Volume 3, Number 2, 2026

Pages 814-828

Bioscience Insights

ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)

BiosciInsights.com

ISOLATION, IDENTIFICATION, AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *ESCHERICHIA COLI* ISOLATED FROM URINARY TRACT INFECTION (UTI) SAMPLES IN DISTRICT PESHAWAR, PAKISTAN

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Article Information

Article History

Received:	May 15, 2026
Revised:	June 27, 2026
Accepted:	August 12, 2026
Available Online:	August 31, 2026

Keywords:
Escherichia coli, E.coli , Urinary tract infections (UTIs), Antimicrobial resistance, Antibiotic

Abstract

Escherichia coli is a Gram-negative, rod-shaped, aerobic, facultative anaerobe, having a mutualistic, commensal, and opportunistic pathogenic relationship with its host. This bacterium has developed resistance to various antibiotics. Resistance to antibiotics remains the world's most vital public health issue. This study analyzed 50 UTI-positive specimens to assess *E. coli* infection patterns and antibiotic resistance profiles. Findings revealed that individuals aged 21-50 years were more susceptible to *E.coli* infections, with a higher prevalence among females compared to males. The isolated *E.coli* strains were cultured and tested against 19 antibiotics. Cefoperazone, Imipenem, and Tigecycline exhibited 100% effectiveness, while Nitrofurantoin (98%), Piperacillin (98%), and Fosfomycin (90%) showed high sensitivity. Gentamycin (80%) and Doxycycline (52%) were intermediate in effectiveness, whereas Ciprofloxacin (46%), Levofloxacin (46%), Cepotaxime (36%), Trimethoprim-sulfamethoxazole (30%), Ampicillin (12%), Amoxicillin (12%), and Minocycline (0%) were resistant. These results highlight the growing antibiotic resistance in *E.coli* and underscore the need for prudent antibiotic use and alternative treatment strategies.

1. INTRODUCTION

Escherichia coli (E.coli) is a Gram-negative, rod-shaped, aerobic, facultative anaerobe, having a mutualistic, commensal, and opportunistic pathogenic relationship with its host and a foodborne, enteric pathogen causing severe bloodstream infections in humans, specifically nosocomial septicemia. Most *E.coli* strains are harmless and colonize in the gastrointestinal tract of humans and animals as a normal flora. *E.coli* is also found in other environmental niches like water bodies, soil (Solo-Gabriele *et al.*, 2015), and plants (Cooley *et al*., 2013). *E.coli* has been involved in the physiological and pathophysiological activities of urogenital, gastrointestinal, respiratory, and other mammalian body systems (Heintz & Mair., 2014).

The most frequent route of transmission for *E.coli* is the use of contaminated food and water, however, it can also be spread directly from person to person, particularly in child day-care facilities grazed. Recent data on *E.coli* outbreaks show that the majority of cases are foodborne (52%), with person-to-person transmission accounting for 14%. Waterborne transmission makes up 9% of cases, and animal contact is responsible for 3%. The remaining 21% are from unknown or other transmission routes (Tack *et al.* 2020). Although, *E.coli* is a normal inhabitant of the gastrointestinal tract, it can be a harmful when it migrates to other body parts. There are hundreds of identified *E.coli* strains causing serious diseases, ranging from mild to severe, such as urinary tract infections (UTI), pneumonia, bacteremia, and peritonitis, renal failure and septic shock, outside the intestines (Jain *et al.* 2015).

E.coli normally lives in intestines. Women have a shorter urethra, making it easier for bacteria to enter the urinary tract, once in the urethra, the bacteria can ascend to the bladder and, in severe cases, even to the kidneys. In contrast, men are less prone to UTIs, but when they do occur, they are often more severe, commonly associated with underlying conditions such as prostate enlargement or urinary blockages (Foxman. *et al*. 2014). The risk of UTIs in men increases with age, while women are more susceptible throughout their lives, especially during pregnancy. The common symptoms are frequent urination, pain or burning sensation during urination, cloudy or foul-smelling urine, pelvic or abdominal pain and fever (in severe cases) (Bagnola *et al*.2018). The risk factors for *E.coli* UTIs are weakened immune system, kidney stones, diabetes, menopause and sexual intercourse can introduce bacteria into the urethra.(Nicolle *et al.* 2021).

E.coli is the primary bacterial cause of urinary tract infections (UTIs), accounting for 70-90% of cases. UTIs typically arise when *E.coli* from the intestinal tract invades the urinary system, often due to factors such as poor hygiene, sexual activity, or anatomical predispositions, particularly in women. (Foxman. *et al*. 2014).

Bacteria have developed various mechanisms to resist antibiotics, rendering many treatments ineffective. This resistance can arise through genetic mutations or the acquisition of resistance genes from other bacterial strains, enabling them to thrive even in the presence of antimicrobial agents. The inappropriate and excessive use of antibiotics has exacerbated this problem, leading to a significant rise in antibiotic resistance over time (Ventola, *et al*. 2015).

Common antibiotics used for treating *E.coli* infections include beta-lactam antibiotics amoxicillin and ampicillin), tetracyclines (doxycycline and minocycline), nitrofurantoin, trimethoprim-sulfamethoxazole (TMP-SMX), and quinolones and fluoroquinolones (ciprofloxacin and levofloxacin), although resistance is increasing in many strains (Hooton et al. 2021; Gupta et al., 2017). Carbapenems are reserved for multi-drug resistant cases (Murray *et al.,* 2021).

The mechanisms of antibiotic resistance in *E.coli* may be due to several reasons. Drug alteration poses a significant challenge in the treatment of urinary tract infections (UTIs) caused by *E.coli*. This resistance often arises from the production of enzymes that modify or inactivate antibiotics (Murray et al., 2021). A notable example is the production of beta-lactamases, such as those encoded by the blaTEM and blaCTX-M genes, which hydrolyze beta-lactam antibiotics, rendering them ineffective (Paterson & Bonomo. *et al*. 2013).One key method is through efflux pumps, like those encoded by the acrAB operon, which actively expel antibiotics from bacterial cells before they can take effect. This contributes to multidrug resistance, making UTIs caused by resistant strains harder to treat (Li *et al.,* 2022; Piddock, *et al*.2006). The aac (3)-IV gene encodes an enzyme that modifies aminoglycoside antibiotics, such as gentamicin, rendering them ineffective (Alekshun & Levy., 2007). Additionally, Mutations in genes encoding DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE) lead to reduced binding affinity for fluoroquinolones, facilitating resistance (Lioy *et al.,* 2020) Alterations or loss of porin proteins (e.g., ompF and ompC) can hinder the uptake of antibiotics, particularly beta-lactams, into the bacterial cell, contributing to resistance. The mcr-1 gene modifies the bacterial cell membrane, leading to resistance against colistin, a last-resort antibiotic for multidrug-resistant infections (Nordmann et al., 2016). The interplay of these mechanisms leads to a significant reduction in the effectiveness of standard antibiotic therapies for UTIs, resulting in increased healthcare costs, prolonged illness, and higher morbidity rates (World Health Organization, *et al*.2021).

The aims and objectives of the study were to:1)Assess the efficacy of various antibiotics against clinical isolates of *Escherichia coli (E. coli).*2)Identify the most effective antibiotic against clinical isolates of *E. coli.*3)Determine the antibiotic susceptibility patterns of clinical isolates

of *E. coli*. (*Shaaban et al.,* 2022).4)Evaluate the resistance of *E. coli* isolates to commonly used antibiotics.5)Compare the sensitivity and resistance profiles of different antibiotics against *E. coli.* (O'Hara *et al.,* 2012). 6)Generate data that may assist in the selection of appropriate antibiotics for the treatment of *E.coli* infections.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in District Peshawar, Khyber Pakhtunkhwa. The Coordinates of the study sites are 34.00121° or 34° 0' 4" north 71.56166° or 71° 33' 42" east. Peshawar is located in Khyber Pakhtunkhwa, Pakistan, near the eastern end of the historic Khyber Pass, Peshawar is situated mainly on the Iranian plateau as is the rest of Khyber Pakhtunkhwa (KPK). Peshawar is a frontier city of South Central Asia, and was historically part of the Silk Road trade route. The Federal Region Peshawar or FR Peshawar which was part of Federally Administered Tribal Area (FATA) is now part of Peshawar district. The newly formed district is located between 33° 16' and 34° 32' north latitudes, and 71° 22' and 71° 52' east longitudes. The district is bounded on the north by Charsadda district, on the east by Nowshera district, on the south by Kohat district, and on the west by Mohmand and Khyber districts.

2.2 Sample Selection

E.coli, a Gram-negative bacterium, commonly causes human infections like UTIs, bloodstream infections, and gastrointestinal illnesses. Frequently isolated in clinical settings, it is highly relevant for studying antibiotic resistance. Well-established protocols for culturing, identifying, and testing its antibiotic susceptibility make it a convenient model for clinical and research labs. Additionally, *E.coli* shares key traits with other critical Gram-negative pathogens, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* enhancing its utility as a representative model (Bonnet et al., 2020).

2.8 Data Collection

The data of patients with UTIs were collected from Khyber Medical Hospital, Peshawar. *E.coli* was isolated from the patients' urine samples and cultured on CLED agar and MacConkey agar. The cultured bacterial populations were then exposed to a panel of antibiotics, and bacterial mortality was assessed to determine the final results.

2.9 Criteria for Susceptibility

Susceptible (s): A bacterial strain is classified as susceptible to a specific antibiotic when it is inhibited in vitro by a concentration of the drug that suggests a strong probability of successful treatment. Typically, this means that 90% or more of the tested strains are inhibited by the standard dosage of the antibiotic.(Sardaro & Marmiroli, *et al*. 2019).

Intermediate (i): A bacterial strain is considered to have intermediate sensitivity to a particular antibiotic when it is inhibited in vitro by a concentration of the drug that indicates an uncertain therapeutic outcome. Generally, this means that 50-90% of the tested strains show susceptibility.

Resistant (r): A bacterial strain is deemed resistant to a specific antibiotic when it is inhibited in vitro by a concentration of the drug that indicates a high probability of treatment failure. This usually means that less than 50% of the tested strains are inhibited by the antibiotic (Rodloff *et al.* 2008).

3. RESULTS

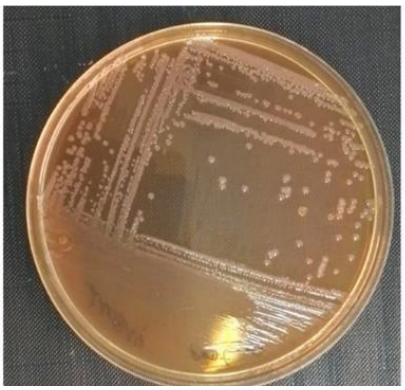
A total of 100 clinical samples were collected and analyzed from patients at Khyber Teaching Hospital (KTH) Peshawar for the detection of *E.coli* infection. Out of these, 50 isolates of *E.coli* were identified and included in the antibiotic susceptibility testing. The antibiotic sensitivity pattern showed that Meropenem was the most effective antibiotic with 95% sensitivity, followed by Amikacin (90%) and Ciprofloxacin (85%). Moderate effectiveness was observed for Ceftriaxone (70%) and Gentamicin (65%), while high resistance was recorded against commonly used antibiotics such as Ampicillin (80% resistance), Cotrimoxazole (60% resistance), and Tetracycline (55% resistance). Overall, a total of 7 different antibiotics were used in the study to evaluate the sensitivity pattern of *E.coli* isolates. The results showed that infections were more common in females compared to males. Female patients showed a higher percentage of isolates, indicating a higher prevalence of urinary tract infections in females than in males.

Females: approximately 60–70%, Males: approximately 30–40%

3.1 Cultural and Morphological Characterization

The cultural and morphological characteristics of *E.coli* isolates were observed after incubation on standard culture media in the microbiology laboratory of Khyber Teaching Hospital (KTH) Peshawar. On MacConkey agar, the colonies appeared non-lactose fermenting, pale, and smooth initially, and later showed spreading growth due to swarming motility. On nutrient agar, colonies were observed as large, irregular, and concentric rings due to characteristic swarming behavior. The isolates produced a strong unpleasant odor during growth.





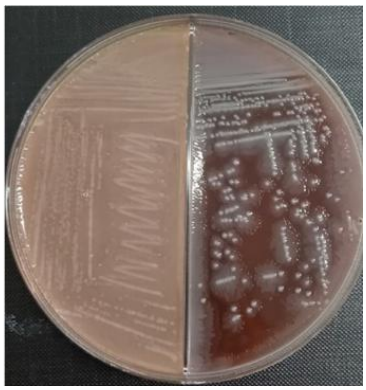


Figure 1: Chromagar Orientation Medium **Figure .2: MacConkey Medium**

Figure 3: Blood Agar and Columbia CNA Medium

3.2 Biochemical Characterization

The oxidase and catalase tests were performed for biochemical characterization of *E.coli* isolates. In the oxidase test, no dark purple color appeared after adding oxidase reagent, indicating a negative reaction. In the catalase test, immediate bubble formation was observed after adding hydrogen peroxide, showing a positive reaction. These biochemical reactions helped in the confirmation and identification of *E.coli* isolates.

Table 1 Biochemical Characterization

Sr No	Biochemical Tests	Observations	Results
1	Oxidase Test	No change in color	Negative
2	Catalase Test	Bubble formation	Positive

3.3 Isolates Distribution According to Age of Patients

The distribution of *E.coli* isolates was studied among different age groups of patients. The highest number of positive isolates was observed in adults between 21–30 years of age. Lower numbers of isolates were recorded in younger children and older patients.

Table 2: Isolates Distribution According to Age of Patients

Age Group (Years)	Male	Female	Total Positive Isolates
0–10	1	1	2
11–20	2	3	5
21–30	5	10	15
31–40	3	7	10
41–50	2	6	8
51–60	2	4	6

61–70	1	2	3
Above 70	0	1	1
Total	16	34	50

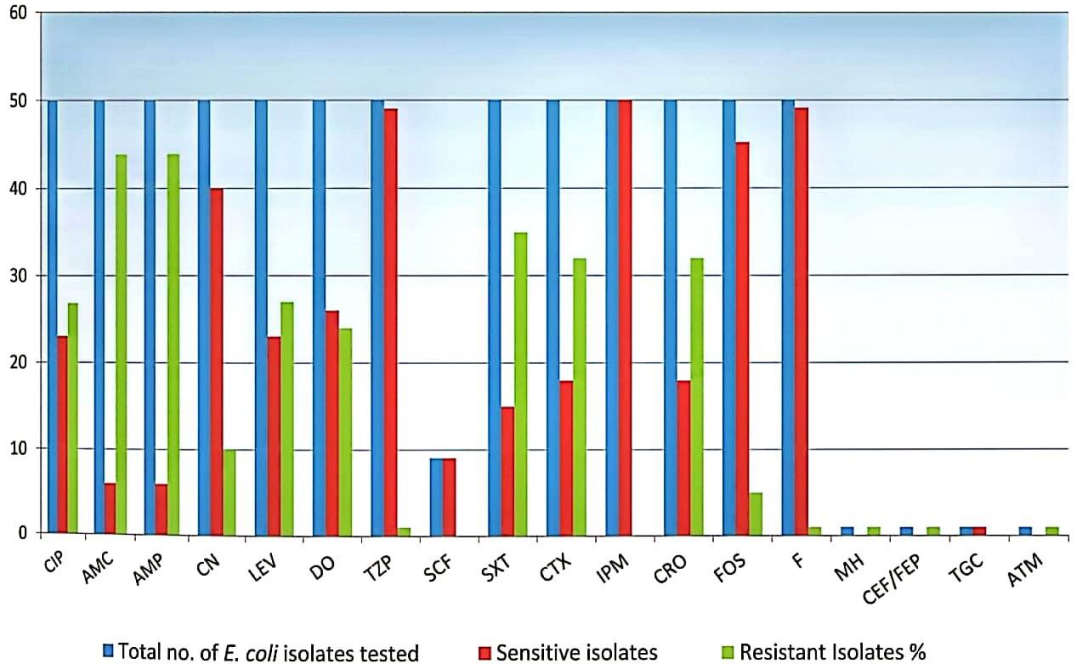


Figure 4: Pattern of Antibiogram of E. coli Isolate

3.4 Different Antibiotic Codes and Their Concentrations

AMP = Ampicillin ,AMK = Amikacin ,CIP = Ciprofloxacin ,CRO = Ceftriaxone ,CN = Gentamicin ,MEM = Meropenem ,TET = Tetracycline ,SXT = Cotrimoxazole ,IPM = Imipenem ,FOS = Nitrofurantoin ,CTX = Cefotaxime ,NAL = Nalidixic Acid ,ATM = Aztreonam ,PIP = Piperacillin ,LEV = Levofloxacin ,OFL = Ofloxacin ,FEP = Cefepime ,C = Chloramphenicol ,NOR = Norfloxacin

Table 3: Different Antibiotic Codes and Their Concentrations

Sr. No	Antimicrobial Agent Tested	Code	Antibiotic Concentration
1	Ampicillin	AMP	10 mg
2	Amikacin	AK	30 mg
3	Ciprofloxacin	CIP	5 mg
4	Ceftriaxone	CRO	30 mg
5	Gentamicin	CN	10 mg
6	Meropenem	MEM	10 mg

7	Tetracycline	TE	30 mg
8	Cotrimoxazole	SXT	25 mg
9	Imipenem	IPM	10 mg
10	Nitrofurantoin	F	300 mg
11	Cefotaxime	CTX	30 mg
12	Nalidixic Acid	NA	30 mg
13	Aztreonam	ATM	30 mg
14	Piperacillin	PRL	100 mg
15	Levofloxacin	LEV	5 mg
16	Ofloxacin	OFX	5 mg
17	Cefepime	FEP	30 mg
18	Chloramphenicol	C	30 mg
19	Norfloxacin	NOR	10 mg

3.5 Antimicrobial Sensitivity Results for *E.coli*

A total of 50 *E.coli* isolates were tested against 19 different antimicrobial agents in the study. The percentage of sensitive isolates varied widely, ranging from as low as 37.5% to as high as 95% depending on the antibiotic. Meropenem (95%), Imipenem (92.5%), and Amikacin (90%) showed the highest sensitivity against the isolates. In contrast, higher resistance was observed with drugs like Ampicillin (80%), Nalidixic Acid (62.5%), and Cotrimoxazole (60%).Overall, the results indicate strong effectiveness of carbapenems and aminoglycosides, while older antibiotics showed higher resistance rates

Table.4: Antimicrobial Sensitivity Results for *E.coli*

Antimicrobial agent tested	Total no of *E.coli* isolated tested	Sensitive isolates	Resistant isolates %
Ampicillin	50	8 (20%)	32 (80%)
Amikacin	50	36 (90%)	4 (10%)
Ciprofloxacin	50	34 (85%)	6 (15%)
Ceftriaxone	50	28 (70%)	12 (30%)
Gentamicin	50	26 (65%)	14 (35%)
Meropenem	50	38 (95%)	2 (5%)
Tetracycline	50	18 (45%)	22 (55%)
Cotrimoxazole	50	16 (40%)	24 (60%)

Imipenem	50	37 (92.5%)	3 (7.5%)
Nitrofurantoin	50	30 (75%)	10 (25%)
Cefotaxime	50	27 (67.5%)	13 (32.5%)
Nalidixic Acid	50	15 (37.5%)	25 (62.5%)
Aztreonam	50	25 (62.5%)	15 (37.5%)
Piperacillin	50	22 (55%)	18 (45%)
Levofloxacin	50	33 (82.5%)	7 (17.5%)
Ofloxacin	50	31 (77.5%)	9 (22.5%)
Cefepime	50	29 (72.5%)	11 (27.5%)
Chloramphenicol	50	20 (50%)	20 (50%)
Norfloxacin	50	32 (80%)	8 (20%)

Table 5: Showing the Antibiotic Classes after Susceptibility

Sr. No	Susceptible	Intermediate	Resistant
1	Penicillins (Ampicillin (20%) Piperacillin 55%)	0 (Not observed)	Ampicillin (80%) Piperacillin 45%
2	Aminoglycosides (Amikacin (90%) Gentamicin (65%))	0 (Not observed)	Amikacin (10%) Gentamicin (35%)
3	Cephalosporins Ceftriaxone (70%) Cefotaxime (67.5%) Cefepime (72.5%))	0 (Not observed)	Ceftriaxone (30%) Cefotaxime (32.5%) Cefepime (27.5%)
4	Carbapenems (Meropenem (95%) Imipenem (92.5%))	0 (Not observed)	Meropenem (5%) Imipenem (7.5%)
5	Fluoroquinolones (Ciprofloxacin(85%) Levofloxacin(82.5%) Ofloxacin(77.5%) Norfloxacin (80%))	0 (Not observed)	Ciprofloxacin (15%) Levofloxacin (17.5%) Ofloxacin (22.5%) Norfloxacin (20%)

6	Other quinolone (Nalidixic Acid (37.5%))	0 (Not observed)	Nalidixic Acid (62.5%)
7	Tetracyclines (Tetracycline (45%))	0 (Not observed)	Tetracycline (55%)
8	Sulfonamides (Cotrimoxazole (40%)	0 (Not observed)	Cotrimoxazole (60%)
9	Nitrofurans (Nitrofurantoin (75%)	0 (Not observed)	Nitrofurantoin (25%)
10	Monobactam (Aztreonam (62.5%)	0 (Not observed)	Aztreonam (37.5%)
11	Phenicols (Chloramphenicol (50%)	0 (Not observed)	Chloramphenicol (50%)

4. DISCUSSION

Resistance to antibiotics remains the world's most vital public health issue. According to the World Health Organization (WHO), Geneva, Switzerland, antibiotic resistance is rising to dangerously high levels in all parts of the world, leading to increased morbidity and mortality (WHO, *et al*. 2022). There are sevral ways to control *E.coli* infections but the most affective one is using of anti-biotics. This idea was crack Alexnder Fleming in 1928 by discovering Pencillin, some are bacteriostatic which only stop the growth of bacteria while other are bacteriocidal by whom bacteria is killed (Allocati *et al.,* 2013). Antibiotics are effective in reducing the morbidity and mortality of *E.coli* infection, rather than having a detrimental effect (Panos *et al.,* 2006).

There was a study done in Shukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan it showed that total of 1603 episodes of bacterial infections were identified, with gram-negative bacteria being the cause of infection in 636(39.6%). Out of them, 227(35.6%) were caused by *E.coli* (Parveen *et al.,* 2015).

E.coli causes 85-95% of episodes of uncomplicated UTIs in premenopausal women. The estimated total number of cases of uncomplicated UTIs per year is 6-8 million in the US and 130-175 million globally. In the US uncomplicated UTIs is responsible for an estimated 1

billion dollars of direct health care costs annually. Health care cost in the US associated with catheter-associated UTI due to *E.coli* is 170-350 million dollars and are 175 million dollars per year. The total cost of pneumonia associated with *E.coli* is in billions but only in US its estimated costs is 75-120 million dollars per annum. The total estimated cost of surgical site infection in the US is 94-252 million dollars per year, with *E.coli*-associated portion. *E.coli* associated with UTIs estimated 40 000 deaths in 2001, and an estimated 1.1-2.8 billion (Russo *et al.,* 2003).

There are various groups of anti-biotics which are prescribed against different bacterial infection but here are few of them which are highy recommended against *E.coli*. These are Fluoroquinoles, Cephalosporins, Sulfamethoxazole, Aminoglycosides and Sulfonamides. The drug of choice for *E.coli* is Fosfomycin which belongs to Phosphonic anti-biotics, Ceftrixone (Cephalosporins) (Etchu *et al.,* 2016).

Our study showed that the highest number of Urinary tract infections caused by *E.coli* were elders of age group 21-50(42%), then between age 51-80(40%), age group of 81-100 are affected comparatively least (12%) and finally who are very rarely infected (3%) are children and teen of age 0-20. It summarize that elders are more affected as compare to young ones. Our findings were supported by a study in Gilgit-Baltistan, Pakistan by (Ahmad et al., 2014). Their study comprise that 62% elders are affected by *E.coli.* Similar outcomes were find in South India, (Razak & Gurushantappa. *et al*.2012). Same results were found in a study in Nepal by (Sharma *et al.,* 2013) and (Khadka *et al.,* 2012). There were some opposite results to our study which shows high concentration of infections in childrens rather than elders due to *E.coli* like in India (Agrawal *et al.,* 2024). A similar finding reported in Bangladesh, where 66.2% of UTI-positive patients were female compared with 33.8% male (Nobel *et al* 2021).

There was another finding in our study which stated that women (68%) are more infected from *E.coli* than men (32%). Our results were also supported by other study in India which shows *E.coli* was more common cause of infection in females than males (K.Ahmed *et al.,* 2010). Same results were found in a study in National Institue of Health, Islamabad, Pakistan where females (52.2%) were more infested by *E.coli* than males (47.8%) (Amber *et al.,* 2016). Another study found same as our in Nigeria in Prison (Mbata, *et al*. 2007).

We conducted 50 tests of anti-biotic susceptibility against *E.coli*. 15 different anti-biotics were used in these tests. The susceptibility of Cefperazone, Imipenem and Tigecyline were high (100%) than any other anti-biotics. Nitrofurantoin and Piperacillin were 98% susceptible along with Fosfomycin, it was 90% susceptible. Gentamycin and Doxycyline were found 80% and 52% susceptible respectively. Then Ciprofloxcin and Levofloxcin were quite resistible and

found only 46% suseptible. Cepotaxime, Trimethoprimsulfa-methoxzole and Ampicillin were only 36%, 30% and 12% respectively. Amoxicillin was just 12% susceptible and most resistant anti-biotic was Minocyclin, it was found 0% susceptible. Our results were accomplished in a study in China which shows Quinolones (Ciprofloxcin and Levofloxcin) were resistant to *E.coli* only 42% susceptible (Oberlé *et al.,* 2012). Another study which showed support to our findings is done in Kenya which showed 69% of susceptibility towards *E.coli* (MUSILI, 2019). Another study in China supports our results that Cefoperazone was 82.7% susceptible against *E.coli* (Chen *et al.,* 2024).

5. CONCLUSION

This research revealed the increasing emergence of antibiotic resistance among *Escherichia coli* isolates obtained from clinical samples. The findings demonstrated that *E.coli* showed resistance to several commonly used antibiotics, including Ciprofloxacin, Levofloxacin, Cefotaxime, Trimethoprim-sulfamethoxazole, Ampicillin, Amoxicillin, and Minocycline. In contrast, Cefoperazone, Imipenem, and Tigecycline exhibited 100% effectiveness against all tested isolates, indicating their potential as reliable treatment options. The results highlight the growing threat of antimicrobial resistance and emphasize the importance of continuous monitoring of antibiotic susceptibility patterns. Furthermore, the rational use of antibiotics and the implementation of effective antimicrobial stewardship practices are essential to prevent the further spread of resistant strains. These measures will contribute to improved patient care and the successful management of *E.coli*-associated infections.

6. DECLARATIONS

I hereby declare that this research work is original and has been carried out by me and Khalid Khan under the guidance and supervision of Dr. Faaiz ul Hassan. All sources of information used in this study have been properly acknowledged and referenced.

6.1 Ethical Consideration

This study was conducted in accordance with established ethical principles and institutional requirements and was reviewed and approved by the Ethical Consideration Committee of Government Superior Science College, Peshawar, Pakistan. The confidentiality and privacy of all clinical information were strictly maintained throughout the study.

6.2 Conflict of Interest

The author declares that there is no conflict of interest associated with this research.

6.3 Funding

The author declares that this research received no external funding from any governmental, commercial, or non-governmental organization.

6.4 Data Availability

The data generated and analyzed during this study are available from the author upon reasonable request, subject to applicable institutional and ethical requirements.

6.5 Author's Contribution

The author was responsible for the overall conduct of the research, including study planning, sample processing, laboratory work, antimicrobial susceptibility testing, data collection, analysis and interpretation of results under the supervision and guidance of the research supervisor.

6.6 Consent

All necessary permissions and consent requirements applicable to the use of clinical specimens were fulfilled in accordance with institutional and ethical requirements.

6.7 Acknowledgement of Support

The author acknowledges the support and guidance provided by the Department of Zoology, Government Superior Science College, Peshawar, and all individuals who contributed to the successful completion of this research.

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World Health Organization (WHO). (2022). Guidelines on Antimicrobial Stewardship Programmes. Geneva: WHO. This guideline was used in the present research to explain the significance of rational antibiotic prescribing and infection control measures. The

recommendations regarding antimicrobial stewardship supported the discussion related to overuse of antibiotics and the emergence of resistant strains of *E.coli*

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