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ORIGINAL RESEARCH

Phenotypic and Genotypic Assessment of Antibiotic Resistance of *Staphylococcus aureus* Bacteria Isolated from Retail Meat

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Background: Resistant *Staphylococcus aureus* (*S. aureus*) bacteria are determined to be one of the main causes of foodborne diseases.

Purpose: This survey was done to assess the genotypic and phenotypic profiles of antibiotic resistance of *S. aureus* bacteria isolated from retail meat.

Methods: Four-hundred and eighty-five retail meat samples were collected and examined. *S. aureus* bacteria were identified using culture and biochemical tests. The phenotypic profile of antibiotic resistance was examined using the disk diffusion method. The genotypic pattern of antibiotic resistance was determined using the polymerase chain reaction.

Results: Forty-eight out of 485 (9.89%) raw retail meat samples were contaminated with *S. aureus*. Raw retail buffalo meat (16%) had the highest incidence of *S. aureus*, while raw camel meat (4%) had the lowest. *S. aureus* bacteria exhibited the uppermost incidence of resistance toward tetracycline (79.16%), penicillin (72.91%), gentamicin (60.41%), and doxycycline (41.666%). The incidence of resistance toward chloramphenicol (8.33%), levofloxacin (22.91%), rifampin (22.91%), and azithromycin (25%) was lower than other examined antibiotics. The most routinely detected antibiotic resistance genes were *blaZ* (58.33%), *tetK* (52.08%), *aacA-D* (33.33%), and *ermA* (27.08%). *Cat1* (4.16%), *rpoB* (10.41%), *msrA* (12.50%), *grlA* (12.50%), *linA* (14.58%), and *dfrA1* (16.66%) had the lower incidence rate.

Conclusion: Raw meat of animals may be sources of resistant *S. aureus* which pose a hygienic threat about the consumption of raw meat. Nevertheless, further investigations are essential to understand supplementary epidemiological features of *S. aureus* in retail meat.

Keywords: *Staphylococcus aureus*, incidence, antibiotic resistance pattern, antibiotic resistance genes, retail meat

Introduction

Meat is a nutrient foodstuff with highly beneficial effects on human health. It is a rich source of protein, fat, and some kinds of vitamins essential for a healthy life.[1–5] Nevertheless, considering the low hygienic conditions of slaughterhouses, numerous outbreaks of foodborne diseases owing to the consumption of contaminated raw or undercooked meat have been reported in diverse parts of the world.[1–5]

Staphylococcus aureus (*S. aureus*), a Gram-positive and catalase-positive bacterium, is considered a substantial cause of foodborne diseases identified by a short incubation period, weakness, vomiting, nausea, abdominal cramps, and toxic shock syndrome.[6] Raw or undercooked foodstuffs, particularly foods with animal origins, are determined as reservoirs of *S. aureus*.[7]

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The bacterium has an emergence of resistance toward diverse kinds of antibiotic agents.[8] Resistant *S. aureus* bacteria are responsible for about 100,000 cases of infectious diseases with about 20–30% mortality per annum in the United States.[9] Resistant *S. aureus* bacteria caused more complicated diseases for a longer period of time.[10] They are responsible for higher costs of control and treatment.[10] Furthermore, a high incidence of resistance toward diverse kinds of antibiotics, particularly penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides, and fluoroquinolones, has been reported for *S. aureus* bacteria isolated from foods with animal origin.[8,11]

The phenotypic presence of antibiotic resistance of *S. aureus* bacteria is mostly associated with the presence of antibiotic resistance genes.[12] A high presence of *tetK* and *tetM, ermA* and *msrA, gyrA* and *grlA, blaZ, dfrA, rpoB, aacA-D, linA*, and *cat1* antibiotic resistance genes in the *S. aureus* bacteria caused the occurrence of resistance against tetracyclines, macrolides, fluoroquinolones, penicillins, folate inhibitors, ansamycins, aminoglycosides, lincosamides, and phenicols, respectively.[12]

Considering the high consumption rate of meat, the high importance of *S. aureus*, and unknown microbial and epidemiological aspects of the bacterium in raw meat samples, an existing survey was carried out to assess the incidence and the phenotypic and genotypic patterns of antibiotic resistance of *S. aureus* bacteria isolated from diverse kinds of raw retail meat samples.

Materials and Methods

Ethical Consideration

The survey was confirmed by the Ethical Council of Research of the Department of Food Hygiene, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

Samples

From May to October 2018, a total of 485 numerous kinds of raw meat samples including camel (n=100), buffalo (n=100), sheep (n= 85), beef (n=100), and goat (n=100) were randomly collected from 65 different retail centers of Isfahan province, Iran. Samples (100 g, femur muscle) were directly transferred to the Food Hygiene Research Center. Transmission was carried out by cool boxes.

Isolation and Identification of *S. aureus*

Twenty-five grams of each collected meat sample were blended with 225 mL of buffered peptone water (Merck, Germany). At that time, solutions were homogenized using Stomacher (Interscience, Saint-Nom, France). Then, 5 mL of the achieved solution was transferred into 50 mL Trypticase Soy Broth (TSB; Merck, Germany) supplemented with 10% NaCl and 1% sodium pyruvate, and incubated for 18 h at 35 °C. Then, a loopful of the culture was transferred into Baird-Parker agar supplemented with egg yolk tellurite emulsion (Merck, Germany) and incubated at 37 °C for about 24 h.[5,7] Black shiny colonies surrounded by 2 to 5-mm clear zones were further identified on the basis of Gram staining, hemolytic activity on sheep blood agar (Merck, Germany), catalase activity, coagulated test (rabbit plasma), oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol salt agar (Merck, Germany), urease activity, nitrate reduction, phosphatase, deoxyribonuclease (DNase; Merck, Germany) test, Voges–Proskauer (Merck, Germany) test, and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests.[8,13]

Phenotypic Assessment of Antibiotic Resistance

The phenotypic pattern of antibiotic resistance of *S. aureus* bacteria was investigated using the disk diffusion method on Mueller–Hinton agar (Merck, Germany).[13] Principles of the Clinical Laboratory Standard Institute (CLSI) were used for this purpose.[14] Diverse kinds of antibiotic agents including aminoglycosides (amikacin (30 µg/disk) and gentamicin (10 µg/disk)), fluoroquinolones (levofloxacin (5 µg/disk) and ciprofloxacin (5 µg/disk)), lincosamides (clindamycin (2 µg/disk)), macrolides (erythromycin (15 µg/disk) and azithromycin (15 µg/disk)), penicillins (penicillin (10 µg/disk)), tetracyclines (doxycycline (30 µg/disk) and tetracycline (30 µg/disk)), phenicols (chloramphenicol (30 µg/disk)), folate pathway inhibitors (trimethoprim–sulfamethoxazole (25 µg/disk)), and ansamycins (rifampin (5 µg/disk)) were used for this goal (Oxoid, UK). The method was completed using the protocol characterized previously.[14] *S. aureus* (ATCC 43300 and ATCC 29213) was used as the quality control organism in antimicrobial susceptibility determination.

Genotypic Assessment of Antibiotic Resistance

S. aureus isolates were subcultured on TSB media (Merck, Germany) and further incubated for 48 h at 37 °C.

Table 1 PCR Protocol Used for Genotypic Assessment of Antibiotic Resistance

Target Gene	Primer Sequence (5′–3′)	PCR Product (bp)	PCR Programs	PCR Volume (50μL)
AacA-D	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227		
ermA	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190		
tetK	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360		
tetM	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	1 cycle: 94 °C — 6 min 34 cycles: 95 °C — 50 s 55 °C — 70 s 72 °C — 60 s 1 cycle: 72 °C — 8 min	5 μL PCR buffer 10× 2 mM $MgCl_2$ 200 μM dNTP (Fermentas) 0.5 μM of each primer F
& R 1.5 U Taq DNA polymerase (Fermentas) 5 μL DNA template *msrA*	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	1 cycle: 94 °C — 6 min 34 cycles: 95 °C — 60 s 50 °C — 70 s 72 °C — 70 s 1 cycle: 72 °C — 8 min	5 μL PCR buffer 10× 2 mM $MgCl_2$ 150 μM dNTP (Fermentas) 0.75 μM of each primer F
& R 1.5 U Taq DNA polymerase (Fermentas) 3 μL DNA template *linA*	F: GGTGGCTGGGGGGTAGATGTATTAACTGG R: GCTTCTTTTGAAATACATGGTATTTTTCGA	323	1 cycle: 94 °C — 6 min 30 cycles: 95 °C — 60 s 57 °C — 60 s 72 °C — 60 s 1 cycle: 72 °C — 10 min	5 μL PCR buffer 10× 2 mM $MgCl_2$ 150 μM dNTP (Fermentas) 0.75 μM of each primer F
& R 1.5 U Taq DNA polymerase (Fermentas) 3 μL DNA template *blaZ*	F: ACTTCAACACCTGCTGCTTTC R: TGACCACTTTTATCA CAACC	490	1 cycle: 94 °C — 5 min. 30 cycles: 94 °C — 20 s 60 °C — 30 s 72 °C — 90 s 1 cycle: 72 °C — 5 min	5 μL PCR buffer 10× 2 mM $MgCl_2$ 150 μM dNTP (Fermentas) 0.75 μM of each primer F

(*Continued*)

Table 1 (Continued).

Target Gene	Primer Sequence (5′–3′)	PCR Product (bp)	PCR Programs	PCR Volume (50µL)
& R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template *cat I*	F: AGTTGCTCAATGTACCTATAACC R: TTGTAATTCATTAAGCATTCTGCC	547	1 cycle: 94 °C — 8 min 32 cycles: 95 °C — 60 s 55°C — 70 s 72 °C — 2 min 1 cycle: 72 °C — 8 min	5 µL PCR buffer 10× 2 mM $MgCl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primer F
& R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template *gyrA*	F: AATGAACAAGGTATGACACC R: TACGCGCTTCAGTATAACGC	223	1 cycle: 94 °C — 10 min 25 cycles: 94 °C — 20 s 52 °C — 20 s 72 °C — 50 s 1 cycle: 72 °C — 5 min	5 µL PCR buffer 10× 2 mM $MgCl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primer F
& R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template *grlA*	F: ACTTGAAGATGTTTTAGGTGAT R: TTAGG AAATCTTGATGGCAA	459		
dfrA	F: CTCACGATAAACAAAGAGTCA R: CAATCATTGCTTCGTATAACG	201	1 cycle: 94 °C — 2 min 30 cycles: 94 °C — 60 s 50 °C — 60 s 72 °C — 60 s 1 cycle: 72 °C — 5 min	5 µL PCR buffer 10× 2 mM $MgCl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primer F
& R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template *rpoB*	F: ACCGTCGTTTACGTTCTGTA R: TCAGTGATAGCATGTGTATC	460	1 cycle: 94 °C — 5 min 32 cycles: 94 °C — 60 s 56 °C — 45 s 72 °C — 60 s 1 cycle: 72 °C — 10 min	5 µL PCR buffer 10× 2 mM $MgCl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primer F
& R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template				

Note: Data from references 15–21.

Table 2 Incidence of *S. aureus* in Diverse Kinds of Retail Meat Samples

Types of Retail Meat Samples	No. of Samples Collected	No. of Samples Positive for *S. aureus* (%)
Camel	100	4 (4)
Buffalo	100	16 (16)
Goat	100	6 (6)
Beef	100	13 (13)
Sheep	85	9 (10.58)
Total	485	48 (9.89)

Genomic DNA was extracted from the bacterial colonies using the DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany) according to the manufacturer's instructions. The purity (A_{260}/A_{280}) and concentration of extracted DNA were then checked (NanoDrop; Thermo Scientific, Waltham, MA, USA). The quality of extracted DNA was assessed using electrophoresis of DNA on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany).

Table 1 presents the polymerase chain reaction (PCR) protocol used for genotypic assessment of antibiotic resistance.[15–21] A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330; Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) was used in all PCRs. Amplified samples were analyzed by electrophoresis (120 V/208 mA) in 2.5% agarose gel. The gel was stained with 0.1% ethidium bromide (0.4 µg/mL). The UVI doc gel documentation system (Grade GB004; Jencons PLC, London, UK) was applied for analysis of images.

Statistical Analysis

Statistical analysis was done using SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). The chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between the prevalence of *S. aureus* and the phenotypic and genotypic properties of antibiotic resistance. *P* value <0.05 was considered a statistically significant level.

Results

Table 2 shows the incidence of *S. aureus* in diverse kinds of raw retail meat samples. Forty-eight out of 485 (9.89%) raw retail meat samples were contaminated with *S. aureus* bacteria. Raw retail camel meat (4%) had the lowest incidence of *S. aureus*, while raw retail buffalo meat (16%) had the highest. A statistically significant difference was found between types of retail meat samples and incidence of *S. aureus* ($P<0.05$).

Table 3 shows the phenotypic profile of antibiotic resistance of *S. aureus* bacteria isolated from diverse kinds of retail meat samples. *S. aureus* bacteria disclosed the highest incidence of resistance toward tetracycline (79.16%), penicillin (72.91%), gentamicin (60.41%), and doxycycline (41.66%) antibiotic agents. Lower incidence of resistance was obtained toward chloramphenicol (8.33%), levofloxacin (22.91%), rifampin (22.91%), and azithromycin (25%) antibiotic agents. A statistically significant difference was found between types of raw retail meat samples and incidence of antibiotic resistance ($P<0.05$). Moreover, a statistically significant difference was found for the incidence of resistance between gentamicin and amikacin ($P<0.05$), azithromycin and erythromycin ($P<0.05$), tetracycline and doxycycline ($P<0.05$), and ciprofloxacin and levofloxacin ($P<0.05$) antibiotic agents.

Table 4 shows the genotypic profile of antibiotic resistance of *S. aureus* bacteria isolated from diverse kinds of retail meat samples. *BlaZ* (58.33%), *tetK* (52.08%), *aacA-D* (33.33%), and *ermA* (27.08%) were the most commonly recognized antibiotic resistance genes amongst the *S. aureus* isolates. Incidences of *cat1* (4.16%), *rpoB* (10.41%), *msrA* (12.50%), *grlA* (12.50%), *linA* (14.58%), and *dfrA1* (16.66%) were lower than other detected antibiotic resistance genes. A statistically significant difference was found between types of raw retail meat samples and the incidence of antibiotic resistance genes ($P<0.05$). Furthermore, a statistically significant difference was found between the incidence of *tetK* and *tetM* ($P<0.05$), *msrA* and *ermA* ($P<0.05$), and *gyrA* and *grlA* ($P<0.05$) antibiotic resistance genes.

Discussion

S. aureus, a pathogen involved in severe gastrointestinal disorders and foodborne diseases, has an emergence of antibiotic resistance. Contaminated meat of animal species is considered one of the likely causes of transmission of antibiotic-resistant *S. aureus* to the human population.[22]

An existing survey addressed the incidence rate and phenotypic and genotypic profiles of antibiotic resistance of *S. aureus* bacteria recovered from raw beef, sheep, goat, camel, and buffalo retail meat samples. The incidence of *S. aureus* in the examined samples was 9.89%. The higher incidence rate was found in buffalo meat samples (16%), while the lower was found in camel meat samples (4%).

Table 3 Phenotypic Profile of Antibiotic Resistance of *S. aureus* Isolates Recovered from Diverse Kinds of Retail Meat Samples

Origins (No. of *S. aureus* Strains)	No. (%) of Isolates Resistant to Each Antibiotic												
	Penicillins	Aminoglycosides		Macrolides		Tetracyclines		Fluoroquinolones		Lincosamides	Folate inhibitors	Phenicols	Ansamycins
	P10	Gen	Amk	Azi	Ert	Tet	Dox	Cip	Lev	Clin	Tr-Sul	C30	Rif
Camel (4)	3 (75)	2 (50)	1 (25)	2 (50)	2 (50)	3 (75)	2 (50)	2 (50)	2 (50)	2 (50)	3 (75)	–	1 (25)
Buffalo (16)	12 (75)	11 (68.75)	7 (43.75)	4 (25)	8 (50)	13 (81.25)	7 (43.75)	7 (43.75)	4 (25)	6 (37.50)	6 (37.50)	2 (12.50)	4 (25)
Goat (6)	4 (66.66)	3 (50)	2 (33.33)	1 (16.66)	1 (16.66)	4 (66.66)	2 (33.33)	1 (16.66)	1 (16.66)	2 (33.33)	2 (33.33)	–	1 (16.66)
Beef (13)	10 (76.92)	8 (61.53)	6 (46.15)	4 (30.76)	6 (46.15)	11 (84.61)	5 (38.46)	5 (38.46)	3 (23.07)	3 (23.07)	5 (38.46)	1 (7.69)	4 (30.76)
Sheep (9)	6 (66.66)	5 (55.55)	3 (33.33)	1 (11.11)	1 (11.11)	7 (77.77)	2 ()	1 (11.11)	1 (11.11)	2 (22.22)	2 (22.22)	1 (11.11)	1 (11.11)
Total (48)	35 (72.91)	29 (60.41)	19 (39.58)	12 (25)	18 (37.50)	38 (79.16)	20 (41.66)	16 (33.33)	11 (22.91)	15 (31.25)	18 (37.50)	4 (8.33)	11 (22.91)

Abbreviation: P10, penicillin (10 µg/disk); Gen, gentamicin (10 µg/disk); Amk, amikacin (30 µg/disk); Azi, azithromycin (15 µg/disk); Ert, erythromycin (15 µg/disk); Tet, tetracycline (30 µg/disk); Dox, doxycycline (30 µg/disk); Cip, ciprofloxacin (5 µg/disk); Lev, levofloxacin (5 µg/disk); Clin, clindamycin (2 µg/disk); Tr-Sul, trimethoprim–sulfamethoxazole (25 µg/disk); C30, chloramphenicol (30 µg/disk); Rif, rifampin (5 µg/disk).

Table 4 Genotypic Profile of Antibiotic Resistance of *S. aureus* Isolates Recovered from Diverse Kinds of Retail Meat Samples

Origins (No. of *S. aureus* Strains)	No. (%) of Isolates Harbored Each Gene											
	Aminoglycosides	Tetracyclines		Macrolides		Lincosamides	Penicillins	Folate inhibitors	Fluoroquinolones		Ansamycins	Phenicols
	aacA-D	*tetK*	*tetM*	*msrA*	*ermA*	*linA*	*blaZ*	*dfrA*	*gyrA*	*grlA*	*rpoB*	*cat1*
Camel (4)	1 (25)	2 (50)	1 (25)	1 (25)	2 (50)	1 (25)	3 (75)	1 (25)	1 (25)	1 (25)	1 (25)	–
Buffalo (16)	8 (50)	10 ()	4 (25)	2 (12.50)	5 (31.25)	3 (18.75)	9 (56.25)	3 (18.75)	4 (25)	2 (12.50)	2 (12.50)	1 (6.25)
Goat (6)	1 (16.66)	2 (33.33)	1 (16.66)	–	1 (16.66)	1 (16.66)	3 (50)	1 (16.66)	1 (16.66)	–	–	–
Beef (13)	4 (30.76)	7 (53.84)	3 (23.07)	2 (15.38)	4 (30.76)	1 (7.69)	8 (61.53)	2 (15.38)	3 (23.07)	2 (15.38)	1 (7.69)	1 (7.69)
Sheep (9)	2 (22.22)	4 (44.44)	1 (11.11)	1 (11.11)	1 (11.11)	1 (11.11)	5 (55.55)	1 (11.11)	1 (11.11)	1 (11.11)	1 (11.11)	–
Total (48)	16 (33.33)	25 (52.08)	10 (20.83)	6 (12.50)	13 (27.08)	7 (14.58)	28 (58.33)	8 (16.66)	10 (20.83)	6 (12.50)	5 (10.41)	2 (4.16)

Table 5 Summarizing the Isolation Rate of *S. aureus* in Different Types of Meat Samples Obtained from Previous Research

Type of Meat Samples	Isolation Rate of S. aureus (%)	Studied Region/ Year	References
Camel	14.50	Egypt/2019	[35]
Camel	11.70	Ethiopia/2017	[36]
Buffalo	16.70	India/2017	[37]
Poultry	17.24	Saudi Arabia/2016	[38]
Camel	50		
Lamb/mutton	20.83		
Beef	13.04		
Camel	10.70	Saudi Arabia/2019	[39]
Beef	20.50	United States/2011	[40]
Camel	45	Egypt/2019	[41]
Meat products	15.30–61.50	Iran/2019	[42]
Red meat	18	Libya/2019	[43]
Chicken	40		
Beef	57.50	Iran/2013	[44]
Lamb	68.80		
Goat	47.50		
Camel	46		
Chicken	40.50	Japan/2005	[45]
Camel	40	Nigeria/2016	[46]
Pork	47.70	China/2018	[47]
Beef	50.40		
Poultry	67.90		
Mutton	54.50		
Beef	24.50	United States/2017	[34]
Chicken	13.50		
Pork	22.60		
Turkey	50.90		
Beef	68	Denmark/2017	[31]
Beef	10.60	The Netherlands/ 2009	[26]
Veal	15.20		
Lamb	6.20		
Mutton	6.20		
Pork	10.70		
Chicken	16		
Turkey	35.30		
Fowl	3.40		
Game	2.20		
Beef	17.30	Turkey/2010	[48]
Poultry	63.60		

This finding is possibly owing to the different diet of diverse animal species which may affect the incidence of bacteria. Furthermore, higher manipulation of buffalo carcasses by contaminated veterinarians through inspection in the slaughterhouse is another imperative risk factor for contamination. Moreover, the low number of camel slaughtering which can reduce the risk of cross-contamination may be another reason for the low incidence of *S. aureus* in camel retail meat. Foodstuff contamination with *S. aureus* may occur straight from infected food-producing animals (or their products such as meat) or may result from poor hygiene throughout production processes, or the retail and storage of food, since humans may also harbor microorganisms. Diverse surveys have been conducted in this field in Japan,[23] Korea,[24] Italy,[25] and the Netherlands.[26] The incidence of *S. aureus* bacteria in raw retail meat samples collected from Brazil,[27] Turkey,[28] Egypt,[29] Germany,[30] and Denmark[31] was 21.72%, 30%, 40.80%, 71.50%, and 52.00%, respectively, which all were much higher than our findings. Additionally, the role of raw retail meat as a reservoir of *S. aureus* bacteria has been reported in surveys performed in Australia,[32] the United Kingdom,[33] and the United States.[34] Hasanpour Dehkordi et al[8] conveyed that the incidence of *S. aureus* bacteria in raw retail beef, sheep, goat, and camel meat samples was 16.00%, 24.00%, 20.40%, and 10.00%, respectively. Table 5 summarizes the isolation rate of *S. aureus* in different types of meat samples obtained from previous research.[35–48]

The contamination rate of raw meat samples with *S. aureus* varies between diverse research studies. The difference in data advises that the time, season, place of sampling, method of sampling, types of samples, and even laboratory techniques applied in research may affect the outcomes of surveys. Moreover, different hygienic levels of butchers and retail centers may affect the incidence of bacteria in diverse investigations. Otherwise, the sample sizes, sample types, and geographic locations of research may be the reason for these differences. Compared to the results of other scientists, the comparatively low rate of *S. aureus* isolation was reported in our survey. This is expected because in fresh meat *S. aureus* is not a good competitor with normal microflora. The reasons for this finding are not clear, but since antibiotic prescription is higher than required, a lower incidence of *S. aureus* is possible.

Our survey also disclosed considerable incidence of resistance toward diverse groups of antibiotics, particularly aminoglycosides, fluoroquinolones, lincosamides, macrolides, penicillins, tetracyclines, phenicols, folate pathway inhibitors, and ansamycins, which was accompanied with the presence of *aacA-D, gyrA* and *grlA, linA, msrA* and *ermA, blaZ, tetK* and *tetM, cat1, dfrA1,* and *rpoB* antibiotic resistance genes, respectively. Thus, the phenotypic pattern of antibiotic resistance of *S. aureus* bacteria was confirmed by the genotypic profile. Furthermore, our outcomes disclosed that some *S. aureus* bacteria exhibited higher incidence of resistance

toward antibiotics used for human beings which can indirectly signify their anthropogenic source. Reversely, some others exhibited higher incidence of resistance toward antibiotics used for animal beings which can indirectly demonstrate their animal origins. Similar resistance profiles of *S. aureus* bacteria isolated from dissimilar kinds of foodstuffs and clinical specimens have also been determined toward aminoglycosides,[5,13,49-52] cephems,[5,13,49–51] penicillins,[5,13,49–51] macrolides,[5,13,49–51] tetracyclines,[5,13,49,50] fluoroquinolones,[5,13,49–52] lincosamides,[5,13,49–51] folate inhibitors,[5,13,49–52] phenicols,[5,13,49] and ansamycins[5,13,49,50]. Momtaz et al[5] reported that the *S. aureus* strains with meat origins harbored the highest prevalence of resistance against tetracycline (97.50%), methicillin (75.60%), sulfamethoxazole (31.70%), trimethoprim (31.70%), streptomycin (31.70%), gentamicin (29.20%), enrofloxacin (28.00%), ampicillin (26.8%), chloramphenicol (20.70%), and cephalothin (17.00%) antibiotic agents. Hasanpour Dehkordi et al[8] determined that *S. aureus* bacteria isolated from raw meat samples had a high incidence of resistance toward ampicillin (100%), ceftriaxone (80.00%), amoxicillin–clavulanic acid (50.00%), lincomycin (61.20%), tetracycline (55.00%), gatifloxacin (96.80%), minocycline (51.20%), cotrimoxazole (45.60%), clindamycin (54.30%), azithromycin (48.10%), erythromycin (37.50%), oxacillin (76.20%), and penicillin (100%) antibiotic agents. Fowoyo and Ogunbanwo[53] disclosed that the *S. aureus* bacteria recovered from ready to eat foodstuffs exhibited a high incidence of resistance toward trimethoprim–sulfamethoxazole (74.90%), ampicillin (86.70%), cefotaxime (3.50%), amoxicillin–clavulanic acid (52.50%), ciprofloxacin (23.90%), oxacillin (35.70%), gentamicin (11.40%), erythromycin (15.70%), and ofloxacin (7.10%). The high incidence of resistance toward chloramphenicol (8.33%) may be due to its unlawful and unselective prescription especially in veterinary medicine. Akanbi et al[54] reported that *blaZ, mecA, rpoB, ermB,* and *tetM* were the most commonly identified antibiotic resistance genes amongst the *S. aureus* bacteria recovered from food samples in South Africa. High distribution of *mecA, gyrA, grlA,* and *cfr* genes was also described in the *S. aureus* bacteria recovered from chicken meat in Egypt.[55] Another Iranian survey[56] disclosed that oxacillin-, gentamicin-, penicillin-, tetracycline-, and erythromycin-resistant *S. aureus* bacteria isolated from milk and dairy products harbored high incidence of *blaZ, aacA-aphD, mecA, tetK* and *tetM, ermB, ermA, ermT, ermC, msrB,* and *msrA* antibiotic resistance markers likewise to our survey. A similar phenotypic profile of antibiotic resistance was also reported from Iran[57] and China.[58] Differences in the opinion of medical and veterinary practitioners in antibiotic prescription, observation of ethics and rules in the use of antibiotics, availability or lack of antibiotics, and their prices are probable reasons for differences found in the incidence of resistance of *S. aureus* strains in numerous investigations. In a survey which was conducted by Bantawa et al,[59] *S. aureus* bacteria isolated from buffalo, chicken, pork, and goat meat samples in eastern Nepal harbored a high incidence of resistance (10–100%) against amoxicillin, tetracycline, cefotaxime, and nalidixic acid.

Our findings also disclosed higher incidence of a phenotypic profile of resistance than a genotypic profile. For example, all of the penicillin-resistant *S. aureus* bacteria did not harbor *blaZ* antibiotic resistance genes. This matter also existed for other antibiotic agents and resistance genes. This finding may be owing to the fact that the presence of antibiotic resistance genes is one of the known procedures for the occurrence of antibiotic resistance in bacteria. On the other hand, several mechanisms have been identified to induce antibiotic resistance in bacteria including reduced permeability of bacteria to antibiotics, efflux antibiotic active pumps out of the bacterial cell, change in antibiotic target site, inactivation of antibiotics through hydrolysis or changes in their structure, occurrence of genetic mutations, and access of bacteria to the secondary metabolic pathways that compensate the antibiotic-inhibited reactions.

Conclusion

In conclusion, a high incidence of *S. aureus* in examined samples, particularly raw retail buffalo and beef meat samples, was accompanied with a high incidence of resistance toward diverse classes of antibiotic agents and also dissimilar antibiotic resistance genes. An existing survey is an initial report of the genotypic evaluation of antibiotic resistance of the *S. aureus* bacteria isolated from raw retail buffalo and camel meat samples. High incidence of resistance of *S. aureus* bacteria toward tetracycline, penicillin, gentamicin, and doxycycline antibiotic agents and also high frequency of *blaZ, tetK, aacA-D,* and *ermA* antibiotic resistance genes may pose an imperative menace regarding the role of raw or undercooked meat consumption on the transmission of antibiotic-resistant *S. aureus*. Incidence of resistance toward human-based and also animal-based antibiotics can indirectly show the origin of *S. aureus* isolates. It seems that tetracycline, penicillin, gentamicin, and doxycycline are not effective therapeutic agents in the cases of *S. aureus* foodborne diseases in Iran. Slaughterhouses can be severely

contaminated with foodborne pathogens,[46–49] so the maintenance of slaughter hygiene, regular microbiological monitoring of carcasses, implementation of good manufacturing practices, and a food safety system such as the HACCP system are essential to minimize the risk to the consumer. Additionally, appropriate cooking of raw meat before consumption, prevention from cross-contamination, and antibiotic prescription based on the outcomes of disk diffusion can diminish the risk of transmission of resistant *S. aureus* bacteria from meat to the human population. However, supplementary surveys are essential to determine more epidemiological features of the *S. aureus* bacteria in raw retail meat of animal species. Our research highlights the importance of controlling the antibiotic susceptibility of *S. aureus* in foodstuffs such as food-producing animals, retail foods, and even human beings, and this information could be used proactively to assist Iranian industries to progress better-quality food safety measures. Otherwise, on the basis of these observations, we recommend that attention should be paid by governments and individuals to prevent the further spread of antibiotic-resistant *S. aureus*.

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Disclosure

The authors report no conflicts of interest in this work.

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