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ANTIBIOTIC RESISTANCE PATTERN OF ESCHERICHIA COLI WITH DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCERS BY PHENOTYPIC AND GENOTYPIC METHODS FROM VARIOUS CLINICAL SAMPLE IN NORTHEAST INDIA.

Medical Microbiology

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ABSTRACT

Background: Resistance to broad-spectrum beta-lactams, mediated by extended-spectrum beta-lactamase (ESBL) an increasing problem in the present world. It is disseminated along with resistant E. coli strains especially IIIrd generation cephalosporins resistant strains and extended spectrum beta-lactamase strains (ESBLs) in combination with genetic subtype. **Aims & Objectives:** Drug resistance among E. coli organisms become a challenge in the medical field. To determine the phenotypic and genotypic characters ESBL producer E.coli from clinical samples comes to our diagnostic center for investigation. **Methods:** This descriptive study was carried out in our laboratory estimation of ESBL by Phenotypic methods Screening test by Double disc synergy test (DDST) and confirmatory method, Phenotypic confirmatory disc diffusion test (PCDD). That drug resistance patterns and genotypic method by RT PCR molecular method. **Results:** Out of 400 E. coli isolates in all clinical samples, the urine sample is most common in my study (46.5%), and less common is the blood sample (10.5%). The phenotypic confirmatory test for detection of ESBLs producing E. coli by DDST and PCDDT is 68.8% & 62.5% respectively. Showed resistance in total isolates to the third generation Cephalosporins Cefotaxime, Ceftazidime, 250 (62.5%), and Imipenem 74 (18.4%). The genotyping results after phenotype of ESBL producing isolates obtained by PCR amplification of NDM, SHV, CTX-M, KPC and TEM genes. Out of ESBL positive E.coli, 189 (75.7%) were carrying NDM, 129 (51.6%) blaKPC, 123 (49.2%) and (87.1%) blaTEM genes, while less gene identify by genetically 99 (39.5%) blaCTX-M and 54 (21.5%) blaSHV isolates included in this study. **Conclusions:** Other than phenotypic methods for screening of ESBL E.coli can be done by RT-PCR molecular method which gives confirm the result with genetic variation and gives 99.99% specificity and sensitivity.

KEYWORDS

ESBL, phenotypic, genotypic, RT-PCR, antibiotic resistance, specificity, and sensitivity.

INTRODUCTION

One of the most important resistant mechanisms in Gram-negative bacteria against beta-lactam antibiotics third-generation cephalosporin is induced by the production of beta-lactamase enzymes [1]. The new broad-spectrum antibiotics such as Cephalosporin and carbapenems used in the treatment of pathogenic infections have led to the production of a new class of broad-spectrum enzymes called beta-lactamase [2].

In the current situation, the occurrence of bacterial genetic mutations in the sequence of the primary beta-lactamase gene results in the production of different enzymes [3]. Beta-lactamase enzymes are classified into mainly four groups A, B, C, and D based on their inhibitory process and mechanism, type of substrate, and physical characteristics such as molecular weight and isoelectric point. According to this classification, broad-spectrum beta-lactamases are categorized in group A [4-5]. The group of Enterobacteriaceae bacteria has rapidly and fastly expanded resistance to broad-spectrum beta-lactam antibiotics during the past two decades[6].

Antimicrobial resistance genes of the ESBL type are mostly plasmid associated and therefore can spread among bacteria and they also harbor resistant genes to other antimicrobial classes with resulting multidrug-resistant isolates[7-8].

The detection of E. coli is of importance for infection control, reduction in the use of antimicrobial agents, and epidemiological surveillance. The ESBL-producing E. coli detection by the phenotypic method or the genotypic method by PCR. Since different results have been achieved by different phenotypic methods used [9], the genotypic methods seem to be necessary for the accurate identification of such resistant strains.

This study was to detect third-generation cephalosporin resistance-producing E. coli isolated and detect ESBL producer *E.coli* from all clinical samples and compare the beta-lactam gene variations obtained by the phenotype and genotype methods.

MATERIALS AND METHODS

Sample Collection

A total of 400 E. coli samples were obtained from the various cultural samples on blood agar medium and another culture medium, collected from patients with various infectious diseases. The sample collection was done through Medicure diagnostic and research centre located in Hyderabad, all patients were suffering from a high level of various types of infections like urinary tract infection, pyogenic infection, blood stream infection, etc. The *E. coli* strains were confirmed using IMViC biochemical reaction.

ANTIBIOTIC SUSCEPTIBILITY TEST

Isolation and identification of *E. coli* by biochemically, antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method according to follows as CLSI guidelines 2021. Antibiotic discs including Cefotaxime (30 µg), Imipenem, Meropenem, Ciprofloxacin, Colistin, and Ceftazidime (30 µg) (Himedia) were applied for susceptibility test. Ceftazidime and Clavulanic acid were considered potential ESBL producers of E. coli and were further investigated for confirmation of ESBL production by a combination disc diffusion test by PCDDT.

SCREENING FOR ESBL PRODUCING ISOLATES

A Ceftazidime and a Ceftazidime + Clavulanic acid (30 µg/10 µg) discs (Himedia) were placed at a distance of 20-25 mm on a Mueller-Hinton Agar (MHA) (Himedia) plate inoculated with a bacterial suspension with peptone water and compare, 0.5 McFarland turbidity standards and incubated overnight at 37 ºC. Increase zone size and diameter of the inhibitory zone for the combination (Clavulanic acid 10µg) disc versus Ceftazidime disc about more than 5 that is ESBL production. The most antibiotic resistance of all samples to other antibiotics including Imipenem (10µg), Gentamycin (30µg), and Ciprofloxacin (5µg) (Himedia) was also determined.[12]

GENOTYPIC ASSAY

The Boiling method was used to extract DNA from *E. coli* samples in our laboratory. NDM, *bla*SHV, *bla*TEM, *bla*KPC, and *bla*CTX-M beta-lactamase genes were detected by PCR. PCR was carried out using a thermal cycler (Bioserve-India) in a total volume of 25 µl containing 10 pmol of each three pair of primers (Bioserve-India), 25 µmol of dNTPs, 5 µl of template DNA, 2.5 µl of 10X Taq buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3)], 2 mM MgCl2 and 2.5 U of Taq polymerase (Bioserve-India). The Primer sequences and a total of 43 cycling conditions were used in this study. In order to confirm the accuracy of genes amplified in this study, a PCR product of each gene was sent for sequencing to the Medicure diagnostic and research center

in Hyderabad and the result was confirmed by ct values.[10]

RESULTS

Total of 400 *E. coli* isolates was found in male and female patient 70 (17.5%) and 330 (82.5%). Out of *E. coli* isolates collected from different clinical samples, data included, urine samples, 186 (46.5%), pus 66 (16%), swab 14 (14%), and sputum 50 (12.5%). Third generation Cephalosporins Cefotaxime or Ceftazidime to be resistant were included in ESBL. Positive *E. coli*, and further confirmation by combined disk test done on DDST and PCDDT out of 68.8%, & 62.5% respectively. Showed resistance in total isolates to the third generation Cephalosporins Cefotaxime, Ceftazidime, 250 (62.5%), and Imipenem 74 (18.4%). The results also showed that 346 out of 400 (25.2%) isolates were sensitive to all antibiotics tested. The combined disk test was done on 275 isolates resistant to third-generation Cephalosporins and also showed that 250 isolates were ESBL-producing strains.

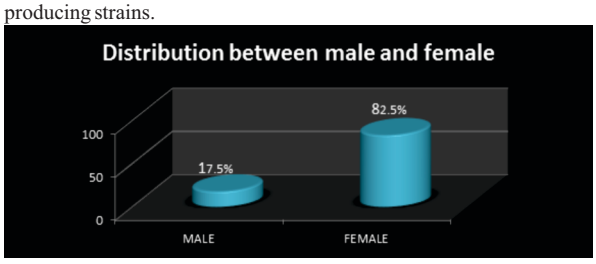

Fig. No 1: Distribution Between Male And Female.

Table No. 1: Distribution According Samples.

S.N.	Sample	Number of Samples	Percentage
1	Urine	186	46.5%
2	Blood	42	10.5%
3	Pus	66	16.5%
4	Swab	56	14%
5	Sputum	50	12.5%

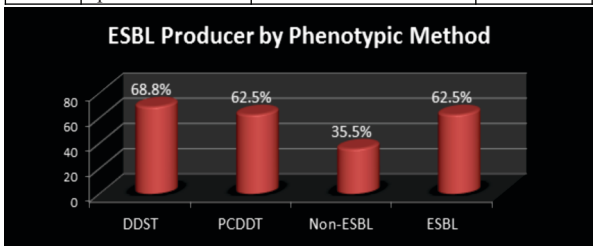

Fig. No. 2: Distribution Between ESBL and Non-ESBL Producer By DDST And PCDDT Method.

The genotyping results after the phenotype of ESBL-producing isolates were obtained by PCR amplification of NDM, SHV, CTX-M, KPC, and TEM genes. Out of ESBL positive E.coli, 189 (75.7%) were carrying NDM, 129 (51.6%) blaKPC, 123 (49.2%) and (87.1%) blaTEM genes, while less gene identify by genetically 99 (39.5%) blaCTX-M and 54 (21.5%) blaSHV isolates included in this study.

Table No. 2: Distribution Between ESBL Producer E. Coli By Genetic Variation.

S.N.	ESBL producer Gene	Total %	P value
1	NDM	75.7%	0.02
2	*bla*KPC	51.8%	0.06
3	*bla*TEM	49.2%	0.07
4	*bla*CTX-M	39.5%	0.01
5	*bla*SHV	21.5%	0.02
P value is significant by statically. (*bla*KPC & *bla*TEM is slightly significant)			

Among total ESBL producers of Escherichia coli, 75.7% showed the presence of at least one ESBL gene. The distribution of at least one gene was seen in isolates having a combination of pairs with primer amplification of genes NDM, blaCTX-M, blaSHV, blaTEM, and blaKPC in one strain. (Table no. 2)

And add length and optical density in all genes (Table No. 3). In PCR Conditions for amplification of NDM, blaCTX-M, blaSHV, blaTEM, and blaKPC genes base pair with SYBR dye for detection of all genes (Table No. 4).

Table No. 3: Primers Used For Amplification Of NDM, *bla*TEM, *bla*SHV, *bla*CTX-M, And *bla*KPC Genes.

S. N.	Gene Detected	Primer (R'-3'5')	Length	ODU
1	NDM	AGCCACCAAAAGCGATGTC	19 Tm_{52}	10.8
2	*bla*KPC	GTTGACGCCCAATCCCTC	18 Tm_{53}	8.5
3	*bla*TEM	AGAAGTAAGTTGGCAGCAGTG	21 Tm_{52}	12.6
4	*bla*CTX-M	GAAGGTCATCAAGAAGGTGCG	21 Tm_{54}	10
5	*bla*SHV	GTCTTATCGGCGATAAACCAAG	21 Tm_{52}	11.5

Table No. 4: PCR Conditions For Amplification Of NDM, *bla*CTX-M, *bla*SHV, *bla*TEM And *bla*KPC Genes Base Pair With SYBR Dye For Detection Of All Gene.

S.N.	Gene Detected	Denaturation Time/temp	Annealing Time/temp	Extension Time/temp	No. of Cycles
1	NDM	94°C-1min	58°C-1½ min	72°C -1min	42
2	*bla*KPC	94°C-1min	58°C-1½ min	72°C -1min	42
3	*bla*TEM	94°C-1min	58°C-1½ min	72°C -1min	42
4	*bla*CTX-M	94°C-1min	58°C-1½ min	72°C -1min	42
5	*bla*SHV	94°C-1min	58°C-1½ min	72°C -1min	42

Table No 5: Distribution Between Antibiotic Sensitive And Resistance Pattern In Total Isolates, ESBL And Non-ESBL Producer By Phenotypic Method.

S.N.	Antibiotic	Total E. Coli%		ESBL %		NON-ESBL %		P Value
		S	R	S	R	S	R	
1	Imipenem	81.5	18.5	80.4	19.4	83.3	16.7	0.03 Signi ficant
2	Meropenem	86.5	13.5	86	14	87.2	12.8	
3	Polymixin-B	100	00	100	00	100	00	
4	Colitin	100	00	100	00	100	00	
5	Ceftazidime	37.5	62.5	00	100	100	00	
6	Ceftriaxone	35	65	06	94	62	38	
7	Gentamycin	48.6	51.4	48	52	50	50	
8	Amoxycillin	47.5	52.5	40	60	63.3	36.7	
9	Cefotaxime	33.7	66.3	00	100	70	30	
10	Tetracycline	49.7	50.3	46.4	53.6	55.3	44.7	
Significant value								

A total of 400 E. coli isolates were found in male and female patients 70 (17.5%) and 330 (82.5%).

Out of E. coli isolates collected from different clinical samples, data were included, urine samples, 186 (46.5%), pus 66 (16%), swab 14 (14%), and sputum 50 (12.5%). Third generation Cephalosporins Cefotaxime or Ceftazidime to be resistant were included in ESBL positive E. coli, and further confirmation by combined disk test done on DDST and PCDDT out of 68.8%, & 62.5% respectively.

Showed resistance in total isolates to the third generation Cephalosporins Cefotaxime, Ceftazidime, 250 (62.5%), and Imipenem 74 (18.4%). The results also showed that 346 out of 400 (25.2%) isolates were sensitive to all antibiotics tested. The combined disk test was done on 275 isolates resistant to third-generation Cephalosporins and also showed that 250 isolates were ESBL-producing strains. (Table no.5)

DISCUSSION

E. coli isolates were collected from different clinical samples, so data were included, urine samples, 186 (46.5%), pus 66 (16%), swab 14 (14%), and sputum 50 (12.5%). Third generation Cephalosporins Cefotaxime or Ceftazidime to be resistant were included in out of E. coli isolates collected from different clinical samples, so data were included, urine pus 66 (16%), swab 14 (14%), and sputum 50 (12.5%). In this study, E. coli isolates were found male and female patients 70 (17.5%) and 330 (82.5%). This study is another study with similar results and is most prevalent in female patients. [11]

A very high rate of MDR phenotype was observed for urine isolates of the organism, which was samples, 186 (46.5%), for E. coli. This study was compared to another study, this result is 68.8%, and a majority of ESBL isolates. [12]

Prevalence of MDR phenotypes in E. coli, ESBL positive and further confirmation by combined disk test done on DDST and PCDDT out of 68.8%, & 62.5% respectively, majority of them ESBL producers. Previous reports from other parts of India also indicated the high

Among MDR E. coli isolates, the prevalence of MDR E. coli.[13,15] Further to another study, the prevalence of MDR, ESBL producer E. coli appears to be increasing resistance ratio the worldwide.[16]

Carbapenems are often the most effective treatment available for infections caused by ESBL and MDR E.coli.[17] In the present study, Imipenem and Meropenem were found to be the most active antimicrobial agents, including the ESBLs producing isolates. However, the incidence of carbapenem resistance in E. coli (18.5% and 13.5%) in total isolates reveals the most serious threat to the clinician. Although carbapenem-resistant E.coli isolates were negative in CLSI phenotypic confirmatory test for ESBL production, out of the isolate was found to possess more than one ESBL gene in genotypic detection.

Increasing resistance to cephalosporin and carbapenems and the event of superbug (multidrug resistance) in Indian hospital settings and community-acquired has now spurred the primary government and regulatory health care societies to impose strict statutory guidelines for rational use of antibiotics.[18]

By following the phenotypic screening method for ESBL production, overall 62.5% of E. coli isolates were screened for detection of ESBL production. The presence of more than one ESBL gene in all the phenotypic screening methods for positive isolates indicated the high prevalence of ESBL-producing E. coli (62.5%) in this northern region. However, the true prevalence of ESBL-producing E. coli isolates in the community cannot be deduced as the samples were collected from only one own center. [19]

Gene detection by PCR with oligonucleotide primers that are specific to the ESBL gene is the easiest and most reliable method and confirm used to detect the presence of ESBL E.coli for further high-level antibiotic treatment for multidrug resistance. However, following PCR amplification of the NDM, blaTEM, blaKPC blaCTX-M, and blaSHV genes with oligonucleotide primers and sequencing is needed to discriminate different variants of the gene in ESBLs.[20]

Among five ESBL genotypes included in this study, the most prevalent genotype was found to be NDM, blaTEM, blaKPC blaCTX-M and blaSHV genes, 75.7%, 51.8%, 49.2%, 39.5% and 21.5% respectively, ESBL producing E.coli. It was observed that NDM and blaTEM alone were more prevalent in E. coli, while blaCTX-M and blaSHV together were more prevalent in ESBL producer E.coli. A previous study from India reported NDM, blaTEM and blaCTX-M together predominated in E. coli (62.5%, 42.9% and 39.2%), while blaSHV and blaKPC together predominated in ESBL producer E.coli (42.6% & 40.8%).[21]

Previous studies from Indian hospitals and medical settings also documented the presence of these ESBL genotypes in combination among the majority of third-generation cephalosporin-resistant E. coli isolates.[17] The occurrence of NDM, blaTEM, blaKPC, blaSHV, and blaCTX-M along with impermeability can cause resistance to carbapenems; this is a worrisome situation, especially in India where the ESBL prevalence is very high.[21]

CONCLUSION

Present scenario knowledge of the multidrug resistance patterns and resistance genes of E. coli isolates in a geographical distribution is of paramount importance for surveillance and control of antimicrobial resistance as well as for microbiologist and clinician guidance on appropriate and rational antibiotic usage. And finally, other studies are needed from other health care settings of Northeast India to determine the immensity of the problem of antimicrobial resistance exiting in emerging pathogenic bacteria like E. coli. In the current situation of multidrug resistance treatment with the combination of antimicrobial agent treatments and new antimicrobial agents, for example, CLSI guidelines might be more helpful against such multidrug gene resistance in E. coli strain.

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