Egypt. J. Vet. Sci. **Vol. 52,** No.1, pp. 55-62 (2021)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Antimicrobial Resistance and Biofilm Encoding Genes Amongst the *Staphylococcus aureus* Bacteria Isolated From Meat and Meat Products

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ROLE of meat and meat products as reservoirs of biofilm formation and antimicrobial-resistant *Staphylococcus aureus* is not clearly observed. The present research was conducted to assess the incidence, antimicrobial resistance and distribution of *icaABCD* biofilm formation genes amongst the *S. aureus* bacteria recovered from raw meat and meat products. One-hundred and sixty raw meat and meat product samples were collected and *S. aureus* bacteria were isolated using microbial culture. Antimicrobial resistance of *S. aureus* bacteria was assessed by disk diffusion. PCR was used to assess the distribution of *icaABCD* genes. Twenty-six out of 160 (16.25%) examined samples were contaminated with *S. aureus*. Raw beef meat (40%) and raw chicken burger (12.50%) samples had the highest *S. aureus* contamination rate. Bacteria harbored the highest resistance toward penicillin (69.23%), ampicillin (61.53%), tetracycline (57.69%) and gentamicin (57.69%) antimicrobials. *IcaD*, *icaA*, *icaB* and *ica C*genes were detected in 65.38%, 57.69%, 50%, and 42.30% *S. aureus* bacteria, respectively. Roles of raw beef, chicken, beef burger and chicken burger samples as reservoirs of antimicrobial resistant-and biofilm formation-*S. aureus* bacteria have been demonstrated in the current research. Nevertheless, supplementary investigations should perform to understand other roles of *icaABCD* biofilm formation genes in *S. aureus* originated from meat and meat products.

Keywords, *Staphylococcus aureus*; Incidence; Antibiotic resistance; Biofilm formation; Meat.

Introduction

Staphylococcus aureus (*S. aureus*) a pathogenic bacterium accountable for emerging foodborne diseases globally. Food related outcomes of the staphylococcal diseases characterized by nausea, weakness, abdominal cramps, vomiting, and toxic shock syndrome (TST) [1,2]. Contaminated food products, predominantly those originated from animals (especially meat and meat products), are known as *S. aureus* reservoirs [3-5].

Staphylococcal foodborne diseases are mostly treated with antimicrobial-based therapeutic options. Nevertheless, newly-launched researches announced the high incidence of resistance of *S. aureus* bacteria originated from animal resources toward diverse classes of antimicrobial agents, particularly cephalosporins, penicillin, macrolides, aminoglycosides, tetracyclines, and fluoroquinolones [6,7].

From a molecular view, biofilm formation is one of the most critical ways of *S. aureus* bacteria to become virulence and resist toward diverse antimicrobial agents [8]. Polysaccharide intracellular adhesion (PIA) factor, which is encoded by the *ica* genes (*icaA*, *icaB*, *icaC* and *icaD*). These genes are involved in biofilm activities and caused intracellular adhesion and protected *S. aureus* bacteria from immune response and antimicrobial agents [8].

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(Received 16/08/2020, accepted 22/09/2020)
DOI. 10.21608/ejvs.2020.39385.1186

Rendering the boost importance of biofilm formation *S. aureus* bacteria as a potential food-borne pathogen, The current survey was performed to assess the antimicrobial resistance and frequency of biofilm formation genes of the *S. aureus* bacteria recovered from meat and meat products.

Materials and Methods

Sampling

In the current cross sectional descriptive survey, a total of 160 raw meat and meat product samples including beef (n= 40) and chicken (n= 40) meat and meat burger (n= 40) and chicken burger (n= 40) samples were randomly collected from supermarkets of Shahrekord city, Iran. Meat samples (100 g) were collected from the femur muscle. Meat product samples (100 g) were collected from valid brands. Samples were transferred to research center using small refrigerator.

Isolation and identification of S. aureus

Twenty-five grams of each sample were merged with 225 mL of buffered peptone water (Merck, Germany) and homogenized by the Stomacher device (Interscience, Saint-Nom, France). At that point, 5 mL of the achieved sample was inoculated on 50 mL Trypticase Soy Broth (TSB, Merck, Germany) medium (with 10% NaCl and 1% sodium pyruvate). Media were incubated at 35 °C for 18 h. At that point, a loopful of an achieved culture was inoculated on the Baird-Parker agar (BPA, Merck, Germany) (contained egg yolk tellurite emulsion). Media were incubated at 37 °C for about 24 h. *S. aureus* colonies were determined as a black shiny color with 2 to 5-mm clear marginal zones. *S. aureus* identification was accompanied using standard tests including Gram-staining, catalase, oxidase, mannitol fermentation, DNase activity, and sugar fermentation tests [6].

Antimicrobial resistance determination

Guidelines of the Clinical Laboratory Standard Institute (CLSI) [9] were applied to determine the antimicrobial resistance pattern of isolated *S. aureus* bacteria. *S. aureus* bacteria were cultured on the Muller Hinton Agar (MHA, Merck, Germany) media and antimicrobial disks (penicillin (10 µg/disk) (P10), tetracycline (30 µg/disk) (T30), methicillin (5 µg/disk) (Met5), ampicillin (10 µg/disk) (AM10), oxacillin (1 µg/disk) (Ox1), vancomycin (5 µg/disk) (V5), gentamicin (10 µg/disk) (G10), rifampin (5 µg/disk) (Rif5) and erythromycin (15 µg/disk) (Er15) (Himedia, India)) were placed on plate media. Media contained *S. aureus* bacteria (0.5 McFarland concentration) and disks were incubated at 37 °C for 24 h. After incubation, diameters of the growth inhibition zone surround the bacteria were measured and compared with those of CLSI. Diameters of the growth inhibition zones of bacteria were compared with those presented in CLSI and then resistance or sensitive strains were determined. *S. aureus* (ATCC 29213) was cultured as control.

Determination of biofilm formation encoding-genes

S. aureus isolates were sub-cultured on TSB and incubated for 48 h at 37 °C. Genomic DNA was extracted from the colonies using the DNA extraction kit (Thermo Fisher Scientific, Germany) rendering the guideline. Quality and quantity of extracted DNA were assessed by nanodrop device (NanoDrop, Thermo Scientific, USA) and electrophoresis (2% agarose gel). Table 1 described the list of primers and PCR circumstances applied for detection of biofilm formation genes [10, 11].

DNA thermo-cycler (Eppendorf, Germany) was applied. Fifteen microliters of the amplified samples were subjected to gel electrophoresis (2% gel contained 0.1% ethidium bromide (0.4 µg/ml)). Results of electrophoresis were checked by the UVdoc (London, UK).*S. aureus* (ATCC 29213) and PCR -grade water (Thermo Fisher Scientific, Germany) were applied as positive and negative controls, respectively[6].

Statistic

Data obtained from the research were transferred to Excel software and analyzed by SPSS 21.0 software (Chicago, USA) using Chi-square and Fisher's exact tests. *P* value <0.05 was determined as significant level.

Results

Table 2 described the incidence of S. aureus amongst examined meat and meat product samples. Twenty-six out of 160 (16.25%) examined samples were contaminated with S. aureus. Beef (40%) had the higher contamination rate than chicken (5%) (P<0.05). Chicken burger (12.50%) had the higher contamination rate than beef burger (7.50%). However, it was not statistically significant (P>0.05).

TABLE 1. Primers and PCR circumstances applied for detection of biofilm formation genes [10, 11].

Target genes	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
icaA	F, ACACTTGCTGGCGCAGTCAA R, TCTGGAACCAACATCCAACA	188	1 cycle, 94 ^{0C} ---------- 5 min. 30 cycles, 94 ^{0C} ---------- 60 s 55 ^{0C} ---------- 60 s 72 ^{0C} ---------- 60 s 1 cycle, 72 ^{0C} ---------- 10 min	5 µL PCR buffer 10X 1.5 mM $Mgcl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Fermentas) 2.5 µL DNA template
icaB	F, AGAATCGTGAAGTATAGAAAATT R, TCTAATCTTTTTCATGGAATCCGT	900	1 cycle, 94 ^{0C} ---------- 5 min. 30 cycles, 94 ^{0C} ---------- 60 s 52^{0C} ---------- 30 s 72 ^{0C} ---------- 90 s 1 cycle, 72 ^{0C} ---------- 10 min	
icaC	F, ATGGGACGGATTCCATGAAAAAGA R, TAATAAGCATTAATGTTCAATT	1100	1 cycle, 94 ^{0C} ---------- 5 min. 30 cycles, 94 ^{0C} ---------- 60 s 55 ^{0C} ---------- 30 s 72 ^{0C} ---------- 30 s 1 cycle, 72 ^{0C} ---------- 10 min	
icaD	F, ATGGTCAAGCCCAGACAGAG R, AGTATTTTCAATGTTTAAAGCAA	198	1 cycle, 94 ^{0C} ---------- 5 min. 25 cycles, 94 ^{0C} ---------- 30 s 55 ^{0C} ---------- 30 s 72 ^{0C} ---------- 60 s 1 cycle, 72 ^{0C} ---------- 10 min	

DNA thermo-cycler (Eppendorf, Germany) was applied. Fifteen microliters of the amplified samples were subjected to gel electrophoresis (2% gel contained 0.1% ethidium bromide (0.4 µg/ml)). Results of electrophoresis were checked by the UVdoc (London, UK).*S. aureus* (ATCC 29213) and PCR -grade water (Thermo Fisher Scientific, Germany) were applied as positive and negative controls, respectively[6].

Table 3 described the antimicrobial resistance pattern of *S. aureus* isolates. *S. aureus* isolates exhibited the uppermost incidence of resistance toward penicillin (69.23%), ampicillin (61.53%), tetracycline (57.69%) and gentamicin (57.69%). Statistically significant variance was obtained between types of samples and incidence of antimicrobial resistance ($P<0.05$).

Table 4 describes the incidence of biofilm formation genes amongst the *S. aureus* bacteria recovered from meat and meat products. Total distribution of *icaD*, *icaA*, *icaB* and *icaC* amongst the *S. aureus* isolates were 65.38%, 57.69%, 50%, and 42.30%, respectively. *IcaD* had the uppermost incidence amongst biofilm formation genes, while *icaC* had the lowermost. *S. aureus* bacteria recovered from both beef and chicken burger samples had the higher distribution of biofilm formation genes than those of beef and chicken ($P<0.05$).

TABLE 2. Incidence of *S. aureus* amongst examined meat and meat product samples.

Samples	N. collected	N (%). Positive for *S. aureus*
Beef	40	16 (40)
Chicken	40	2 (5)
Beef burger	40	3 (7.50)
Chicken burger	40	5 (12.50)
Total	160	26 (16.25)

TABLE 3. Antimicrobial resistance pattern of *S. aureus* isolates recovered from meat and meat products.

Samples (N. *S. aureus*)	N. (%) *S. aureus* harbored resistance to each antibiotic								
	P10	T30	Met5	AM10	Ox1	V5	G10	Rif5	Er15
Beef (16)	12 (75)	9 (56.25)	9 (56.25)	10 (62.50)	8 (50)	4 (25)	9 (56.25)	7 (43.75)	7 (43.75)
Chicken (2)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	-	1 (50)	1 (50)	1 (50)
Beef burger (3)	2 (66.66)	2 (66.66)	2 (66.66)	2 (66.66)	1 (33.33)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)
Chicken burger (5)	3 (60)	3 (60)	2 (40)	3 (60)	2 (40)	1 (20)	3 (60)	2 (40)	2 (40)
Total (26)	18 (69.23)	15 (57.69)	14 (53.84)	16 (61.53)	12 (46.15)	6 (23.07)	15 (57.69)	11 (42.30)	11 (42.30)

TABLE 4. Incidence of biofilm formation genes amongst the *S. aureus* bacteria recovered from meat and meat products.

Samples (N. *S. aureus*)	N. (%) *S. aureus* harbored each gene			
	icaA	*icaB*	*icaC*	*icaD*
Beef (16)	8 (50)	7 (43.75)	5 (31.25)	10 (62.50)
Chicken (2)	1 (50)	1 (50)	-	1 (50)
Beef burger (3)	2 (66.66)	2 (66.66)	2 (66.66)	2 (66.66)
Chicken burger (5)	4 (80)	3 (60)	4 (80)	4 (80)
Total (26)	15 (57.69)	13 (50)	11 (42.30)	17 (65.38)

Discussion

The importance of biofilm formation *S. aureus* as an emerging resistant bacteria in foodstuffs, particularly those with animal origins, is not documented well. Nevertheless, growing consideration has been focused on understanding staphylococcal biofilms as imperative factors for the occurrence of antimicrobial resistance [12].

An existing research was completed to assess the antimicrobial resistance pattern and distribution of biofilm formation genes amongst the *S. aureus* bacteria recovered from meat and meat products. Findings revealed that 16.25% of examined samples were contaminated with *S. aureus*. Incidence of *S. aureus* bacteria amongst raw meat samples examined in surveys conducted on Denmark [13], Egypt [14], Turkey [15], Germany [16] and Brazil [17] were 52%, 40.80%, 30%, 71.50% and 21.72%, respectively. Incidence of *S. aureus* in meat products in Iran [18], China [19], India [20], USA [21], Iraq [22], and Nigeria [23] were 9.86%, 35%, 21.81%, 27.90%, 54.20%, and 9.70%, respectively. Total incidence of *S. aureus* amongst the beef, camel meat, chicken, mutton, beef burger, beef sausage, ground beef, chicken burger, ground chicken, and chicken sausage samples collected from Libya were 20%, 23.80%, 40%, 0%, 25%, 33%, 36.40%, 50%, 20%, and 10%, respectively [24]. Brazilian survey [25] revealed that the incidence of *S. aureus* amongst the beef burger, beef sandwich, chicken burger and chicken sandwich samples were 64%, 20%, 72% and 8%, respectively. Variances in types of samples, method of experiment, geographical and seasonal circumstances may cause such difference in the incidence of *S. aureus* in diverse researches. Primary presence of *S. aureus* in meat samples through slaughtering and transmission of *S. aureus* from workers and staff of the slaughterhouses, veterinarians and butchers are two imperative routes of meat and meat products contamination.

S. aureus isolates harbored the considerable incidence of resistance toward penicillin, ampicillin, tetracycline and gentamicin which may partly be owing to the high prescription of these antimicrobial agents. Additionally, irregular and boost use of these antimicrobial, particularly in aviculture and veterinary caused significant occurrence of antimicrobial resistance. Excessive use of disinfectants, self-medication with antimicrobials and administration of single-dose therapeutics may be other probable reasons. Similar to our findings, boost incidence rate of resistance of *S. aureus* bacteria toward penicillin, ampicillin, tetracycline and gentamicin was reported from China [19], India [26], South Africa [27], Iran [28], Pakistan [29], and United Kingdom [30]. Similar to our survey, Fowoyo and Ogunbanwo [31]described that the considerable incidence of resistance of *S. aureus* bacteria recovered from foodstuffs toward erythromycin (15.70%), oxacillin (35.70%), ampicillin (86.70%), gentamicin (11.40%), and trimethoprim–sulfamethoxazole (74.90%). Diverse views of veterinary physicians about type of prescribed antimicrobials, viability of rules about limitation of uses of antimicrobials, and prices of antimicrobial agents may affect the incidence of antimicrobial resistance in different regions.

High distribution of *icaABCD* biofilm formation genes increased the importance of isolated S. *aureus* strains. The *icaABCD* genes encrypt a Polysaccharide Intercellular Adhesion (PIA), which causes severe protection of *S. aureus* bacteria toward contrary environmental circumstances such as presence of antimicrobials and antiseptic agents and also immune responses. Furthermore, the *ica* biofilm formation gene acts as an imperative factors in nosocomial infections [32] and foodborne diseases [33, 34]. In a similar survey conducted by Ferreira, Tette, Mendonça, Soares and Carvalho [35], *icaA* and *icaD* genes were detected in 97% of the *S. aureus* bacteria recovered from poultry products. It seems that the *S. aureus* carrying *icaABCD* biofilm formation genes may be more important because of its ability to cause antimicrobial-resistant diseases. However, its role as an important foodborne agent has been overlooked. Diverse kinds of food-borne bacteria are responsible for food poisoning due to the consumption of raw or undercooked meat and meat products [36-42].

Conclusion

Boost incidence of *S. aureus* resists toward diverse antimicrobials and carrying *icaABCD* biofilm formation genes was reported in the current survey. High incidence of resistance toward routinely used antimicrobials and high distribution of *icaABCD* biofilm formation genes may reflect emerging public health issues rendering the consumption of contaminated beef, chicken, beef burger and chicken burger in raw or undercooked circumstances. However, further researches are mandatory to obtain other

epidemiological importance of biofilm formation *S. aureus* in meat and meat products.

Acknowledgements

Many thanks from staffs of the Food Hygiene Research Unit, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

Conflict of interest : The authors declared that no conflict of interest.

Funds statement **:** Self-funding

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