



Antibacterial Activity of *Lippia alba* (Mill) NEBr, *Mentha x piperita* L. and *Ocimum gratissimum* L. Essential Oils Against Multiresistant *Salmonella enterica* Serovars

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ABSTRACT

This study evaluated the sensitivity of 20 *Salmonella enterica* serovars, isolated from poultry, bovine meat and bone meal, to commercial antibiotics and essential oils of *Lippia alba* (Mill) NEBr, *Mentha x piperita* L. and *Ocimum gratissimum* L. as alternative antimicrobials. The assays were conducted using disk diffusion and the microdilution technique. The disk diffusion method tested pathogen susceptibility to seven commercial antimicrobial agents and showed that all the strains were resistant to at least two antibacterial agents. The isolates were frequently resistant to streptomycin (95%), nalidixic acid (75%) and gentamicin (70%) and sensitive to norfloxacin (45%), ciprofloxacin (20%) and chloramphenicol (20%). The microdilution technique assessed the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oils. The action of *O. gratissimum* and *L. alba* essential oils showed a small variation, with MIC ranging from 2,5 to 10 mg mL⁻¹ and MBC from 5 to 10 mg mL⁻¹. For *M. piperita* essential oil, MIC ranged from 16 to 40 mg mL⁻¹ and MBC from 40 to 320 mg mL⁻¹. Therefore, essential oils of *O. gratissimum* and *L. alba* showed the highest activity against *S. enterica* serovars isolated from different sources, revealing their potential as natural antimicrobial agents.

Keywords: Antibiotics; Minimum inhibitory concentration; Minimum bactericidal concentration; Poultry; Animal meal

INTRODUCTION

Salmonellosis is a major worldwide public health concern, accounting for 93.8 million foodborne illnesses and 155,000 deaths per year. From 2,500 *Salmonella* serotypes identified, more than half of them belong to *Salmonella enterica* subsp. *enterica*, which accounts for the majority of *Salmonella* infections in humans [1]. *Salmonella enterica* diseases are usually associated with contaminated animal-derived foods [2]. The current tendency to adopt natural renewable products for animal husbandry has motivated the use of natural antimicrobials, which have been increasingly used along with food and medicine [3]. They are a valuable alternative because, although food additives are important to increasing the microbiological safety of animal products in the food industry, the inappropriate use of antibiotics causes pathogenic microorganisms to develop resistance against these drugs [4]. There has been growing global concern in the last 30 years in relation to multi-resistant *Salmonella* serotypes, such as *S. typhimurium*, *S. enteritidis* and *S. newport* [2]. For instance, the development of antimicrobial resistance by bacteria that contaminate poultry makes this product a potential vehicle for transmission of foodborne diseases caused by resistant *Salmonella*, resulting in a number of public and consumer health problems [5]. The emergence of multi-

drug-resistant *Salmonella* serotypes is having a great impact on the efficacy of antibiotic treatment, and an increasing prevalence of these strains may lead to an increase in mortality rates of *Salmonella* infections [1].

Plant species used as spices and condiments for food seasoning may exhibit antimicrobial activity and thus serve as food preservatives [6]. This is the case of essential oils, which have become a potential substitute for dietary antibiotics due to their positive impact on growth, gut microbiota and animal welfare [7]. Essential oils have great potential in the field of biomedicine as they effectively destroy several bacterial, fungal, and viral pathogens [8].

In case of *Salmonella* serovars for example, extracts and essential oil of *Lippia alba* (Mill) N.E.Br are promising microbial agents against *Salmonella* sp. [9,10]. Machado et al. [11] reported the antimicrobial activity from the essential oil of *Lippia alba* against *Salmonella* Choleraesuis and microorganisms that cause food spoilage and food-borne disease. Another plant, *Mentha x piperita* L., popularly known as peppermint, showed antibacterial activity against *Escherichia coli* and, to a lesser extent, against *Salmonella* Enteritidis [12]. Antibacterial activity was also found in extract and essential oil of *Ocimum gratissimum* L., which is efficient against different serovars of *Salmonella* such as *S. enteritidis* [13], *S. thyphimurium* [14] and *S. typhi* [14-16].

Considering the importance of the essential oils biological potential and the problem from salmonellosis/multidrug resistance, the present study evaluated the sensitivity of 20 *Salmonella enterica* strains isolated from poultry, bovine meat and bone meal to commercial antimicrobial agents, to define the resistance or multi-resistance and sensitivity to alternative antimicrobials: essential oils of *L. alba*, *M. piperita* and *O. gratissimum*.

EXPERIMENTAL SECTION

Microorganisms

The 20 isolates of *S. enterica* were obtained from chilled poultry (14 isolates), mechanically separated poultry (2 isolates), poultry viscera meal (2 isolates), in addition to bovine meat and bone meal (2 isolates). The presence of pathogens species was confirmed in a laboratory accredited by the Ministry of Agriculture of Brazil and located in Lajeado, Rio Grande do Sul state (UNIANÁLISES / UNIVATES).

Antigenic characterization and serovar identification was performed at the Laboratory of Enteric Bacteria of the Oswaldo Cruz Institute, in Rio de Janeiro, Rio de Janeiro state.

Sensitivity of *Salmonella enterica* Strains to Commercial Antimicrobial Agents

The susceptibility of *S. enterica* strains to antimicrobial agents was evaluated in triplicate, using the disk diffusion technique recommended by the Clinical and Laboratory Standards Institute [17]. The antimicrobial agents (OXOID[®]) tested were chloramphenicol (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), norfloxacin (10 µg), streptomycin (10 µg) and tetracycline (30 µg). *Escherichia coli* ATCC 25922 was evaluated as reference strain.

Plant Material

L. alba, *M. piperita* and *O. gratissimum* specimens were collected from the Medicinal Plant Collection of Embrapa Amazônia Ocidental in Manaus, Amazonas State, Brazil. The plants were identified and deposited in the herbarium of the Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, with the following registry numbers: 13,886, 13,890 and 13,889, respectively.

Distillation and Analysis of the Essential Oils

The essential oils were distilled from leaves of *L. alba*, *M. piperita*, and *O. gratissimum* harvested from the garden of Medicinal Plants Collection at Embrapa Amazônia Ocidental in Manaus. The extraction was performed in a Clevenger hydrodistillation apparatus for 2 h. The oil was separated from the aqueous phase, dried with anhydrous sodium sulfate (CRQ, Diadema, Brazil), filtered and stored in closed containers at -20°C until used in the assays.

The chemical composition of the essential oils was determined using a 7890A Agilent gas chromatograph (Palo Alto, USA) equipped with an HP-5MS capillary column (5% -diphenyl-95% - dimethyl silicone, 30 m × 0.25 mm × 0.25 µm). Oven temperature was raised from 60 to 240°C at 3°C min⁻¹. Hydrogen was used as carrier gas (1.5 mL min⁻¹). One microliter of a 1% solution of essential oil diluted in dichloromethane (Merck Millipore, Darmstadt, Germany) was injected in split mode (1:100). The injector was kept at 250°C, and the detector (FID) operated at 280°C. Mass spectra were obtained in an Agilent 5973N mass selective detector, operating in electron ionization mode (EIMS, 70 eV), coupled to an Agilent 6890 gas chromatograph using the same column and conditions described above. Helium was used as carrier gas (1.0 mL min⁻¹). Identification of the oil components was based on a computer search using the 6th edition of the Wiley Registry of Mass Spectral Data [18] and comparisons between calculated linear retention indices [19] and literature data [20].

MIC and MBC Determination

The antimicrobial activity of the three essential oils tested was based on the minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determined by the broth microdilution method in 96-well plates, according to the Clinical and Laboratory Standards Institute (20). The plates were loaded, in triplicate, with the essential oils at different concentrations ranged between 20 and 0,625 mg mL⁻¹, using standard antimicrobial chloramphenicol (Flucka BioChemika, St. Gallen, Switzerland) as a positive control.

For *M. piperita* oil, the amount of stock solution prepared was increased if they were inactive using the same conditions adopted for the other oils evaluated. Bacterial growth was identified by the cloudy aspect of the well, whereas clear wells indicated growth. The first well without visible bacterial growth corresponded to MIC, that is, the lowest oil concentration in which the bacteria did not grow.

Bacterial growth was confirmed by injecting sterile aqueous solution with 0.5% triphenyltetrazolium chloride (TTC) (Nuclear-CAQ, Diadema, Brazil) into the wells. This solution forms a red complex with bacterial respiration enzyme, indicating the presence of living organisms. For MBC determination, cell suspension was sown in the plates with Tryptone Soya Agar (TSA) (Himedia, Mumbai, India), only in the cells where bacterial growth was not observed. The plates were incubated at 35 °C ± 1°C for 24 hours. Bacterial growth was then observed, followed by determination of the lowest concentration of essential oil that inhibited growth.

Statistical Analysis

ANOVA and Duncan's test were applied to data to compare the antimicrobial potential of the essential oils tested and assess the susceptibility of the different isolates/serovars of *Salmonella enterica* to the oils.

RESULTS

Antimicrobial Resistance

Antimicrobial resistance is shown in Table 1. All the serovars were resistant to at least two of the antibiotics tested.

Table 1: Isolate serovar, source and antibiotics resistance pattern

Sample / Isolate source	Identification	Resistance pattern
#1 / chilled poultry	<i>S. schwarzengrund</i>	GEN, STR
#2 / chilled poultry	<i>S. enteritidis</i>	GEN, STR, CIP, CHL, NAL
#3 / chilled poultry	<i>S. mbandaka</i>	GEN, STR, CIP, CHL, NAL
#4 / chilled poultry	<i>S. enterica subs enterica (O:4,5:-:1,2)*</i>	GEN, NAL
#5 / chilled poultry	<i>S. enteritidis</i>	EST, CHL, NAL
#6 / chilled poultry	<i>S. enterica subs enterica (O:4,5:-:1,2)*</i>	STR, TET, NAL
#7 / chilled poultry	<i>S. heidelberg</i>	STR, NAL
#8 / chilled poultry	<i>S. enterica subs enterica (O:4,5:-:1,2)*</i>	GEN, NOR, STR, TET, CHL,
#9 / chilled poultry	<i>S. enterica subs enterica (O:6,7)*</i>	STR, CHL, NAL
#10 / chilled poultry	<i>S. typhimurium</i>	GEN, STR, TET
#11 / chilled poultry	<i>S. schwarzengrund</i>	GEN, STR, TET, NAL
#12 / chilled poultry	<i>S. agona</i>	GEN, STR, NAL
#13 / chilled poultry	<i>S. mbandaka</i>	GEN, STR, CHL, NAL
#14 / chilled poultry	<i>S. mbandaka</i>	GEN, STR, TET, NAL
#15 / poultry viscera meal	<i>S. montevideo</i>	NOR, STR, CHL, NAL
#16 / poultry viscera meal	<i>S. mbandaka</i>	GEN, NOR, STR, TET
#17 / bovine meat, bone meal	<i>S. enterica subs enterica (rugosa)</i>	GEN, STR, TET
#18 / bovine meat, bone meal	<i>S. orion</i>	GEN, STR
#19 / poultry cuts**	<i>S. agona</i>	GEN, STR, NAL
#20 / poultry cuts**	<i>S. senftenberg</i>	STR, TET, NAL

CHL = chloramphenicol, CIP = ciprofloxacin, GEN = gentamicin, NAL = nalidixic acid, NOR = norfloxacin, STR = streptomycin, TET = tetracycline *undetectable flagellar structure; **mechanically separate

Chemical Composition of Essential Oils

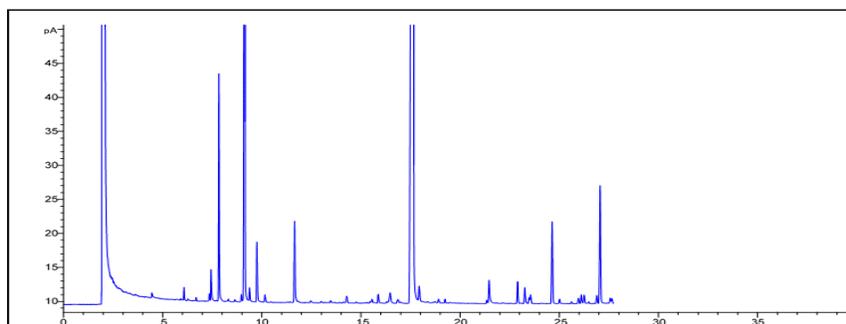
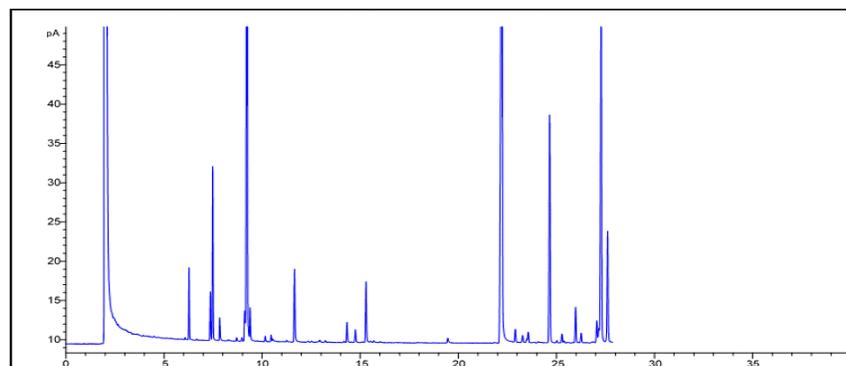
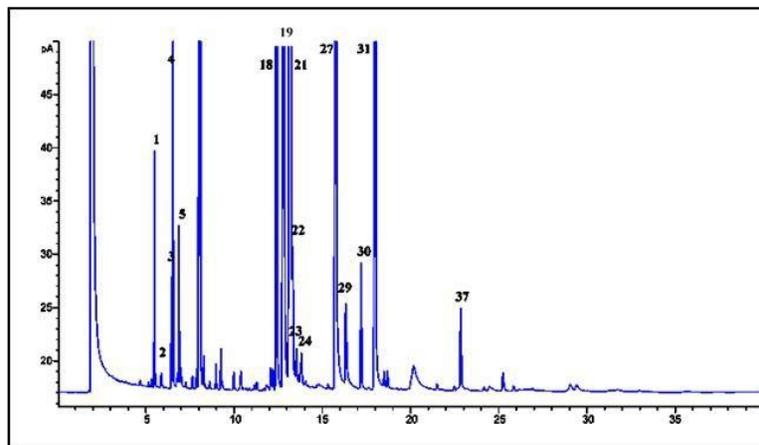
The composition of the essential oils of *L. alba* (LA), *M. piperita* (MP) and *O. gratissimum* (OG) is shown in Table 2.

Table 2: Chemical composition of *L. alba* (LA), *M. piperita* (MP) and *O. gratissimum* (OG) essential oil

Identified Components	LRI ^a	LRI ^b	LA (%)	MP (%)	OG (%)
α -pinene	932	932	-	0.8	1
3-methyl-cyclohexanone	953	945	-	0.1	-
sabinene	971	969	-	0.4	0.7
β -pinene	975	974	0.7	1.3	2.8
myrcene	988	988	3.5	0.6	0.7
3-octanol	994	988	-	0.1	-
<i>p</i> -cymene	1022	1020	-	0.1	-
limonene	1026	1024	17.5	3.5	-
1,8-cineole	1028	1026	-	-	28.2
(<i>Z</i>)- β -ocimene	1033	1032	0.3	0.1	3.7
(<i>E</i>)- β -ocimene	1044	1044	1.1	-	-
γ -terpinene	1055	1054	-	0.1	-
<i>cis</i> -sabinene hydrate	1066	1065	-	0.2	-
terpinolene	1089	1086	-	0.1	-
linalool	1097	1095	1.6	0.1	1.3
<i>neo</i> -isopulegol	1143	1144	-	0.1	-
<i>p</i> -menth-3-en-8-ol	1146	1145	-	0.1	-
menthone	1153	1148	-	11	-
menthofuran	1163	1159	-	22.5	-
δ -terpineol	1166	1162	-	-	0.4
menthol	1174	1167	-	27.5	0.4
terpinen-4-ol	1176	1174	-	1	-
isomenthol	1182	1179	-	0.2	-
α -terpineol	1186	1186	-	0.3	1.1
<i>trans</i> -dihydro-carvone	1200	1200	0.2	-	-
<i>trans</i> -carveol	1214	1215	0.4	-	-
pulegone	1238	1233	-	12.8	-
carvone	1242	1239	61.7	-	-
piperitone	1249	1249	0.6	0.6	-
<i>neo</i> -menthyl acetate	1274	1271	-	0.7	-
menthyl acetate	1294	1294	-	12.5	-
piperitenone	1335	1340	0.7	-	-
eugenol	1354	1356	-	-	43.3
α -copaene	1372	1374	0.5	-	-
β -bourbonene	1380	1381	0.4	-	0.9
β -elemene	1387	1389	0.3	-	0.8
(<i>E</i>)- β -caryophyllene	1414	1417	1.8	0.5	3.7
α -humulene	1450	1452	0.2	-	0.6
germacrene D	1477	1480	2.7	-	0.9
β -selinene	1481	1489	-	-	5.5
α -selinene	1490	1498	-	-	1.7
α -muurolene	1497	1500	0.2	-	-
germacrene A	1507	1508	0.5	-	-
cubebol	1516	1514	0.4	-	-
7- <i>epi</i> - α -selinene	1517	1520	-	-	0.4
nerolidol	1556	1561	0.4	-	-
caryophyllene oxide	1574	1582	0.3	-	-
Total			96	97.3	98.1

^a Calculated linear retention indices on a HP-5 column (Van den Dool and Kratz, 1993); ^b Literature linear retention indices (Adams, 2007).

Carvone (61.7%) and limonene (17.5%) and were the major compounds found in the oil of *L. alba*. In *M. x piperita* oil, from the 27 compounds identified, menthol (27.5%), menthofuran (22.5%), pulegone (12.8%), menthyl acetate (12.5%) and menthone (11.0%) were the most abundant. In *O. gratissimum* oil, the major components were eugenol (43.3%) and 1,8-cineole (28.2%). The GC-FID chromatographic profile of the essential oils from the leaves of *L. alba* (LA), *M. piperita* (MP) and *O. gratissimum* (OG) is shown in Figures 1-3 respectively.

Figure 1: GC chromatogram of essential oil from *Lippia alba*Figure 2: GC chromatogram of essential oil from *Ocimum gratissimum*Figure 3: GC chromatogram of essential oil from *Mentha x piperita*

Antibacterial Activity

The antibacterial activity of the essential oils, as determined by MIC and MBC, is shown in Table 3. Irrespective of the *Salmonella* isolate, MIC and MBC showed a small variation. For *O. gratissimum* and *L. alba* oil, MIC ranged from 2, 5 to 10 mg mL⁻¹ and MBC from 5 to 10 mg mL⁻¹. For *M. piperita*, MIC ranged from 40 to 160 mg mL⁻¹ and MBC from 40 to 320 mg mL⁻¹. MIC and MBC values (Table 3) of *O. gratissimum* and *L. alba* were similar, and both showed higher antimicrobial activity than *M. piperita* oil ($p < 0.05$).

Table 3: Susceptibility of *Salmonella enterica* serovars against *O. gratissimum*, *L. alba* and *M. piperita* L essential oils (N=3)

Serovar	<i>O. gratissimum</i>		<i>L. alba</i>		<i>Mentha x piperita</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
#1Schwarzengrund	5	6.7	5	5	40	40
#2 Enteritidis	10	10	5	5	40	40
#3 Mbandaka	5	5	10	10	40	160
#4 non-identified*	5	5	5	10	80	80
#5 Enteritidis	5	10	5	6.7	40	40
#6 non-identified*	5	10	5	5	80	80
#7 Heidelberg	5	5	5	5	160	320
#8 non-identified*	5	5	5	5	20	20
#9 non-identified*	2.5	10	10	10	80	106.7
#10 Typhimurium	5	5	5	6.7	40	40
#11 Schwarzengrund	5	5	5	5	40	106.7
#12 Agona	5	5	5	5	40	40
#13 Mbandaka	5	6.7	5	5	80	80
#14 Mbandaka	5	10	10	10	80	80
#15 Montevideo	5	5	5	5	40	53.3
#16 Mbandaka	5	5	5	5	40	53.3
#17 non-identified*	5	6.7	5	6.7	80	160
#18 Orion	5	5	5	5	40	40
#19 (MecPo) Agona	5	5	5	5	80	80
#20 Senftenberg	2.5	5	5	5	80	80
MIC: minimum inhibitory concentration, in mg mL ⁻¹ ; MBC: minimum bactericidal concentration, in mg mL ⁻¹ ; * undetectable flagellar structure.						

DISCUSSION

In Brazil few cases of outbreaks have been reported with serovar *S. mbandaka*. In the USA, however, the 2013 outbreaks caused by this serovar were related to contact with birds and consumption of sesame spread [21,22]. In addition, this serovar was isolated from poultry carcass in the USA and chicken fingers in Canada [23-25]. In the present study, *S. mbandaka* (samples #3, #13, #14 and #16) was resistant or mildly resistant to all the antimicrobial agents tested, except for sample #3, which was affected by tetracycline. Similarly, *S. enteritidis* (samples #2 and #5) was resistant to the antimicrobials tested. This is an alarming result, given that it is one of the most virulent serovars and the primary cause of *Salmonella* outbreaks in Brazil [26,27].

By contrast, *S. typhimurium* (sample #10) is sensitive to norfloxacin, ciprofloxacin and chloramphenicol, despite its high association with foodborne outbreaks [28,29]. *S. enteritidis* exhibited multi-resistance and *S. typhimurium* low resistance to antimicrobials [30]. In the present study, norfloxacin, ciprofloxacin and chloramphenicol were the most efficient antimicrobials against the *Salmonella* serovars tested.

In Spain, 133 *Salmonella* isolates from poultry in Spain were sensitive to chloramphenicol and ciprofloxacin [30]. In a similar study, was observed low resistance of *Salmonella* isolates from different matrices to norfloxacin and chloramphenicol, and like the present study, isolates from humans, food and chilled poultry were sensitive to norfloxacin [31]. Studies on *S. Hadar* [32] and 64. *S. enterica* isolates from different sources in Sudan [33] also detected high susceptibility of the bacteria to norfloxacin, ciprofloxacin and chloramphenicol. In Northern India, it was reported that *Salmonella* isolates from poultry are sensitive to ciprofloxacin and chloramphenicol, but they did not test norfloxacin [5]. In Brazil, a recent assessment of the resistance of *Salmonella* isolated from poultry in São Paulo state found 100% sensitivity to norfloxacin and chloramphenicol [34]. Similar to our findings, Calixto et al.

[35] reported that *Salmonella* isolates from animal-derived meal are highly sensitive to chloramphenicol and significantly resistant to nalidixic acid. Norfloxacin and ciprofloxacin belong to the same antibiotic class (fluoroquinolones) and derive from nalidixic acid. However, while both showed antimicrobial activity, this effect was not found using nalidixic acid against the isolates. Chloramphenicol was very efficient against *Salmonella* strains, due to the little exposure to this drug, since its use in animal feed has been prohibited in Brazil since 2003. The variability among *S. enterica* serovars isolated from the matrices was significant. It is important to underscore the high resistance of new serovars (*S. Mbandaka*) and serovars isolated or associated with global outbreaks (*S. enteritidis*) that are highly resistant to the antibiotics tested. The serovars isolated in the present study were more sensitive to norfloxacin, ciprofloxacin and chloramphenicol, considered the most efficient antibiotics.

In the present study, we found that the essential oils studied can provide a feasible alternative to the use of conventional drugs against the *Salmonella* serovars tested. According to the MIC values of *O. gratissimum* essential oil, isolate #2 was the most resistant and isolates #9 and #20 were the most sensitive. The MBC value showed that isolates #2, #5, #6, #9 and #14 were the most resistant to the oil (Table 3). Other studies show different antibacterial activity of *O. gratissimum* oil against the *Salmonella* species tested. For example, Matasyoh et al. [15], using the agar disc diffusion method, found higher MIC (107 mg mL⁻¹) against *S. typhi*. This activity was related to the presence of eugenol, which represented 68.8% of the oil content, a higher percentage than that observed in the essential oil evaluated in the present study. Against *S. enteritidis*, Nakamura et al. [13], also using the agar disc diffusion method, found MIC of 3 µg mL⁻¹ and MBC of 6 µg mL⁻¹, which is considerably lower than our findings. Sartoratto et al. [4], using the microdilution technique, obtained a MIC of mg mL⁻¹ against *S. Choleraesuis*. The lower MIC value may be explained by the eugenol content (93.9% of the oil), which is more than two-fold higher that was detected in the present study. In tests with *L. alba* essential oil, the most resistant isolates were #3 and #9, based on MIC, and #3, #4, and #9, based on MBC (Table 3). Similarly, in a study with *L. alba* essential oil against *S. choleraesuis*, Machado et al. [11], using the microplate method, reported MIC and MBC of 9.4 mg. mL⁻¹ for oils distilled from fresh leaves, and 5.3 mg.mL⁻¹ for oil obtained from dried leaves. Fabri et al. [10], also using the microplate method, reported a MIC of 5 mg.mL⁻¹ for methanol extract of *L. alba* against *S. typhimurium*. Using the disc diffusion method, Aquino et al. [9] detected a MIC value of 6.25 µg mL⁻¹ for *L. alba* essential oil against 4 out of 5 *Salmonella* strains isolated from beef.

Isolate #7 was the most resistant to *M. piperita* essential oil (MIC from 40 to 160 mg mL⁻¹; MBC from 40 to 320 mg mL⁻¹). Iscan et al. Tested essential oils of *M. x piperita* with different compositions against pathogenic microorganisms. Despite the evidence of menthol being the component responsible for the bioactivity of *M. x piperita* essential oil, they did not observe an association between oil composition and microbial activity. The authors found MIC between 1.25 and 2.5 mg mL⁻¹ against *S. typhimurium*, much lower than that obtained in the present study, although menthol content was similar (27.5%).

At first sight, isolates from poultry samples seem to be more resistant to the oils with the highest MIC and MBC values. However, this conclusion is unreliable because most of the samples were poultry (14 of the 20 isolates tested). As observed for the antibiotics, samples #2, #3, #5, #13 and #14, corresponding to the serovars Mbandaka and Enteritidis, were the most resistant to *O. gratissimum*, *L. alba* and *M. piperita* essential oils, whereas samples #13 and #6 were the most sensitive to the oils but not to the standard antimicrobials. Other studies show a wide fluctuation in MIC and MBC, likely because of the variation in oil composition. Indeed, essential oil composition is affected by several factors such as climate, soil, geographical region, length of the day and night, plant age and stress. Therefore, oil action likely results from the synergism of its components, including a number that are found in low levels, such as menthone, which accounted for only 11% of oil content in the present study. Another important consideration regarding differences in the results is the lack of standardization of the techniques used to assess the microbiological activity of the oils. This results in large discrepancies among the results, even if the oils exhibit similar composition. The antibacterial effectiveness of the dietary use of essential oils should be tested because it can be affected by synergetic interactions between oil compounds, food items and ingredients. Therefore, antimicrobial activity should be assessed in mixtures of essential oils with mediums that simulate different food models.

CONCLUSION

Different *Salmonella enterica* serovars were found in samples of chilled poultry, mechanically processed poultry and animal-derived meals. The predominant serovars were *S. mbandaka* (4/20), *S. enteritidis* (2/20), *S. agona* (2/20), *S. schwarzengrund* (2/20) and *Salmonella enterica* subsp. *enterica* (O:4,5:-:1,2) (2/20).

In relation to resistance to antimicrobial drugs, all the *S. enterica* isolates were resistant to at least two compounds. All the samples were resistant or mildly resistant to gentamicin, streptomycin and nalidixic acid. The most efficient antibiotics against the serovars isolated were norfloxacin, ciprofloxacin and chloramphenicol. The most resistant serovars were *S. mbandaka* and *S. enteritidis*. The essential oils of *O. gratissimum* and *L. alba* exhibit higher bacterial activity against isolates of enteric salmonella from different sources, but the susceptibility of the isolates to the oils was variable. These results suggest that these essential oils have potential antimicrobial for future use. Nevertheless, procedures to prevent contamination and epidemiological studies on Salmonellae must be carried out. In addition, antibiotics must be used with caution in animal feed and health treatments in order to avoid the development of new serovars that are more resistant to antibiotics or that develop resistance to those that are currently efficient.

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