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Original Research Article

# ANTIBIOTIC RESISTANCE AND ESBL DETECTION IN ESCHERICHIA COLI STRAINS FROM CASES OF URINARY TRACT INFECTIONS

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**ABSTRACT**

**Background:** The present study aimed to determine the antibiotic susceptibility pattern of uropathogenic Escherichia coli (E.coli) in our region along with detection of prevalence of ESBL producers and biofilm production. This study is also evaluating colistin resistance among uropathogenic Escherichia coli.

**Materials & Methods**: A total of 100 *Escherichia coli* isolates were obtained from mid stream urine samples. Each sample was inoculated within 4 hr on CLED agar and incubated at $37^0$ C aerobically for 16-18 hr. All the isolates which were resistant to ceftazidime and cefotaxime were further tested for ESBL production by confirmatory phenotypic double disc synergy method using ceftazidime, ceftazidime-clavulanic acid and cefotaxime, cefotaxime-clavulanic acid discs. More than 5mm increase in zone diameter for cetfazidime or cefotaxime tested in combination with clavulanic acid versus its zone when tested alone confirmed an ESBL producing organism.

**Results:**In the present study, out of 100 cases, higher incidence of *E.coli* was observed among females (58%) than in males (42%).Majority of the *E.coli* isolates were sensitive to chloramphenicol (90%), nitrofurantoin (72%) and ertapenem (69%) and imipenem (58%). High resistance was detected with cefotaxime (90%) ciprofloxacin (90%), ampicillin(83%) and ceftazidime (72%).Carbapenems showed low resistance when compared to other group of drugs.53% of *E.coli* isolates were ESBL producers by double disc synergy method. No colistin resistance was observed.

**Conclusion:**Drug resistance in uropathogenic *E.coli* (UPEC)is increasing, which leads to increased morbidity, mortality and economic burden on patients, hence the need for appropriate antibiotic use with a local antibiotic policy and establishment of antimicrobial stewardship in every healthcare facility.

**Keywords:** Antibiotic susceptibility, uropathogenic *E.coli*, colistin resistance, disc elution method, ESBL.

## INTRODUCTION

Urinary tract infections (UTI) are one of the most frequent bacterial infection, burdening more than 150 million of new cases every year.[1]*Escherichia coli* accounts for 70-95% of UTI which is normal gut flora in human beings.[2]

The increasing drug resistance in uropathogens is posing a great therapeutic challenge in the treatment of UTI.

Extended spectrum betalactamases (ESBL) are enzymes that mediate resistance to 3rd generation cephalosporins, such as cefotaxime, ceftriaxone, ceftazidime and monobactum. Such enzymes are

commonly found in *Escherichia coli* and other enterobacteriales.[3]

ESBL producers are also gaining resistance to other classes of antibiotics leading to multi drug resistance which are resistant to more than two groups of drugs rendering the treatment difficult posing challenges in clinical practice.[4]

Resistance to aminogylcosides, flouroquinolones, betalactams and carbapenems lead to consideration of colistin in the treatment of UTI. There is very low resistance encountered in gram negative bacteria towards colistin. The occurrence of ESBL producers changes from time to time and also place to place rapidly.[5]

Increasing prevalence of ESBLs is creating a mandatory need for laboratory to identify the antibiotic susceptibility pattern & ESBL producer.

Biofilm forming UPEC prevalence ranged from 60%to70%.Biofilm production in *E.coli* increases the rate of UTI and also exhibit multidrug resistance. Bacterial biofilm formation is considered as one of the important virulence factor because to kill the organism in the biofilm,1000-fold antibiotic concentration is needed.

Present study aimed to determine the antibiotic susceptibility pattern in our region along with detection of prevalence of ESBL producers and biofilm production. This study is also evaluating colistin resistance among uropathogenic *E.coli.*

## MATERIAL AND METHODS

This is a prospective study done in the Department of Microbiology, Siddhartha Medical College, Vijayawada for a period of one year after the approval of the institutional ethical committee.

A total of 100 Escherichia coli isolates obtained from mid-stream urine samples obtained from patients with suspected urinary tract infection before the administration of antibiotics were included in the study. Each sample was inoculated within 4 hr on CLED agar and incubated at $37^0$C aerobically for 16-18 hr. Significant growth of *E.coli* i.e $10^5$CFU/ml according to KASS criteria was processed further. Lactose fermenting colonies were subjected to further identification by standard conventional biochemical methods.

Antibiotic susceptibility testing of all the isolates was done by Kirby Bauer disc diffusion method for all the antibiotics except colistin for which MIC was done by disc elution method according to CLSI guidelines. The list of antibiotics tested were ampicillin, amoxycillin-clavulanic acid, ciprofloxacin, trimethoprim-sulphamethoxazole, imipenem, meropenem, ertapenem, cefepime, cefotaxime, ceftazidime, tetracycline, chloramphenicol, gentamicin and nitrofurantoin.

All the isolates which were resistant to ceftazidime and cefotaxime were further tested for ESBL production by confirmatory phenotypic double disc synergy method using ceftazidime, ceftazidime-clavulanic acid and cefotaxime, cefotaxime-clavulanic acid discs. More than 5mm increase in zone diameter or cetfazidime or cefotaxime tested in combination with clavulanic acid versus its zone when tested alone confirmed an ESBL producing organism.

Biofilm production was tested by congo red agar method.All the isolates that showed black colored colonies on congo-red agar were considered as biofilm producers.

## RESULTS

Out of 100 cases, higher incidence of *E.coli* was observed among females (58%) than in males (42%). Majority of the *E.coli* isolates were sensitive to chloramphenicol (90%), nitrofurantoin (72%) and ertapenem (69%) and imipenem (58%).High resistance was detected with cefotaxime (90%) ciprofloxacin (90%), ampicillin(83%) and ceftazidime (72%).[Table 1]

Carbapenems showed low resistance when compared to other group of drugs. Though presumptive ESBL were 91%, only 53% of *E. coli* isolates were confirmed to be ESBL producers by double disc synergy method.

Though presumptive ESBL were 91%, only 53% of *E. coli* was confirmed ESBL producers by combined disc diffusion method.

11% showed intermediate resistance (that is ≥2µ l) to colistin and remaining 89% were sensitive (showed more than ≥1µ l).There was no resistance detected to colistin (≥4µ l)

72% *E.coli* were biofilm produces, but they did not show much difference in the distribution between ESBL & Non ESBL producers.

**Table 1: Results of Antibiotic sensitivity testing**

| Antibiotic | Sensitive% | Intermediate% | Resistance % |
|---|---|---|---|
| Amoxycillin-ClavulanicAcid | **36** | **31** | **33** |
| Ampicillin | **14** | **3** | **83** |
| Cefepime | **14** | **23** | **63** |
| Cefotaxime | **1** | **9** | **90** |

| | | | |
|---|---|---|---|
| Ceftazidime | **10** | **18** | **72** |
| Chloramphenicol | **90** | **4** | **6** |
| Ciprofloxacin | **0** | **10** | **90** |
| Ertapenem | **69** | **13** | **18** |
| Gentamicin | **20** | **34** | **46** |
| Imipenem | **58** | **20** | **22** |
| Meropenem | **56** | **23** | **21** |
| Nitrofurantoin | **72** | **8** | **20** |
| Tetracycline | **44** | **2** | **54** |
| Trimethoprim-sulphamethoxazole | **50** | **2** | **48** |

## DISCUSSION

*Escherichia coli* is a well-known uropathogen. UTI infections are more common in female due to anatomical factors. This study showed 58% isolates of *E.coli* were from females and 42% were from male patients. Samiyah *et al*., (2023) study collective count of 1644 incidents of *Escherichia coli (E. coli )* was observed, where in *E. coli* constituted 85% of the cases, while the remaining 15% comprised *E. coli* ESBL producers. Majority of the *E.coli* isolates were sensitive to chloramphenicol (90%), nitrofurantoin(72%), ertapenem (69%) imipenem (58%). Samiyah et al., (2023) study shows that *E. coli* ESBL were sensitive to colistin, tigecycline, amikacin, meropenem, imipenem, and nitrofurantoin by 100% and 93.3–100%, 95–99.6%, 95–99.06%, and 81–91%, respectively and in Niranjan V.*et al*., (2013) study, of the total 311 *E. coli* isolates, 91 (76.51%) were multi drug resistant (MDR). The isolates showed high levels of resistance to ampicillin (88.4%), amoxicillin-clavulanic acid (74.4%), norfloxacin(74.2%), cefuroxime (72.2%), ceftriaxone (71.4%) and co-trimoxazole (64.2%). The isolates were sensitive to amikacin (82.6%), piperacillin-tazobactum (78.2%), nitrofurantoin (82.1%) and imipenem (98.9%).[6-7]

Though chloramphenicol is not recommended as a first-line choice for treating complicated urinary tract infections, it can be alternative for infections caused by MDR and/or XDR pathogens in renal transplant patients where in other study ceftriaxone was most commonly used for empirical therapy for UTI among inpatients in hospital.

High resistance was detected with cefotaxime (90%) ciprofloxacin (90%), ampicillin (83%) and ceftazidime (72%). Imipenem (22%), meropenem (21%), nitrofurantoin (20%) showed low resistance when compared to other group of drugs.Though presumptive ESBL were 91%, our present study confirmed only 53% of *E. coli* were ESBL producers by combined disc diffusion method. Sheriff *et al* reported 57% ESBL production in *E.coli* production by Phenotypic Confirmation Disc Diffusion Test. Higher percentage of resistance to ciprofloxacin in the current study (90%) was correlating to Rajeswari Pilli *et al*. (95%), which can be attributed to inappropriate, excessive use and over counter issue of oral formulations of fluoroquinolones . Roshan Pandit *et al*., (2019) shows that the *Escherichia coli* (154, 62.1%) was the key uropathogen, and majority (~64.9%) of them were multidrug resistant (MDR). Among MDR *E. coli* isolates, 40.3% were producing extended-spectrum β-lactamases (ESBLs). bla-TEM (83.8%), bla-CTX-M (66.1%), and bla-SHV (4.8%) were common ESBL genotypes. Nitrofurantoin, gentamycin, and imipenem were the most effective antibiotics for ESBL-producing *Escherichia coli* isolates.[8]

Samiyah *et al*., (2023) The prevalence of *E. coli* ESBL was observed to be 64.7% in females and 35.3% in males, with a majority (67%) of the affected individuals being over the age of 50.The prevalence of ESBL ranged from 19-60% from various hospitals in India.[6-13]

72% *E.coli* were biofilm producers , but they did not shown much difference in the distribution between ESBL & Non ESBL.

Colistin resistance detected by minimum inhibitory concentration by disc elution method according to CLSI guidelines 0% of resistance detected (≥4µl) and 11% showed intermediate resistance that is (≥2µl) and remaining 89% showed more than (≥1µl). Shresta *et al*,[9] and Sheriff *et al*,[10] reported 100% colistin sensitivity. According to Narayan Ahirwar *et al.*, (2024) Colistin susceptibility was observed in cases of multi drug resistance (MDR) and pan-drug resistance (PDR).[11-16]

## CONCLUSION

In the present study, out of 100 cases, higher incidence of *E.coli* was observed among females (58%) than in males (42%). Majority of the *E.coli* isolates were sensitive to chloramphenicol (90%), nitrofurantoin (72%) and ertapenem (69%) and imipenem (58%). High resistance was detected with cefotaxime (90%) ciprofloxacin (90%), ampicillin (83%) and ceftazidime (72%).Carbapenems showed low resistance when compared to other group of drugs. 53% of *E.coli* isolates were ESBL producers by double disc synergy method. No colistin resistance was observed. Drug resistance in uropathogenic *E.coli* is increasing, which leads to increased morbidity, moratlity and economic burden on patients, hence the need for appropriate antibiotic use with a local antibiotic policy and establishment of antimicrobial stewardship in every healthcare facility and strict guidelines for over counter use of antibiotics.

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