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Antibiotic Sensitivity Pattern and ESBL Detection among Various Clinical Isolates of *Escherichia coli*

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A B S T R A C T

The ESBL producing isolate of *E.coli* among other clinical isolates has been increasing over the past few years resulting in limitation of therapeutic options. Bacteria produces ESBL poses a major problem for clinical therapautics. A cross sectional study was carried out in the Department of Microbiology Sharda Hospital School of Medical Science and Research, Sharda University, Greater Noida. 204 *E.coli* were isolated from various clinical samples which consists of urine, pus, sputum, blood, stool, swab, and different body fluids from both IPD & OPD patents were included in this study. Sample were processed & identified as per routine laboratory protocol. ESBL screening & confirmatory along with AST was done by Kirby - Bauer disc diffusion method. The following antibiotic disc were used Cefotaxime, Ceftriaxone, Ceftazidime, Ampicillin, Meropenem, Fosfomycin, Tetracycline, Cefepime, Cotrimoxazole, Nalidixic acid according to the clinical laboratory standard institution (CLSI) guideline 2019. The most common isolates were found from urine 153 (75%) followed by Pus 27 (13%). In the present study used two different confirmatory methods for the detection of ESBL producing *E.coli*. Combined disc test & Double disk synergy test. During the study period of total no. of *E.coli* isolates were 204, out of which 168 *E.coli* isolates were resistant to 3rd generation cephalosporin,(51%) isolates were ESBL producer by double disc synergy test & (55%) isolates were ESBL producer by combined disc test. ESBL producer of *E.coli* isolates showing a great degree of resistance to antibiotics. The study reveals higher percentage of isolates were resistant to 3rd generation cephalosporin and ESBL producer were more. This study conclude that resistant to cephalosporin were due to extended spectrum beta lactamase production in our isolates & Combined disk test was found to be a better test as compared to double disk synergy test.

Introduction

Resistant bacteria are emerging world wide as a threat to favor- able outcomes of treatment of common infections in com- munity and hospital settings. The common hospital- acquired infections caused by members of Enterobacteriaceae such as Urinary tract, gastrointestinal, and pyogenic infections. Among Enterobacteriaceae, *Escherichia coli* is the most commonly isolated species. *E.coli* is very well known to show multidrug resistance. Prolonged antibiotic exposure, overstay in hospital, severe illness, unprecedented use of third generation

cephalosporin and increased use of intra venous device or catheters are important risk factor for infection with multidrug resistant *E.coli*. Extended spectrum -lactamases (ESBLs), which is defined as increased hydrolysis of oxyimino - β-lactams, cefotaxime, ceftriaxone, ceftazidime, and aztreonam, have been reported increasingly in recent years.

They have also been found in other members of Enterobacteriaceae. Major outbreak involving these resistant organisms has been reported all over the world in many members of the Enterobacteriaceae (Sridhar *et al.,* 2014).

Treatment of infections caused by *E.coli* is becoming increasingly difficult because of antibiotic resistance. The ESBL enzyme are inhibited by beta-lactamase inhibitors such as clavulanic acid (Dinesh Kumar, Yogesh chander, 2014).

Materials and Methods

E.coli isolates recovered 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. Ethical approval was obtained from Ethical Committee, School of Medical Sciences & Research, Greater Noida, India.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method. The following antibiotic disks were used, ampicillin (10µg), ceftazidime (30µg), 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.

Phenotypic screening of ESBL

Antibiotic disks of ceftazidime, cefotaxime, and Cefodoxime were used More than one agent of these was used for screening to enhance the sensitivity of ESBL detection, according to CLSI (2019)

Phenotypic confirmatory methods

Confirmatory test was done by two methods

Cephalosporin 3rd gneration/clavulanate combination disks

Cefotaxime (30 µg) or ceftazidime disks (30 µg) with or without clavulanic acid 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

According to Chi-square test is used for statistical analysis of the data. A '*P* value' less than 0.05 was considered statistically significant. A chi square tests revealed that there was no significance association between Ceftazidime & Cefotaxime.

Results and Discussion

Total samples 13,639, 12,380 culture isolates were found as no growth. Out of which, 2930 (79.3%) isolates were found from IPD, 9450 (95%) isolates were found from

OPD & 1,259 culture isolates were found as growth 762(20.6%) isolates were found from IPD & 497(4.9%) isolates were found from OPD. Out of 1,259 growth isolates, 915 isolates were Gram negative baclli & 334 isolates were Gram positive cocci. Out of 915 GNB isolates, 204 isolates were identified & confirmed as *E.coli.* 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

Maximum no. of *E.coli* stains were recovered from Urine (75%) followed by Pus (1%). (Table 2)

Most of the patients from whom *E.coli* isolates 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-3). Maximum no. of culture positive case in the present study were found in the age group age group 0 – 30 year.

Most of the isolates were obtained from OPD i.e (85%) & in IPD the maximum no. of the isolates were received from patients n General Surgery (7%) followed by NICU (1%) in table 4.

The antimicrobial susceptibility pattern of the various isolates are depicted in the (table 5).

There was resistance to 3rd generation cephalosporin i.e cefotaxime (52%) & Ceftazidime (30%). The isolates exhibited a high degree of resistance to Ceftazidime (52%). Imipenem (54%), Meropenem (49%) isolates were sensitive. There was a sensitivity to Fluroquiolones isolates (51%) were sensitive to ciprofloxacin, (48%) were sensitive to Levofloxacin. In case of aminoglycosides, Gentamicin sensitive was seen in (45%) isolates. Tetracycline (52%) isolates were sensitive & Colistin were 100% sensitive.

Screening & Confirmatory for ESBL production

All isolates were screened for ESBL production by Kirby bauer disc diffusion method using 3rd generation cephalosporin Shown in table 6.

Resistant strains, Cefotaxime (55%), Ceftazidime (51%) were confirmed for ESBL producing strains by Combined disc diffusion test (CDT) Shown in table 7.
Resistant strains, Cefotaxime (51%), Ceftazidime (49%) were confirmed for ESBL producing strains by double disc synergy test shown in the table 8.

On Comparison between the two method Combined disc test & Double disc synergy test using 3rd generation cephalosporin. Combined disc method was found to be a better test for phenotypic confirmatory of ESBL production with no significance p vaule shown in table 8.

P value is .61. A chi square test showed that there was no significant association between CDT & DDST.

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 profile of ESBL producing *E.coli* isolates from various clinical samples. (Dinesh Kumar and Yogesh chander, 2014)

Table.1 Total sample received during the study period

IPD		
Growth N (%)	**No Growth N (%)**	**Total**
762(20.6%)	2,930(79.3)	3,692
OPD		
Growth N (%)	**No Growth N (%)**	**Total**
497(4.9%)	9,450(95%)	9,947
1,259	12,380	13,639

In the present study 1,259 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.,* (2013). A low prevenence rate of 7.15%was reported by Alippour, Nilifar *et al.,* (2010) whereas higher prevalence rate of 26.45 % *E.coli* isolates was reported by 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.

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 A. suneetha *et al.,* (2017), most isolated *E.coli* were from urine (26.79%).

Table.2 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	0.98%
swab	2	0.98%
Brachial aspirate	2	0.98%
Ascitic fluid	2	10.98%
BAL	1	0.49%
Total	204	-

Table.3 Age wise Distribution of *E.coli*

Age in years	No. of isolates (N)	Percentage (%)
0-30 year	112	54%
31-50 year	40	19%
51-70 year	35	17%
71- 90 year	17	8%
Total	204	-

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

Ward	No. of isolates	%
General surgery	16	7%
NICU	3	1%
Paedritics	2	0.9%
psycology	2	0.9%
MICU	2	0.9%
Orthopaedic	2	0.9%
ICCU	1	0.4%
Gynaecology	1	0.4%
OPD	175	85%
Total	204	-

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 Positive Screened Isolates

Total *E.coli* isolates	*E.coli* screened positive for Cefotaxime N (%)	*E.coli* screened positive for Ceftazidime N (%)
204	60 (76%)	108 (73%)

Table.7 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.8 ESBL Positive Confirmed Isolates by double disc synergy test

E.coli	Total (204)	ESBL Positive Isolates	ESBL Negative Isolates
Cefotaxime	60 (76.47%	31 (51%)	29 (48%)
Ceftazidime	108 (73.10%)	53 (49%)	55(50%)

Most of this study *E.coli* was isolated from patients admitted in General surgery (7%). A simiar prevalence of 26.1% & 29% was reported by Fatima Jummai *et al.,* (2019) respectively.

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 the present 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).

Table.9 On Comparison between the two method Combined disc test & Double disc synergy test using 3rd generation cephalosporin

	Total	Positive	Negative	P value
Cefotaxime by Combined disc test	60	33	27	
Ceftazidime by combined disc test	108	56	52	
Cefotaxime with Amoxyclav by Double disc synergy test	60	31	29	**.61**
Ceftazidime with Amoxyclav by double disc synergy test	108	53	55	

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.,* (2019) & disagreement to this pattern was observed by Roshene (2015) were resistant rate to Gentamicin was (64.6 %)

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 our 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. Out of 168 screened isolates, (55%) were ESBL positive by combined disc test while (51%) were positive by Double disc synergy test. Our study showed that Combined disc test & Double disc synergy test both are inexpensive, simple, convenient & have a good sensitivity & specificity for the detection of ESBL in *E.coli* (Meeta Sharma *et al.,* 2013).

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

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.

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