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Study of Antibacterial Activity of Gentamicin-Cetirizine on Uropathogenic *Escherichia coli* Isolates

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ABSTRACT

Background: Urinary tract infections (UTIs) cause a wide range of infections in individuals; they are common nosocomial infections that have recently become difficult to treat because of the increased emergence of multidrug-resistant bacteria. The present study aimed to determine and compare the minimum inhibitory concentration of gentamicin alone and in combination with cetirizine against *Escherichia coli* strains isolated from hospitalized patients with UTI.

Materials & Methods: This study was performed on 76 *E. coli* strains isolated from a total of 103 samples of patients admitted to three hospitals in Gonbad-e Kavus. Kirby Bauer disk diffusion and broth microdilution tests were used to determine antibiotic susceptibility and the minimum inhibitory concentration (MIC) of gentamicin alone and in combination with cetirizine according to CLSI M100-S25 (2015) criteria.

Findings: Evaluation of the minimum inhibitory concentration of gentamicin-cetirizine combination against *E. coli* isolates showed that none were able to grow at a concentration of 8 µg/mL. The concentration of gentamicin in combination with cetirizine, inhibiting 90% of *E. coli* isolates (MIC_{90}), was 4 µg/mL, which was 16 times lower than that of gentamicin alone (MIC_{90}= 64 µg/mL) (p=.02).

Conclusion: Gentamicin in combination with cetirizine was found to be more potent in inhibiting *E. coli* isolates than gentamicin alone. Therefore, the results of this study could provide a clear perspective for dealing with drug-resistant pathogens.

Keywords: *Escherichia coli*, Drug resistance, Gentamicin, Cetirizine.

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Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases, affecting approximately 150 million people worldwide each year. Uropathogenic *Escherichia coli* (UPEC) is responsible for 70-90% of UTIs in humans. Most UTIs are non-serious; however, lack of timely diagnosis or treatment could cause serious complications such as urinary tract disorders, uremia, and abortion in pregnant women. UTIs are more common in women than in men, so that about half of women experience this infection at least once in their lifetime. Studies in various communities have shown that among Gram-negative bacilli, *E. coli* is the most common etiologic cause of UTI [1-2]. Treatment of patients with UTI is currently facing the problem of drug-resistance. The basis of proper treatment of patients with UTI is the selection of a suitable antibiotic with high efficiency and effectiveness [3-5]. Unfortunately, excessive and sometimes incorrect use of antibiotics provides the conditions for the survival of bacteria and plays an important role in reducing the efficacy of some antibiotics in treating infections. Among drug-resistant *E. coli* strains, the prevalence of aminoglycosides-resistant bacterial strains is high. Gentamicin is an aminoglycoside that inhibits protein synthesis. Aminoglycoside presence in the cytosol generally disturbs peptide elongation at the 30S ribosomal subunit, giving rise to inaccurate mRNA translation and therefore biosynthesis of proteins [6-7]. Given these problems, the use of new antimicrobial compounds or the study of the synergistic effects of two antimicrobial compounds is a promising solution for the management of drug-resistance. Cetirizine is an antihistamine with aromatic and piperazine rings, whose effective antimicrobial activity may be attributed to these structural components. Therefore, it is possible that cetirizine could be used as an antimicrobial key to cover microbial resistance and bacterial infections [8].

Objectives: The present study aimed to investigate the synergistic effects of an aminoglycoside (gentamicin) with cetirizine on Uropathogenic *E. coli* isolates.

Materials and Methods

Bacterial isolation: Urine samples were collected from 103 patients with UTI in three hospitals in Gonbad-e Kavus, northeast of Iran, from October 2018 to February 2019. The patients, either symptomatic or asymptomatic, were hospitalized for more than five days. Diagnosis of UTI was based on positive urine culture, i.e., the presence of 10^5 colonies in asymptomatic patients and 10^4 colonies in symptomatic patients [9]. The specimens were taken from first-morning urine and then cultured on blood agar, eosin methylene blue agar, and MacConkey agar, purchased from Merck Co., Germany. The cultured samples were incubated at 37 °C for 24-18 hrs. *E. coli* isolates were identified based on Gram staining and biochemical assays, including glucose and lactose fermentation, gas production, indole, Voges-Proskauer, Triple Sugar Iron (TSI) agar, sulfide indole motility, and methyl red tests.

Antibiotic susceptibility: Antibiotic resistance pattern of *E. coli* isolates was determined by the disk diffusion (Kirby-Bauer) method using the following antibiotic disks: tetracycline (30 µg), gentamicin (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ofloxacin (5 µg), nitrofurantoin (100 µg), chloramphenicol (30 µg), and nalidixic acid (10 µg). All antibiotic disks were purchased from Padtan Teb Co., Iran. The results were analyzed according to the standards described by the Clinical and Laboratory Standards Institute (M100-S25) in 2015 [10]. *E. coli* ATCC25922 was used as a control.

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Table 1) Antibiotic resistance pattern of *E. coli* isolates based on the hospital ward

Antibiotics	Ward	Internal (n=26) N (%)	Surgery (n=7) N (%)	ICU (n=29) N (%)	Obstetrics and Gynecology (n=14) N (%)	P-Value
Tetracycline	Resistant	10(38.5)	5(71.4)	7(24.1)	6(42.9)	.075
	Intermediate	13(50)	-	18(62.1)	5(35.7)	
	Susceptible	3(11.5)	2(28.6)	4(13.8)	3(21.4)	
Gentamicin	Resistant	5(19.2)	3(42.9)	2(6.9)	4(28.6)	.026*
	Intermediate	13(50)	-	21(72.4)	7(50)	
	Susceptible	8(30.8)	4(57.1)	6(20.7)	3(21.4)	
Ofloxacin	Resistant	5(19.2)	1(14.3)	7(24.1)	1(7.1)	.038*
	Intermediate	12(46.1)	3(42.9)	12(41.4)	5(35.7)	
	Susceptible	9(34.6)	3(42.9)	10(34.5)	8(57.1)	
Ceftazidime	Resistant	5(19.2)	1(14.3)	6(20.7)	1(7.1)	.01*
	Intermediate	5(19.2)	5(71.4)	11(37.9)	5(35.7)	
	Susceptible	16(61.5)	1(14.3)	12(41.4)	8(57.1)	
Ceftriaxone	Resistant	6(23.1)	1(14.3)	9(31.1)	2(14.3)	.02*
	Intermediate	10(38.5)	3(42.9)	10(34.5)	5(35.7)	
	Susceptible	16(61.5)	3(42.9)	10(34.5)	7(50)	
Chloramphenicol	Resistant	9(34.6)	3(42.9)	11(37.9)	5(35.7)	.066
	Intermediate	11	2(28.6)	11(37.9)	2(14.3)	
	Susceptible	6(23.1)	2(28.6)	7(24.1)	7(50)	
Nalidixic acid	Resistant	9(34.6)	4(57.1)	14(48.3)	7(50)	.071
	Intermediate	10(38.5)	3(42.9)	9(31.1)	6(42.8)	
	Susceptible	7(26.9)	-	6(20.7)	1(7.1)	
Nitrofurantoin	Resistant	7(26.9)	3(42.9)	9(31.1)	3(21.4)	.05
	Intermediate	10(38.5)	-	9(31.1)	6(42.8)	
	Susceptible	9(34.6)	4(57.1)	11(37.9)	5(35.7)	

$p<.05$ (*significant)

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Table 2) Distribution of MIC of gentamicin and combination form of gentamicin-cetirizine against *E. coli* isolates

Gentamicin-Resistant Isolates	Organism Identification Number	MIC ($\frac{\mu g}{mL}$) G	MIC ($\frac{\mu g}{mL}$) G + C
14 isolates of *E. coli*	EC_{11}	64	4
	EC_{17}	64	4
	EC_{29}	32	4
	EC_{33}	64	4
	EC_{27}	64	4
	EC_{33}	64	8
	EC_{34}	64	4
	EC_{45}	64	4
	EC_{49}	64	4
	EC_{56}	64	4
	EC_{63}	64	1
	EC_{67}	64	4
	EC_{69}	32	2
	EC_{72}	64	4

Determination of gentamicin minimum inhibitory concentration (MIC): Based on the protocol of the Clinical and Laboratory Standards Institute [10], to prepare the drug stock solution using the microdilution method, gentamicin powder (Sigma-Aldrich, USA) was added to water solution. The antibiotic with an initial concentration was inoculated to 96-well microplates containing Mueller-Hinton broth (Merck, Germany). The MIC of gentamicin at different concentrations of 0.06-64 µg/mL was determined. Then bacterial suspension of gentamicin-resistant *E. coli* isolates (with a turbidity of 0.5 McFarland) was separately inoculated into each well. After overnight incubation at 37 °C, the growth rate was measured and compared with positive (without antibiotic) and negative (without bacterial suspension) controls. The inhibitory effect was assessed by reading absorbance at 630 nm using a Plate Reader (BioTec, Germany). The minimum concentration which inhibits bacterial growth up to 90% in comparison with positive control is considered as MIC_{90}.

Determination of MIC of gentamicin in combination with cetirizine: In order to prepare stock solutions, 0.64 g of cetirizine and gentamicin powders were dissolved in water to obtain a concentration of 64 µg/mL. To prepare serial dilutions, 50 µL of cetirizine and gentamicin were added to the first well of a 96-well microplate containing 50 µL of Mueller-Hinton broth. After adding 50 µL of gentamicin-resistant bacterial suspension (1.5×10^8 CFU/mL) and 24 hours of incubation at 37°C, MIC values were determined and interpreted.

Findings

Demographic specifications of bacterial isolates: Among 103 patients with UTI (average age: 48 ±21 years), the highest prevalence rate of infection was observed in individuals ≥ 65 years of age (36.36%), while the lowest prevalence rate of infection (9.09%) was observed in 15 and 25-year-old patients. Among isolates, 76 (73.8%) isolates were identified as *E. coli*, most of which were isolated from women (62.1%) and intensive

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Table 3) Mean MIC of gentamicin and combination form of gentamicin-cetirizine on *E. coli* isolates

Strains 1.5×10^8 CFU/mL	Gentamicin	Gentamicin - Cetirizine	P-Value
MIC_{50} (µg/mL)	32	1	.063
MIC_{90} (µg/mL)	64	4	.02*

$p<.05$(*significant)

care unit (ICU) patients (38.2%).

Susceptibility to antibiotics: Based on the antibiotic susceptibility testing results, the frequency of resistance to gentamicin was 14 cases (18.4%). The highest rates of resistance and susceptibility were observed against nalidixic acid (average=44.7%) and ceftazidime (average=48.7%), respectively (Table 1).

Minimum inhibitory concentration: Evaluation of the effects of different concentrations of gentamicin (0.06-64 µg/mL) on the growth of *E. coli* strains showed that the most significant growth changes occurred at concentrations of 4 and 8 µg/mL. According to Table 2-3, the concentration of gentamicin in combination with cetirizine, inhibiting 90% of *E. coli* isolates (MIC_{90}), was 4µg/mL , which was 16 times lower than that of gentamicin alone (MIC_{90}= 64 µg/mL) (p=.02).

Discussion

With the emergence of multidrug-resistant bacteria, the management of infections caused by *E. coli* strains has become challenging in the community. Epidemiological studies have shown that the *Enterobacteriaceae* family is the most important group of bacteria isolated from UTIs, and *E. coli* accounting for more than 75% of UTIs is the main leading cause of these infections [11]. In this study, out of a total of 76 *E. coli* isolates, 62.1% were separated from women, and older groups were more infected. In studies conducted in Iran in 2013 [12] and 2017 [13], UTIs were shown to be more common in women than in men, which is consistent with the results of this study. Another variable in this study was different hospital wards. The results of the present study showed that the most *E. coli* isolates (38.2%) were isolated from the ICU ward, which is consistent with the results of a previous study in Iran in 2012 [14]. It seems that long-term hospitalization in this ward, the patient's serious condition, the use of invasive therapeutic tools such as tracheal tube, ventilator, and urinary catheter are among the main reasons for the prevalence of resistant organisms in this ward [15]. Today, one of the most significant global health problems is the increasing prevalence of antibiotic-resistant pathogens in humans. The main cause of increased resistance in pathogenic bacteria is the overuse of antibiotics, leading to the emergence and spread of resistant pathogens [3]. In the present study, nalidixic acid had the least effect, while gentamicin had a moderate effect on *E. coli* isolates, similar to a study in Iran in 2015 [16]. Accordingly, the isolation of *E. coli* strains from nosocomial infections, especially UTIs, was put on the agenda of this study to investigate the behavior of these bacteria in relation to antibiotics and the use of new ways. In combination with other antibiotics synergistically, the treatment protocol could be improved by increasing the effectiveness of the drug and reducing the effective dose of the drug.

Extensive studies by researchers around the

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world have reported antimicrobial effects of a variety of drugs belonging to different classes of non-antibiotic drugs such as antihistamines 'bromodiphenhydramine (Bromazine) and diphenhydramine', all of which have shown antimicrobial effects of various drugs from the past to the present. Cetirizine dihydrochloride is one of these non-antibiotic drugs, which inhibits the effect of histamine on H1 receptor of muscles [17-18]. Evaluation of the minimum inhibitory concentration (MIC) of gentamicin-cetirizine combination against *E. coli* isolates showed that with increasing the concentration from 2 to 4 µg/mL, the inhibitory power of the antibiotic increased. In a study by Martins et al. (2008), cetirizine was introduced as an antibacterial agent with inhibitory effects against *Staphylococcus aureus* and *Salmonella typhi* [19]. Based on experiments conducted by Maji et al. (2017), it was found that cetirizine at a concentration of 1000 µg/mL has more inhibitory effects against *E. coli* isolates [20], which was lower than that of a wide range of antibiotics, and its effectiveness was evaluated as more effective. In another study, ibuprofen-fluconazole combination was shown to be more potent against candidiasis as a fungal infection [21].

Conclusion

Improper use of antibiotics in recent years and lack of awareness of proper use have caused many problems due to their effects and especially the emergence of multidrug-resistant bacteria. It was found that the combined use of gentamicin with cetirizine has a greater inhibitory effect than the use of gentamicin alone. It seems that alternative way for controlling drug-resistant bacteria is the combined use of non-antibiotics and antibiotics with synergistic effects. Therefore, the use of this combination requires more attention and research in order to minimize the rate of UTIs in the future.

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