e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2024; 16(3); 238-244

Original Research Article

Antimicrobial Susceptibility Pattern of E. Coli Causing Urinary Tract Infection with Special Reference to Fluoroquinolone Resistance in a Tertiary Care Teaching Hospital in South India

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Received: 25-12-2023 / Revised: 23-01-2024 / Accepted: 26-02-2024

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Conflict of interest: Nil

Abstract:

Background: Antimicrobial resistance among Escherichia coli causing urinary tract infection (UTI) is a public health concern.

Objectives: 1. To find the prevalence of E. coli causing urinary tract infections from urine samples and to determine the antimicrobial susceptibility pattern of E. coli isolates by Kirby Bauer's disc diffusion method. 2. To study about the minimum inhibitory concentration (MIC) fluoroquinolone resistant E. coli isolates by Microbroth dilution method

Method: The Prospective study was conducted among 150 UTI Patients attending Tertiary care centre for treatment. Ethical principles were adhered. Their socio - demographic details were collected and received urine samples were processed using standard methods and antibiotic susceptibility was done by Kirby-Bauer disk diffusion test and minimum inhibitory concentration of fluoroquinolone resistant E. coli isolates by Microbroth dilution method. Data was analysed using SPSS Software version 23.0.

Results: The mean age of the participants was 48.55 + 14.37. Females were predominant. Majority, 49.3% belonged to 31-60 years age group. Among the 241 isolated organisms from culture, the most common were E. coli 62.2% (150) and Klebsiella pneumoniae was found to be 10.4% (25) of the participants and candida was found in 6.5%. Highest resistance rate of E. coli growth was found in Co-trimoxazole (81%), Nalidixic acid (80 %), Ampicillin (78%), Ciprofloxacin (70%) and levofloxacin (60%). It was found 32.26% of participants who are having Levofloxacin-resistant Escherichia coli with 32µg/ml Minimum Inhibitory concentration (MIC). 40.91% of participants showed Ciprofloxacin resistant Escherichia coli with 64µg/ml MIC.

Conclusion: In the present study, 62.2% of E.coli growth in their culture who was complained as urinary tract infection. The empirical guidelines of UTI treatments and prophylactics that are optimized against uropathogens without altering the normal development of microflora.

Keywords: E coli, UTI, MIC, Antimicrobial Resistance.

Introduction

The public health issue of urinary tract infections (UTI) is a bacterial illness that affects both community and hospital settings all over the world. [1] They present a significant problem for medical personnel because of their high prevalence, recurrence, complications, and rising antibiotic resistance. Antimicrobial resistance is an example of a public health issue that the World Health Organization says must be handled with the utmost urgency. [2] A concern to world health and development is antimicrobial resistance (AMR). The Sustainable Development Goals (SDGs) must be attained urgently through multi - sectoral action. AMR has been listed as one of the top 10 worldwide public health dangers to humanity by the WHO. The main causes of the emergence of infections that are resistant to medication are antimicrobial misuse and overuse. 80–90% of community-acquired urinary tract infections (CA-UTI) and 30–50% of nosocomial acquired urinary tract infections are caused by Escherichia coli (E. coli) (NA-UTI). [3] Fluoroquinolones are preferred as the first line treatment

for UTI. [4,5] Due to frequent exposure to fluoroquinolones and prolonged antimicrobial therapy, especially in older patients, the minimal concentration of fluoroquinolones has considerably increased with patient's age.

The therapy of UTI is predicted to become challenging due to the decreasing trends of antibiotic resistance in E. coli. [6-8] which led to fewer available therapeutic alternatives. [4,5] From this given context, the study's goals were established as follows, which will enable us to create an appropriate strategy for making antibiotic recommendations:

Objectives

1. To find the prevalence of E. coli causing urinary tract infections from urine samples.
2. To determine the antimicrobial susceptibility pattern of E. coli isolates by Kirby Bauer's disc diffusion method.
3. To study the fluoroquinolone resistant E. coli isolates by disc diffusion method
4. To find out the MIC of Ciprofloxacin & Levofloxacin for E. coli by Microbroth dilution method as per CLSI guidelines.

Methodology

Study Design and Description of participants

The present Institutional based cross-sectional prospective study was conducted at Tertiary care hospital, Trichy by the Microbiology department for a period of 6 months (January 2016 to June 2016).

The participants were selected based on inclusion criteria, Patients of any age with symptoms of UTI, Antenatal case, Diabetes, those who receiving antibiotics treatment, irrespective of age and sex who came through outpatient or inpatient departments and whose routine urine examination revealed numerous pus cells on microscopy were included.

The participants were excluded those who are not willing.

Sample size and sampling

It was calculated using the formula = ⌊DEFF*Np (1-p)]/ $[(d^2/Z^2_{1-\alpha/2}*(N-1) +p*(1-p)]$, Based on the 56.8% prevalence of E.coli growth among UTI patients in the study conducted by Niranjan V et al [9] with 95% confidence interval and 8% absolute precision the sample size was calculated to be 148 which was rounded off to 150. Consecutive sampling was applied to select the study participants.

Methods

After obtaining institutional ethical clearance, the study was preceded. The nature and purpose of the study was explained to all the participants. Urine samples were collected through aseptic measures from suspected urinary tract infected cases as per followed protocol.

Samples were cultured on Nutrient agar, MacConkey agar media, using calibrated loop of delivering 0.01ml of the sample and incubated aerobically for 18 to 24 hours at 37°C. As per Kass count the plates showing significant growth were processed further. [9] Identification of E. coli was done by standard method depending on observation of colony characteristics, gram-stain as well as using biochemical tests for further identification. Susceptibility to antimicrobial agents was determined by Kirby-Bauer disk diffusion method and Minimum Inhibitory Concentration (MIC) of fluoroquinolone resistant E. coli isolates by Microbroth dilution method on Muller-Hinton agar as described by the Clinical Laboratory Standard Institute (CLSI) 2015.

Antibiotics used for antibiogram determination of the collected strains among FQ were: The antimicrobial agents are: Ampicillin 10µg (AMP), Gentamicin 10µg (GEN), Amikacin 30µg (AK), Cotrimoxazole 25µg (COT), Nalidixic acid 30µg (NA), Nitrofurantoin 300µg (NIT), Ciprofloxacin 5µg (CIP), Levofloxacin 5µg (LE), Cefotaxime 30µg (CTX), Cefepime 30µg (CPM), Imipenem 10µg (IPM) and Piperacillin/Tazobactum 100/10µg (PIT). Minimum Inhibitory Concentration of Ciprofloxacin and Levofloxacin for E. coli isolates were determined by Microbroth dilution method. The data was entered in Microsoft excel and analysed using SPSS windows version 20.0 software.

Ethical clearance

Institutional Research and Ethics Committee clearance were obtained. IEC NO: CMCH&RC/IEC-NO:137/26.11.2015. Ethical principles such as respect for the persons, beneficence, justice and ensuring confidentiality was adhered to the throughout study. Informed written consent was obtained from all participants.

Results

Socio - demographic details

The mean age of the participants was 48.55 + 14.37. Female was predominant. Majority of them, 95.2% were married. More than half of them were illiterates and lived in joint families.

One fourth of the participants were suffering from one or more chronic morbidities. Of 150 participants, 25.6% were overweight and 34.4% were obese.

Table 1: Distribution of study participants based on age category

Age category (Years)	Frequency	Percentage
0-5	1	0.7
6-10	2	1.3
11-18	2	1.3
19-30	21	14.0
31-60	74	49.3
61-75	43	28.7
>76	7	4.7
Total	150	100.0

(Table 1) Among 150 participants majority, 49.3% belong to working age group (31-60 years) followed by 28.7% belonged to geriatric age group (61 – 75 years)

Table 2: Distribution of study participants based on isolated organism among the culture

Organism growth	Frequency	Percentage
Candida species	21	8.7
Citrobacter.spp	2	0.8
Escherichia coli	150	62.2
Enterobacter.spp	1	0.4
Klebsiella oxytoca	15	6.2
Klebsiella pneumoniae	25	10.4
Non fermenting Gram Negative Bacilli	3	1.2
Pseudomonas aeruginosa	20	8.2
Proteus mirabilis	1	0.4
Proteus vulgaris	1	0.4
Staphylococcus aureus	1	0.4
Serratia. spp.	1	0.4
Total	241	100.0

From Table 2 it was found the isolated organism among the culture, the most common were 62.2% E.coli and 10.4% Klebsiella pneumoniae was found in the participants and candida was found in 6.5% of the participants.

Table 3: Antibiotic Resistance pattern of Escherichia coli (% resistance)

Resistance to E.coli	Frequency	Percentage
Nalidixic Acid (NX)	120	80.0
Gentamicin(Gen)	96	64.0
Amikacin (AK)	16	10.7
Nitrofurantoin (NIT)	45	30.0
Piperacillin- Tazobactum (PIT)	30	20.0
Cefaperazone Sulbactum (CFS)	48	32.0
Ampicillin (AMP)	117	78.0
Cefoxitin (CX)	120	80.0
Cefixime (CFM)	115	76.7
Cefotaxime (CTX)	90	60.0
Ceftriaxone (CTR)	87	58.0
Cefepime (CPM)	80	53.3
CO-trimoxazole (COT)	122	81.3
Aztronem (AZT)	10	6.7
Ciprofloxacin (CIP)	110	73.3
Levofloxacin (LEV)	90	60
Total	150	100

Among 150 participants it was found 80% of participants who are having E.coli growth were resistance to NX and CX 30, 78% were resistant to AMP. The other resistant group of antibiotics were GEN (64%), AK (10.7%), COT (81.3%), Nalidixic acid (NA) 30µg (80%), NIT (30%), Ciprofloxacin (CIP) 5µg (73.3%), LE (60%), CTX (60%) and CPM (53.3%). All E.coli isolates sensitive to Imipenem (100%) (Table 3)

Table 4: Distribution of Ciprofloxacin resistant Escherichia coli as per their Minimum Inhibitory Concentration

CIP-MIC	Frequency	Percentage
4	2	1.82
8	3	2.73
16	12	10.91
32	27	24.55
64	45	40.91
128	18	16.36
256	3	2.73
Total	110	100

(Table 4) Among 110 participants it was found 40.91% of participants who are having ciprofloxacin resistant E.coli growth with 64 Minimum Inhibitory Concentration.

Table 5: Distribution of Levofloxacin resistant Escherichia coli as per their Minimum Inhibitory Concentration

LEV-MIC	Frequency	Percentage
8	22	24.44
16	23	25.6
32	30	33.33
64	17	18.89
128	1	1.11
256	0	0
Total	90	100

Among 90 participants it was found 33.33% of participants who are having Levofloxacin-resistant Escherichia coli with 32 Minimum Inhibitory Concentration. (Table 5)

Table 6: Distribution of the Ciprofloxacin resistant Escherichia coli as a function of Age and MIC group among In/Out patients

	CIP-MIC CAT	Age Category						
		0-5	6-10	11-18	19-30	31-60	61-75	>76
	4-16	0	0	0	1	0	1	0
OP		0	0	0	50.0%	0.0%	50.0%	0
	32-64	0	0	0	0	5	4	0
		0	0	0	0.0%	55.6%	44.4%	0
	>128	0	0	0	1	2	0	0
		0	0	0	33.3%	66.7%	0.0%	0
IP	4-16	1	0	0	2	8	3	1
		6.7%	0.0%	0.0%	13.3%	53.3%	20.0%	6.7%
	32-64	0	1	1	5	32	23	1
		0.0%	1.6%	1.6%	7.9%	50.8%	36.5%	1.6%
	>128	0	1	0	2	7	5	3
		0.0%	5.6%	0.0%	11.1%	38.9%	27.8%	16.7%

From Table 6 it was found among the OP patients 50% of the participants belonging to 19 – 30 years of age group had growth of Ciprofloxacin resistant Escherichia coli with 4-16 MIC when compared to IP Patient (13.3%).

Table 7: Distribution of the Levofloxacin resistant Escherichia coli as a function of Age and MIC group among In/Outpatients

	LEV-MIC CAT	Age Category						
		0-5	6-10	11-18	19-30	31-60	61-75	>76
	8-16	0	0	0	0	4	4	0
OP		0	0	0	0.0%	50.0%	50.0%	0
	32-64	0	0	0	1	3	0	0
		0	0	0	25.0%	75.0%	0.0%	0

	>128	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
IP	4-16	0	1	1	1	20	13	1
		0.0%	2.7%	2.7%	2.7%	54.1%	35.1%	2.7%
	32-64	0	1	0	6	18	15	3
		0.0%	2.3%	0.0%	14.0%	41.9%	34.9%	7.0%
	>128	0	0	0	0	1	0	0
		0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%

Among the OP patients 25% of the participants belonging to 19 – 30 years of age group had growth of Levofloxacin-resistant Escherichia coli with 32-64 MIC whereas in IP Patient 14% of the participants belonging to 19 – 30 years of age group had growth of Levofloxacin resistant Escherichia coli with 32-64 MIC. (Table 7)

Discussion

Key findings of the study

In the present study, prevalence of E.coli infection were found in 62% of UTI patients in their culture. The second most common is Klebsiella pneumoniae which was found in 10.4% of the participants. It was found 80% of participants who are having E Coli growth were resistance to NX and CX30 which is almost like the GLASS report8 (Global Antimicrobial Resistance and Use Surveillance System) which demonstrates the prevalence of ciprofloxacin resistance, a common antibiotic used to treat urinary tract infections, with rates ranging from 8.4% to 92.9% for Escherichia used to treat urinary tract infections.

Gender and age distribution of the participants

In the current study 49.3% of the participants belong to the working age group (31-60 years), which might be because the lack of concentration of water intake during working hours and usage of common latrine with poor hygiene. Most of the participants were females. During the reproductive age, UTI commonly affects females. [11] The main reason is that Women have a shorter urethra than men. As a result, there is less distance for bacteria to travel to reach the bladder. Being sexually active tends to more UTIs. After menopause, a decline in circulating oestrogen causes changes in the urinary tract. The changes can increase the risk of UTIs.

The resistant pattern of E.coli organism

In the present study 80% of participants who are having E.coli growth were resistance to NX and CX 30, 78% were resistant to AMP. The other resistant group of antibiotics were GEN (64%), AK (10.7%), COT (81.3%), Nalidixic acid 30µg (NA), NIT (30%), Ciprofloxacin 5µg (CIP), LE (60%), CTX (60%) and CPM (53.3%). which is almost similar to the GLASS report [8]

Comparison of OP and IP patient - Escherichia coli isolates showed ciprofloxacin and Levofloxacin growth in MIC

Our study showed that when Escherichia coli isolates were placed into three groups based on MIC of ciprofloxacin (4 to 16, 32 to 64, and ≥ 128 µg/ml) similarly Escherichia coli isolates were placed into three groups based upon levofloxacin MIC (8 to 16, 32 to 64, and ≥ 128 µg/ml) which showed 32.26% of participants having Levofloxacin resistant Escherichia coli growth in 32 Minimum Inhibitory concentration. 50% of the participants belonging to 19 – 30 years of age group had growth of Ciprofloxacin-resistant Escherichia coli with 4-16 MIC when compared to IP Patient (13.3%) which might be due to the fact of infection control practices and catheter associated factor which is contrast to study conducted by Niranjan V et al [9] similarly 25% of OP patient belonging to 32 – 64 years of age group had growth of Levofloxacin resistant Escherichia coli with 4-16 MIC whereas in IP Patient only 14% were resistant to levofloxacin. With rise in the age of patient levofloxacin resistance to E coli increases which might be due to weakened immunity or overuse of antibiotics which is similar to the study conducted by Sangeeth K et al. [3]

Thus, these findings clearly indicate that MIC of Escherichia coli to ciprofloxacin and levofloxacin has increased and also the resistance to other antibiotics has increased as the MIC increases.

Escherichia coli in the Era of Antibiotic Resistance

The greatest risk is posed by antimicrobial resistance. [12] Recurrent infections are brought on by the development of biofilms inside the bladder, which also increases the risk of MDR strains.13 Resistance is being shown because of a recent source of worry. Antibiotics are frequently prescribed to patients with symptomatic UTIs; nevertheless, these medications have the potential to permanently alter the normal microbiota of the vagina and gastrointestinal tract as well as to foster the growth of multidrug-resistant microbes. [13,14] The possibility of being colonized by multidrug-resistant uropathogens can rise due to the existence of niches that the changed microbiota is no longer able to fill. Importantly, the "golden age" of antibiotics is coming to an end, necessitating preventa-

tive measures Therefore, a number of organizations are making multiple efforts to address this issue.

Global Action Plan on Antimicrobial Resistance (GAP)

During the 2015 World Health Assembly, nations made global commitments to the framework outlined in the Global Action Plan1 (GAP) 2015 on AMR as well as to the creation and execution of multi - sectoral national action plans.

The World Organization for Animal Health (WOAH) and the Food and Agriculture Organization of the United Nations (FAO) subsequently endorsed it (OIE). Countries must assure costing and the execution of national action plans across all sectors to guarantee sustainable progress. GAP outlines the objectives: to improve antimicrobial resistance awareness and understanding through valuable communication, education and training; to strengthening the knowledge and evidence base through surveillance and research; to reduce the occurrence of infection through effective sanitation, hygiene and infection prevention measures; Optimizing the antimicrobial medicines use in health of human and animal and to develop the economic case for sustainable investment that takes account of all countries needs and to increase investment in new medicines, diagnostic tools, vaccines and other interventions [15].

The WHO Global Strategy for Containment of Antimicrobial Resistance, which was created in 2001 and provides a framework of actions to limit the emergence and reduce the spread of AMR, was one of the global initiatives to control it prior to the endorsement of the GAP in 2015.

In order to continue bridging knowledge gaps and informing initiatives at all levels, WHO created the Global Antimicrobial Resistance and Use Surveillance System (GLASS) in 2015. GLASS was designed to gradually include data from AMR surveillance. GLASS-AMR requires AMR data to be collected through a case-finding comprehensive surveillance system, which gathers results from susceptibility testing for priority human bacterial pathogens isolated from clinical specimens (blood, urine, stool and cervical and urethral specimens) sent routinely to laboratories for clinical purposes.

Pathogens currently included in GLASS-AMR are: Acinetobacter spp., E. coli, Klebsiella pneumoniae, Neisseria gonorrhoeae, Salmonella spp., Shigella spp., Staphylococcus aureus, and Streptococcus pneumoniae. [16]

Limitations of the study

The strength of the study was that it included both OPD patients and inpatients which helped us in comparing the resistance rates. This study was cross-sectional, a design that does not permit establishing cause-effect relationships.

Since it is hospital-based study with smaller sample size findings of the study may not be generalized. The resistance genes were not genotyped in this investigation, nor ESBLs tested.

Conclusion

In the present study 62% of UTI patients had E.coli growth in their culture. Furthermore, a significant proportion of recurring UTIs suggests that not all UTIs respond well to antibiotic treatment. To establish empirical guidelines for UTI treatments and prophylaxis that are optimal against uropathogens without changing the normal microflora, more research must be done to determine the physiological mechanisms of virulence of normal flora and the causes of resistance. Antibiotic overuse and systematic misuse must be eliminated. The main objective of this approach is to ensure that infectious diseases are treated and prevented with quality-assured, safe, and effective medicines. As a result, antimicrobial resistance awareness and understanding are required through effective communication, education, and training; the incidence of infection through effective sanitation, hygiene, and infection prevention measures also needs to be scaled up.

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