positive

In discordance with our study Arindam Chakraborty *et al*,.[5] (2012) reported 6% prevalence of NDM-1 E.coli; Kumari Seema *et al*,.[14] (2011) reported 4% prevalence of NDM-1 *E.coli* in a tertiary care hospital; Deshpande P *et al*,.[15] reported 6-8% prevalence of NDM-1 *E.coli*.

In present study, we obtained 16(59%) positivity with phenotypic test for MBL ESBL *E.coli* and 27(27%) positivity with genotypic test for MBL. Sensitivity of phenotypic test for MBL is 72% and Specificity of test is 99%. This observation is comparable with Diana Doyle *et al*,.[16] (2012) reported that Modified Hodge Test had sensitivity of 58% and Specificity of 93% ; Anjana Shenoy *et al*,.[13] (2014) conducted a study at Dept of Neuromicrobiology NIMHANS and concluded that MHT is 81% sensitive and specificity 99%. Also Nirav P Pandya *et al*,.[17] (2011) conducted a study and told that DDST results is more subjective as it depends upon expertise to discriminate true synergism from the intersection of inhibition zones.

Based on these findings, genotypic assay could be considered in the diagnostic workflow as confirmatory method for carbapenemase production and/or as an identification tool for the most important different carbapenemase genes. When the presence of a carbapenemase is suspected, PCR is the fastest way to determine which family of β-lactamase is present. This finding is supported by Simone Ambretti *et al,*.[18] (2012); Anjana Shenoy K. *et al*[13] (2014); J. Hrabak *et al*,.[19] (2014 ). *E.coli* with NDM-1 carbapenamase is highly resistant to many antibiotic fluoroquinolones - main classes of antibiotic classes for treatment of Gram negative bacterial infections.

In this current study, genotypic detection of blaCTX-M gene among blaNDM-1 positve ESBL *E.coli* isolates was detected by Polymerase Chain Reaction, in which 17(62.96%) were Positive and 10(37.03%)

were Negative respectively. Among them 12(44.44%) isolates - Urine, followed by 02(7.40%) isolates - Endotracheal secretions and 02 isolates – Pus.

In concordance to our study, Jesus Rodriguez Bano *et al*,.[21] (2004) reported *bla*CTX-M type ESBL *E.coli* - 70%; Meeta Sharma *et al*,.[22] (2013) in the study detected 80% of *bla*CTX-M gene in *E.coli* by PCR. According to another study by Vaida *et al,.*[23] who reported *bla*CTX-M encoding genes in majority of ESBL *E.coli* -96%. In discordant with our study, Bali et al,.[119]detected 22.75% *bla*CTX-M gene in *E.coli*. According to King -Ting Lim *et al.*[121], majority of the ESBL-positive isolates from Malaysia harbored TEM-1 (88%), which is quite high as compared to this study, but they reported prevalence of *bla*CTX-M (20%) and SHV (8%) which is very low as compared to this study.

Enterobacteriaceae contribute to a major part of the gut flora. Just like other bacteria, blaNDM-1 producing *E.coli* are capable of colonising the gut of patients. They in turn serve as reservoirs for spreading infection or contaminating the environment and fomites, especially in healthcare settings. In order to control the spread, disinfection measures need to be followed as contact isolation of these infected/colonised patients is not routine and may not be feasible in all healthcare institutions, especially in the developing countries. Microbiological surveillance of the rectal flora at the time of admission (especially in patients who have already been exposed to antibiotics and healthcare interventions) and contact isolation of potentially colonised/infected patients will go a long way in preventing contamination of the environment and spread to other patients. In addition, appropriate use of carbapenems will also prevent selecting resistant bacteria in a given geographical area.

## CONCLUSION:

*NDM-1* producing strains appear to be an emerging worldwide problem. Based on our findings, we conclude that genotypic assay could be considered in the diagnostic workflow as confirmatory method for carbapenemase production and/or as an identification tool for the most important different carbapenemase genes. When the presence of a carbapenemase is suspected, PCR is the fastest way to determine which family of β-lactamase is present. Hence timely detection of *NDM-1* would help in the appropriate antibiotic therapy.

**Acknowledgment:** We are great full to the JSS University, Mysore, for providing financial Assistance

**Image 1: Showing Modified Hodge Test:**

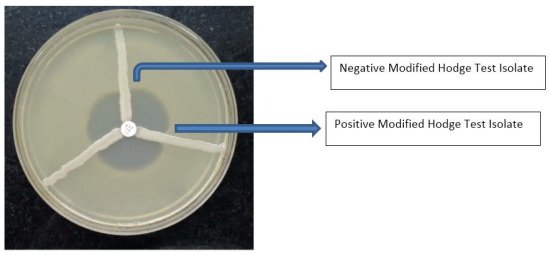



**Image 2: Showing Double Disk Synergy Test:**

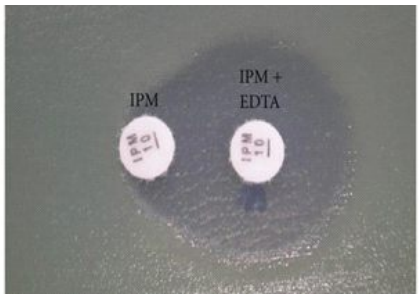
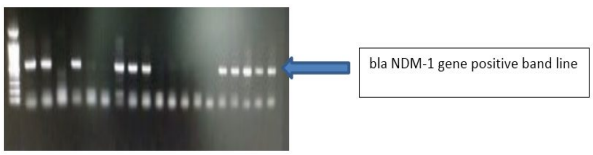



**Image 3: Showing bla NDM-1gene positive Gel electrophoresis:**




## References:


1. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR (December 2009). "Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India.
2. Clinical Laboratory Standards Institutes.(2013.) Performance Standards for antimicrobial susceptibility testing, XVI International Supplement (M100-S16). Wayne, Pennsylvania, USA: National Committee for Clinical Laboratory Standards 2013.
3. Centers for disease control and prevention. Modified Hodge test for carbapenemase detection in enterobacteriaceae. Available at http://www.ndhealth.gov/microlab/Uploads/HodgeTest.pdf.
4. Bora A, Ahmed G U, Hazarika N K, Prasad K N, Shukla S K, Randhawa V, Sarma J B.(2013) Incidence of blaNDM-1 gene in Escherichia coli isolates at a tertiary care referral hospital in Northeast India. Indian J Med Microbiol ;31:250-6
5. Vijaya Doddaiah, Dhanalakshmi Anjaneya. (2014) Prevalence of ESBL, AmpC and Carbapenemase among Gram Negative Bacilli Isolated from Clinical Specimens. American Journal of Life Sciences. Vol. 2, No. 2, , pp. 76-81. doi: 10.11648/ j.ajls. 20140202.17
6. Arindam Chakraborty, Prabha Adhikari, Shalini Shenoy, Shrikala Baliga, Sathish Rao, Dhanashree B.(2012) Extra-intestinal β-lactamase Producing Escherichia coli Infection-an Emerging Infection in a South Indian Tertiary Care Hospital ID: JCDR/ 2012/4156:2471.
7. Wadekar MD, Anuradha K, Venkatesha D.(2013) Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. Int J Current Res Acad Rev. 2013; 1(3):89-5.
8. Chauhan K, Pandey A, Asthana AK, Madan M.(2015) Evaluation of phenotypic tests for detection of Klebsiella pneumoniae carbapenemase and metallo-beta- lactamase in clinical isolates of Escherichia coli and Klebsiella species. Indian J Pathol Microbiol 2015;58:31-5.
9. Nirav P. Pandya, Sweta B. Prajapati, Sanjay J. Mehta, Kunjan M. Kikani and Pratima J. Joshi.( 2011). Evaluation of various methods for detection of metallo-lactamase (MBL) production in gram negative bacilli. Int. J. Biol. Med.Res. 2(3): 775-777.
10. Rai S, Manchanda V, Singh NP, Kaur IR.(2011) Zinc-dependent carbapenemase in clinical isolates of family Enterobacteriaceae. Indian J Med Micobiol. 2011; 29:275-9.
11. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al.(2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan and the UK: a molecular, biological and epidemiological study. Lancet Infect Dis, 2010; 10 (9): 597-602.
12. Martha Fidelis Mushi (2013)- Carbapenem Resistance Determining Genes Among MDR Gram Negative Bacterial Clinical Isolates From Bugando Medical Cetre - Tanzania, 2013
13. Bora A, Ahmed G U, Hazarika N K, Prasad K N, Shukla S K, Randhawa V, Sarma J B. (2013) Incidence of blaNDM-1 gene in Escherichia coli isolates at a tertiary care referral hospital in Northeast India. Indian J Med Microbiol 2013;31:250-6
14. Shenoy KA, Jyothi EK, Ravikumar R.(2014) Phenotypic identification & molecular detection of blandm-1 gene in multidrug resistant Gram-negative bacilli in a tertiary care centre. The Indian Journal of Medical Research. 2014; 139(4): 625-631.
15. Seema K, Ranjan Sen M, Upadhyay S, Bhattacharjee A.(2011) Dissemination of the New Delhi metallo-β lactamase-1 (NDM-1) among Enterobacteriaceae in a tertiary referral hospital in north India. J Antimicrob Chemother 2011; 66:1646-7.
16. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R.(2010) New Delhi Metallo-beta lactamase (NDM-1) in Enterobacteriaceae: Treatment options with carbapenems compromised. J Assoc Physicians India 2010; 58:147-9.
17. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JDD.(2012) Laboratory Detection of Enterobacteriaceae That Produce Carbapenemases. Journal of Clinical Microbiology. 2012;50(12):3877-3880. doi:10.1128/JCM.02117-12. 129.
18. Nirav P. Pandya, Sweta B. Prajapati, Sanjay J. Mehta, Kunjan M. Kikani and Pratima J. Joshi. (2011). Evaluation of various methods for detection of metallo- -lactamase (MBL) production in gram negative bacilli. Int. J. Biol. Med.Res. 2(3): 775-777.
19. Ambretti S, Gaibani P, Berlingeri A et al.(2013) Evaluation of phenotypic and genotypic approaches for the detection of class A and class B carbapenemases in Enterobacteriaceae. Microb Drug Resist2013;19: 212-5
20. Hrabak J, Chudackova E, Papagiannitsis CC.(2014) Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. Clin Microbiol Infect 2014; doi: 10.1111/1469-0691.12678.
21. Rodríguez-Baño J, Navarro MD, Romero L, Martinez-Martinez L, Muniain MA, et al,.(2004). Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing Escherichia coli in nonhospitalized patients. J Clin Microbiol 42:1089-1094.10.1128/ JCM.42.3. 1089-1094.2004
22. Sharma M, Pathak S, Srivastava P.(2013) Prevalence and antibiogram of Extended Spectrum β-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing Escherichia coli and Klebsiella spp. Journal of Clinical and Diagnostic Research : JCDR. 2013;7(10):2173- 2177. doi:10.7860/ JCDR/ 2013/6460.3462.
23. Vaida S, Marius L, Aurelija B, Justas P, Rita P, Agne G, et al.(2010) Molecular characterization of extended-spectrum b-lactamase producing Escherichia coli and Klebsiella pneumoniae isolates from hospitals in Lithuania. Journal of Medical Microbiology. 2010;59(10):1263-65
24. Bali EB, Ac KL, Sultan N.(2010) Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum-lactamase produced by Escherichia coli, Acinobacter baumannii and Klebsiella isolatesin a Turkish hospital. African Journal of Microbiology Research. 2010 April; 4(8):650-54.
25. Lim KT, Yasin R, Yeo CC, Puthucheary S, Thong KL.(2009) Characterization ofa Multidrug Resistant ESBL-Producing Escherichia coli Isolates from Hospitals in Malaysia. J Biomed Biotechnol. 2009;165637:10.pages.