

## PREVALENCE OF MULTI DRUG RESISTANT *E. COLI* FROM PATIENT WITH SUSPECTED URINARY TRACT IN NAROWAL

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### Abstract

*Escherichia coli* (*E. coli*) is one of the most common bacteria related to bloodstream infections and Urinary Tract Infections (UTIs). UTIs are one of the not unusual place infections that can occur in community settings. UTIs rank among the most prevalent bacterial infections known to cause 1.7 million deaths, annually according to WHO. UTIs are estimated to affect 150-200 million people each year and about 40% of women and 12% of men will experience at least one symptomatic infection during a lifetime. The aim of this investigation was focused on the detection rate of MDR *E. coli* in the urine samples and the level of resistance to antimicrobials. Altogether 230 mid-stream urine specimens were obtained through mid-stream urine sample method from the patients who were admitted with clinical suspicion of UTI. These samples were grown on CLED agar and then identified with both Indole test and Triple Sugar Iron agar. Of the 230 urine samples, 109 of them, which was 47 percent, were positive to *E. coli*, whereas 121 samples, which was 52 percent, were negative. A higher level of *E. coli* incidence was revealed in age 51 to 60 years' age group. More so, although the percentage distribution of *E. coli* isolates was relatively close to the percentage distribution of both *P. aeruginosa* and *K. pneumonia* isolates, *E. coli* was predominantly more frequent amongst female patients (65%). Resistance profile of *E. coli* isolates show 19 (39%) were MDR resistant to Ciprofloxacin = 89%, Resistant to Sulfamethoxazole – Trimethoprim = 89%, Resistant to Levofloxacin = 83%. On the other hand, amikacin was established to be the most active antibiotic against these isolates. The study presented here has shown a high percentage of drug-resistant *E. coli* that is emerging as a threatening factor in countries like Pakistan. This underlines the necessity of worldwide programmed of rational use of antimicrobial drugs and the search for measures to prevent the development of resistance to antibiotics in such areas.

### INTRODUCTION

*Escherichia coli* (*E. coli*) is a member of the Enterobacteriaceae family (Korbel et al., 2017) and the gram-negative bacterium that causes BSI and

can spread (UKHSA, 2022). Usual inhabitant of the human and animal intestine, *E. coli* is a versatile organism that also has pathogenic

potential leading to both intestinal and extra-intestinal infections (Foster-Nyarko & Pallen, 2022).

*Escherichia coli* (*E. coli*) is the most commonly reported bacteria for development of bacteremia and is mostly manifested in elderly patients (Bonten et al., 2021). This bacterium is especially known to emerge in the initial stages in the gastrointestinal tracts and then may flank out throughout the system to include the urinary systems. In the case of such dissemination, one can get UTIs and septicemia, while the newborn get neonatal meningitis. In children, *E. coli* is the most common cause of both bacteremia and meningitis as have been demonstrated by Bryce and his team. UTIs, particularly those originating from the invasive *Escherichia coli* pathogens, continue to be considered among the commonest bacterial infections that are treatable under the ambulatory care setting (Córdoba et al., 2017). These infections are one of the components of the acquired bacterial diseases, indicating that *E. coli* is the main pathogen of acute UTIs and other related infections (Medina & Castillo-Pino, 2019). UTI (Urinary tract infection) is infection that occur in bladder (cystitis), kidney (pyelonephritis), and urethra (urethritis) and one of the common infectious disease (McCann et al., 2020). UTI are caused by bacteria and other microorganism such as viruses and fungi. Most common fungi are *Candida albicans* and common are herpes simplex virus and cytomegalovirus that cause UTIs (Olin & Bartges, 2015). The lower (cystitis) and upper (pyelonephritis) UTIs these infections can be characteristic with the patients of clinical situation and that are more serious microorganism invasion (Smelov et al., 2016).

However, it is believed to be the same across sex but found slightly more common in women of the same group's age as the men (O'Brien et al., 2015). Most acute uncomplicated cystitis occur in healthy non pregnant women Foxman and et al., 2000. The bacteria that are most likely to be responsible for UTIs include those that are gram negative and are known to be *E. coli*, *Proteus mirabilis* and *Klebsiella pneumoniae*. UTIs are classified into two types: straightforward, they are those UTIs, which are not linked to any comorbid conditions

which are labeled as complicated, in patients with immunosuppression, diabetes mellitus or with anatomical and functional abnormalities of the urinary system (Nitzan et al., 2015).

UTIs are generally recognized to be the second most prevalent infection in human beings and only after respiratory tract infection (Mobley & Alteri, 2015). Bacteriuria in Inpatient UTIs these bacteria are responsible for 30-50% and for Outpatient UTIs 80-90% by uropathogenic *E. coli* (Ejrnaes, 2011). Some patients are thought to be at increased risk for development of UTI; they are among the most common infections that occur while in hospital and are among the most common causes of bacteremia and Nosocomial infections namely; 25-35%. These infections happen in all age groups (Akram et al., 2007). Previous data suggested that UTIs were predominantly acquired in hospital care settings, however, the latest data points to patients developing UTIs from the community (Frazee, et al., 2018). Some of the high risk individuals are; children, old persons, pregnant women, those with spinal cord injuries, catheter users, multiple sclerosis, diabetic patient and AIDS patients. Of the uropathogens, *E. coli* is the most common bacteria found in the patients with community acquired UTIs (Bonkat et al., 2017).

The WHO estimates that UTI is a causative factor in approximately seven hundred thousand deaths each year (Organization, 2020). UTI most worldwide prevalence of C 150 to 200 million /year; female 40% for at least one symptomatic episode in life time; male 12%. Hospital-acquired UTIs account for 40-50% of all cases of bacterial associated complications which results to increased morbidity, length of stay and cost (Karam et al., 2019).

UTIs are a global concern with seven million, five hundred thousand people affected each year and considerable strain on the healthcare costs. They remain a global health priority similar to what was postulated by Bano et al., (2012). Of particular concern for the patient, UTIs are associated with 34 percent of catheter-associated health care infections (Fink et al., 2012). Research also indicates that first-time UTI patients are likely to experience the same in future; therefore, the

expenses increase further; particularly women who are above 30 years of age.

UTIs affect humans and animals and is the second most common diagnosed infection due to careless use of antibiotics and spectral antimicrobials (Bischoff et al., 2018). E. The most frequent ESBL producer according to Alizade and her team in 2015 is E.coli. It is produced by multidrug resistant (MDR) phenotype with clinical impact (Health & Services, 2019). The constantly increasing appearance of ESBL-producing E. coli with an increased resistance level to trimethoprim/sulfamethoxazole (SMX-TMP) is a significant problem (Critchley et al., 2019). ESBL enzymes decrease beta lactam antibiotics' efficiency by breaking its beta-lactam ring and decreases its ability to bind target proteins; however, carbapenems and cephamycin are not inhibited by this enzyme (Barguigua et al., 2011). Since the year 2000, distantly related ESBLs of the CTX-M type have tended to replace nearer ones of the TEM, and SHV types (Barguigua et al., 2011). This has led to raises in the use of carbapenem antibiotics particularly within clinical practice (Blair et al., 2015).

UTIs are amongst the common bacterial infection often treated in outpatient clinics and the fourth most leading cause of antibiotic-resistant infections in primary care patients (Bryce, et al., 2016). These results for both urinary and fecal bacteria definitely suggest cross resistance and infection spread. Infrequently E. coli is cultured from urine and its yield may be variable, similar to values found in fecal samples of normal individuals (Hay, Birnie, et al., 2016). One of the most important reasons is that children including adolescents facilitate dissemination and development vectors of AMR infections and play the role of Amplifiers (Hay, Sterne, et al., 2016). Increased rate of multidrug resistant especially uropathogenic E.coli is prevalent and it is associated with the abuse of antibiotics (Miles et al., 2006). The repeated UTIs are also unable due to ability of uropathogens to avoid antibiotics (O'Brien et al., 2015). Conservative approach to UTI treatment involves the use of empirical antimicrobial therapy but there misuse results to formation of microbial resistance and subsequent

spread of the resistant strains aissue of major public health concern (De Francesco et al. 2007, Arjunan et al., 2010).

The samples suggest that in the last few years antibiotic resistance of Enterobacteriaceae isolates has become a major public health issue. According to WHO in its 2014 global report, E.coli was considered to be a major concern in both, HC associated and community acquired infections (Group, 1983). The current global world is wetting worse levels of antibiotic resistance, which are expected to cause higher morbidity and mortality ratios thus a major health concern (Dyar et al., 2012). The today average level of antimicrobial resistance correlates with their application and is considered a novel problem (Toval et al., 2014).

## METHODOLOGY

### Study Design

It is a cross sectional and observational study.

### Study Settings

This study has been performed at Sughra Shafi Medical Complex.

### Study Duration

Study duration for this research work was 6 months W.E.F July 2025 to December 2025.

### Sample Size

Calculated via using a formula  $n = z^2 \times p(1-p) / E^2$  where P is an estimated prevalence=16% E is margin of error taken as 5%. And Z for Confidence level of 95% is 1.96.

A sample of 230 patients will be selected among the population presenting with at Sughra Shafi Medical Complex.

### Sampling Technique

Non-Probability Convenient Sample technique.

### Sample Selection

#### Inclusion Criteria:

Admitted patients and those patients who's are suspected for Urinary Tract Infection (UTI). Those patients who are capable of providing a mid-stream urine specimen using the "clean catch" method.

## Exclusion Criteria

**Non-E. coli Isolates:** While other bacteria like *P. aeruginosa*, *K. pneumoniae*, and *S. aureus* were identified in some samples, the primary focus of the resistance analysis and prevalence reporting was restricted to *E. coli*.

**Contaminated Samples:** The procedure emphasized instructing patients on how to avoid contamination risk during collection; samples that did not follow the "mid-stream" protocol (eliminating the first and last drops) were likely excluded to ensure accuracy.

## Data Collection Procedure

Self-collected urine samples were prepared in total of two hundred and thirty. The patients were provided with sterile, dry, wide necked, no leakage container with advice to donate 10 – 20 ml of their urine. Patients were informed of contamination risk and told on how to make clean catch midstream sample.

The patients were asked to wash their hands and then provide a mid-stream urine sample. This was done by eliminating the first and the last urine drops, because the middle ones were taken to act as a control sample.

Earlier, before midstream, male patients were requested to wash their hands before transferring

## 2 Identification of *E. coli* Colonial Morphology

### 3. Gram staining

Gram staining was done for 109 isolates which were grown on CLED agar. They showed pink

### 4. Indole test

Of total, 109 bacterial isolates and test indicate by the formation of a pink to red (cherry- red ring).

### 5. Triple sugar ion test

Of total, 109 bacterial isolates, in positive TSI test indicates the results yellow by yellow colour and yellow butt on the bottom.

the specimen during the flow. Female patients we instructed to: Make them wash their hands, then use clean water to clean around the urethral opening called the perineum and use a sterile gauze pad to wipe it, prepare for the collection of urine as they open their labia.

When the sample was taken, the container had written the name of the patient, the identification number assigned to the patient, date as well as the time of collection of the sample. The specimen was placed in the next available box after completing the request form and sent to the laboratory.

## Data Analysis Procedure:

The data will be analyzed through SPSS 2.4 version.

## Ethical Consideration:

The Sughra Shafi Complex in Narowal Ethical Review committee provided its clearances before the study's sample collection initiated.

## RESULT

### 1 Prevalence of collected samples

During the study, 230 urine samples were collected from suspected UTI patients. Who have visited the Sughra Shafi Medical Complex, Narowal, Punjab Pakistan during the period 6 months.

230 samples were cultured on CLED agar. Of total, 109 (47%) exhibit round, usually golden colonies after overnight incubation at 37 C .

colored bacilli which were rod in shape under the microscope .

**6. Citrate Test:**

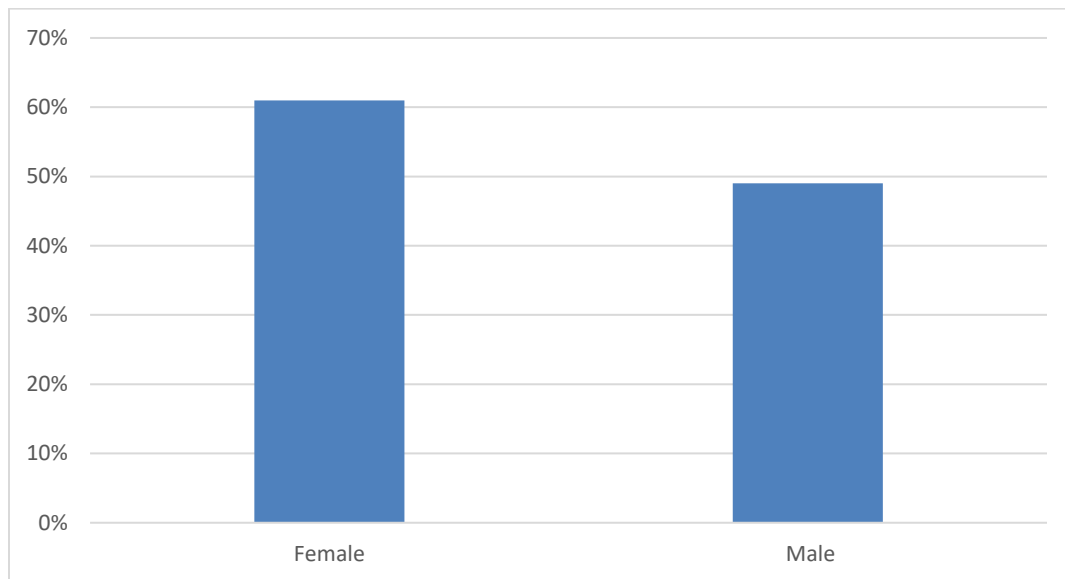
Citrate produce green color to intense blue along the slant.

**7. Gender based distribution of Samples**

230 samples are collected were 109(47%) were positive for *E. coli*, 121(53%) whereas were negative. As shown in fig 7.

**Table 1: Gender based distribution of samples**

Gender	Frequency	Percentage
Female	140	61%
Male	90	49%
Total	230	100%



**Fig 1: Gender wise distribution of samples**

**8. Gender wise distribution of *E. coli*:**

Out of 109 (47%), 71(65%) were females and 38(34%) were male. Gender based analysis showed

the prevalence of *E. coli* was more among female patients than in male as shown in fig 8.

**Table 2: Gender wise distribution of *E. coli***

Gender	Frequency	Percentage
Male	38	34%
Female	71	65%
Total	109	100%

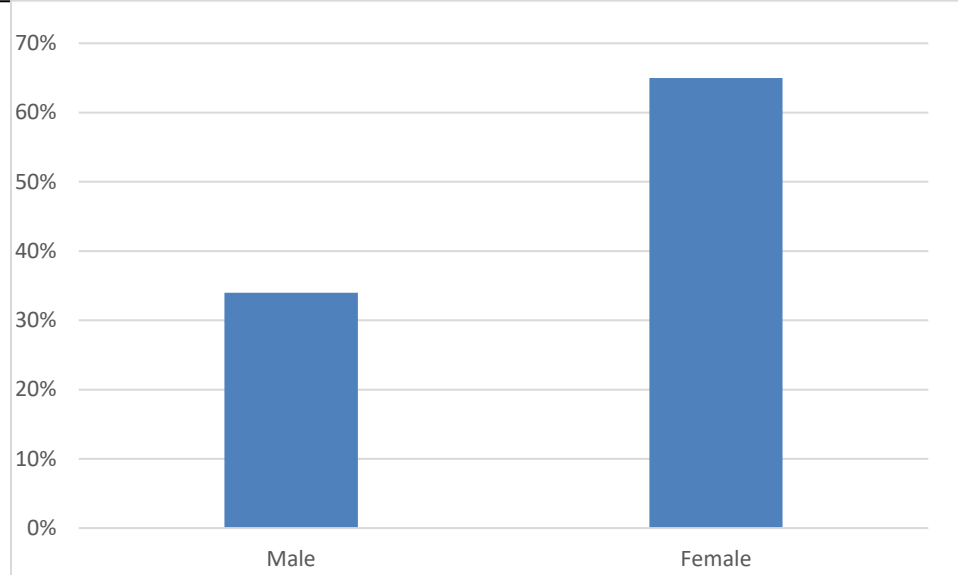


Fig 2: Gender wise distribution of *E. coli*

**9. Age wise distribution of *E. coli*:**

Out of 109, the highest prevalence of *E. coli* was in 51 to 60 age group followed by 61 to 70 age group,

31 to 40 age group, 21 to 30 age group and 1 to 10 age group as shown in fig 9.

Table: age wise distribution of *E. coli*

Age (Years)	Quantity	Percentage
1-10	4	3%
11-20	5	4%
21-30	8	7%
31-40	16	14%
41-50	9	8%
51-60	35	32%
61-70	24	22%
71-80	5	4%
81-90	3	2%
Total	109	100%

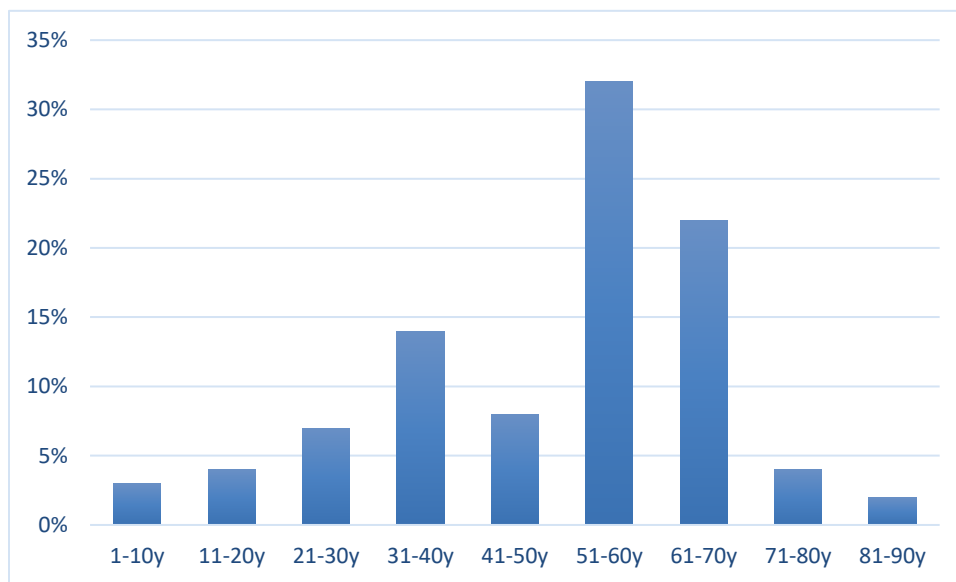


Fig 3: age wise distribution of *E. coli*

**10. Antimicrobial susceptibility testing:**

Antimicrobial resistance revealed 93% resistance against *E. coli*. The highest prevalence was due to MDR (39%) *E. coli* of total, *E. coli* showed resistance against ciprofloxacin (93%), sulfamethoxazole-trimethoprim (89%),

Levofloxacin (83%) followed by Ceftazidime (83%) and tetracycline (80%). Whereas most effective was Fosfomycin and Amikacin. Antibiotic susceptibility was carried out following CLSI recommended guidelines. As shown in table 12.

Class	Drugs	Resistance	Sensitive
Fluoroquinolones	Ciprofloxacin	93%	8%
Sulfonamides	Sulfamethoxazole-trimethoprim	89%	11%
Fluoroquinolones	Levofloxacin	83%	17%
Cephalosporin	Ceftazidime	83%	17%
Carbapenems	Imipenem	18%	82%
Tetracycline	Tetracycline	80%	20%
Cephalosporin	Ceftriaxone	78%	22%
Carbapenems	Meropenem	24%	76%
Cephalosporin	Cefepime	72%	28%
Aminoglycosides	Tobramycin	65%	35%
Aminoglycosides	Gentamycin	65%	35%
Penicillin	Amoxicillin-Clavulanic acid	24%	76%
Phosphoric	Fosfomycin	18%	82%
Aminoglycosides	Amikacin	16%	84%

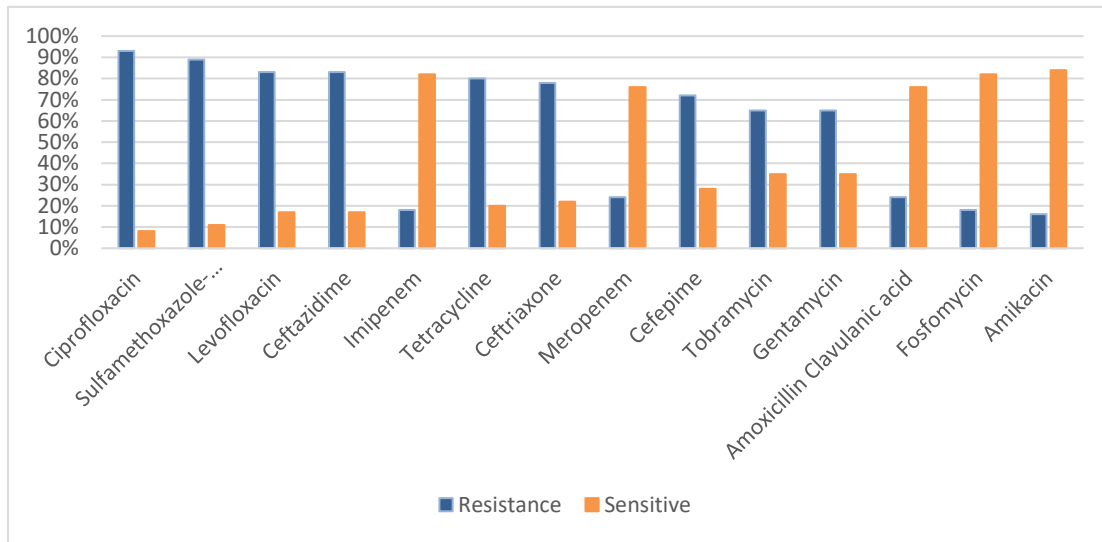


Fig 4: Antibiogram of *E. coli*

**DISCUSSION**

He also said: Being one of the premier UTIs due to *E.coli* responsible for one of the most significant world problems, while developing countries like Pakistan are most affected. Out of all the cohort observational comparative study conducted in the present study of antibiotic resistance UTI isolates, 58 studies with 77,783 *E.coli* isolates of urine samples were included. Specifically detected overall resistance rates were 53.4% for ampicillin, 23.6% for ciprofloxacin and least 1.3% for nitrofurantoin the study conducted by Bryce et al., 2016 in the member countries of OECD. In the present study, we conducted in Narowal our own research with MDR-*E. coli* in patients with suspected UTI. Of 230 samples, which were taken from different sources, 109 samples were examined in more detail those being positive samples, which grew golden colonies when inoculum on the CLED media.

Another study was conducted in Iran to determine the bacteriological Type of UTI patients and their antimicrobial resistance pattern. Of 3,798 patients, 568 had positive UTI samples that was a 14.96%. The bacterial organisms most frequently isolated in the present study were *E.coli*, *Pseudomonas* as well as *Staphylococci* sp. These bacteria had high susceptibility rate to amikacin 91.57% and imipenem 99.1%, but had high resistance rate to ampicillin 89.29% and

methicillin 99.1 Mihankhah et al., 2017. A cross sectional study done from a tertiary care hospital tried to identify frequency and antibiotics resistance of uropathogens among outpatients. When addressing the study question, The authors found out that among 804 urine samples collected during the same study period, 290 were positive for urinary pathogens yielding a prevalence of 36.1%. Surprisingly, antibiotic resistance higher among the elderly patients these discovered in Muhammad et al., (2020).

*E. coli* has been reported for the first time in 2002 in Islamabad Pakistan. *E. coli* is distributed locally, but unfortunately some cases have been reported from other countries from which patients travel to Pakistan. The rise of *E. coli* is a critical concern that could affect the already fragile health care system and lead to high mortality rates in women. Pathogens that are resistant to carbapenems are also entering our health care system. While pervious research in Pakistan, unites states and Iran classified *E. coli* as the most frequent 100% carbapenems-resistant infection, *Enterococcus* was shown as the most typical carbapenems-resistant bacterium. In our study *E. coli* were observed, the highly resistance for Cefepime (93%), tetracycline (90%), Sulfamethoxazole-trimethoprim (98%), Ciprofloxacin (87%), Levofloxacin and

Ceftazidime (83%), Ceftriaxone (78%), Amoxicillin Clavulanic acid (76%) and the sensitives drugs rates are Amikacin (84%), Imipenem (82), Meropenem (76%). The Imipenem and amikacin are most effective drug.

## CONCLUSION

UTIs are considered to be a multiplier disease in the population and affect approximately 52% of people of all age groups; they rank second among the bacterial infections. Recent findings in antibiotic-resistant infections and most especially those caused by *Escherichia coli* in the urine samples have been a worry to public health departments. In this study, susceptibility testing was performed using the guideline set by the Clinical and Laboratory Standards Institute (CLSI) for antibiotic testing. Antibiotic susceptibility was carried out following CLSI recommended guidelines. According to our study the resistance is very high in district Narowal due to the reason of self-medication, misuse of drugs and not follow up proper instruction of doctor. The national antimicrobial resistance action plan (AMR) must be vigorously and effectively implemented by the Pakistani Ministry of Health. Active monitoring research to guide future public health initiatives and local, national and international controls. Additionally, by encouraging individuals to practice hygiene practices, we can reduce the danger of *E. coli* contamination.

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