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Original Research Article

PREVALENCE OF *Staphylococcus aureus* AND *Salmonella* SPECIES IN RETAILED CHICKEN MEAT WITH A REDUCTION TRIAL USING *Nigella sativa* AND ROSEMARY ESSENTIAL OILS

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Abstract: Chicken meat represents an importance source of animal derived proteins, vitamins, and minerals. However, chicken meat might act as a potential source of human exposure to foodborne pathogens such as *Staphylococcus aureus (S. aureus)* and *Salmonella* species. The objectives of the present study were first to investigate the prevalence rates of *S. aureus* and *Salmonella* species in the retailed chicken breast and thigh muscles at Zagazig city, Egypt. Second, serological identification of the isolated bacteria was followed. Third, screening of *S. aureus* enterotoxin coding genes (*sea, seb, sec, sed,* and *see*) and methicillin resistance (*mecA*) gene, as well as *Salmonella* virulence associated genes including *Salmonella hyper-invasive locus* (*hilA*)*,* and *Salmonella* enterotoxin (*stn*) was done using PCR. Thereafter, the inhibitory effects of *Nigella sativa*, and rosemary essential oils were investigated against *S. aureus*, and *Salmonella* Typhimurium. The obtained results revealed isolation of *S. aureus* from the examined breast and thigh muscles at percentages of 23.3%, and 26.6%, respectively, whereas percentages of *Salmonella* spp. isolation from both samples were 33.3%, and 16.6%, respectively. Four *Salmonella* serotypes namely, *S.* Typhimurium, *S.* Enteritidis, *S.* Kentucky, and *S.* Anatum were further identified. The recovery rates of *S.* Typhimurium from breast and thigh muscles were 23.33% and 13.33%, respectively followed by *S.* Enteritidis (3.33% each). Staphylococcus enterotoxin genes *(sea,* and *see*), and *mecA* gene were detected in *S. aureus* isolates. Besides, Salmonella *hilA* and *stn* genes were also detected in *Salmonella* Typhimurium isolates. *Nigella sativa* and rosemary essential oils at 0.1%, and 0.5% could significantly reduce *S. aureus*, and *Salmonella* Typhimurium in chicken breast meat.

Key words: chicken muscles; *Staphylococcus aureus; Salmonella* species; *Nigella sativa*; rosemary essential oils

Introduction

Chicken meat is regarded as an important source of animal-derived protein, essential amino acids, polyunsaturated fatty acids, vitamins, and minerals. Economically, chicken meat plays an important role in solving food security issues related to the shortage in red meat industry because of its wide availability, and relatively low price compared with the red meat (1, 2).

Microbial contamination of chicken meat might take place during any step of the manufacture process starting from the slaughter, defeathering, deplucking, evisceration, distribution, and storage. Therefore, chicken meat is regarded as a potential source for spreading of food poisoning pathogens such as *Staphylococcus aureus* (*S. aureus*) and *Salmonella* spp. (3, 4).

Ingestion of foods contaminated with *S. aureus*-enterotoxins is the major cause of food poisoning cases that characterized by their rapid onset (1-6 hours post ingestion of contaminated foods), nausea, vomiting, abdominal cramps, and diarrhea (5).

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S. aureus and staphylococcal enterotoxins (SEs) were isolated and detected in retailed chicken giblets in Egypt (1, 6), chicken meat and giblets retailed in US markets (7), and in chicken breast and thigh retailed in Cambodian markets (8).

Non-typhoidal salmonellosis is a foodborne disease caused by ingestion of foods contaminated with *Salmonella* spp. The incubation period of this disease is 12 to 36 h post-ingestion of contaminated foods. The major symptoms of this foodborne infection include nausea, vomiting, fever, severe diarrhea, abdominal cramps, and malaise (9). *Salmonella* spp. was isolated from chicken meat in Vietnam (10), Japan (11), and chicken giblets in Egypt (3).

There is an increasing demand for natural antimicrobials as food preservatives to avoid the undesirable effects of the chemical preservatives. In this regard, plant essential oils offer friendly alternatives for chemical preservatives. Black seed (*Nigella sativa*) essential oil has a documented antimicrobial effect, particularly against *Listeria monocytogenes* (12). Besides, rosemary (*Rosmarinus officinalis L.*) essential oil has recorded antimicrobial activities against several food poisoning microorganisms (13).

Insight of the previous facts, this study was undertaken to investigate the prevalence rates of two food poisoning organisms, including *S. aureus*, and *Salmonella* spp., in the retailed chicken meat (breast, and thigh) in Egypt. Furthermore, detection of enterotoxin coding genes and virulence-associated genes were screened using PCR. In addition, the inhibitory effects of *Nigella sativa*, and rosemary essential oils against *S. aureus*, and *Salmonella* spp. were examined.

Materials and methods

Sampling and samples preparation

Sixty random chicken muscle samples, including breast and thigh muscles (30 each) were collected directly after slaughter from chicken butchery shops with different sanitation levels at Zagazig city, Egypt. The samples were cooled and transferred without delay to Animal Health Research Institute, Zagazig, Egypt for bacteriological examination.

Samples were prepared according to the recommended guidelines (14). In brief, ten grams from each sample were mixed with 90 mL of 1% sterile peptone water (Oxoid CM9, UK) and blended for 2.5 min at 2000 rpm. The mixture was then allowed to stand for 15 min at room temperature.

Isolation and identification of S. aureus

S. aureus isolation and identification were done according to Animal and Plant Health Agency (APHA) (14) procedures. In short, 0.1 mL of each prepared homogenate was spread over a Baird Parker agar (Oxoid, UK) plate supplemented with egg yolk emulsion using a sterile glass spreader. Plates were incubated on inverted positions at 37°C for 48 h. *S. aureus* colonies appear as black, shiny, circular, smooth, and convex with narrow white margin and surrounded by a clear zone extending into the opaque medium. Five suspected *S. aureus* colonies were picked up and purified on nutrient agar (Oxoid, UK) slopes. *S. aureus* colonies were subjected to morphological, biochemical (catalase, coagulase, and mannitol sugar fermentation tests), and serological identification (14).

Isolation and identification of Salmonella species

The procedures of ISO 6579 (15) for *Salmonella* isolation and identification were followed. In brief, ten mL of the previously prepared meat homogenate from each sample were incubated at 37°C for 18 ± 2 h as pre-enrichment on non-selective liquid medium. Selective enrichment was done on Rappaport Vassiliadis soya broth (Oxoid, UK) and incubation at 41.5°C for 24 ± 2 h. A loopful from the previously enriched culture was streaked on the surface of xylose lysine desoxycholate (XLD) agar (Oxoid, UK) plate and incubated 37°C for 24 ± 2 h. Suspected colonies (non-lactose fermenters) were red with or without black centers. Such colonies were purified and sub-cultured onto nutrient agar slopes and incubated at 37°C for 24 h. The purified colonies were subjected to morphological, biochemical (indole, methyl red, Voges Proskauer, citrate utilization, hydrogen sulfide production, and urease, tests), and serological identification (15).

Table 1: Oligonucleotide primer sequences used in the study

Gene	Primer sequence (5'-3')	Product size (bp)	Annealing temperature (°C)	Reference
mecA	GTAGAAATGACTGAACGTCCGATAA CCAATTCCACATTGTTTCGGTCTAA	310	50	(16)
sea	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	50	(17)
seb	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164	50	
sec	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451	50	
sed	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTTCGTTC	278	50	
see	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209	50	
hilA	CGGAAGCTTATTTGCGCCATGCTGAGGTAG GCA TGG ATC CCC GCC GGC GAG ATTGTG	854	60	(18)
stn	TTGTGTCGCTATCAC TGG CAACC ATTCGTAACCCGCTCTCGTCC	617	60	(19)

Molecular identification of Staphylococcal enterotoxins and Salmonella-associated virulence genes

DNA isolation from the cultured and identified bacteria was done using Genomic DNA extraction kit according to the instructions of the manufacturer (Alliance Global, Dubai, UAE). Primer pairs for *S. aureus* enterotoxin genes *(sea, seb, sec, sed* and *see*), methicillin resistance (*mecA*) gene for detecting methicillin resistant *S. aureus* (MRSA), and *Salmonella hilA,* and *stn* virulence associated genes were purchased from Metabion International, Gmbh, Germany, and displayed in Table 1. PCR amplification reactions and cycling conditions were performed (16-19) on a Thermal Cycler (Master cycler, Eppendorf, Germany) using uniplex PCR approaches. Amplified PCR products were run on 1.5% agarose gel electrophoresis (AppliChem, GmbH, Germany) in 1x Tris Borate EDTA (TBE) buffer stained with ethidium bromide then visualized on a UV transilluminator. DNA Ladder (100 bp, Qiagen, GmbH) was used to determine the fragment sizes.

An experimental trial to investigate the inhibitory effects of Nigella sativa and rosemary essential oils against S. aureus and *Salmonella* species

The antibacterial effects of *Nigella sativa*, and rosemary essential oils extracted, prepared, and purchased from National Research Center, Dokki, Giza, Egypt were tested at two concentrations for each (0.1 and 0.5% prepared in pure corn oil (100%)) based on pre-experimental trials (Data are not shown). Two parallel experimental trials were conducted to test the inhibitory effects of these oils, one against *S. aureus*, and the other against *Salmonella* Typhimurium. In the two experiments, microbiologically negative breast muscles were prepared as slices (20). Each slice was four cm width, six cm length, 1.0 cm thickness and 25 g in weight. Five groups for each experiment were planned (n = 3/group). Group 1 was employed as a control, where breast muscles were artificially inoculated with the tested organism (10^7 CFU/mL) (21), group 2 treated with *Nigella sativa* oil 0.1%, group 3 treated with *Nigella sativa* oil 0.5%, group 4 treated with rosemary oil 0.1%, and group 5 treated with rosemary oil 0.5%. Treatment groups were soaked in the tested oils for 30 min at room temperature and examined for bacterial counts (22).

Statistical analysis:

All microbial counts were transferred into base-10 logarithms of CFU/g. Data were analyzed using one-way ANOVA procedure of SPSS v.23 (SPSS Inc., Chicago, Illinois, The USA). Tukey's multiple comparison tests were used to detect significant variations. Data were expressed as means ± SD, with a *P*-value of 0.05 is considered significant.

Results

The results revealed that isolation percentages of *S. aureus* from the examined breast and thigh muscles were 23.3 and 26.6%, respectively, whereas *Salmonella* spp. was isolated from these samples with percentages of 33.3, and 16.6%, respectively (Figure. 1).

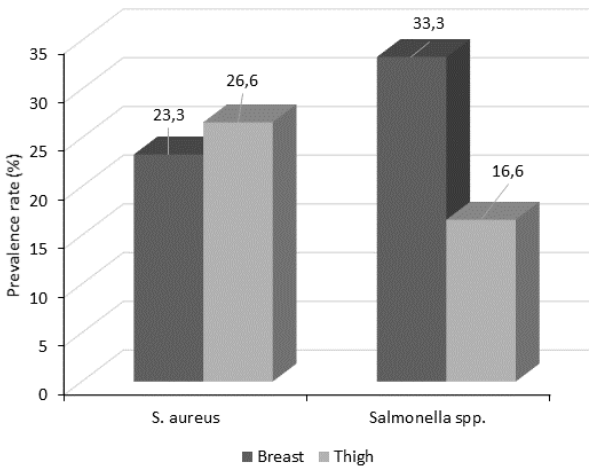

Figure 1: Prevalence rates (%) of *S. aureus* and *Salmonella* spp. in the examined chicken breast and thigh muscles retailed in Zagazig city, Egypt

Four *Salmonella* species were recovered namely, *S.* Typhimurium, *S.* Enteritidis, *S.* Kentucky, and *S.* Anatum. *S.* Typhimurium and *S.* Enteritidis were isolated at 23.33%, and 3.33% from breast muscles; and at 13.33% and 3.33% from thigh muscles. *S.* Kentuckyand *S.* Anatum were isolated only from breast muscles at 3.33% (Table 2).

PCR testing of three randomly selected *S. aureus* isolates for harboring *mecA* and *Staphylococcal* enterotoxins revealed detection of *mecA* in two of the tested isolates and only *sea* and *see* in one *S. aureus* isolate. Similarly, PCR testing of four selected *S.* Typhimurium isolates for harboring *hilA*, and *stn* virulence associated genes revealed detection of *hilA* in two of the isolates; while *stn* was detected in only one *S.* Typhimurium isolate (Data are not shown). These PCR screened isolates were subjected to the two essential oils under study.

Nigella sativa and rosemary essential oils were used to reduce *S. aureus*, and *Salmonella* spp. in an experimental trial using chicken breast meat. The obtained results revealed significant inhibitory effects for the two used oils against *S. aureus* in a concentration-dependent manner and against *Salmonella* spp. without clear effect for the dose (Table 3). At the same time, the used oils did not change the sensory characteristics (blue-whitish color, firm in consistency, and fresh odor) of the breast meat at the two tested oil concentrations (0.1%, and 0.5%) (Data are not shown).

Discussion

Staphylococcus aureus is a major foodborne pathogen (23-25). It is capable of producing heat-stable enterotoxins such as *sea, seb, sec, sed*, and *see*. Moreover, MRSA is responsible for many nosocomial infections worldwide. Herein, *S. aureus* was isolated from retailed chicken breast and thigh at variable rates. In addition, some of the identified *S. aureus* isolates harbored *mecA, sea*, and *see*-coding genes, indicating that chicken breast and thigh muscles might be considered potential sources for enterotoxigenic MRSA. In agreement with the obtained results, toxin producing MRSA were isolated from retailed chicken meat and meat products in Egypt (1, 6), United states (7, 26), and Canada (27).

Table 2: Serological identification of *Salmonella* spp. isolated from breast and thigh muscles

Salmonella serotypes	Breast muscles		Thigh muscles		Antigenic structure		
	No.	%	No.	%		O	H
S. Typhimurium	7	23.33	4	13.33	B	1,4,5,12	i : 1,2
S. Enteritidis	1	3.33	1	3.33	D	1,9,12	g,m : -
S. Kentucky	1	3.33	-	-	E1	8,20	i:Z_6
S. Anatum	1	3.33	-	-	E1	3,10,	e,h : 1,6
Total	10	33.33	5	16.66			

Table 3: Inhibitory effects of *Nigella* sativa and rosemary essential oils against *S. aureus*, and *S.* Typhimurium growth in chicken breast

Experimental trials	*S. aureus*		*S.* Typhimurium	
	Mean ± SD (log 10 cfu/g)	Reduction (%)	Mean ± SD (log 10 cfu/g)	Reduction (%)
Control	8.39 ± 0.01[a]	0	8.18 ± 0.02[a]	0
Nigella sativa 0.1%	6.58 ± 0.09[bc]	21.57	6.09 ± 0.03[b]	25.52
Nigella sativa 0.5%	5.69 ± 0.60[d]	32.10	5.89 ± 0.03[b]	27.92
Rosemary 0.1%	6.81 ± 0.02[b]	18.81	6.06 ± 0.03[b]	25.94
Rosemary 0.5%	6.22 ± 0.01[c]	25.82	5.92 ± 0.05[b]	27.56

Values within the same column carrying different letter are significantly different at $P < 0.05$

Staphylococci can be found on the skin, hair, and nails of food handlers. Additionally, washing water used in the cleaning of chicken carcasses is also considered as a source for MRSA (1). Such sources might explain the isolation of *S. aureus* from retailed chicken products in the present study. Detection of enterotoxins in the identified *S. aureus* isolates agrees with Shawish and Al-Humam (28) who detected at least one of the *S. aureus* enterotoxins (*sea, seb, sec,* and *sed*) in the examined meat products sold in Egypt and Saudi Arabia. *S. aureus* is responsible for many cases of food poisoning worldwide. For instances, Center for Disease prevention and Control (CDC) reported a food poisoning outbreak in a military unit, US, 2012. This outbreak was due to *S. aureus* (29). Furthermore, in Europe, European Food Safety Association reported that 293 food poisoning outbreaks in 2011 were due to *Staphylococcus* spp (30).

Salmonella spp. was isolated from the examined chicken breast and thigh muscles at different rates. The Egyptian legislation prohibits the presence of *Salmonella* spp. in meat, and this reflects the poor hygienic practices adopted during processing of chicken carcasses in chicken retail markets in Egypt (31). In agreement with the recorded results in the present study, high prevalence rates for *Salmonella* spp. in retailed chicken meat were recently reported in Malaysia (32), and Bangladesh (33). Four *Salmonella* serotypes were identified in the present study. Of these serotypes, *S.* Typhimurium and *S.* Enteritidis are major food poisoning agents. These serotypes were responsible for several food poisoning outbreaks worldwide. For instances, *Salmonella* enterica serovar Typhimurium -associated outbreak was reported in Switzerland during May-June 2008 (34). In addition, seven S. Typhimurium outbreaks were reported in three Australian states and territories (35). Two virulence factors namely *Salmonella* enterotoxin (*Stn*) and hyper-invasive locus *(hilA)* coding were detected in the isolated *S.* Typhimurium. *Salmonella* enterotoxin (*Stn*), which is contributed to the pathogenicity process of *Salmonella*, primarily diarrhea had been identified in *Salmonella* serovars Typhi, Typhimurium and Enteritidis (36). The *hilA* gene encodes a regulator that activates the expression of invasion genes in response to both environmental and genetic regulatory factors (37).

There are continuous efforts to find alternatives to the chemical preservatives with antimicrobial activities (38-41). In this regard, a trial to investigate the inhibitory effects of *Nigella sativa*, and rosemary essential oils against *S. aureus* and *Salmonella* spp was performed. Interestingly, the two used essential oils had significant inhibitory effects against the examined microorganisms. In agreement with these findings, rosemary oil significantly retarded growth of *Listeria monocytogenes* in mozzarella cheese (42). Besides, *Nigella sativa* oil had significant antibacterial activities against Gram-positive bacteria (43).

Conclusion

The obtained results in the current investigation revealed isolation of *S. aureus*, and *Salmonella* spp. from retailed chicken breast and thigh muscles at variable rates. This indicates unsatisfactory hygienic measures adopted during slaughtering, defeathering, and processing of chicken carcasses. Therefore, strict hygienic procedures should be followed in chicken processing plants. In addition, using *Nigella sativa* and rosemary es-

sential oils at 0.1%, and 0.5% is of value in reducing the microbial load of chicken carcasses, particularly *S. aureus*, and *Salmonella* spp.

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References

1. Darwish WS, Atia AS, Reda LM, et al. Chicken giblets and wastewater samples as possible sources of methicillin-resistant *Staphylococcus aureus*: Prevalence, enterotoxin production, and antibiotic susceptibility. J Food Safety 2018; 38: e12478.

2. Morshdy AEMA, Nahla BM, Shafik S, et al. Antimicrobial effect of essential oils on multidrug-resistant *Salmonella* Typhimurium in chicken fillets. Pak Vet J 2021; http://dx.doi.org/10.29261/pakvetj/2021.055.

3. Abd-Elghany SM, Sallam KI, Abd-Elkhalek A, et al. Occurrence, genetic characterization and antimicrobial resistance of Salmonella isolated from chicken meat and giblets. Epidemiol Infect 2015; 143: 997–1003.

4. Darwish WS, Saad Eldin WF, Eldesoky KI. Prevalence, molecular characterization and antibiotic susceptibility of *Escherichia coli* isolated from duck meat and giblets. J Food Safety 2015; 35: 410–415.

5. Hennekinne JA, De Buyser ML, Dragacci S. *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. FEMS Microbiol Rev 2012; 36: 815–836.

6. Abolghait SK, Fathi AG, Youssef FM, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. Int J Food Microbiol 2020; 328: 108669.

7. Neyaz L, Rajagopal N, Wells H, et al. Molecular characterization of *Staphylococcus aureus* plasmids associated with strains isolated from various retail meats. Front Microbiol 2020; 11: 223.

8. Rortana C, Nguyen-Viet H, Tum S, et al. Prevalence of *Salmonella* spp. and *Staphylococcus aureus* in chicken meat and pork from Cambodian markets. Pathogens 2021; 10: 556.

9. Sams AR. Poultry meat processing Chap. 9, ISBN–0120-3, CRC Press LLC. New York, USA. 2001.

10. Luu QH, Fries R, Padungtod P, et al. Prevalence of Salmonella in retail chicken meat in Hanoi, Vietnam. Ann N Y Acad Sci 2006; 1081: 257-61.

11. Iwabuchi E, Yamamoto S, Endo Y, et al. Prevalence of Salmonella isolates and antimicrobial resistance patterns in chicken meat throughout Japan. J Food Prot 2011; 74: 270-273.

12. Mouwakeh A, Telbisz Á, Spengler G, et al. Antibacterial and resistance modifying activities of *Nigella sativa* essential oil and its active compounds against *Listeria monocytogenes*. In Vivo 2018; 32: 737-743.

13. Chraibi M, Farah A, Elamin O, et al. Characterization, antioxidant, antimycobacterial, antimicrobial effects of Moroccan rosemary essential oil, and its synergistic antimicrobial potential with carvacrol. J Adv Pharm Technol Res 2020; 11: 25-29.

14. American Public Health Association (APHA) Compendium of methods for the microbiological examination of food, 4th Ed., Washington. 2001.

15. ISO (International Standard Organization) 6579: 4th ed. Microbiology - General guidance on methods for the detection of *Salmonella*, International Organization for Standardization, Geneve, Switzerland. 2002(E).

16. McClure JA, Conly JM, Lau V, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from-resistant staphylococci. J Clin Microbiol 2006; 44: 1141-114.

17. Mehrotra M, Wang G, Johnson WM. Multiplex PCR for detection ofgGenes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol 2000; 38: 1032-1035.

18. Cardona-Castro N, Restrepo-Pineda E, Correa-Ochoa M. Detection of *hilA* gene sequences in serovars of *Salmonella enterica* subspecies *enterica*. Mem Inst Oswaldo Cruz 2002; 97: 1153-1156.

19. Murugkar HV, Rahman H, Dutta PK. Distribution of virulence genes in Salmonella serovars isolated from man & animals. Indian J Med Res 2003; 117: 66-70.

20. Tang H, Darwish WS, El-Ghareeb WR, et al. Microbial quality and formation of biogenic amines in the meat and edible offal of *Camelus dromedaries* with a protection trial using gingerol and nisin. Food Sci Nutr 2020; 8: 2094-2101.

21. Govaris A, Solomakos N, Pexara A, et al. The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella* Enteritidis in minced sheep meat during refrigerated storage. Int J Food Microbiol 2010; 137: 175-180.

22. Maturin L, Peeler JT. 2001. Chapter 3. Aerobic Plate Count," In: Food and Drug Administration (FDA), Bacteriological Analytical Manual Online, 8th Edition, Silver Spring, Berlin, 1998.

23. Ammar AM, Attia AM, Abd El-Aziz, et al.

Class 1 integron and associated gene cassettes mediating multiple-drugresistance in some food borne pathogens. Int Food Res J; 23: 332-339

24. Abd El-Aziz NK, Abd El-Hamid MI, Bendary MM, et al. Existence of vancomycin resistance among methicillin resistant S. aureus recovered from animal and human sources in Egypt. Slov Vet Res *2018;* 55: 221–230.

25. Abd El-Aziz NK, Tartor YH, Gharib AA, et al. Propidium monoazide quantitative real-time polymerase chain reaction for enumerationof some viable but nonculturable foodborne bacteria in meat and meat products. Foodborne Pathog Dis; 15: 226--234

26. Ge B, Mukherjee S, Hsu CH, et al. MRSA and multidrug-resistant *Staphylococcus aureus* in U.S. retail meats, 2010-2011. Food Microbiol 2017; 62: 289-297.

27. Crago B, Ferrato C, Drews SJ, et al. Prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in food samples associated with foodborne illness in Alberta, Canada from 2007 to 2010. Food Microbiol 2012; 32: 202-205.

28. Shawish RR, Al-Humam NA. 2016. Contamination of beef products with staphylococcal classical enterotoxins in Egypt and Saudi Arabia. GMS Hyg Infect Control 2016; 11: Doc08.

29. Centers for Disease Control and Prevention (CDC). Outbreak of staphylococcal food poisoning from a Military Unit Lunch Party - United States, July, 2012. 2013; 62: 1026-1028.

30. European Food Safety Authority (EFSA). European Centre for Disease Prevention and Control. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009. EFSA J 2011; 9(3): 2090.

31. Ammar AM, Abd El-Aziz NK, Hanafy MS, et al. Serotypes profile of avian *Salmonellae* and estimation of antibiotic residues in chicken muscles using highperformance liquid chromatography. Advances in Environmental Biology (2016); 10: 173-179

32. Zakaria Z, Hassan L, Sharif Z, et al. Analysis of *Salmonella enterica* serovar Enteritidis isolates from chickens and chicken meat products in Malaysia using PFGE, and MLST. BMC Vet Res 2020; 16: 393.

33. Parvin MS, Hasan MM, Ali MY, et al. Prevalence and multidrug resistance pattern of *Salmonella* carrying extended-spectrum β-Lactamase in frozen chicken meat in Bangladesh. J Food Prot 2020; 83: 2107-2121.

34. Schmid H, Hachler H, Stephan R, et al. Outbreak of *Salmonella enterica* serovar Typhimurium in Switzerland, May-June 2008, implications for production and control of meat preparations. Euro Surveill 2008; 13: pii: 19020.

35. Ford L, Wang Q, Stafford R, et al. Seven *Salmonella* Typhimurium outbreaks in Australia linked by trace-back and whole genome sequencing. Foodborne Pathog Dis 2018; 15: 285-292.

36. Prager R, Fruth A, Tschape H. Salmonella enterotoxin (stn) gene is prevalent among strains of *Salmonella enterica*, but not among *Salmonella bongori* and other Enterobacteriaceae. FEMS Immunol MedMicrobiol 1995; 12: 47-50.

37. Boddicker JD, Knosp BM, Jones BD. Transcription of the Salmonella invasion gene activator, *hilA* requires HilD activation in the absence of negative regulators. J Bacteriol 2003; 185: 525-533.

38. Tartor YH, Hassan FAM. Assessment of carvacrol for control of avian aspergillosis in intratracheally challenged chickens in comparison to voriconazole with a reference on economic impact. J Appl Microbiol. 2017;123(5):1088-1099. doi: 10.1111/jam. 13557.

39. Gharieb, R.M.A.; Saad, M.F.; Mohamed, A.S.; Tartor, Y.H. Characterization of two novel lytic bacteriophages for reducing biofilms of zoonotic multidrug-resistant Staphylococcus aureus and controlling their growth in milk. *LWT* **2020**, *124*, doi:10.1016/j.lwt.2020.109145.

40. Mahboub HH, Tartor YH. Carvacrol essential oil stimulates growth performance, immune response, and tolerance of Nile tilapia to Cryptococcus uniguttulatus infection. Dis Aquat Organ. 2020;141:1-14. doi: 10.3354/dao03506. PMID: 32940246.

41. Abd El-Aziz NK, Ammar AM, El-Naenaeey EYM, et al. Antimicrobial and antibiofilm potentials of cinnamon oil and silver nanoparticles against Streptococcus agalactiae isolated from bovine mastitis: New avenues for countering resistance. BMC Vet Res *2021*; 17, 1–14.

42. Han JH, Patel D, Kim JE, et al. Retardation of *Listeria monocytogenes* growth in mozzarella cheese using antimicrobial sachets containing rosemary oil and thyme oil. J Food Sci 2014; 79: E2272-8.

43. Ugur AR, Dagi HT, Ozturk B, et al. Assessment of *in vitro* antibacterial activity and cytotoxicity effect of *Nigella sativa* Oil. Pharmacogn Mag 2016; 12: S471-S474.