

# Effects of Omeprazole and Caffeine Alone and in Combination with Gentamicin and Ciprofloxacin Against Antibiotic Resistant *Staphylococcus Aureus* and *Escherichia Coli* Strains

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## Key Words

antimicrobial resistant, omeprazole, caffeine, *Escherichia coli*, *Staphylococcus aureus*, antibiotics

## Abstract

**Objective:** Antibiotic resistance is a global health problem and threatens health of societies. These problems have led to a search for alternative approaches such as combination therapy. The aim of the present study was to investigate the effect of caffeine and omeprazole in combination with gentamicin or ciprofloxacin against standard and clinically resistant isolates of *Staphylococcus aureus* and *Escherichia coli*.

**Methods:** The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of different agents against bacterial strains were determined. The interaction of non- antibiotic drugs with gentamicin and ciprofloxacin was studied *in vitro* using a checkerboard method and calculating fraction inhibitory concentration index (FICI). Verapamil as ef-

flux pump inhibitor was used to evaluate the possible mechanism of bacterial resistance to antibiotics.

**Results:** The MIC and MBC values of gentamicin against bacterial strains were in the range of 20- 80 µg/ml and 40-200 µg/ml, respectively. Caffeine and omeprazole had no intrinsic inhibitory activity against tested microorganisms. However, upon combination of caffeine with antibiotics, the synergistic effects were observed. Verapamil was able to reduce the MIC values of gentamicin (4 folds) only in some bacterial strains.

**Conclusion:** These findings indicated that caffeine was effective in removing bacterial infection caused by *S. aureus* and *E. coli*. The relevant mechanisms of antibiotic resistance were not related to the drug efflux.

## 1. Introduction

Antibiotic resistance has been considered as serious problem in treating bacterial infections [1, 2]. Serious consequences have been reported for infection by resistant pathogens such as higher doses of antibiotics administration, prolonged hospitalizations and also associated with increased morbidity and mortality [3].

Received: Sep 19, 2018 Reviewed: Jan 29, 2019 Accepted: Feb 11, 2019

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Gentamicin and ciprofloxacin are potent antibiotic and used for the treatment of both type of bacteria [4, 5]. Despite the wide range of application, the antibiotic shows some drawbacks which are limited the routine administration like increased resistance to it [6].

To date, different approaches have been employed to overcome bacterial resistances [7, 8]. The combination therapy of antibiotics is useful and desirable ones and shows certain advantages like increasing the antibiotic activity, reducing the risk of resistance to antibiotics [9]. Repurposing and revival of non-antibiotic drugs which having potential antibacterial activity would be desirable for reducing the risk of increasing resistance and also toxicity.

Methylxanthines are potent bronchodilator agents and widely used as acute asthma treatment. They show wide range of pharmacological activities such as antimicrobial activities against some pathogens [10-13]. For instance, aminophylline and caffeine increased the antibiotic activities of carbenicillin, ceftizoxime and gentamicin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Previously, it was confirmed that caffeine was able to decrease the MIC values of gentamicin against *S. aureus* and *P. aeruginosa* [14, 15]. Additionally, the antimicrobial properties of caffeine against *Candida albicans* were reported [16, 17]. Different mechanisms have been mentioned about antibacterial activity of caffeine such as inhibiting incorporation of adenine and thymidine in the synthesis of DNA via inhibition of thymidine kinase and also inhibiting the synthesis of DNA [18, 19].

The extrusion of antibiotics via efflux pump is primary mechanism by which bacteria can resist the action of antibiotics. These pumps are complexes within the bacterial cell envelope and are mainly used to export toxic substances such as antibiotics and found in wide range of multi- drug resistant nosocomial pathogens [20]. Therefore, targeting bacterial efflux pumps could effectively re-sensitize resistant bacterial strains to broad spectrum of antibiotics. Omeprazole as proton pump inhibitors (PIPs) and a class of efflux pump inhibitor (EPIs) is used to overcome antibiotic resistance in *S. aureus* [21, 22].

So that, the aim of the present study was to evaluate the antibacterial activities of gentamicin and ciprofloxacin in combination with omeprazole and caffeine against resistant human pathogens.

## 2. Materials and methods

### 2.1. Materials

Caffeine, omeprazole, ciprofloxacin and gentamicin were obtained from Darou Pakhsh Pharmaceutical Company (Tehran, Iran). Muller Hinton broth (MHB) and Tryptone Soya Agar plates (TSA) were purchased from Hi media (India). TTC (triphenyl tetrazolium chloride) was purchased from Merck (Germany). All of the original samples were used on arrival. Water used was double-distilled water.

### 2.2. Bacterial strains

The antibacterial activity was evaluated against four clinically resistant Gram positive bacteria: *Staphylococcus au-*

*reus* (*S. aureus*) and two isolated of Gram negative bacteria: *Escherichia coli* (*E. coli*) which were received from the Clinical Microbiology Laboratory, University Hospital of Imam Reza, in Mashhad, Iran, as multidrug resistant isolates.

The standard strain of *S. aureus* ATCC43300 which is MRSA (methicillin resistance *S. aureus*) was used as control strain. Bacterial strains were maintained by subculture on TSA to their growth at 37°C for 24 h.

### 2.3. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values

The MIC values of gentamicin, omeprazole and caffeine against pathogens was determined as previously described [23]. Briefly, approximately  $10^6$  CFU/mL cells from overnight bacterial cultures were used as inoculum. Serial dilutions of each compound were prepared in MHB in 96- well microtiter plates. Then, the inoculum was added to each well to obtain  $10^5$  CFU/mL at final bacterial concentration. The inoculated microplates were incubated at 37°C for 24 h under aerobic condition. MIC was determined by adding 20  $\mu$ L of 5 mg/mL of TTC to each well plus incubating at 37°C for 30 min.

For MBC determination, an aliquot of 10  $\mu$ L from all wells and tubes without growth was seeded in TSA. The plates were then incubated for overnight at 37°C. As definition, MBC is the lowest concentration of antimicrobial agent that kills > 99.9% of bacteria.

### 2.4. Synergism testing

To evaluate the synergistic effect of the combination of antibiotics, gentamicin and ciprofloxacin, and non-antibiotic drugs, caffeine and omeprazole, the checkerboard method was used [8]. The MIC was assayed as mentioned above and finally to assess the synergistic activity the fractional inhibitory concentration index [24] value was used.

FICI (fractional inhibitory concentration index) of the antibacterial agent combination was FIC (drug A) + FIC (drug B), and also the FIC is equal to (MIC of drug A in combination) / (MIC of drug A alone). The combination effects were analyzed according to the following criteria; < 0.5 denoting synergy; 0.5-0.75 denoting partial synergy; 0.76-1 denoting an additive effect; 1-4 denoting indifference; and > 4 denoting antagonism [25].

### 2.5. Statistical analysis

All experiments were performed three times. Results were presented as mean  $\pm$  SD.

## 3. Results

### 3.1. Determination of MIC and MBC values

The MIC and MBC values of gentamicin, omeprazole and caffeine for each bacterial strains were illustrated in Table 1. As seen, these values for caffeine and omeprazole were more than 200  $\mu$ g/mL.

**Table 1** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of gentamicin against *Staphylococcus aureus* and *Escherichia coli* strains.

	Strain	Gentamicin	
		MIC ( $\mu\text{g/ml}$ )	MBC
<i>Staphylococcus aureus</i>	1	>80	>80
<i>S. aureus</i>	2	20	40
<i>S. aureus</i>	3	>80	>80
<i>S. aureus</i>	4	>80	>80
<i>S. aureus</i> (ATCC 43300)	5	50	200
<i>Escherichia coli</i>	1	100	200
<i>E. coli</i>	2	50	200

**Table 2** Results of the combination of gentamicin and caffeine against *Staphylococcus aureus* and *Escherichia coli*.

Bacterial strains	Strain	Agent	MIC ( $\mu\text{g/ml}$ )		FIC	FICI	Outcome
			Alone	gentamicin + caffeine			
<i>Staphylococcus aureus</i>	1	gentamicin	100	100	1	2	Indifference
		caffeine	200	200	1		
<i>S. aureus</i>	2	gentamicin	12.5	6.25	0.5	0.75	Partial synergy
		caffeine	200	50	0.25		
<i>S. aureus</i>	3	gentamicin	100	100	1	2	Indifference
		caffeine	200	200	1		
<i>S. aureus</i>	4	gentamicin	100	100	1	2	Indifference
		caffeine	200	200	1		
<i>S. aureus</i> (ATCC 43300)	5	gentamicin	50	25	0.5	1.5	Indifference
		caffeine	200	200	1		
<i>Escherichia coli</i>	1	gentamicin	100	50	0.5	1	Partial synergy
		caffeine	200	50	0.25		
<i>E. coli</i>	2	gentamicin	50	50	1	2	Indifference
		caffeine	200	200	1		

### 3.2. Synergism of gentamicin and non-antibiotic drugs

The combined effects of gentamicin and non-antibiotic drugs were shown in Tables 2 and 3.

Based on these results, for two clinical isolates the partial synergistic effect was observed. The FICI values were 0.75

for *S. aureus*, strain 2, and 1 for *E. coli*, strain 1, respectively.

As seen, The FICI values for all microorganisms were 2 and the “indifference” outcome reflected in the table. The results of this part of study indicated that combination of gentamicin with omeprazole was not effective and omeprazole was ineffective against bacterial strains both administered alone or in combination with antibiotic.

The results of combination of ciprofloxacin with caffeine

**Table 3** Results of the combination of gentamicin and omeprazole against *Staphylococcus aureus* and *Escherichia coli*.

Bacterial strains	Strain	Agent	MIC (µg/ml)		FIC	FICI	Outcome
			Alone	gentamicin +			
<i>Staphylococcus aureus</i>	1	gentamicin	100	100	1	2	Indifference
		omeprazole	200	200	1		
<i>S. aureus</i>	2	gentamicin	12.5	12.5	1	2	Indifference
		omeprazole	200	200	1		
<i>S. aureus</i>	3	gentamicin	100	100	1	2	Indifference
		omeprazole	200	200	1		
<i>S. aureus</i>	4	gentamicin	100	100	1	2	Indifference
		omeprazole	200	200	1		
<i>S. aureus</i> (ATCC 43300)	5	gentamicin	50	50	1	1.5	Indifference
		omeprazole	200	200	1		
<i>Escherichia coli</i>	1	gentamicin	100	100	0.5	2	Indifference
		omeprazole	200	200	1		
<i>E. coli</i>	2	gentamicin	50	50	1	2	Indifference
		omeprazole	200	200	1		

and omeprazole showed that enhanced antibacterial activities were not observed by combination therapy (data not shown).

In order to investigate the role of efflux pump in bacterial resistance; verapamil, as a potent efflux pump inhibitor, was used in combination with gentamicin in some of the clinical isolates. The results showed that in presence of verapamil, the synergistic effects were observed, (FICI = 0.37), and the MIC values of gentamicin were reduced 4 folds only in specific bacterial strains; mainly *S. aureus*.

#### 4. Discussion

Clinical isolates of bacteria are mainly causes of nosocomial infections and currently considered as clinical and public health problem. These problems have led to develop alternative approaches such as combination therapy. By this approach therapeutic outcomes were enhanced and also the adverse effects of antibiotic drugs were also reduced. Therefore, in the present study the combination of antibiotics, gentamicin and ciprofloxacin with non-antibiotic drugs, caffeine and omeprazole, was evaluated.

The MIC and MBC values of gentamicin against bacterial strains (Table 1) indicated that isolated strains were resistant to antimicrobial agent and higher concentration of antibiotics is needed to remove bacterial infections. So that, combination of non-antibiotic drugs with antibiotics was used as an approach to enhance the antibacterial activity and also reduce the concentration of the antibiotic.

To date, some studies indicated the antibacterial activity

of methylxanthines [10, 15]. In the present study, the combinatorial effect of caffeine and gentamicin was investigated (Table 2). These data indicated that the synergistic effects were only observed in some of the clinical isolates. These results were in line with previous published data which showed that methylxanthines did not show promising results when combined with antibiotics. However, it was shown that caffeine was more effective in Gram-positive bacteria in comparison with Gram-negative ones [1]. In the present study, this combination showed a synergistic effect against both types of Gram-negative and -positive bacteria and which confirmed that type of species plays an important role in evaluating the combination effects. Additionally, it was shown that combination of cell wall-targeting antibiotics such as penicillin and cephalosporin with caffeine yield synergistic effects. These observations might be due to the antibiotics facilitated the diffusion of caffeine into microorganisms and therefore better interaction with DNA [15]. However, both gentamicin and caffeine affected on DNA and consequently the antibiotic activity did not alter remarkably.

Efflux-mediated resistance is a well-known mechanism in bacterial resistance and therefore finding a solution to remove or bypass efflux systems is attractive. Efflux pump inhibitors (EPIs) drugs have been shown promising results since a single EPI that is active against efflux pumps of bacteria and can be used as combination therapy. Several of them are non-antibiotic drugs and have demonstrated efflux pump inhibitory properties on NorA and other resistance pumps (MDR pumps) of Gram-positive

organisms. Omeprazole, as an EPI, and some analogs of it have been used to evaluate the antibacterial activity. The results indicated that some analogs were able to restore the bactericidal activity of norfloxacin [22]. Additionally, omeprazole which is usually used in treatment of *Helicobacter pylori* infection, can reduce the MICs of the antibiotics in MDR strains of *H. pylori* [26]. The results of present study were in contrast with Multidrug these findings and the antibacterial activity of antibiotics were not changed by combination with omeprazole (Table 3). Based on these observations, it can be assumed that the efflux pump was not active in tested bacteria. In order to deep investigate, verapamil as potent EPI was combined with gentamicin and the synergistic effects were tested [27].

The MIC values of gentamicin were reduced in only some of the bacterial strains. So that, it can be assumed that the antibiotic resistance of the strains which were used in the present study might be related to the other resistance mechanisms and the mechanism of drug efflux was not mainly effective.

## 5. Conclusion

Present findings indicated that caffeine, a bronchodilator agent, was able to reduce the drug resistance of clinically isolates of *S. aureus* and *E. coli*, but omeprazole as a EPIs was not effective in removing bacterial infection. The relevant mechanisms of antibiotic resistance were not related to the drug efflux in these bacteria.

## Conflict of interest

All authors declared that there is no conflict of interest.

## Acknowledgment

The authors are thankful to the Vice Chancellor of Research, Mashhad University of Medical Sciences for financial supports. The results described in this paper are part of a Pharm. D. thesis.

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