ORIGINAL RESEARCH PAPER

Microbiology

DETECTION OF CTX-M AND NDM-1 GENE IN CLINICAL ISOLATES OF E.COLI.

KEY WORDS: Carbapenems, Extended spectrum beta-lactamase(ESBL), Modified Hogde Test(MHT), Polymerase Chain Reaction(PCR).

Dr. Tejashree A. Professor, Department of Microbiology, JSS Medical College, Mysore.

Dr. Deepashree R. M.D. Post graduate, Department of Microbiology, JSS Medical College, Mysore.

Dr. Devanad D Assistant Professor, Department of Biochemistry, JSS Medical College, Mysore.

Archana Hegde M Research Scholar, Department of Microbiology, JSS Medical College, Mysore. - Corresponding author

ABSTRACT

During the last few years, CTX-M has become the predominant ESBL family and CTX-M-producing *E. coli* has spread globally and has been involved in nosocomial outbreaks and community acquired infections. The emergence of bacterial strains that produce carbapenemases further limits the therapeutic options available to clinicians. Currently, bacteria receiving the most attention are New Delhi metallo-beta-lactamase-1(NDM-1) producing superbug that confers resistance to most antibiotics including carbapenems. This study was undertaken to know the prevalence of MBL *E.coli* in tertiary care centre: 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.

Objectives: The present study was undertaken to assess the Phenotypic Identification of ESBL and MBL *E.coli* and to detect *bla*CTX-M & *bla*NDM-1 gene in a tertiary care hospital.

Materials and 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. Urine, Pus, ET secretions, BAL, Blood and other Body fluids received in the laboratory were subjected to routine processing as per standard operating procedures. Antimicrobial susceptibility testing was performed as per the CLSI guidelines (2013)2 by modified Kirby Bauer method. Double disk synergy test (DDST), Modified Hodge test, EDTA-Disk Diffusion Synergy Test as phenotypic tests. Genotypically PCR was carried out for the detection of *bla*CTX-M and *bla*NDM-1 genes.

Result: Total of 1,579 *E.coli* were isolated from various clinical samples. Out of these, 190 randomly selected isolates were tested for phenotypic identification of ESBL. Among the 190 isolates, 142(74%) were positive for ESBL production. In these, 100 non repetitive isolates were randomly selected for *bla*CTX-M and blaNDM-1gene detection. In which, 67(67%) *bla*CTX-M gene and 26(26%) NDM-1 gene were detected by conventional PCR from ESBL E.coli isolates. 16(16%) of Metallo-β lactamase (MBL) production was detected by phenotypic tests for ESBL *E.coli* isolates.

Conclusion: PCR analysis determined that 67(67%)ESBL E.coli isolates were positive for *bla*CTX-M. 16(16%) of ESBL *E.coli* were positive for phenotypic tests for metallo-β lactamase production by Modified Hodge Test and Disc Diffusion Synergy test. By PCR analysis NDM-1 was found to be positive in 26(26%) of ESBL *E.coli*. The increasing frequency of carbapenemase-producing bacteria underlined the necessity of having tools available to monitor the emergence and the spread of each family of carbapenemase gene types. Phenotypic tests though specific, do not differentiate between chromosomal and plasmid encoded genes and hence genotypic characterization should be considered.

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.

The first β-lactamase, Penicillinase was identified in *Escherichia coli* prior to the release of penicillin for use in medical practice. Beta lactamases seen in *E.coli* are SHV, VEB-1, OXA, CTX-M, NDM-1. During the last few years, CTX-M has become the predominant ESBL family and CTX-M-producing E. coli has spread globally and has been involved in nosocomial outbreaks and community acquired infections.

Carbapenem antibiotics are considered the drugs of choice for the treatment of extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and other multidrug resistant bacteria.[1] The emergence of bacterial strains that produce carbapenemases further limits the therapeutic options available to clinicians. Currently, 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. 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 in tertiary care centre: 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. The present study was undertaken to assess the Phenotypic Identification of ESBL and MBL E.coli and to detect *bla*CTX-M & blaNDM-1 gene in a tertiary care hospital in Karnataka.

Materials and Methods :

This was a hospital based prospective study, undertaken in the department of Microbiology, This study was undertaken as part of the JSS University funded Project. 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 only are included for molecular detection of CTX-M and NDM-1 gene. The samples collected were processed as per standard methods. The study protocol was approved by the ethics committee of the institute.

Urine, Pus, ET secretions, BAL, 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 with Beta haemolytic, grey, moist, opaque colony. Followed by MacConkey Agar showing flat, dry, pink colonies. 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.

Antimicrobial susceptibility testing was performed as per the CLSI guidelines (2013)2 by modified Kirby Bauer method. Mueller-Hinton agar (Hi-media laboratories Pvt, Ltd. Mumbai) was prepared from dehydrated base as per manufacturer's recommendations. Commercially available antibiotic disks (Hi-media laboratories Pvt. Ltd. Mumbai) with appropriate diameter and potency were used. All the strains were tested for their sensitivity to antimicrobial drugs using recommended CLSI guidelines (2014) 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 methods of ESBL detection:[2]

Screening method: Isolate considered a potential ESBL producer if the test results were as follows, Antibiotic with its respective zone size: Ceftazidime(≤ 22 mm), Cefotaxime(≤ 27 mm) and Ceftriaxone(≤ 25 mm). Followed by confirmatory methods like CLSI Phenotypic confirmatory Disk diffusion test : Combined disk method was used to confirm the presence of ESBL. The isolates were tested for ESBL production by the combination disk diffusion method using Ceftazidime (30µg), Ceftazidime/Clavulanic acid (30/10 µg). ≥ 5 mm increase in the zone diameter for either antimicrobial agent tested in combination with Clavulanic acid versus its zone when tested alone confirms production of ESBL by isolate. Double disk synergy test (DDST): Double disk synergy test (DDST) was performed on an agar plate with a disk containing Cefotaxime (30µg) and a disk containing Piperacillin / Tazobactam (100µg/10µg respectively), placed 30 mm apart (centre to centre). Extension of the inhibition zone around the Cefotaxime disk towards the Piperacillin / Tazobactam disk indicates production of ESBL. In some cases, the ESBL positivity was further confirmed by the double disk diffusion method. Escherichia coli strains ATCC 25922 was used as quality control strains for the DST.

Phenotypic Detection of MBL - Detection methods :

I. Modified Hodge test: 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 MHA plate and allow to dry 3–5 minutes. Place a 10 µg 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.

CTX - M and NDM-1 Gene Detection:

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 blaCTX-M and blaNDM-1 gene was identified by PCR using primers.

i.*bla*CTX-M : 5 ---> 3 550bp
CGCTTTGCGATGTGCAG
ACC GCG ATA TCG TTG GT

ii.*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, AnnealingTemperature: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%) were positive for ESBL production. In these, 100 non repetitive isolates were randomly selected as sample size.

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

Age	Number	Percentage
0-1mo	5	5
1mo-18yrs	16	16
18-30yrs	10	10
31-45yrs	21	21
46-60yrs	27	27
>60yrs	21	21
Total	100	100

Out of total 100 ESBL E.coli clinical isolates subjected for resistance pattern analysis, maximum number were from patients in the age group of 46- 60 years, accounting for 27 %, which was followed by the age groups 31-45 years and >60 years which accounting for 21% each respectively.

Table 2: Department wise Distribution of clinical isolates

Serial Number	WARDS/ Department	Number Number	Percentage Percentage
1	OPD	13	13
2	Medicine Ward	14	14
3	MICU	4	4
4	ICCU	7	7
5	RICU	1	1
6	Pulmonology Ward	1	1
7	Nephrology Ward	3	3
8	Emergency Ward	3	3
9	Neurology Ward	2	2
10	Ortho ward	5	5
11	SICU	6	6
12	Surgery ward	14	14
13	Urology	6	6
14	Gastroenterology unit	1	1
15	PICU	1	1
16	Paediatric ward	11	11
17	OBG	5	5
18	NICU	3	3
Total	100	100	

Maximum clinical isolates were from medicine and surgery ward (14%),

Outpatient department (13%) followed by Pediatric ward (11%), ICCU (7%), SICU(6%), Urology (6%), OBG (5%), Nephrology (3%), Emergency ward (3%),NICU(3%), PICU (1%), Gastroenterology unit (1%), Pulmonology ward (1%).

Table 3: ICU/Non ICU Distribution of ESBL E.coli isolates:

Serial Number	Ward	Number	Percentage
1	ICU	22	22
2	Non- ICU	78	78
Total	100	100	

Among the clinical isolates received 78% were from NON ICU and 22% from ICU.

Table 4: Sample wise Distribution of Isolates

Serial number	Sample	Number	Percentage
1	Urine	63	63
2	Exudates	23	23
3	ET secretions	5	5
4	Blood	4	4
5	Bile	2	2
6	Ascitic Fluid	2	2
7	BAL	1	1
	Total	100	100

Among the isolates maximum were from urine (63%), Exudates (23%),
followed by Endotracheal secretions (5%), Blood (4%), Bile (2%), Ascitic Fluid (2%), BAL (1%),

Fig 1 : Genotypic Detection of blaCTX-M in ESBL *E.coli*

In the present study, in 67% of ESBL E.coli isolates blaCTX-M gene was detected by conventional PCR.

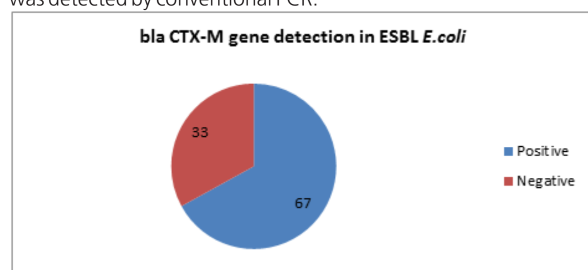


Fig 2 : Phenotypic Detection of MBL in ESBL *E.coli*

In present study, MBL was detected by phenotypic tests in 16% of ESBL *E.coli* isolates.

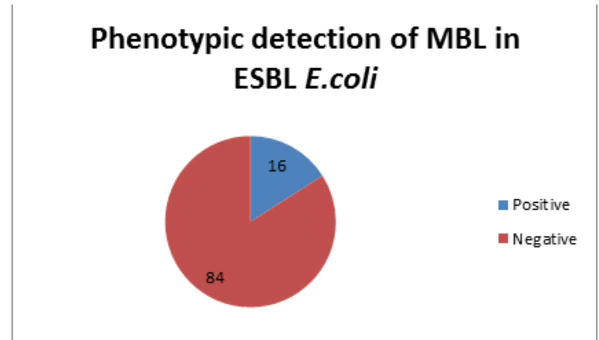
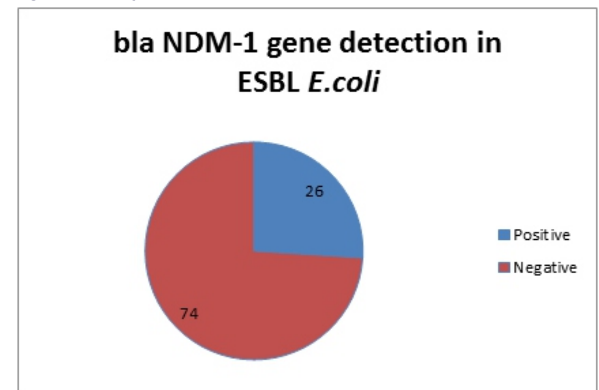


Fig 3 : Genotypic Detection of blaNDM-1 in ESBL *E.coli*



In the present study, in 26% of ESBL E.coli isolates NDM-1 gene was detected by conventional PCR.

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.

In the present study, an attempt was made to know the drug resistance pattern of *E.coli* in various clinical isolates in tertiary care hospital. Most of the samples we received were from the patients in the age group of 46-60 years accounting for 27%. This was followed by age group of 31-45 & >60years accounting for 21% each. Among clinical samples included in the study, males contributed to 65% of the cases and females accounting for 35%. Our studies is comparable with observations made by Arindam Chakraborty et al[5] (2012) who reported that age group between 45-60years contributed to 30% & age group between 19-45years contributed to 23% of ESBL producers. In same study it was also

reported that 56% of patients were male and 44% were female.

Concurrent observations were made in two other studies; Mark Melzer et al.[3] (2005) reported that age group 50-70years - 32.6% & Male - 60.9%, Female -39.1% and Metri Basavaraj et al.[4] (2011) reported maximum ESBL producers were seen in age group of 41-60years.Most of the samples in our study were from Medicine and Surgery ward (14%) followed by Outpatient department (13%), Paediatrics (11%), ICCU (7%), SICU & Urology (6%).

Complementary findings were shown by study of Prabha Adhikari et al.[5] (2012) who reported that majority of ESBL clinical samples were from Medicine -16%, Surgery - 14%. Similar observations were made by Metri Basavaraj et al.[4] (2011) Medicine 10.9%, Surgery 8.9%, and ICCU & SICU 5%. In present study randomly selected 100 patients with Extraintestinal ESBL E.coli infections were included. Among them 25% cases were diagnosed of urinary tract infections, 18% had metabolic disorders, 15% with skin and soft tissue infections, 5% Respiratory infections, 37% Others.

Corresponding observations was made by Shalini Shenoy et al., (2012)[5] reported 53% urinary tract infections, 13% skin and soft tissue infections, 6.3% Respiratory infections, 27% others. Maximum ESBL E.coli isolates were from Urine (63%), Exudates (23%),Endotracheal secretions (5%), Blood (4%), Bile (2%), Ascitic Fluid (2%) and BAL (1%).

Concurrent observations were seen in the study conducted by John N Kateregga et al,.[6] (2014), isolates from urine (63.9%), Exudates (9%), Blood (4%). Another study Vipul M Khakhkhar et al.[7] (2011) reported that ESBL isolates were from Urine (77%), Exudates (25%), and other body fluids (20%). In the last few years, the emergence and wide dissemination of *E. coli* strains showing resistance to broad-spectrum of antimicrobial agents has been reported.

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria.42-46

According to the European Centre for Disease Prevention and Control (ECDC) and the Centres for Disease Control and Prevention (CDC), multi-drug resistant (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial categories. MDR bacteria are the principal cause of failure in the treatment of infectious diseases.[8] Of the 100 ESBL E.coli isolates tested, 61 (61%) isolates were Multidrug Resistant. MDR in Urinary isolates - 41%, Exudates - 56%, endotracheal aspirate - 60%, Blood - 50%, Bile - 100%.

Our findings are correlating with Ibrahim ME et al.[8](2011) reported prevalence of *E.coli* recovered from various clinical specimens - Urine (65.1%) and wound (22%) specimens represented the majority of specimens. Concurrent studies by Niranjan V et al.[9] (2014) reported 56.8% uropathogens are MDR; Hanna E Sidjabat et al.[10] (2015) reported 60-79% of MDR *E.coli.*

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 was randomly selected as sample size.

In other studies of Sridhar PN Rao et al,[11] conducted a multicentric study across Karnataka - part of India SENTRY surveillance (May 2009 - September 2012)-estimated that 61.4% of *E.coli* and 46.21 % of K.pneumoniae was positive for phenotypic confirmatory tests for ESBL which is correlating with the present study. Collateral studies by Umadevi , Kandhakumari G et al,.[12] (Feb 2008 - Jan 2009) concluded that 81% of *E.coli* and 74% of K.pneumoniae are ESBL producers. Bhavanarushi Sreekanth et al.,[13] conducted a study in DM Wayanad institute of medical sciences, concluded that 72% were ESBL producers.

In our study we found 74% isolates produced ESBL. Arindam Chakraborty et al,.[14] conducted a study (2013-14) in Govt Medical College, Uttar Pradesh and Manipal University to know the prevalence of ESBL. They observed that 70.5% isolates were found to be ESBL producers. Arjit Bora et al,.[15] conducted a study in a tertiary care teaching hospital in Northeast India between August 2009 - July 2010 and concluded that 73.5% of E.coli isolates were positive for phenotypic confirmatory tests.

In contrary to our study, Vijaya Doddaiah et al,.[16] (2014), reported ESBL prevalence of 30.71% and Irene Petersen et al,.[18] (2007) concluded that 13% were ESBL producers. CTX-M-type ESBLs have emerged within the community, particularly among E. coli and is responsible in causing wide array of infections. Livermore et al.,[19,20] stated in separate studies that the CTX-M gene is the most prevalent ESBL- encoding gene worldwide and is replacing TEM and SHV types as the predominant ESBL in many European and Asian countries.

By Polymerase Chain Reaction analysis 67(67%) isolates were positive for blaCTX-M. Among them 43 isolates - Urine, 15 isolates - Exudates, followed by 3 isolates - Endotracheal secretions , 2 isolates - Blood, 2 isolates - Bile, 2 isolates - Ascitic fluid, 1 isolate - BAL.

In concordance to our study, Jesus Rodriguez Bano et al,.[21] (2004) reported blaCTX-M type ESBL *E.coli* - 70%; Meeta Sharma et al,.[22] (2013) in the study detected 80% of blaCTX-M gene in E.coli by PCR. According to another study by Vaida et al,.[23] who reported blaCTX-M encoding genes in majority of ESBL *E.coli* -96%. In discordant with our study, Bali et al,.[119] detected 22.75% blaCTX-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 blaCTX-M (20%) and SHV (8%) which is very low as compared to this study.

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% of ESBL E.coli was positive for phenotypic tests for metallo-β lactamase production by Modified Hodge Test and Disc Diffusion Synergy test.

Our study is comparable with Arjit Bora et al,.[26] (2012) ascertained that 18.98% were positive for carbapenemase production ; Vijaya Doddiah et al,.[16] (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,.25 (2013) - 18% of Enterobacteriacae were MBL producers, [13] 4% of *E.coli* were MBL producers; Kalpana Chauhan et al,.[27] (2014) 15% of *E.coli* were carbapenemase producers. Discordant with our study, Pandya et al,.[28] (2011) carbapenemase *E.coli* 2.87%, Rai et al,.[29] (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 blaNDM-1was found to be 26% of ESBL *E.coli*.

We observed that the carbapenem resistant ESBL *E.coli* was mainly from 18 Urine isolates, 4 isolates from exudates, 2 from endotracheal secretions, 1 each from Blood and Bile.

Identical to our study Karthikeyan Kumaraswamy et al,.[30] investigated and reported that 25.3% of *E.coli* were NDM-1producers; Martha Fidelis Mushi et al,.31 (2012) concluded that 22% of *E.coli* isolates were positive for blaNDM-1 and A.Bora et al,.[15] (August 2009- July 2010) and told that all the screen positive 14 isolates were found to be positive for plasmid mediated blaNDM-1 gene by PCR. In another study Shenoy et al,.[32] (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,.33 (2011) reported 4% prevalence of NDM-1 E.coli in a tertiary care hospital; Deshpande P et al,.34 reported 6-8% prevalence of NDM-1 *E.coli*.

In present study we obtained 16% positivity with phenotypic test for MBL ESBL E.coli and 26% 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,.35 (2012) reported that Modified Hodge Test had sensitivity of 58% and Specificity of 93% ; Anjana Shenoy et al,.32 (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,.36 (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 -lactamase is present. This finding is supported by Simone Ambretti et al,.37 (2012); Anjana Shenoy K. et al32 (2014); J. Hrabak et al,.38 (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 our study NDM-1 isolates were resistant to most classes of antibiotics except few isolates were sensitive to aminoglycosides and all isolates were sensitive to Glycylglycines and Polymyxins group of antibiotics. This coinciding with other studies Karthikeyan Kumarakrishnan et al,.[30] (2010); S Nagaraj et al,.[39] (2014) ; Webster PC et al,.[40] (2010) ; Deshpande P et al,.[34] (2010); Nordmann P et al,.[41] (2011), Meeta Sharma et al,.[22] (2013).

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.

SUMMARY & CONCLUSION

Total of 1579 E.coli was isolated from various clinical samples over a period of one year from November 2013 - December 2014. 190 isolates was tested for phenotypic identification of ESBL. Out of which 142 (74%) was positive for ESBL production. In these, 100 non repetitive isolates was randomly selected as sample size. Maximum samples were received from were from the patients in the age group of 46-60 years. Most of the samples in our study were from Medicine and Surgery ward (14%). Majority of ESBL E.coli isolates were from Urine (63%), followed by Exudates (23%). Of the 100 ESBL *E.coli* isolates tested, 61 isolates were Multidrug Resistant. By PCR analysis we determined that 67 isolates were positive for blaCTX-M. 16% of ESBL *E.coli* was positive for phenotypic tests for metallo-β lactamase production by Modified Hodge Test and Disc Diffusion Synergy test. By PCR analysis NDM-1was found to be positive in 26% of ESBL *E.coli*.

The increasing frequency of carbapenemase-producing bacteria underlined the necessity of having tools available to monitor the emergence and the spread of each family of carbapenemase gene types. Phenotypic tests though specific, do not differentiate between chromosomal and plasmid encoded genes and hence genotypic characterization should be considered.

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