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PHENOTYPIC CHARACTERIZATION OF ANTIBIOTIC RESISTANCE PATTERN OF ESCHERICHIA COLI WITH SPECIAL REFERENCE OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) FROM VARIOUS CLINICAL SAMPLE IN TERTIARY CARE CENTRE

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ABSTRACT **BACKGROUND:** Increase the resistance to antimicrobials in E. coli has become a serious global health concern complicating treatment strategies and increasing medical centres costs. 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:** In this study carried out in Medicure diagnostic and research center in Hyderabad This study is cross sectional & descriptive type 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). **RESULTS:** Total of 400 E. coli isolates was found in male and female patient 70 (17.5%) and 330 (82.5%). Out of total isolates to age group distribution in ESBL and Non-ESBL E. coli more age group 16-30 yrs. total, Most of E. coli isolates collected from different clinical samples, in total, ESBL, and non-ESBL urine samples, 186 (46.5%), 117 (46.8%), 69 (46%) and less than samples pus 39 (9.7%), 30 (12%), 09 (06%) respectively. Third generation Cephalosporins, Cefotaxime or Ceftazidime to be resistant were included in ESBL positive E. coli were 62.5% and non-ESBL 37.5%. And ESBLs positive E. coli, 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 Ceftriaxone 67% Gentamycine 63%, Ceftazidime, 75% and ESBL resistance Ceftriaxone 87%, Gentamycine 74%, Ceftazidime, 100%, & non-ESBL resistance E. coli were 51% Ceftriaxone, & Gentamycine etc. **CONCLUSIONS:** It is essential to report ESBL production along with routine susceptibility, which will aid the clinicians in prescribing antibiotics.

KEYWORDS : ESBL, phenotypic, antibiotic resistance, specificity, and sensitivity.

INTRODUCTION

Escherichia coli is a member of the family Enterobacteriaceae and it is a short, straight gram-negative bacillus that is non-sporing, generally motile with peritrichous flagella, often fimbriate and occurs singly, or in pairs in rapidly growing liquid cultures. A capsule or microcapsule is often present and a few strains produce profuse polysaccharide slime [1].

E. coli is a facultative anaerobe capable of fermentative and respiratory metabolism. Its optimum temperature is 37°C and it grows readily on a wide range of simple culture media and on simple synthetic media. A soluble α- hemolysin may be demonstrated in erythrocyte containing media and some strains possess a cell-associated β hemolysin that may be released when the cell is lysed. As a non-pathogenic inhabitant of the intestine of many mammals, including humans, E. coli exists as part of the indigenous flora, often contributing to the vital tasks performed by the intestinal micro-flora [2-4].

Traditionally, commensal E.coli have been described as colonizers that rarely cause infection and categorized as belonging to phylogroup A and B1, while ExPEC isolates are mostly derived from phylogroup B2 and D[5].

All four phylogroups can, however, cause intestinal and extra intestinal infections and phylogroup B2 and D have been found as regular colonizing strains in healthy individuals [6].

ESBLs are Ambler class A penicillinases enzymes, which confer resistance to and hydrolyze the expanded spectrum of cephalosporins like ceftazidime, cefotaxime, monobactam-azteronam, and related oxyimino β-lactams as well as older penicillins and cephalosporins[7]. They arise from mutations in the genes for common plasmid-mediated β-lactamases, especially Temoniera (TEM) and sulfhydryl variable (SHV) enzymes, which alter the configuration of the enzyme near its active site to increase the affinity and hydrolytic ability of the β-lactamase for oxyimino compounds while simultaneously weakening the overall enzyme efficiency. Widespread use of third-generation cephalosporins and aztreonam is the major cause of the mutations leading to the emergence of ESBLs [8-10].

Antimicrobial resistance is associated with high morbidity, mortality, increased length of hospitalization, and cost of health care. Bacterial resistance to third generation Cephalosporins, poses a great challenges, in a developing country like India. Enterobacterial resistance to third-generation Cephalosporins is typically caused by the production of an Extended spectrum of beta lactamase (ESBL) [11].

MATERIALS AND METHODS

Bacterial isolates

A total of 400 isolates in cross-sectional study in E. coli were recovered from various clinical specimens at a tertiary care hospital in Northeast India.

Antibiotic susceptibility testing

Kirby-Bauer disk diffusion method was performed on Mueller-Hinton agar (MHA) plates to determine the susceptibilities of different Beta lactam and non beta lactam antibiotics and results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2021[7].

The following antibiotics were tested:

Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Amikacin (30 µg), Gentamycin (10 µg), Imipenem, (10 µg), Meropenem (10 µg). Polymyxin and Colistin.

All the antibiotic discs and media were procured from HiMedia Laboratories Pvt, Ltd., Mumbai, India. E. coli ATCC 25922 strains were used for quality control [7].

Screening for ESBL production

By disc diffusion susceptibility testing, any isolate with a zone diameter of ≤17 mm for Cefotaxime, 22 mm for Ceftazidime, ≤25 mm for Ceftriaxone was considered to be screening positive isolate for ESBL production [7].

Phenotypic confirmatory test for ESBL production by PCDDT method.

For this purpose, following antibiotic discs were used:

Cefotaxime (30 g), and Ceftazidime-Clavulanic acid (30/10 g) (HiMedia Laboratories Pvt, Ltd., Mumbai, India). Discs were placed 25 mm apart on a MHA plate inoculated with 0.5 McFarland suspension of the test isolate [7].

Plates were incubated at 35°C for 18-24 hrs. at ambient atmosphere. After incubation the zone diameters around each of the disc were measured. A difference of ≥5 mm between the zone diameters of either of the cephalosporin discs and their respective Cephalosporin /Clavulanic acid disc was considered as positive phenotypic confirmatory test for ESBL production. E. coli ATCC 25922 was used as negative control [7].

RESULTS

Total of 400 E. coli isolates was found in male and female patient 70 (17.5%) and 330 (82.5%). (Table no. 1).

Out of total isolates to age group distribution in ESBL and Non-ESBL E. coli more age group 16-30 yrs. total, ESBL and Non-ESBL 50.3%, 54 and 44 respectively. And less than isolates in total, ESBL, and non-ESBL E. coli in 46-60, 31-45, and 46-60 age group 12.3%, 10.8% and 10% respectively (Table no 2).

Most of E. coli isolates collected from different clinical samples, in total, ESBL, and non-ESBL urine samples, 186 (46.5%), 117 (46.8%), 69 (46%) and less than samples pus 39 (9.7%), 30 (12%), 09 (06%) respectively (Table no. 03).

Third generation Cephalosporins, Cefotaxime or Ceftazidime to be resistant were included in ESBL positive E. coli were 62.5% and non-ESBL 37.5%. And ESBLs positive E. coli, further confirmation by combined disk test done on DDST and PCDDT out of 68.8%, & 62.5% respectively (figure no.01).

Showed resistance in total isolates to the third generation Cephalosporins Ceftriaxone 67% Gentamycine 63%, Ceftazidime, 75% and ESBL resistance Ceftriaxone 87%, Gentamycine 74%, Ceftazidime, 100%, & non-ESBL resistance E. coli were 51% Ceftriaxone, & Gentamycine.

GENDER WISE

Table no. 1 Distribution between male and female in ESBL and non-ESBL with total isolate

S.N.	GENDER	TOTAL	%	ESBL	%	NON-ESBL	%
1.	**MALE**	70	17.5	49	19.6	21	14
2.	**FEMALE**	330	82.5	201	80.4	129	86

AGE GROUP WISE

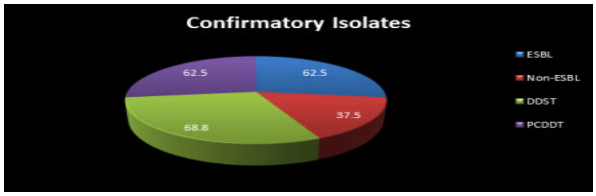
Table no. 2 Distribution between age group wise in ESBL and non-ESBL with total isolate

S.N.	AGE GROUP	TOTAL	%	ESBL	%	NON-ESBL	%
1.	**0-15**	53	13.3	23	9.2	30	20
2.	**16-30**	201	50.3	135	54	66	44
3	**31-45**	46	11.5	27	10.8	19	12.4
4	**46-60**	49	12.3	34	13.6	15	10
5	**>61**	51	12.8	31	12.4	20	13.3
6	**TOTAL**	400		250		150	

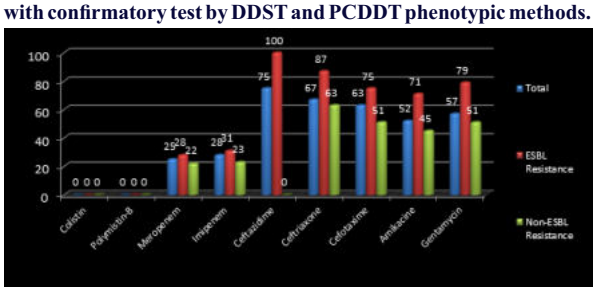
SAMPLE WISE

Table no. 3 Distribution between samples in ESBL and non-ESBL with total isolate

S.N.	SAMPLE	TOTAL	%	ESBL	%	NON-ESBL	%
1.	**URINE**	186	46.5	117	46.8	69	46
2.	**PUS**	39	9.7	30	12	09	6
3	**SWAB**	49	12.3	29	11.6	20	13.33
4	**SPUTUM**	56	14	25	10	31	20.7
5	**BLOOD**	49	12.3	35	14	14	9.33
6	**OTHER**	21	5.3	14	5.6	07	4.4
7	**TOTAL**	400	100%	250	100	150	100



Graph:-1 Distribution of total isolates in ESBL and Non- ESBL with confirmatory test by DDST and PCDDT phenotypic methods.



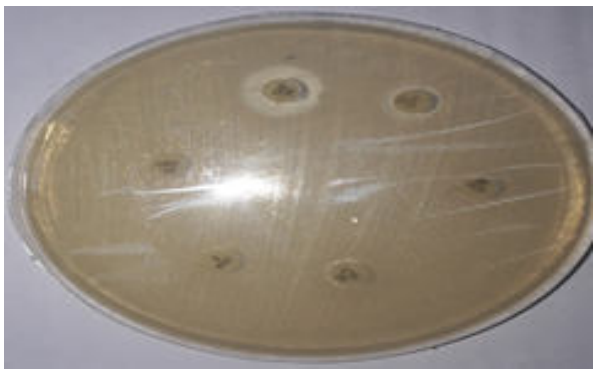
Graph:-2 Antibiotic resistance pattern in EBL and Non-ESBL E. coli isolates, compared with total E. coli isolates.

Table no.:-3 Antibiotic resistance pattern in EBL and Non-ESBL E. coli isolates, compared with total E. coli isolates.

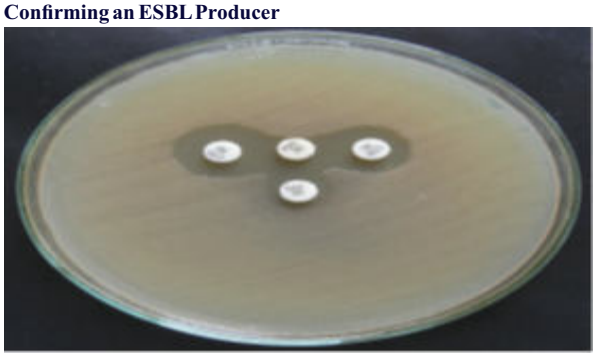
S.N.	Antibiotics	Total %	ESBL Resistance %	Non-ESBL Resistance %
1	**Colistin**	00	00	00
2	**Polymyxin-B**	00	00	00
3	**Meropenem**	25	28	22
4	**Imipenem**	28	31	23
5	**Ceftazidime**	75	100	00
6	**Ceftriaxone**	67	87	63
7	**Cefotaxime**	63	75	51
8	**Amikacine**	52	71	45
9	**Gentamycin**	57	79	51

Table No. 4: Antibiotic resistance pattern in total isolated samples.

S.N.	Antibiotics	Urine	Pus	Swab	Sputum	Blood	Other
1	**Colistin**	00	00	00	00	00	00
2	**Polymyxin-B**	00	00	00	00	00	00
3	**Meropenem**	21	24	21	23	24	25
4	**Imipenem**	25	26	25	23	27	25
5	**Ceftazidime**	67	61	63	63	62	70
6	**Ceftriaxone**	70	66	65	64	68	66
7	**Cefotaxime**	71	59	60	61	69	72
8	**Amikacine**	48	54	55	49	51	56
9	**Gentamycin**	50	51	50	44	47	50



Figer:1 Double Disc Synergy Test showing the Zone of Inhibition of Ceftazidime (CAZ) Cefotaxime (CTX) and Ceftriaxone (CTR) enhancing towards the Amoxicillin/Clavulanic Acid (AMC) disc Confirming an ESBL Producer



Figer:2 Phenotypic Confirmatory Disc Diffusion Test. ESBL production confirmed by an increase in Zone of ≥5 mm for Ceftazidime (CAZ) and Ceftazidime/Clavulanic Acid (CAC) and

Cefotaxime (CTX) and Cefotaxime/Clavulanic Acid (CEC)

DISCUSSION

Escherichia coli remain the major ESBL producing organisms isolated worldwide which are recommended to be routinely tested and reported by the Clinical and Laboratory Standards Institute.

In the present study, Escherichia coli was the most predominant ESBL among Escherichia coli isolates was also reported in a study by Ananthakrishnan et al, [12] and Kumar Mulley et al., [13]. A similar study from central India reported the incidence of 69% ESBL producing isolates of E. coli respectively Rudresh SM et al [14]

Most E. coli isolates were collected from different clinical samples, in total, ESBL, and non-ESBL urine samples, 186 (46.5%), 117 (46.8%), and 69 (46%) respectively. The highest number of samples in our study was urine and the most commonest organism was E. coli. The study by Andrews B et al [15] and another by Pawan et al [16] also showed a dominance of ESBL isolates among E. coli from urine samples. Among urine samples, ESBL prevalence was 39.8% among E. coli. This was in accordance with studies by Shobha K L et al [17]] and Kumar A et al [18].

In the present study, ESBL detection was carried out by the Phenotypic Confirmatory Disc Diffusion test (PCDDT) on 250 (68.8%) isolates of E. coli. Imipenem was found to be the most effective (75.83%) antibiotic followed by Colistin, Polimyxin-B (100%) and Meropenem (75%). Our findings correlate with a similar study done by Basavaraj M et al.,[19] and Dutta H et al.,[20] where Imipenem sensitivity was reported to be 84.6% and 96.2% among the isolates. The isolates of E. coli found in another study to be most resistant to and Ceftazidime which coincides well with studies done by Kannaiyan M et al.,[21]

Every health care institution must develop its own antimicrobial strategy program, which is based on the local epidemiological data and international guidelines to optimize the antimicrobial use among the hospitalized patients and to improve patient outcomes [22].

CONCLUSION

ESBL production is generally accompanied by multi-drug resistance. Hence knowledge of their clinical samples will aid in averting the inessential use of antibiotics, especially the third-generation cephalosporins. Such information will also highlight the importance of taking steps to curtail their spread in this institute. Preventive measures like continuous surveillance and strict implementation of infection control practices can go a long way in containing the menace of drug resistance in health care settings.

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