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Control the outgrowth of *Staphylococcus aureus* and *Escherichia coli* in minced chicken meat using *Nigella sativa* seeds water extract during refrigerated storage

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ABSTRACT

The present investigation examined *Staphylococcus aureus* and *Escherichia coli* infection, their virulence genes in chicken meat samples, and evaluated the antioxidant activity (total phenolic, total flavonoid content, and DPPH) of *Nigella sativa* seeds (black cumin) water extract with its impact on the general acceptability and the isolated strains in minced chicken meat. The results showed that *Staph. aureus* and *E. coli* were isolated from 58% and 6% of the examined samples, respectively. Eight strains of coagulase positive *Staph. aureus* screened by PCR were positive for *23S rRNA* and *tsst* virulence gene, but only two strains were positive for *femA* virulence gene. Otherwise, all 3 *E. coli* strains were positive for *eaeA* virulence gene, whereas only one strain (*E. coli* O117) had *stx1* virulence gene and *stx2* was undetected. The addition of *Nigella sativa* seeds extract to minced chicken meat play a vital role in maintaining the meat's sensory qualities (color, texture, and odor), as well as preserving pH, TVBN, and TBARS within acceptable limits of Egyptian standards till six days of cold storage. Also, reducing *Staph. aureus* and *E. coli* growth that were inoculated in minced chicken meat, particularly when using 2% *Nigella sativa* seeds extract in comparison to the inoculated control and 1.5% *Nigella sativa* seeds extract groups. Additionally, the impact of *Nigella sativa* seeds extract on reducing *Staph. aureus* growth was more pronounced than on *E. coli*. Therefore, *Nigella sativa* seeds extract may be utilized as a type of preservatives and antibacterial agent in food-related products.

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INTRODUCTION

Among many vital nutritional characteristics of poultry flesh are reduced fat content and a comparatively substantial amount of polyunsaturated fatty acids (Nkukwana et al. 2014). Despite the fact that chicken flesh is regarded as a low-calorie diet because of its low fat level, the high unsaturation level of its muscle lipids makes them particularly susceptible to oxidation. Meat color, texture, flavor, nutritional loss, and shelf life, all affected as a result of oxidation. Furthermore, poultry meats are excellent sources of protein, such meats' protein content is subjected to oxidation after slaughter, that can result in a secondary oxidation of lipids and ultimately result in the loss of protein's functional characteristics and quality (Estévez, 2015).

The shelf life of chicken flesh is extremely limited due to its high susceptibility to microbial deterioration. Because of its high protein and moisture content it is considered as a perfect medium for microorganism development (Vaithyanathan et al. 2011). Pathogens frequently contaminate poultry carcasses and their components through their intestinal tract or by faeces relating food and feathers (Dincer and Baysa, 2004). Food-related infection and intoxication have grown in importance as health risks. The most prevalent and frequent food-borne bacteria that cause food poisoning are *Staph. aureus* and *E. coli* (Pires et al. 2012). *Staph. aureus* is a significant bacterium that has been recognized globally as the fifth reason of food-borne illness in humans (Mork et al. 2005).

The regular intestinal flora of mammals, including humans contains a significant amount of *E. coli*, which is typically safe for the host causing illness only in immunosuppressed hosts or once the gastrointestinal tract is violated. Nevertheless, certain particular *E. coli* strains are main pathogens with increased capacity to spread illness after gaining particular virulence features (Li et al. 2005).

According to Kerry et al. (2006), the rise of moisture, pH value, and protein content make chicken flesh more perishable and a fa-

vorable medium for the growth of microbes that cause disease and spoiling. Therefore, using antioxidants is among the most crucial techniques for preventing both fat and protein oxidation within feed, raw meat, and flesh products (Descalzo and Sancho, 2008). Various phytochemical substances found in plants, such as alkaloids, flavonoids, terpenoids and tannin, have been demonstrated to exhibit antibacterial activity in vitro (Srinivasan et al. 2001). *Nigella sativa* or black cumin had the greatest effective radical scavenging antioxidant activity in comparison to synthetic antioxidants (Singh et al. 2014). *Nigella sativa* or black cumin is a member of the Ranunculaceae family, and is well known for its therapeutic benefits. Alkaloids, volatile and fixed oils in addition to a number of pharmacologically potent compounds, including carvacrol, thymol, thymoquinone, dithymoquinone, hedrin, nigellidine and nigellicine-N-oxide are found in the seeds of *Nigella sativa* (Azeem et al. 2014). Numerous scientific studies on *Nigella sativa* seeds reveal the plant has a variety of bioactivities, including antioxidant, antibacterial and antifungal properties (Toma et al. 2015).

Consequently, The aim of this current study aimed for assessing the microbial contamination of fresh, commercially available chicken cuts by *Staph. aureus* and *E. coli*. and evaluated *Nigella sativa* seeds (water extract) antioxidant activity (total phenolic, total flavonoid content and DPPH), with investigation of the effectiveness of adding water extract of *Nigella sativa* seeds on the overall acceptability of minced chicken flesh during chilled storing as well as reducing the development of *Staph. aureus* and *E. coli* inoculated in minced chicken meat samples.

MATERIALS and METHODS

Samples collection and preparation

50 samples of chicken breast and thigh (25, each) were obtained from various retail stores located in Kafrelshiekh city, Egypt. Every sample was sent to the lab in strictly sterile conditions, where immediately subjected to bacteriological analysis. Samples were prepared according to APHA (1992).

***Staph. aureus* and *E. coli* isolation and identification**

One ml from prepared samples was added to Tryptic soya broth containing 6.5% NaCl, which was aerobically incubated at 37°C for 18–24 hours prior to streaking onto Baird–Parker's agar plates. Following a 24-hour aerobic incubation period at 37 °C, the suspected *Staph.aureus* colonies (black, shiny, convex colonies having narrow white edges and distinct zones surrounding it) were chosen and confirmed on mannitol salt agar plates (yellow colonies) (APHA, 2004) before performing additional morphological and biochemical confirmation (Cheesbrough, 2006).

In order to isolate *E. coli* of faecal type, enriched tubes of MacConkey broth augmented by inverted Durham's tubes containing 1ml of prepared samples were incubated at 44°C for 24 hours (Eijkman test) (Tubes that are positive showed acid and gas production) and then plated on EMB agar (ISO, 2001). Following 24 hours of incubation at 37°C, characteristic metallic green colonies from the EMB agar plates were identified biochemically as *E. coli* (Quinn et al. 2002). The isolates were serologically identified utilizing diagnostic quick *E. coli* antisera kits (DENKA SEIKEN Co., Japan, Kok et al. (1996).

Detection of *Staph. aureus* species-specific 23S rRNA gene and virulence genes (*tsst* and *femA*) by using Polymerase Chain Reaction (PCR)

Extraction of DNA:

The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was modified from the producer's guidelines in order to extract DNA from the strains. In brief, the sample suspension of 200 µl was incubated for 10 min at 56 °C with 20 µl proteinase K and 200 µl lysis buffer. Next, 200 µl of 100% ethanol were added to the lysate following incubation. Then, the sample washed and centrifuged according to the producer's guidelines. The nucleic acid was eluted using 100 µl of the kit's elution buffer.

Oligonucleotide Primer:

The primers utilised were given by Metabion (Germany) and are listed in table (1).

Amplification of PCR:

Uniplex PCR:

Primers were used in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer at 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. 2720 thermal cycler of an Applied Biosystems was utilised to perform the reaction .

PCR Products analysis:

PCR products were split up utilizing 5 V/cm gradient, electrophoresis done at room temperature on agarose gel 1.5% (Applichem, Germany, GmbH) in 1x TBE buffer. Each gel slot was loaded with 20 µl of uniplex products and 30 µl of the multiplex products prior to gel analysis. Fragments sizes were determined with Gelpilot 100 bp plus Ladder (Qiagen, Germany, GmbH) and generuler ladder 100 bp (Fermentas, thermo, Germany). Photographs of gel were taken by a gel documentation system (Alpha Innotech, Biometra) and finally, software for data analysis was used.

Table 1. Target genes, primers sequences, amplicon sizes and conditions of cycling

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
<i>Staph. aureus 23S rRNA</i>	ACGGAG-TTACAAAGGACGAC	1250 bp	94°C 5 min.	94°C 30 sec.	55°C 1 min	72°C 1.2 min.	72°C 12 min.	Bhati et al., 2016
	AGCTCAGCCTTAAC-GAGTAC							
<i>tst</i>	ACCCCTGTTCCCTTATC ATC	326 bp	94°C 5 min.	94°C 30 sec.	50°C 30 sec.	72°C 30 sec.	72°C 10 min.	Mehrotra et al., 2000
	TTTTCAGTATTT-GTAACGCC							
<i>femA</i>	AAAAAA-GCACATAACAAGCG	132 bp	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 45 sec.	72°C 10 min.	Mehrotra et al., 2000
	GATAAAGAAGAAAC-CAGCAG							

Detection of *E. coli* virulence genes by using Polymerase Chain Reaction (PCR)

Extraction of DNA:

The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was utilized, and strain DNA extraction was done by following the producer's instructions with making adjustments. In brief, 200 µl of sample suspension mixed with 10 µl of proteinase K and 200 µl of lysis buffer and the mixture was incubated at 56 °C for 10 minutes. Incubation was followed by adding of 200 µl of ethanol (100%) to lysate. Subsequently, the sample washed and centrifuged in compliance with the producer's guidelines. Nucleic acid elution is performed using the elution buffer (100 µl) that comes with the kit.

Oligonucleotide Primers:

Table (2) includes a list of primers that were being supplied by Metabion (Germany).

Amplification of PCR:

The use of primers was carried out in a 25-µl reaction which contained 12.5 µl EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer at 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. Re-

action was carried out with 2720 thermal cycles of an Applied Biosystems.

Analysis of the PCR Products:

Electrophoresis was used to separate the PCR products on 1.5% agarose gel (Appllichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. 20 µl of the product were injected into each gel slot for analysis of gel. Sizes of the fragments were established by a generuler ladder 100 bp (Fermentas, Germany). Photographs of the gel were taken through a gel documentation system (**Alpha Innotech, Biometra**), then the data was analysed using computer software.

Table 2. Target genes, sequences of primers, amplicon sizes and conditions of cycling

Target gene	Primers sequences	Amplified segment (bp)	Prim. Den.	Amplification (35 cycles)			Final extension	Reference
				Sec. den.	Ann.	Ext.		
<i>eaeA</i>	ATGCTTAG- TGCTGGTTTAGG GCCTTCATCATTTCGCTT TC	248	94°C 5 min.	94°C 30 sec.	51°C 30 sec.	72°C 30 sec.	72°C 7 min.	Bisi-Johnson et al., 2011
<i>Stx1</i>	ACACTGGATGATCTCAG- TGG CTGAATCCCCCTCCATTA TG	614	94°C 5 min.	94°C 30 sec.	58°C 40 sec.	72°C 45 sec.	72°C 10 min.	Dipineto et al., 2006
<i>Stx2</i>	CCATGACAACGGACAG- CAGTT CCTGTCAACTGAGCAG- CACTTTG	779	94°C 5 min.	94°C 30 sec.	58°C 40 sec.	72°C 45 sec.	72°C 10 min.	Dipineto et al., 2006

Nigella sativa seeds (water extract) preparation

Nigella sativa seeds were bought at a regional store in Kafrelshiekh city, Egypt. The seeds after cleaning were rinsed slowly using tap water to remove any dust particles, before being air-dried for 24 hours at room temperature (Faujan et al. 2007). The seeds were then processed via an electric grinder to obtain a fine powder. In brief, an exact weight of 15 g of powder was placed inside a conical flask in order to prepare the water extract, then addition of 100 ml of sterilized distilled water were done. The entire contents was held overnight at room temperature, shaken continuously, and then centrifuged (Remi Centrifuge, Bombay, India) at 5,000 rpm for 10 minutes. The supernatant had been preserved till it was used (Chauhan et al. 2018).

Determination of the phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH radical scavenging activity) in *Nigella sativa* seeds water extract

Determination of the total phenolic, total flavonoid content, and antioxidant activity (DPPH) were done according to Sembiring et al. (2018) at NRC (National Research Centre), Egypt.

Milligrams of gallic acid equivalent (GAE) per gramme (gm) was used to express the total phenolic content.

The flavonoids content were represented as milligrams of quercetin equivalent (QUE) per 1 gramme of dry extract.

The following equation was used to calculate the % DPPH scavenging activity:

DPPH scavenging activity (%) or inhibition % = $A_0 - A_1 / A_0 \times 100$.

Where A_0 is the absorbance of control reaction and A_1 represent the absorbance in presence of test sample.

The experimental design

The fresh chicken fillet utilized in this experiment weighed a total of 1800g, and it was obtained from chicken's butcher shops in Kafrelshiekh city, Egypt. Subsequently, minced by an electric grinder and split into two parts: the first part (1500g) subdivided into 3 groups (500g each), include control group without any additives, second group supplied with *Nigella sativa* seeds extract 1.5% and third group supplied with *Nigella sativa* seeds extract 2%, then all groups refrigerated at 4 °C for six days and examined daily for sensory assessment and physicochemical analysis (pH, TVBN and TBARS) every 2 days till the end of storage period.

The second portion (300g) of minced chicken meat used for examination the effect of *Nigella sativa* seeds aqueous extract on the isolated strains which partitioned into 3 categories (100g each), include control positive group inoculated with the isolated strains of *Staph. aureus* and *E.coli* (1.5×10^6 cfu/g, each), second group inoculated with *Staph. aureus* and *E.coli* + *Nigella sativa* seeds extract 1.5% and third group inoculated with *Staph. aureus* and *E.coli* + *Nigella sativa* seeds extract 2%, prior to being refrigerated at 4 °C and examined for *Staph. aureus* and *E.coli* count every 2 days span through six days storage period.

Sensory analysis

The sensory features (color, odor and texture) of the minced chicken flesh samples were assessed to determine their general acceptance. 9 expert panelists were asked to assign a score indicating how well-liked each sample. A nine-points descriptive grade was adopted with a score of 9 being the highest and a score of 1 being the lowest (Horwitz, 1982).

Physicochemical examination

1-pH measurement: An electrical pH metre (Bye model 6020, USA) had been used to monitor pH rate, as stated by Pearson (2006).

2- Total volatile basic nitrogen (TVBN) measurement: had been measured in accordance with ES: 63-9 (2006).

3-Thiobarbituric acid reactive substances (TBARS) measurement: According to ES: 63-10 (2006), Malonaldehyde (MDA) is a byprod-

uct from lipid peroxidation, and this testing depends on detecting it.

Bacteriological Analysis

Staph. aureus and *E.coli* count

Aseptically, 90 ml of peptone water 0.1% was poured into a stomacher containing 10 g of minced chicken flesh samples. To obtain 1/10 dilution, samples of minced chicken meat were homogenized for 60 seconds in the stomacher under sterile conditions. Serial dilutions were prepared to be used for the counting of *Staph.aureus* on Baird Parker agar plates (APHA, 2004) and *E. coli* on EMB (Eosin methylene blue agar plates) (ISO, 2001). The bacterial colonies on all plates were counted as cfu/g after 48hr incubation at 37°C.

Statistical analysis

Each measurement was analysed using SPSS (Statistical Package for the Social Sciences) 22.0 (IBM Corp., Armonk, NY, USA). ANOVA (one-way analysis of variance) followed LSD (Least Significant Differences) test had been applied to ascertain the significance for every parametric data, which was expressed as mean \pm SE (Standard Errors). At $P < 0.05$, mean comparisons among the groups were deemed significant.

RESULTS

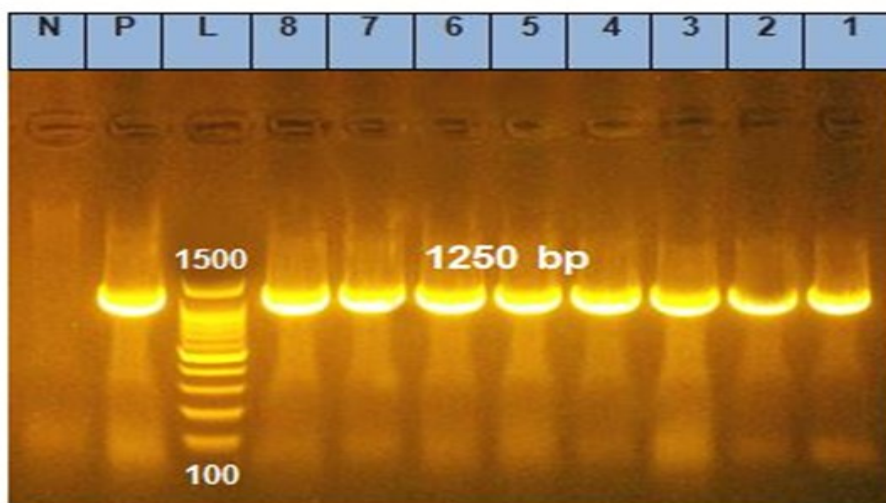
Table 3. Incidence of *Staph. aureus* and *E.coli* in chicken meat samples (n= 50)

Chicken meat samples	No. examined samples	No. +ve samples for <i>Staph.aureus</i>		No. +ve coagulase <i>Staph.aureus</i> isolates		No.+ve samples for <i>E.coli</i>	
		No.	%	No.	%	No.	%
Breast	25	14	56	14	100	2	8
Thigh	25	15	60	15	100	1	4
Total	50	29	58	29	100	6	3

Table 4. Serological identification of *E. coli* strains

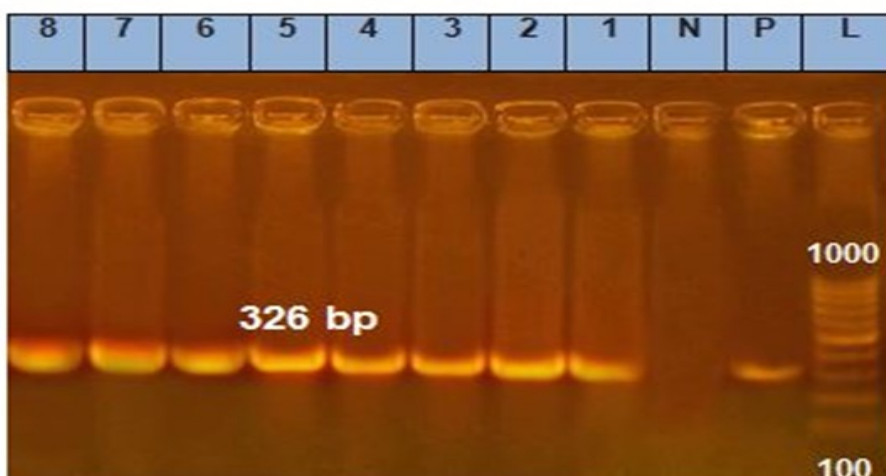
Chicken meat sample	Serogroup	No. of strains
Breast	O117	1
Breast	O44	1
Thigh	O15	1

Fig (1). Uniplex PCR amplification products of *Staph. aureus 23S rRNA* on agarose gel electrophoresis



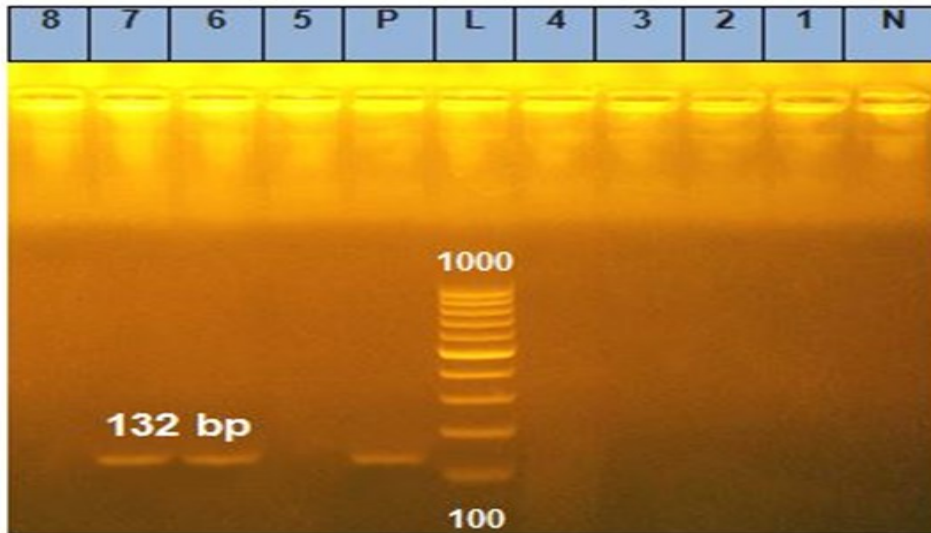
Lane L: 100-1500 bp molecular size marker.
 Lane P: Control positive *Staph. aureus 23S rRNA* at 1250 bp .
 Lane N: Control negative *Staph. aureus 23S rRNA*.
 Lanes 1, 2, 3, 4, 5, 6, 7 and 8: Positive *Staph. aureus 23S rRNA* (100% of the examined strains).

Fig 2. Uniplex PCR amplification products of *Staph. aureus tsst* virulence gene on agarose gel electrophoresis



Lane L: 100-1000 bp molecular size marker.
 Lane P: Control positive *Staph. aureus tsst* virulence gene at 326 bp.
 Lane N: Control negative *Staph. aureus tsst* virulence gene.
 Lanes 1, 2, 3, 4, 5, 6, 7 and 8: Positive *Staph. aureus tsst* gene (100% of the examined strains)

Fig 3. Uniplex PCR amplification products of *Staph. aureus femA* virulence gene on agarose gel electrophoresis



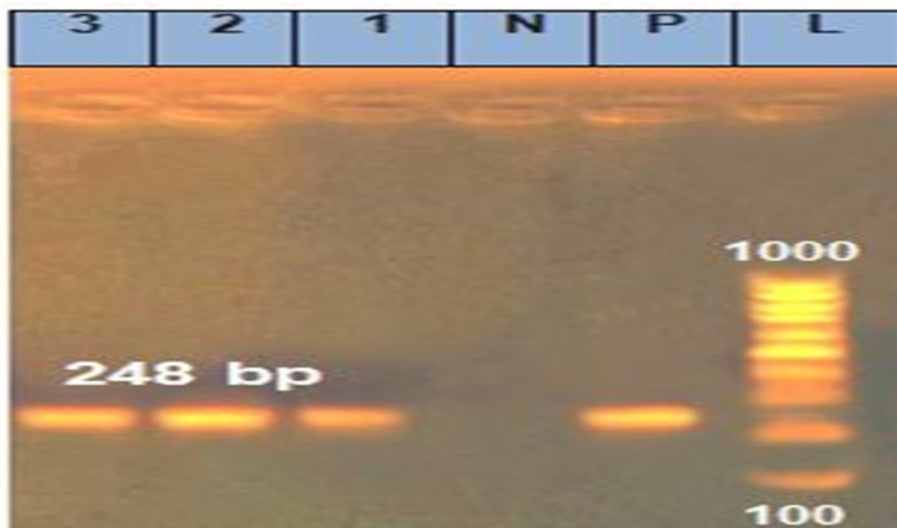
Lane L: 100-1000 bp molecular size marker.

Lane P: Control positive *Staph. aureus femA* virulence gene at 132 bp.

Lane N: Control negative *Staph. aureus femA* virulence gene.

Lanes 6 and 7: Positive *Staph. aureus* strains for *femA* gene (25% of the examined strains).

Fig 4. Uniplex PCR amplification products of *E. coli eaeA* virulence gene on agarose gel electrophoresis



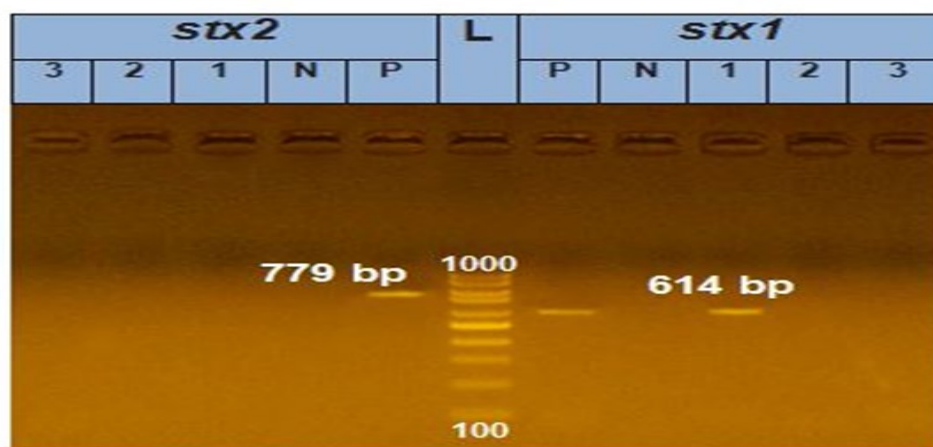
Lane L: 100-1000 bp molecular size marker.

Lane P: Control positive *E. coli eaeA* virulence gene at 248 bp.

Lane N: Control negative *E. coli eaeA* virulence gene.

Lanes 1, 2 and 3: Positive *E. coli eaeA* gene (100% of the examined strains).

Fig 5. Uniplex PCR amplification products of *E. coli Stx1* and *Stx2* virulence genes on agarose gel electrophoresis



Lane L: 100-1000 bp molecular size marker.

Lane P: Control positive *E. coli Stx1* and *Stx2* virulence genes at 614 and 779 bp respectively.

Lane N: Control negative *E. coli Stx1* and *Stx2* virulence genes.

Lanes 1: Positive only for *E. coli Stx1* gene (33.33% of the examined strains).

Table 5. Determination of total phenolics, flavonoids content, and antioxidant activity of *Nigella sativa* seeds (water extract) (Mean ± SE)

	Phenolic contents (mg GAE/g)	Flavonoids contents (mg QUE/g)	Antioxidant activity by DPPH (µg TE/g)
<i>Nigella sativa</i> seeds (water extract)	71.053 ± 0.68	53.753 ± 0.03	0.979 ± 0.000 DPPH scavenging%: 39.5 IC50: 11.14 µg/ml

Table 6. Overall acceptability (color, odor and texture) of minced chicken meat supplied with various concentrations of *Nigella sativa* seeds extract during refrigerated storage.

Groups/ time	Control without additives	<i>Nigella sativa</i> seeds extract 1.5 %	<i>Nigella sativa</i> seeds extract 2%
0 day	9	9	9
1 st day	8	9	9
2 nd day	7	8	8
3 th day	6	8	8
4 th day	5	7	7
5 th day	4	6	7
6 th day	3	5	6

9: Excellent, 8: veryv.good, 7: v. good, 6: good, 5: medium, 4: fair, 3: poor, 2: v. poor, 1: v. v. poor.

Table 7. Statistical analytical results of pH, TVBN and TBARS values of minced chicken meat supplied with various concentrations of *Nigella sativa* seeds extract during refrigerated storage.

Time/Groups	0 day	2 nd day	4 th day	6 th day
	PH			
Control without additives	5.75±0.13 ^a	6.44± 0.01 ^a	6.48± 0.01 ^a	6.53± 0.01 ^a
<i>Nigella sativa</i> seeds extract 1.5 %	5.78± 0.14 ^a	6.39±0.06 ^a	6.44±0.01 ^b	6.51±0.01 ^b
<i>Nigella sativa</i> seeds extract 2%	5.74± 0.16 ^a	6.38±0.01 ^a	6.42±0.01 ^c	6.46±0.01 ^c
	TVBN			
Control without additives	15.07±0.94 ^a	16.70±0.67 ^a	17.53±0.54 ^a	20.30±0.58 ^a
<i>Nigella sativa</i> seeds extract 1.5 %	10.63±0.69 ^b	11.31±0.67 ^b	12.77±0.55 ^b	16.80±0.58 ^b
<i>Nigella sativa</i> seeds extract 2%	10.31±0.67 ^b	11.20±0.69 ^b	12.44±0.60 ^b	14.00±0.58 ^c
	TBARS			
Control without additives	0.07± 0.01 ^a	0.13± 0.01 ^a	0.23± 0.01 ^a	0.50± 0.06 ^a
<i>Nigella sativa</i> seeds extract 1.5 %	0.02± 0.01 ^b	0.11± 0.01 ^a	0.20± 0.06 ^a	0.43±0.01 ^{ab}
<i>Nigella sativa</i> seeds extract 2%	0.04± 0.01 ^b	0.10± 0.01 ^a	0.19± 0.01 ^a	0.30± 0.06 ^b

Averages of various letters within a single column, differ considerably at (P < 0.05)

Table 8. Statistical analysis results of minced chicken meat inoculated with *Staph.aureus*(1.5 x 10⁶cfu/g= 6.18, log10) and supplied with various concentrations of *Nigella sativa* seeds extract throughout refrigerated storing.

Time/Groups	0 day	2 nd day	4 th day	6 th day
Control+ve (inoculated)	6.72 ± 6.28 ^a	6.96 ± 6.28 ^a	7.19± 6.09 ^a	7.95 ± 5.46 ^a
Inoculated+ <i>Nigella sativa</i> seeds extract 1.5 %	6.09± 5.69 ^{ab}	5.60 ± 5.08 ^b	5.24 ± 4.82 ^b	4.93 ± 4.08 ^b
Inoculated+ <i>Nigella sativa</i> seeds extract 2%	6.01 ± 5.65 ^b	5.34 ± 4.83 ^b	5.09± 4.74 ^b	4.60 ± 4.29 ^b

Averages of various letters within a single column, differ considerably at (P < 0.05)

Table 9. Statistical analysis results of minced chicken meat inoculated with *E.coli* (1.5×10^6 cfu/g = 6.18, log 10) and supplied with various concentrations of *Nigella sativa* seeds extract throughout refrigerated storing.

Time/Groups	0 day	2 nd day	4 th day	6 th day
Control+ve (inoculated)	6.44 ± 6.27 ^a	7.09 ± 7.04 ^a	7.56 ± 6.63 ^a	8.01 ± 6.58 ^a
Inoculated+ <i>Nigella</i> <i>sativa</i> seeds extract 1.5 %	6.18 ± 5.36 ^a	5.97 ± 5.72 ^a	5.73 ± 5.26 ^b	5.90 ± 4.74 ^b
Inoculated+ <i>Nigella</i> <i>sativa</i> seeds extract 2%	6.24 ± 5.79 ^a	5.79 ± 5.47 ^a	5.68 ± 5.34 ^b	5.67 ± 4.24 ^b

Averages of various letters within a single column, differ considerably at (P < 0.05)

DISCUSSION

Table (3) explained that out of 50 chicken meat specimen (Breast and Thigh), 29 (58%) were positive for *Staph. aureus* and also were coagulase-positive. Similar finding detected by **Herve and Kumar (2017)** where 53% of retail chicken meat was *Staph. aureus* contaminated. Whereas, a lower prevalence (14%) of *Staph. aureus* was found in poultry carcasses in Romania as stated by **Cretu et al. (2018)**. Additionally, our results explained that thigh samples were 60% for *Staph. aureus* while breast samples were 56%, almost identical outcomes were reported with **Shahjada et al. (2022)** that found *Staph. aureus* in 67.5% and 47.5% of chicken thigh and breast samples, respectively.

Also, our results clarified that 3 (6%) samples (include 8% breast and 4% thigh) out of 50 analysed chicken flesh samples were revealed the presence of *E.coli* (Table 3). High occurrence (87.5%) of *E.coli* in poultry meat was stated by **Elzاهر et al. (2018)**. Furthermore, *E. coli* prevalence in breast specimens (8%) was more than in the thigh specimens (4%), however, **Shahjada et al. (2022)** noticed higher *E. coli* isolations in thigh and breast samples (77.5% and 57.5%), respectively. **Ayçiçek et al. (2004)** clarified that improper handling, less of hygiene during production and distribution may be the reason for existence of *E. coli* in the specimens under examination.

According to Table (4), three *E. coli* isolates were found in the samples of chicken meat under investigation. These isolates identi-

fied serologically to O117, O44 and O15 *E. coli*.

In the present study, eight isolates of coagulase-positive (CoPS) *Staph. aureus* from the examined chicken flesh samples were identified using *23S rRNA* gene analysis, and the result revealed that all isolates (100%) were positive for *Staph. aureus 23S rRNA* gene (Fig. 1). Similar results were observed by **Sunagar et al. (2013)**. **Salauddin et al. (2020)** verified that *23S rRNA* was a useful approach for identifying *Staph. aureus* isolates and determining its evolutionary characteristics.

tsst virulence gene was present in 100% of *Staph. aureus* isolates in our research (Fig. 2).

Since earlier studies have shown that *tsst*-producing *Staph. aureus* has been linked to human infections, handlers of chickens are at risk of contracting zoonotic hazards due to the high prevalence of *Staph. aureus* virulence gene *tsst* (**Sharma et al. 2018**). In contrast, *femA* virulence gene was present in only two isolates (25%) (Fig. 3). According to **Mebkhout et al. (2018)**, *Staph. aureus* strains were recovered from chicken carcasses with higher *femA* results (85.71%). Moreover, **Abd El Tawab et al. (2016)** found all 5 *Staph. aureus* investigated strains isolated from chicken meat had *femA* virulence gene.

The virulence gene *E.coli eaeA* was found in all three of the tested *E. coli* strains (100%) (Fig. 4), however only one strain had *stx1* virulence gene (serogroup O117) by 33.33% and none of the three isolates had virulence gene

stx2 (Fig. 5). Different data of *E. coli* virulence genes were identified by **Boudjerda and Lahouel (2022)**, they reported that *eaeA*, *stx1* and *stx2* did not amplified from 85 isolates of *E. coli* obtained from chicken flesh. Conversely, **Abd El Tawab et al. (2015)** reported that gene *eaeA* was found in O128, O55, O1, and O2 of *E. coli* strains using multiplex PCR, while gene *stx1* was not found in each of the *E. coli* serogroups, and gene *stx2* was identified in O114 and O128 *E. coli* strains in both presumably healthy and diseased broiler chickens.

The values of the total phenolics (TPC), flavonoid (TFC) components, and antioxidant activity by DPPH in aqueous extract of *Nigella sativa* seed sare demonstrated in Table 5. The total phenolic compounds were 71.053 ± 0.68 mg GAE/g. Different values of TPC were obtained by **Chauhan et al. (2018)** (TPC 4.4 ± 1.2 mg GAE /g), and **Zwolan et al. (2020)** (TPC 24.89 ± 0.61 mg GAE/g). The extraction of soluble phenolic acids (both nonpolar and semipolar) may be the cause of the increased phenolic acid levels (**Thippeswamy and Naidu, 2005**). According to **Chauhan et al. (2018)**, there could be a range of reasons for the difference in TPC levels of black cumin seeds, including variations in climate, plant types, and cultivars that use various extraction techniques and solvents. The present study determined that the overall content of flavonoids was 53.753 ± 0.03 mg QUE/g. *Nigella sativa* seed water extract displayed antioxidant activity by DPPH 0.979 ± 0.000 μ g TE/g, DPPH scavenging percent 39.5, and IC₅₀: 11.14 μ g/ml, which is almost the same as the result of **Chauhan et al. (2018)** (33.96 percent radical scavenging activity). Additionally, our results closely match those of **Das et al. (2015)**, who found that extracts of black cumin had a 20–49% DPPH radical scavenging activity. According to **Bourgou et al. (2008)**, there was a linear correlation between total polyphenols and radical inhibition, and the antiradical activity of *Nigella sativa* extracts increased in direct proportion to the polyphenol concentration.

Sensory evaluation depends on the product's general acceptability and organoleptic qualities like color, texture and odor (**Haq et al. 2013**). The acquired results (Table,

6) demonstrated that the highest level of the sensory quality (color, odor and texture) was obtained in minced chicken meat samples supplied with 2% *Nigella sativa* seeds extract, followed by 1.5% *Nigella sativa* seeds extract and still acceptable for more than 6th days storage. In contrast, the sensory grades of the control group started to decline after 4th day of refrigerated storage.

Rahman et al. (2021) revealed that the black cumin seed extract possess high antioxidant content that prevents the myoglobin content from autoxidizing, minimizes rancidity and lipid oxidation, improve tenderness and helps to reduce microbial load when used in meat preservation. Also, **Lorenzo et al. (2018)** mentioned that adding natural antioxidants to flesh products improve their sensory characteristics by maintaining them during storing by preventing oxidation of proteins and lipids.

The utilizing of extract of *Nigella sativa* seeds possessed highly notable impact ($p < 0.05$) on pH of samples of minced chicken flesh (Table, 7) where pH mean value varies significantly ($P < 0.05$) between control and supplied groups. Since pH is among the parameters linked to meat's lipid oxidation, the pH of the supplied minced chicken meat groups with *Nigella sativa* seed extract decreased with time due to the antioxidant impact of plant extracts to reach 6.46 ± 0.01 and 6.51 ± 0.01 in *Nigella sativa* seeds extract 2% and 1.5%, respectively, in contrast to control samples (6.53 ± 0.01) at 6th day of cold storage. According to **Cortez-Vega et al. (2012)**, the increased number of psychotropic bacterial colonies and accumulation of microbial metabolic products such as amines and ammonia during cold storage may be the cause of the higher pH values in control samples compared to samples of the supplied groups with *Nigella sativa* seed extract.

TVBN is frequently employed as a meat-decomposition indicator. The samples supplied with *Nigella sativa* seeds extract 2% and 1.5% had TVBN values 14.00 ± 0.58 and 16.80 ± 0.58 , respectively at 6th day of cold storage remained below the acceptable levels of TVBN stated by EOS (2005, TVBN 20mg/100g). In contrast, TVBN mean values

of control samples significantly ($p < 0.05$) increased from the initial time (15.07 ± 0.94) to reach the rejection level (20.30 ± 0.58) (Table, 7) as reported by **EOS (2005)** at the 6th day of cold storage. *Nigella sativa* seeds extract treatment of chicken meat samples resulted in lower levels of TVBN, which may be explained through either a more rapid decline in the number of bacteria or a reduced ability of microbes to oxidatively deaminate non protein nitrogen molecules, or both (**Mexis et al. 2009**). According to **Irimia et al. (2020)**, TVBN values of chicken breasts supplied with 1 and 2% *Nigella sativa* oil solutions were differ significantly after 3 days of refrigerated storage from the control, whereas only the samples of 2% *Nigella sativa* oil solutions after six days of refrigeration were significantly differ from control.

TBARS (Thiobarbituric Acid Reactive Substances) are frequently utilised to evaluate the oxidation of lipid in the products. TBARS readings of the control group substantially differed from those of the 2% *Nigella sativa* supplied group ($p < 0.05$), where the control samples had the greatest TBARS level (0.50 ± 0.06) as opposed to 0.43 ± 0.01 and 0.30 ± 0.06 in the 1.5% and 2% *Nigella sativa* seeds extract supplied groups, respectively (Table 7). **Zwolan et al. (2020)** found that chicken meatballs treated with water extract of *Nigella sativa* seeds exhibited a slower rate of oxidative changes demonstrated by reduced TBARS results in comparison to control meatballs. Black cumin's phenolic component was believed to be accountable to its antioxidant effect on TBARS level. The high redox potential of phenolic components, that would enable it to function as reducing factors and hydrogen donors, is known to be associated with their antioxidant activity (**Singh et al. 2014**).

Changes in the count of *Staph. aureus* inoculated samples of minced chicken meat throughout refrigeration are shown in Table (8). Counts decreased from initial count (6.18, log10) until 6th day of storage to reach 4.60 ± 4.29 and 4.93 ± 4.08 (log10) in groups provided with 2 and 1.5% *Nigella sativa* seeds extract, respectively. In contrast the count significantly increased ($p < 0.05$) in the control group

to 7.95 ± 5.46 (log10) at 6th day of the cold storage.

Aqueous extract of *Nigella sativa* seeds were shown to be efficient versus several examined bacteria as staphylococcus despite the bacteria's resistance to various antibiotics by **Ali and Blunden (2003)**. Additionally, **Bakathir and Abbas (2011)** reported that *Nigella sativa* seeds (concentration of 300 mg/ml) extracted in distilled water suppressed the development of *Staph. aureus* in vitro clearly.

Reduction in *E. coli* count inoculated in minced chicken flesh samples through the cold storage (Table, 9) revealed a substantial difference among the control samples and the supplied groups with *Nigella sativa* seeds extract ($P < 0.05$), where *Nigella sativa* seeds extract 2% and 1.5% resulted in reducing *E. coli* count from the initial count (6.18, log10) to reach 5.67 ± 4.24 and 5.90 ± 4.74 (log10), respectively at 6th day of cold storage, while the count in the control samples significantly rise from the start of the test to reach 8.01 ± 6.58 (log10) at 6th day of cold storage.

According to **Al Sultani et al. (2021)**, Aqueous extracts of *Nigella sativa* demonstrated a maximal inhibitory zone against *E. coli*. Also, **Takma and Korel (2019)** mentioned that the impact of adding black seed oily extract to active packaging film on lifespan and quality of chicken flesh kept for 5 days at 4 °C showed antibacterial activity toward *E. coli* and *Staph. aureus*.

Both *Staph. aureus* and *E. coli* have been rendered inactive by the antibacterial action of black seeds. Studies using survival curves have shown that *Staph. aureus* is more sensitive compared to *E. coli*, which was obviously demonstrated by the decline in the number of survival bacteria. Such action would be clear from its capacity to focus on the bacterial cell membrane producing the damage. Due to the loss of target bacterial organelles and/or components of bacteria, this could cause bacterial lysis (**Najib et al. 2014**).

The majority of plant antioxidant activity is attributed to phenolic compounds, whose re-

dox potential enables them to function as donors of hydrogen, reducing agents, metal chelators and singlet oxygen quenchers (Belhachat et al. 2017). Also, *Nigella Sativa* seeds have antioxidant characteristics that make it suitable for use in the manufacturing of meat products. Despite this, adding its dark-colored seeds to the finished product had a negative effect on its appearance (Zwolani et al. 2019), so extracts appear to be a valid option.

CONCLUSION

The results of this investigation indicate that *Staph. aureus* and *E. coli* are prevalent in the analyzed chicken meat specimens, with predominant infection of *Staph. aureus*. Additionally, PCR proved the capacity to detect the species-specific *23S rRNA* gene, the virulence genes *tsst* and *femA* in *Staph. aureus* strains and *E. coli* genes (*eaeA*, *stx1* and *stx2*) with remarkable risks of chicken flesh contamination. Therefore, hygienic food handling, preparation, proper cooking and refrigeration should be followed in order to prevent food borne illness. In addition, this study revealed a distinct and irrefutable antioxidant and antibacterial impact of ground seeds of *Nigella Sativa* (water extract) on sensory, physicochemical characteristics and development of *Staph. aureus* and *E. coli* in minced chicken meat, especially in using 2% *Nigella Sativa* seed extract compared to control and 1.5% *Nigella sativa* seed extract groups, and the degree of reduced count in *Staph. aureus* was higher than in *E. coli*. So, *Nigella sativa* seeds extract may be chosen for usage as possible food bio preservatives as well as anti-*Staph. aureus* and anti-*E. coli* agents in foods.

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