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Original Research

Antibiotic resistance trends in UTI-causing *E. coli*

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Abstract

The aim of this study was to find out the prevalence of multi-drug resistance E coli in urinary tract infection patients visiting the General Medicine Department at Pakistan Institute of Medical Sciences. 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. Fifty samples were not MDR, while 100 out of 150 samples were positive for MDR. Amoxicillin/clavulanic acid (74.7%), Cefepime (70%) and levofloxacin (62.7%) exhibited the highest resistance among the agents tested, whereas Fosfomycin (15.3%), amikacin (16%) and tazobactam (18.7%) 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 [1].

Like all other members of the Enterobacteriaceae family, Escherichia coli has grown more resistant to antibiotics over time [2]. 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 [1].

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 [3]. *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 [4]. 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 [5].

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) [6]. 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 [7]. These bacteria persist and continue to spread because they are resistant to treatment 150 million UTIs are estimated to cost the global healthcare system more than $6 billion a year. 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 bacteraemia, 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 [8]. 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 [9]. 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 [10]. 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 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 [11].

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 [12]. 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 [13].

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 [14]. 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 microorganisms increase problems and decrease the effectiveness of high-quality treatments [16]. The spread of resistant bacteria outside of hospitals and into the network, which includes nursing homes, makes it difficult to control UTIs caused by multidrug resistant pathogens. This is primarily because resistance to new antimicrobials is becoming more widespread [17]. These facilities may serve as reservoirs for these MDROs, which may then be transferred to acute hospitals in susceptible populations. These populations include nursing home residents, where UTIs may be more likely to develop resistance to antibiotics such as ciprofloxacin, cephazolin, and nitrofurantoin, in addition to the point remedy of asymptomatic bacteraemia, which may be a particular concern. After bacteraemia, 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 [18]. 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 [19]. 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 [20]. Healthcare-associated risk factors 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 [21].

Literature review: According to a study done in India by V. Viranjan and A. Malini (2014), 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 [22]. 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 [23]. Twenty separate European centres were asked to provide a total of 1000 isolates; however, some centres were unable to provide the necessary quantity of isolates, therefore a total of 887 isolates were obtained. Moreover, not a single centre 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 [24].

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 [23].

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 employed three different types of culture 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. Composition of CLED agar media is Agar 15 gm/L, Lactose 10.0 gm/L, Pancreatic Digest of Casein 04 gm/L, Pancreatic Gelatin 04 gm/L, Beef Extract 03gm/L, Bromothymol Blue 0.128 gm/L, L-Cystine 0.02 gm/L and Distilled Water 1 liter.

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. The ingredients of MacConkey agar include Agar (13.5 gm/L), Peptone (Pancreatic Digest of Gelatine) (17 gm/L), Neutral Red (0.03 gm/L), Lactose Monohydrate (10 gm/L), Sodium chloride (05 gm/L), Bile salts (1.5 gm/L), Crystal violet (0.001 gm/L), and Distilled Water (1 litter) are the ingredients.

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. Randomly one plate was used for sterility testing to incubate at 37°C for 24 hours. Mueller Hinton's composition Agar is made of distilled water (1 L), beef extract (2 gm/L), starch (1 gm/L), acid hydrolysate of casein (17.50 gm/L), and Agar (17.O mg/L).

Sampling Technique and Principal: Staining technique is confirmatory technique used for the detection of bacterial cells such as rods, cocci, spiral etc. when viewed under a microscope. The methods employed to distinguish between Gram-positive and Gram-negative bacterial colonies. The underlying idea of this mechanism is that gram-positive bacteria have thick peptidoglycan layers in their cell walls that allow them to retain crystal violet dye and turn purple, whereas gram-negative bacteria have thin peptidoglycan layers along with extra lipid layers. Gram-negative bacteria's lipid layer causes their cell walls to stain with a counterstain, like safranin, rather of the principal stain, crystal violet, which is removed by the process's decolorizing agent. Following the gram staining process, isolates of every type of bacteria used in this investigation were analysed.

Procedure: A drop of normal saline was placed on a slide, and 2–3 bacterial colonies were mixed to prepare a smear. After air drying and heat fixation, the smear was sequentially stained with crystal violet (1 min), Gram's iodine (1 min), decolorized with ethanol/acetone (10–15 sec), and counterstained with safranin (1 min). The slide was rinsed with water between each step, air-dried, and examined under a light microscope at 100× oil immersion.

Biochemical tests: The different biochemical test was used for identification and differentiation of bacterial species the enzymatic reaction of bacterial cells, different types of color ere 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. By following procedure; Flooded 3 ml water in API tray to maintain moisture for the bacterial growth. Picked the pure colony form the plates and mixed in sterile distal water. Mixed slowly to avoid contamination. Inoculated the equal bacterial suspension in the tubes from ONPG to NO2 except Citrate. For citrate test fill both tube and cupule from the suspension. Avoided bubbles formation in the tubes. Applied mineral oil to create anaerobic condition in LDC, ODC, H2S and URE. Incubated API strep at 37C for 24 hours.

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 3.1. 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 labeled with plus (+) sign and negative were labelled as minus (-) sign. In each group added the positive value and then checked in the literature.

Kirby-Bauer disc diffusion method was used to measure the antibiotic susceptibility of bacterial species. According to (CLSI) Clinical and Laboratory Standard Institute 2019 guidelines the antibiotic used mentioned in the table 3.1 & 3.2**.**

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 in table 4.1, 4.2 & 4.3 respectively. Shows the distribution of the analysed samples' age status, with 116 samples (n=150) being adults and 34 samples (n=150) being under the age of 14 shown in table 4.1.

Shows the gender frequency among the tested samples, of which 44 (n=150) are female and 106 (n=150) are male shown in table 4.2.

Shows the frequency of both positive and negative samples based on each examined sample's MDR status. Of the 150 samples collected, 48 samples (32%) are MDR positive and 101 samples (67.3%) are multi drug resistant negative shown in table 4.3.

Shows the resistivity and sensitivity of all 150 examined samples. The table above provides the overall rates of resistance for the isolates of *E. coli* that were analysed. Amoxicillin/clavulanic acid (74.7%), cefepime (70%) and levofloxacin (62.7%) exhibited the highest resistance among the agents tested, whereas Fosfomycin (15.3%), amikacin (16%) and tazobactam (18.7%) showed the lowest resistance as shown in figure 4.4.

Discussion: The purpose of this study was to find out 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. The lowest rates of resistance were found for Fosfomycin (15.3%), amikacin (16%), and tazobactam (18.7%); the highest rates were found for amoxicillin/clavulanic acid (74.7%), cefepime (70%), and levofloxacin (62.7%). *Escherichia coli* is shed in feces and can contaminate the surrounding environment. In this study, the susceptibility patterns of *E. coli* strains were assessed to evaluate the efficacy of commonly prescribed antibiotics. 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*. Furthermore, our study found that *E. coli* showed 77.3% sensitivity 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 β-lactam antibiotics such as Amoxicillin/clavulanic acid and Ampicillin, followed by cephalosporins and fluoroquinolones.

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.

Among other antibiotics tested in our study, Cotrimoxazole showed 47.3% resistance, Sulbactam 24.7%, Cefepime 70%, Ampicillin 54%, Ceftriaxone 43.3%, Fosfomycin 15.3%, and Norfloxacin 54% resistance. 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. This situation highlights a growing public health challenge necessitating urgent attention.

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Table 3.1. Measuring Antimicrobial sensitivity pattern

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

Table 3.2. List of antibiotic discs used in the present study

Name of antibiotics	**Concentration (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 4.1. Age status

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 4.2. Gender status

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 4.3. MDR status

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 4.4. *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
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