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The Detection of ESBL-Producing *Escherichia coli* in Various Clinical Sample Using 3rd Generation Cephalosporin by Different Diffusion Methods

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A B S T R A C T

This study aimed to determine the prevalence of extended spectrum of beta lactamases (ESBLs), to compare two antibiotic i.e Cefotaxime & Ceftazidime by different phenotypic methods for ESBL confirmation and to evaluate the antibiotic resistance patterns among ESBL-producing *Escherichia coli*. Total no. of *E.coli* isolates were obtained from various clinical samples. They were subjected for the antibiotic susceptibility pattern by Kirby bauer disc diffusion method. 3rd generation cephalosporin resistant isolates were detected for ESBL production. In our isolates we have found increased percentage (100%) isolates showed sensitivity to colistin followed by cefepime which showed sensitivity of (54%). (80 - 90 %) *of E.coli* isolates showed resistance to cephalosorin group of drugs. (51%) of *E.coli* isolates were found to be extended spectrum beta lactamase producers using cefotaxime (30µg). Cefotaxme/ Clavulanic acid (30/10 µg) & (55%) os isolates were shown to be positive ESBL using ceftazdime (30 µg), Ceftazidime /Clavulanic acid (30/10 µg). This study found a high rate of ESBL production among cefotaxime antibiotic. Clinical microbiology laboratories should routinely incorporate ESBLdetection methods in their laboratory producers for continous surveillance of drug resistance isolates & antibiogram to guide eprical theapy.

Introduction

Despite the widespread availability of antibiotics, urinary tract infections (UTIs) remain the most common bacterial infections in humans (Sharma, 1997). Among the wide array of available antibiotics, þ-lactams are the most varied and most widely used agents, accounting for over 50% of all systemic antibiotics in use (Bronson and Barrett, 2001). The most common cause of bacterial resistance against þ-lactam antibiotics is the production of þ-lactamases (Medeiros, 1997). Many of the second- and third-generation cephalosporins were specifically designed to resist the hydrolytic action of major þ-lactamases. However, the evolution of extended-spectrum þ- lactamases (ESBLs) has added another weapon to the arsenal of these enzymes.

ESBLs are commonly produced by many members of *Enterobacteriaceae*, especially *Escherichia coli* and *Klebsiella pneumoniae*. These organisms efficiently hydrolyze oxyimino-cephalosporins, conferring resistance to third-generation cephalosporins and

monobactams (Jacoby and Medeiros, 1991). The detection of ESBLs is a challenge for routine clinical microbiology laboratories in resource- limited settings, and the detection of a decrease in susceptibility to oxyimino-cephalosporins is not sufficiently sensitive to detect all ESBL-producing strains.

The guidelines developed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2010) (EUCAST) recommend screening for ESBL-producing isolates based on decreased susceptibility to extended-spectrum cephalosporins in primary antibiotic disc diffusion tests along with one additional confirmatory test.

However, the most sensitive method for the phenotypic detection of ESBL remains unknown (Garrec *et al.,* 2011). Existing phenotypic methods of ESBL detection include disc diffusion-based screening, the double disc synergy test (DDST) confirmatory tests.

As per the Clinical and Laboratory Standards Institute (CLSI) guidelines, an initial screen for reduced susceptibility to more than one of the five indicator cephalosporins followed by a confirmatory test can improve the sensitivity of detection. The further identification of specific gene.

Materials and Methods

E.coli strain were isolated from clinical samples including pus, urine, blood, cerebrospinal fluid (CSF), stool, sputum, ear swab, and different body fluids received in the bacteriology laboratory in the department of microbiology, School of Medical Sciences & Research, Greater Noida from in- patient and out-patient departments of Sharda Hospital during the period from December 2019 to November 2020 were included in the study.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method. The following antibiotic disks were used, ampicillin (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefepime (µg), norfloxacin (10 µg), and nitrofurantoin (300 µg), Meropenem (20 µg), Amoxyclav (30 µg), Fosfomycin (200 µg), Tetracycline (30 µg), Cefodoxime (10 µg), Cefuroxime (30 µg), Cotrimoxazoe (25 µg), Nalidixic acid (30 µg) (CLSI guidelines, 2019).

ESBL detection methods

E. coli were first screened for ESBL production by phenotypic method and then phenotypic confirmatory test was done as per CLSI guidelines 2019.

Disc susceptibility test to screen for ESBLs

All isolates were screened for ESBL production using three indicator cephalosporins, namely ceftazidime (30 µg), cefotaxime (30 µg) and cefpodoxime (30 µg). The isolates were considered to be resistant if the diameter of the inhibition zone for ceftazidime, cefotaxime or cefpodoxime was 22 mm, 27 mm or 17 mm, respectively.

The strains that showed resistance to at least one of the three cephalosporins were tested further using phenotypic confirmation methods.

Phenotypic confirmatory methods

Confirmatory test was done by two methods

Cephalosporin 3rd gneration/clavulanate combination disks

Cefotaxime (30 µg) or ceftazidime (30 µg) disks with or without clavulanate was used for phenotypic confirmation of the presence of ESBL as recommended by CLSI 2019 guidelines. A lawn culture of *E. coli* was made on the MHA plate and disks were placed at an appropriate distance from each other and incubated aerobically overnight at 37°C. A difference in zone of inhibition of ≥5 mm of either of cephalosporin disks and their clavulanate containing disks indicates production of ESBL.

Double disk synergy test

Double disk synergy (DDST) is a disk diffusion test in which antibiotic disks of ceftazidime (30 µg), cefotaxime (30 µg) are placed on the lawn culture plate of *E. coli* on MHA, 15 mm (center to center) from the Amoxyclav (10 µg) disk.

This plate is incubated aerobically overnight at 37°C and examined for an extension of the edge of zone of inhibition of antibiotic disks toward the disk containing clavulanate giving a dumbbell shape. It is interpreted as synergy, indicating the presence of an ESBL.

Statistical analysis

The results were analyzed with descriptive statistics wherever appropriate. The *Chi*-square test was used to evaluate the statistical significance of differences in the results. A *p* value of <0.05 was considered statistically significant. Statistical analysis was found <.05. A chi square test showed that there was no significant association between Ceftazidime & cefotaxime.

Results and Discussion

Out of 1,264 total samples, 204(16%) isolates were identified & confirmed as *E.coli.* These *E.coli* isolates were isolated from IPD & OPD. Total no. of specimen received during the study period from urine samples. Urine (75%) was the most common followed by Pus (13.2%).

In OPD, most common samples was received from urine (86%), while in the IPD most common sample was received from pus (50%). The Table given below depicts the no. of patient's sample received in the bacteriology laboratory for culture & sensitivity during the study period.

Demorphic profile

Most of the patients from whom *E.coli* were isolated were in the age group of 0 -30 year (54%), followed by 31-50 year (20%), 51-70 year (17%), 71-90 year (8%) respectively (table 2). Maximum no. of culture positive case in the present study had Male (32%) & Female (68%) were found in the age group 0 – 30 year.

Maximum no. of *E.coli* stains were recovered from Urine (153) followed by Pus (27). (Table 3)

Most of the isolates were obtained from OPD i.e (77%) & In IPD the maximum no. of the isolates were received from patients in Gerenal surgery (7%) followed by SISU (1%) shown in table 4

The antimicrobial susceptibility pattern of the various isolates are depicted in the (table 5). (52 %) & (30 %) isolates were resistant to 3rd generation cephalosporin which is Cefotaxime Ceftazidime respectively.

The isolates exhibited a high degree of resistance to Ceftazidime (52%). (54%) & (49%) isolates were sensitive to Carbepenems which is Imipenem and Meropenem respectively. There was a sensitivity to Fluroquiolones isolates (51%) were sensitive to ciprofloxacin, (48%) were sensitive to Levofloxacin. In case of aminoglycosides, with Gentamicin sensitive was seen in (45%) isolates. Tetracycline (52%) isolates were sensitive & Colitin were 100% sensitive.

ESBL Producers among Cefotaxime and Ceftazidime resistant *Escherichia coli*

A difference of ≥ 5mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin / clavulanate disk was taken to be phenotypic confirmation of ESBL production.

Among the 204 *Escherichia coli* strains isolated, 60 (76.47 %) strains were found to be resistant to Ceftotaxime and 108 (73.10%) strains were found to be resistant to Ceftazidime shown in the table 6.

Total no of *Escherichia coli* strains isolated were 204 (16%), Among the (16%) *E.coli* isolates, (76%) were resistant to Cefotaxime In which (51%) were Synergy positive and (73%) were resistant to Ceftazidime in which (49%) were Synergy positive shown in the table 7.

(55%) Cefotaxime isolated strains were confimed ESBL producing strain (51%) Ceftazidime isolated strains were confirmed ESBL producing strain by combined disc test shown in the table 8.

3rd generation cephalosporin revealed that (73%) isolates were resistant to Ceftazidime, (76%) were resistant to Cefotaxime as indicated in Table 9.

In a disc-based ESBL screening test, (82%) isolates were resistant to at least one of the three indicator cephalosporins. Resistance was most frequently observed for ceftazidime (51%) and cefotaxime (55%). Among these cephalosporins, ceftazidime was found to be the best antibiotic for the ESBL phenotypic confirmatory tests when using either the DDST or the CDDT, as shown in Table 10. Extended spectrum β – lactamase (ESBL) producing *Escherichia coli* has tremendously increased worldwide and it is one of the most common cause of morbidity and mortality associated with hospital – acquired infections. This could be attributed to association of drug resistance in ESBL producing isolates. The present study was to determine the sensitivity profie of ESBL producing *E.coli* isolates from various clinical samples (Dinesh Kumar *et al.,* 2014).

In this study 868 bacterial isolates cultured from various (13,639) clinical specimens over a period of 12 months, 204 (16%) isolates were identified as *E.coli*. Similar prevelance of (13.7%) of *E.coli* isolates was reported in a study conducted by Anand Kumar *et al.,* in 2013 (Anand kumar *et al.,* 2013). A low prevenence rate of 7.15%was reported by Alippour, Nilifar *et al.,* in 2014 whereas higher prevalence rate of 26.45 % *E.coli* isolates was reported by Md Rana *et al.,* in 2014 (Alipourfard Niufar *et al.,* 2010; Md Rana *et al.,* 2014).

In present study *E.coli* infection was predominantly observed in female (68%) than male (32%). Most of the male & female patients were in the age group of 0 – 30 year (54%) was in concurrence with studies conducted by Fatima jummai *et al.,* (2019) where female (59%) & male (29%) respectively (Fatima jummai *et al.,* 2018).

Maximam no. of *E.coli* isolates in this study were isolated from urine (75%) followed by pus (13%), sputum (7%). A similar observation has been reported in the study done by Getnet Tesfaw *et al.,* (2018). In another study conducted by Kavita *et al.,* (2017), most isolated *E.coli* were from urine (26.79%).

Most of the *E.coli* was isolated from patients admitted in General surgery (7%).A similar prevalence of 26.1% & 29% was reported by Fatima jummai *et al.,* in 2019 respectively (Fatima jummai *et al.,* 2018).

The antimicrobial susceptibility profile of the *E.coli* isolates were resistant to 3rd generation cephalosporin such as ceftazidime (52%) & cefotaxime (30%) this was concordance with study done by Roshene *et al.,* (2015) which showed 52.15 % respectively towards cephalosporin. In this study the fluroquilonones, such as ciprofloxacin (51%) conferred slightly greater sensitive than levofloxacin (48%) which agreed with a study done by Roshene *et al.,* (2015) & disagreed with Tahira Fatima *et al.,* in 2019 who observed sensitivity of 72% to ciprofloxacin.

In the present study, it was found that *E.coli* exhibited moderate sensitivity towards aminoglycosides which includes Gentamin (45%) & tobramycin (41%). This data was agreement with studies conducted by Shobha prasada *et al.,* in 2019 & disagreement to this pattern was observed by Roshene were resistant rate to Gentamicin was (64.6 %) Roshene *et al.,* (2015).

The spread of ESBL producing bacteria has become rapid worldwide & therapeutic option for these organisms have become increasely limited, *E.coli* is one of the most common ESBL producing bacteria currenty.

In the present study (55%) isolates were ESBL producer correlating with studies done by Ranjan *et al.,*. (62.1%) & Silvia Munoz *et al.,* (2019) (36.3%) reported lesser ESBL producer in their studies (Silvia Munoz *et al.,* 2019).

Out of 168 screened isolates,(55%) were ESBL positive by combined disc test using cefotaxime, Ceftazidime alone & Cefotaxime, Ceftazidime /Clavulanic acid while (51%) were positive by Double disc synergy test using same antibiotic.

In the present study showed that Cefotazime & ceftazidime both are 3rd generation cephalosporin &have a good sensitivity & specificity for the detection of ESBL in *E.coli* (Meeta Sharma *et al.,* 2013).

Table.1 Total sample received during the study period

IPD		
Positive N (%)	**Negative N (%)**	**Total**
201(26%)	546 (71%)	767
OPD		
Positive N (%)	**Negative N (%)**	**Total**
122 (24%)	380 (75%)	502

Table.2 Age wise Distribution of *E. coli*

Age in years	No. of isolates (N)	Percentage (%)
0-30 year	112	54%
31-50 year	41	20%
51-70 year	35	17%
71- 90 year	17	8%

Table.3 Sample wise distribution of *E. coli*

Sample	No. of isolates (N)	Percentage %
Urine	153	75%
Pus	27	13%
Sputum	7	3%
Stool	6	2%
Blood	4	1%
BAL	2	1%
Brachial aspirate	2	1%
Ascitic fluid	2	1%
Swab	2	1%

Table.4 Ward wise Distribution of *E. coli*

Ward	No. of isolates	%
General surgery	16	7%
SISU	3	1%
NEROICU	2	0.4%
Paedritics	2	0.9%
psycology	1	0.4%
MICU	1	0.4%
Orthopaedic	1	0.4%
Economy	1	0.4%
ICCU	1	0.4%
Gynaecology	1	0.4%

Figure.1 Age wise distribution of *E. coli*

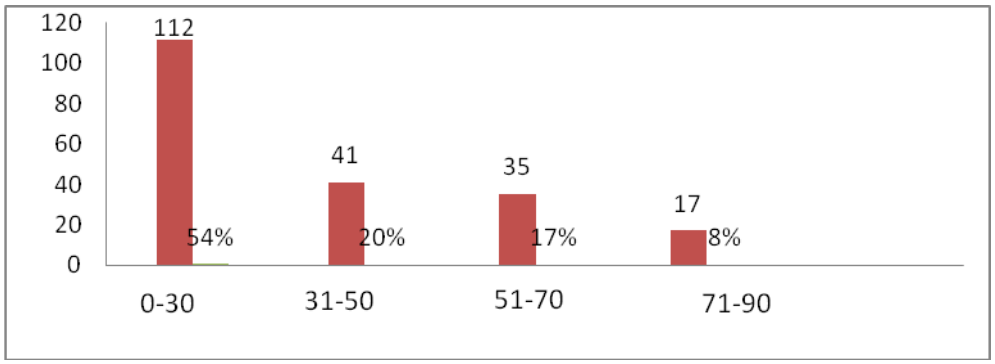

Table.5 Antibiotic Susceptibility Pattern of *E. coli*

Antibiotic	Sensitive N (%)	Resistant N (%)
Cefotaxime (CTX)	93 (45%)	60 (30%)
Cefepime (CPM)	112(54%)	49(24%)
Cefuroxime (CXM)	105(51%)	43(21%)
Ceftazidime (CAZ)	35(17%)	108(52%)
Levofloxacin (LE)	98(48%)	52(25%)
Teracycline (TE)	108(0.5%)	49(24%)
Ampicillin (AMP)	101(49%)	85(41%)
Gentamicin (GEN)	92(45%)	44(21%)
Imipenem (IMP)	111(54%)	34(16%)
Meropenem (MRP)	100(49%)	33(16%)
Ceftriaxone (CTR)	97(47%)	59(28%)
Cefodoxime(CPD)	96(47%)	47(23%)
Ciproflaxacin (CIP)	106(51%)	51(25%)
Nitrofurantoin(NIT)	89(43%)	31(15%)
Colistin (CL)	204 (100%)	0 (0)

Table.6 ESBL producers among Ceftazidime & Cefotaxime resistant *Escherichia coli*

E. coli	Total (204)	% of ESBL PRODUCTION
Cefotaxime resistant	60 (76.47%)	33 (55%)
Cefazidime resistant	108 (73.10%)	56 (51%)

Table.7 ESBL detection by double disk synergy test using cefotaxime & ceftazidime**.**

E. coli	Total (204)	Positive
Cefotaxime +Amoxyclav	60 (76.47%)	31 (51%)
Cefazidime+ Amoxyclav	108 (73.10%)	53 (49%)

Table.8 ESBL Positive Confirmed Isolates by combined disc test

E. coli	Total (204)	ESBL Positive Isolates	ESBL Negative Isolates
Cefotaxime	60 (76.47%	33 (55%)	27 (45%)
Ceftazidime	108 (73.10%)	56 (51%)	52 (48%)

Table.9

Right –Ceftotaxime	Left side – Ceftotaxime + Clavulanic acid
Right –Ceftazidime	Left side – Ceftazidime + Clavulanic acid

Table.10 Comparison of resistance pattern in *E. coli* between ESBL positives and negative strains.

Pattern	Resistance pattern (n = 168)	ESBL positives strains (n =89)	ESBL negative strains (n = 79)
Ceftazidime (CA)	108 (73.10%)	56 (51%)	
Cefotaxime (CE)	60 (76.47%	33 (55%)	27 (45%)

Figure.2 Sample wise Distribution of *E. coli* isolate

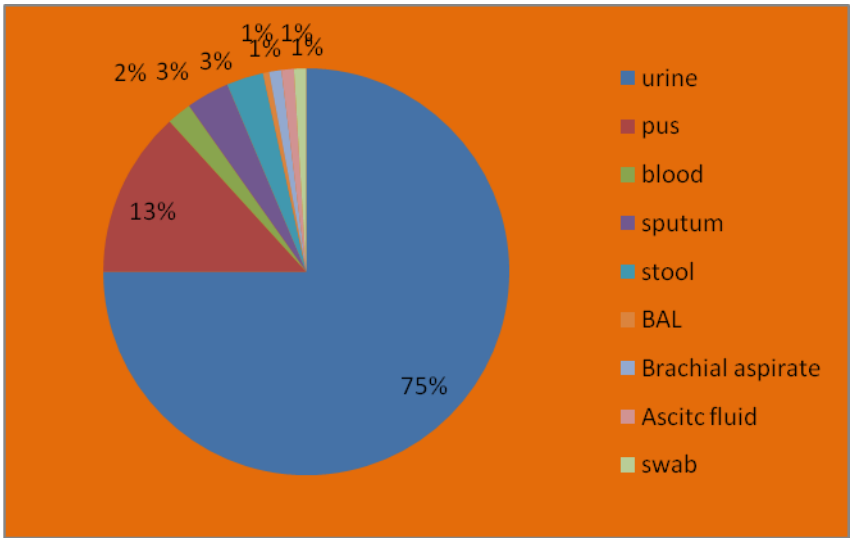

Figure.3 Ward wise distribution of *E. coli*

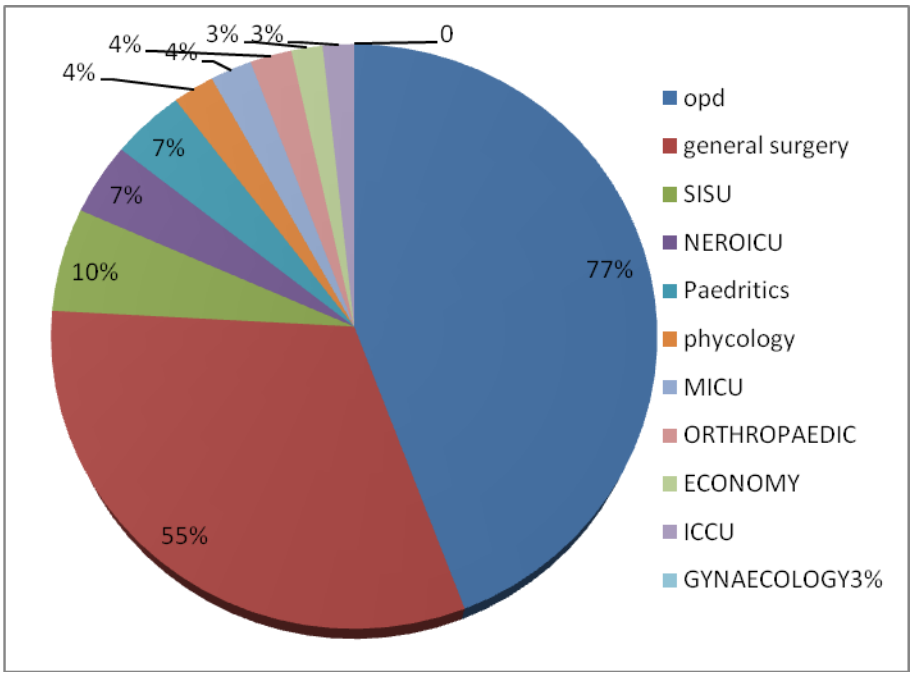

Table.11 Comparison of screening and confirmatory methods for the detection of extended spectrum þ-lactamases.

Cephalosporins	Screening test[a]		Confirmatory tests[b]	
	DDST		DDCT test	
Ceftazidime (CA)	108	56	53	
Cefotaxime (CE)	65	33	31	
ESBL positive	168	89	84	

[a]Disc diffusion test.

[b] Strain showing resistance to at least one cephalosporin indicator antibiotic is selected for confirmatory test.

Figure.4 Antibiotic Sensitibility Patterns of *Escherichia Coli*

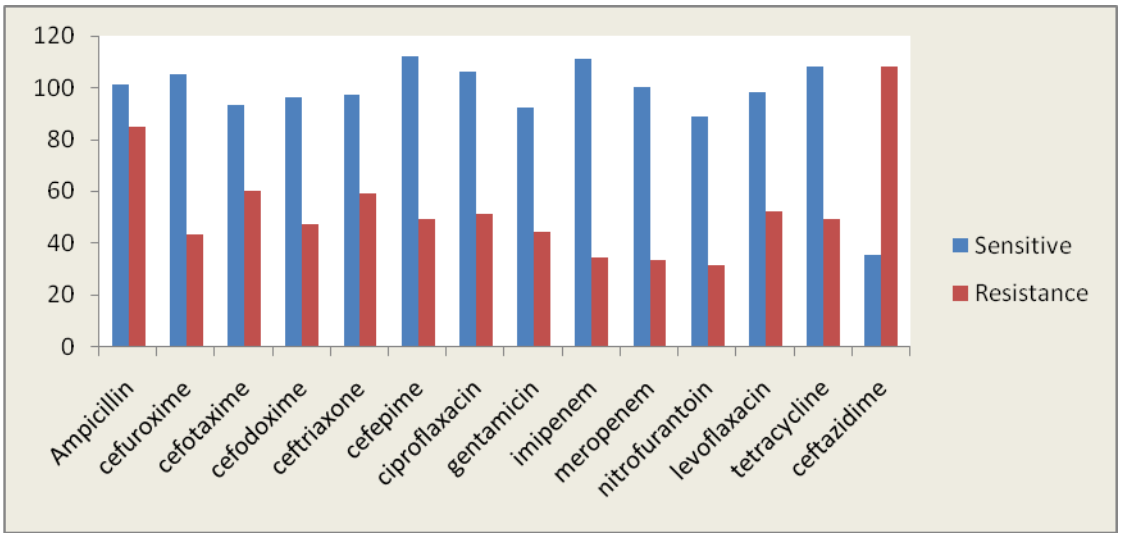

Figure.5

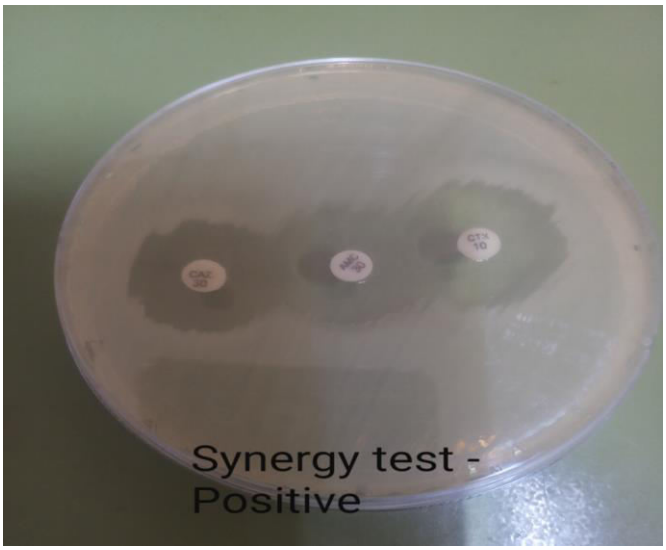

Figure.6

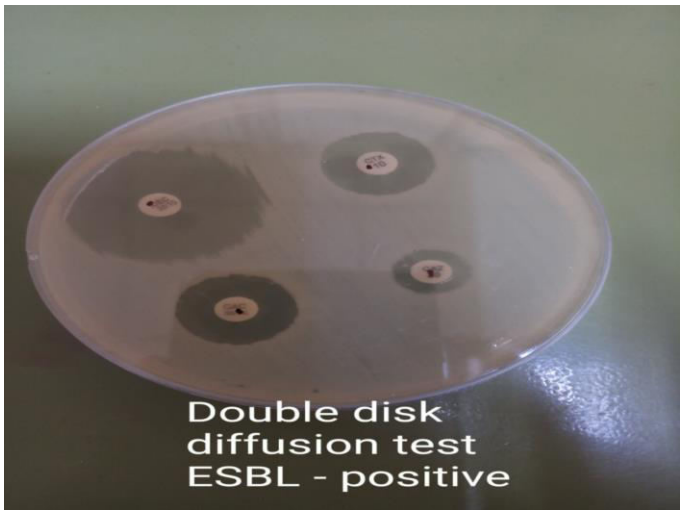

Figure.7

In the present study found that an increased percentage of isolates were resistant to most of the routinely used antibiotics. However, a good sensitivity was observed to colitin. Most of the isolates were resistant to 3rd generation cephalosporin group of antibiotics. These isolates were found to exhibit extended spectrum beta lactamase. The present conclude that *E.coli* isolates were ESBL producers**.** The present study suggest that both test combined disc test (CDT & Double disc synergy test (DDST) using Cefotaxime & Ceftazidime is a simple & easy to perform in the laboratory & helpful in ESBL detection in any setup but Cefotaxime was found to be better drug as compared to Ceftazidime.

Author Contribution

Aalia Amin: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.
Conflict of Interest The authors declare no competing interests.

References

Alipourfard Niufar *et al.,* (2010) Antibiogram of extended β – lactamase producing *E.coli* isolates from Hospital sample,04 (01):32 -36.

Anand kumar Harwalkar *et al.,* (2013) The detection of ESBL – producing *Escherichia coli* in patients with symptomatic urinary tract infections using different diffusion methods in a rural setting 6,108 -114.

Bronson J J, Barrett J F. Quinolone, everninomycin, glycyl-cycline, carbapenem, lipopeptide and cephem antibacterials in clinical development. Current Medicinal Chemistry 2001;8:1775—93. https://doi.org/10.2174/0929867013371653

Dinesh Kumar, Yogesh chander *et al.,* Antimicrobial Susceptibiliy Profile of Extended spectrum β – Lactamase (ESBL) producing *E.coli* from various clinical sample 2014 :7 1 – 8 https://doi.org/10.4137/IDRT.S13820

European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Vaxjo, Sweden: European Committee on Antimicrobial Susceptibility Testing; 2010.

Fatima jummai Giwa *et al.,* (2018) ESBL production & Antimicrbial pattern of uropathogens in aTertiary Hospital in Northwestern Nigeria 2018,9,11-6 https://doi.org/10.4103/atp.atp_39_17

Garrec H, Drieux-Rouzet L, Gilmard J, Jarlier V, Robert J. Comparison of nine phenotypic methods for detection of extended spectrum þ-lactamase production by *Enterobacteriaceae*. Journal of Clinical Microbiology 2011;49(3):1048—57.

https://doi.org/10.1128/JCM.02130-10

Getnet Tesfaw *et al.,* Isolation of extended – spectrum β – lactamase – (ESBL) producing *E.coli* from patient with community – onset urinary tract infection injimma university specialized Hospital Southwest Ethiopia 2018,10,11 – 55. https://doi.org/10.1155/2018/4846159

Jacoby G A, Medeiros A A. More extended þ-lactamases. Antimicrobial Agents and Chemotherapy 1991;35:1697—704. https://doi.org/10.1128/aac.35.9.1697

Kavita A. Suneetha C. *et al.,* Molecular Detection of CTX-M Genes in ESBL producing *Escherichia coli* isolated from various clinical samples volume (8),issue(2),year *2017.*

Md Rana *et al.,* (2014) Prevalence of ESBL positive bacteria in radialogically positive urinary tract infection. https://doi.org/10.1186/2193-1801-3-216

Medeiros A A. Evolution and dissemination of beta lacta- mases accelerated by generation of beta lactam antibiotics. Clinical Infectious Diseases 1997;24:S19—45. https://doi.org/10.1093/clinids/24.supplement_1.s19

Meeta Sharma *et al.,* Prevalence & antibiotic of ESBL (ESBL producing Gram negative bacilli producing *E.coli* 2013,6460 -3462

Ranjan K. P. *et al.,* Prevalence of extended spectrum β – lactamases producing gram negative bacterial septicaemic neonates in a tertiary care hospital J.Med.

Roshene R *et al.,* Antibiotic Sesitivity Pattern & ESBL detection among cinical isolation of *E.coli* vol 7 (10), 2015, 878 – 881

Sharma S. Current understanding of pathogenic mechanisms in UTIs. Annals of the National Academy of Medical Sciences 1997;33(1):31—8

Shobha Prasada, Bhat A, Bhat S, Shenoy Mulki S, Tulasidas S. Changing antibiotic susceptibility pattern in uropathogenic *Escherichia coli* over a period of 5 years in a tertiary care center. Infect Drug Resist. 2019 May 29;12:1439-1443. https://doi.org/10.2147/IDR.S201849.

Silvia Munoz, L., David C Hoopes, Allyson Bloom *et al.,* Extended – spectrum β – lactamases 15 oct 2019.

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