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Original Research Article
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PREVALENCE, PHENOTYPIC-GENOTYPIC RESISTANCE AND BIOFILM FORMATION OF *Staphylococcus aureus* IN CHICKEN MEAT WITH REFERENCE TO ITS PUBLIC HEALTH HAZARD

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Abstract: The purpose of this study was to determine the prevalence, antimicrobial resistance in *S. aureus* and presence of enterotoxigenic as well as biofilm-forming genes in *S. aureus* bacteria isolated from broiler meat from traditional shops and supermarkets in Sharkia, Egypt. For this determination, two hundred fresh raw chicken meat cotton swabs were collected from the breast and thighs (100 each). *S. aureus* was highlighted through a coagulase test, and then phenotypic and genotypic characterizations were studied. Uniplex PCR was used to identify the occurrence of enterotoxin genes in the selected isolates. Finally, they were subjected to a biofilm formation test using 96-well flat bottom polystyrene microtiter plates; besides, biofilm-forming genes were investigated. Nineteen isolates out of the 200 samples tested positive for *S. aureus* (9.5 percent), No Vancomycin resistance strains were obtained, nor did any ciprofloxacin isolates for *S. aureus* while, all isolates were 100 % resistant for streptomycin, amoxicillin- clavulanic acid and sulfamethoxazole-trimethoprim *S. aureus* isolates were found resistant to at least one antibiotic. The multiple antibiotic resistance (MAR) index of *S. aureus* isolates was ranged from 0.3 to 0.7 with an average of 0.4. In The mecicillin resistant *S.aureus* (MRSA) strains carried *mec*A gene with a percentage of 5%, while the *bla*Z gene was distributed with a percentage of 5.5%, 11/200). The obtained isolates gave 60% strong biofilm formation and 40% were non-formers, with nil results for moderate or weak production isolates. The *ica*A gene was 100%, in comparison to *ica*D which was zero. It should be noted that strong biofilm former strains were only 100% positive for *sea* and *seb*. This study pointed out the higher prevalence of MDR isolates of *S. aureus* isolates in chicken meat due to inadequate handling and insufficient sanitary equipment and post microbial contamination. This finding highlighted the importance of broiler meat as a reservoir for antimicrobial-resistant strains of *S. aureus* and biofilm-forming of *Staphylococcus aureus*.

Key words: prevalence; antimicrobial resistance; *Staphylococcus aureus*; biofilm producer

Introduction

Owing to its low cost, lack of religious obstructions and high quantity of required amino acids, poultry meat production and consumption have grown rapidly all over the world. Moreover, given the obvious scarcity of red meat, broiler meat is seen as a valuable source of protein (1). The annual chicken meat production in Egypt is estimated around 1.5 million tons in 2020 and represented a slight increase to reach 1.55 million tons in 2022. To inspect microbiological security, cleanliness conditions throughout processing, besides product storage conditions, *S. aureus* had been tested in meat as well as fowl products (2). Bacterial foodborne illness accounted for almost 68 percent of all incidences in the last ten years, with *S. aureus* being the third most common pathogen after *Salmonella* (3). Staphylococci have been categorized into 50 dissimilar species and subspecies based on their competency to make coagulase thus far. Coagulase-positive staphylococci (CPS) are

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those that produce coagulase, while coagulase-negative staphylococci (CNS) are those that do not. Only CPS strains, on the other hand, have been linked to food poisoning (4). Food poisoning caused by *S. aureus* is an intoxication triggered by the intake of foodstuffs containing adequate preformed enterotoxins (one or more). Nausea, intense vomiting, stomach cramps, with or without diarrhoea, are all symptoms of it, which appear quickly (2–8 hours). The sickness is normally self-limiting and disappears within 24–48 hours of commencement. It is frequently serious enough to necessitate hospitalization, especially when newborns, the aged, or disabled are involved (5).

Staphylococcus aureus bacterium's foodborne disorders are caused by the presence of multiple enterotoxins, that are produced by staphylococci and streptococci that are functionally related and have similar sequences (6). These bacterial proteins are known to be pyrogenic; in addition, they are interrelated to serious human illnesses like food poisoning and toxic shock syndrome. Although further species have been shown to be enterotoxigenic , such as: *S. hyicus*, *S. intermedius*, *S. epidermidis* and *S. xylosus* (7,8). The majority of these toxins are produced by *S. aureus* (7). Staphylococcal enterotoxins (SEs) are single-chain, low-molecular-weight proteins that have high heat tolerance. They are characterized into twenty-three types. The most critical types with severe clinical implications are SEA, SEB, SEC, SED, and SEE(6)

Antimicrobial resistance (AMR) is a significant hazard to global health security and has emerged as a significant concern in clinical practice and healthcare. Higher costs and reduced efficacy of therapy for common diseases are seen in healthcare settings as a result of antibiotic resistance and a lack of synthesis of newer antibiotic drugs (9). Through carrying the *mec*A gene, which encodes the penicillin binding protein 2a (PBP2a), Methicillin Resistant *S. aureus* (MRSA) obtains resistance to penicillin and other β-lactam drugs (10). In clinical testing, most MRSA strains were found to be multidrug resistant (MDR) (11). The occurrence of *S. aureus* and MRSA in retail meat has gotten a lot of attention recently (10). Between May 2002 and August 2003, researchers looked for MRSA in 444 raw chicken flesh samples (165 thighs and breasts, as well as other organs) sold in 145 stores across 47 prefectures in Japan. *S. aureus* was found in 292 (65.8%) of the 444 samples and 131 of the 145 markets (12).

Biofilms, as opposed to planktonic cells, increase bacterial resistance to environmental stresses such as cleaning, disinfection, and inhibition, allowing these microorganisms to remain on surfaces and processing facilities (13). *S. aureus* biofilms are known to be one of the bacteria's main virulence factors as well as a major source of clinical infection. The most essential step in *S. aureus* biofilm establishment is the synthesis of the polysaccharide intercellular adhesin (PIA), which modulates the adhesion of bacterial cells to one another in the biofilm (14). The icaADBC operon encodes the enzymes necessary for PIA production. The ica operon was discovered and investigated extensively in *S. epidermidis* before being discovered in *S. aureus*. In spite of indications to the contrary, most *S. aureus* strains appear to contain the whole ica operon (15). Therefore, the aim of this study was to investigate the prevalence, phenotypic-genotypic antimicrobial resistance, and existence of biofilm-forming genes in *S. aureus* bacteria isolated from broiler meat.

Materials and methods

Sample Collection

From September to November 2021, 200 cotton swabs of broiler chicken meat from the breast and thigh regions (100 each) were acquired from retail outlets in Alsharqia province, including traditional open markets and supermarkets. The obtained swabs were immediately transferred under aseptic conditions to pre prepared buffered peptone water (0.1 %), then to the laboratory of the Faculty of Veterinary Medicine, Zagazig University, in an icebox under the same conditions for bacteriological analysis.

Bacterial isolation and identification

All samples were examined bacteriologically for the presence of *S. aureus*. Isolation and identification of coagulase-positive Staphylococci was done according to standard methods (16)

Antimicrobial susceptibility testing

The disk diffusion test, as described by the Clinical and Laboratory Standards Institute was performed using Mueller-Hinton agar plates with disks containing the following 10 antimicrobial agents for *S. aureus*. The interpretive class for each isolate (resistant, intermediate, or susceptible) was obtained according to the CLSI recommendations (17). Isolates showing resistance to three or more antibiotics were defined as MDR isolates.

Molecular detection of antimicrobial resistance and virulence genes

The genomic DNA was extracted from 19 biochemically identified *S. aureus* isolates with phenotypic resistance and isolated from poultry meat using the QIAamp DNA mini kit (Takara Kit, Catalogue no. 51304, Japan). From Table 1, The selected primer sequences and cycling conditions were carried out to amplify the resistance genes to methicillin (*mec*A) and beta-lactamase (*blaZ*) in *S. aureus* isolates using uniplex PCR (18,19). Moreover, the oligonucleotides sequences and PCR cycling conditions to identify enterotoxin genes (*sea*, *seb, sec*, *sed*, and *see*) in *S. aureus* were performed by multiplex PCR (20). The electrophoresis of PCR products was performed on a 1.5% agarose gel with ethidium bromide (0.5 µg/ml) and a gel pilot 100 bp ladder (QIAGEN, USA). A positive control isolate of *S. aureus* was included in each reaction.

Detection of Biofilm Formation Ability and Biofilm Genes

Five *S. aureus* isolates (n = 5) to generate biofilms were tested and studied using 96-well flatbottom polystyrene microtiter plates. (Techno Plastic Products, Switzerland) as formerly termed (21). Each isolate's fresh culture in TSB (200 µL) was injected into wells of a sterile microtiter plate and incubated at 37°C for 24 hours. Negative and positive controls were TSB without bacteria *and S. aureus* ATCC 25923, respectively. On the way to eliminate non-adherent cells, the contents of every well were removed and splashed three times with 200 µL of phosphate buffer saline (PBS, pH 7.3).

For 15 minutes, the plates were drained and air-dried. The biofilms were dyed for 30 minutes with 150µL of 0.1 percent crystal violet (Fluka AG, Buchs, Switzerland), then rinsed twice with PBS and air-dried. The dye bound to the cells was resolubilized for 45 minutes in 150 µL of 95 percent ethanol, and the optical density (OD) was quantified using an ELISA reader at a wavelength of 570nm. (Awareness Technologies stat fax 2100, CA, United States). The samples were examined in triplicate, and the procedure was repeated three times. As a result, the average OD values and standard deviations (SD) for the tested isolates and negative controls were estimated. For evaluation of biofilm development, the cut-off value of the OD (ODc) was calculated as follows: ODc = average OD of negative control + (3× SD of negative control), and the isolates were classified as follows: strong biofilm producers (4× ODc< OD), moderate (2× ODc< OD≤ 4×ODc), weak (ODc< OD≤ 2× ODc) and non-producer (OD≤ ODc). Additionally, Biofilm producing isolates were further investigated for biofilm-related genes (*ica*A and *ica*B) (22, 23).

Results

Prevalence of S. aureus in broiler meat

Table (1) showed the prevalence of *S. aureus* in the chicken samples. *S. aureus* was found in 19 of the tested 200 samples (9.5 %).The broiler thigh had slightly higher incidence (10%) of *S. aureus* compared with the broiler breast (9%).

Among the total recovered isolates of *S.aureus*, the distribution of *S.aureus* in the thigh region was the highest (52.63%, 10 out of 19) in comparison to that distribution in chicken breast (47.36%, 9 out of 19).

Determination of enterotoxin genes in S. aureus

The ratio of enterotoxin-encoding genes *sea, seb*, *sec*, and *sed* in *S. aureus* isolates were screened by using multiplex PCR. Each of *sea* and *seb* genes exhibited the highest distribution (31.57%, 6 out of 19) among the recovered isolates of *S.aureus*; while all recovered isolates were negative for other enterotoxin genes (*sec*, *sed* &*see*) as illustrated in Table (2). The PCR products of *sea* and *seb* genes were 102 and 164 bp, respectively (Figure 1).

Phenotypic resistance of S. aureus

The present study revealed that all isolates of *S.aureus* showed the peak resistance (100%) to methicillin and amoxicillin- clavulanic acid followed by moderate resistance percentage (63.15%) to doxycycline (Table 3). A lower resistance (26.31%) was detected to sulfamethoxazole-trimethoprim followed by gentamicin, clindamycin (15.78%, each) and chloramphenicol (10.52%). The resistance of *S. aureus* isolates to imipenem was the lowest (5.26%). Neither vancomycin nor ciprofloxacin resistant isolate were found (Table 3).

The multiple antibiotic resistance was determined for 19 isolates of *S.aureus* based on results of disc diffusion method. The MAR index of the isolates was ranged from 0.3 to 0.7 with an average of 0.4 (Table 4). The predominant MAR index (0.3) was found in 4 isolates of *S. aureus isolates* which were resistant to 3 antibiotics. One isolate of S. aureus of chicken meat origin was found to have the highest MAR index of 0.7 which was resistant to 7 antimicrobials out of 10 tested antibiotics. Moreover, slightly higher MAR index (0.5) was detected in one isolate of S. aureus as this strain was resistant to 5 out of 10 tested antimicrobials (Table 4).

Genotypic resistance of S. aureus

The phenotypic resistance of *S. aureus* finding was confirmed through molecular detection of antimicrobial resistance genes (*blaZ*, *mec*A and *van*A). Concerning *mec*A gene, 10 out of 19 *S. aureus* isolates was positive to *mec*A gene with distribution percentage of 52.6%; while the distribution of *blaZ* gene was 57.89% (11/19) (Table 5). It was noticed that all *S. aureus* isolates were negative for *van*B gene. It was shown from that *blaZ* gene was amplified with PCR product of 610 bp (Figure 2). The PCR product of *mec*A gene was 310 bp (Figure 3).

Analysis of biofilm formation by the micro-titer plate method

This study showed three out of five isolates were strong biofilm producers, which were the same isolates that produce *sea* and *seb*, representing 60% of the total, while the other 40% were non-biofilm producers. A zero percentage was obtained for both weak and moderate biofilm producer isolates (Table 6)

Determination of biofilm formation encoding genes.

S. aureus isolates were screened for the total distribution of biofilm-related genes *ica*A and *ica*D. The biofilm-related gene *ica*A was found in 100% of the highly biofilm-producing *S. aureus* strains. However, the *ica*D gene was not negative in all isolates (Figure 4).

Table 1: Prevalence of *S. aureus* in chicken meat (n=19)

Source	No. of examined samples	No. of infected samples	Percent of infection (%)
Broiler breast	100	9	9
Broiler thigh	100	10	10
Total	200	19	9.5

Table 2: Distribution of pathogenic enterotoxin genes in *Staphylococcus aureus* of chicken meat (n=19)

Enterotoxin genes	No of positive isolates	% of distribution
sea	6	31.57
seb	6	31.57
sec, sed, see	0	0

Table 3: Phenotypic resistance of *S. aureus* strains isolated from broiler meat using disk diffusion method (n= 19)

Antimicrobials (disc concentration/µg)	R		S		I	
	NO	%	NO	%	NO	%
ME (10)	19	100	0	0	0	0
AMC(30)	19	100	0	0	0	0
CN (10)	3	15.78	16	84.21	0	0
DA (2)	3	15.78	16	84.21	0	0
IPM(10)	1	5.26	18	94.73	0	0
CIP(5)	0	0	16	84.21	3	15.78
SXT (25)	5	26.31	12	63.15	2	10.52
C (30)	2	10.5	14	73.68	2	10.52
VA (30)	0	0	18	94.73	1	5.26
DO(30)	12	63.15	3	15.78	4	21.05

R: resistant; I: intermediate; S: sensitive, N: Number of examined isolate, NO: Number of positive, %: Percentage; Data were represented by No (%); ME: methicillin; AMC: Amoxicillin- clavulanic acid; CN: gentamicin; DA: clindamycin; CIP: ciprofloxacin; SXT: sulfamethoxazole-trimethoprim; C: chloramphenicol; VA: vancomycin; IPM: Imipenem; DO: Doxycycline.

Table 4: Multiple antibiotic resistance profile (MAR index) of *S. aureus* isolated from chicken meat (n=19)

Resistance pattern	Antimicrobial resistance profile	Number of isolates	Number of antibiotics	MAR index
I	ME, AMC ,CN,DA, SXT, IMP, DO	1	7	0.7
II	ME, AMC, CN, DA, DO	1	5	0.5
II	ME, AMC, DA, SXT	1	4	0.4
IV	ME, AMC, CN	1	3	0.3
V	ME, AMC, SXT	3	3	0.3
VI	ME, AMC, DO	10	3	0.3
VII	ME, AMC, C	2	3	0.3

Table 5: Distribution of antimicrobial resistance genes in *S. aureus* stains (n=19)

Gene	No. of isolates	Percentage%
mecA	10	52.63
blaZ	11	57.89
vanA	0	0

Table 6: Analysis of biofilm formation in *S. aureus* isolates by the microtiter plate method (n=5)

Degree of biofilm formation	No. of isolates	Percentage%
None	2	40%
Weak	0	0
Moderate	0	0
Strong	3	60%

Discussion

The purpose of this study was to look for the incidence, antimicrobial resistance patterns in *S. aureus* in addition to studying enterotoxins and the distribution of biofilm-forming genes in *S. aureus* bacteria obtained from broiler meat. Food products of animal source, particularly poultry, have been recognized as the primary transmitters of *Salmonella* illnesses in individuals in epidemiological data (24). *Staphylococcus aureus* is an opportunistic human and animal pathogen that causes food poisoning and a wide range of infections, from the skin and soft tissue infections to catastrophic illnesses like endocarditis, septicemia, osteomyelitis, and pneumonia (25). Inadequate handling, unsuitable storage conditions, insufficient sanitary procedures, and post-production microbial contamination could all contribute to *S. aureus* contamination of food (26).

S. aureus was found in 9.5% of the investigated samples, with 47.36 % in the broiler breast and 52.63 % in the thigh region. It is, however, less common than the *S. aureus* bacterium found

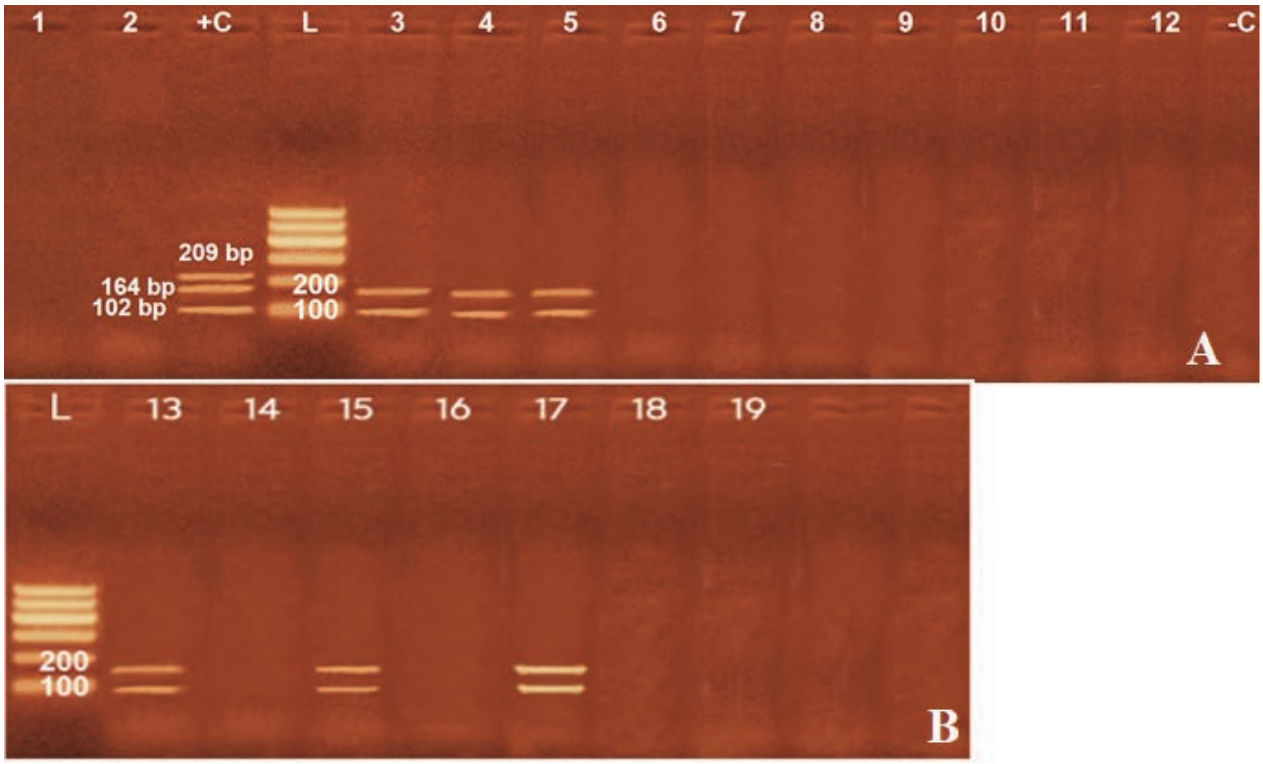

Figure 1: Agarose gel electrophoresis of multiplex polymerase chain reaction for detection of enterotoxin genes in *Staphylococcus aureus* isolated from chicken meat. Enterotoxin genes of *S. aureus*; L: Ladder: 100bp. Lanes (3, 4, 5, 13, 15 and 17) were positive strains for *sea* gene (102 bp) and *seb* (164 bp)

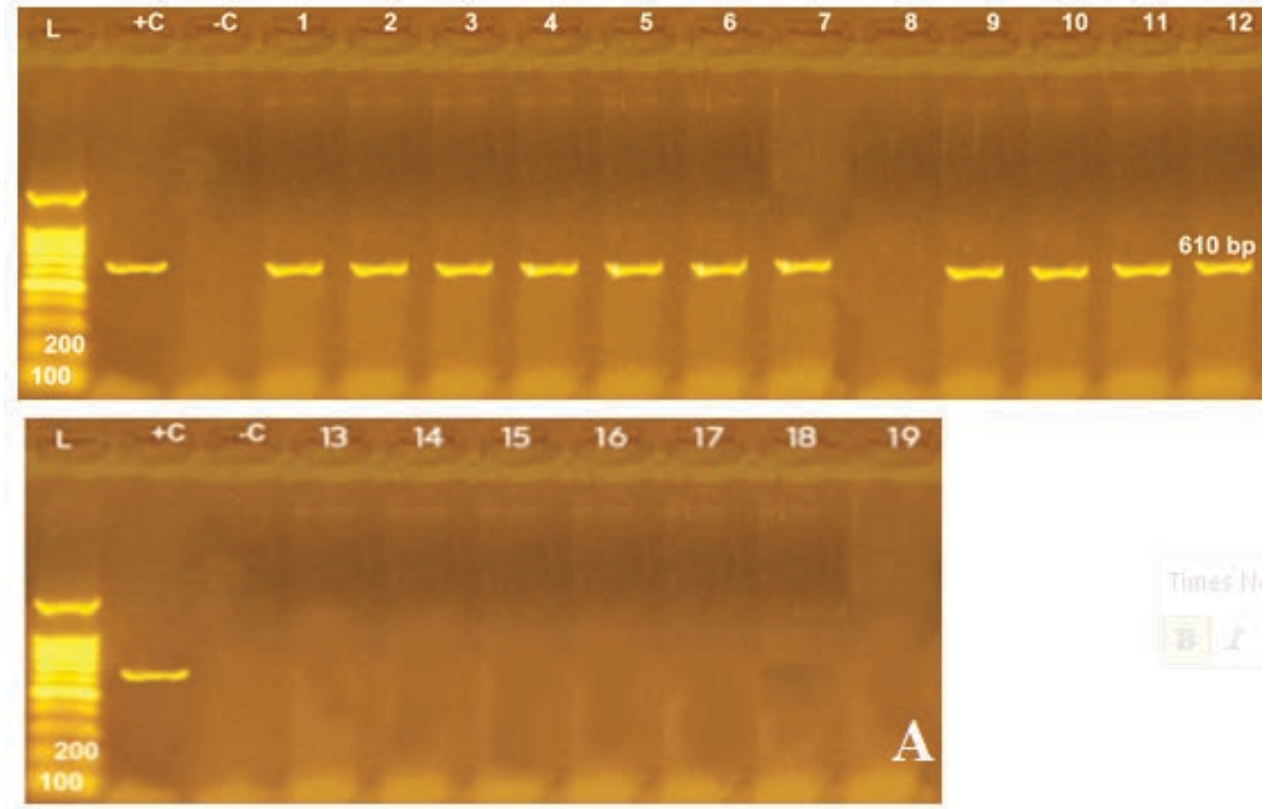

Figure 2: Uniplex PCR to amplify *blaZ* gene in 19 *S. aureus* strains from poultry meat. C+: positive control isolate; C-: negative control isolate of *S. aureus*; L: Ladder 100 bp; lanes 1,2,3,4,5,6,7,9,10,11 and 12 were positive PCR product at 610 bp

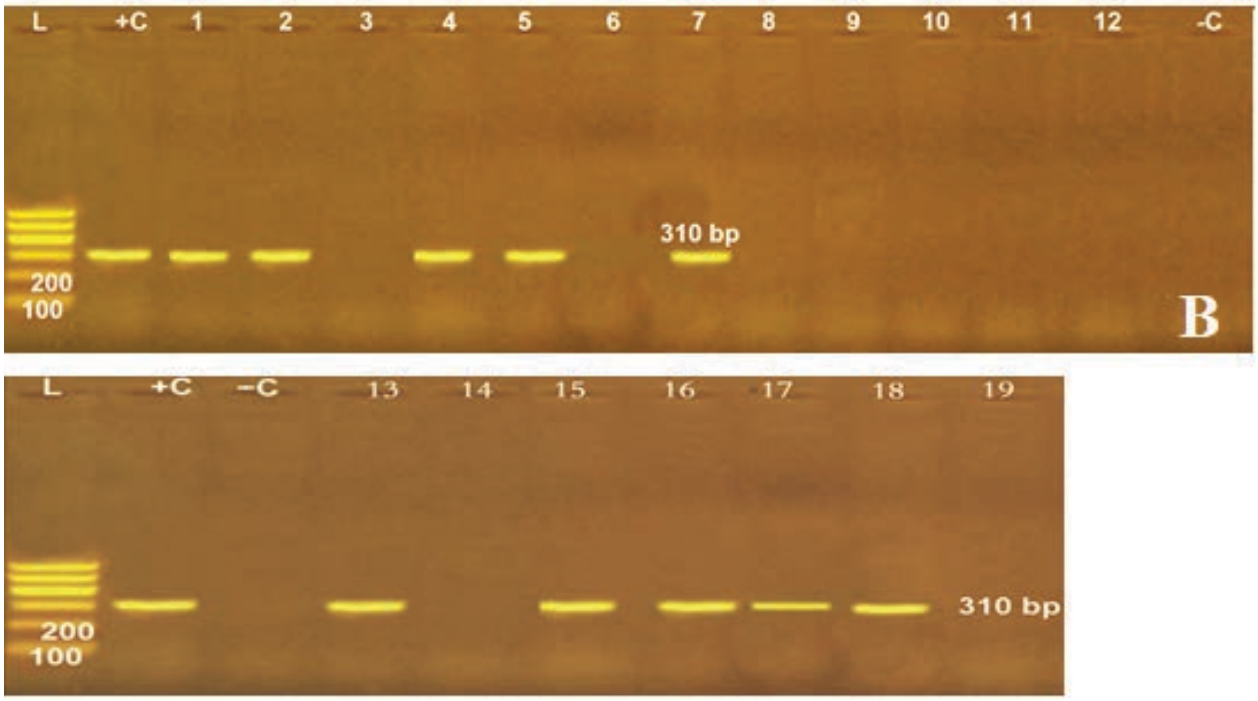

Figure 3: Uniplex PCR to amplify *mec*A gene in 19 *S. aureus* strains from poultry meat. C+: positive control isolate; C-: negative control isolates of *S. aureus*; L: Ladder 100 bp. Lanes 1,2, 4,5, 7,13,15,16, 17 and 18 were positive PCR product at 310 bp

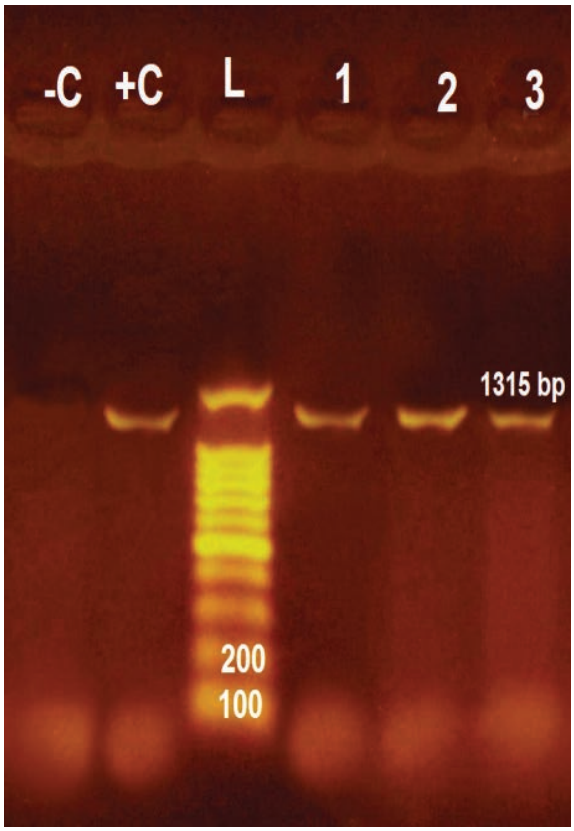

Figure 4: Gel electrophoresis indicating the presence of *ica*A gene in *S. aureus* isolates. C+: Positive control isolate; C-: Negative control; L: Ladder 100 bp; lanes(1,2&3) were positive for *ica*A gene

in raw broiler meat samples; 70% and 80% for the breast and thigh meat, respectively (27). Although, lower percentage of thighs (27.5%) and breasts (22.5%) were obtained (28). Furthermore, Abd El Tawab *et al.* (29) found that 34.3% of the fresh broiler samples were contaminated with *S. aureus*. Another study stated a much higher prevalence of *S. aureus* reached 52% of coagulase *S. aureus* detected in fresh chicken portions (30). The disparity between the estimated isolation rates, including those previously recorded in Egypt could be ascribed to the identification of *S. aureus* isolates in the abovementioned investigations utilizing just biochemical identification. Staphylococci phenotypic identification has already been documented to be inadequate, inconsistent, and unreliable (31). Thighs always had a larger contamination proportion than the breasts, which could be related to the higher quantity of fat (6gm vs. 4gm in the breast). Also, Saadati *et al.* (6) found that *S. aureus* was present in 12 % of the investigated chicken specimens in Iran, according to**,** which nearly matched our findings. Additionally, Momtaz *et al.* (32) reported that 28.05% of *S. aureus* was detected in fresh raw chicken flesh in the same country. In Japan, however, Kitai *et al.* (12) found 41.2 percent of

S. aureus in chicken thighs and 37.3 % in chicken breasts. It's worth noticing that the findings in Portugal indicated that *S. aureus* was the most common infection found in 38.5 % of the poultry flesh (33). *S. aureus* can be found in a variety of environments, involving human body parts that can contaminate food. It is regarded as one of the most important pathogenic species for foodborne diseases. When it's found in food, it's a sign of reduced cleanliness and bad storage (34).

An additional goal of this study was to use specific primers to determine the prevalence of SEs in fresh poultry meat. Because of its propensity to create enterotoxins, *Staphylococcus aureus* is key in poultry meat hygiene (28). The existence of one or more types of enterotoxigenic genes at the same time is a secondary but crucial result of the current study. SEs generated by coagulase-positive staphylococci (CPS), primarily *Staphylococcus aureus*, exhibit super-antigenic and emetic properties, resulting in toxic shock syndrome and staphylococcal food poisoning. Therefore, SEs are deemed to represent a concern to public health, and reporting food poisoning outbreaks has been mandatory (35). The seriousness of food poisoning signs depends on the amount of SE consumed in food and the human health level. Individuals of all ages typically suffer from more sophisticated ailments (36). Among the *S. aureus* bacteria isolated from raw poultry specimens, the furthermost commonly found enterotoxigenic genes were *sea* and *seb*. The prevalence of *sea* and *seb* was 31.57% each in combination. SEC, SED, or SEE were not found in any of the isolates in our research, either alone or in combination. Compared to Darwish *et al*. (37) who concluded that *sea* was the most common gene (64.28 %), afterward *sed* (57.14 %), *sec* (50%), and lastly *seb* (7.14 %). However, Fasiku *et al*. (38) declared a higher percentage for *seb*, which was 75% without combinations of more than one of the tested *se* genes (38).

In India, however, *seb* was the most prevalent gene in isolates from chicken flesh, accounting for 80.95% of the isolates, while none of the isolates in the study had *sea* or *see* (39). Furthermore, Sundararaji *et al*. (40) reported that four isolates from chicken samples tested positive for *sea* (40%), and three tested positive for *seb* as well as *sec* gene (30%). In Iran, on the other hand, Madahi *et al*. (41) who achieved in their study that, eight *S. aureus* isolates (33.33 %) generated *sea*, one (4.16 %) *seb*, three (12.50 %) *sec*, two (8.33 %) *sed,* three (12.50 %) *sed,* and no *see* was detected from chicken nuggets in their study. Staphylococcal enterotoxins cause the signs of staphylococcal foodborne illness and might be linked to other staphylococcal illnesses. These enterotoxins are resistant to heat, and once released, they are difficult to eliminate, even after heat processing. As a result, momentous hygiene precautions ought to be taken once handling and processing chicken flesh (37).

The unregulated use of medications such as growth boosters in animals has been linked to the establishment of antimicrobial-resistant S. aureus; thus, usage should be limited to the field scale (31). The antibiotics chosen were routinely used in humans and animals to treat infections caused by S. aureus and Salmonella. For instance, Penicillin (e.g., amoxicillin-clavulanic acid and methicillin) are b-lactam antibiotics, and their mode of action involves inhibiting bacterial peptidoglycan layer formation (42). As a result , It is challenging to handle infections generated by resistant strains (11). In our investigation, the antimicrobial resistance profile for *S. aureus* strains isolated from fresh chicken meat revealed that the strains exhibited the highest antibiotic resistance to Methicillin and Amoxicillin-clavulanic acid; moreover, all the isolates were resistant to at least one antibiotic, except for Vancomycin and Ciprofloxacin, which had zero resistance results. Besides, the most of the isolates, in this recent study, were multi drug resistant especially to methicillin, amoxicillin-clavulanic acid and doxycycline. In line with our results, El Bayomi *et al*. (31) demonstrated that no isolates were vancomycin resistant; nevertheless, penicillin was only 53.3% resistant, and ciprofloxacin resistant isolates were 33.3% in contract to ours, which was negative (37). In Pakistan, results vary for gentamicin and chloramphenicol being 13.15% and 21.05%, correspondingly , as stated by Akbar and Anal (34), which contradict our results. Further, Safarpoor *et al* (39) featured that the highest prevalence of resistance were to penicillin (60 %) and trimethoprim–sulfamethoxazole (50%), which was obtained from chicken-containing foods. Additionally, resistance to chloramphenicol was found to be (50%) from foods of chicken origin (39). Also, Kim *et al* (43) in Korea said that penicillin resistance was found in a high percentage of

S. aureus isolates (51% percent) isolated from chicken meat. The present study revealed that MAR index of *S. aureus* isolates was ranged from .3 to 0.7 with an average of 0.4. In Comparison with a previous study in India, MAR index of different S. aureus isolates indicates an overall range from 0.06-0.56 where in raw chicken meat and ready to eat (RTE) chicken products range differs from 0.06 to 0.56 and 0.06-0.37, respectively (44). Our finding confirmed that S. aureus isolates revealing more than 0.2 MAR index exhibits that these are from a high-risk source of contamination where antimicrobials are frequently used (45).

Methicillin-resistant *S. aureus* (MRSA) is a pathogen that colonizes and attacks a variety of host species. It has been discovered in the avian farming system, increasing worries regarding probable transfer from farm to plate (46). All MRSA strains have a genetic determinant called *mec*A or *mec*C, which encodes for low affinity penicillin binding proteins called PBP2a. The *mec*A gene is found on the Staphylococcal cassette chromosome mec (SCCmec), a genomic island that contains resistance genes to -lactam ATBs in addition to other resistance genes (11). In the present study, 52.63% of the phenotypic resistant isolates were MRSA and 57.89% had the *bla*Z encoding virulence gene, whereas, *mec*A represents 5% of the total samples and *bla*Z is 5.5%. 100% of the tested strains. This finding was coordinated with Abd El tawab *et al.* (29). However, Saadati *et al.* (6) obtained 5% of MRSA and 100% for *bla*Z encoding gene. The subgroup evaluation done by Ribeiro *et al.* (46) revealed a pooled occurrence of 5% of MRSA in chicken meat. Two years later, Abolghait *et al.* (47) showed that MRSA was found in 5.5 % of the chicken samples (8/144) and the *seb* gene was found in most of MRSA isolates (75 %).

Microorganisms use biofilm development as an environmental adaptation method besides the production of it, is aided by high humidity or moisture content in the environment. Consequently, it is frequently difficult to be eliminated using standard disinfection methods, such as detergents or sanitizing agents (48). In our study, most of the MRSA strains are biofilm producers (60 %, 3/5) and harbored 76% *ica*A biofilm-forming gene. Results that obtained by Saber *et al.* (25) revealed that 50 (83.3 %) of the 60 MRSA isolates produced biofilms, while 10 (16.6 %) were non producer. In china, Chen *et al.* (49) stated that biofilms were generated by around 72% of the isolates. Precisely, 54.6 % of these isolates created weak biofilms, whereas 14.4 % and 3.09 % developed moderate as well as strong biofilms, correspondingly with 100% *ica*A positive. Meanwhile, Ou *et al.* (50) avowed that, 64.8% of all the 165 isolates had strong biofilm formation ability and 20.0% were moderate producers. Two years before, Wang *et al.* (51) reported that, six isolates obtained from chicken meat were classified as strong biofilm formers and none were negative producer in contrast to our conclusions. The importance of isolated *S. aureus* strains was boosted by the *ica*ABCD gene development genes, which encode a polysaccharide intercellular adhesion (PIA), resulting in significant protection of *S. aureus* bacteria against adverse environmental conditions such as the presence of antimicrobials and antiseptic chemicals, as well as immunological responses (52). In A study revealed that 50% of *S.aureus* isolates from chicken meat were positive for *ica*A gene as was previously reported by Abbasi *et al.* (52). A nearly parallel results to our finding was achieved by Eftekhar and Dadaei (15), who declared that by colony morphology, 53.3 % of clinical MRSA isolates demonstrated the potential to produce biofilm, with 75 % carrying the ica operon. Biofilms can prevent antibiotics from reaching bacteria and boost bacterial resistance, that bacterium in biofilms exhibiting 10 to 1500 times' greater antibiotic resistance than bacteria in free cells (53). As a result, a better knowledge of the molecular evolution of staphylococcal biofilms is required to create innovative solutions for biofilm associated contamination.

Conclusion

This study confirmed higher prevalence of multidrug resistant isolates of *S. aureus* isolates in chicken meat due to inadequate handling and insufficient sanitary equipment and post microbial contamination. A higher MAR index of *S. aureus* from chicken meat origin more than 0.2 exhibits that these isolates are derived from a high-risk source of contamination where antibiotics are frequently used. The strong biofilm former strains were only 100% positive for *sea* and *seb* genes. This study highlighted the importance of broiler meat as a reservoir for antimicrobial-resistant strains foe *S. aureus* and biofilm-forming of

Staphylococcus aureus, and these MDR isolates represents a hazard for food safety and public health. Further studies are required to create innovative solutions for biofilm-associated MDR isolates of *Staphylococcus aureus*.

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