

IN VITRO INTERACTION OF CIPROFLOXACIN AND SOME NATURAL COMPOUNDS AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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Received: 05 July 2016, Revised and Accepted: 23 July 2016

ABSTRACT

Objective: This study was designed to determine the effect of the combination of some natural compounds with ciprofloxacin (CIP) against methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

Methods: About 15 (MRSA) strains were selected of patients suffer from different infections. Natural compounds included flavonoids rutin, quercetin, polyphenolic acids gallic acid, tannic acid, and phenylpropanoids such as 4-hydroxy coumarin, thymol, caffeine, and ethyl cinnamate were applied alone and in combination with CIP.

Results: Most natural products showed activity against MRSA strains when tested alone. The combination was decreased the activity of CIP. Quercetin and tannic acid were the most effective compounds, but rutin, gallic acid, 4-hydroxy coumarine, thymol, and caffeine had no activity against (MRSA) strains.

Conclusion: The relationship between the natural compounds and CIP was antagonism, wherein the compounds had reduced the antibacterial effects of CIP in combination.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Natural products, Ciprofloxacin, Quercetin, Tannic acid.

INTRODUCTION

Staphylococcus aureus is a Gram-positive, coagulase positive coccus in the family Staphylococcaceae, found on the mucus membranes and the human skin [1]. A number of strains have developed resistance to more than 20 other antimicrobial drugs which they represent more than 60% of *S. aureus* isolates, and they are classified as methicillin-resistant *S. aureus* (MRSA). They are an opportunist strains because they have acquired a gene that makes them resistant to all beta-lactam antibiotics [2-4]. They can cause various invasive conditions such as pneumonia, endocarditis, septic arthritis, osteomyelitis, meningitis, septicemia, and nosocomial infections associated with indwelling medical devices and surgical sites [5]. Human community-acquired-MRSA strains are mainly associated with superficial skin or soft tissue disease, although they have also caused sepsis, necrotizing fasciitis, necrotizing pneumonia, and other conditions [6,7]. The morbidity and mortality associated with MRSA infections is high in spite of antimicrobial therapy. Therefore, the search for new structural ligands and novel targets of attack as a means to overcome bacterial resistance is an important research goal [8]. Combination therapy is a new way, which may improve the efficacy of antimicrobial treatment for resistant microbes [9]. Most recent studies showed that natural compounds have been explored a strong antimicrobial activity against Gram-positive bacteria when they were used in combination with antibiotics [10].

Natural compounds are chemical compounds or substances produced by a living organism such as plants, animals, or bacteria and fungi [11]. There are various chemical components with antimicrobial effect including saponins, flavonoids, thiosulfonates, glucosinolates, phenolics, and organic acids [12]. The antimicrobial efficacy of components in plants depends on the chemical structure of active components and their concentration [13]. However, phenolic compounds such as terpenes, aliphatic alcohols, aldehydes, ketones, acids, and isoflavonoids are the main components in plants with antimicrobial activity. Flavonoids such as myricetin, rutin, kaempferol, quercetin and flavones such as luteolin

have inhibited the growth of MRSA. Furthermore, alkyl gallates (methyl, ethyl, propyl, and butyl gallates) and gallic acid, phenylpropanoids such as 3,4-dimethoxycinnamic, ethyl cinnamate, 2,4,5-trimethoxycinnamic acid, 4-hydroxy coumarin, caffeine, chlorogenic, ferulic acids constitute a large part of our daily diet and there is a possibility that they may act as antibacterial agents [14,15].

The combination of natural compounds with antibiotics may be appeared as synergistic, additive, or antagonistic type. Most of the antibiotics in combination with natural compounds have a synergistic activity, but some antibacterial agents have antagonistic effects [16]. The compound (ciprofloxacin [CIP]), a fluoroquinolone compound, has a good antibacterial activity on dangerous infections. It is used commonly in the treatment of many infections especially urinary tract infections, sexually transmitted diseases, respiratory infections, and gastrointestinal infections [17]. The combination of CIP with antibiotics or natural compounds is used to dispose of the resistant of MRSA strains [18].

METHODS

MRSA isolates collection

About 15 clinical strains were collected from patients of Aleppo University Hospital.

Bacterial culturing medias such as nutrient agar (NA; CM0003B), Muller Hinton agar (MHA; CM0337B), were from Oxoid, UK. Mannitol salt agar (MSA; LAB007) was obtained from Lab M Limited, UK.

Isolates identification were performed using Gram-staining, catalase test, coagulase test, and MSA differentiation [19].

Isolated colonies of *S. aureus* from MSA plates were aseptically inoculated in sterile nutrient broth and incubated overnight at 37°C. Thereafter, turbidity of inoculum was adjusted to 0.5 McFarland using

0.9% (w/v) sterile normal saline and was used to prepare bacterial lawns on sterile MHA plates. Methicillin discs were applied on seeded plates and incubated overnight at 37±1°C. Following incubation, plates with zones of inhibitions <10 mm diameter or no zone of inhibition were considered to be MRSA strains [20].

Natural compounds

Different natural compounds, Table 1, were used in combination with standard CIP (Sigma-Aldrich, UK). Stock solutions of the compounds

Table 1: Natural compounds

Natural compounds	Chemical group	Source
Quercetin	Flavonoid	Sigma-Aldrich, UK
Rutin	Flavonoid	Sigma-Aldrich, UK
4-hydroxy coumarin	Phenylpropanoid	Himedia (India)
Tannic acid	Phenolic acids	Himedia (India)
Gallic acid	Phenolic acids	SRL
Thymol	Phenylpropanoid	SRL
Caffeine	Alkaloid	Himedia (India)
Ethyl cinnamate	Phenylpropanoid	Himedia (India)

Table 2: Diameters of inhibition zones (in mm) of natural compounds alone and CIP against MRSA strain

MRSA isolates	Natural compounds and CIP								
	Q	R	T	G	HQ	TH	CA	EC	CIP
1	25	Ne*	22	Ne*	Ne*	Ne*	Ne*	Ne*	30
2	22	Ne*	22	Ne*	25	Ne*	Ne*	Ne*	30
3	22	Ne*	22	Ne*	22	Ne*	Ne*	Ne*	28
4	22	Ne*	21	Ne*	20	Ne*	Ne*	Ne*	29
5	22	Ne*	22	Ne*	Ne*	Ne*	Ne*	Ne*	30
6	20	Ne*	20	Ne*	Ne*	Ne*	Ne*	Ne*	27
7	20	Ne*	20	Ne*	Ne*	Ne*	Ne*	Ne*	26
8	20	Ne*	22	Ne*	Ne*	Ne*	Ne*	Ne*	29
9	20	Ne*	20	Ne*	Ne*	Ne*	Ne*	Ne*	28
10	20	Ne*	21	Ne*	Ne*	Ne*	Ne*	Ne*	27
11	20	Ne*	21	Ne*	Ne*	Ne*	Ne*	Ne*	30
12	22	Ne*	20	Ne*	11	Ne*	Ne*	Ne*	Ne*
13	23	18	22	Ne*	15	Ne*	Ne*	Ne*	Ne*
14	23	Ne*	20	Ne*	15	Ne*	Ne*	Ne*	Ne*
15	20	Ne*	20	Ne*	20	Ne*	Ne*	Ne*	Ne*

Ne*: Negative result, Q: Quercetin, R: Rutin, G: Gallic acid, HQ: 4-hydroxy coumarin, TH: Thymol, CA: Caffeine, EC: Ethylcinnamate, CIP: Ciprofloxacin, MRSA: Methicillin-resistant *Staphylococcus aureus*

made at concentrations of 6 g/l by dissolving the powder in dimethylsulfoxide. CIP solution was prepared at concentration 0.05 g/l.

Natural compounds and CIP sensitivity tests

Susceptibility tests of clinical strains to CIP, natural compounds, and CIP+natural compounds combinations were tested using well diffusion method on MHA plates [21]. Every well was injected with 500 µg/100 µl, and in the combination, it was 500 µg/50 µl of every natural compound with 5 µg/50 µl of CIP. Following overnight incubation at 37°C diameters of inhibition zones were recorded. Test substances were considered to have activity if diameter of inhibition zone was >10 mm, whereas ≤10 mm zone of inhibition were regarded as inactive [22].

RESULTS

The studied compounds showed different activity against the MRSA strains. Some natural compounds had antibacterial effects when were applied alone. Quercetin and tannic acid had the best activity compound against the MRSA strains and the diameter of inhibition zones ranged from 20 to 25 mm of quercetin and ranged from 20 to 22 mm of tannic acid. Rutin, ethyl cinnamate, caffeine, gallic acid, and thymol had no activity at all. 4-hydroxy coumarin had no activity against some MRSA strains but showed activity against other MRSA strains. Table 2 showed the diameters of inhibition zones of the compound CIP and natural compounds alone.

The results of the combination of natural compounds with CIP showed antibacterial activity against most MRSA strains, but the natural studied compounds had decreased the activity of CIP. Quercetin and CIP combination led to reduce the inhibition zone from 30 to 25 mm. Furthermore, tannic acid decreased the diameter of inhibition zone to 25 mm. Gallic acid, rutin, thymol, caffeine, ethyl cinnamate had no effect on CIP in the combination. 4-hydroxy coumarin with CIP showed activity against some strains. Table 3 represented the diameters of inhibition zones of the combination of CIP with natural compounds.

DISCUSSION

The search for new antibacterial compounds become popular with the spread of multidrug-resistant bacteria, and less antibiotics are effective to counteract those bacteria including multidrug MRSA. Natural compounds have been proposed as alternative drugs because of their antibacterial effects. Moreover, natural compounds have been reported as drug modulating or modifying agents when they are given together with other drugs [23]. Natural compounds have different mechanisms

Table 3: Diameters of inhibition zones (in mm) of natural compounds in combination with CIP against MRSA

MRSA isolates	Natural compounds and CIP							
	Q+	R+	T+	G+	HQ+	TH+	CA+	EC+
	CIP (500+5) µg	CIP (500+5) µg	CIP (500+5) µg	CIP (500+5) µg	CIP (500+5) µg	CIP (500+5) µg	CIP (500+5) µg	CIP (500+5) µg
1	25	30	25	30	Ne*	30	29	30
2	30	30	25	28	25	29	30	30
3	28	28	25	28	22	28	28	28
4	25	28	25	28	20	29	29	29
5	28	28	22	28	Ne*	30	30	30
6	25	25	22	25	Ne*	27	27	27
7	25	25	25	25	Ne*	26	26	26
8	25	28	21	28	Ne*	29	29	28
9	25	25	20	25	Ne*	28	28	28
10	25	25	22	25	Ne*	27	27	27
11	25	25	25	28	Ne*	30	30	29
12	22	Ne*	20	Ne*	11	Ne*	Ne*	Ne*
13	22	Ne*	18	Ne*	15	Ne*	Ne*	Ne*
14	20	Ne*	18	Ne*	15	Ne*	Ne*	Ne*
15	20	Ne*	20	Ne*	20	Ne*	Ne*	Ne*

Ne*: Negative result, Q: Quercetin, R: Rutin, G: Gallic acid, HQ: 4-hydroxy coumarin, TH: Thymol, CA: Caffeine, EC: Ethylcinnamate, CIP: Ciprofloxacin, MRSA: Methicillin-resistant *Staphylococcus aureus*

of action as antibacterial agents. Flavonoids act in many ways to inhibit bacteria. Quercetin has been identified as topoisomerase Type II as CIP, so there is a competition between this two compounds on the goal enzyme [24]. The combination of CIP and quercetin led to reduce the antibacterial activity of CIP. Tannic acid achieves the antibacterial effect, mainly targeting on bacterial cell wall such as complex with cell wall protein, membrane disruption metal ion complexation, and binds to adhesions. However, the specific mechanism of tannic acid how it works together with antibiotics is still needed to be approved [25]. The present results revealed that the CIP activity was decreased in combination with tannic acid. There is a suggestion that tannic acid modulates the over activity of efflux pumps in MRSA strains, so it affects on CIP and causes to decrease its activity. Other natural compounds had no antibacterial activity, so there was no influence on the activity of CIP.

In this study, it was found that the combination of CIP with natural compounds could decrease the antibacterial activity against MRSA strains. Therefore, combination CIP with natural compounds should be taken in consideration.

The present research was limited to *in-vitro* studies only because of nonavailability of animal models facilities, which remained the major limitation of these studies. Since the findings are promising, therefore, they can be extended to *in-vivo* stage.

CONCLUSION

From the antibiotic sensitivity tests, it is evident that natural compounds quercetin, and tannic acid had an antibacterial effect, but gallic acid, 4-hydroxy coumarin, thymol, caffeine, and ethyl cinnamate had no inhibition effect against MRSA strains. Some natural compounds decreased the antibacterial activity of CIP. Antagonism effect showed between CIP and natural compounds in combination.

ACKNOWLEDGMENT

The authors would like to thank the Aleppo University for financial support for this work and would like to thank Dr. Ammar Yahia from Microbiology Laboratory (Aleppo University Hospitals, Syria) for supporting this work. There is no known conflict of interest associated with the publication.

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