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INHIBITION OF MULTIDRUG-RESISTANT BACTERIA ISOLATED FROM FRESH CHICKEN MEAT AND SAUSAGE BY NATURAL ANTIBACTERIAL AGENTS

Amany A. Abd-Allah, Nahed A. El-Wafai, S.A.M. Mahgoub* and Zakia A. El-Kenawy

Agric. Microbiol. Dept., Fac. Agric., Zagazig Univ., Egypt

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ABSTRACT: A total of 66 samples of fresh chicken meat (n=42) and sausage samples (n= 24) were collected from various meat shops of Zagazig and Kafr Saker cities (El-Sharkia Governorate) to examine the presence of pathogenic multidrug-resistant bacteria mainly *Staphylococcus aureus* and to evaluate some natural antimicrobial agents against some bacterial strains. About 127 bacterial isolates were isolated onto Baird Parker agar from the samples. Identification to species and strain levels of these isolates was accomplished by morphological, biochemical characteristics as well as 16S rDNA gene analysis. Among these, 42 were staphylococci isolates, 16 (38.08%) of them were positive in coagulase reaction. The most frequently observed genus identified by 16S rDNA sequencing analysis was *Staphylococcus* since 28.6% and 33.3% of sausage and fresh chicken meat samples were found to be positive for *S. aureus*, respectively. The highest percentages of antibiotic resistance were to ofloxacin and oxacillin (100%) while this value was 96.42% and 82.14% with tetracycline and doxycycline, respectively. Multidrug resistance was also found in most bacterial isolates obtained. The natural antibacterial (chitosan, curcumin and two essential oils) agents showed the strongest antibacterial activity against all multidrug resistant bacteria strains tested. An inhibitory effect was shown against either total bacterial counts or staphylococci counts when fresh chicken skin was treated with lemongrass essential oil with 5 µg/l and stored at 4°C for 6 days. This study revealed a high prevalence of *S. aureus* bacteria in fresh chicken and sausage samples, consequently this study reflected the poor hygienic conditions of slaughtering and handling of chicken meat as well as during manufacture of sausage that available in local markets.

Key words: *Staphylococcus aureus*, chicken meat, antibiotic sensitivity tests, multidrug resistance, sausage.

INTRODUCTION

Consumers now buy fresh chilled chicken, which originates from slaughter plants, through shops and supermarkets. Hence, the demand for these products has increased markedly, and safety problems have become a public health concern. The safety issues associated with chilled chicken have been based mostly on the presence of toxicant and pathogenic bacteria in food, which might be influence public health. It was noteworthy that according to Regulation 178/2002 of the European Parliament and Commission, a foodstuff was regarded as unsafe not only if it was harmful to consumer health but also if it was not fit for human consumption (Nowak *et al.,* 2012). In this sense, spoiled food, which means food with an appearance, taste or flavor leading to its rejection, was also considered unsafe. Chicken meat is prone to deterioration in a short time, even under chilled conditions (Patsias *et al.,* 2008). Microbiological contamination was one of the most important factors contributing to quality loss, resulting in slime, colony formation, compromised food texture, off-flavors and off-odors (Grama *et al.,* 2002; Hyldgaard *et al.,* 2015). Therefore, it became imperative that meat supplies to

*** Corresponding author: Tel. :** +201099341197
E-mail address: samahgoub@zu.edu.eg

consumers should be of best quality, with high standards of production, processing and handling from the place of production up to the consumer table.

Food pathogens were more serious than spoilage organisms because the food product may not actually look or smell spoiled. The most important pathogens associated with meat include *Listeria monocytogenes, Salmonella*, verotoxigenic *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens, Campylobacter jejuni/coli, Yersinia enterocolitica* and *Aeromonas hydrophila*. Outbreaks associated with these pathogens have been reported in meat and meat products (Mead *et al.*, 1999; Lahti *et al.*, 2001; CDC, 2007; Eurosurveillance, 2007). The emergence of multidrug-resistant bacteria was a world health problem. *Staphylococcus aureus* was one of the most opportunistic pathogens associated with hospital and community-acquired infections and septicemia (Cardozo *et al.*, 2013; Diarra *et al.*, 2013). *Staphylococcus aureus* was a serious threat to human health, due to its ability to cause a multitude of skin and respiratory infections and foodborne illnesses. It was a part of the normal microbiota on human skin and in mucous, and was the main cause of *Staphylococcus* infections in hospitals (Figueiredo and Ferreira, 2014) and food contamination during handling (Wattinger *et al.*, 2012). Its ability to form biofilms can lead to persistent contamination of food processing (Herrera *et al.*, 2007; Gutiérrez *et al.*, 2012; Spanu *et al.*, 2013) and hospital environments (Otto, 2013). Recently, the exponential increase in livestock associated methicillin-resistant *S. aureus strains* (LA-MRSA), such as clone CC398, have become a concern due to their emergence along the whole farm to fork chain (farm animals, meat products and humans) (Fluit, 2012). Over the few decades, the number of methicillin-resistant *S. aureus* (MRSA) infections has increased in many countries due to the rise of epidemics in humans (Cardozo *et al.*, 2013). MRSA and methicillin-susceptible *S. aureus* (MSSA) ranked as the second most common cause of hospital-associated blood stream infections (Purrello *et al.*, 2014). MRSA was also a substantial contributor to hospitalized patients with complicated skin and soft tissue infections (Nathwani *et al.*, 2014). The methicillin-resistant *S. aureus* (MRSA) has been detected in retail meats products (Ge *et al.*, (2017). Therefore, the aim of this study was 1) to evaluate the bacteriological quality of chilled chicken and sausage products within periods of refrigeration at 4°C, 2) to determine the prevalence of *S. aureus* in chicken and sausage products from different plants as well as the profile of resistant bacteria in these products 3) to identify some pathogenic bacteria, 4) to evaluate the antibacterial activity of curcumin, chitosan and essential oil against *S.aureus in vitro* and 5) to evaluate the antibacterial activity of essential oil against multidrug-resistant bacteria *in situ*.

MATERIALS AND METHODS

Sampling Procedure

Sixty six samples of sausage (n =24) and chicken meat (n = 42) including skin, breast and legs were collected in this study. The samples of sausage samples were purchased from different supermarkets that were produced from different companies (A,B,C,D) while chicken meat samples were purchased from different shops (*i.e.*, E,F,G,H,I,J,K,L,M,N). All samples were collected from Zagazig and Kafr Saker cities, El-Sharkia Governorate, Egypt during 2014-2015. The samples were kept in ice box and were immediately returned to the Laboratory of Microbiology Dept. Fac. Agric. Zagazig Univ. and refrigerated at 4ºC under aseptic conditions to avoid any change in their quality due to any chemical or microbial action. An inhibitory effect was shown against both total bacterial count (TBC) and total staphylococci count (TSC) when chicken skin were treated with lemongrass essential oil with 5µg/l and stored at 4^{o}C for 6 days.

Natural Antibacterial Agents

Lemongrass (*Cymbopogon citrates* L.) and geranium (*Pelargonium graveolens*) leaves were collected from different Hyper-markets, Zagazig city in Egypt. Plant materials were stored in cool and dry place for extraction of oil. According to Guenther (2013), the essential oil was extracted by hydro distillation using a Clevenger type apparatus for 4 hr., and evaporated the solvent under reduced pressure at 40ºC using rotary

evaporator. The essential oils obtained were sterilized by filtration using Millipore cellulose filter membrane (0.45 µm) and stored at low temperature.

Curcumin from *Curcuma longa* L. (Turmuric powder)- $C_{21}H_{20}O_6$; purity 97%; C1386; Sigma, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) $(CH_3)_2SO$ and was stirred for 24 hr., to ensure total solubility. The final concentration of curcumin prepared in the solution was 1% (*W/V*).

Chitosan of medium molecular weight (Mw 190,000 - 310,000 Da, 75-85% deacetylation, Sigma, Aldrich, Germany) was dissolved in 1% (*V/V*) acetic acid solution and was stirred for 24 hr., to ensure total solubility. The final concentration of chitosan in the solution was 1% (*W/V*).

Strain Used

Bacterial strain of *Staphylococcus aureus* subsp. *aureus* ATCC 6538 was obtained from Egyptian Culture Collection at Cairo, Ain Shams University, Faculty of Agriculture (MERCIN).

Microbiological Analyses

For microbiological analyses, the samples (25 g) were transferred aseptically to a blender; 225 ml of sterile buffer peptone water (0.1% *W/V*) was added and homogenized for 60 sec., at room temperature. Decimal solution in buffer peptone water (BPW) was prepared and duplicate 0.1 ml samples of appropriate dilutions were spread on non-selective and selective agar plates. Determinations were carried out as follows: TBC on plate count agar (PCA; Merck, 1.05463) incubated at 25ºC for 72 hr.; staphylococci on Baird Parker agar (Biolife, Milano, Italy) supplemented with egg yolk incubated at 37ºC for 48 hr. *S. aureus* was detected by examining the plates for typical black colonies, convex colonies, with a light halo, and these were tested for positive coagulase reaction (Bactident Coagulase Biolife, Milano, Italy). Multidrug resistant bacteria were counted and isolated from nutrient agar supplemented with neomycin or amoxicillin or ciprofloxacin or spiramycin plates incubated at 37ºC for 48 hr. For experimental purposes, the lowest detection limit of the above techniques was 2 log CFU/g. Populations of bacteria shown were the mean of three replicates and converted to $\log_{10}$ CFU/g.

Identification of Twenty Eight Bacteria Isolates

After being isolated and purified, putative staphylococci isolates were subjected to some microscopically and biochemically tests routine for identification as recommended in Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Also, they were identified again mass spectrometry as advanced strategy and confirmed by MALDI-TOF (Matrix Assisted Laser Desorption/Ionization Time of Flight) in Clinical Pathology Department at Zagazig University Hospitals, while three isolates were identified in Sigma Scientific Services Company, Giza, Egypt using 16S rRNA according to the protocol of Maniatis *et al*. (1989), using the GeneJet genomic DNA purification Kit (Thermo K0721) and two primers: F:- AGA GTT TGA TCC TGG CTC AG, R:- GGT TAC CTT GTT ACG ACT T

Antibiotics Susceptibility Test

The inhibition of *S. aureus* ATCC 6538 strain, *S. aureus* (n=16)., *S. epidermidis* (n=4)., *S. cohnii* (n=1), *S. hemoliticus* (n=4), *S. mominis* (n=1), *Macrococcus caseolticus* (n=1) and *Bacillus pumilus* (n=1) strains by various antibiotics were tested by standard disc diffusion technique (Bauer, *et al*., 1966; NCCLS 2003 and 2004). The cultures were grown in nutrient broth overnight and plated on Muller Hinton agar (Hi-Media, Mumbai). The following antibiotic discs with their concentrations indicated in parenthesis were used; spiramycin (SP: 100 µg), clindamycin (DA: 2 µg), doxycycline (DO: 30 µg), ampicillin (AM: 10 µg), tetracycline (TE: 30 µg), ciprofloxacin (CIP: 5 µg), neomycin (N: 30 µg), ofloxacin (OFX: 5 µg), amoxicillin (AMC: 30µg), penicillin G (P: 10 µg) oxacillin (OX: 10 µg), methaicillin (ME: 5 µg). The antibiotic discs were dispensed with a sufficient separation from each other so as to avoid overlapping of inhibition zones. After 30 min, the plates were inverted and incubated at 37ºC for 18–24 hr. Results were recorded by measuring the diameter of the inhibition zones (IZ) in millimeters.

Multiple Antibiotic Resistance Index

Multiple antibiotics resistance (MAR) index was calculated by using the following formula: MAR Index = Number of antibiotics to which the isolate was resistant/Total number of antibiotics tested (Raja and John, 2015).

Measurements of Antibacterial Activity of Curcumin, Chitosan and Essential Oils *In vitro*

Antimicrobial activity of curcumin was examined by agar well diffusion method (Deans and Ritchie, 1987) against *S. aureus* ATCC 6538, *S. aureus, S. epidermidis, S. cohnii, S. hemoliticus, Macrococcus caseolticus* and *Bacillus pumilus*. The cultures organisms were grown in Tryptic Soy Agar (TSA) (Biolif, Milan, Italy) at 37°C for 24 hr., and were maintained in TSA at 4°C. The pure cultures of the tested bacteria were subcultured on Mueller-Hinton broth (MHB, M1657, India) for 24 hr., at 37°C. Using 0.5 McFaland standards, the bacterial suspension was adjusted to a density of bacterial cells of 1.5×10^8 CFU/ml. A sterile swab immersed in this bacterial suspension was used to inoculate the entire surface of Mueller-Hinton Agar (MHA: M173, India) plates. Wells of 6-mm diameter were made on MHA plates using gel puncture. About 20 µl of two curcumin concentrations (0.25and 0.5% *V/V*) diluted in dimethyl sulfoxide DMSO $(CH_3)_2$ SO were transferred to each well of all plates, then, the plates were incubated for 24 hr., at 37°C, the inhibition zones (IZ) were measured in millimeters. A positive control was prepared with amoxicillin antibiotic. About 20 µl of two chitosan concentrations (0.25 and 0.5% *V/V*) diluted in acetic acid were transferred to each well of all plates, then, the plates were incubated for 24 hr., at 37°C, the inhibition zones (IZ) were measured. A positive control was prepared with amoxicillin antibiotic. Also, about 20µl of each essential oil of different concentrations (0.25 and 0.5% *V/V*) diluted in DMSO were transferred onto each well of all plates then, plates were incubated for 24 hr., at 37°C. The inhibition zones were measured. A positive control was prepared with a 100 mg/l chloramphenicol solution. All experiments were done in triplicate.

Measurement of Antibacterial Activity of Lemongrass Essential Oil *in situ*

Chicken skin meat was cut into 4 cm^2 and treated as follows: Group 1 (control): 50 ml sterilized distilled water were used for treating the skin meat. Group 2 (control with lemongrass): 5 µg/l lemongrass essential oil were used for treating the skin meat. Group 3 (seven treatments): the skin meat were artificially inoculated separately with 7 different bacterial strains then 5 µg/l lemongrass essential oil were used for treating each treatment. All samples were sterilized put in special bages and stored at 4°C for further analyses. Total bacterial count (TBC) and total staphylococci count (STC) were performed at different storage time (0 to 6 days). At the time of determination, the content of the bags was then diluted in 90 ml of 0.1% peptone water and homogenized for 2 min. Homogenates samples were then ten-fold serially diluted in the peptone water. Plate count agar (Merck, Germany) was used for counting TBC. To count STC, Baird parker agar plates were used and incubated at 37◦C for 48 hr. All colonies were finally, enumerated and the results were represented as CFU/g. For each condition, three independent samples were analyzed in duplicate.

Statistical Analysis

Data from microbiological analyses were entered into Excel 2010 and transformed into log CFU/g for all experiments. Analysis of variance was used to determine the significant difference ($P<0.05$) in bacterial count and antibacterial activity.

RESULTS AND DISCUSSION

This study was conducted to investigate the prevalence and some characteristics of antibiotic-resistant bacteria isolated from fresh white/red chicken meat and processed beef sausage samples on either plate counts or Baird Parker agar. A total of 66 samples of fresh chicken meat (n=42) and sausage samples (n= 24) were collected from various meat shops of Zagazig and Kafr Saker cities (El-Sharkia Governorate) to examine the bacterial load in the previous products. In addition, identification the isolated bacteria to species and strain level was accomplished by morphological, biochemical

characteristics as well as 16S rDNA gene analysis. Also, in order to study antibiotic sensitivity pattern and the presence of multidrug-resistant of a pathogenic *Staphylococcus aureus*. Moreover, evaluate some natural antimicrobial agents against different species of bacteria.

Total Bacterial and Staphylococci Counts of Sausage and Chicken Meat

The results of total bacterial count (TBC) and total staphylococci count (TSC) (log CFU/g) found in sausage, white chicken meat (*i.e.* skin, breast and leg parts) and red chicken meat (*i.e.* skin, breast and leg parts) are shown in Tables 1, 2 and 3. There were no significant ($P > 0.05$) differences in the TBC and TSC (log CFU/g) between sausage samples produced by A, C and D companies, but the significant ($P < 0.05$) load in TBC and TSC were detected between B company and the others (Table 1). The TBC and TSC were higher ($P < 0.05$) in sausage produced by A, C and D companies than those of sausage produced by B company. The levels of TBC and TSC ranged between 3.91 to 4.50 Log CFU/g and 3.44 to 4.38 Log CFU/g in sausage samples, respectively. The percentage of the incidence of staphylococci count over the total bacteria count in sausage samples from four different companies ranged between 88.0 to 97.3%.

These results revealed that the staphylococci group were predominant spoilage bacteria in sausage products. The counts of total aerobic bacteria were similar to those reported by other authors in processed meat. Ismail *et al.* (2000) reported that the initial population of aerobic bacteria ranged from 3.32 to 5.77 log CFU/g of meat products. Results in Table 2 show that there were significant ($P <0.05$) differences in TBC and TSC counts in white chicken meat parts (*i.e.* skin, breast and leg) between the shops (*i.e.* E to K). TBC and TSC counts of skin were significantly ($P < 0.05$) different between the shops of E, I and K and shops F, H and J but not significantly ($P > 0.05$) different between those of F, H and J (Table 2). The levels of TBC and TSC in different parts of white chicken meat samples ranged between 3.26 to 5.46 Log CFU/ g and 1.26 to 5.26 Log CFU/g, respectively. The highest TBC and TSC were recorded in skin (5.46 and 5.26 log CFU/g, respectively) comparing to the leg and breast. Also, the lowest counts of TBC and TSC were observed in leg and breast meat giving log CFU/g (3.59 and 2.46) and (3.26 and 1.26), respectively. The percentage of TSC over TBC in chicken meat parts ranged between 38.7 to 98.5%. These results concluded that the percentage of TSA over TBC was varied and this is mean that the TSC was not the main of cross-contamination of chicken meat samples.

The counts of total bacteria and total staphylococci in red chicken meat parts were collected from three different markets are shown in Table 3. Total bacterial count and total staphylococci count of the skin of red chicken meats were varied significantly ($P < 0.05$) between the shops L, M and N. Also, the values of TBC and TSC of breast and leg meats were significantly ($P < 0.05$) lower than those of the skin parts. The levels of TBC and TSC in red chicken meat samples ranged between 3.0 to 5.5 Log CFU/g and 1.4 to 5.3 Log CFU/g, respectively. The highest level of TBC were found in skin (3.6 and 5.5 Log CFU/g) compared to the leg and breast meat which showed the lowest number of TBC and TSC (3.0 and 1.4 Log CFU/g, respectively). The percentage of TSC over TBC ranged between 45.2 to 97.2% in red chicken meat parts. These results indicated that the percentage of TSA over TBC were varied and this is mean that the TSC was not the main of cross-contamination of chicken meat samples. In this study the highest bacterial count was found in the samples obtained from the shops F, H, J, M and N and the lowest count was found in the others.

The presence of staphylococci may have been due to the fact that these foods were often prepared by hand in final packaging and this direct contact may lead to an increase of contamination with *Staphylococcus* (Colombari *et al.*, 2007). The present investigation assessed the microbiological quality of chicken and sausage products throughout refrigeration. The results of this study indicated that there were some poor handling practices during the preparation of chicken and turkey products which require more attention. The counts of TBC and TSC in raw chicken meat were found in the range of the microbiological standards of the Council (94/65/EC) (1994) and Egyptian

Table 1. Total bacterial count (TBC) and total staphylococci count (TSC) in sausage samples from four different companies (log CFU/g)

Sausage samples company*	TBC	TSC	TSC/TBC (%)
A	4.50±0.03[a]	4.38±0.05[a]	97.3
B	3.91±0.03[b]	3.44±0.03[b]	88.0
C	4.24±0.01[a]	3.90±0.04[a]	92.0
D	4.31±0.01[a]	4.16±0.05[a]	96.5

All values reflect the mean values of 3 replicates and standard errors. Values in the same column bearing different letters are significantly different ($P < 0.05$).*The name of the companies: A, B , C and D.

Table 2. Total bacterial count (TBC) and total staphylococci count (TSC) in white chicken meat from seven different markets (log CFU/g)

Shop*	TBC			TSC			TSC/TBC (%)		
	Skin	**Leg**	**Breast**	**Skin**	**Leg**	**Breast**	**Skin**	**Leg**	**Breast**
E	4.17±0.06[b]	4.99±0.03[a]	4.11±0.03[b]	3.89±0.01[c]	4.61±0.02[b]	3.29±0.03[c]	93.3	92.4	80.0
F	5.03±0.02[a]	3.62±0.12[c]	3.26±0.20[c]	4.50±0.02[b]	3.14±0.06[c]	1.26±0.20[f]	89.5	86.7	38.7
G	3.66±0.08[c]	5.02±0.02[a]	4.23±0.01[b]	3.56±0.02[c]	4.91±0.01[a]	3.95±0.04[c]	97.3	97.8	93.4
H	5.46±0.01[a]	4.96±0.01[a]	4.35±0.04[b]	4.37±0.05[b]	2.46±0.04[e]	4.05±0.05[cd]	80.0	49.6	93.1
I	4.57±0.03[b]	3.65±0.02[c]	4.52±0.03[b]	4.14±0.06[bc]	3.30±0.05[c]	4.16±0.05[cd]	90.6	90.4	92.0
J	5.46±0.01[a]	3.62±0.03[c]	4.55±0.03[b]	5.26±0.01[b]	3.52±0.06[c]	4.16±0.06[c]	96.3	97.2	91.4
K	4.63±0.02[b]	3.59±0.03[c]	4.52±0.02[b]	4.56±0.02[b]	3.52±0.04[c]	4.05±0.05[cd]	98.5	98.1	89.6

All values reflect the mean values of 3 replicates and standard errors. Values in the same column bearing different letters are significantly different ($P < 0.05$) * The name of the shops (E-K)

Table 3. Total bacterial count (TBC) and total staphylococci count (TSC) in red chicken meat from three different markets (log CFU/g)

Shop*	TBC			TSC			TSC/TBC (%)		
	Skin	**Leg**	**Breast**	**Skin**	**Leg**	**Breast**	**Skin**	**Leg**	**Breast**
L	3.6±0.09[b]	3.6±0.01[a]	3.1±0.09[b]	3.5±0.04[c]	3.2±0.26[a]	1.4±0.03[c]	97.2	88.9	45.2
M	5.5±0.01[a]	3.0±0.07[b]	4.3±0.04[a]	4.3±0.07[b]	2.5±0.09[b]	4.1±0.01[a]	78.2	83.3	95.3
N	5.5±0.01[a]	3.7±0.02[a]	4.5±0.01[a]	5.3±0.01[a]	3.2±0.06[c]	4.1±0.03[a]	96.4	86.5	91.1

All values reflect the mean values of 3 replicates and standard errors. Values in the same column bearing different letters are significantly different ($P < 0.05$). *The name of the shop (L-N).

Food Codex for raw meat. According to these standards acceptable levels of total viable counts, *Escherichia coli* and *Staphylococcus aureus* are 5×10^6, 5×10^2, and 5×10^3 log CFU/g, respectively. The most probable reason of high microbial count in sausage meat might be the poor hygienic quality of raw meat, inadequate storage and thawing conditions, contamination from grinder, and the time between mincing and mixing. Minced beef poses more risk compared to intact muscle tissue because it could be contaminated throughout increased surface area and mixing during the mincing operation. For raw meat products, potential safety and quality can be estimated with the use of indicator microorganisms including aerobic plate count, staphylococci count and *E. coli* count. To ensure the microbiological quality of the final product, raw meat and ingredients must be inspected prior to entering the plant. Certified suppliers must be selected. Strong criteria for hygienic quality of raw meat must be set for suppliers. After receiving, raw meat and ingredients must be stored in appropriate conditions until use. Effective cleaning and sanitation programs must be performed in the plant. Personnel should follow the standard hygienic procedures and personnel health conditions must be monitored regularly. Finally, proper time and temperature settings for cooking should be selected.

Total Bacterial and Staphylococci Count on Solid Media Supplemented with Antibiotics

In order to compare the results from the study in question with the above results, the percentages of antibiotic-resistant bacteria from the total heterotrophic bacteria growing on solid media supplemented with antibiotics were calculated. The results are presented in Tables 4 and 5. The number of staphylococci population and total bacterial count onto Baird Parker agar and nutrient agar ranged between (3.9 to 4.5 log CFU/g) and (3.4 to 4.4 log CFU/g) in sausage samples and (4.1 to 5.5 log CFU/g) and (3.9 to 4.1 log CFU/g) in chicken meat samples, respectively. The percentage values of neomycin, amoxicillin, ciprofloxacin and spiramycin-resistant bacteria in the sausage samples and chicken meat samples were ranged from (66.1 to 97.5%), (76.2 to 88.6%), from (59.1 to 89.8%) and (41.2 to 86.1%), respectively. Meanwhile, the high percentage values of neomycin, amoxicillin, ciprofloxacin and spiramycin-resistant bacteria in the sausage samples and chicken meat samples were 97, 89, 90 and 86%, respectively. Huang *et al.* (2011) evaluated the level of antibiotic tolerance of heterotrophic bacteria and investigated the distribution of bacterial resistance to six different antibiotics (penicillin, ampicillin, cephalothin, chloramphenicol, tetracycline, rifampicin) in the secondary effluent of the wastewater treatment plant to provide useful information about antibiotic-resistant bacteria and suspected risk of antibiotic resistance bacteria to natural waters. They added that the highest percentages of ampicillin and spiramycin-resistant heterotrophic bacteria were 88.6 and 91.8, respectively.

Identification of Bacteria Grown onto Baird Parker Agar

Antibiotic resistant bacteria are a well-known public health problem. This study was conducted to investigate the prevalence and genetic characteristics of antibiotic-resistant bacteria isolated and enumerated on the Baird Parker agar from fresh chicken meat and processed beef sausage samples. A total of 42 samples of fresh chicken meat (n=30) and sausage samples (n= 12) were collected from various meat shops of Zagazig and Kafr Saker cities (El-Sharkia Governorate) to examine the presence of multidrug-resistant bacteria and pathogenic bacteria onto nutrient agar supplemented with 4 different separately antibiotic and selective media, respectively. After enrichment and inoculation onto nutrient agar supplemented with antibiotic and selective media, the colonies grown onto these media were identified by the microscopic and some biochemical tests. The bacterial isolates were belonged to 3 genera *i.e. Staphylococcus*, *Bacillus* and *Macrococcus*. The total bacterial isolates included 127 isolates, then only 28 isolates, were selected and confermed. The isolates (n=28) were identified to species and the identified bacteria including: *S. aureus* (n=16)., *S. epidermidis* (n=4)., *S. cohnii* (n=1), *S. hemoliticus* (n=4), *S. mominis* (n=1) *Macrococcus caseolticus* (n=1) and *Bacillus pumilus* (n=1) and in 4 out of the 12

Table 4. Number (log CFU/g) of resistant bacteria in sausage and chicken meat samples

Sample		NA	PB	NA+N	NA+Amc	NA+Cip	NA+ SP
Sausage	A	4.5	4.4	ND	3.9	3.8	3.7
	B	3.9	3.4	ND	3.1	2.3	1.6
	C	4.3	4.0	ND	3.3	3.8	3.9
	D	4.3	4.1	ND	3.5	3.9	3.1
Chicken meat	E	4.1	3.9	3.9	3.6	3.2	3.1
	F	5.0	4.5	4.2	4.4	4.0	4.1
	G	5.0	3.9	3.3	4.0	4.2	4.3
	H	5.5	4.1	5.4	4.7	5.1	4.1

NA, Nutrient agar; BP, Baird Parker agar; N, Neomycin; Amc, Amoxicillin; Cip, Ciprofloxacin; SP, Spiramycin; ND: Not determined.(A – D): Sausage samples (E – H): Chicken meat samples

Table 5. Percentage values of resistant bacteria in the tested samples

Sample		N (%)	Amc (%)	Cip (%)	Sp (%)
Sausage	(A)	ND	86.2	84.7	83.1
	(B)	ND	76.2	59.1	41.2
	(C)	ND	77.7	88.9	91.8
	(D)	ND	80.5	89.1	67.2
Chicken meat	(E)	94.9	87.0	77.9	75.7
	(F)	83.5	88.6	80.0	81.6
	(G)	66.1	80.1	84.6	86.1
	(H)	97.5	85.2	89.8	75.7

N, Neomycin; Amc, Amoxicillin; Cip, Ciprofloxacin; SP, Spiramycin; ND : Not detected

sausage samples were coagulase positive in *S. aureus*. The number of *S. aureus* positive from all samples were 16 out from 42 isolates. These results revealed that a contamination of fresh chicken meat and sausage with *S. aureus*, *S. hemoliticus* and *S. epidermidis* was confirmed in this study.

16S rDNA Gene Sequence Similarity and Phylogenetic Analysis

Molecular identification of the three selected isolates were carried out based on 16S rRNA sequence analysis. The partial sequences of 16S rRNA obtained from isolates were aligned with all the presently available 16S rRNA sequences in the GenBank data base. The sequences were deposited in NCBI-GenBank and in the obtained Accession Numbers (Table 6). As a result, a phylogenetic tree was mapped using the neighbor joining method. Phylogenetic analysis using the 16S rRNA sequences indicated that the isolates belonged to the genera *Staphylococcus* and *Stenotrophomonas* according to blast results shown three isolates were identified as *S. aureus*, *S. hemoliticus* and *Stenotrophomonas terrae*.

Table 6. Genotyping identification results of the isolated bacteria from fresh chicken meat and sausage

Bacterial code and source	Description	Accession number	Maximum identity (%)
(C 76) Chicken meat	*Staphylococcus aureus* strain ATCC 12600 UvrA and HprK genes, partial cds	AF195962	97
(S 73) Sausage	*Staphylococcus haemolyticus* strain JCM 2416 16S ribosomal RNA gene, partial sequence	NR_113345	99
(C66) Chicken meat	*Stenotrophomonas terrae* strain R-32768 16S ribosomal RNA gene, partial sequence	NR_042569	98

Antibiotic Sensitivity Test

The presence of antibiotic resistant bacteria in meat may have important public health consequences. Antibiotic sensitivity test helps in determining and selecting effective antibiotics against a particular disease caused by a particular bacterium. Further, selection of appropriate antibiotic reduces the cost of treatment as well as the time of recovery. The highest percentages of antibiotic resistance (Table 7) were to ofloxacin and oxacillin (100%) while this value was 96.42% and 82.14% with tetracycline and doxycycline, respectively. However, all the isolates were sensitive to penicillin G (100%). *S. aureus* strains (C121 and C120) showed highest resistance (66.67%) against the most antibiotic tested followed by *S. cohnii* (C14) with resistant 58.33% and *S. epidermidis* (C8) with 50% resistant .Furthermore, all strains were resistant to ofloxacin and oxacillin. The evaluation of antibiotic resistance profile was performed to promote the safety evaluation. Consequently, twelve different antibiotics were used against *S. aureus* isolates (Table, 8). The antibiotic resistance profile of the tested *S. aureus* to different antibiotics revealed that all *S. aureus* isolated from chicken meat and sausage samples (n = 13 and n = 3, respectively) were sensitive to penicillin G and neomycin (100%) (Table 8). Otalu, *et al.* (2011) observed that some degree of resistance towards the antibiotics in poultry meat where they found that 84.6% of the isolates were sensitive to the antibiotics. In the current study, 94 -100% of *S. aureus* were found to be resistant to spiramycin, tetracycline, ofloxacin and oxacillin and 69-88% of *S. aureus* were found to be resistant to methaicillin (13/17), doxycycline (15/17) and clindamycin . In this respect, Heo *et al.* (2008) who reported that 50% of *S. aureus* isolates from poultry meat samples were resistant to ampicillin. In contrast, in our study 25% of *S. aureus* isolates were resistant to ampicillin. Further, only 17% of the isolates from different meat samples were reported to be resistant to ampicillin by Kelman *et al.* 2011. Results of this study also represent high sensitivity of isolates towards penicillin G. Yurdakul *et al.* (2013) found that 25% of *S. aureus* isolates from chicken meat were resistant to erythromycin. The antibiotic resistant results for tetracycline (96.42%) in the present study were in accordance to the study by Lin *et al.* (2009) and Kelman *et al.* (2011) they observed lesser percentage of 66.7% and 69%, respectively of resistant isolates. However, Heo *et al.* (2008) and Otalu *et al.* (2011) found the same percent of resistant isolates as compared to the present study as they observed 92.9 and 100 % *S. aureus* isolates resistant to tetracycline, respectively. Yurdakul *et al.* (2013) also found all strains of *S. aureus* were resistant to tetracycline in their study on chicken meat. The finding of a large number of *S. aureus* isolates resistant to methaicillin, oxacillin, ofloxacin, tetracycline, doxycycline, spiramycin, clindamycin and ampicillin is of considerable concern as these drugs are commonly used in veterinary medicine in Egypt. The indiscriminate use of antibiotics especially, in developing countries has evoked serious bacterial resistance and emergence of new and highly resistant strains of bacteria to commonly used antibiotics (Harakeh *et al.,* 2006). Multidrug-resistance which has been defined as resistance to 3 or more antimicrobial agents was found in all the isolates used in the present

Table 7. Effect of different antibiotics on the studied bacteria based on the diameter of inhibition zone (mm)

Strain	Diameters of inhibition zone (mm)												MAR* index
	SP	DA	DO	AM	TE	CIP	N	OFX	AMC	P	OX	ME	
***S.aureus* (C123) ATCC 6538**	0	9	0	12	0	21	10	0	29	17	0	9	0.41
***S. aureus* (C11)**	0	1	0	10	8	0	8	0	29	20	0	9	0.41
***S.aureus* (C12)**	0	9	0	12	0	21	10	0	29	17	0	9	0.41
***S.aureus* (C13)**	0	9	0	10	0	30	8	0	29	15	0	0	0.50
***S.aureus* (C45)**	0	9	0	10	0	21	8	0	29	16	0	9	0.41
***S.aureus* (C66)**	0	8	0	0	0	21	11	0	29	17	0	0	0.58
***S.aureus* (C76)**	0	0	0	11	0	21	11	0	29	17	0	0	0.58
***S.aureus* (S85)**	0	0	0	10	0	21	9	0	29	17	0	0	0.38
***S.aureus* (S87)**	0	0	0	12	0	21	8	0	29	17	0	0	30.38
***S.aureus* (S88)**	0	0	0	10	0	21	10	0	29	17	0	0	0.58
***S.aureus* (C94)**	0	0	0	11	0	21	10	0	29	17	0	0	0.58
***S.aureus* (C117)**	0	0	0	0	0	21	10	0	29	17	0	0	0.58
***S.aureus* (C118)**	0	0	9	12	0	21	10	0	29	17	0	0	0.50
***S.aureus* (C119)**	0	0	9	10	0	21	10	0	29	17	0	0	0.50
***S.aureus* (C120)**	0	0	0	0	0	21	10	0	29	17	0	0	0.66
***S.aureus* (C121)**	0	0	0	12	0	27	10	0	0	17	0	0	0.66
***S.aureus* (C122)**	12	0	0	0	0	34	13	0	32	20	0	0	0.58
***S.epidermidis* (C5)**	1	0	0	13	0	35	9	0	32	20	0	9	0.41
***S.epidermidis* (C6)**	1	0	12	12	0	35	9	0	32	20	0	9	0.33
***S.epidermidis* (C7)**	1	0	12	10	0	35	9	0	32	20	0	9	0.33
***S.epidermidis* (C8)**	0	0	0	11	0	35	9	0	32	20	0	9	0.50
***S.hemoliticus* (C15)**	9	9	0	0	0	32	9	0	32	20	0	9	0.41
***S.hemoliticus* (C16)**	9	0	0	10	0	32	9	0	32	20	0	0.9	0.41
***S.hemoliticus* (C17)**	9	0	0	12	0	32	9	0	32	18	0	9	0.41
***S.hemoliticus* (C67)**	9	0	0	13	0	32	9	0	30	18	0	9	0.41
***S. mominis* (C24)**	19	0	0	13	0	32	10	0	31	19	0	9	0.41
***Ma.caseolyticus* (S1)**	13	0	0	15	0	32	10	0	29	16	0	0	0.50
***B.pumilus* (C93)**	1	0	0	14	0	35	11	0	26	18	0	9	0.41
***S.cohnii* (C14)**	9	0	15	0	0	30	0	0	0	18	0	9	0.58

MAR* = Multiple antibiotics resistance index. Spiramycin (SP: 100 µg), Clindamycin (DA: 2 µg), Doxycycline (DO: 30 µg), Ampicillin (AM: 10 µg), Tetracycline (TE: 30 µg), Ciprofloxacin (CIP: 5 µg), Neomycin (N: 30 µg), Ofloxacin (OFX: 5 µg), Amoxicillin (AMC: 30µg), Penicillin G (P: 10 µg) Oxacillin (OX: 10µg), Methaicillin (ME: 5 µg).

Table 8. Antibiotic resistance pattern of *S. aureus* isolates from chicken meat and sausage

No.	Antibiotic agent	Resistance	Intermediate resistance	Susceptible
1	**Spiramycin(100)**	15/16 (93.75%)	1/16 (6.3%)	0.0 (0.0%)
2	**Clindamycin(2)**	11/16(68.75%)	5/16(31.3%)	0.0 (0.0%)
3	**Doxycycline(3)**	14/16(87.5%)	4/16(25%)	0.0 (0.0%)
4	**Ampicillin(10)**	4/16 (25%)	12/16(75%)	0.0 (0.0%)
5	**Tetracycline(30)**	15/16(93.8%)	1/16(6.3%)	0.0 (0.0%)
6	**Ciprofloxacin(5)**	1/16 (6.3%)	0.0 (0.0%)	15/16 (93.8%)
7	**Neomycin (3)**	0.0 (0.0%)	16/16 (100%)	0.0 (0.0%)
8	**Ofloxacin(5)**	16/16(100%)	0.0 (0.0%)	0.0 (0.0%)
9	**Amoxicillin(30)**	1/16 (6.3%)	0.0 (0.0%)	15/16(93.8%)
10	**Penicillin(10)**	0.0 (0.0%)	0.0 (0.0%)	16/16 (100%)
11	**Oxacillin(10)**	16/16 (100%)	0.0 (0.0%)	0.0 (0.0%)
12	**Methaicillin(5)**	13/16 (81.3%)	3/16(18.8%)	0.0 (0.0%)

study. In another study (Waters *et al.*, 2011) multidrug resistance was common among *Staphylococcus* isolates (52%). Multidrug-resistant *S. aureus* isolates were especially one of the greatest public concerns since the treatment of infections is more difficult when encountering resistance (Heo *et al*., 2008), especially, in developing countries where widespread and uncontrolled use of antibiotics is common.

Antibacterial Activity of Curcumin

From results presented in Table 9, it is clear that two different concentrations of curcumin (0.25% and 0.50%) showed strong antibacterial activity against *Staphylococcus*, *Macrococcus caseolticus* and *Bacillus pumilus* with an inhibition zone of 32 to 36 mm closely to amoxicillin concentration 30 µg/ml (26 to 32 mm). The curcumin at 0.5% showed the largest inhibition zone (IZ) of 36 mm with *S. cohnii* compared to amoxicillin with IZ of 30 mm. The minimum inhibitory concentration (MIC) against the studied bacteria was about 0.25% (*V/V*). Curcumin possesses strong antimicrobial activity against a wide range of microorganisms including fungi, Gram-positive and Gram-negative bacteria (Liang, *et al*., 2008; Rudrappa and Bais, 2008; Neelofar, *et al*., 2011; Mun, *et al*., 2013; Betts and Wareham, 2014). The antibacterial activity of curcumin against *B. subtilis*, *E. coli* and *Pseudomonas aeruginosa* occurs through the inhibition of bacterial cell proliferation, affecting virulence, quorum sensing and biofilm initiation as well as membrane damages of bacterial cell (Rudrappa and Bais, 2008; Kaur *et al*., 2010; Tyagi *et al*., 2015).

Antibacterial Activity of Chitosan

The antibacterial effect of two different concentrations of chitosan prepared with acetic acid (0.25 and 0.5% *V/V*), measured against *Staphylococcus*, isolates *M. caseolticus* and *B. pumilus* strains by disc assay was evaluated during 24 hr., at 37°C (Table 9). The addition of 0.5% chitosan exhibited a significant inhibitory effect on the isolated bacteria of *Staphylococcus*, isolates *M. caseolticus* and *B. pumilus* by forming inhibition zones (IZ) with different sizes around the acidified-impregnated discs. A concentration dependent increase in the diameter of the inhibition zone was observed, the widest zones were recorded at 0.5% concentration of chitosan against all tested strains (21 to 29 mm) and were less more than those of amoxicillin 30

µg/ml concentration (26 to 32 mm). From the experimental results, it was clearly emerges that chitosan exhibited antimicrobial effect against the tested bacteria (Table, 9). The application of different concentrations of chitosan caused an immediate reduction in the tested strains. The observed antimicrobial activity of chitosan on *Staphylococcus*, isolates *M. caseolticus* and *B. pumilus* is in accordance with the results reported by other authors. Chitosan activity can be explained by changes in cell permeability, the interaction between the amino groups of chitosan and the electronegative charge on the cell surface which leads to leakage of the intracellular protein and electrolyte (Varaldo, 1991; Rabea, *et al*., 2003). Moreover, according to Brodelius *et al*. (1989), a high concentration of chitosan may cause cell death due to membrane permeabilization. Antimicrobial potential increased with the increase in chitosan concentrations. According to Petrou *et al.* (2012) chitosan applied alone or in combination with oregano essential oil can extend the shelf-life of chicken filets packed in a modified atmosphere for 6 or 14 days. Vasilatos and Savvaidis (2013) examined the antimicrobial activity of chitosan with the addition of 0.25% (*V/W*) rosemary essential oil on the growth of various microorganisms on turkey filets.

Antibacterial Activity of Lemongrass and Geranium Essential Oil (LO and GO)

As shown in Table 9, the two tested concentrations (0, 0.25 and 0.5% *V/V*) of two different essential oil constituents, including lemongrass (*Cymbopogon citrates* L.) and geranium (*Pelargonium graveolens* L.) essential oils, each known to have anti-staphylococcal effects (Burt, 2004). Both essential oils exhibited a significant inhibitory effect on the isolated bacteria of *Staphylococcus, Macrococcus caseolticus* and *Bacillus pumilus* by forming inhibition zones (IZ) with different sizes around the acidified-impregnated discs. A concentration dependent increase in the diameter of the inhibition zone was observed with both oils. The widest zones were recorded at the 0.5% concentration of both essential oil against all tested strains (32 to 41 mm) and were higher than those of amoxicillin 30 µg/ml concentration (26 to 32 mm). Essential oil-derived compounds are proposed to compromise the integrity of bacterial cytoplasmic membrane, leading to leakage of essential cellular constituents (Burt, 2004). However, the antimicrobial activities of essential oil-derived compounds vary depending on the lipophilic properties of their hydrocarbon skeleton and the hydrophilicity of their functional groups (Faleiro, 2011). These essential oils might be beneficial against the isolated *S. aureus* strains compared to stranded strains of *S. aureus* ATCC 6538 (Table 9). Prashar *et al*. (2003) and Si *et al*. (2006) found that geranium oils have strong antimicrobial activitiy and it could be related to its major component citronellol. Also, the essential oil contains the oxygenated monoterpene linalool which known to have superior antimicrobial activity (D'auria *et al.*, 2005). In addition, Jirovetz *et al*. (2006) showed that essential oil with floral-rosy scent, such as geranium, possess high antimicrobial activities against a wide range of microorganisms and these effects are mainly the outcome of a blend of some biologically active principal aroma compounds (citronellol, isomenthone, linalool, and many of their derivatives). Recently, Boukhatem *et al.* (2013) confirmed the components in lesser quantity such as, pinene, linalool, rose oxide, geranyl formate and caryophyllene could also contribute to the antimicrobial activity of these oils. In the same line, the antimicrobial activity of LO may be linked to its chief component citral. Also, Yang *et al*. (2016) reported that ginger EO, the main compound was citral, has strong antimicrobial properties.

Effects of Lemongrass Essential Oil on the Survival of Bacteria and Different Isolates of Staphylococci

Results in Table 10 reveal that an inhibitory effect was shown against both total bacterial count (TBC) and total staphylococci count (TSC) when chicken skin was treated with lemongrass essential oil with 5µg /l and stored at 4°C for 6 days. Moreover, when the samples were not treated with oil, the number of TBC increased from 5.63 to 6.77 log CFU/g, while, it was increased from 3.90 to 4.95 log CFU/g in the treated group. When the samples were not treated with oil, the number of TSC increased from 4.51 to 5.79 log CFU/g, while, it was increased

Table 9. Diameter of zones of inhibition of some bacterial strains caused by curcumin, chitosan and two essential oils compared to amoxicillin

Natural antibacterial agent	Diameter of inhibition zone (mm) ± SD								
	Amoxicillin 30 µg/ml	Curcumin		Chitosan		Lemongrass oil		Geranium oil	
Strain	Control	0.25%	0.5%	0.25%	0.5%	0.25%	0.5%	0.25%	0.5%
***S. aureus* ATCC 6538**	30±0.3	23±0.2	31±0.2	14±0.1	23±0.0	35±0.1	37±0.1	31±0.1	36±0.1
S. aureus	29±0.3	25±0.1	32±0.2	13±0.3	24±0.1	30±0.1	35±0.2	29±0.1	36±0.1
S. epidermidis	32±0.2	31±0.1	33±0.1	15±0.0	26±0.1	35±0.3	36±0.2	34±0.2	41±0.3
S. cohnii	30±0.2	32±0.0	36±0.1	19±0.0	29±0.2	31±0.0	36±0.1	33±0.1	40±0.3
S. hemoliticus	32±0.0	30±0.2	34±0.2	18±0.2	27±0.2	33±0.1	38±0.1	35±0.3	41±0.2
Macrococcus caseolticus	29±0.1	29±0.2	32±0.2	16±0.1	25±0.3	31±0.2	35±0.3	35±0.2	40±0.3
Bacillus pumilus	26±0.3	25±0.1	35±0.1	14±0.4	21±0.2	28±0.2	32±0.2	27±0.3	32±0.1

Table 10. Survival and growth of total bacterial count (TBC) and different strains of staphylococci (Mean population recovered (Log CFU/g)) in chicken skin affected by essential oil stored at 4°C.

Treatment (Lem.)	0 Day	1 Day	2 Days	3 Days	4 Days	5 Days	6 Days
Media used			**Plate Count Agar (TBC)**				
Control 1	5.63	5.75	5.83	5.86	6.68	6.76	6.77
Control 2	5.30	4.63	4.40	4.63	4.59	4.49	4.40
S.aureus (S85)	3.70	4.30	4.45	4.48	4.57	4.69	4.75
S. aureus (C12)	4.11	4.65	4.77	4.80	4.85	4.94	5.02
S.aureus (C13)	3.90	4.04	4.28	4.62	4.80	4.89	4.96
S.aureus (C76)	3.48	3.70	3.95	4.08	4.34	4.52	4.69
S. aureus (C85)	4.36	4.86	4.96	5.11	5.24	5.29	5.33
S. hemoliticus (S73)	4.26	4.52	4.62	5.06	5.13	5.23	5.30
St. terrae (C66)	3.48	3.95	4.04	4.23	4.36	4.46	4.60
Average	3.90	4.29	4.44	4.63	4.76	4.86	4.95
Media used			**Baird Parker Agar (TSC)**				
Control 1	4.51	4.65	4.81	5.75	5.67	5.73	5.79
Control 2	4.33	3.66	3.45	3.23	3.51	3.19	3.19
S.aureus (S85)	1.95	2.36	2.95	2.97	2.97	3.00	3.05
S. aureus (C12)	1.00	1.30	1,30	2.23	2.90	3.05	3.19
S.aureus (C13)	1.00	1.30	1.48	2.20	3.00	3.28	3.31
S.aureus (C76)	1.00	1.30	1.95	2.54	3.05	3.30	3.47
S. aureus (C85)	1.00	1.30	1.90	2.30	2.99	3.05	3.28
S. hemoliticus (S73)	1.00	1.30	1.70	2.23	2.36	2.60	2.97
St. terrae (C66)	1.00	1.30	1.48	2.04	2.79	2.91	3.00
Average	1.14	1.45	1.82	2.36	2.87	3.03	3.18

Control 1 = without lemongrass, Control 2= with Lemongrass

S. =Staphylococcus, St.= Stenotrophomonas, Lem. = Lemongrass essential oil (5µg/L).

from 1.14 to 3.18 log CFU/g as an average in the treated groups. Before 6 days of storage at 4°C, Log CFU/g of TBC was decreased from 5.63 to 3.90 at 0 day and after treatment with essential oil then decreased from 6.77 to 4.95 after 6 days. A reduction in TSC was observed at 0 time when chicken skins were treated with essential oil. The survival of staphylococci strains was inhibited by lemongrass essential oil. Essential oil of plants as a natural food additive with proven antimicrobial effects, has been demonstrated to inhibit the growth of spoilage inducer and food-borne pathogenic bacteria. Lemongrass essential oil has been used as preservatives for its antimicrobial (Prashar *et al*., 2003). Further studies are needed to elucidate transmission routes of MRSA in relation to meat and other foods and to provide the tools for preventing the spread of MRSA. At present the high prevalence of MRSA in meat has not been shown to contribute significantly to the dissemination of MRSA to humans and the possible health hazard for consumers of the presence of MRSA in foods should be further elucidated.

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تثبيط البكتيريا متعددة المقاومة للمضادات الحيوية والمعزولة من لحوم الدجاج الطازجة والسجق بواسطة المضادات البكتيرية الطبيعية

أماني أحمد السيد عبدالله - ناهد أمين الوفائي - سمير أحمد مرغني محجوب- زكية علي القناوي

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة الزقازيق – مصر

تم جمع ٦٦ عينة من لحوم الدجاج الطازج (ن = ٤٢) وعينات السجق (ن = ٢٤) والتي جمعت من محلات اللحوم المختلفة من مدينة الزقازيق ومدينة كفر صقر (محافظة الشرقية) لفحص وجود المكورات العنقودية الذهبية المقاومة للعديد من المضادات الحيوية والمسببة للأمراض، ولتقييم بعض مضادات الميكروبات الطبيعية المختلفة ضد بعض أنواع هذه البكتريا وتم عزل ١٢٧ عزلة بكتيرية علي بيئة بيردباركر من العينات تحت الدراسة وقد تم تحديد الأنواع وعلي مستوى السلالة باستخدام الصفات المورفولوجية والاختبارات الكيموحيوية، وتحليل الجينات 16S rDNA ومن بين هذه العزلات تم الحصول علي ٤٢ عزلة من البكتيريا العنقودية ومن بينها ١٦ عزلة (بما يوازي ٣٨.٠٨%) كانت موجبة لاختبار الكوأجيوليز، وكان أكثر الأجناس التي تم تحديدها من خلال تحليل الحمض النووي كانت المكورات العنقودية، وقد تم تحديد نسبة ٢٨.٠٦ و٣٣.٣٣% في عينات السجق ولحم الدجاج الطازجة لتكون إيجابية للعنقوديات الذهبية علي التوالي، أعلى نسبة من السلالات كانت مقاومة للمضادات الحيوية ووصلت النسبة إلى ١٠٠%، مع الافلاكسين والاوكسالين بينما كانت المقاومة للتتراسيكلين والدوكسي سلين (٨٢.١٤% و٩٦٤٢%) علي التوالي كما لوحظ مقاومة اغلب البكتريا المعزولة لعديد من المضادات وكانت المضادات الطبيعية المضادة للبكتيريا (الشيتوزان، والكركم واثنين من الزيوت الطيارة) لها نشاط أقوى من معظم المضادات الحيوية ضد البكتيريا متعددة المقاومة للعديد من المضادات الحيوية، وأظهرت الدراسة تأثير تثبيطي علي كل من أعداد البكتيريا الكلية والعنقوديات عند معاملة جلد الدجاج الطازج بزيت الليمون الطيار بتركيز ٥ ميكرو ملليتر في اللتر عند تخزينها لمدة ٦ أيام على درجة ٤ درجة مئوية، وتعكس الدراسة سوء حالة النظافة والذبح والتعامل مع اللحوم الطازجة وكذلك السجق في العينات المتوفرة في الأسواق.

المحكمـون:

١- أ.د. همت محمد محمد عبدالهادي أستاذ الميكروبيولوجي – كلية الزراعة – جامعة عين شمس.

٢- أ.د. هـويـــدا محمـد لـــبيب أستاذ الميكروبيولوجي المتفرغ – كلية الزراعة – جامعة الزقازيق.