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Antimicrobial activity of essential oils of *Origanum vulgare* L. and *Origanum majorana* L. against *Staphylococcus aureus* isolated from poultry meat

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ABSTRACT

One of the microorganisms present in poultry meat products responsible for the most usual foodborne intoxication is *Staphylococcus aureus*. Essential oil of spices has been used as a bio-preservative due to its antimicrobial activity against foodborne pathogens. The aim of this study was to evaluate the antimicrobial activity *in vitro* of the essential oils of oregano and marjoram against *S. aureus* isolated from poultry meat marketed in Pelotas/RS—Brazil, and to carry out the phenotypic and molecular characterization of these oils. For this, twenty samples of poultry meat were analyzed. *Staphylococcus* spp. was isolated from Baird Parker agar, and its molecular characterization was performed by PCR. All isolates of *S. aureus* were tested for the presence of genes coding for staphylococcal enterotoxins. Antimicrobial activity of essential oils of oregano and marjoram was evaluated by disk diffusion, minimal inhibitory concentration and minimal bactericidal concentration. The results showed that seventeen samples were contaminated by *Staphylococcus* positive-coagulase and nine were positive to acriflavine resistance and thermonuclease tests, and in the last ones *S. aureus* was confirmed. The genes coding for staphylococcal enterotoxins detected were *sea* (6) and *seb* (3). All isolates were sensitive to essential oils tested, indicating that these can be an alternative to food preservation.

1. Introduction

Poultry and poultry products are a highly perishable food, and their shelf-life varies from 4 to 10 days under refrigeration (Marenzi, 1986). Deterioration depends mainly on the microbiological quality of the poultry carcasses, as poultry meat offers the perfect environment, pH, nutrients and humidity conditions for microorganism growth (Al-Nehlawi et al., 2014). One of the microorganisms present in poultry meat products responsible for the most usual foodborne intoxication is *Staphylococcus aureus*.

Staphylococcus positive-coagulase is one of the common pathogens responsible for nosocomial infections (Jansen et al., 2013) as well as food poisoning incidents (Huong et al., 2010). In appropriate conditions, the microorganism multiplies in the food, reaching high concentrations ($\geq 10^5$ microorganisms/g), producing enterotoxins from four to six hours later. Staphylococcal enterotoxins (SEs) are resistant to proteases, thus maintaining their activity in the digestive tract after ingestion (Franco and Landgraf, 2008). They remain in the food after cooking, favoring the establishment of a clinical case of food poisoning (Neto et al., 2002). So far, twenty two SEs have been described. The classical enterotoxins (enterotoxin A, enterotoxin B, enterotoxin C, enterotoxin D and enterotoxin E) are the most studied ones and are responsible for 95% of staphylococcal food poisoning cases (Al-Tarazi et al., 2009; Johler and Stephan, 2010). The main reservoirs are the mucosa of the nasopharynx and the skin of humans and animals (Kloss and Schleifer, 1986).

S. aureus has been shown to be a bacterium that is able to acquire resistance very quickly over time with the indiscriminate and prolonged use of antibiotics (Abdel-massih and Abraham, 2014). This can be a problem because the genes that mediate antibiotic resis-

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tance can be rapidly disseminated to other bacteria (Morris et al., 1998). In addition, there is a strong debate about the safety aspects of synthetic preservatives, since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity when used for long time (Moreira et al., 2005).

For these reasons, many studies have been performed in search of naturally occurring antimicrobials as an economic and effective option (Vargas et al., 2004). Antimicrobial compounds derived from plants are used because they are able to inhibit the growth and proliferation of pathogenic microorganisms (Souza et al., 2000). Another factor that also influences the search for plant-derived compounds with medicinal properties is that they are unlikely to cause side effects or develop resistance to certain microorganisms (Cowan, 1999).

Along with the plant-derived compounds are the essential oils (EO), characterized as aromatic oily liquids. It has long been recognized that some EO have antimicrobial properties (Boyle, 1955; Guenther, 1948). They can be synthesized by all plant organs, including flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes (Bakkali et al., 2008).

One of the most popular spices used around the world is *Origanum vulgare* L. (oregano). It is used in cooking and also as a medicinal therapy, arousing interest not only in the use of its leaves in folk medicine, but also in relation to its essential oil for therapeutic purposes (Cleff et al., 2008). Another natural antimicrobial produced from spices used in cooking is the essential oil of *Origanum majorana* L. (marjoram). This compound has aroused interest among researchers in recent times because it shows biological activities including antimicrobial, antifungal and antioxidant, and it may have the greatest potential for use in industrial applications (Baâtour et al., 2011, 2013).

In this context, the aim of this study was to evaluate the antimicrobial activity *in vitro* of the essential oils of oregano and marjoram against *S. aureus* isolated from poultry meat marketed in Pelotas, Rio Grande do Sul state, Brazil, and to carry out the phenotypic and molecular characterization of these oils.

2. Material and methods

2.1. Isolation and phenotypic characterization of Staphylococcus spp.

Twenty samples of poultry meat were collected from two establishments (I—sold in a supermarket, II—from a butchery) of Pelotas/RS—Brazil, randomly. To isolate *Staphylococcus* spp., 25 g of poultry meat was homogenized in 225 mL of Tryptic Soy Broth (TSB, Acumedia®) with 10% NaCl (sodium chloride, Synth®). The mixture was incubated at 36 °C for 24 h. After that, an aliquot was spread in Baird-Parker Agar (BPA, Acumedia®). Then, the plates were incubated at 36 °C for 48 h. The typical colonies were sub-cultured and identified by Gram staining and coagulase test (American Public Health Association—APHA, 2002). The isolates characterized as Gram-positive cocci and positive-coagulase were submitted to sensitivity to acriflavine (Harley and Prescott, 1996) and thermonuclease tests (Silva et al., 1997). The ones which showed acriflavine resistance and positive thermonuclease were submitted to genotypic identification.

2.2. Genotypic identification of Staphylococcus spp.

2.2.1. DNA extraction

Genomic DNA extraction was performed according to the protocol proposed by Matthews et al. (1997), with modifications. Briefly, colonies from TSA culture (24 h) were transferred to 100 μL of TE-A buffer solution (10 mM Tris (tris (hydroxymethyl) aminoethane) ®, Synth and 5 mM EDTA (ethylenediaminetetraacetic acid), Synth®) until 1.0 McFarland. The cell lysis of peptidoglycan occurred by the addition of 100 μL of lysostaphin (100 μg/mL, Sigma–Aldrich®) and incubation at 37 °C for 45 min, followed by addition of 20 μL solution TE-B buffer (50 mM Tris and 20 mM EDTA with SDS (20%), (Sodium dodecyl sulfate, Invitrogen®), and 3 μL of proteinase K (2 mg/100 μL, Invitrogen®) with incubation at 37 °C for an hour. Then, 200 μL of NaCl (5 M, sodium chloride, Synth®) was added and mixed gently for 15 s followed by centrifugation (10,000 × *g*, 4 °C 15 min). The upper layer was transferred to a sterile tube and extracted with phenol: chloroform: isoamylalcohol (25:24:1) by centrifugation (16,000 × *g*, 4 °C 15 min). The DNA precipitation was performed with twice the volume of 95% of cold ethanol and it was left to settle overnight (−20 °C). Then, centrifugation was performed (16,000 × *g*, 4 °C for 10 min) and the pellet was washed twice with ethanol (70%) and dried for 30 min. The pellet was re-suspended in 30 μL of ultra-pure water. Then, 2 μL of RNAse (10 mg/mL, Invitrogen®) was added and incubated at 37 °C for an hour. The DNA was maintained at −20 °C and later it was quantified by spectrophotometric method in an Eppendorf BioSpectrometer kinetic® (Eppendorf).

Table 1
Primers used for molecular identification of *Staphylococcus* spp. isolated from poultry meat.

Target gene	Primer (5′–3′)	Product size (bp)	Reference
NUC1	ATGAAGTCAAATAAATCGCT	458	Baron et al. (2004)
NUC2	TTTGGTGAAAAATACTTCTC		
16S1	GGACGGGTGAGTAACACGTGG	252	Baron et al. (2004)
16S2	TCCCGTAGGAGTCTGGACCGT		

2.2.2. Biplex—PCR

Genotypic identification of *Staphylococcus* spp. was performed by identifying the 16S rRNA to genus-specific level and the *nuc* gene that encodes to thermonuclease species-specific level (Baron et al., 2004). The primers used for detection of 16S rRNA and *nuc* genes are shown in Table 1.

The reaction mixture was prepared with 12.5 μL of GoTaq® GreenMaster Mix 2X (DNA polymerase supplied 2 X Green GoTaq® Reaction Buffer (pH 8.5), 400 μM dATP, 400 μM dGTP, 400 μM dCTP, 400 μM dTTP and 3 mM $MgCl_2$, Promega Corp.®), 2 μL of each primer (10 pmol for thermonuclease gene and 1 pM for 16S rRNA gene, 100 μM/μL, Eurofins MWG operon®), 2 μL of DNA (50 ng) and 6,5 μL of ultra-pure water (Promega Corp.®), to complete final volume of 25 μL. Amplification reactions were performed under the following conditions: initial cycle of 94 °C for 4 min; 32 cycles of 95 °C for 2 min, annealing temperature of 52.7 °C for 2 min, 72 °C for 2 min; a final extension step of 72 °C for 7 min. DNA amplifications were performed in DNA thermal cycler model MJ Research PTC 100. The total DNA obtained was then mixed with GelRed™ and submitted to electrophoresis in 1.5% (w/v) agarose gel in 0.5 X TBE (Tris borate—EDTA®) buffered for 70 min at 80 V using a molecular weight marker of 1 Kb (Invitrogen®). The gels were photographed on an UV trans-illumination (Loccus®, L-Pix Touch). *S. aureus* ATCC 25923 was used as positive control and *Escherichia coli* ATCC 11229 was used as negative control for the genus.

2.2.3. Detection of genes coding for staphylococcal enterotoxins

For the genotypic characterization, primers that target the genes of classical staphylococcal enterotoxins were used (*sea, seb*, *sec,sed* and *see*). The primers and references used are shown in Table 2.

PCR mixes were performed using 12.5 uL of GoTaq® GreenMaster Mix 2 X (Promega, Corp.®), 1 uL of each primer (100 μM/μL, Eurofins MWG operon®) at a concentration of 10 pmol, except in

Table 2
Primers used to detect enterotoxin classic genes.

Primer	Sequence 5′–3′	pb	Reference
SEA1	ACGATCAATTTTTACAGC	544	Rosec and Gigaud (2002)
SEA2	TGCATGTTTTCAGAGTTAATC		
SEB1	ATTCTATTAAGGACACTAAGTTAGGGGA	404	Jarraud et al. (2001)
SEB2	ATCCCGTTTCATAAGGCGAGT		
SEC1	GACATAAAAGCTAGGAATTT	257	Rosec and Gigaud (2002)
SEC2	AAATCGGATTAACATTATCCA		
SED1	CAAATATATTGATATAATGA	330	Zocche et al. (2009)
SED2	AGTAAAAAAGAGTAATGCAA		
SEE1	CAAAGAAATGCTTTAAGCAATCTTAGGC	482	Jarraud et al. (2001)
SEE2	CACCTTACCGCCAAAGCTG		

the primer for the *sec* gene, in which we used 50 pmol, 2 uL of DNA (50 ng), 8.5 uL and ultra-pure water (Promega, Corp.®) to complete the final volume of 25 uL. Amplification was carried out in a thermocycler model MJ Research PTC 100 under the following conditions: an initial 5- min denaturation at 95 °C; followed by 37 cycles of denaturation for 1 min at 95 °C,1 min of annealing at 44.5 °C, and 1 min of extension at 72 °C for 1 min; and a final extension step of 72 °C for 10 min. Amplification was carried out on a thermocycler model MJ Research PTC 100. After amplification, the total DNA obtained was mixed with GelRed™ and submitted to electrophoresis in 1.5% (w/v) agarose gel in 0.5 X TBE (Tris borate—EDTA®) buffered for 70 min at 80 V using a molecular weight marker of 1 Kb (Invitrogen®). The gels were photographed on an UV transillumination (Loccus®, L-Pix Touch). The positive controls used in the reactions to the genes coding for classical enterotoxin were *S. aureus* FRIS6 strains (*sea* and *seb*), ATCC 19095 (*sec*), FRI361 (*sed*) and FRI326 (*see*). The negative control used in the reactions was *E. coli* ATCC 11229.

2.3. Antimicrobial activity of essential oils from oregano and marjoram against Staphylococcus aureus

The susceptibility of *S. aureus* isolates from poultry meat to essential oils of oregano and marjoram provided by Bioessência® was determined by the disk diffusion, minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). According to the certificates of analysis issued by the company, both essential oils originate from Brazil and were extracted from leaves of the plants by steam distillation. High- resolution gas chromatography (Gas Chromatograph HP5890) was used to perform the composition of the essential oils. The major compounds of the essential oil of oregano are carvacrol and thymol, and of the essential oil of marjoram it is terpineol.

2.3.1. Disk diffusion method

Isolates were grown from freezer stocks overnight in Brain Heart Infusion broth (BHI, Acumedia®) at 36 °C, adjusted to 0.5 McFarland standard (1.5×10^8 cfu mL^{-1}), swabbed in BHI agar (Acumedia®). Paper disks with 6 mm diameter soaked with 10 μL of oregano and marjoram essential oil (raw) were laid on the surface of BHI agar. The plates were incubated at 36 °C for 24 h. The diameter of the zone of inhibition of bacterial growth was measured (CLSI, 2012b).

2.3.2. Minimal inhibitory concentration (MIC)

To determine MIC, the microdilution technique was used (CLSI, 2012a). In order to carry out subsequent serial dilutions, two parts of essential oil were added to the culture medium to obtain final concentrations of 1562–100 μL mL^{-1}. To each cavity were added 180 μL of BHI broth (Acumedia®) with 1% Tween 80 (Sigma®) and 20 μL of *S. aureus* inoculum. To evaluate any possible contamination of the culture media only BHI with Tween 80 (negative control) was used, and as positive control the medium with Tween 80 and the culture of *S. aureus* was used. Subsequent serial decimal dilutions were performed until 3×10^4 cells/cavity final concentrations. The plates were incubated at 36 °C for 24 h. To each cavity were added 20 μL of 0.5% 2–3–5 Triphenyl Tetrazolium Chloride. The plates were incubated at 36 °C for 20 min. The test result was achieved by observing the color reaction. The color change (from transparent to red) was indicative of bacterial metabolic activity.

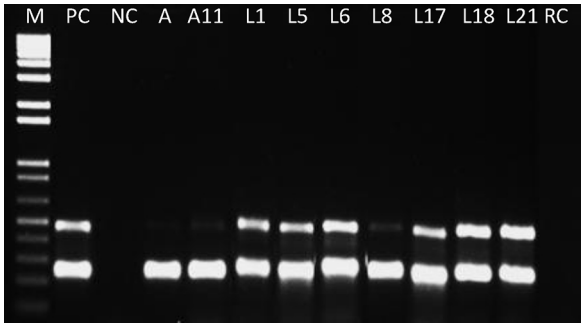

Fig. 1. Electrophoresis in 1.5% (w/v) agarose gel with PCR products by *nuc* gene of isolates identified as *Staphylococcus* positive-coagulase. M: molecular weight marker (1 Kb); PC: positive control – *S. aureus* ATCC 25923; NC: negative control – *E. coli* ATCC 11229; A, A11, L1, L5, L6, L8, L17, L18 and L21: isolates of *Staphylococcus* positive-coagulase; RC: reaction control (ultra- pure water).

2.3.3. Minimal bactericidal concentration (MBC)

The determination of minimal bactericidal concentration was performed according to the technique developed by Duarte (2006) with modifications. From the cavities that showed inhibition of microorganisms in the MIC test, aliquots of 5 μL were collected, transferred to BHI agar and incubated at 36 °C for 24 h. In cases where there was no bacterial growth in the culture medium it can be seen that the essential oils tested showed bactericidal activity but not bacteriostatic.

2.4. Antibiotic susceptibility

The test for antibiotic susceptibility to *S. aureus* was performed by disk diffusion method. Six different antibiotics were tested: ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), sulphazotrin (25 μg) and tetracycline (30 μg), provided by Laborclin® (Clinical Laboratory and Pathology, Brazil). *S. aureus* ATCC 25922 was used as positive control. For this, isolates were grown from freezer stocks overnight in BHI broth at 36 °C and adjusted with 0.5 McFarland standard (1.5×10^8 cfu mL^{-1}), and swabbed onto the Muller–Hinton agar. Paper disks of antibiotics were laid on the surface of Muller–Hinton agar (Acumedia®). The plates were incubated at 36 °C for 24 h. The diameter of the zone of inhibition of bacterial growth was measured and the resistance profile was assessed in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2011).

3. RESULTS

3.1. Isolation and identification of Staphylococcus spp.

It was verified that seventeen samples of poultry meat were contaminated by *Staphylococcus* positive-coagulase. Nine isolates showed positive tests for acriflavine resistance and thermonuclease. All isolates were identified by PCR as *S. aureus* (Fig. 1).

3.2. Detection of classical staphylococcal enterotoxins

From the nine isolates of *S. aureus*, six (L1, L5, L6, L8, L17 and L18) showed genes coding for enterotoxin A (*sea*) (Fig. 2) and three

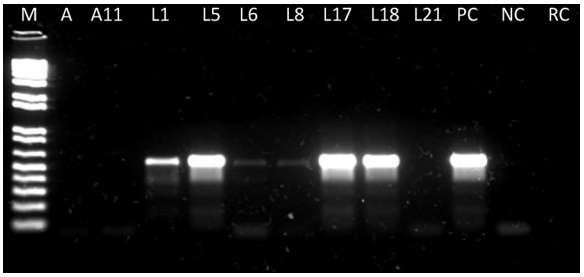

Fig. 2. Electrophoresis in 1.5% (w/v) agarose gel with PCR products for enterotoxin A (*sea*) of isolates identified as *Staphylococcus aureus*. M: molecular weight marker (1 Kb); PC: positive control – *S. aureus* FRIS6 strains; NC: negative control – *E. coli* ATCC 11229; A, A11, L1, L5, L6, L8, L17, L18 and L21: isolates of *S. aureus*; RC: reaction control (ultra-pure water).

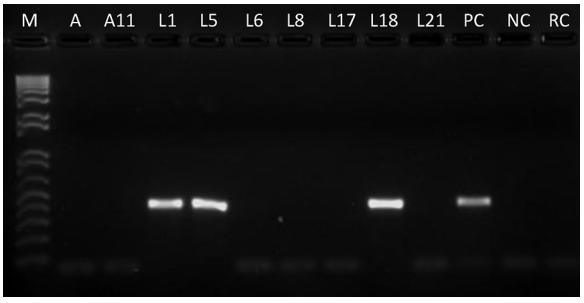

Fig. 3. Electrophoresis in 1.5% (w/v) agarose gel with PCR products for enterotoxin B (*seb*) of isolates identified as *Staphylococcus aureus*. M: molecular weight marker (1 Kb); PC: positive control – *S. aureus* FRIS6 strains; NC: negative control – *E. coli* ATCC 11229; A, A11, L1, L5, L6, L8, L17, L18 and L21: isolates of *S. aureus*; RC: reaction control (ultra-pure water).

Table 3
Antimicrobial activity *in vitro* tests (disk diffusion, minimal inhibitory concentration—MIC and minimal bactericidal concentration—MBC) of essential oils of *Origanum vulgare* L. (oregano) and *Origanum majorana* L. (marjoram) against *Staphylococcus aureus* isolated from poultry meat.

Isolate	Essential oil	Disk diffusion (mm)	MIC ($\mu L\,mL^{-1}$)	MBC ($\mu L\,mL^{-1}$)
A	*O. majorana*	41	50	>CMT
	O. vulgare	35	25	50
A11	*O. majorana*	13	50	>CMT
	O. vulgare	16	12.5	25
L1	*O. majorana*	12	100	>CMT
	O. vulgare	24	25	100
L5	*O. majorana*	9	25	>CMT
	O. vulgare	46	25	50
L6	*O. majorana*	13	25	>CMT
	O. vulgare	50	25	100
L8	*O. majorana*	17	25	100
	O. vulgare	31	6.25	100
L17	*O. majorana*	14	6.25	100
	O. vulgare	24	12.5	100
L18	*O. majorana*	9	6.25	50
	O. vulgare	25	25	100
L21	*O. majorana*	13	50	100
	O. vulgare	33	6.25	>CMT

CMT: Concentration of essential oil Maximum Tested.

isolates (L1, L5 and L18) showed genes coding for enterotoxin B (*seb*) (Fig. 3). No isolates showed genes coding for enterotoxins C–E.

3.3. Antimicrobial activity

The results of antimicrobial activity of EO of oregano and marjoram against *S. aureus* isolated from poultry meat are shown in Table 3 and Fig. 4. All isolates (6) were sensitive to the EO tested.

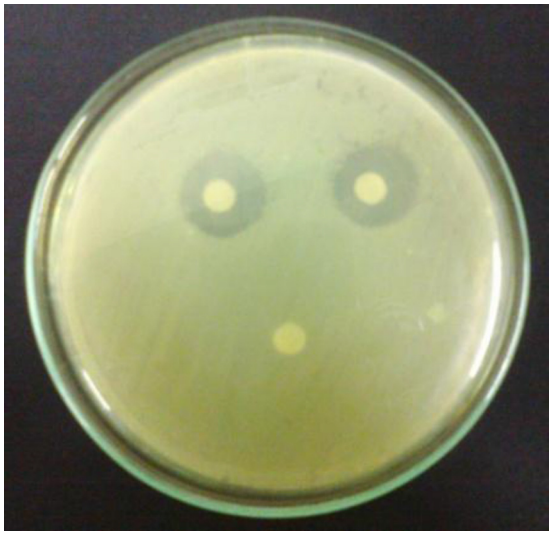

Fig. 4. The inhibitory effect of essential oil of *Origanum majorana* L. on the growth of *Staphylococcus aureus*. Antimicrobial activity of essential oil was carried out according to disk diffusion method by measuring the inhibitory zone size.

3.4. Antibiotic susceptibility

The results of the antibiotic susceptibility tests are shown in Table 4. All isolates were sensitive to ciprofloxacin, chloramphenicol, gentamicin and sulphazotrin. Four isolates were resistant to ampicillin and one to tetracycline.

4. Discussion

Staphylococcus positive-coagulase is known for colonizing surfaces, equipment and utensils with inadequate hygiene (Gava et al., 2009). Furthermore, the hands of individuals involved in the production of foods are the main vehicles for this microorganism (Ho et al., 2014). In search of *Staphylococcus* positive-coagulase, 17 samples of poultry meat were contaminated and 9 confirmed *S. aureus* species. This can be associated with the production conditions to which the samples were submitted.

It was noted that samples from establishment II were more contaminated than samples from establishment I. It is possible to infer that the sanitary conditions of establishment II are precarious (deficiency in the processing of raw materials, inadequate temperatures and handling of foods), providing optimal conditions for the growth of microorganisms (Franco and Landgraf, 2008).

It was observed that from nine isolates of *S. aureus*, six showed genes coding for *sea* and three for *seb*. These results were interesting because enterotoxin A is the most commonly reported in foods and also considered the main cause of staphylococcal food poisoning worldwide. However, enterotoxin B is known for promoting poisoning with more severe symptoms than other enterotoxins (Argudin et al., 2010; Valihrach et al., 2013; Carfora et al., 2015). Song et al. (2015) also detected the prevalence of these two enterotoxins in *S. aureus* isolates from raw and processed foods, of which 5.6% showed the gene *sea* and 3.5% *seb*. Gencay et al. (2010) identified 2.9% of isolates of *S. aureus* carrying the genes of *sea* in their study.

These results emphasize that three isolates (L1, L5 and L18) are carriers of both *sea* and *seb* genes, and can be considered potential enterotoxigens. Silva et al. (2005) highlight that the presence of two genes of staphylococcal enterotoxins in the same isolate maximizes their toxigenic capacity. When they are present in food the expression of two genes can occur at the same time, and thus they are involved in cases and/or food poisoning outbreaks.

Table 4
Antibiotic susceptibility of *Staphylococcus aureus* isolated from poultry meat.

Isolate	Antibiotic Ampicilin 10 μg	Ciprofloxacin 5 μg	Chloramphenicol 30 μg	Gentamicin 10 μg	Sulphazotrin 25 μg	Tetracyclin 30 μg
A	S	S	S	S	S	S
A11	S	S	S	S	S	S
L1	R	S	S	S	S	R
L5	R	S	S	S	S	S
L6	R	S	S	S	S	S
L8	S	S	S	S	S	S
L17	S	S	S	S	S	S
L18	R	S	S	S	S	S
L21	S	S	S	S	S	S
Sensitive—S (*n*)	5	9	9	9	9	8
Intermediate—I (*n*)	0	0	0	0	0	0
Resistant—R (*n*)	4	0	0	0	0	1

All isolates were sensitive to the two EO tested, but oregano EO produced a zone of inhibition in the disk diffusion test greater than the zones of inhibition for the marjoram EO. Even then, it needed a higher concentration of marjoram EO to inhibit the growth of isolates than oregano EO, except for isolates L17 and L18 (MIC). In the MBC test, the maximum concentration tested of marjoram EO was not enough to cause a reduction of 99.9% in the number of viable cells of three isolates (L1, L5, L6). On the other hand, the oregano EO was efficient in reducing viable cells in concentrations between 25 and 100 $\mu L\,mL^{-1}$, except for the isolate L21, where the maximum concentration tested was not effective for bactericidal action.

There has been a substantial increase in the number of reports where authors screened plants for antibacterial properties and mode of action. Essential oils and their constituents, such as the terpenoids, carvacrol and thymol, occur widely in nature, contributing to the characteristic of plant flavors and aromas. Their mechanism of action against bacteria is yet not fully understood, but it is speculated to involve membrane disruption through lipophilic products (Simões et al., 2009). The most frequent inhibitions involve phenolic compounds of oils which sensitize the phospholipid bilayer of the cell membrane, causing an increase in the permeability and leakage of vital intracellular constituents (Kim et al., 1995).

Regarding their biological properties, we know that essential oils are complex mixtures of numerous molecules, and one might wonder if their biological effects are the result of a synergism of all molecules or if they reflect only those of the main molecules present at the highest levels according to gas chromate-graphical analysis (Bakkali et al., 2008). Generally, the major compounds determine antimicrobial activity. Carvacrol, terpinen-4-ol and thymol are majority compounds in oregano EO (Barros et al., 2009), and terpinen-4-ol is the majority compound in marjoram EO (Busatta et al., 2008; Freire et al., 2011).

Exposure of bacterial cells to carvacrol has resulted in increases in the membrane fluidity and leakage of protons and potassium ions, leading to a decrease in pH gradient across the cytoplasm membrane, a collapse of the membrane potential, an inhibition of ATP synthesis and ultimately cell death (Ultee et al., 1998). On the other hand, Cox et al. (2000) affirms that terpinen, the majority compound of marjoram EO, inhibits oxidative respiration, providing evidence of a lethal action related to cytoplasmic membrane damage. In general, an EO possessing the strongest antibacterial properties contains a high percentage of carvacrol and/or thymol, such as oregano or thyme (Burt, 2004).

Barros et al. (2009) reported that *S. aureus*, isolated from foods when exposed to oregano EO, reduced the colony-forming ability and salt tolerance. Souza et al. (2000) concluded that oregano EO can be applied in foods so as to inhibit the growth of *S. aureus* by suppressing the synthesis of staphylococcal enterotoxin.

Ličina et al. (2013) prepared many extracts of oregano and observed high antimicrobial activity against *S. aureus* and other pathogens. Oliveira et al. (2009) observed that marjoram and oregano EO showed antimicrobial activity against clinical isolates of *S. aureus*. Busatta et al. (2008) and Freire et al. (2011) analyzed antimicrobial activity of marjoram EO and observed that it inhibits the growth of *S. aureus*. These results corroborate this study.

Currently consumers are demanding the use of natural foods with a low level of synthetic preservatives (Freire et al., 2011). Another factor is that pathogenic microorganisms are showing resistance to the antibiotics that are usually used in combating foodborne illness (Aydin et al., 2011).

About 50 years ago, antibiotics were introduced for the treatment of microbial diseases. Since then, the greatest threat to the use of antimicrobial agents for therapy of bacterial infections has been the emergence of antimicrobial resistance in pathogenic bacteria (Mathur and Singh, 2005). Emergence of antibiotic resistance in bacteria is mainly based on two factors, the presence of resistance genes and selective pressure from the use of antibiotics (Levy and Marshall, 2004). Resistance to a given antibiotic can be intrinsic to a bacterial species or genus (inherent or natural resistance) that results in an organism's ability to thrive in the presence of an antimicrobial agent due to an inherent characteristic of the organism. Intrinsic resistance is not horizontally transferable, and poses no risk in non-pathogenic bacteria. In contrast, acquired resistance is present in some strains within a species that is usually susceptible to the antibiotic under consideration, and might be horizontally spread among bacteria. Acquired resistance to antimicrobial agents can arise either from mutations in the bacterial genome or through the acquisition of additional genes encoding for a resistance mechanism (Mathur and Singh, 2005).

Xu et al. (2014) analyzed 78 isolates of *S. aureus* from different foods and observed that 29 were resistant to ciprofloxacin, 19 to tetracycline and 9 were intermediately resistant to gentamicin. Aydin et al. (2011) studied 1070 samples of food and isolated 154 *S. aureus*. From these, 10 were resistant to gentamicin, 24 to tetracycline and 12 to chloramphenicol. Still, one isolate had intermediate resistance to gentamicin, 18 to tetracycline and 6 to chloramphenicol. In the present study the sensitivity was observed of all isolates (6) to ciprofloxacin, chloramphenicol, gentamicin and sulfazotrim. One (1) isolate (L1) was resistant to tetracycline and four isolates (L1, L5, L6 and L18) were resistant to ampicillin. These results differ from the earlier referenced papers.

5. Conclusion

In the present study, *S. aureus* was isolated and genotypically identified from poultry meat. Nine isolates showed genes coding for enterotoxin A and three for enterotoxin B. Meanwhile, it was found that four isolates of *S. aureus* were antibiotic-resistant. Natural antimicrobials, essential oils of *O. vulgare* L. and *O. majorana* L., can be an alternative tool for food preservation when it comes to growth inhibition of *S. aureus,* because all isolates were sensitive to the EO tested.

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