

Potentiating Effect of *Mentha arvensis* and Chlorpromazine in the Resistance to Aminoglycosides of Methicillin – Resistant *Staphylococcus aureus*

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Abstract. *Background:* This is the first report testing the antibiotic resistance-modifying activity of *Mentha arvensis* against MRSA (methicillin – resistant *Staphylococcus aureus*). *Materials and Methods:* In this study an ethanol extract of *Mentha arvensis* L. and chlorpromazine were tested for their antimicrobial activity alone or in combination with conventional antibiotics against MRSA strains. *Results:* A potentiating effect of this extract on gentamicin, kanamycin and neomycin was demonstrated. Similarly, a potentiating effect of chlorpromazine on the same aminoglycosides was observed, indicating the involvement of an efflux system in the resistance to these antibiotics. *Conclusion:* It is therefore suggested that extracts from *M. arvensis* could be used as a source of plant-derived natural products with resistance-modifying activity, such as in the case of aminoglycosides, constituting a new weapon against bacterial resistance to antibiotics, as with chlorpromazine.

Staphylococcus genus is widely spread in nature, being part of the indigenous microbiota of skin and mucosa of animal and birds. Some *Staphylococcus* species are frequently recognized as etiological agents of many animal and human

opportunistic infections (1). *S. aureus*, *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus* are the most important species as community and nosocomial human infection causing agents. In addition of causing different kinds of intoxications, *S. aureus* has been the most common etiological agent of festering infections that attack different tissues and/or organs (e.g. furuncle, carbuncle, abscess, myocarditis, endocarditis, pneumonia, meningitis, bacterial arthritis) (2, 3). Capsule, peptidoglycan, teichoic acids, adesins and synthesis of enzymes and extracellular toxins are some virulence attributes present in/on the *S. aureus* cell (1).

With an increased incidence of resistance to antibiotics, natural products from plants could be interesting alternatives in the therapy of such infections (4, 5). Some plant extracts and phytochemicals are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to demonstrate such efficacy (6-8). Many plants have been evaluated not only for direct antimicrobial activity, but also as a resistance-modifying agent (9, 10). Several chemical compounds, synthetic or from natural sources, such as the phenothiazines, have direct activity against many species of bacteria, enhancing the activity of a specific antibiotic, reversing the natural resistance of specific bacteria to given antibiotics, promoting the elimination of plasmids from bacteria and inhibiting transport functions of the plasma membrane with regards to a given antibiotic. The inhibition of plasma membrane-based efflux pumps has also been observed (11, 12). The enhancement of antibiotic activity or the reversal of antibiotic resistance by natural or synthetic non-conventional antibiotics affords the classification of these compounds as modifiers of antibiotic activity.

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Mentha arvensis (Labiatae) is a herbaceous plant that occurs in all South America. This plant, and particularly its essential oils, are commonly used in folk medicine, exploiting a wide range of biological and pharmacological activities. Several compounds have been isolated from these oils, mainly menthol, *p*-menthone, menthol acetate and other phytochemicals (13, 14).

Aminoglycosides are potent bactericidal antibiotics targeting the bacterial ribosome and the increase in cases of bacterial resistance to aminoglycosides is widely recognized as a serious health threat (15). The main mechanisms of resistance to aminoglycosides are active efflux and enzymatic inactivation (16).

In this work, an ethanol extract of *Mentha arvensis* and chlorpromazine was tested as a resistance modifying agent in an aminoglycoside-resistant strain of *S. aureus*.

Materials and Methods

Strains. The experiments were performed with the clinical isolate *Staphylococcus aureus* 358 (SA358), resistant to several aminoglycosides (17). The strain was maintained in heart infusion agar slants (HIA, Difco), and prior to assay, the cells were grown overnight at 37°C in brain heart infusion (BHI, Difco).

Plant material. Leaves of *Mentha arvensis* were collected in the county of Crato, Ceará State, Brazil. The plant material was identified and a voucher specimen was deposited with the number 2886 at the Herbarium "Dárdano de Andrade Lima" of Universidade Regional do Cariri – URCA.

Preparation of ethanol extract of *Mentha arvensis* (EEMA). A quantity of 200 g of aerial parts were dried at room temperature and powdered. The powdered material was extracted by maceration using 1 L of 95% ethanol as solvent at room temperature and the homogenate was allowed to stand for 72 h at room temperature. The extracts were then filtered and concentrated under vacuum in a rotary evaporator (18). For the tests, the dry extract material was dissolved in DMSO.

Drugs. Chlorpromazine, gentamicin, tobramycin, kanamycin, amikacin and neomycin were obtained from Sigma Chemical Company. All drugs were dissolved in sterile water.

Drug susceptibility test. The minimum inhibitory concentration (MIC) of EEMA, antibiotics and chlorpromazine (CPZ) were determined in BHI by the microdilution assay using suspensions of 10⁵ cfu/mL and a drug concentration range of 1024 to 1 µg/mL (twofold serial dilutions) (19). MIC was defined as the lowest concentration at which no growth was observed. For the evaluation of EEMA as a modulator of antibiotic resistance, MICs of the antibiotics were determined in the presence of EEMA (32 µg/mL) and CPZ (16 µg/mL) at sub-inhibitory concentrations, and the plates were incubated for 24 h at 37°C. CPZ was used as positive control for efflux pump inhibition.

Results

The addition of EEMA to the growth medium at 32 µg/mL produced a dramatic reduction in the MIC for gentamicin,

Table I. MIC values (µg/mL) of aminoglycosides in the absence and presence of EEMA and CPZ in *Staphylococcus aureus* 358.

	SA358		
Antibiotics	MIC	EEMA (32 µg/ml)	CPZ (16 µg/ml)
Amikacin	8	32	8
Gentamicin	8	<1	4
Kanamycin	≥1024	64	8
Neomycin	16	8	8
Tobramycin	8	8	8
EEMA	≥1024	-	-
Chlorpromazine	16	-	-

EEMA: ethanolic extract of *Mentha arvensis*; CPZ: chlorpromazine; MIC: minimal inhibitory concentration.

kanamycin and neomycin in the strain *S. aureus* 358, demonstrating a potentiating effect of EEMA on aminoglycoside activity. However, the MIC of amikacin was enhanced (Table I).

A MIC reduction for kanamycin, gentamicin and neomycin was also observed when CPZ was added to the growth medium at 16 µg/ml, which indicates the involvement of an efflux pump in the resistance to these antibiotics (Table I).

A potentiating effect of CPZ on amikacin or tobramycin was not observed, which suggests the occurrence of other resistance mechanisms (Table I).

Discussion

Studies on interactions of natural products from plants of the genus *Mentha* have been reported. *M. piperita* and *M. pulegium* have been shown to possess anti-genotoxicity activity when combined with H₂O₂ (20), and the former has shown an additive effect when combined with oxytetracycline (21). Another plant of this genus, *M. spicata*, has demonstrated enhanced antimicrobial activity when combined with nitrofurantoin (22). The combined effects of natural products of *Mentha arvensis* and drugs has been shown for anti-anaphylactic and antibiotic activity in *E. coli* (23, 24), however, natural products of *Mentha arvensis*, or of any plants from the genus *Mentha*, having a potentiating effect on gentamicin or other aminoglycosides in MRSA strains have not been previously reported.

Phenothiazines, such as chlorpromazine, probably act on the plasma membrane of bacteria affecting the efflux pumps (25). This modification of permeability could enhance the activity of antibiotics that act within the cell, such as the aminoglycosides.

The results obtained indicate that *Mentha arvensis* (and broadly Labiateae) could serve as a source of plant-derived natural products with antibiotic resistance-modifying activity to be used against multiresistant and MRSA *Staphylococcus aureus*.

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