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PJMLS

Print ISSN: 2707-4471. Online ISSN: 2707-448X

Short Communication	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v6i2.1443	Copyright © All rights are reserved by Corresponding Author
Vol. 6 No. 2, 2023: pp. 155-160 www.readersinsight.net/pjmls	
	Revised: June 23, 2023 **Accepted:** June 29, 2023
Submission: March 31, 2023	**Published Online:** June 30, 2023

ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *ESCHERICHIA COLI* AMONG URINARY TRACT INFECTED PATIENTS IN QUETTA CITY

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Abstract
Drug resistance is becoming a cause of difficult situations around the globe. This resistance causes major difficulties in treatment. This entire study was carried out to detect the current level of Antibiotic Resistance in E. coli among urinary tract infected patients in Quetta. A total of 100 urine samples were collected randomly from different diagnostic laboratories of Quetta during May-June 2022. Organism detection was done by using specific media and various biochemical test including gram stain, catalase test, urease test and methyl red test according to standard procedures. Antibiotic susceptibility test was done using some commonly used antibiotics including Amoxicillin-clavulanic acid, Ceftriaxone (CRO), Amikacin (AK), Ofloxacin (OFX), Imipenem (IPM) and Cefotaxime-Avibactam (CZA) on Mueller Hinton agar. Results indicated that E. coli isolates were more susceptible to Amikacin followed by Ceftazidime-avibactam, Imipenem, Ofloxacin and Ceftriaxone, whereas high resistance was observed against ceftriaxone followed by Ofloxacin, Ceftazidime-avibactam, Imipenem and Amikacin, respectively. It showed 100% resistance in E. coli against ceftriaxone which indicates a rise in resistance as compared to the previous studies conducted in the area. This most common UTI pathogen represents major multidrug resistance against commonly prescribed antibiotics concluding the need of proper antimicrobial test before using antibiotics for treatment.
Key words: *Antimicrobial resistance, E. coli, Multi drug resistance, Urinary tract infections*

INTRODUCTION

Urinary tract infections are reported as a prevalent category of infectious diseases among females (1). Large number of antibiotics are prescribed by doctors and health practitioners on a daily basis for treatment of UTI in females. As *Escherichia coli* is the cause of 80% community-acquired uncomplicated UTIs, this pathogen must be in focus, when using empirical antibiotics (2).

Urinary tract infections are usually occurring in communities. They are classified into two types named as upper urinary tract infections also called pyelonephritis and lower urinary tract infections also called cystitis, prostatitis. Type of UTI depends on infection site Clinical indications of urinary tract infections are different; these may be asymptomatic bacteriuria or septic shock. Proper understanding of symptoms and use of suitable antibiotics are essential to avoid severe complications (2).

Urinary tract infections are a major reason of morbidity in older men, as well as boys and females of all age groups. Consequences include pyelonephritis with sepsis, pre-birth term, frequent recurrences and kidney damage in young children (3). It is linked with the small urethral tube of female and close to the anus proximity or urethra; it allows easy access of pathogen to the urethra. One of estimation indicates that 50% women face frequent access of acute cystitis in adult life (4).

Escherichia coli are reported as the most common bacterium responsible for urinary tract infections in various regions and a cause of both hospital and community- acquired urinary tract infections (5, 6). Some

other pathogens including *Proteus morganella, Providencia* sp., *Citrobacter* sp., *Enterobacter* sp., *Klebsiells* sp., *Staphylococcus saprophyticus* and *Enterococcus* sp. may occur (7).

Spread and emergence of drug resistant bacteria is a warning for human health. Drug resistant bacteria, mainly *Enterobacteriaceae* members exhibit resistance against high doses of antibacterial drugs by exhibiting various mechanisms of action and chemical structures (7).

Bacteria of *Enterobacteriaceae* family including *E. coli* show some major mechanisms to avoid high doses of drugs including overexpression of efflux proteins, beta lactamase enzyme for drug degradation, decreased uptake, target alteration and aminoglycoside degrading enzyme (8).

Antibiotics are generally used to treat symptomatic urinary tract infections. These antibiotic treatments can cause long term changes in normal microflora of vagina and GIT and also cause increased multi drug resistance against microorganisms (9).

MATERIALS AND METHODS

STUDY POPULATION

This study was conducted at Center for Advance Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta. Urine samples were collected from different diagnostic laboratories of Quetta.

SAMPLE COLLECTION

One hundred 100 urine samples were collected in sterile containers and sent to the laboratory for examination.

LABORATORY PROCEDURES

Loop full of samples were streaked on Eosine Methylene Blue Agar and incubated for 24 hours at 37 °C to obtain Green metallic sheen. Samples observed with green metallic sheen were further streaked on nutrient media using sterile loop. Microscopic examination and various biochemical tests were performed to confirm the suspected organism (10). Antimicrobial susceptibility test was done to analyze the antimicrobial susceptibility of *E. coli*. 0.5 McFarland standard solution was used as a reference solution to adjust turbidity of testing isolates and streaked on Mueller Hinton agar with commonly used antibiotics and incubated at 37 °C for 24 hours. Results were interpreted using Clinical Laboratory Standard Institute (CLSI) protocol. The brief summary of the whole protocol is as follows (Fig. 1).

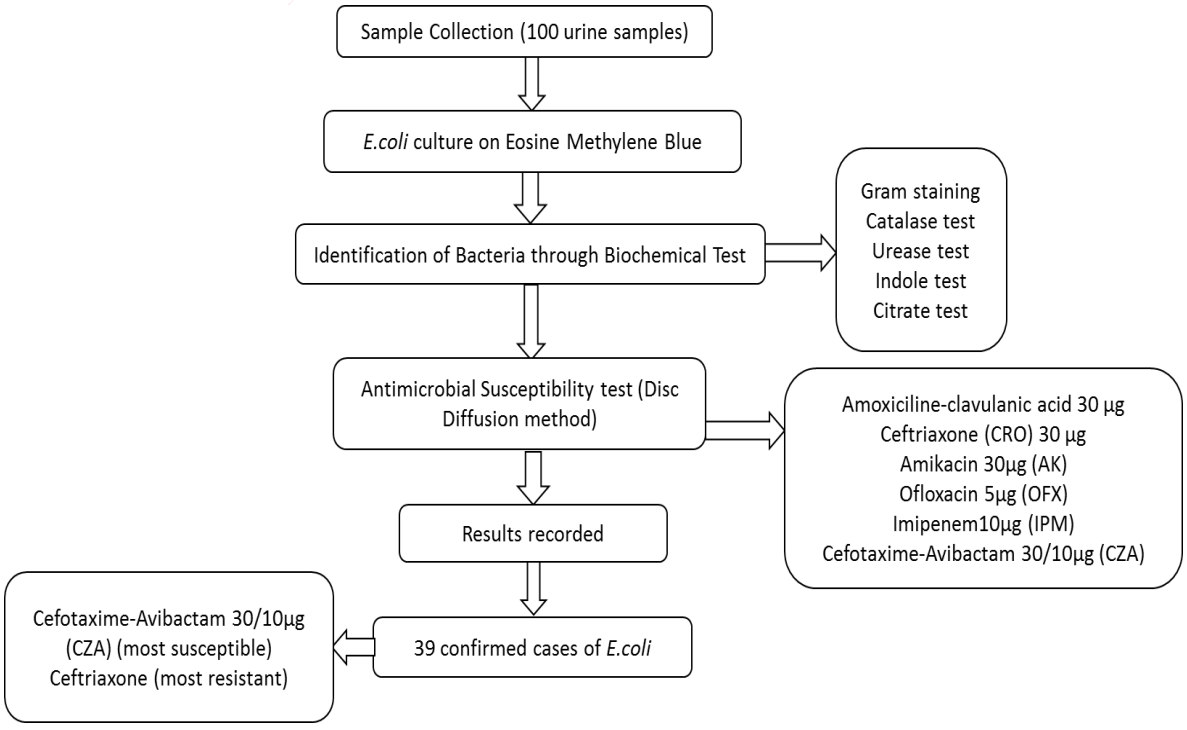

Fig. 1. Schematic diagram of experimental design

RESULTS

Out of hundred (n=100) urine samples n=39 (39%) samples showed green metallic sheen on EMB Agar indicating presence of *E. coli*. Further confirmation of these positive isolates was done using various biochemical tests including Gram staining, catalase test, citrate test, urease test and indole test. Ideal results of biochemical tests were obtained. The confirmed isolates were processed for antimicrobial susceptibility test with commonly used antibiotics including Amoxiciline-clavulanic acid 30 µg, Ceftriaxone (CRO) 30 µg, Amikacin 30µg (AK), Ofloxacin 5µg (OFX), Imipenem 10µg (IPM), Cefotaxime-Avibactam 30/10µg (CZA) (Fig. 2). Taking CLSI as a standard most of the isolates were susceptible to amikacin 36/39 (92%) followed by Ceftazidime-avibactam 29/39 (74%) and imipenem 28/39 (72%), Ofloxacin 5/39 (12%) and ceftriaxone 0/39 (0%). All of the strains were resistant to ceftriaxone (100%) followed by Ofloxacin 33/39 (86%), Ceftazidime-avibactam 10/39 (26%), Imipenem 3/39 (8%) (Table I; Fig. 3).

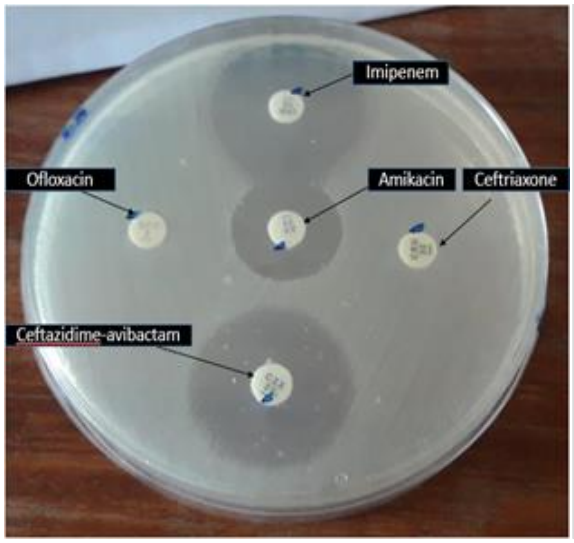

Fig. 2. Results of antimicrobial susceptibility test depicts susceptibility of isolated E. coli against Ceftazidime-avibactam (CZA), Imipenem (IPM) and Amikacin (AK), and Resistance against Ceftriaxone (CRO) and Ofloxacin (OFX)

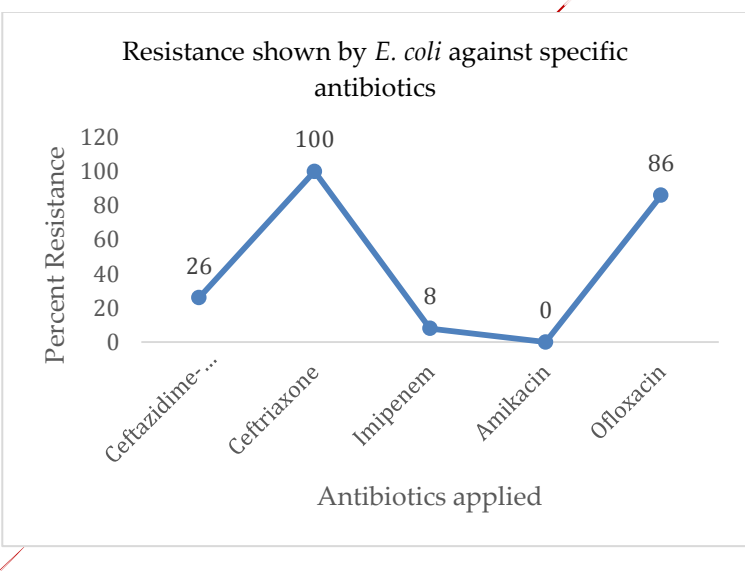

Fig. 3. Percent resistance of different antibiotics against *E. coli* isolates

Taking CLSI as a standard all of the isolates were resistant to ceftriaxone (100%) followed by Ofloxacin 33/39 (86%), Ceftazidime-avibactam 10/39 (26%), Imipenem 3/39 (8%) (Table I; Fig. 3).

DISCUSSION

Latest information of antibiotic susceptibility pattern of infection causing organisms is important in treatment of urinary tract infections. Throughout the research one hundred urine samples were collected from different diagnostic laboratories and transported to the research laboratory within two hours for analysis of samples. Thirty-nine (39%) samples out of hundred showed growth of *E. coli* on Eosine methylene blue Agar, same medium was used (11) to obtain *E. coli.* This positive rate was low as compared to other reports in country. This difference might be due to sample size variation, but on the other hand this work is in line with research done in Quetta, Pakistan (12), has a low *E. coli* rate among UTI patients (23.75%).

Table I. Results showing antimicrobial resistance pattern against *E. coli* from UTI patients (n=39)

Antibiotic	Disc concentration (µg)	Resistant (%)
Ceftazidime-avibactam	30/10	10 (26)
Ceftriaxone	30	39 (100)
Imipenem	10	3 (8)
Amikacin	30	0 (0)
Ofloxacin	5	33 (86)

Species identification was done using different biochemical tests including gram staining, catalase test, citrate test, urease test and indole test. *E. coli* isolates were gram negative in Gram staining which is due to thin peptidoglycan layer and lipid membrane separated by periplasmic space. Crystal violet iodine complex (CV-I) attached with outer and inner wall but during the process of decolorization outer layer of lipopolysaccharide loosed reliability due to this, inner membrane exposed. Washing of crystal violet iodine complex and safranin presented the color wall pink as done earlier (13).

According to our study 39% samples (n=39) were indole positive. This is due to the mechanism of production of tryptophanase enzyme; this enzyme is produced by isolates which break down amino acid tryptophan into indole. In this test when indole reacts with dimethylaminobenzaldehye (Kovac's reagent) producing pink color indicating indole positive organism (14).

Highest antimicrobial resistance is observed which is the serious threat to the population with the passage of time. Up-to-date knowledge about antimicrobial resistance is needed for management of UTIs (15, 16).

Overuse and misuse of different types of antibiotics are major causes of resistance in *E. coli* against various drugs. This resistance is making *E. coli* challenging in public health and acts as a superbug. Susceptibility tests against various antibiotics must be performed before their recommendation for treatment. If some of these precautions and management will not been followed, then the current death rates of 700,000/year can be reached up to ten million death rate per year around the globe by the year 2050 (17).

This research work imitates high antibiotic resistance in *E. coli* against commonly prescribed antibiotics. This study showed 100% resistance in *E. coli* against ceftriaxone antibiotic. This resistance rate is alarming as previous study (18) in Quetta indicated 22.4% resistance against ceftriaxone. High resistance in present research work is may be due to misuse, overuse or self-prescription of antibiotics. In Pakistan antibiotics can easily be purchased from medical stores without recommendation by the doctors. This easy drug availability in developing countries causes high resistance in pathogenic organisms.

In this study, a very low level of resistance is detected against Ceftazidime-avibactam. While amikacin exhibited 26% and 0%, respectively.

Present study shows about 92% susceptibility of Amikacin against *E. coli*, likewise previous studies indicated the susceptibility of Amikacin UTI isolates was >96% (19). These results clearly indicate thebeffectiveness of Amikacin in treatment of UTI. Another study conducted by Bashir M. F et al., (2008) indicated susceptibility of Amikacin against *E. coli* was 98.1% show effectiveness of Amikacin in dealing with urinary tract diseases caused by gram negative bacteria (20).

Avibactam is a class of non- β lactam antibiotics. Combination of avibactam with ceftazidime is manufactured specially to treat different infections of gram negative bacteria. This combination of ceftazidime-avibactam showed great activity against Enterobacteriaceae family (21). The Dias Neto JA et al., (2003) reported that imipenem was effective to treat urinary tract infections because causative pathogens of UTI were sensitive to imipenem 83% (22). Our study complies with these findings representing 72% sensitivity of imipenem against UTI.

Study presented by Ferdosi-Shahandashti et al., (2015) indicated that Ofloxacin was effective against *E. coli* showing susceptibility of about 87.7% but this study indicated the 86% resistance level of Ofloxacin (23). There are various reasons behind this difference, it may be due to misuse, self-recommended, overuse of antibiotics. Or it may be due to differences in regions.

Levofloxacin is a third generation antibiotic, it exhibits broad spectrum activity against pathogens. This antibiotic has capability to stop the growth of bacteria by damaging the enzyme -known as DNA gyrase. This enzyme functions in replication and duplication of bacteria. Ofloxacin used in current study belongs to class levofloxacin. It is commonly used in treatment of various types of infections caused by gram positive and gram negative bacteria (24).

CONCLUSION

Escherichia coli are the most common organism observed in urinary tract infection. Continuous use of some drugs cause resistance in these pathogens results in complications during treatment. In order to reduce this issue advanced knowledge about resistance patterns of pathogenic organisms is important. This study concludes that the most resistance was showed by E.coli against Ceftriaxone antibiotic followed by

Ofloxacin. Furthermore, current study recommends that either Amikacin or combination of Ceftazidime-avibactam can be used in Quetta city for the treatment of urinary tract infections and proper susceptibility testings before treatment of UTIs.

Conflict of Interest:

All authors have no conflict of interest.

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