

## Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Pakistan

Sarmad Zahoor<sup>1\*</sup>, Naveed Shehzad Ahmad<sup>1</sup>, Sadia Asif<sup>1</sup>, Hafiz Muhammad Sajid Jehangir<sup>1</sup>, Asad Ali<sup>1</sup>, Muhammad Sohail Afzal<sup>2</sup>, Muhammad Mohsin Ali<sup>1</sup>, Saroosh Zahoor<sup>2</sup>

<sup>1</sup>Department of Internal Medicine, King Edward Medical University, Mayo Hospital Lahore, Pakistan

<sup>2</sup>Department of Life Sciences, University of Management and Technology, Lahore, Pakistan

\*Corresponding author E-mail: [drsarmadzahoor@gmail.com](mailto:drsarmadzahoor@gmail.com)

### Abstract

Background: Urinary track infection (UTI) is a common infection in Pakistan. Poor knowledge about personal hygiene and lack of awareness are major reasons of this infection. All over the world and in Pakistan *E. coli* and *K. pneumoniae* are main causative agents of UTI.

Objectives: To analyze Antibiotic susceptibility pattern of bacterial strains isolated from urinary tract infections.

Methods: In present study total 227 samples from Genomic Research Lab and Diagnostic Center Rawalpindi, Pakistan were tested for presence of different microbial infectious agents and their antibiotic resistance and susceptibility pattern. Biochemical test such as Gram staining, Lactose fermentation test, Motility test, Catalase test and Colony formation on MacConkey agar, Indole test, and Citrate utilization test were applied. The antibiotic susceptibility and resistance pattern was evaluated by using fourteen different antibiotics on Muller-Hinton agar. Top ten antibiotics were selected at a cut off value of 100 samples for *E. coli* and 20 samples for *Klebsiella*.

Results: Against *E. coli* and *K. species* the most susceptible antibiotic was Imipenem with 97.5% and Amikacin with 94.9% susceptibility respectively. Moreover, against *E. coli* and *K. species* the most resistance antibiotic was Ciprofloxacin 81.7% and Tobramycin with 76.1% resistance respectively. The comparison of present study with previous studies had shown an increase in resistance against various antibiotics.

Conclusion: It is concluded from the present study that antibiotic resistance is on rise in Pakistan and the effect of each antibiotic was different with respect to type of infectious agent so it is recommended that the doctor should determine the dominant type of infectious agent and its susceptibility response before prescription of any antibiotic.

**Keywords:** Antibiotic Resistance; Antibiotic Susceptibility; *Escherichia Coli*; *Klebsiella Specie*; Urinary Tract Infection.

### 1. Introduction

It is a well established fact that major causative agents of Urinary Tract Infections (UTI) are bacteria; in some studies other microbes and fungal infections had also been reported (10). *Escherichia coli* incident was highest followed by *Klebsiella pneumoniae* in cases of UTI, other bacterial strains reported in UTI are *Staphylococcus*, *Enterococcus*, *Proteus*, *Pseudomonas* (2).

Random data analysis and studies indicate that UTI cannot be explained on the basis of age, gender or region of the world (WHO, 2017). It was noticed that women had higher incidence of UTI in most studies, nearly twenty percent women had recurrent UTI attacks (3). In males higher incidence of UTI was reported in late fifties due to different prostatic disorders, children generally suffer less UTI attacks. It was also found that fifty percent cases of UTI are hospital acquired (4).

Urinary track is made up of kidneys, ureters, urinary bladder, urethra and associated glands. It is very important for osmoregulation. Any infection in UTI can damage kidneys function and could impart fetal consequences (3, 5).

Antibiotic resistance is increasing all over the world particularly in developing countries (6, 7). Pakistan being a developing country is facing the same problem. Increased resistance against cephalosporin had been reported, mainly due to over exposure to drug (8, 9). Statistics revealed that in Pakistan 18,596 standard units per 1000 people of antibiotics were used in 2014 that was doubled as compared to other neighboring countries in China it was 9894, in Bangladesh it was 9537, in Sri Lanka it was 10388 standard units per 1000 people (10).

Different studies across the globe had proven that same antibiotic had different resistance or susceptibility pattern for different sites of infection. Most commonly used antibiotic for UTI are Amoxicillin + Clavulanate, Imipenem, Piperacillin + tazobactam, Amikacin etc (11). To the best of our knowledge there is no current report regarding antibiotic resistance pattern from twin cities (Rawalpindi and Islamabad); therefore present study was designed to find the current scenario of antibiotic resistance patterns against different bacterial strains isolated from UTI patients in twin cities of Pakistan.

## 2. Materials and methods

The study was carried out in twin cities, in total 227 individuals suffering for UTI who visited Naveed Laboratory, Pakistan were enrolled. This study was conducted during October 2019 to June 2020 and was approved by the ethical committee of Department of Life Sciences, University of Management and Technology, Lahore, an informed written consent was taken by each participant. Midstream urine samples were taken and different biochemical test such as Gram staining, Lactose fermentation test, Motility test, Catalase test and Colony formation on MacConkey agar, Indole test, and Citrate utilization test were applied for the isolation of different microbial strains. MullarHigton agar was used for the determination of susceptibility and resistance pattern. The effect of each antibiotic had been tested on all isolates and results were compiled to determine which antibiotic was most effective against UTI.

## 3. Results

In present study total 227 UTI samples were tested. Among the tested samples *Citrobacter* spp. (1), Aerobic Gram Negative Bacteria (1), *Candida* spp. (11), Coagulase negative *Staphylococcus* (6), *Enterobacter* spp. (1), *Enterococcus* spp (2), *Escherichia coli* (143) and *Klebsiella* (63) were isolated. According to results *Escherichia coli* (62.7%) had highest incidence as causative agent of UTI followed by *Klebsiella* species (27.6%). In some patients co-infection of *Escherichia coli* and *Klebsiella* (9.2%) was also found. As both *Klebsiella pneumoniae* and *Escherichia coli* had high tendencies of antibiotic resistance; in this study their resistance and susceptibility response was evaluated against each antibiotic separately as well.

According to present study Amikacin was found to be the most sensitive or susceptible antibiotic with 93.5% susceptibility followed by Imipenam with 87.4% and Piperacillin+ tazobactam with 79.8% susceptibility. Where as, Ciprofloxacin was the most resistant drug with 76.8% resistance followed by Cefixime 75.8 and Ceftriaxone 66.6% respectively (Table 1).

**Table 1:** Antibiotic Susceptibility and Resistance Pattern of Bacteria Associated with UTI in Pakistan

Antibiotic	TST	R	% age	S	% age
Amoxicillin + Clavulanate	196/227	102/196	52	94/196	47.9
Ceftriaxone	198/227	134/198	67.6	64/198	32.3
Ciprofloxacin	164/227	126/164	76.8	38/164	23.1
Imipenam	199/227	25/199	12.5	174/199	87.4
Piperacillin+ tazobactam	189/227	38/189	20.1	151/189	79.8
Amikacin	184/227	12/184	6.5	172/184	93.5
Ceftazidime	194/227	123/194	63.4	71/194	36.6
Levofloxacin	100/227	64/100	64	36/100	36
Tobramycin	113/227	72/113	63.7	41/113	36.2
Cefixime	149/227	113/149	75.8	36/149	24.2

TSR= Total Sample Tested, R= Resistance, S= Susceptibility.

More over, %age antibiotic resistance and susceptibility of each antibiotic was calculated against *E.coli* and *K. pneumoniae* separately as well. In current study total 143 *E.coli* samples were tested, antibiotic which were tested against minimum 100 samples was included in the analysis. Imipenam was found to be the most effect drug with 97.5% susceptibility, followed by Amikacin with 94.5% and Piperacillin+ tazobactam with 84.2% susceptibility respectively.

Ciprofloxacin was most resistant drug with 81.7% resistance followed by Cefixime 74.4% and Ceftriaxone 66.1% resistance respectively (Table 2).

**Table 2:** Antibiotic Susceptibility and Resistance Pattern of *Escherichia Coli* Associated with UTI in Pakistan

Antibiotic	TST	R	% age	S	% age
Amoxicillin + Clavulanate	131/143	64/131	48.8	67/131	51.1
Ceftriaxone	133/143	88/133	66.1	45/133	33.8
Ciprofloxacin	104/143	85/104	81.7	19/104	18.2
Imipenam	121/143	13/121	10.7	118/121	97.5
Piperacillin+ tazobactam	127/143	20/127	15.7	107/127	84.2
Amikacin	122/143	8/122	6.5	115/122	94.5
Ceftazidime	131/143	82/131	62.5	49/131	37.4
Levofloxacin	73/143	44/73	60.2	29/73	39.7
Tobramycin	86/143	51/86	59.3	35/86	40.6
Cefixime	98/143	73/98	74.4	25/98	25.5

TSR= Total Sample Tested, R= Resistance, S= Susceptibility.

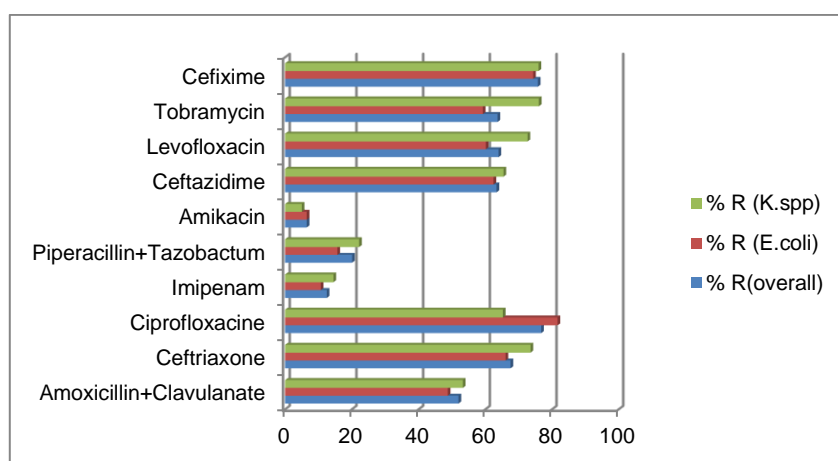
The same ten antibiotics were selected for *K. pneumoniae* which were tested against *E. coli* on the basis of cut off value at minimum tested against 20 samples. Total 43 samples of *K. pneumoniae* were tested. It was found that Amikacin was the most susceptible drug with 94.9% susceptibility, followed by Imipenem with 85.4% and Piperacillin+ tazobactam 77.7% susceptibility against *K.pneumoniae* in UTI isolates. Tobramycin was the most resistant drug with 76.1% resistance followed by Cefixime 76% and Ceftriaxone with 73.6% resistance respectively (Table 3).

**Table 3:**Antibiotic Susceptibility and Resistance Pattern of Klebsiella PneumoniaAssociated with UTI in Pakistan

Antibiotic	TST	R	% age	S	% age
Amoxicillin + Clavulanate	56/63	31/56	53.3	25/56	44.6
Ceftriaxone	57/63	42/57	73.6	15/57	26.3
Ciprofloxacin	52/63	34/52	65.3	18/52	34.6
Imipenem	62/63	9/62	14.5	53/62	85.4
Piperacillin+ tazobactam	54/63	12/54	22.2	42/54	77.7
Amikacin	59/63	3/59	5	56/59	94.9
Ceftazidime	61/63	40/61	65.5	21/61	34.4
Levofloxacin	22/63	16/22	72.7	6/22	27.2
Tobramycin	21/63	16/21	76.1	5/21	23.8
Cefixime	46/63	35/46	76	11/46	23.9

TSR= Total Sample Tested, R= Resistance, S= Susceptibility.

The comparison of over all %age antibiotic resistance and individual %age of *E. coli* and *K. pneumoniae* clearly indicated that drug resistance against different antibiotics varies with the type of pathogenic bacteria (Fig 1).



**Fig. 1:**Comparison of Overall Resistance, Resistance Against E.Coli and KlebsiellaSpp.Associated with UTI in Pakistan.

## 4. Discussion

In present study antibiotic resistance and susceptibility patterns of *Klebsiella* and *E. coli* against top ten antibiotics was calculated. The study results highlighted that in a developing country like Pakistan it is very important to evaluate the efficacy of prescribed antibiotic. This study is a small contribution to evaluate change in resistance and susceptibility patterns with the type of disease causing bacterial stains, as very limited data in this regard is available from Pakistan.

In present study *E. coli* and *Klebsiella* were the main causative agents for UTI and their incidence was 62.7% and 27.6%. In another study from Pakistan the incident of *E. coli* was reported as 40% in 2015 (11). In a study carried out at tertiary care hospital of Pakistan the incidence of *E. coli* was reported as high as 80% in UTI patients (12). In Iran it was reported as 64% in 2007 and in next year it was reported as 60.9% (13). In USA overall incidence of *E.coli* in UTI was found from 75-90% (14).

In a study from Liaquat University Hospital Jamshoro reported incident of *Klebsiella* 39.1% in UTI patients [9]. In India, Nepal, Ethiopia and Nigeria the incidence of *Klebsiella* was 14.4%, 15.7%, 31.5% and 3.5 % respectively in cases of UTI (15-18).

For the treatment of *E. coli* and *Klebsiella* different antibiotics are prescribed in Pakistan and all over the world. In this study Ceftriaxone, Ciprofloxacin, Imipenem, Amikacin, Ceftazidime, Levofloxacin, Tobramycin, Cefixime and combination of antibiotics like Amoxicillin + Clavulanate and Piperacillin+ tazobactam were used. Studies indicated that Imipenem was among most widely used antibiotic in countries such as Iran, India, Estonia, Lebanon, Nigeria, Jordan Turkey, Saudi Arabia and Pakistan against bacterial infections including UTI (11,19-29).

It was found in present study that over all and against *E.coli* and *Klebsiella*, Imipenem has susceptibility 87.4%, 97.5% and 85.4% respectively in UTI infection. In other studies across the globe sensitivity was 100 % in Nigeria, 90 % in Estonia, India and Iran while 70% in Jordan (11,19-20, 22-25, 27-29). Different studies from Pakistan, India and Turkey showed variable results but in all studies susceptibility of Imipenem was reported high; it was reported between 95% to 98% in these regions which is comparable to our results(9, 26, 29-31).

In present study Ciprofloxacin resistance was 81.7%, 76.8% and 65.3 against all isolates, *E. coli* and *Klebsiella* respectively. Other similar studies on UTI isolates carried out in Uganda (87%), Ethiopia (80%), Nepal (49.4%) and Jordan (75%) which showed higher sensitivity than present study (11, 15-17, 23-24, 27, 29, 32-33).

In present study against Amikacin over all susceptibility was 93.5%, against *E. coli* % it was 94.5% and against *Klebsiella* sensitivity was reported as 85.4%. Our findings were similar to other susceptibility reports from India (74%), Estonia with 98%, Nepal with 87 %, Nigeria, Iran with 100% (17, 23-24, 33-37). For *E.coli* susceptibility was also high and it was reported over 90% in most studies (9, 26, 29-31).

Tobramycin was resistant in 63.7% isolates in present study. These results were quite contrary to other finding in which %age susceptibility was much higher approximately 70% in Pakistan, India and Iran (13, 26, 38).

Resistance against Ceftriaxone was 67.6%, whereas % susceptibility ranges from 30-60% in different regions (13, 18, 26-27, 32, 36, 39). In present study against E.coli resistance was 66.1%.

In current study over all UTI causing isolates showed 63.4% resistance against Ceftizidime, for Klebsiella resistance was 65.5% and for E. coli it was 62.5%. Its resistance was much higher similar to other studies reported from Nigeria, Iran, India and Lebanon where it was as high as 80% (8, 15-16, 18, 20, 24, 35).

It was found in this study that Cefixime and Levofloxacin were 75.8% and 65% resistant respectively. These results were similar to other studies in the region. Similar patterns were reported from other regions like Nepal, India, Iran and Nigeria for E. coli and Klebsiella both strains (11, 15-16, 24, 31, 35).

It was found that against Amoxicillin + Clavulanate 52% samples showed resistance. In our study against E.coli % resistance was 48.8% and against Klebsiella it was 53.3%. Other studies from Pakistan and Saudi Arabia showed higher resistance than reported in present study (11, 30).

Our results showed that Piperacillin and Tazobactam were 78.9% susceptible against UTI isolates and against E.coli and Klebsiella it was 84.2% and 77.7% in other studies it was reported from 53% to 95% susceptibility in different regions of the world (11, 19, 30).

## 5. Conclusion

Pakistan ranks highest in antibiotic consumption per 1000 people in South Asia, such large scale antibiotic consumption makes Pakistan vulnerable for developing multi drug resistance (MDR). Since no study was reported on MDR pattern in isolates from UTI in last five years from Islamabad, this study was an effort to highlight the need of pre-screening for the type of pathogen before prescribing any drug. Results showed that same antibiotics had different susceptibility response for different pathogens in UTI isolates. Therefore, it is very important to prescribed targeted drug in case of UTI infection. In 2017 WHO announced antibiotic resistance among top ten emerging health issues. It is recommended that this study should be extended on national level and patients should be screened not only for UTI but all other type of infection as well. Pakistan is a poor country with low literacy rate and poor awareness about contact hygiene so it is very important that increasing drug resistance response of gram negative disease causing agents should be controlled.

## Declarations section

Ethics approval and consent to participate

The study protocols and informed consent documents were approved by the Institutional Bioethics Review Committee (IBRC).

## Consent to publish

Informed written consent for publication was obtained from each participant.

## Availability of data and materials

The datasets used and/or analyzed during the study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

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