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RESEARCH ARTICLE

Antimicrobial Effect of Essential Oils on Multidrug-Resistant *Salmonella typhimurium* in Chicken Fillets

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A B S T R A C T

Chicken meat, which could be a healthy and nutritionally food, is regrettably incriminated as a source of *Salmonella typhimurium* which has a great ability to cause human salmonellosis. This study aimed to assess the prevalence of multidrug-resistant *S. typhimurium* in chicken meat and the effects of essential oils on its viability. A total of 300 chicken meat and its products samples were streaked on XLD agar plates, which was followed by identification of the isolates based on biochemical and serological tests. Ten isolates were serotyped as *S. typhimurium* then assayed for susceptibility to 14 antimicrobials by the single diffusion method. Eight isolates (80%) showed multiple antimicrobial resistance (MAR) for 3 or more antimicrobials with MAR index of 0.4857 in average. Serotyped *S. typhimurium* strain with the highest antimicrobial resistance, confirmed by 16s RNA sequencing, was selected for studying the effects of thyme, oregano, and lemon essential oils with concentrations of 0.5, and 1% on its viability after inoculating into chicken fillets by intensity of 3.0×10^6 and on sensory traits of chicken fillets on 2nd, 4th, 6th, and 8th days of inoculation during cold storage (4ºC). All results showed a significant reduction of *S. typhimurium* counts with highest inhibition obtained using 1% lemon essential oil. The sensory properties of treated chicken fillets were improved by all used essential oils, compared to the control samples after 6th day, and 8th day of the storage period. The samples treated with 0.5, and 1% lemon essential oil revealed the highest improvement of sensory attributes. This study proved that the majority of *S. typhimurium* existing in chicken meat are Multidrug-resistant and have no negative effect on sensory traits, hence, posing a public health hazard. Natural essential oils have, also, great antimicrobial effect on *S. typhimurium*, thus it could replace chemical antimicrobials.

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INTRODUCTION

Besides being a healthy, nutritionally valuable food, chicken meat can also act as a source of food-borne pathogens (Kahraman *et al.,* 2016; Heredia and García 2018). Chicken is one of the most important *Salmonellae* reservoirs that can be transmitted to humans through the food chain, making the food chain from farm to table unsafe (Swaggerty *et al.,* 2019). *Salmonellae* are most commonly isolated bacterial agents of food-borne disease outbreaks. Although over 2610 *Salmonellae* serovars have been identified, human infections have been caused by a few serotypes with *S. typhimurium* being one of the most common (Mezal *et al.,* 2013).

Among healthy humans, the infectious dose is usually 10^6 to 10^8, but in some circumstances, and in infants and the elderly, lower bacterial counts can cause disease (Chen *et al.,* 2013). While uncommon, life-threatening invasive infections with bacteraemia (5-10% of infected individuals) and/or other extra-intestinal infections can occur, especially affecting the risk groups (infants, elderly, and immunocompromised patients) (Crump *et al.,* 2015). Effective antimicrobial agents are necessary in severe cases. *Salmonella*'s strains that are antimicrobial-resistant are especially threatening because they may compromise the effective treatment of human salmonellosis (Berrang *et al.,* 2006).

Multidrug-resistant *S. typhimurium* rose and became a significant health concern. One of the contributing factors to the widespread emergence of multidrug-resistant *S. typhimurium* has been the indiscriminate prophylactic and therapeutic use of antimicrobials in food animals (Angulo *et al.,* 2004).

Reducing antibiotic use and developing natural, safe, sustainable intervention strategies, such as, the use of essential oils (EOs), might help to stem antibiotic resistance. Essential oils are aromatic oily liquids obtained from plants having diverse components with antimicrobial activity, notably phenolic compounds. EOs' antimicrobial activity has been investigated successfully *in vitro* against various gram-positive and gram-negative pathogens, including *S. typhimurium* (Burt, 2004).

Misuse of antimicrobials in humans and animals is a major health concern in Egypt, resulting in the emergence of multidrug-resistant serotype of *Salmonella*. The current research was therefore intended to investigate the prevalence of multidrug-resistant *S. typhimurium* being one of the most frequently isolated *Salmonella* serovars from chicken meat and its products with a trial to control using essential oils.

MATERIALS AND METHODS

Collection of Samples: Total 300 samples of chicken cuts (raw thigh, frozen thigh, raw breast, and frozen breast), chicken giblets (gizzard, liver, and heart), and chicken products (pane, luncheon, and burger) were collected. Thirty samples from each category were collected from different poultry shops, and supermarkets having different sanitation levels in Mansoura city, Egypt. The collected samples were packed, identified, transferred immediately as possible in an icebox, and processed in the Laboratory of Animal Health Research Institute, Mansoura lab.

Isolation and Identification of *S. typhimurium*: *S. typhimurium* was isolated and identified according to the established protocols (Lapage, 1976; Vassiliadis, 1983; Popoff *et al.,* 1997). All samples were streaked on XLD agar plates. Suspected isolates of *Salmonella* were biochemically identified then Serotyped according to Kauffman White scheme for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co., Japan).

Antimicrobial resistance profile and 16S rRNA gene sequencing: The single diffusion method for *S. typhimurium* was used for antimicrobial susceptibility (Srivani, 2011). Sensitivity discs with variable concentrations were used to determine the susceptibility of the isolated *S. typhimurium* strains (Oxoid Limited, Basingstoke, Hampshire, UK). Multiple Antibiotic Resistance (MAR) index for each strain was determined as following (Singh *et al.,* 2010):

MAR index = No. of resistance / Total No. of tested antibiotics

S. typhimurium strain with the highest antimicrobial resistance was selected for molecular confirmation by performing 16S rRNA gene sequencing. 16S rRNA gene of selected isolate was amplified using the universal primers 27f and 1492r (Goda *et al.,* 2010). Amplicons of 16S rRNA amplification (~1500 bp) were sequenced and submitted to NCBI for obtaining GenBank accession number. The sequence was subjected to online software CLUSTAL O (1.2.4) for sequence alignment and the aligned sequence was processed to draw the phylogenetic tree.

Preparation of samples, essential oils, and *S. typhimurium* strain for studying the antibacterial effect of different essential oils on *S. typhimurium*: Approximately, 3.5 Kg of chicken fillet samples were divided into 7 groups, and 5 samples represented each group (100g for each). The first group was prepared as control (Untreated group) and the other 6 groups were treated individually with thyme, oregano, and lemon essential oils at two concentrations (0.5 and 1%). The tested essential oils were obtained in pure form from the Faculty of Agriculture, Benha University.

The molecularly confirmed strain of *S. typhimurium* (MNTAH1) in this study with MK680211.1 accession number was grown on XLD agar plates. Accurately, five colonies of the tested strain were picked up and inoculated into tubes containing 0.1% sterile peptone water (5 mL) and incubated at 37°C for 24 h from which dilutions up to 10^7 were prepared. Further, such dilutions were cultivated on XLD plates to determine the cell concentration. The cell count was adjusted to 3×10^6 cfu/ mL by tube dilution method (Kantachote and Charernjiratrakul, 2008). Such count was used as an infective dose inoculated into chicken fillet samples.

Inoculation of chicken fillets with *S. typhimurium*: At room temperature (25ºC), the fillet samples were dipped in 150 mL of sterile peptone water (0.1%) containing *S. typhimurium* at a dose of 3×10^6 cfu/ mL for 15 min. After dipping, the fillet samples were kept at room temperature for 30 minutes to allow attachment and absorption of bacteria (Tassou and Nychas, 1995). *S. typhimurium* was enumerated to get the initial load before dipping in the prepared essential oils.

The inoculated samples with known *S. typhimurium* count (3×10^6) were divided into 7 groups (100 gm of each). All the control and treated groups were dipped at room temperature (25ºC) for 30 minutes then removed and properly packed in polythene bags, labelled, cold stored at 4±1°C in refrigerator, and subjected to bacteriological and sensory examinations. Sensory evaluation (overall acceptability) and *S. typhimurium* counts were conducted at 0, 2, 4, 6, and 8 days. Actually, the experiment was performed 5 times for each trial.

Sensory Evaluation: The examined samples of chicken fillets were analysed for the quantification of the final sensory profile according to procedures of the World's Poultry Science Association (1987). Five trained panellists applied the proposed organoleptic method of raw chicken meat analysis. The different attributes were quantified on a rating scale from 1 to 3. The sensory evaluation was performed in the form of External aspect (Slime), odour, colour, and muscular elasticity, where samples from different treatments were randomly

subjected to judgment by 5 trained panellists. Overall impression was judged where 1-4 corresponded to ''Unacceptable", 4-8 to ''Acceptable", and 8-12 to "Excellent". Each mark was described in the supplementary materials. Differences between the variables were tested for significance by one-way ANOVA with Tukey's post-test using SPSS ver. 25. Differences at $P<0.05$ were significant.

RESULTS

The results of present study showed that the *S. typhimurium* harbours in all different chicken cuts and products, except frozen breast, heart, and burger. The obtained results in Table 1 revealed that examination of raw chicken thigh, frozen raw chicken thigh, raw chicken breast, raw chicken gizzard, raw chicken liver, chicken pane, and chicken luncheon appeared to harbour *S. typhimurium* with percentages of 10, 3.33, 3.33, 6.67, 3.33, 3.33 and 3.33%, respectively. *S. typhimurium* was not found in each of frozen raw chicken breast, raw chicken heart, and chicken burger.

Table 1: Incidence of isolated *S. typhimurium* strains from the examined chicken meat and its products samples.

Samples	Incidence of *S. typhimurium*		
	Number of examined samples	Number of positive samples	Percentage of positive samples
Raw thigh	30	3	10%
Frozen thigh	30	1	3.33%
Raw breast	30	1	3.33%
Frozen breast	30	-	-
Gizzard	30	2	6.67%
Liver	30	1	3.33%
Heart	30	-	-
Pane	30	1	3.33%
Luncheon	30	1	3.33%
Burger	30	-	-
Total	300	10	3.33%

Table 2: Antimicrobial susceptibility of *S. typhimurium* strains isolated from the examined chicken meat and its products samples (n=10)

Antimicrobial agents	Susceptible		Resistant	
	No	%	No	%
Streptomycin (S)	-	-	10	100
Erythromycin (E)	-	-	10	100
Norocillin (NO)	2	20	8	80
Cephalothin (CN)	3	30	7	70
Penicillin G (P)	3	30	7	70
Nalidixic acid (NA)	4	40	6	60
Cephradine (CE)	5	50	5	50
Sulphamethoxazol (SXT)	6	60	4	40
Clindamycin (CL)	6	60	4	40
Tetracycline (T)	8	80	2	20
Ampicillin (AM)	8	80	2	20
Amikacin (AK)	9	90	1	10
Doxycycline (DO)	9	90	1	10
Gentamicin (G)	9	90	1	10

The isolated 10 *S. typhimurium* strains were assayed for susceptibility to 14 antimicrobials as displayed in Table 2. The antimicrobial resistance percentages for the *S. typhimurium* isolates were the highest for streptomycin and erythromycin (100% each) followed by norocillin (80%), cephalothin (70%), penicillin G (70%), nalidixic acid (60%), cephradine (50%), sulfamethoxazole (40%), clindamycin (40%), tetracycline (20%), ampicillin (20%), amikacin (10%), doxycycline (10%) and gentamicin (10%) as shown in Table 3.

Out of *S. typhimurium* 10 isolates, 8 (80%) showed multiple antimicrobial resistance (MAR) for 3 or more antimicrobials. It was cleared that the MAR index ranged from 1 to 0.143 with an average of 0.4857.

Sequencing results of the 16S rRNA gene amplicons from the selected highly resistant *S. typhimurium* isolate demonstrated 99.8% identity with *S. typhimurium* strain MNTAH1 which published under MK680211.1 accession number in GenBank and the phylogenetic tree was shown in Fig. 1.

The antibacterial activity of thyme, oregano, and lemon essential oils (0.5%) on viability of *S. typhimurium* inoculated into chicken fillets by intensity of 3.0×10^6. At the control group, *S. typhimurium* counts were 2.92×10^6, 2.84×10^6, 2.79×10^6, and 2.76×10^6 cfu/g with reduction percentages of 2.7, 5.3, 7 and 8% after 2nd day, 4th day, 6th day, and 8th day of inoculation, respectively. By using 0.5% thyme essential oil, *S. typhimurium* counts were 2.16×10^6, 1.31×10^6, 6.62×10^5, and 4.17×10^4 cfu/g with reduction percentages of 28, 56.33, 77.93, and 98.61% after 2nd day, 4th day, 6th day, and 8th day of inoculation, respectively. While, by using 0.5% oregano essential oil, *S. typhimurium* counts were 1.44×10^6, 7.98×10^5, and 9.63×10^3 cfu/g with reduction percentages of 52, 73.4, and 99.7 after 2nd day, 4th day, and 6th day of inoculation, respectively, but the count of *S. typhimurium* was not detected after 8th day. By using 0.5% lemon essential oil, *S. typhimurium* counts were 9.81×10^5, and 4.49×10^4 cfu/g with reduction percentages of 67.3 and 98.5% after 2nd day, and 4th day of inoculation, respectively, but the counts of *S. typhimurium* were not detected after 6th day, and 8th day as shown in Table 4.

The antibacterial activity of thyme, oregano, and lemon essential oils (1%) on viability of *S. typhimurium* inoculated into chicken fillets by intensity of 3.0×10^6. At the control group, *S. typhimurium* counts were 2.92×10^6, 2.84×10^6, 2.79×10^6, and 2.76×10^6 cfu/g with reduction percentages of 2.7, 5.3, 7, and 8 after 2nd day, 4th day, 6th day, and 8th day of inoculation, respectively. By using 1% thyme essential oil, *S. typhimurium* counts were 1.95×10^6, 5.21×10^5, and 1.73×10^4 cfu/g with reduction percentages of 35, 82.6, and 99.4% after 2nd day, 4th day, and 6th day of inoculation, respectively, but the count of *S. typhimurium* was not detected after 8th day. While, by using 1% oregano essential oil, *S. typhimurium* counts were 8.53×10^5, and 6.07×10^4 cfu/g with reduction percentages of 71.6, and 98 after 2nd day, and 4th day of inoculation, respectively, but the counts of *S. typhimurium* were not detected after 6th day, and 8th day. By using 1% lemon essential oil, *S. typhimurium* counts were 7.12×10^4, and 1.30×10^3 cfu/g with reduction percentages of 97.6%, and 99.9% after 2nd day, and 4th day of inoculation, respectively, but the counts of *S. typhimurium* were not detected after 6th day, and 8th day as shown in Table 5.

The results showed the sensory properties of different treated chicken fillets samples during cold storage (4 ºC) were improved by using both 0.5 and 1% concentrations of thyme, oregano, and lemon essential oils, compared to the control samples after 6th day, and 8th day of the storage period. The samples treated with 0.5 and 1% lemon essential oil revealed the highest improvement of sensory attributes, while the samples treated with 1% oregano essential oil demonstrated the lowest one as shown in Table 6.

Table 3: Antimicrobial resistance profile of *S. typhimurium* strains isolated from the examined chicken meat and its products samples (n=10)

No	*Salmonella* strains	Antimicrobial resistance profile	MAR index
1	*S. typhimurium*	S, E, NO, CN, P, NA, CE, SXT, CL, T, AM, AK, DO, G	1
2	*S. typhimurium*	S, E, NO, CN, P, NA, CE, SXT, CL, T, AM	0.786
3	*S. typhimurium*	S, E, NO, CN, P, NA, CE, SXT, CL	0.643
4	*S. typhimurium*	S, E, NO, CN, P, NA, CE, SXT, CL	0.643
5	*S. typhimurium*	S, E, NO, CN, P, NA, CE	0.500
6	*S. typhimurium*	S, E, NO, CN, P, NA	0.428
7	*S. typhimurium*	S, E, NO, CN, P	0.357
8	*S. typhimurium*	S, E, NO	0.214
9	*S. typhimurium*	S, E	0.143
10	*S. typhimurium*	S, E	0.143
		Average 0.4857	

S: Streptomycin, P: Penicillin-G, CL: Clindamycin, E: Erythromycin, NA: Nalidixic acid, T: Tetracycline, NO: Norocillin, CE: Cephradine, AM: Ampicillin, CN: Cephalothin, SXT: Sulphamethoxazol, AK: Amikacin, DO: Doxycycline, G: Gentamicin, MAR: Multiple Antibiotic Resistance.

Table 4: Antibacterial activity of various essential oils (0.5%) on the viability of *S. typhimurium* inoculated into chicken fillets by intensity of 3.0×10^6 (n=5)

Treatment	Control		Thyme oil		Oregano oil		Lemon oil	
Storage time	Count	R %	Count	R %	Count	R %	Count	R %
Zero time	$3.0\times10^6\pm0.28\times10^{6a}$	--	$3.0\times10^6\pm0.28\times10^{6a}$	--	$3.0\times10^6\pm0.28\times10^{6a}$	--	$3.0\times10^6\pm0.28\times10^{6a}$	--
2nd day	$2.92\times10^6\pm0.25\times10^{6b}$	2.7	$2.16\times10^6\pm0.19\times10^{b}$	28.00	$1.44\times10^6\pm0.15\times10^{6a}$	52.0	$9.81\times10^5\pm1.46\times10^{5c}$	67.3
4th day	$2.84\times10^6\pm0.22\times10^{6b}$	5.3	$1.31\times10^6\pm0.14\times10^{6a}$	56.33	$7.98\times10^5\pm1.32\times10^{5d}$	73.4	$4.49\times10^4\pm0.70\times10^{4c}$	98.5
6th day	$2.79\times10^6\pm0.23\times10^{6a}$	7.0	$6.62\times10^5\pm1.05\times10^{b}$	77.93	$9.63\times10^3\pm1.57\times10^{3c}$	99.7	ND	--
8th day	$2.76\times10^6\pm0.18\times10^{6a}$	8.0	$4.17\times10^4\pm0.82\times10^{b}$	98.61	ND	--	ND	--

R %= Reduction % D= Not detected

Table 5: Antibacterial activity of various essential oils (1%) on viability of *S. typhimurium* inoculated into chicken fillets by intensity of 3.0×10^6 (n=5).

Treatment	Control		Thyme oil		Oregano oil		Lemon oil	
Storage time	Count	R %	Count	R %	Count	R %	Count	R %
Zero time	$3.0\times10^6\pm0.28\times10^{6a}$	------	$3.0\times10^6\pm0.28\times10^{6a}$	------	$3.0\times10^6\pm0.28\times10^{a}$	------	$3.0\times10^6\pm0.28\times10^{a}$	------
2nd day	$2.92\times10^6\pm0.25\times10^{6b}$	2.7	$1.95\times10^6\pm0.17\times10^{6a}$	35.0	$8.53\times10^5\pm1.64\times10^{d}$	71.6	$7.12\times10^4\pm1.39\times10^{c}$	97.6
4th day	$2.84\times10^6\pm0.22\times10^{6b}$	5.3	$5.21\times10^5\pm0.80\times10^{5c}$	82.6	$6.07\times10^4\pm0.54\times10^{c}$	98.0	$1.30\times10^3\pm0.11\times10^{a}$	99.9
6th day	$2.79\times10^6\pm0.23\times10^{6a}$	7.0	$1.73\times10^4\pm0.16\times10^{4a}$	99.4	ND	------	ND	------
8th day	$2.76\times10^6\pm0.18\times10^{6a}$	8.0	ND	------	ND	------	ND	------

R %= Reduction % ND= Not detected

Table 6: Influence of addition of essential oils on sensory traits of the examined samples of chicken fillets (n=5)

Treatments		External aspect (3)	Odor (3)	Color (3)	Muscular elasticity (3)	Overall Score (12)	Sensorial Quality
Control	0 Time	3±0 a	3±0 a	3±0 a	3±0 a	12±0 a	Excellent
	2nd D	2.8±0.2 c	2.8±0.2 c	3±0 b	2.8±0.2 a	11.4±0.4 c	Excellent
	4th D	2.4±0.24 c	2.2±0.2 b	2.6±0.24 b	2.4±0.24 a	9.6±0.4 b	Excellent
	6th D	1.6±0.24 a	1.6±0.24 a	2±0 b	1.8±0.2 a	7±0.32 a	Acceptable
	8th D	1±0 a	1±0 a	1.4±0.24 a	1±0 a	4.4±0.24 a	Acceptable
0.5% Thyme oil	0 Time	3±0 a	3±0 a	3±0 a	3±0 a	12±0 a	Excellent
	2nd D	3±0 d	2.6±0.24 b	3±0 b	3±0 b	11.6±0.24 d	Excellent
	4th D	3±0 d	2.4±0.24 c	3±0 c	3±0 d	11.4±0.24 d	Excellent
	6th D	3±0 d	2.2±0.37 c	3±0 e	2.8±0.2 e	11±0.55 e	Excellent
	8th D	2.6±0.24 c	1.2±0.2 b	2.2±0.2 c	2.4±0.24 e	8.4±0.4 d	Excellent
0.5% Oregano oil	0 Time	3±0 a	3±0 a	3±0 a	3±0 a	12±0 a	Excellent
	2nd D	2.6±0.24 b	2.6±0.24 b	3±0 b	3±0 b	11.2±0.2 b	Excellent
	4th D	2.2±0.37 b	2.4±0.24 c	3±0 c	3±0 d	10.6±0.6 c	Excellent
	6th D	1.8±0.37 b	2±0.32 b	3±0 e	2.8±0.2 e	9.6±0.6 d	Excellent
	8th D	1.2±0.2 b	1.4±0.24 c	2.2±0.2 c	2.4±0.24 e	7.2±0.37 c	Acceptable
0.5% Lemon oil	0 Time	3±0 a	3±0 a	3±0 a	3±0 a	12±0 a	Excellent
	2nd D	3±0 d	3±0 d	2.6±0.24 a	3±0 b	11.6±0.24 d	Excellent
	4th D	3±0 d	3±0 d	2.6±0.24 b	2.8±0.2 c	11.4±0.24 d	Excellent
	6th D	3±0 d	3±0 d	2.4±0.24 c	2.6±0.24 d	11±0.32 e	Excellent
	8th D	2.8±0.2 d	2.6±0.24 d	1.6±0.24 b	2.4±0.4 e	9.4±0.5 e	Excellent
1% Thyme oil	0 Time	3±0 a	3±0 a	3±0 a	3±0 a	12±0 a	Excellent
	2nd D	3±0 d	2.4±0.24 a	3±0 b	2.8±0.2 a	11.2±0.37 b	Excellent
	4th D	3±0 d	2±0.32 a	3±0 c	2.6±0.24 b	10.6±0.4 c	Excellent
	6th D	2.8±0.2 c	1.6±0.24 a	2.8±0.2 d	2.2±0.37 b	9.4±0.51 c	Excellent
	8th D	2.6±0.24 c	1±0 a	2.2±0.2 c	1.8±0.2 b	7.6±0.51 c	Acceptable
1% Oregano oil	0 Time	3±0 a	3±0 a	3±0 a	3±0 a	12±0 a	Excellent
	2nd D	2.4±0.24 a	2.6±0.24 b	2.6±0.4 a	3±0 b	10.6±0.51 a	Excellent
	4th D	2±0.32 a	2.2±0.2 b	2±0.45 a	2.8±0.2 c	9±0.45 a	Excellent
	6th D	1.8±0.37 b	1.6±0.24 a	1.8±0.2 a	2.4±0.24 c	7.6±0.51 b	Acceptable
	8th D	1.2±0.2 b	1.2±0.2 b	1.6±0.24 b	2.2±0.2 d	6.2±0.2 b	Acceptable
1% Lemon oil	0 Time	3±0 a	3±0 a	3±0 a	3±0 a	12±0 a	Excellent
	2nd D	3±0 d	3±0 d	2.6±0.24 a	3±0 b	11.6±0.24 d	Excellent
	4th D	3±0 d	3±0 d	2.6±0.24 b	2.8±0.2 c	11.4±0.24 d	Excellent
	6th D	3±0 d	3±0 d	2.4±0.24 c	2.6±0.24 d	11±0.32 e	Excellent
	8th D	2.8±0.2 d	2.8±0.2 e	1.4±0.24 a	2±0.45 c	9±0.45 e	Excellent

1->4: Unacceptable; 4-> 8: Acceptable; 8- 12: Excellent.

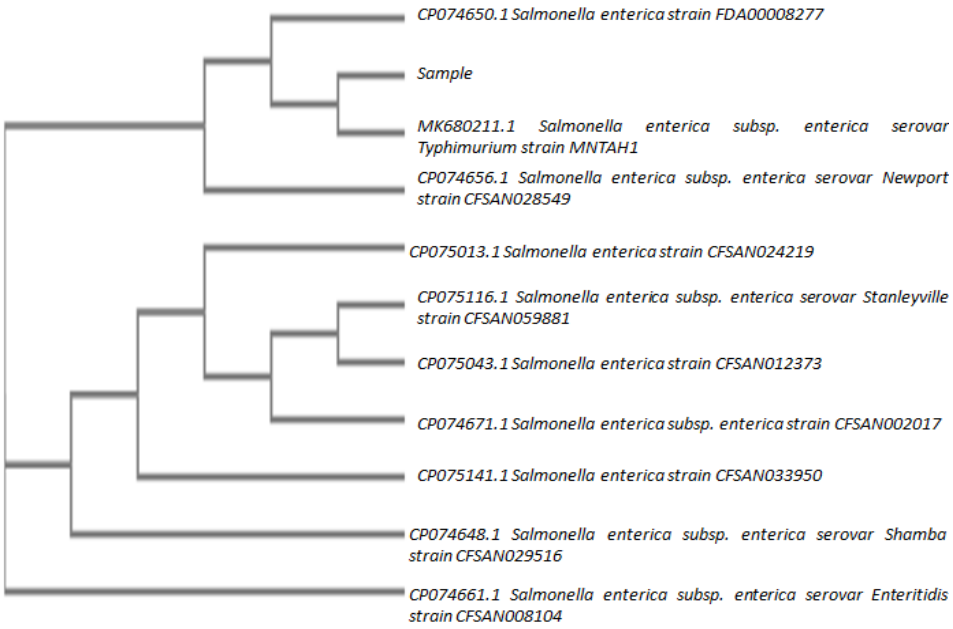

Fig. 1: Phylogenic tree of the *S. typhimurium* isolate detected in the current study and other related isolates in gene bank.

Table 7: Correlation between *S. typhimurium* Count and Overall sensory score of chicken fillets under effect of various essential oils treatments in refrigerator storage

		0 Time	2nd D	4th D	6th D	8th D	Correlation coefficient
0.5% Thyme oil	Overall SS (12)	12	11.6	11.4	11	8.4	0.827163
	S. typhimurium Count	3.0×10^{6}	2.16×10^{6}	1.31×10^{6}	6.62×10^{5}	4.17×10^{4}	
0.5% Oregano oil	Overall SS (12)	12	11.2	10.6	9.6	7.2	0.6257611
	S. typhimurium Count	3.0×10^{6}	1.44×10^{6}	7.98×10^{5}	9.63×10^{3}	ND	
0.5% Lemon oil	Overall SS (12)	12	11.6	11.4	11	9.4	0.629611
	S. typhimurium Count	3.0×10^{6}	9.81×10^{5}	4.49×10^{4}	ND	ND	
1% Thyme oil	Overall SS (12)	12	11.2	10.6	9.4	7.6	0.8515376
	S. typhimurium Count	3.0×10^{6}	1.95×10^{6}	5.21×10^{5}	1.73×10^{4}	ND	
1% Oregano oil	Overall SS (12)	12	10.6	9	7.6	6.2	0.842889
	S. typhimurium Count	3.0×10^{6}	8.53×10^{5}	6.07×10^{4}	ND	ND	
1% Lemon oil	Overall SS (12)	12	11.6	11.4	11	9	0.4855317
	S. typhimurium Count	3.0×10^{6}	7.12×10^{4}	1.30×10^{3}	ND	ND	

SS= Sensory score. ND= Not Detected.

The results also shown varying degrees of positive correlation between *S. typhimurium* count and sensory score of chicken fillets under effect of various essential oils treatments in refrigerator storage as shown in Table 7.

DISCUSSION

Chicken meat and its products are exposed to contamination with *Salmonella* from many sources, primary during pre-processing, and processing steps and secondary after processing through packaging, marketing and storage. This contamination may render it harmful to consumers.

Several previous reports found different *S. typhimurium* incidences in chicken meat, and lower incidences of *S. typhimurium* have also been reported (Zaki and Hadad, 2019).

Meanwhile, multiple antimicrobial resistant *S. typhimurium* is recognized as an environmental hazard to the food supply and human health (Berrang *et al.*, 2006; Medeiros *et al.*, 2011; and Abd-Elghany *et al.*, 2015). The current results may be linked to the widespread use of several antimicrobials in the mass production of chickens for treatment, prophylaxis, and/or promotion of growth (Agyare *et al.*, 2019). Resistant *S. typhimurium* can be transmitted from animals to humans through the ingestion of contaminated food and thus pose a public health risk.

Regarding our findings, similar results were reported by Medeiros *et al.* (2011), and Soomro *et al*, (2011) with *S. typhimurium* isolates resistant to streptomycin (100%), ampicillin (83.3%), tetracycline (38.9%), nalidixic acid (11.1%), and gentamicin (11.1%). Inversely, previous reports found that *S. typhimurium* had a complete resistance to streptomycin, erythromycin, sulfamethoxazole, oxytetracycline, amoxicillin, penicillin, and nalidixic acid, albeit within a lower (4%) gentamicin resistance (Abd-Elghany *et al.*, 2015). A different study reported differences in the anti-bacteriogram pattern of the chicken (Li *et al.*, 2017).

Results in this study showed the reduction percentages of *S. typhimurium* counts obtained by treatment with different concentrations of thyme, oregano, and lemon essential oils. All results showed a significant reduction of *S. typhimurium* counts with the highest inhibition obtained using 1% lemon essential oil. Essential oils with the strong antibacterial properties against food-borne pathogens contain an exorbitant number of phenolic compounds (Burt, 2004; Chouhan *et al.*, 2017). It would thus appear reasonable to have a similar antimicrobial mechanism of action with other phenolic compounds based on their ability to disintegrate the outer membrane of Gram-negative bacteria, release lipopolysaccharides and increase cytoplasmic membrane permeability to ATP (Lambert *et al.*, 2001). The composition of essential oils is

based on a variety of factors like extraction method, harvesting season, and geographic sources (Sadeh *et al.*, 2019). The strongest antimicrobial action has been demonstrated in essential oils produced from the herbs harvested during or after flowering (Burt, 2004). This may explain the findings obtained from this study, which may be attributed to differences in the quantity of phenolic compounds in used essential oils with the highest quantity in lemon essential oil followed by essential oils of oregano and thyme, respectively.

In details, thyme oil exhibits excellent inactivation effect against *Salmonella* (Mazzarrino *et al.*, 2015). And therefore, the application of thyme oil in food preservation is highly utilized. Likewise, oregano essential oils have been reported to have antimicrobial activity against a variety of Gram-positive, Gram-negative bacteria, yeasts and molds *in vitro* conditions (Viuda-Martos *et al.*, 2007). The efficacy of oregano essential oil has been tested in food systems such as meat, taramasalad, and aubergine salad (Skandamis *et al.*, 2002). Furthermore, the effects of essential oils and their components on bacterial growth and survival have been studied for many years. The components of citrus essential oils carvacrol, citral, and geraniol had strong activity against *S. typhimurium* and its rifampicin resistant mutant *in vitro*. The fractions were more active against all bacteria than were the oils themselves. These citrus oils did not reduce populations of *S. Senftenberg*, but lime terpeneless, terpineol, and geraniol reduced it 100%. Lime terpeneless oil, orange terpeneless oil, lemon terpeneless oil, and d-limonene reduced growth from 100 to 50% ranked from the most to the least (O'Bryan *et al.*, 2008).

Decreasing *S. typhimurium* count is not responsible for increasing sensory score and that it does not express itself by changing chicken meat sensory characteristics. Herein lies its great hazard to public health, as it is not possible for the consumer to recognize its presence physically. A previous result found that a value-added dried meat product obtained by using oregano essential oil to enhance food safety received an acceptable sensory response from consumers (Hernández *et al.*, 2017).

Preservative effects of these essential oils may be due to their antioxidant effect in agreement with Fratianni *et al.* (2010) who recorded minimal chicken meat oxidative deterioration of fatty acids during the first 4 days of storage and increased at 8th of storage with essential oils treatment. These essential oils, also, inhibit food spoilage microorganisms (Gutierrez *et al.*, 2009). Oregano essential oil inhibited the behaviour of *S. typhimurium* in sterile and naturally contaminated beef fillets stored under aerobic, modified atmosphere consisting of and a vacuum packaged environment during storage at 5°C (Skandamis *et al.*, 2002).

Conclusions: We aimed to evaluate occurrence of multidrug-resistant *S. typhimurium* in chicken meat, to study the effects of different essential oils on its viability. Results of the current study showed the potential importance of chickens as a source of multiple antimicrobial-resistant *S. typhimurium* for human infections. Essential oils of thyme, oregano, and lemon, mainly the essential oil of lemon, were effective in mitigating the growth of *S. typhimurium* attached to chicken breast. Most importantly, the tested essential oils, not only inhibited *S. typhimurium* inoculated into chicken fillets but also improved their sensory features within 8 days of cold storage. A 1% lemon essential oil was found to be the greatest one with a soundest antimicrobial and acceptable sensory score. We demonstrated that the natural essential oils could displace chemical antimicrobials.

Authors contribution: Alaa Eldin M.A. Morshdy and Mohamed A. Hussein conceived and designed the study. Boshra M. Nahla; Saleh Shafik; Alaa Eldin M.A. Morshdy, and Mohamed A. Hussein executed the experiment. Mohamed A. Hussein; Alaa Eldin M.A. Morshdy, and Boshra M. Nahla analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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