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Resistance Pattern of Pathogenic *E. coli* Against Ciprofloxacin, Cefotaxime, and Gentamicin

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ABSTRACT

Objective: The objective of this study was to evaluate current susceptibility of pathogenic isolates of *E. coli* against ciprofloxacin, cefotaxime and gentamicin. **Methodology:** Antibacterial activity was performed using Kirby Bauer Technique. **Results:** The average zone values were compared by using ANOVA and result found to be significant with p-value <0.001. Highest zone values were found to be 19.31±9.30 for ciprofloxacin followed by cefotaxime 16.46±9.86. The lowest zone value were found to be for gentamicin *i.e.* 12.57±2.19. Resistance against ciprofloxacin and cefotaxime were 82.5% and 85 % respectively. While high degree of resistance was observed among isolates against gentamicin (97.5 %). **Conclusion:** Increasing resistance against ciprofloxacin and cefotaxime demands coordinated monitoring of activity and rational use of these antibiotics and development of new, safe and effective therapeutic agents.

Key words: Antibacterial activity, Bacterial resistance, *E. coli*, Ciprofloxacin

INTRODUCTION

Escherichia coli members of the family *Enterobacteriaceae* are major cause of urinary tract infections in women[1]. These infections are complicated by the wide distribution and increasing prevalence of antibiotic-resistant strains of *Escherichia coli*[2]. The development of some resistance is almost certainly an inevitable consequence of the clinical use of antimicrobial drugs[3]. The variety of mechanisms by which bacteria acquire resistance to antimicrobial drugs is astonishing. Microbial resistance develops through random mutation, drug inactivation, decreased drug uptake, decreased drug receptor sites, and modification of metabolic pathways formally attacked by the drug[4].

Resistance development occurs primarily among bacteria already resistant to one or more antimicrobial agents[5]. High resistance to ciprofloxacin was detected among *Escherichia coli*[6]. Most ciprofloxacin-resistant strains were multidrug resistant[7]. Ciprofloxacin resistance among *E. coli* isolates was found to be 12%[8]. Multiple antibiotic resistances have increased dramatically in some hospital isolates, and appear to be associated with hospital cross-infection. Resistance to gentamicin among *E. coli* isolates varied 0.4% to 3.2%[9].

The underlying resistance problems are largely due to socioeconomic and behavioral factors[10]. High efficacy and relative lack of adverse effects has resulted in overuse in many situations, and increasing resistance to available drugs has become a worldwide problem[11]. An urgent need exists for more appropriate selection and use of antimicrobial drugs in the developed as well as in developing countries. The focus in developing countries should be on the availability of safe and effective drugs[12].

The current study was conducted to check the resistance status of *E. coli* isolates obtained from different patients against ciprofloxacin, cefotaxime and gentamicin.

MATERIALS & METHODS

Collection of specimen

Isolates of pathogenic *Escherichia coli* were randomly taken from the urine & blood samples of the patients at microbiology lab, Fauji Foundation Hospital and Pakistan Institute of Medical Sciences, Islamabad. The sample size was calculated at 5% level of significance and 80% power of test. These parameters were calculated by using proportions of expected resistance for different drugs.

Drugs

Standard Antibiotic discs of ciprofloxacin (CIP 5), cefotaxime (CTX 30), and gentamicin (CN 10) were used for comparison.

Determination of antibacterial activity

Nutrient agar and nutrient broth were made[13] and media was poured into disposable Petri plate @ 10-15 ml / plate. Nutrient broth was dispensed into sterilized glass test tubes @ 5 ml/ test tube. Broth test tubes were inoculated with bacterial isolates and placed in incubator at 37°C for 24 hours. The antibacterial activity of drugs was determined by the Kirby Bauer Technique. Nutrient agar plates were inoculated with the selected isolates @ 200 µl/ plate. Three standard drug discs were placed on each plate. The plates were incubated at 37°C for 18-24 hrs. The relative antibacterial potency of drugs was calculated by comparing zones of inhibition[14].

Statistical analysis

Data was entered and analyzed by using SPSS. 14.0. Quantitative variables,zone of inhibition were measured in mm and were compared using **ANOVA**. Qualitatively sensitivity and resistance for each drug were reported by frequency and percentages. Comparisons for sensitivity and resistance were done using Chi-Square.

RESULTS

Total number of *E. coli* isolates samples were 100 % (40). Out of which 60% (24) *E. coli* were isolated from female patients and 40% (16) *E. coli* were isolated from male patients. This shows more prevalence of *E. coli* infections in female than male. Ages of the patients were between 14 and 56 years in both the sexes, with a mean age of 33.78 and SD of 12.38. Table 1 show that *E. coli* infects all age groups in both the sexes.

When Zone values for different drugs were compared, zone values appeared for 18, 23 and 40 cases for ciprofloxacin, cefotaxime and gentamicin respectively. Rest of the cases gave completely no response. The average zone values were compared by using ANOVA and result found to be significant with $p < 0.001$. The lowest zone value were found for gentamicin i.e. 12.57±2.19. While ciprofloxacin and cefotaxime average zone values were 19.31±9.30 and 16.46 ± 9.86 respectively (Table 2, Fig. 1). After Post hoc Test, i.e. Tukey's test gentamicin was significantly lower than ciprofloxacin and cefotaxime. But ciprofloxacin and cefotaxime were having no significant difference.

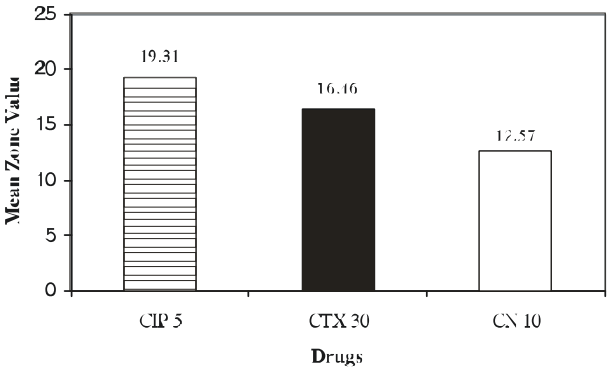

Fig. 1: Average zone values of various drugs against *E. coli*.

Difference was significant among all drugs, when a comparison was made for patients who are resistant, sensitive, giving no response and having response but then re-growing., all with p-values <0.001. On basis of cut off value, numbers of resistant cases were 10 from ciprofloxacin, 14 from cefotaxime and 39 from gentamicin, showing gentamicin to be the most resistant and ciprofloxacin the most sensitive one. When comparisons were made among sensitive cases the results were exactly opposite to that of resistant. When comparison were made among the group of No response, there was a significant percentage of cases for ciprofloxacin and cefotaxime i.e., 55 and 42.5%, respectively,

Table 1: Distribution of patients and age by status and gender

Pathogen	Gender	N	%	Mean	S.D	Min.	Max.
Escherichia coli	Male	16	40.00	32.56	12.84	15	55
	Female	24	60.00	34.58	12.28	14	56
	Total	40	100.00	33.78	12.38	14	56

Table 2: Comparison of zones of inhibition of drugs against *E. coli* via statistical data.

Drug	N	Mean	Std. Deviation	Std. Error	95% Confidence interval for mean		Min	Max
					Lower bound	Upper bound		
Ciprofloxacin	18	19.31	9.30	2.19	14.68	23.93	8.50	33.30
Cefotaxime	23	16.46	9.86	2.06	12.20	20.72	7.80	33.20
Gentamicin	40	12.57	2.19	0.35	11.87	13.27	9.20	18.30
Total	81	15.15	7.39	0.82	13.52	16.78	7.80	35.20

Table 3: Frequency of resistance and sensitivity of drugs against *Escherichia coli.*

	Ciprofloxacin		Cefotaxime		Gentamicin	
	No.	%	No.	%	No.	%
Resistant	10	25.00	14	35.00	39	97.50
Sensitive	7	17.50	6	15.00	1	2.50
No Response	22	55.00	17	42.50	0	0.00
Re-growth	1	2.50	3	7.50	0	0.00
Chi-Square	**31.20**		**17.33**		**149.60**	
P-Value	**0.0000**		**0.0006**		**0.0000**	

showing complete resistance (Table 3, Fig. 2). The overall gentamicin resistance was 97.5%.

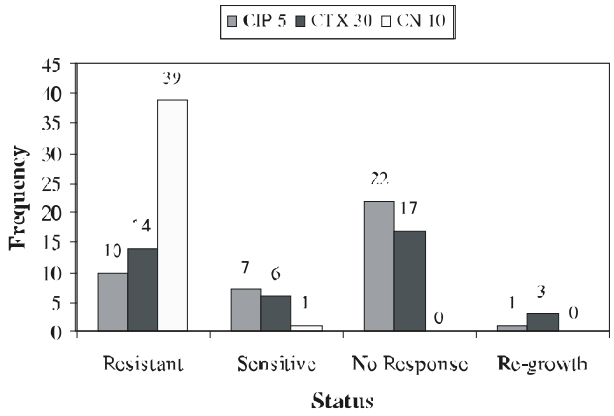

Fig. 2: Resistance status of each drug against *E. coli*.

DISCUSSION

Escherichia coli are most common cause of community-acquired urinary tract infections and gastrointestinal infections in women patients. There is increasing prevalence of antibiotic-resistant strains of *Escherichia coli* [1, 2]. Our studies also confirmed more prevalence of *E. coli* in females and less prevalence in males. *E. coli* infections were found common in patients either who were taking unhygienic food or were hospitalized with catheters. The drugs used to treat *E. coli* infections most frequently now-a-days are ciprofloxacin and cefotaxime. Most of the patients were having the past history of use of these drugs.

All the conditions during antibacterial testing using Disc diffusion method were standardized.

Conditions such as temperature, composition of culture medium, size of inoculum, time of incubation may interfere in the results of resistance tests to drugs against pathogens[14]. In our study, increase in resistance rates was observed to ciprofloxacin, indicating emerging ciprofloxacin resistance among urinary tract infection isolates. 55 % and 42.5% cases of ciprofloxacin and cefotaxime respectively, showed complete resistance with no response against *E. coli*. Increase in resistance trends to ciprofloxacin were also observed for the most prevalent gram-negative agents: *Escherichia coli* in previous studies[6-8]. The reason for this resistance was the most frequent and indiscriminate use of broad-spectrum drugs ciprofloxacin and cefotaxime against *E. coli*. Lack of adverse effects has increased the use of cefotaxime by paramedics. Although several previous reports have revealed that *E. coli* isolates resistant to one antimicrobial agent are likely to be resistant to other antimicrobial agents[5, 7]. Increasing resistance against ciprofloxacin and cefotaxime demands coordinated monitoring of activity and rational use of these antibiotics. Few studies reported much less increase in acquired resistance in *Escherichia coli* organisms against gentamicin increasing from 0.4% to 3.2% for *E. coli*[9]. But in our study gentamicin was found to be the most resistant drug against *E. coli* isolates as in 97.5 %. Gentamicin is most commonly used in birds and animals. And its residues pass in milk, meat and eggs to human being.

CONCLUSION

More research is urgently needed to define mechanisms of resistance, to look for new targets for antimicrobial drugs, to discover more effective ways of using our existing drugs, to minimize the development of resistance, to ascertain the most useful therapy for infections due to multidrug-resistant organisms. But new antibiotics by themselves will not alter the kinetics of the cycles of resistance development. Indeed, wider and more indiscriminate use could actually shorten the cycle time unless behaviour changes, which are difficult but not impossible to achieve, occur with regard to valuing antibiotics as precious and finite resources.

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