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INCIDENCE OF ANTIMICROBIAL-RESISTANT ESCHERICHIA COLI IN UROPATHOGENIC INFECTIONS: AN EMERGING THERAPEUTIC DILEMMA

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Abstract

Background: *The purpose of this study was to determine the frequency of multi-drug-resistant E. coli in patients with UTIs who were seen at the Pakistan Institute of Medical Sciences' General Medicine Department.*

Methodology: *During the course of six months, a total of eight hundred urine samples were taken from both indoor and outdoor patients attending the PIMS laboratory. Of these samples, 150 tested positives for urinary tract infections. After that, these samples were examined utilizing various culture media. Urine samples were cultured on CLED Agar media to determine the presence of bacteria, and then the biochemical test API 10, which is often used to identify gram negative bacteria, was performed. After bacterial growth was observed in the samples, MHA medium was added to determine the antibiotics' sensitivity or susceptibility using an antibiotic disc. Following that, the results were documented, with over three medications exhibiting resistance to the tested antibiotics being ruled out as MDR.*

Results: *Out of 150 samples, 100 tested positive for MDR, whereas the remaining 50 did not. Of the agents examined, levofloxacin (62.7%), amoxicillin/clavulanic acid (74.7%), and cefepime (70%) showed the most resistance, whereas tazobactam (18.7%), amikacin (16%), and Fosfomycin (15.3%) showed the lowest resistance. The other examined medicines in the current investigation included ceftriaxone 43.3%, ampicillin 54%, cefepime 70%, sulbactam 24.7%, and cotraimaxloe 47.3%. Resistance to norfloxacin is 54% and Fosfomycin is 15.3%, respectively.*

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INTRODUCTION

UTIs (urinary tract infections) are a significant public health issue. Known as nosocomial infections, they are among the most common bacterial diseases that affect both the general public and hospitalized patients. The main cause of UTIs is Escherichia coli, which is frequently found as a commensal resident of the gastrointestinal system. Children's illnesses and mortality are linked to infections produced by members of the Enterobacteriaceae family, which are also one of the main causes of hospital admission. Antibiotics that are easily obtainable and reasonably priced have proven to be beneficial in curing infections caused by these bacteria in low-income or developing nations [20].

Like all other members of the Enterobacteriaceae family, Escherichia coli has grown more resistant to antibiotics over time [19]. Due to the overgrowth of strains of bacteria that are resistant to multiple drugs, the effectiveness of antibiotics that were once effective has significantly decreased. This has led to the development of broad-spectrum antibiotics such as fluoroquinolones and third-generation cephalosporins, which are typically too expensive for most developing or low-income nations [20].

E. COLI

Gram-negative, rod-shaped bacteria from the Enterobacteriaceae family is called Escherichia coli. It is, of course, a commensal that lives in human colons. It also coexists with higher organisms and animal colonies, including lizards and birds [1]. E. Coli often exterminates its host by excreting faeces into an area where it can live for several days or months. E. Coli's ability to survive in its secondary environment is dependent on a number of factors, including temperature, food, and water humidity [2]. Since E. Coli is primarily an intestinal dweller, its ability to live outside of its normal host is therefore rather limited. If E. Coli is found in food or water, for example, it may be a sign of faecal contamination or poor hygiene [3].

Causes of E. coli

The most prevalent bacterial illness in older adults is urinary tract infections (UTIs), which are most commonly caused by E. coli. The presence of harmful bacteria (E COLI) (105 CFU/mL) in voided urine is known as a urinary tract infection (UTI) [7]. Extra-intestinal pathogenic E. coli, or "ExPEC," is another extra-intestinal illness that can be caused by Escherichia coli. This category is essentially based on the knowledge that the bulk of extra-intestinal disorders are caused by genotypic and phenotypic differences among E. coli. These diseases include urinary tract infections (UTIs), meningitis in newborns, hospital-acquired pneumonia, biliary and GIT infections, septic arthritis, etc. A straightforward infection must first establish itself in a non-sterile area, such the gastrointestinal tract, before it can attack a sterile place. In addition, non-pathogenic E. coli strains can cause infections anytime the host's regular immune system is weakened. such as peritonitis, catheter-related UTIs, etc. [18].

150 million UTIs are estimated to cost the global healthcare system more than $6 billion a year [4]. Most people, especially adults, have UTIs, which only need to be treated briefly with antibiotics. However, those with pyelonephritis, especially those who are older, may develop bacteremia, require prolonged hospital stays, require antimicrobial medication and treatments, have reduced functional status, or even die. A study estimates that each year, around 150 million UTIs are reported worldwide [4]. It involves the most prevalent hospital acquired infection, accounting for over 35% of all hospital acquired infections, in the vast majority of hospitals. Simple UTI isolates contained E. Coli; the most prevalent bacterium associated with UTIs [5].

There have been reports worldwide that E. coli infections, which cause UTIs, are becoming resistant to traditional medications. Resistance to more recent medications has also surfaced [6]. Antimicrobial resistance and observation are crucial in determining the extent of the issue, ruling out potential solutions, and directing the empirical selection of antibacterial medicines for the treatment of afflicted persons. Our goal is to determine the current prevalence of multidrug resistant strains of E. coli that cause UTIs between isolates and commonly used antibiotic pathogens. Antibiotics and other drugs have been used to eradicate the bacteria that is the source of sickness and illness. The development of antibiotics

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has significantly improved human health. Many infections that formerly killed people decades ago can today be easily cured with antibiotics. Microbes that are resistant to antibiotics are those that they cannot stop or eliminate. They continue to make a living and, when antibiotics are present, even multiply. Multidrug resistant organisms are bacteria that can withstand more than one antibiotic [8].

How Bacteria Show Resistance to Antibiotic

The use of antibiotics causes microbes to evolve resistance to such drugs. Every time a person takes antibiotics, the sensitive bacteria are killed, but some bacteria that are resistant to the drugs may remain behind and proliferate and multiply. Self-medication and frequent use the fundamental and main causes of the rise in the number of microorganisms exhibiting antibiotic resistance are incomplete antibiotic treatments [17]. Antibiotics were traditionally used to treat bacterial infections, but they are frequently ineffective against viral infections that cause illnesses like the flu, colds, and sore throats. Antibiotic resistance spreads as a result of overuse of antibiotics. The first and most important step in stopping the emergence of resistance is to take antibiotics sparingly, avoiding self-medication, partial therapy, and excessive usage [16].

The development of resistance to antibiotics occurs when bacteria undergo certain changes that reduce or even completely eradicate the therapeutic benefits of chemicals, antibiotics, and other medications used to treat infections and prevent infectious diseases. These bacteria persist and continue to spread because they are resistant to treatment. Microorganisms have multiple ways to attempt this. Certain bacteria have the ability to neutralize antibiotics before they cause side effects, while others may suddenly reject the medication. Still other bacteria may be able to alter the site of an antibiotic's attack, which prevents the bacteria from changing their characteristics in any way that would allow the antibiotic to have an impact [10].

Any infectious condition treated with antibiotics usually results in the death of the susceptible microorganisms as well as their inhibition. Sometimes a microbe survives the antibiotic because it possesses the necessary ability to neutralize and block its effects. This allows the microorganism to proliferate and multiply and eventually replace all of the dead bacteria. Antibiotic exposure thus provides a selected strain, which causes the remaining microorganism to develop resistance. Furthermore, bacteria that would not typically be susceptible to antibiotics might develop resistance via genetic mutation or by gaining DNA segments that code for the resistance, which sets them apart from other bacteria. It is possible to combine the DNA responsible for resistance genes into a single, easily transferred package. Because of the mutation of a single DNA strand, bacteria can thus become resistant to numerous antimicrobial agents [15].

MDR

Multi-drug-resistant microbes make matters worse and reduce the efficacy of good treatments [11]. It is challenging to control UTIs caused by multidrug resistant pathogens because resistant germs are spreading outside of hospitals and into the network. This is mainly due to the increasing prevalence of resistance to novel antimicrobials [12]. These establishments might act as holding tanks for these MDROs, which might subsequently be sent to acute hospitals in vulnerable groups. In addition to the point cure of asymptomatic bacteremia, which may be of special concern, these populations include inhabitants of nursing homes, where UTIs may be more likely to acquire resistance to antibiotics including ciprofloxacin, cephazolin, and nitrofurantoin. After bacteremia, mortality is better when there is evidence against antimicrobials than when there is evidence sensitive to antimicrobials. However, this is most likely due to ineffective empirical antimicrobial treatment rather than a correlation with increased virulence of the E. Coli strains [9].

Character factors, predisposing factors, and bacterial factors are terms that can be used to describe threat elements for prognosis of UTIs caused by multidrug resistant microorganisms [13]. Individual and demographic factors that could impact the risk of urinary tract colonization with multidrug-resistant organisms (MDROs) include advanced age, female gender, a history of urinary tract infections, diagnoses of dementia and low functional level, diabetes, and prostatic disease. Healthcare-associated risk factors

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and predisposing factors for prolonged risk of network-obtained MDROs in the urinary tract include intrusive methods such as urine catheterization, prior hospitalization, living in a nursing home, and prior exposure to antibiotics [14].

LITERATURE REVIEW

According to a study done in India, 119 (38.2%) of the 311 E. coli isolates were obtained from in-patients; these isolates were taken into consideration for additional research. The reports that were generated indicated that 91 (76.51%) of the 119 E. coli isolates in total were classified as multidrug resistant. The isolates exhibited resistance to ampicillin (88.4%), amoxicillin-clavulanic acid (76.4%), ceftriaxone (72.4%), and trimethoprim (64.2%) at extremely high levels. (Da names ta ka simply lags shat a makhy). These isolates shown sensitivity to amikacin (83.2%), imipenem (96.9%), nitrofurantoin (84.1%), and piperacillin-tazobactam (79.2%). Typically, ceftriaxone was used as an empirical treatment for urinary tract infections. Following appropriate treatment for all 92 of these MDR-caused UTI cases, 72 infections showed improvement and 13 clearly showed worsening [23].

The most active substance was imipenem. Ipenem was one of the most active agents, with a 100% susceptibility rate. Nitrofurantoin and amikacin had susceptibility rates of 94% for both of these medications, in order of precedence. Moreover, in both types of isolates, there were elevated rates of resistance to ampicillin (82%) and trimethoprim (75%), which were most frequently coupled. However, 64% of the isolates shown resistance to several medication classes. Additionally, 39% of community-acquired isolates and 61% of hospital-acquired isolates were found to be resistant to cefpodoxime, a sign of prolonged β-lactamase synthesis [24].

Twenty separate European centers were asked to provide a total of 1000 isolates; however, some centers were unable to provide the necessary quantity of isolates, therefore a total of 887 isolates were obtained. Moreover, not a single center fit the definition of being underrepresented. Escherichia coli (54.3%), Enterococcus spp. (12.5%), Klebsiella spp. (6%), Proteus spp. (6.8%), Pseudomonas aeruginosa (6.3%), and Enterobacter spp. (4.0%), coagulase negative staphylococci (2.8%), Staphylococcus aureus (2.4%), Candida spp. (1.8%), Citrobacter spp. (1.6%), Acinetobacter spp. (1.3%), Serratia spp. (1.1%), and Morganellamorganii (1.0%) were the most frequently occurring microorganisms causing urinary tract infections [25].

The most active (94 percent susceptible) compound was found to be nitrofurantoin, followed by aminoglycosides. There were substantial reports of ampicillin (55%) and co-trimoxazole (40%) resistance rates. While it was uncommon to find isolates exhibiting resistance to multiple drug classes, 5.7% of community-acquired isolates and 21.6% of hospital-acquired isolates were found to be resistant to ceftriaxone, a sign of Extended spectrum β-lactamase generation [24].

METHODOLOGY

This descriptive Study was conducted at department of General Medicine Pakistan Institute of Medical Sciences from March to August 2023.Male and female patients in various age groups are complaining of burning when they urinate and having a fever, vomiting as well as nausea. Patients who had used antibiotics in the past were excluded. For data analysis we will use SPSS version 23.0 (Statistical Package for Social Sciences) and for the measurement of mean median mode we will use central tendency. For Range variance and standard deviation, we will use measure of dispersion. For the graphical presentation we will use pie charts and graphs. Eight hundred urine samples were collected from all patients who visited PIMS, both indoor and outdoor, and of any gender and age. The study used three kinds of media: MacConkey agar, Mueller Hinton agar, and CLED agar.

CLED

Cystine Lactose electrolyte deficient, according to manufacture composition the agar was prepared. Weight the necessary quantity of media and dissolved in distilled water and then autoclaved such as 15 psi for 15 minutes at 37°C. pH of the medium was change to 7.2 and then pour to sterile plates under the sterile state for hardening. Randomly one plate was used for sterility testing to incubate at 37°C for 24 hours.

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MacConkey agar

According to manufacture composition the agar was prepared. Weight the required quantity of media and dissolved in distilled water and then autoclaved for 15 psi for 15 minutes at 121°C. pH of the medium was regulated to 7.2 and then pour the sterile plates under sterile condition for solidifying.

Mueller Hinton agar

According to manufacture composition the agar was prepared. Weight the required quantity of media and mix up in distilled water and then autoclaved such as 15 psi for 15 minutes at 37°C. pH of the medium was altered to 7.2 and then pour to the plates under the sterile condition for solidifying.

Sampling Technique and Principal:

Staining technique is confirmatory technique used for the detection of bacterial cells. The methods employed to distinguish between Gram-positive and Gram-negative bacterial colonies Following gram staining process, isolates of every type of bacteria used in this investigation were analyzed.

Procedure:

A drop of normal saline putted on the slide Picked 02 to 03 colony from the bacterial growth and mixed. Air dried the smear and then heat fixed. Applied crystal violet on the smear for one minute. Washed slowly with tap water. Gram iodine was applied for one minute. Washed again slowly with tap water. Applied decolorize reagent for 10 to 15 seconds. Washed again slowly with tap water. Applied counter stain (safranin) for one minute. Washed with tap water and then dried the slides. Observed the slides using light microscope under 100X by using oil immersion. The constituents of Gram stain are Ammonium Oxalate, Potassium Iodide, Gram Iodine (Iodine Crystals), Methanol, Gram Decolorize (Ethanol/ Acetone), Safranin (Counter stain).

Biochemical tests:

The different biochemical test was used for identification and differentiation of bacterial species the enzymatic reaction of bacterial cells; different types of colors were produced which is useful for identification. API 10s is used in present study for identification of gram-negative bacteria such as E coli.

API 10S

For identification of the Enterobacteriaceae family API 10s was used. API 10S strep consist of micro tubes having dehydrated substrates. Bacterial suspension is inoculated and different color changes were produced after metabolism.

Reading the Strep

After incubation period the strep were read with the reference table commercially available. Noted color of all the reactions on the results sheets shown in table 1. Put the extra reagent which is required for some tests. Add one drop of TDA reagent to the TDA tube. Add a drop of JAMES reagent to the IND tube. Added one drop of NIT 1 and NIT 2 reagent tube to the GLU tubes.

Interpretation

Identification is through numerical profile.

Test are separate into 4 groups of the results sheet. Every group is further separately divided into 3 subgroups labelled with a value 1, 2 and 4. The positive reaction were labelled with plus (+) sign and negative were labelled as minus (-) sign. In each group added the positive value and then checked in the literature.

Table 1

Test. API 10 S	Negative		Positive	
ONPG	Colorless		Yellow/ Pale Yellow	
Glucose	Blue/Blue Green		Yellow/Yellow-grey	
ARA	Blue/Blue Green		Yellow	
LDCOil	Yellow		Red/Orange	
ODCOil	Yellow		Red/Orange	
CITRATE Full	Pale Green/Yellow		Blue Green/Blue in upper part	
H_2SOil	Colorless/grayish		Black deposit / Thin Line	

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Urea Oil	Yellow		Red/Orange
TDA	Yellow		Reddish Brown immediate
INDOLE	Colorless /Pale Green / Yellow		Pink Immediate
OXIDASE	Colorless		Purple Blue
NO_2	In Glucose add1 drop Nit 1 + 1 drop Nit 2.see after 2-5 minutes		
	Yellow		Red

ONPG	Glu	ARA	LDC	ODC	CIT	H_2S	Urea	TDA	IND	OX	NO_2
1	2	4	1	2	4	1	2	4	1	2	4

Measuring Antimicrobial sensitivity pattern:
Using the Kirby-Bauer disc diffusion method, the antibiotic vulnerability of various bacterial species was assessed.

Table 2: A list of the antibiotics utilized in this investigation

Antibiotics	Amount (µg)
Nitrofurantoin	100
Ampicillin	10
Amoxicillin/clavulanic acid	
Fosfomycin	50
Ceftriaxone	30
Ciprofloxacin	5
Clotrimazole	25
Gentamycin	10
Amikacin	30
Piperacillin Tazobactam	110
Imipenem	10
Ceftazidime	30
Cefepime	30

Table 2 shows a detail list of the antibiotics utilized in this study investigation.

Disc Diffusion Technique:
Kirby Bauer disk diffusion method was used to measuring the susceptibility pattern in –vitro. On sterile MHA plate made lawn of pure culture and placed the antibiotic disc. Incubate the plates for 24 hours and then zones were measured with scale in millimeter according to CLSI guideline.

RESULTS
In order to determine the current scope of multidrug resistant bacteria seen in UTI patients, a total of 150 samples were examined at the microbiology section of the PIMS laboratory. Depending on the age, gender, and MDR status of the 150 samples under analysis, the overall rates of resistance are given.

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Table 3: Age status – cross tabulation

Age Status					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Under 14	34	22.7%	22.7%	22.7%
	Adult	116	77.3%	77.3%	100.0%
	Total	150	100.0%	100.0%	

Table 3 shows the distribution of the analyzed samples' age status, with 116 samples (n=150) being adults and 34 samples (n=150) being under the age of 14.

Table 4: Gender – cross tabulation

Gender					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	106%	70.7%	70.7%	70.7%
	Female	44%	29.3%	29.3%	100.0%
	Total	150%	100.0%	100.0%	

Table 4 shows the gender frequency among the tested samples, of which 44 (n=150) are female and 106 (n=150) are male.

Table 5: MDR status – cross tabulation

MDR status					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	MDR E coli positive	48	32.0%	32.0%	32.0%
	MDR E coli Negative	101	67.3%	67.3%	99.3%
	3	1	.7%	.7%	100.0%
	Total	150	100.0%	100.0%	

Table 5 shows the frequency of both positive and negative samples based on each examined sample's MDR status. Of the 150 samples that were gathered, 101 samples (67.3%) are multidrug resistant negative, and 48 samples (32%) positively reported.

Table 6: Escherichia coli's current antibiotic susceptibility and resistance.

S. No	Antibiotic	Concentration	E coli	
			Sensitive	Resistance
01	Amoxicillin/Clavulanic Acid	30	9.3	74.7
02	Clotrimazole	25	28.7	47.3
03	Gentamicin	10	64.7	33.3
04	Amikacin	30	77.3	16.0
05	Tazobactam	110	74.0	18.7
06	Imipenem	100	75.3	21.3

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07	Cefepime	30	25.3	70.0
08	Levofloxacin	05	35.3	62.7
09	Ampicillin	20	13.3	54.0
10	Nitrofurantoin	300	70.0	21.3
11	Ceftriaxone	30	17.3	43.3
12	Fosfomycin	200	82.0	15.3
13	Norfloxacin	10	36.3	54.0

Table 6 displays the sensitivity and resistivity of each of the 150 samples that were analyzed. The resistance for the E. coli isolates that were examined are shown in the above table.

DISCUSSION

Main objective of this study was to address how common multidrug resistance was in UTI patients visiting the General Medicine Department at Pakistan Institute of Medical Sciences. A total of 150 urine samples were collected, comprising 34 (22.7%) from pediatric patients and 116 (77.3%) from adults. The findings revealed a significant level of resistance among Escherichia coli (E. coli) isolates to various antibiotics tested. Amikacin (16%), tazobactam (18.7%), and Fosfomycin (15.3%) had the lowest resistance rates; Cefepime (70%), levofloxacin (62.7%), and amoxicillin/clavulanic acid (74.7%) had the greatest rates.

Imipenem showed a sensitivity of only 75%, indicating a moderate effectiveness. This contrasts with studies in Turkey where both imipenem and meropenem exhibited 100% sensitivity against E. coli. This research shows that E. coli have 77% more sensitivity compared to Amikacin, which was slightly lower compared to findings from Nepal (87%). Amikacin, an intravenously administered aminoglycoside antibiotic, demonstrated higher sensitivity possibly due to its limited use in severe infections. This contrasts with Gentamicin (64.7%), Nitrofurantoin (70%), and Tazobactam (74%), which showed varying degrees of resistance despite their common prescription for E. coli infections.

The rise of multidrug-resistant organisms poses a significant public health and therapeutic challenge worldwide. Gram-negative bacteria, including E. coli, often develop cross-resistance to multiple antibiotics, thereby compromising treatment efficacy. Our study found that nearly 75% of E. coli isolates were resistant to more than one antibiotic, particularly showing resistance to antibiotics beta-lactam. Similar resistance patterns have been observed in neighboring and distant countries, indicating a global concern. Notably, non-β-lactam antibiotics like Gentamicin showed better activity with 64% sensitivity in our study, higher than reported rates in Israel (29%) and India (36%), possibly due to different antibiotic usage practices.

Fosfomycin demonstrated the lowest resistance rate among tested antibiotics. Previous studies have shown variable resistance trends over time, with increasing resistance reported for Imipenem and Fosfomycin.

CONCLUSIONS

The prevalence of multidrug resistance (MDR) among antibiotics previously effective against E. coli has been gradually increasing, presenting a concerning trend likely attributed to widespread use, incomplete courses of medication, and self-administration, among other factors. It is imperative to create antimicrobial stewardship programs (ASPs) in order to address the issue of antibiotic resistance. These initiatives encourage the proper use of antibiotics, improving treatment results while lowering resistance and side effects. Establishing rules for the prescription of antibiotics, carrying out frequent audits with input, and educating and training medical personnel are important tactics. In the end, multidisciplinary cooperation between clinicians, infectious disease experts, and pharmacists guarantees thorough management and adherence to best practices, maintaining the effectiveness of currently available antimicrobials.

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