



Research Article

RESISTANCE PATTERN IN CLINICAL ISOLATES OF *ESCHERICHIA COLI* AGAINST FOUR GROUPS OF ANTIBIOTICS

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Abstract: *Escherichia coli* are associated with variety of infections in our country. The developing resistance among different strains of *Escherichia coli* against different antibiotics is a serious problem all over the world. *Escherichia coli* are opportunistic pathogens. It is the most common cause of urinary tract infections, neonatal meningitis and diarrhea. During the present study 50 isolates of *Escherichia coli* has been collected from Dr.Ziauddin Hospital Laboratory. The sensitivity pattern of these isolates to Ciprofloxacin, Cefepime, Cefecolor, Cefexime was determined by disc diffusion method. Data was analyzed by using National Committee for Clinical standards (NCCLS). *Escherichia coli* when tested against four groups of antibiotic showed resistance against Cefepime(42%), Ciprofloxacin(64%), Cefexime(60%) and Cefecolor(52%) in hospitalized patients. Present study showed that Cefepime is the most effective antibiotic against *Escherichia coli*. However, *Escherichia coli* has developed resistance against traditionally used antibiotics especially Ciprofloxacin which has reduced the number of choices for physicians in prescribing medicine.

Key words: Resistance, Broad spectrum, *Escherichia coli*.

INTRODUCTION

Escherichia coli are classical specie of coli form group of bacteria. It is facultative anaerobic, Gram positive, non-sporulating bacilli that produce acid and gas from fermentation of lactose¹. Although it is part of normal flora of intestinal tract, certain strains can cause a moderate to severe gastroenteritis in humans and animals. Enteroinvasive strains invade epithelial cells of large intestine and cause diarrhea in older children and adults, enterotoxigenic strains colonize the small intestine and cause acute gastroenteritis in newborns and in infants up to 2 years of age. Other strains of *Escherichia coli* which are usually harmless in their normal habitat(the intestine) can cause disease when they gain access to other sites or tissues². This includes both nosocomial and community acquired urinary tract infection (UTI). The spectrum of disease ranges from cystitis to pyelonephritis. It also causes pulmonary infections, peritonitis, it is an important cause of neonatal meningitis, and it can be isolated from wound infections³.

Cephalosporins are similar to Penicillins chemically and inhibit bacterial cell wall synthesis. They are more stable than Penicillin to many bacterial beta lactamases and therefore usually have a broad spectrum. The explosive growth of the cephalosporin during past decade has made a system of classification by their chemical structure, clinical pharmacology, and resistance to beta lactamase or antimicrobial activity⁴.

Cefecolor is the second generation cephalosporin. It is used orally. The concentration in plasma after oral administration is about 50 of those achieved after an equivalent oral dose of cephalexin. It has been primarily used to treat sinusitis, otitis or lower respiratory tract infections⁵.

Cefexime is a bactericidal and is stable to hydrolysis by many beta-lactamases. It is generally classified as a third generation cephalosporin antibacterial and is given orally for the treatment of susceptible infections. Cefepime is referred to as extended spectrum of fourth generation parental cephalosporin. Cefepime is used in the treatment of infections due to susceptible organisms. They include infections of the urinary tract infection, respiratory tract, and skin,⁶ monotherapy for empirical treatment of fever in granulocytopenic cancer patients, serious bacterial infections such as septicemia, osteomyelitis, other infections.

Ciprofloxacin is a fluoroquinolone which is intended for both oral and parental use. It is one of the second generations of quinolones. It has good tissue penetration and high potency against most Gram negative pathogen, it is less effective against Staphylococci and borderline or poor activity against Streptococci and anaerobes. It is found to be highly effective against majority of Enterobacteriaceae such as *Escherichia coli*, other microorganisms such as *Enterococcus faecalis*, methicillin-susceptible *Staphylococcus epidermidis*, *Staphylococcus*

saprophyticus are usually susceptible to ciprofloxacin. It is considered to be ideal for the treatment of urinary tract sepsis, prostatitis and epididymorrchitis, enteric fever, soft tissue and skin infections, osteomyelitis, bacterial diarrhea, respiratory tract infections, in cystic fibrosis, endocarditis, meningitis, empiric treatment of bacteremia, rickettsial diseases, otitis, sinusitis, ocular brucellosis, mycobacterial infection etc.⁷.

MATERIAL AND METHODS

The present study was carried out to evaluate the sensitivity pattern of 50 isolates of *Escherichia coli* against three Cephalosporins of varied generations that is Cefecolor, Cefexime, Cefepime, and also against flouroquinolone that is Ciprofloxacin. For this purpose clinical isolate have been collected from different laboratories and hospitals and sensitivity pattern of *Escherichiacoli* was determined by disc diffusion method.

Mueller Hinton broth:

Each isolate was grown in 5 ml Mueller Hinton broth at 37 °C for 2-4 hrs. until turbidity reached. 0.5mcfarland standard, if it exceeds, the suspension was diluted with broth till it was usually comparable to the Mcfarlands standard.

Muller Hinton agar:

Mueller Hinton agar was used for disc diffusion susceptibility testing, according to National Committee for Clinical Laboratory Standards (NCCLS) and international guidelines. The medium was poured into 90 mm diameter sterile petri dishes to a depth of 4 mm, agar has to be

solidify at room temperature and sealed in plastic bag and stored at 2-8 °C. Before use it has to be dried in a 35- 37°C incubator for 30 minutes.

Anti-microbial discs:

Commercially available antibiotics discs were used. Cefecolor(CEC 30 OXOID), Cefexime(CFM5 OXOID), Cefepime (CEP 30 OXOID), Ciprofloxacin(CIP 5 OXOID).

Mcfarland Turbidity Standards:

The barium sulphate standard is used to compare the turbidity of the test and control inocula.⁸.

Colonies of *Escherichia coli* were taken by the help of sterile wire loop and inoculated in 3-4ml of Mueller Hinton broth. The broth was incubated at 37°C for 4-6 hrs. Then the turbidity of broth was checked to the turbidity of 0.5 Mcfarland standards. Then by using a sterile swab inoculate plates of Mueller Hinton agar. Remove the excess fluid by pressing and rotating the swab against the side of tube swabbed the plates in (3)three directions, rotating the plate approximately 60°C(to ensure even distribution).The surface of the agar was dried for 3-5 min. Place anti-microbial discs by using sterile forceps. The disc should be about 15mm from the edge of the plates and not closer than 25 mm from disc to disc. The disc should be slightly pressed to ensure its contact with the medium. Incubate the plate at 37°C overnight. The zone of inhibition was measured in mm, according to reference standards(Table 1)⁹.

Table 1:NCCLS Standards of different antibiotics against *E. coli*

Antibiotics	Zone of Inhibition Diameter (mm)			
	Disc Content	Resistance	Intermediate	Sensitive
Ciprofloxacin	5 µg	≤15	16-20	≥21
Cefepime	30 µg	≤14	15-17	≥18
Cefixime	5 µg	≤15	16-18	≥19
Cefecolor	30 µg	≤14	15-17	≥18

Table 2: Total % efficacy of different antibiotics among *Escherichia Coli* isolated (N= 50)

Antibiotics	Disc Code	Resistance	Intermediate	Sensitive	Efficacy
Ciprofloxacin	CIP	32	-	18	36
Cefepime	FEP	21	-	39	58
Cefixime	CFM	30	07	13	26
Cefecolor	CEC	26	10	14	28

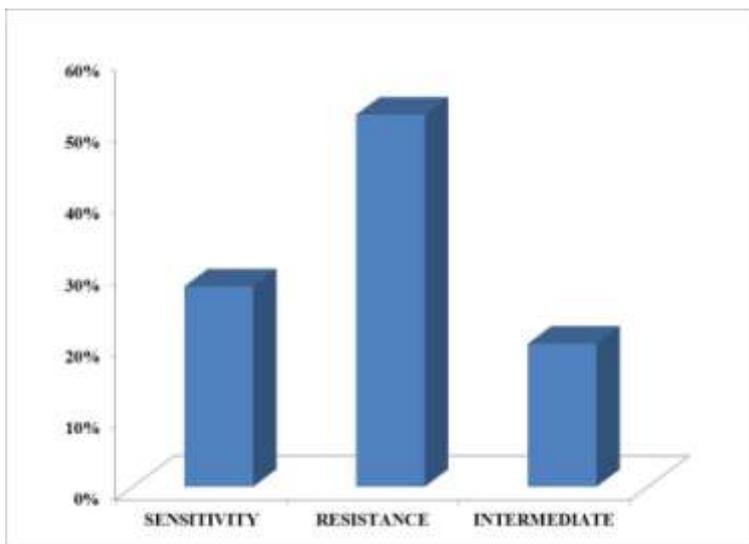


Figure: 1, Susceptibility pattern of *E. coli* against Cefclor.

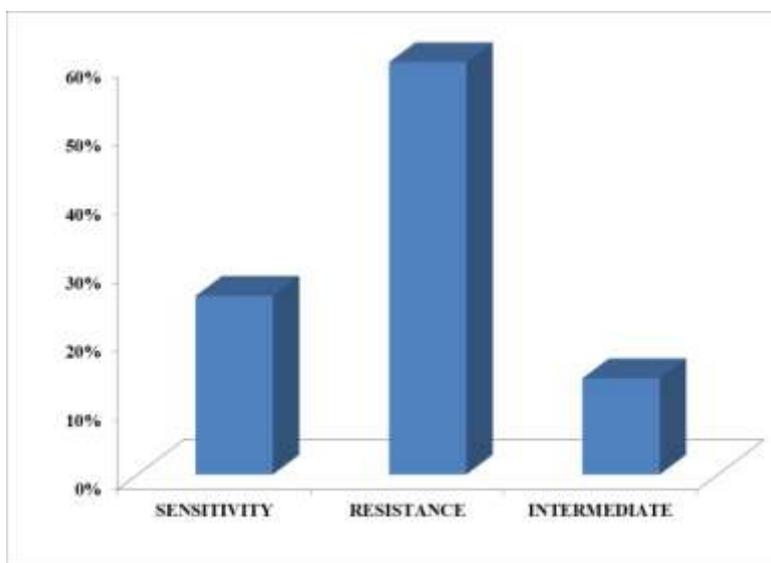


Figure: 2, Susceptibility pattern of *E. coli* against Cefexime

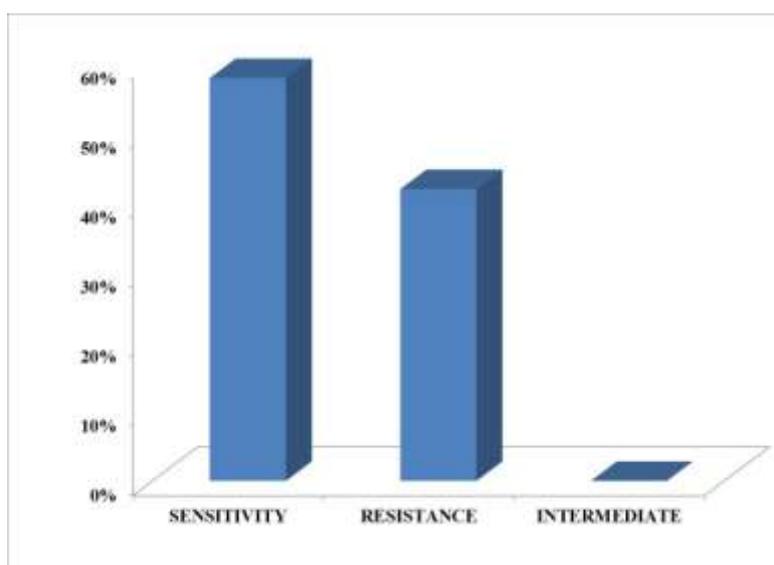


Figure: 3, Susceptibility pattern of *E. coli* against Cefepime

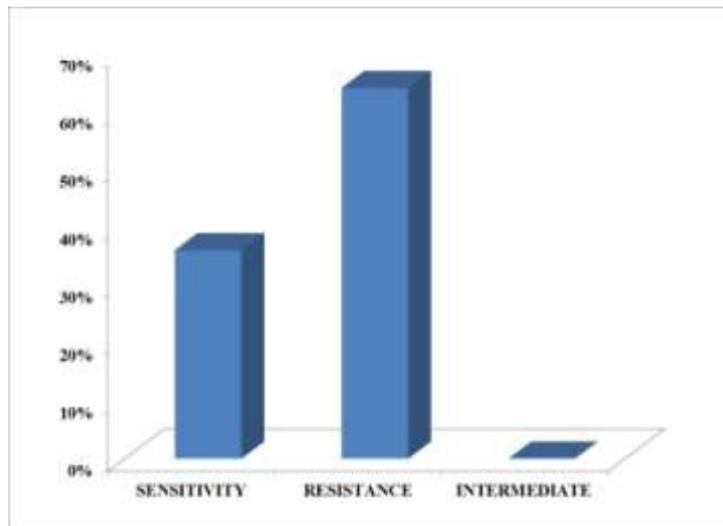


Figure: 4, Susceptibility pattern of Ciprofloxacin against *E. coli*

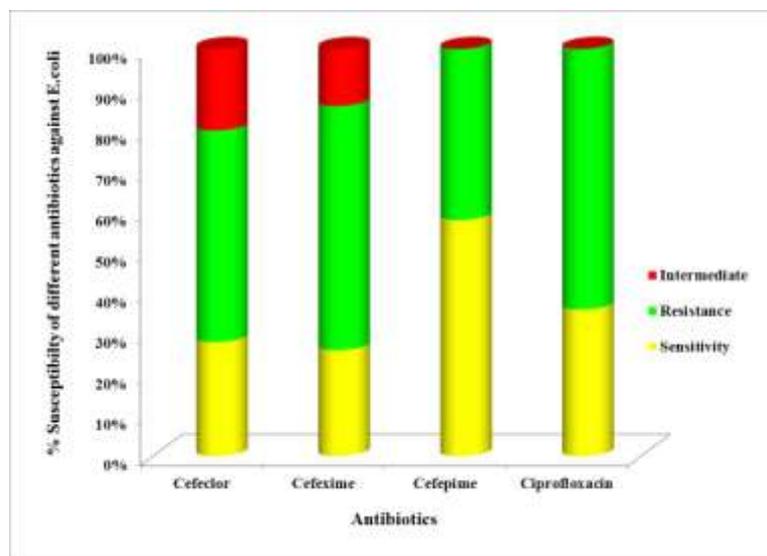


Figure:5, Comparison of susceptibility pattern of *E. coli* against different groups of antibiotics.

RESULTS

During the present study, attempt has been made to evaluate the sensitivity of Ciprofloxacin, Cefepime, Cefclor and Cefexime against *Escherichia coli*. For this purpose clinical isolates were collected from different hospitals and pathological labs and sensitivity was determined by Disc Diffusion Method.

Resistance Pattern Against Antibiotics :

The overall percentage towards different groups in order of resistance being antibiotics resistance, Cefclor(52%), Cefexime(60%),Cefepime(42%), Ciprofloxacin(64%).

Susceptibility Pattern Of Eschericia coli:

Cefepime was found to be most effective showing 58% sensitivity while cefepime and cefclor showed less effectiveness with 26% and 28% sensitive strains respectively whereas ciprofloxacin has intermediate sensitivity of 36%.

The sensitivity pattern of the mentioned drugs against 50 isolates of *Escherichia coli*, also explained in figure 1-4.

DISCUSSION

Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries. The present in vitro study was designed to identify the susceptibility or the resistance profile of the isolates of various strains of *Escherichia coli* from different clinical laboratories and hospitals of Karachi. For this purpose 50 clinical isolates of *Escherichia coli* were isolated and identified by conventional methods. The normal intestinal flora are a reservoir for resistance genes, the prevalence of resistance in commensal *Escherichia coli* is useful indicator of antibiotic resistance in bacteria in the community. The prevalence of strains resistant to antibiotics has been found to be increased that is the resistance gene reservoirs are increasing in healthy persons¹⁰.

Moreover when low doses of antibiotics were used against bacteria, they inhibit the growth of susceptible bacteria and leaving the smaller number of already resistant bacteria to thrive and grow. These bacteria spread their resistance traits to other previously nonresistant cells then eventually affecting other cells¹¹.

The increasing trend of developing resistance among microorganisms has been observed. The trend of developing resistance among various strains of microorganisms is a serious clinical threat. For the past few decades it has been observed that due to various reasons normal flora microorganisms are undergoing genetic mutations and which is the leading cause of developing resistance against traditionally used antibiotics to treat infections caused by those organisms. *Escherichia coli* is found to be the leading cause of urinary tract infections and other infections all over the world and it has also shown resistance whether the source is human or animal and in human being specifically the children and women are found to be most prone to infections caused by *Escherichia coli*. The lower sensitivity rate of *Escherichia coli* to commonly used antibiotics to treat infections caused by it, have drawn attention of physicians and pharmacists towards selection of antibiotics by finding the resistance pattern eradicates the serious illnesses.

The present study was designed to identify the resistance profile of *Escherichia coli*. In order to assist physicians present study has shown increasing trend of resistance against 1st, 2nd and 4th generation cephalosporin and 3rd generation quinolones. The prevalence of resistance to most drugs tested in *Escherichia coli* isolates from apparently healthy human being is within the high range reported previously and has increased from 1986 to 1998¹⁰. *Escherichia coli* are the most common isolate recovered from clinical specimens all over the world¹².

These results are in accordance with the work of Xio YH *et al.* reported 65% resistance of *Escherichia coli* against fluoroquinolones¹³. High resistance rate were shown for *Escherichia coli* against both Cefepime and Ciprofloxacin by Cefepime 81.7% and ciprofloxacin 50.9%¹⁴.

According to results of study reported at Annual Conference on Antimicrobial Resistance, the resistance of uropathogenic *Escherichia coli* to Ciprofloxacin among college students has doubled since 1999¹². Findings are further substantiated by the work of Okeke *et al.*¹⁰ observed a rapid increase in prevalence of resistance in commensal *E.coli* against multiple antibiotics in 1986-1998, Oslon *et al.* reported antibiotic resistance in urinary isolates of *Escherichia coli* from college women with urinary tract infection 11.8% to ciprofloxacin compared to 1.8% among those without prior urinary tract infection¹⁵.

In a respective study it was reported that 38% *Escherichia coli* strain isolated from complicated UTI were found to be resistant to Ciprofloxacin¹⁶.

Saeed *et al.* reported emerging multiple resistance among *E.coli* towards Cefixime 82.8% and Ciprofloxacin 75.9%¹⁷, Ribeirokoch *et al.* reported Ciprofloxacin showing increasing trend of resistance 3.3% per year since 2000-2004¹⁸, Sabate *et al.* reported 38% resistance of *Escherichia coli* to Ciprofloxacin¹⁹. Similarly, Angeliki Mavroidi *et al.* observed increase of resistance to fluoroquinolones²⁰. Our result is also in resemblance to Muhammad Naeem *et al.* reported in their studies that susceptibility of uropathogens including *E.coli* to fluoroquinolones is decreasing inpatients who are hospitalized²¹.

Our result is not in confirmation to the results of Farajnia *et al.* reported 94% susceptibility of *E.coli* to Ciprofloxacin²². The present results were found to be in contradiction to the findings of Andreu *et al.* reported 95.8% susceptibility rate of *E.coli* for Cefixime and 77.2% for Ciprofloxacin²³, Anatoliotaki *et al.* reported antimicrobial resistance of *E.coli* isolate to Cefaclor 22.5%²⁴, Elmanama *et al.* showed *E.coli* resistance to Cefaclor 42.2%²⁵.

Zhang *et al.* compared the resistance rate among Cephalosporins and reported increasing trend since 1990-2004 that is of Cefaclor 33.3%-46.8% and also against fluoroquinolones 11%- 55.4%²⁶. Kumamoto *et al.* reported decrease in susceptibility of *E.coli* to Cefaclor after 2000²⁷. The present results are not in confirmation to Pape *et al.* (2004) reported 24% resistance rate of *E.coli* to Cefaclor²⁸. Saeed *et al.* reported resistance of *E. coli* to Cefixime 82.8% and Ciprofloxacin 75.9%¹⁷, Merino *et al.* reported increasing trend of *E.coli* resistance between 2002-2007 against Cefixime and Ciprofloxacin²⁹. In our study, the most effective antibiotic against *Escherichia coli* is Cefepime showing 58% efficacy (Table-2).

CONCLUSION

Present study has shown increasing trend of resistance against 1st, 2nd and 4th generation cephalosporin and 3rd generation quinolones. In order to assist physicians our studies showed that most effective antibiotics against *Escherichia coli* was found to be Cefepime (fig:4), thus it could be the safer choice for them to opt as first line of treatment. However it is recommended that physicians should determine the sensitivity profile before starting antibiotic therapy. The increasing trend of developing resistance among microorganisms has been observed. The trend of developing resistance among various strains of microorganisms is a serious clinical threat. For the past few decades it has been observed that due to various reasons normal flora microorganisms are undergoing genetic mutations and which is the leading cause of developing resistance against traditionally used antibiotics to treat infections caused by those organisms. *Escherichia coli* is found to be the leading cause of urinary tract infections and other infections all over the world and it has also shown resistance whether the source is human or animal and in human beings specifically the children and women are found to be most prone to the infections caused by *Escherichia coli*. The lower sensitivity rate of *Escherichia coli* to commonly used antibiotics to treat infections caused by it, have drawn attention of physicians and pharmacists

towards selection of antibiotics by finding the resistance pattern to eradicate the serious illnesses.

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