QUINOLONES RESISTANCE AMONG *ESCHERICHIA COLI* ISOLATES FROM CLINICAL SPECIMENS

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ABSTRACT

Objective: This study was aimed to isolates the Quinolones resistance *Escherichia coli* from clinical samples including urine, pus and HVS at Hayatabad Medical complex over a period of 4 months.

Material and Methods: A total of 240 samples were processed for isolation of *E. coli* in which 48 (20%) were positive for *E. coli* isolates. Seven Quinolones antimicrobial drugs namely Nalidixic Acid, Ciprofloxacin, Levofloxacin, Norfloxacin, Ofloxacin, Pefloxacin, and Moxifloxacin were used during the study. Antimicrobial activity was tested by standard Disk Diffusion (Kirby-Bauer) method.

Results: The prevalence of *E. coli* in urine sample was 37.5%, Pus 12.5% and HVS 10%. The gender wise distribution of *E .coli* was higher in females than males. Resistant pattern of *E. coli* shows that it is highly resistant to Nalidixic acid 95.8% and least resistance shown to Ciprofloxacin 54%. Ciprofloxacin is a drug of choice which shows 39.5% sensitivity to all isolates of *E. coli,* whereas, Nalidixic acid showed high resistance of 95.5%.

Conclusion: This study concluded that *E. coli* is a dominant microorganism among the clinical specimens in HMC. The *E. coli* is present in females more than males. More than 60% resistance is shown by all Quinolones toward *E.coli*. Ciprofloxacin is a drug of choice and shows 39.5% sensitivity to all isolates of *E. coli,* whereas, Nalidixic acid showed high resistance 95.5%.

Key words: Quinolones, *E. coli*, Antibiotics, Resistance.

INTRODUCTION

Escherichia coli is a common member of the gastrointestinal flora of most vertebrates, including humans, and may be isolated from a variety of environmental sources. While most strains are nonpathogenic, certain ones can cause a variety of infections[1]. Pathogenic variants of *E. coli* cause intestinal and extra-intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicemia. Therapeutic options vary depending on the type of infection. For example, for urinary tract infections, trimethoprim/sulfamethoxazole and fluoroquinolones are treatments of choice[2]. Fluoroquinolone antimicrobial drugs are highly bioavailable, broad-spectrum agents with activity against gram-negative pathogens, especially those resistant to other classes of antimicrobial drugs[3]. The increasing prevalence of fluoroquinolone resistance in *Escherichia coli*, the most common gram-negative pathogen, is concerning. This is particularly true given the negative impact of fluoroquinolone resistance on clinical outcomes[4]. In the past few years, fluoroquinolones have been prescribed more frequently for the treatment of urinary tract infections. This may have led to an increase in fluoroquinolone-resistant *E. coli* infections, which are difficult to treat[5]. The US Food and Drug Administration (FDA) have banned fluoroquinolone use in poultry, after it was associated with the rapid rise in ciprofloxacin resistance in human Campylobacter infections[6].

There is escalating resistance to fluoroquinolones in some countries i.e. 30% resistance of *E. coli* to fluoroquinolones has been reported in Spain while 64% resistance to ciprofloxacin has been reported in Nigeria[7]. The emergence of multi-drug resistant *E. coli* prompted us to conduct this study to determine the antimicrobial susceptibility pattern among different clinical samples in HMC against selected Quinolones and updating of the antibiogram against these antibiotics.

MATERIALS AND METHODS

This study was conducted at microbiology department of Abasyn University Peshawar from April to July 2012. All clinical samples were collected from Hayatabad Medical Complex (HMC) Peshawar and brought to Abasyn University for further processing.

Clinical samples which include midstream urine was collected in a sterile culture and sensitivity bottles,

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Pus sample and high vaginal swabs were collected through sterile swabs from patients. The sample was brought to laboratory in aseptic conditions within hour for further processing. Each sample was inoculated on blood, Macconkey, CLED and EMB agar with the help of wire loop for bacterial growth. Media was then incubated under 37°C for 24 hours. If growth does not appear, it is further incubated for 24 hours as described by Cheesbrough[8] where every specimen which yielded heavy or pure growth were included in this study.

Suspected bacterial species were characterized and identified according to standard bacteriological methods, Gram Stains and biochemical tests such as oxidase, indole production, citrate utilization, triple iron sugar utilization as highlighted by Cheesbrough[8]. TSI is used for differentiation of microorganisms on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulfide production. Oxidase test based upon the oxidase enzyme which is produced by oxidase positive bacteria. Indole test is performed to determine the ability of microorganisms to degrade the amino acid tryptophan after which indole is released.

ANTIBIOTIC SUSCEPTIBILITY TESTING

For determination of antibiotic susceptibility profile of *E.coli*, the Kirby-Bauer disc diffusion method was used for susceptibility testing[9]. Zone of inhibition around the antibiotics disc activity against the bacteria was observed.The diameters of the zones of inhibitions were then measured. The susceptibility (sensitive, intermediate, or resistance) was determined using the Clinical and Laboratory Standard Institute (CLSI)[9].

Following Quinolones and flouroquinolones antibiotics were used for antibiograms of *E.coli* i.e. Ciprofloxacin, Norfloxacin, Levofloxacin, Moxifloxacin, Pefloxacin, Ofloxacin and Nalidixic acid.

RESULTS

A total number of 240 clinical samples were processed for isolation of *E. coli* out of 240 samples 48(20%) of *E. coli* were isolated from different clinical specimens including Urine, pus and high vaginal swab. The percentage of *E. coli* in Urine was 37.5%, HVS 10% and Pus 12.5%. *E. coli* was mostly prevalent in Urine samples (Table 1).

The sex wise incidence of *E. coli* in different samples shows that in urine the ratio of *E. coli* in male patient was 33.3% and female with high percentage 66.6%. In Pus the ratio was 40.0% from male and 60.0% and female. While HVS was 100% isolated from females (Table 2).

The antibiogram of *E. coli* isolated from urine showed most of the isolate were sensitive to Ciprofloxacin 10 (33.3%) followed by Ofloxacin and Levofloxacin 9(30%), Moxifloxacin 7(23.3%), Norfloxacin 6(20%), Pefloxacin 5(16.3%), Nalidixic acid 1 (3.3%) The highest resistance was shown by Nalid-

Table 1: Prevalence of E. coli in different samples.

Sample	Total Positive	Total	Percentage (%)
Urine	30	80	37.5%
Pus	10	80	12.5%
HVS	8	80	10%

Table 2: Sex wise incidence of *E .coli*

Samples	Male Patients	Female Patients
Urine	33.3%	66.6%
Pus	40.0%	60.0%
HVS	_____	100%

TABLE 3: SENSITIVITY PROFILE OF SELECTED ANTIBIOTICS AGAINST E. COLI ISOLATED FROM URINE SAMPLES.

Serial No	Antibiotics	Susceptible	Intermediate	Resistant
1	Ciprofloxacin	10 (33.3%)	1(3.3%)	19 (63.4%)
2	Nalidixic acid	1 (3.3%)	0	29 (96.7%)
3	Norfloxacin	6 (20%)	0	24 (80%)
4	Ofloxacin	9 (30%)	1(3.3%)	20 (60%)
5	Levofloxacin	9 (30%)	1(3.3%)	20 (60%)
6	Moxifloxacin	7 (23.4%)	6 (20%)	17 (56.6%)
7	Pefloxacin	5 (16.3%)	5	20 (60%)

Table 4: Sensitivity profile of selected antibiotics against *E. coli* isolated from Pus samples.

Serial No	Antibiotics	Susceptible	Intermediate	Resistant
1	Ciprofloxacin	3	1	6
2	Levofloxacin	3	2	5
3	Norfloxicin	3	0	7
4	Pefloxacin	1	3	6
5	Moxifloxacin	2	0	8
6	Nalidixic acid	0	1	9
7	Ofloxacin	2	1	7

Table 5: Sensitivity profile of high vaginal swab

Serial No	Antibiotics	Susceptible	Intermediate	Resistant
1	Ciprofloxacin	6	1	1
2	Levofloxacin	5	1	2
3	Norfloxicin	4	3	1
4	Pefloxacin	3	3	2
5	Ofloxacin	5	1	2
6	Moxifloxacin	3	2	3
7	Ofloxacin	0	0	8

ixic acid 29 (96.7%), followed by Norfloxacin 24(80%), Ofloxacin, Levofloxacin and Pefloxacin 20(60%), Ciprofloxacin 19 (63.3%) and finally least resistant shown by Moxifloxacin 17 (56.6%). Some of the isolate shows intermediate zone of inhibition. The highest ratio shown by Moxifloxacin (20%) (Table 3).

The sensitivity profile of *E. coli* from Pus samples shows that Ciprofloxacin, Levofloxacin, Norfloxicin, was highly sensitive 3 (30%) followed by moxifloxacin 2(20%), Pefloxacin 1 (10%) ,and finally Nalidixic acid was most resistant 9(90%) and least resistant shown by levofloxacin 5(50%). Some of the antibiotic used to determine their efficacy showed an intermediate zone of inhibition of them Pefloxacin 3(10%) Levofloxacin 2(20%), Ciprofloxacin, Nalidixic acid 1(10%). No intermediate zone shown by Moxifloxacin and Norfloxacin (Table 4).

The antibiogram shows of E. coli isolate from HVS showed that most of the isolates 6(75%) are highly sensitive to ciprofloxacin and followed by levofloxacin and Ofloxacin 5(62.5%), Norofloxacin 4 (50%), Pefloxacin and Moxifloxacin 3 (37.5%) While Nalidixicacid showed no susceptibility at all. The highest resistance shown by Nalidixic acid 100% followed by Moxifloxacin 37.5%, Ofloxacin, Levofloxacin and Pefloxacin 25%, Ciprofloxacin and Norfloxacin 12.5%. Least resistance was shown by Ciprofloxacin 12.5%. The Norfloxacin and Pefloxacin showed 3(37.5%) highest intermediate zone of inhibition (Table 5).

DISCUSSION

Various studies on antibiotic resistance are emerging with an intention to bring into light about the resistance that is developing among the microorganism towards the antibiotic. Microorganism shows different responses to older and new generation of antibiotics with time to time. The current study was aimed to isolate *Escherichia coli* from different clinical samples. Findings from routine testing by medical microbiological laboratories indicate growing resistance to several antibiotics in *E. coli* from urinary tract isolates[5]. About 20% of *E. coli* isolates were screened in all samples including Urine, Pus and HVS. The frequency of *E. coli* in urine specimen was 37.5 % but according to Hasan et al.[10] the frequency of *E. coli* in urine was 47.7% and similar results (49%) were obtained by Bhargavi et al.[11], while Okonko et al.[12] reported 13.3% *E. coli* in urine. The frequency of *E. coli* in pus samples was 12.5% while Khan et al.[13] reported 44% of *E. coli* in pus but Hasan et al.[10] recovered 19%, while nearly same results were mentioned by

Mahmood[14] i.e. 14.37%. The frequency of *E. coli* species in high vaginal swabs was 10%. These results are in favor of Mumtaz et al.[15] i.e. 13.7% and similar results (8.9%) were reported by Hasan et al.[10]. Adegok et al.[16] reported 22.9% resistance of *E. coli* from HVS. The difference in susceptibility pattern may be due to genuine population susceptibility since it is known that factors such as low socioeconomic status, sexual intercourse and pregnancy are common among people[17].

It was identified that the frequency of *E. coli* was higher in females than males in all samples. The incidence of urinary tract infections is far more frequent in women than in men by reason of their fundamental physiological differences. The gender wise distribution of *E. coli* in urine samples was 33.3% in males while 66.6% in females. Anuradha et al.[18] isolated 81.75% isolates from females and 18.25% from males. While Iqbal et al.[19] reported 60% isolates from females and 40% from males. The gender wise distribution of *E. coli* in pus was 40% in males while in females it was 60%. Khan et al.[20] reported similar findings i.e. 61.76% from female and 39.24%from males. Females are more prone to UTIs because of anatomical differences including short urethra, absence of prostatic secretion in pregnancy and easy contamination of the urinary tract with fecal flora and the physiological increase in plasma volume during pregnancy decreases urine concentration[21].

The resistance profile of isolated *E. coli* from urine shows that Nalidixic acid is highly resistant 29 (96.7%), followed by Norfloxacin 24(80%), Ofloxacin, Levofloxacin and Pefloxacin 20(60%), Ciprofloxacin 19 (63.3%) and least resistant was shown by Moxifloxacin 17 (56.6%). Bargavi et al.[11] reported that *E. coli* was highly sensitive to Naldixic acid (92.6%), Ciprofloxacin (91.9%) and Norfloxacin (66.7%). Anuradha et al.[18] reported that *E. coli* was 100% resistance to Ciprofloxacin and Norfloxacin while Nakhjawani et al.[22] reported that *E. coli* was 49.3% resistance to Nalidixic acid, Ofloxacin 44.5%, Norfloxacin 41.4% and Ciprofloxacin 40.2%.

Resistance profile of *E. coli* in pus sample shows that Nalidixic Acid is highly resistant 90% followed by Moxifloxain 80%, Norfloxacin and Ofloxacin 70%, Ciprofloxacin and Pefloxacin 60% and Levofloxacin 50% respectively. Kumar et al.[23] also verified that Ciprofloxacin, Ofloxain and Pefloxcin showed highest resistance of 54.1%, 49.1% and 80.3% respectively. Isolates of *E. coli* in HVS showed 100% resistance to Nalidixic Acid, followed by Moxifloxacin 37.5%, Levofloxin, Pefloxin and Ofloxcin 25%, Ciprofloxacin and Norfloxacin 12.5% respectively. Similarly Mumtaz et al.[15] illustrated that Ciprofloxin and Norfloxin were 69% and 52.7% resistant while Adegoke et al.[16] confirmed that Nalidixic acid, ciprofloxacin and Ofloxacin were 56%, 48.5% and 45% resistant respectively.

CONCLUSION

This study concluded that *E. coli* is a dominant microorganism among the clinical specimens in HMC. The *E. coli* is present in females more than males. More than 60% resistance is shown by all Quinolones toward *E.coli*. Ciprofloxacin is a drug of choice and shows 39.5% sensitivity to all isolates of *E. coli,* whereas, Nalidixic acid showed high resistance 95.5%. The present findings are quite alarming to find out that all isolates of *E. coli* showed resistance to Quinolone antibiotics. Guidelines for presumptive treatment should be implemented for this resistance pattern.

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