PREVALENCE AND ANTIBIOGRAM OF UROPATHOGENIC *ESCHERICHIA COLI* AND *ENTEROBACTER CLOACAE*

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Abstract. Resistance to various routinely used antibiotics is rapidly increasing, which has become a serious global problem. The main aim and objective of this study was to determine antibiotics resistance of uropathogenic isolates of *Enterobacter cloacae* and *Escherichia coli* and to determine the prevalence rate of urinary tract infections (UTIs) in different age groups of male and female patients. 250 samples were collected from patients in Ayub Medical Complex Abbottabad and Yahya Hospital Haripur. Urine samples were inoculated Eosin Methylene Blue (EMB) agar and incubated for 24 h. Then further identification of *E. cloacae* and *E. coli* isolates was performed by biochemical tests and microscopy. Antibiotics susceptibility was tested by disk diffusion method against levofloxacin, tobramycin, gentamicin, ofloxacin and imipenem. Our results showed that only 70 samples gave culture positive results in which 31 (12.4%) cases were positive for *E. coli* while only 9 (3.6%) cases were positive for *E. cloacae*. The prevalence of *E. coli* was high in females (66%). All isolates were highly sensitive to imipenem (58%) and highly resistant to levofloxacin (48%). The results of prevalence of uropathogenic *E. coli* and *E. cloacae* were compared with available data to get clear picture of current trend in antibiotic resistance. Data of present study will be helpful in choice of antibiotics for treatment of UTI in Hazara region.
Keywords: *eosin methylene blue, microscopy, biochemical tests, disk diffusion method, prevalence rate*

Introduction

Uropathogenic *E. coli* is the most common cause of urinary tract infections (UTIs). 80%-85% of total UTI isolates is *Escherichia coli* (Totsika et al., 2012). Other causative agents include *Klebsiella*, *Proteus*, *Serratia*, *Enterobacter*. Other organisms such as *Pseudomonas* and *Enterococcus* also greatly contribute to nosocomial and catheter associated UTIs (Nicolle et al., 2005). Uropathogens possess different types of virulence factors that contribute to their pathogenesis and cause damage to urinary tract (Dobrindt and Hacker, 2010). Uropathogenic *E. coli* (UPEC) possess a variety of virulence factors including α-hemolysin (HlyA), cytotoxic necrotizing factor 1 (CNF 1), type 1 pilli (which are essential for colonization), siderophores (aerobactin and

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yersiniabactin of *E. coli*) (Kot, 2017). *E. cloacae* infects hosts and causes illness through a variety of strategies, including invasion, colonization, immune response evasion, and toxin release (Davin et al., 2019). Many virulence factors, including siderophores, fimbriae, biofilm, efflux pump, virulence factors are linked to its pathogenic process (Perez et al., 2012).

Based on the part of urinary tract colonized by bacteria, UTIs are classified as urethritis (inflammation of urethra which is the tube carrying urine out of the body), cystitis (inflammation of urinary bladder) (Chung et al., 2010), ureteritis (inflammation of ureters) and pyelonephritis (inflammation of kidney) (Lane and Takhar, 2011). Initiation of UTIs occurs with periuretheral contamination caused by uropathogen inhabiting gut, subsequent colonization of urethra which is followed by locomotion of uropathogen to the bladder or kidney with the help of flagella or pilli (Werneburg, 2022). In conditions such as *Staphylococcus aureus* bacteremia or endocarditis, UTIs are generated by entry of the organism through blood (Mancuso et al., 2023). Adherence of this microorganism depends on three major features; adhesive mechanism of bacterium, the receptive features of the urothelium of organism and finally the fluid that is present between both surfaces (Murray et al., 2021). Adhesins present on the surface of the bacterial membrane are required for initial attachment onto urinary tract tissues forming a protective layer referred as biofilm. This biofilm formation helps the bacteria to adjust in unfavorable conditions, colonization and persistance (Lila et al., 2023). Symptoms include acute dysuria (burning during urination), increase in incontinence (inability to hold urine), increase in urogency (an increase urge to urinate), increase in frequency, hematuria (bloody urination), pyuria (cloudy urine caused by pus), flank pain (deep pain in sides and back) and low grade fever (Stone, 2012).

Antibiotics used to treat UTIs include many different types. B-Lactam antibiotics (e.g. amoxicillin/clavulanate, cefaclor, cefdinir, cefpodoxime, and ceftriaxone) are another option in managing uncomplicated UTIs. Fluoroquinolones (e.g. levofloxacin or ciprofloxacin) are recommended for treating uncomplicated pyelonephritis and complicated UTIs (Gupta, 2011). Other antibiotics include trimethoprim/sulfamethoxazole. In spite of the fact of availability of many types of antibiotics, UTIs are currently difficult to treat because of resistance to antibiotics. To find the antibiotic resistance pattern is very important for efficacy of treatment of a particular disease (Costelloe et al., 2010). Antibiotic sensitivity and resistivity of uropathogenic *E. coli* depends on serotype of pathogen. *E. coli* O26, O103, O111, O128, and O145 are UTI causing pathogens (CM Schroeder, 2002). *E. coli* has high ability to spread resistance among its population. *E. coli* resistance is increasing to different antimicrobial drugs (Poire et al., 2018). Increase in resistivity to antibiotics decrease the efficacy of treatment. Intra et al. (2023) conducted a twelve years surveillance study on the antibiotic resistance pattern of *E. cloacae* and founds that the community and hospital-related individuals showed a markedly growing trend of resistance to several commonly used antibiotics, most likely as a result of incorrect and unregulated usage.

Mechanisms of drug resistance in bacteria include active efflux of the antibiotic out of the cell, degradation of the antibiotics by enzymes, alteration of antibiotic binding targets, over production of a target enzyme, developing alternative metabolic pathways to those against which antibiotic is made (Van et al., 2011). Several mechanisms are used to acquire antibiotic resistance genes. Genes of resistance are transmitted among bacteria belonging to varied classification groups through transduction, tranposons, conjugation, transformation, mutations (Schwarz et al., 2005). In this way resistance to antibiotics

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spread among uropathogens. Owing to this reason MDR strains are emerging, creating challenges in treatment and spreading outbreaks worldwide (Totsika, 2011). Certainly, this has been the case with uropathogenic *Escherichia coli* (UPEC) and *Enterobacter cloacae*. The susceptibility pattern of *E. coli* and *E. cloacae* to different antibiotics varies worldwide. Therefore, information of local susceptibility pattern (antibiograms) is very important for selecting appropriate empirical antibiotics (Badura et al., 2015). The main aim of the present study was to evaluate the antibiotic resistance patterns of uropathogenic *E. coli* and *E. cloacae* in both female and male of different age groups.

Materials and methods

Inclusion and exclusion criteria

The present study was conducted in Microbiology Laboratory of University of Haripur, Pakistan. 250 mid stream samples from patients of symptoms of UTIs who attended the two Hospitals (Ayub Teaching Hospital and Yahya Hospital) including both male and female of different age groups were collected. Patients were classified into 5 groups according to their age. A detailed clinical history was obtained which included age, sex, previous episodes of UTIs. Patients who were already on antibiotic treatment were excluded from study.

Urine culturing

Urine sample was inoculated on Eosin methylene blue (EMB) agar using a calibrated wire loop (0.001 ml). After overnight incubation, growth count of 10^5 CFU/ml of urine (CFU: Colony Forming Units) was considered as positive and were used further.

Identification and isolation

Single type of bacteria that showed growth with significant count were identified by gram staining and biochemical tests including methyl red (MR) test, voges proskauer (VP) test and citrate utilization (CU) test.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by disk diffusion method (Kirby Bauer disk method). 0.9% saline inoculated colonies were incubated at 30°C until it achieves the turbidity of 0.5 McFarland standard. MHA plates were inoculated with bacterial suspension using sterilized cotton swab. Antibiotic disks were placed using sterile forceps on the surface of plates. After incubating plates at 35°C for 24 h, zone of clearance were measured and results were compared according to CLSI criteria. Then using tables the zone sizes for each drug were classified as sensitive, intermediate, or resistant. The antibiotic discs used were Imipenem, Gentamicin, Tobramycin, Levofloxacin and Ofloxacin.

Results

Urine culture

In the beginning of our study we selected 250 patients and cultured their urine samples on EMB. A Criterion was that monomicrobial culture in a number of 10^5

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CFU/ml must appear after urine culturing. According to selected criterion 70 specimen showed monomicrobial culture and were taken positive. 180 specimens gave mixed culture after culturing and were considered as contaminated. They were excluded from the study (*Fig. 1*).

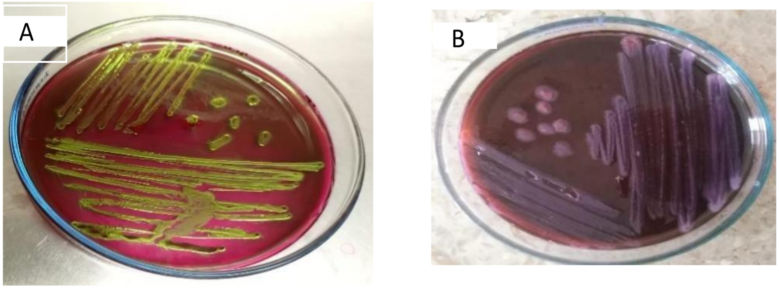

Figure 1. *(A) E. coli on EMB agar; (B) Enterobacter cloacae on EMB*

Gram staining

All the 59 out of 70 specimen were observed as Gram negative under microscope. Both *E. coli* and *E. cloacae* are Gram-negative bacilli, that have peritrichous flagella. Gram positive isolates were excluded from the study (*Fig. 2*).

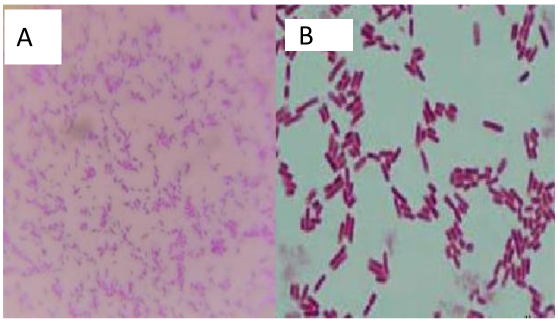

Figure 2. *Microscopic presentation of isolated bacteria. (A) E. coli and (B) Enterobacter cloacae*

Biochemical characterization of E. coli and E. cloacae

Biochemical characterization of 59 colonies were carried out. In the methyl red test out of 59 isolates, 35 isolates on MR-VP broth gave positive results while 23 gave negative results. In Voges-Proskauer test, 33 isolates gave negative results while 20 were positive. Citrate utilization test**,** 31 isolates gave negative results which were identified as *E. coli.* 9 isolates were citrate positive (*Tables 1* and *2*; *Figs. 3* and *4*).

Age and gender wise prevalence of E. coli and Enterobacter cloacae

Our study showed that 51.6% cases of *E. coli* in 21-40 age group which is the highest while only 3.2% cases were reported in above 80. *Enterobacter cloacae* was not consistently distributed across all age groups. Overall, it is found that the percentage of *E. cloacae* isolates (33.33%) were the highest in 1- 40 age groups while no isolate was found above 80.

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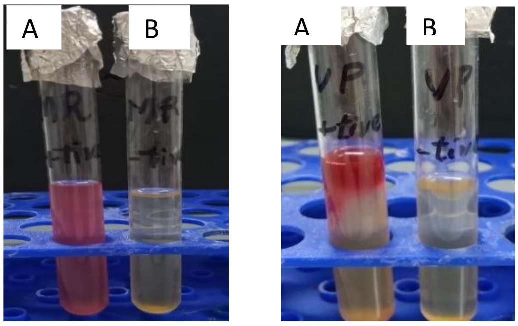

Figure 3. *MR test and VP test of isolated bacteria*

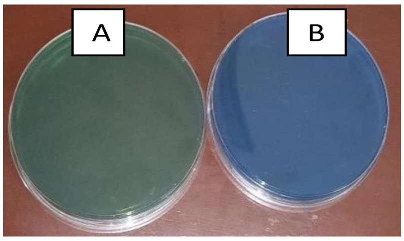

Figure 4. *Simmon citrate test results of isolated bacteria. (A) negative; (B) positive*

Table 1. *Biochemical characterization of isolated strains*

Tests	**Results**	
Gram staining	- (Rods)	- (Rods)
Methyl Red	+	-
Voges-Proskauer	-	+
Citrate Utilization	-	+
Results	*E. Coli* (31)	*E. cloacae* (9)

Table 2. *Gender and age wise prevalence of E. coli and Enterobacter cloacae*

Age group	**Total samples**	**No. of positive cases for *E. coli***	**No. of positive cases for *E. cloacae***
1-20	20 (8%)	03 (9.6%)	3 (33.3%)
21-40	166 (66.4%)	16 (51.6%)	3 (33.3%)
41-60	50 (20%)	08 (25.8%)	2 (22.2%)
61-80	10 (4%)	3 (9.6%)	1 (11.1%)
Above 80	4 (1.6%)	01 (3.2%)	0 (0%)
Total	250	31	9
		No. of male patients 13 (42%) No. of female patients 18 (58%)	No. of male patients 4 (%) No. of female patients 5 (%)

Antibiotic sensitivity pattern of E. coli and Enterobacter cloacae

There were almost same sensitivity pattern towards antibiotics in male and females. This study showed that antibiotic sensitivity and resistivity was independent of age and

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sex. In both gender (male and female), *E. coli* isolates of all age groups were found resistant to antibiotics. The highest resistance was found against levofloxacin (32%) and ofloxacin (32%) while lowest resistance was against imipenem (19%). In uropathogenic *Enterobacter cloacae,* the highest resistance was found against levofloxacin (56%) while lowest resistance was against ofloxacin (22%) (*Table 3; Figs. 5, 6* and *7*).

Table 3. *Antibiotic susceptibility among uropathogenic E. coli and E. cloacae*

Antibiotics	No. of sensitive isolates (%)		No. of intermediate isolates (%)		No. of resistant isolates (%)	
	E. coli	***E. cloacae***	***E. coli***	***E. cloacae***	***E. coli***	***E. cloacae***
Imipenem (IMP-10)	22(71%)	4(45%)	3(10%)	2(22%)	6(19%)	3(33%)
Tobramycin (TOB-10)	19(61%)	3(33%)	5(16%)	2(22%)	7(23%)	4(45%)
Levofloxacin (LEV-5)	12(39%)	2(22%)	9(29%)	2(22%)	10(32%)	5(56%)
Ofloxacin (OFX-5)	18(58%)	5(56%)	3(10%)	2(22%)	10(32%)	2(22%)
Gentamicin (CN-10)	18(58%)	4(45%)	5(16%)	1(10%)	8(26%)	4(45%)

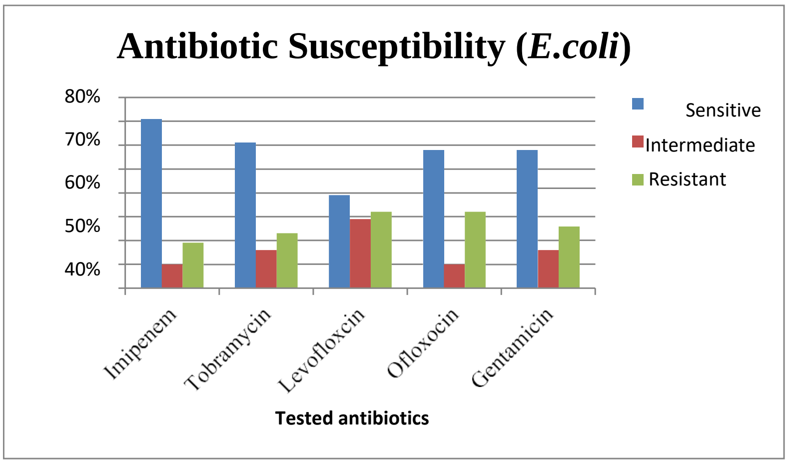

Figure 5. *Graphical presentation of antibiotic susceptibility pattern of E. coli*

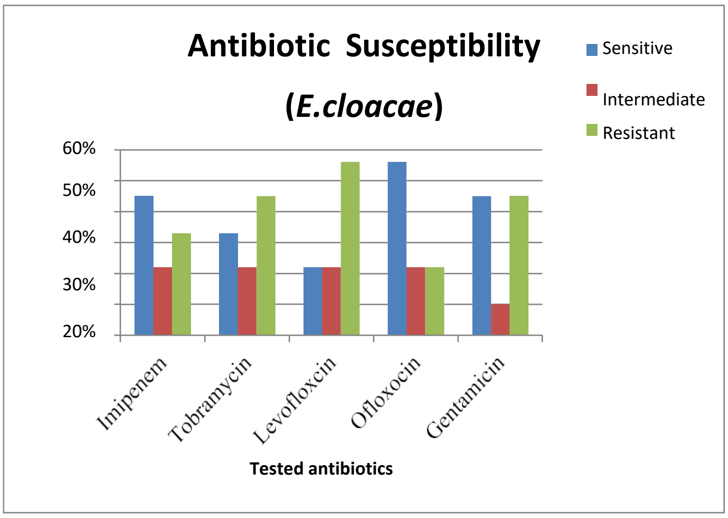

Figure 6. *Graphical presentation of antibiotic susceptibility patterns of E. cloacae*

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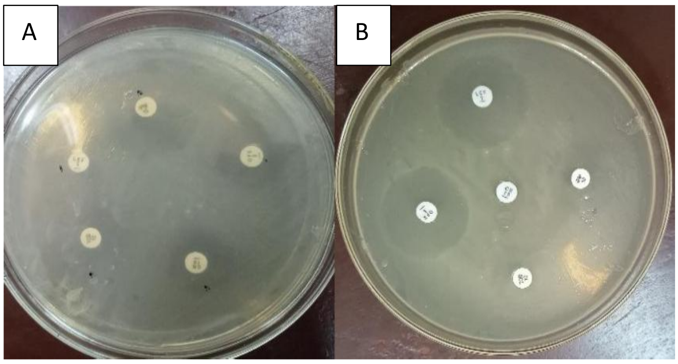

Figure 7. *(A) Resistance pattern of Enterobacter cloacae; (B) resistance pattern of E. coli*

Discussion

Infection of the urinary tract is among the most common bacterial infections worldwide (Najar et al., 2009). The present study provides information about distribution of E. coli and E. cloacae in different age groups of both gender as well as resistance pattern of E. coli and E. cloacae. Thus, this study also allows the comparison of prevalence and resistance of E. coli and E. cloacae isolates in UTI patients belonging to Hazara. The most common causative agent of UTIs in our study was found to be E. coli. Biochemical tests detected 31 (12.4%) E. coli isolates from a total of 250 samples while 9 (3.6%) Enterobacter cloacae isolates from a total of 250 samples. These results were consistent with results obtained from other studies conducted worldwide which suggest that more than 80% of UTIs are caused by E. coli (Sabir et al., 2014). As reported in the previous research (Bashir et al., 2008; Foster, 2008) the current study found the high rate of E. coli in females compared to males. High percentage of E. coli isolates was found in patients aged 21-60 years. Least percentage was found in patients of age above 80 years. This result was comparable to results of Kiffer et al. (2007) finding higher percentage of E. coli isolates in patients aged 13-60 and lower percentage in patients aged below 13 years and aged above 60 years. Urinary tract infections are currently difficult to treat because of resistance of E. coli to antibiotics. It was found through different studies conducted worldwide that resistance pattern varies over time and varies also in different geographical regions (Ventola, 2015). Several reasons of antibiotic resistance have been determined including overuse, incorrect prescription of antibiotics, barriers in usage of novel antibiotics (Klein et al., 2019). Hence, antibiotic treatment should be carried out after local evaluation of antibiotic sensitivity patterns. In our study E. coli showed same sensitivity and resistivity to different antibiotics in people belonging to different age groups. The results of present study revealed that E. coli isolates possessed high resistance to ofloxacin (32%) and levofloxacin (32%). E. cloacae is also considered emerging pathogen contributing widely in UTIs all over the world. Levofloxacin resistance of E. cloacae was 56% and ofloxacin resistance was 22%. It is verified through different studies that resistance of members of family Enterobacteriaceae to levofloxacin has been increased worldwide (Hassanshahi et al., 2020; Jang et al., 2011).

Conclusion

Antibiotic resistance is a devastating problem. The emergence of drug resistance among *E. coli* and *E. cloacae* to these antibiotics has left the physicians with few

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restricted options for choosing the precise antibiotics for the treatment of the infections which are caused by these resistant organisms. It is thus recommended that physicians should rationally prescribe the antibiotics after evaluation of antimicrobial sensitivity reports of the isolates as resistance rates vary geographically. This will be helpful in the rational use of antibiotics and it will also reduce the problem of antimicrobial resistance. Based on the results of this research, we suggest to avoid recommending antibiotics for which *E. coli* and *E. cloacae* isolates were found resistant, i.e. levofloxacin. Present study provides useful information on the current trends of antibiotic resistance which will absolutely lead to the rational selection of antibiotics for empiric treatment of UTIs.

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Conflict of interests. The authors declare no conflict of interests.

Ethical approval. The current research is approved the Institutional Research Ethics Committee of the University of Haripur, KPK. Informed consent was obtained, and ethical guidelines were followed. The approval letter confirms that all necessary precautions were taken to ensure the protection of human subjects and adherence to ethical standards.

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