Molecular typing, phenotypic and genotypic assessment of antibiotic resistance and virulence factors amongst the *Staphylococcus aureus* bacteria isolated from raw chicken meat

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Research article

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Abstract

Background: Staphylococcus aureus is an important cause of foodborne diseases due to the consumption of contaminated raw chicken meat. The present research was performed to evaluate the phenotypic and genotypic properties of antibiotic resistance, virulence factor profiles and molecular typing of *S. aureus* strains isolated from chicken meat. A total of 36 S. aureus strains were isolated from raw chicken meat samples. Phenotypic pattern of antibiotic resistance was assessed by disk diffusion. Distribution of antibiotic resistance and virulence genes was evaluated by PCR. Molecular typing of isolates was perform ed by the ERIC-PCR.

Results: Considering the over than 80% similarity, 36 *S. aureus* isolates were classified in 9 different profiles with 43 to 100% similarities. *S. aureus* strains showed the highest incidence of resistance against penicillin (100%), tetracycline (91.66%), cephalothin (77.77%), ciprofloxacin (75%), erythromycin (75%), mupirocin (63.88%), clindamycin (61.11%) and trimethoprim/sulfamethoxazole (61.11%). The most commonly detected antibiotic resistance genes amid the *S. aureus* isolates were *mecA* (100%), *tetK* (80.55%), *tetM* (66.66%), *aacA-D* (61.11%), *msrA* (55.55%) and *ermA* (55.55%). Total distribution of *etB*, *etA*, *tsst-1*, *clfA* and *coa* virulence factors amongst the *S. aureus* strains was 61.11%, 58.33%, 13.88%, 75% and 100%, respectively.

Conclusions: Genetic cluster of bacteria affected the antibiotic resistance and virulence characters of *S. aureus* strains. *S. aureus* strains with the same ERIC-genetic cluster had similar antibiotic resistance and virulence characters which may show their similar origins. Presence of one or more virulence factors and antibiotic resistance genes amongst the resistant-S. aureus strains signifies an important public health threat rendering the consumption of raw or undercooked chicken meat.

Background

Chicken meat harbors variety of imperative dietary supplements including proteins, carbohydrate, fats, minerals and vitamins with healthy advantageous for human [1–4]. Thus, their regular daily consumption has been widely recommended. In keeping with this, human involvement in the production, inspection and processing of chicken meat increased the risk of microbial contamination and occurrence of foodborne diseases [1–4].

Staphylococcus aureus (*S. aureus*) is a Gram-positive, catalase positive, and cocci-shaped bacterium characteristically originate from nose and respiratory tract and on the skin [5–8]. *S. aureus* is responsible for plain nosocomial and community-acquired infections, foodborne diseases and food poisoning [5–8]. Occurrence of diverse kinds of gastrointestinal diseases known by abdominal cramps, nausea, vomiting, weakness and diarrhea and also toxic shock syndrome (TSS) are accredited to *S. aureus* [5–8].

Foodborne diseases caused by *S. aureus* bacteria are mostly resist toward antibiotic therapy. Currently, resistant-*S. aureus* has become a thoughtful issue in health-care centers and the community [6, 9]. Epidemiological surveys have recognized that the *S. aureus* bacteria isolated from diverse kinds of foodstuffs, particularly those with animal origins, harbored considerable incidence of resistance toward frequently used antibiotic agents, especially aminoglycosides, penicillins, tetracyclines, fluoroquinolones, cephalosporins, and macrolides [6, 9]. Presence of the genes encode resistance toward methicillin (*mecA*), aminoglycosides (*aacA-D*), streptogramins (*vatA*, *vatB* and *vatC*), tetracyclines (*tetK* and *tetM*), lincosamides (*linA*) and macrolides (*ermA*, *ermB*, *ermC*, *msrA* and *msrB*), is one of the main modes for occurrence of antibiotic resistance amongst the *S. aureus* bacteria [6, 9, 10].

Some potential virulence factors are accompanied in the pathogenesis of clinical infections and in some cases foodborne diseases caused by *S. aureus* [8, 11]. Toxic shock syndrome toxin-1 (*tsst-1)*, coagulase encoding gene (*coa*), exfoliative toxins A and B (*eta* and *etb*) and clumping factor (*clfA*) are frequently detected in *S. aureus* strains isolated from human clinical infections and foodstuffs [8, 11, 12].

Genotypic-based molecular analysis of bacterial strains has an imperative role in their classifications especially in outbreaks of nosocomial infections and foodborne diseases. Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) is a technique with high ability to generate DNA fingerprints that differentiate between bacterial strains [13]. Application of diverse kinds of ERIC-PCR methods has been used for molecular typing of *S. aureus* isolates [13].

Notwithstanding the great number of reports and researches on the dynamics of *S. aureus*, supplementary investigations is desirable, particularly in some area like Iran where there is a comparative scarcity of data on the epidemiology of *S. aureus*'s foodborne diseases. Thus, an existing survey was performed to assess the phenotypic and genotypic properties of antibiotic resistance, distribution of virulence factors and molecular typing of *S. aureus* bacteria isolated from raw chicken meat samples in Iran.

Results

Molecular typing of S. aureus strains

The present survey was conducted to assess the phenotypic and genotypic properties, virulence factor profiles and molecular typing of a total of 36 *S. aureus* strains isolated from chicken meat.

Figure 1 reveals the ERIC-based molecular typing of *S. aureus* strains isolated from chicken meat samples. Considering the over than 80% similarity, 36 *S. aureus* isolates were classified in 9 different profiles with 43 to 100% similarities. Isolates No 2, 11, 20, 24, 35, 5, 12, 13 and 36 were classified in diverse profiles. The lowest similarity (43%) was found for isolates No 20, 11, 2 and 24. An achieved major clonal cluster was ERIC-cluster No 3 with 7 *S. aureus* isolates. Diverse ERIC-types were further analyzed for phenotypic and genotypic properties of antibiotic resistance and distribution of virulence factors.

Phenotypic profile of antibiotic resistance

Table 2 reveals the phenotypic pattern of antibiotic resistance of diverse ERIC-types of *S. aureus* strains. *S. aureus* strains harbored the uppermost incidence of resistance toward penicillin (100%), tetracycline (91.66%), cephalothin (77.77%), ciprofloxacin (75%), erythromycin (75%), mupirocin (63.88%), clindamycin (61.11%) and trimethoprim/sulfamethoxazole (61.11%). Isolated bacteria exhibited the lowest incidence of resistance toward nitrofurantoin (16.66%) and rifampin (41.66%) antibiotic agents. Statistically significant difference was found between ERIC-type of *S. aureus* isolates and incidence of antibiotic resistance ($P < 0.05$).

Table 2

Phenotypic pattern of antibiotic resistance of diverse ERIC-types of the *S. aureus* strains isolated from chicken meat.

ERIC Type	Isolates No	Phenotypic profile of antibiotic resistance (%)										
		F300*	SXT25	TE30	RA5	Mup	E15	AZM15	Cln	CIP5	CL30	P10
Type 1	21	-	+	+	+	+	+	+	+	+	+	+
	30	-	-	+	+	+	+	+	+	+	-	+
	27	+	+	+	-	-	+	+	+	+	+	+
	31	-	-	-	+	+	+	+	-	+	+	+
	25	-	+	+	+	-	-	-	-	-	+	+
	26	-	-	+	-	+	+	+	-	+	+	+
	34	-	+	-	-	-	+	+	+	+	+	+
Type 2	9	-	+	+	+	+	+	+	+	+	+	+
	16	-	+	+	+	+	+	+	+	+	+	+
	15	-	+	+	+	+	-	-	+	-	+	+
	18	+	-	-	-	+	-	+	-	-	+	+
Type 3	3	-	+	+	-	+	+	-	-	+	-	+
	8	-	+	+	-	-	+	+	-	+	+	+
	14	-	+	+	+	-	+	-	-	+	+	+
	35	-	+	+	+	+	+	+	-	+	+	+
Type 4	28	-	+	+	-	-	+	-	+	+	+	+
	29	-	+	+	+	+	+	+	+	+	+	+
	12	-	+	+	-	+	+	-	+	+	+	+
	5	-	+	+	+	+	-	+	+	-	+	+
Type 5	4	+	-	+	-	+	+	-	+	+	-	+
	10	-	-	+	-	+	+	-	+	+	+	+
	17	-	-	+	-	+	+	-	+	+	-	+
	13	-	+	+	+	-	-	+	+	-	+	+
Type 6	6	+	-	+	-	+	+	+	+	+	-	+
	19	-	+	+	+	-	-	+	+	-	+	+
	36	-	-	+	-	-	+	+	-	+	+	+
Type 7	1	-	-	+	-	+	+	-	-	+	+	+
	7	-	+	+	+	-	-	+	-	-	+	+
Type 8	32	-	-	+	-	+	+	+	-	+	-	+
	33	-	-	+	-	+	+	-	-	+	-	+
Type 9	22	-	-	+	-	+	+	-	+	+	+	+
	23	+	+	+	+	+		+	+	-	+	+
Type 10	20	-	+	+	-	-	+	-	+	+	+	+

*F300 = Nitrofurantoin, SXT25 = Trimethoprim/sulfamethoxazole, TE30 = Tetracycline, RA5 = Rifampin, Mup = Mupirocin, E15 = Erythromycin, AZM15 = Azithromycin, Cln = Clindamycin, CIP5 = Ciprofloxacin, CL30 = Cephalothin, P10 = Penicillin,

ERIC Type	Isolates No	Phenotypic profile of antibiotic resistance (%)										
		F300*	SXT25	TE30	RA5	Mup	E15	AZM15	Cln	CIP5	CL30	P10
Type 11	24	-	+	+	-	+	+	-	+	+	+	+
Type 12	11	+	+	+	-	-	+	-	+	+	+	+
Type 13	2	-	-	+	-	-		+	-	-	-	+
Total (36)		6 (16.66)	22 (61.11)	33 (91.66)	15 (41.66)	23 (63.88)	27 (75)	21 (58.33)	22 (61.11)	27 (75)	28 (77.77)	36 (100)

*F300 = Nitrofurantoin, SXT25 = Trimethoprim/sulfamethoxazole, TE30 = Tetracycline, RA5 = Rifampin, Mup = Mupirocin, E15 = Erythromycin, AZM15 = Azithromycin, Cln = Clindamycin, CIP5 = Ciprofloxacin, CL30 = Cephalothin, P10 = Penicillin,

Genotypic profile of antibiotic resistance

Table 3 reveals the genotypic pattern of antibiotic resistance of diverse ERIC-types of the *S. aureus* strains isolated from chicken meat. *MecA* (100%), *tetK* (80.55%), *tetM* (66.66%), *aacA-D* (61.11%), *msrA* (55.55%) and *ermA* (55.55%) were the most frequently detected antibiotic resistance genes amongst the *S. aureus* isolates. *VatC* (8.33%), *vatB* (36.11%), *ermC* (38.88%) and *vatA* (41.66%) had the lowermost incidence amongst all examined antibiotic resistance genes. Statistically significant difference was found between ERIC-type of *S. aureus* isolates and incidence of antibiotic resistance genes ($P < 0.05$). Additionally, statistically significant differences was found amid the distribution of *tetK* and *tetM* ($P < 0.05$), *ermA* and *ermC* ($P < 0.05$), *vatA* and *vatB* and *vatC* ($P < 0.05$) and finally *msrA* and *msrB* ($P < 0.05$).

Table 3

Genotypic pattern of antibiotic resistance of diverse ERIC-types of the *S. aureus* strains isolated from chicken meat.

ERIC Type	Isolates No	Profile of antibiotic resistance genes (%)											
		linA	ermC	ermA	vatC	vatB	vatA	tetM	tetK	aacA-D	msrB	msrA	mecA
Type 1	21	+	-	+	-	+	-	+	+	+	-	+	+
	30	-	+	-	-	-	+	+	-	+	+	-	+
	27	+	-	+	-	-	+	-	+	+	+	+	+
	31	+	-	+	-	-	-	+	+	-	-	+	+
	25	-	+	-	+	-	+	+	-	+	+	-	+
	26	-	-	+	-	-	-	+	+	+	+	-	+
	34	+	+	-	-	-	+	-	+	+	+	+	+
Type 2	9	-	-	+	-	-	+	+	-	+	-	+	+
	16	+	-	-	-	+	+	+	+	+	+	-	+
	15	-	+	+	-	-	+	+	+	+	-	-	+
	18	+	-	+	-	-	+	+	-	+	-	+	+
Type 3	3	+	-	+	-	+	-	-	+	-	+	+	+
	8	-	+	-	-	+	-	+	+	+	-	+	+
	14	-	+	-	-	-	-	+	+	+	+	-	+
	35	+	-	+	-	+	-	-	+	-	-	+	+
Type 4	28	-	-	-	-	-	+	-	+	-	+	-	+
	29	-	+	-	-	-	+	+	+	+	-	+	+
	12	-	-	+	-	-	+	-	+	-	+	-	+
	5	-	+	-	-	-	+	+	-	-	-	+	+
Type 5	4	+	-	+	+	-	-	-	+	+	-	+	+
	10	+	+	-	-	+	-	+	+	-	+		+
	17	+	+	-	-	-	+	+	+	-	+	-	+
	13	+	-	+	+	-	-	-	+	+	-	+	+
Type 6	6	-	-		-	+	-	+	+	-	+	-	+
	19	-	-	+	-	-	+	+	+	+	-	+	+
	36	-	-	+	-	-	-	+	+	-	-	+	+
Type 7	1	+	-	+	-	-	-	-	+	+	-	+	+
	7	+	-	+	-	-	-	-	+	+	+	-	+
Type 8	32	-	+	-	-	+	-	+	-	-	+	-	+
	33	-	+	-	-	+	-	-	+	+	-	+	+
Type 9	22	+	-	+	-	-	+	-	+	-	+	-	+
	23	+	-	+	-	+	-	+	+	+	-	+	+
Type 10	20	-	+	-	-	+	-	+	-	+	+	+	+
Type 11	24	+	-	+	-	-	-	+	+	-	-	+	+

ERIC Type	Isolates No	Profile of antibiotic resistance genes (%)											
		linA	ermC	ermA	vatC	vatB	vatA	tetM	tetK	aacA-D	msrB	msrA	mecA
Type 12	11	-	+	-	-	+	-	+	+	+	+	-	+
Type 13	2	+		+	-	+	-	+	+	-	+	-	+
Total (36)		18 (50)	14 (38.88)	20 (55.55)	3 (8.33)	13 (36.11)	15 (41.66)	24 (66.66)	29 (80.55)	22 (61.11)	19 (52.77)	20 (55.55)	36 (100)

Profile of virulence factors

Table 4 reveals the distribution of virulence factors of diverse ERIC-types of the *S. aureus* strains isolated from chicken meat. Total distribution of *etB*, *etA*, *tsst-1*, *clfA* and *coa* virulence factors amongst the *S. aureus* strains was 61.11%, 58.33%, 13.88%, 75% and 100%, respectively. Statistically significant difference was found between ERIC-type of *S. aureus* isolates and incidence of virulence factors ($P < 0.05$).

Table 4
Distribution of virulence factors of diverse ERIC-types of the *S. aureus* strains isolated from chicken meat.

ERIC Type	Isolates No	Profile of virulence factors (%)				
		etB	etA	tsst-1	clfA	coa
Type 1	21	+	+	-	+	+
	30	+	+	-	+	+
	27	+	+	-	+	+
	31	+	+	-	+	+
	25	+	+	-	-	+
	26	+	+	-	+	+
	34	+	+	-	+	+
Type 2	9	+	+	-	+	+
	16	+	+	-	+	+
	15	+	+	-	+	+
	18	+	-	-	+	+
Type 3	3	+	+	-	+	+
	8	-	+	-	+	+
	14	+	+	-	+	+
	35	+	+	-	+	+
Type 4	28	+	+	-	+	+
	29	+	+	-	+	+
	12	-	-	+	-	+
	5	+	-	-	+	+
Type 5	4	-	-	-	+	+
	10	-	-	+	+	+
	17	-	-	+	+	+
	13	-	-	-	+	+
Type 6	6	+	-	-	+	+
	19	-	-	-	+	+
	36	+	+	-	+	+
Type 7	1	+	+	-	+	+
	7	-	-	-	-	+
Type 8	32	-	-	-	+	+
	33	+	-	+	-	+
Type 9	22	-	+	-	-	+
	23	-	-	-	+	+
Type 10	20	+	+	-	-	+
Type 11	24	-	-	+	-	+

ERIC Type	Isolates No	Profile of virulence factors (%)				
		etB	etA	tsst-1	clfA	coa
Type 12	11	-	+	-	-	+
Type 13	2	-	-	-	-	+
Total (36)		22 (61.11)	21 (58.33)	5 (13.88)	27 (75)	36 (100)

Discussion

From the epidemiological prospective, it is essential to know the exact ways of transmission of antibiotic resistant-bacteria to human population. Foods with animal origin have a critical role in transmission of antibiotic resistant-bacteria to human [14–17]. Chicken meat is considered as a ubiquitous source of antibiotic resistant-*S. aureus* [18, 19].

An existing survey was carried out to assess the phenotypic and genotypic properties of antibiotic resistance, characterization of virulence factors and molecular typing of a total of 36 *S. aureus* bacteria isolated from raw chicken meat samples. Our findings described that the *S. aureus* bacteria exhibited the highest incidence of resistance toward penicillin, tetracycline, cephalothin, ciprofloxacin, erythromycin, mupirocin, clindamycin and trimethoprim/sulfamethoxazole which was assisted with attendance of *mecA*, *tetK*, *tetM*, *aacA-D*, *msrA* and *ermA* antibiotic resistance genes. Widespread and unauthorized administration of antimicrobials and disinfectant solutions in both medicine and veterinary have been considered to be a major factor in the emergence of antibiotic resistance amongst *S. aureus* bacteria. Similarly, considerable incidence of resistance of *S. aureus* bacteria recovered from chicken meat samples toward penicillin, tetracycline, cephalothin, ciprofloxacin, erythromycin, mupirocin, clindamycin and trimethoprim/sulfamethoxazole antibiotic agents was conveyed from China [19], Bangladesh [20], Nepal [21], Pakistan [22], United States [23] and Iran [24]. V Govender, E Madoroba, K Magwedere, G Fosgate and L Kuonza [25] stated that the *S. aureus* bacteria isolated from poultry meat in South Africa harbored resistance toward penicillin (27.80%), ampicillin (22.20%), oxacillin (15.30%), cefoxitin (20.80%), ceftiofur (5.60%), oxytetracycline (43.10%), clindamycin (22.20%), gentamicin (18.10%), erythromycin (13.90%), sulfamethoxazole (6.90%), enrofloxacin (1.40%), ciprofloxacin (0%), vancomycin (0%) and florfenicol (0%) antibiotic agents. DG Amoako, AM Somboro, AL Abia, C Molechan, K Perrett, LA Bester and SY Essack [26] reported that the *S. aureus* bacteria isolated from poultry products harbored the highest incidence of resistance toward tetracycline (61.67%), penicillin G (55.83%), erythromycin (54.17%), clindamycin (43.33%), rifampicin (40.83%), doxycycline (36.67%), ampicillin (34.17%), amikacin (30.83%), moxifloxacin (30.83%), trimethoprim–sulfamethoxazole (30.00%), levofloxacin (23.33%), ciprofloxacin (15.83%), gentamicin (8.33%) and cefoxitin (7.50%). Surveys conducted on America, Asia, Africa, and Europe continents revealed the high incidence of antibiotic resistant-*S. aureus* in foods with animal origins [27, 28]. Similar pattern of resistance of *S. aureus* bacteria isolated from different kinds of foodstuff and clinical samples have been reported toward fluoroquinolones, aminoglycosides, macrolides [6, 8, 29–32], tetracyclines [6, 8, 29, 30], cephems [6, 8, 29–31], folate inhibitors [6, 8, 29–32], penicillins [6, 8, 29–31], phenicols [6, 8, 29, 30], lincosamides [6, 8, 29–31], and ansamycins [6, 8, 29, 30] antibiotic agents. 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 of differences found in the incidence of resistance of *S. aureus* strains in numerous investigations. Genotypic pattern of antibiotic resistance of *S. aureus* strains isolated from chicken meat samples has less evaluated. Our findings revealed that *mecA*, *tetK*, *tetM*, *aacA-D*, *msrA* and *ermA* were the main antibiotic resistance genes amongst the *S. aureus* isolates. Thus, phenotypic pattern of antibiotic resistance of *S. aureus* strains was confirmed by the genotypic pattern. However, our findings disclosed higher incidence of phenotypic profile of resistance than genotypic profile. For example, all of the tetracycline-resistant *S. aureus* bacteria didn't harbored *tetK* or *tetM* antibiotic resistance genes. This matter was also existed for other antibiotic agents and resistance genes. It is maybe owing to the fact that presence of antibiotic resistance genes is one of the known procedures for occurrence of antibiotic resistance in bacteria. Otherwise, several mechanisms have been identified to induce antibiotic resistance in bacteria including reduced permeability of bacteria to antibiotics, efflux antibiotic's active pumps to 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. OE Akanbi, HA Njom, J Fri, AC Otigbu and AM Clarke [33] described that *mecA*, *ermB* and *tetM* were the most commonly detected antibiotic resistance genes amongst the *S. aureus* bacteria recovered from food samples which was relatively similar to our findings. Similar to our results, high incidence of *mecA*, *tetK*, *tetM*, *vatA*, *ermA*, and *msrA* was also described in the *S. aureus* bacteria recovered from chicken meat in Egypt [34], bovine mastitis milk and pig carcasses and clinical infections in Germany [35, 36], ruminants in Iran [37], and raw milk in Switzerland [38]. Another Iranian survey [39] represented the high distribution of *aacA-aphD*, *mecA*, *tetK* and *tetM*, *ermB*, *ermA*, *ermT*, *ermC*, *msrB* and *msrA* antibiotic resistance markers likewise to our survey. H Hizlisoy, NE Onmaz, F Karadal, A Serhat, Y Yildirim, Z Gonulalan and H Kilic [40] reported that the distribution of *aacA-aphD*, *tetK*, *tetM*, *vatA*, *vatB*, *blaZ*, *ermA*, *ermC*, *mecA*, *vanA* and *vanB* antibiotic resistance genes amongst the *S. aureus* strains isolated from foods with animal origin in Turkey was 100%, 44%, 37%, 0%, 100%, 91%, 53%, 47%, 100%, 0%, and 11%, respectively. A total of 61.11% of the *S. aureus* strains

harbored the *aacA-D* gene which encode resistance toward gentamicin. Similar findings were also reported by (Australia), RM Gomes, MRQ Bomfim, MJ Trindade, LM Farias and SG Santos [42] (Brazil), G Adwan, K Adwan, N Jarrar and A Amleh [43] (Palestine), and L Oksuz, C Dupieux, A Tristan, M Bes, J Etienne and N Gurler [44] (Turkey). *ErmA* and *ermC* genes are mainly attributed to occurrence of resistance toward erythromycin. Total distribution of *ermA* and *ermC* genes amongst the *S. aureus* strains were 55.55% and 8.33%, respectively. Likewise, T Zmantar, K Chaieb, FB Abdallah, AB Kahla-Nakbi, AB Hassen, K Mahdouani and A Bakhrouf [45] conveyed that the incidence of *erm*A and *erm*C amongst the *S. aureus* strains isolated from Tunisia were 22.80% and 17.10%, respectively. Similar to our findings, higher incidence of *ermA* than *ermC* antibiotic resistance genes was also reported previously [5, 6, 46, 47]. However, higher distribution of *ermC* (74.50%) than *ermA* (30.90%) gene was reported in a study conducted by G Adwan, K Adwan, N Jarrar and A Amleh [43]. *MecA* gene was detected in all examined *S. aureus* bacteria. The distribution of the *mecA* gene in the *S. aureus* bacteria isolated from foods with animal origins in India [16], Korea [48] and Turkey [49] were 77%, 61.90% and 57%, respectively which all were lower than our report. The high detection rate of the *mec*A gene found in the present survey, might be due to its horizontal transmission amid the *S. aureus* strains obtained together in food processing environment. *VatA*, *vatB* and *vatC* antibiotic resistance genes were detected in 41.66%, 36.11% and 8.33% of *S. aureus* strains. These genes are mainly encoded resistance against quinupristin-dalfopristin and streptogramin antibiotic agents. R Ranjbar, MHS Shahreza, E Rahimi and N Jonaidi-Jafari [50]reported that the total incidence of *vatA*, *vatB* and *vatC* antibiotic resistance genes amongst the S. aureus strains isolated from foods with animal origins were 38.55%, 19.27% and 9.63%, respectively. Our findings addressed the higher distribution of *tetK* (80.55%) than *tetM* (66.66%) antibiotic resistance genes. Similarly, higher incidence of *tetK* than *tetM* antibiotic resistance genes was also reported previously [5, 6, 8, 10, 40, 46, 50, 51]. Higher incidence of antibiotic resistance genes reported in some previously published papers is due to the fact that they examined the distribution of antibiotic resistance genes amongst the methicillin-resistant *S. aureus* (MRSA) strains.

Toxins are usually regarded as one of the major factors in the virulence of *S. aureus* globally, and hence, it is significant to evaluate their distribution amongst isolates from food with respect to measuring public health risks. Results of an existing survey revealed that the distribution of *coa*, *clfA*, *etB*, *etA* and *tsst-1* virulence factors amongst the *S. aureus* isolates were 100%, 75%, 61.11%, 58.33% and13.88%, respectively. Presence of virulence *S. aureus* amongst the chicken meat samples exhibited an imperative public health threat regarding the consumption of raw or undercooked chicken meat samples. In the same way, these genes were predominant in the S. aureus strains isolated from foods with animal origins [52–54]. The genes encoding exfoliative toxin isoforms (*etA* and *etB*) were the most important factors associated with the pathogenesis of the *S. aureus* infections. Exfoliative toxins play a role in host colonization and the invasion of injured mucosa and skin. Thus, the high distributions of *etA* and *etB* genes in examined chicken meat samples could be due to the transmission of *S. aureus* strains from the skin of workers and staffs of slaughterhouses [55]. H Li, P Andersen, M Stegger, R Sieber, H ngmer, N Staubrand, A Dalsgaard and J Leisner [56] reported that the *S. aureus* strains isolated from poultry meat samples didn't harbor *etA* and *etB* virulence factors, while the distribution of the *tsst-1* gene was 3.40%. The tsst-1 gene is a super-antigen that can cause a diversity of clinical complications. X Yang, S Yu, Q Wu, J Zhang, S Wu and D Rong [57] reported that the distribution of *tsst-1*, *etA* and *etB* virulence factors amongst the *S. aureus* strains isolated from foods with animal origins were 7.24%, 10.14% and 10.14%, respectively. It has been reported that *S. aureus* clones containing *eta*, *etb*, and *tsst* are increasingly responsible for severe infections. CB Waryah, J Gogoi-Tiwari, K Wells, KY Eto, E Masoumi, P Costantino, M Kotiw and T Mukkur [58] reported that the incidence of *clfA* gene amongst the *S. aureus* strains isolated from human origins on Australia was 83.87%. Additionally, *coa* and *clfA* virulence genes were detected in 63.41% and 76.82% of S. aureus strains isolated from chicken meat in previous Iranian survey [8]. Clumping factor is a significant adhesion protein of *S. aureus* that is governed by *clf*A gene. This virulence factor is critical for colonization and establishment of infections caused by the *S. aureus*. It contributes in the pathogenesis of diseases caused by *S. aureus* by facilitating bacterial binding via soluble or immobilized fibrinogen as fibrinogen plays an important role in platelet thrombus formation [59]. Coagulase protein has the capability to convert fibrinogen to fibrin and has enough potentials to be a virulence factor in infections caused by the *S. aureus*. It is codified by the *coa* gene that holds a preserved and a recurrent polymorphic region that can be used to evaluate relatedness amid *S. aureus* isolates [60]. High distribution of *clfA* and *coa* virulence factors amongst the *S. aureus* strains isolated from foods with animal origins was reported from Brazil [53], Thailand [61], Switzerland [62] and India [63].

Our survey also showed that the *S. aureus* strains of the same molecular cluster (ERIC-type) had the same profiles of antibiotic resistance and virulence factors. This matter maybe show the common source of contamination of chicken meat samples with *S. aureus* with the same molecular cluster.

Conclusions

In conclusion, presence of *S. aureus* in examined samples which was accompanied with the high incidence of resistance toward diverse classes of antibiotic agents and also dissimilar antibiotic resistance genes and virulence factors was reported in the current survey. High incidence of resistance of *S. aureus* bacteria toward penicillin, tetracycline, cephalothin, ciprofloxacin, erythromycin, mupirocin, clindamycin and trimethoprim/sulfamethoxazole which was assisted with attendance of *mecA*, *tetK*, *tetM*, *aacA-D*, *msrA* and *ermA* antibiotic resistance genes may pose an imperative menace regarding the role of raw or undercooked chicken meat consumption on transmission of antibiotic-

resistant *S. aureus*. Incidence of resistance toward human-based antibiotics can indirectly show the origin of *S. aureus* isolates. It seems that penicillin, tetracycline, cephalothin, ciprofloxacin, erythromycin, mupirocin, clindamycin and trimethoprim/sulfamethoxazole are not effective therapeutic agents in the cases of *S. aureus* foodborne diseases in Iran. Simultaneous presence of one or more antibiotic resistance genes and virulence factors in the antibiotic-resistant *S. aureus* bacteria specify an imperative public health threat about the consumption of raw or undercooked chicken meat samples. Thirty-six *S. aureus* strains were classified into the 9 diverse genetic clusters according to the ERIC-PCR. *S. aureus* strains with similar genetic cluster had similar phenotypic and genotypic properties of antibiotic resistance and distribution of virulence markers. Thus, they may have the same origin. Poultry slaughterhouses can be severely contaminated with foodborne pathogens, 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 chicken 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 chicken meat to human population. Our research highlights the importance of control the antibiotic susceptibility of *S. aureus* in the foodstuffs such as food producing animals, retail foods, and even human beings, and these 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*. However, supplementary surveys are essential to determine more epidemiological features of the *S. aureus* bacteria in raw chicken meat.

Materials And Methods

Samples and bacteria isolation and identification

From March 2019 to November 2019, a total of 36 *S. aureus* strains were isolated from 300 raw chicken meat samples collected from Isfahan, Iran. Isolation of S. aureus was performed according to method described by F Safarpoor Dehkordi, H Gandomi, A Akhondzadeh Basti, A Misaghi and E Rahimi [6]. Briefly, 25 g of each collected samples were blended with 225 mL of buffered peptone water (Merck, Germany). At that moment, solutions were homogenized using Stomacher (Interscience, Saint-Nom, France). At that time, five milliliters 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. At that moment, 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. Black shiny colonies enclosed with significant zones were identified using biochemical tests including Gram staining, oxidase test, catalase activity, resistance to bacitracin (0.04 U), coagulated test (rabbit plasma), urease activity, glucose O/F test, voges-proskaver (Merck, Germany) test, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, mannitol fermentation, hemolysis activity on blood agar (Merck, Germany) and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests [6].

Phenotypic evaluation of antibiotic resistance

Patterns of antibiotic resistance of the *S. aureus* bacteria was assessed using the simple disk diffusion according to the Kirby-Baur technique. The Mueller–Hinton agar (Merck, Germany) was used for this goal. Susceptibility of *S. aureus* isolates were examined toward 11 diverse antibiotic disks (Oxoid, UK) including penicillin (10 µg/disk), trimethoprim/sulfamethoxazole (25 µg/disk), cephalothin (30 µg/disk), tetracycline (10 µg/disk), ciprofloxacin (10 µg/disk), nitrofurantoin (300 µg/disk), azithromycin (15 µg/disk), rifampin (5 µg/disk) and erythromycin (15 µg/disk). clindamycin (10 µg/disk) and mupirocin (5 µg/disk). Instructions of the Clinical and Laboratory Standards Institute [64] was used to assess the susceptibility of bacteria toward antibiotic agents. The plates containing the discs were allowed to stand for at least 30 min before incubated at 37 °C for 24 h. The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standards [64]. *Staphylococcus aureus* ATCC 25923 was used as quality control organism in antimicrobial susceptibility determination.

Genotypic assessment of antibiotic resistance and virulence factors

S. aureus isolates were sub-cultured on TSB media (Merck, Germany) and further incubated for 48 h at 37 ^{o}C. Genomic DNA was extracted from bacterial colonies using the DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany) according to manufacturer's instruction. Purity (A260/A280) and concentration of extracted DNA were then checked (NanoDrop, Thermo Scientific, Waltham, MA, USA). The truth of the DNA was assessed on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany). Table 1 represents the list of primers and PCR conditions used for amplification of antibiotic resistance genes and virulence factors in the *S. aureus* bacteria isolated from raw chicken meat [5, 8, 65, 66]. A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. 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 systems (Grade GB004, Jencons PLC, London, UK) was applied for analysis of images.

Table 1

Target genes and PCR circumstances used for detection of antibiotic resistance genes and virulence factors in the *S. aureus* bacteria isolated from raw chicken meat (5,12,67,68).

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50 µL)
AacA-D	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227	1 cycle: 94 ^{0C} ——— - 5 min. 25 cycle: 94 ^{0C} ——— - 60 s 55 ^{0C} ——— - 70 s 72 ^{0C} ——— - 60 s 1 cycle: 72 ^{0C} ——— - 10 min	5 µL PCR buffer 10X 1.5 mM $Mgcl_2$ 200 µM dNTP (Thermo Fisher Scientific, St. Leon-Rot, Germany) 0.5 µM of each primers 1.25 U Taq DNA polymerase (Thermo Fisher Scientific, St. Leon-Rot, Germany) 2.5 µL DNA template
ermA	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190		
ermC	F: AATCGTCAATTCCTGCATGT R: TAATCGTGGAATACGGGTTTG	299		
tetK	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360		
vatC	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGCAGTACCGGATTTGC	467		
tetM	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	1 cycle: 94 ^{0C} ——— - 6 min. 34 cycle: 95 ^{0C} ——— - 50 s 55 ^{0C} ——— - 70 s 72 ^{0C} ——— - 60 s 1 cycle: 72 ^{0C} ——— - 8 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 200 µM dNTP 0.5 µM of each primers 1.5 U Taq DNA polymerase 5 µL DNA template
vatA	F: TGGTCCCGGAACAACATTTAT R: TCCACCGACAATAGAATAGGG	268		
msrA	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	1 cycle: 94 ^{0C} ——— - 6 min. 34 cycle: 95 ^{0C} ——— - 60 s 50 ^{0C} ——— - 70 s 72 ^{0C} ——— - 70 s 1 cycle: 72 ^{0C} ——— - 8 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP 0.75 µM of each primers 1.5 U Taq DNA polymerase 3 µL DNA template
vatB	F: GCTGCGAATTCAGTTGTTACA R: CTGACCAATCCCACCATTTTA	136	1 cycle: 94 ^{0C} ——— - 6 min. 35 cycle: 95 ^{0C} ——— - 50 s 55 ^{0C} ——— - 70 s 72 ^{0C} ——— - 80 s 1 cycle: 72 ^{0C} ——— - 10 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP 0.75 µM of each primers 1.5 U Taq DNA polymerase 3 µL DNA template

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50 µL)
linA	F: GGTGGCTGGGGGGTAGATGTATTAACTGG R: GCTTCTTTTGAAATACATGGTATTTTTCGA	323	1 cycle: 94 ^{0C} _____ - 6 min. 30 cycle: 95 ^{0C} _____ - 60 s 57 ^{0C} _____ - 60 s 72 ^{0C} _____ - 60 s 1 cycle: 72 ^{0C} _____ - 10 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP 0.75 µM of each primers 1.5 U Taq DNA polymerase 3 µL DNA template
mecA	F: ACGAGTAGATGCTCAATATAA R: CTTAGTTCTTTAGCGATTGC	293	1 cycle: 94 ^{0C} _____ - 3 min. 30 cycle: 94 ^{0C} _____ - 30 s 60 ^{0C} _____ - 30 s 72 ^{0C} _____ - 30 s 1 cycle: 72 ^{0C} _____ - 10 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP 0.75 µM of each primers 1.5 U Taq DNA polymerase 3 µL DNA template
msrB	F: TATGATATCCATAATAATTATCCAATC R: AAGTTATATCATGAATAGATTGTCCTGTT	595	1 cycle: 94 ^{0C} _____ - 2 min. 25 cycle: 94 ^{0C} _____ - 60 s 50 ^{0C} _____ - 60 s 72 ^{0C} _____ - 90 s 1 cycle: 72 ^{0C} _____ - 8 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP 0.75 µM of each primers 1.5 U Taq DNA polymerase 3 µL DNA template
clfA	F: GGCTTCAGTGCTTGTAGG R: TTTTCAGGGTCAATATAAGC	980	1 cycle: 94 ^{0C} _____ - 4 min. 35 cycle: 94 ^{0C} _____ - 1 min 57 ^{0C} _____ - 1 min 72 ^{0C} _____ - 1 min 1 cycle: 72 ^{0C} _____ - 5 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 5 µL DNA template

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50 µL)
coa	F: CGAGACCAAGATTCAACAAG R: AAAGAAAACCACTCACATCA	970	1 cycle: 95 ^{0C} —— - 2 min. 30 cycle: 95 ^{0C} —— - 30 s 58 ^{0C} —— - 2 min 72 ^{0C} —— - 4 min 1 cycle: 72 ^{0C} —— - 7 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 5 µL DNA template
tsst-1	F: ATGGCAGCATCAGCTTGATA R: TTTCCAATAACCACCCGTTT	350	1 cycle: 94 ^{0C} —— - 6 min. 30 cycle: 94 ^{0C} —— - 2 min 55 ^{0C} —— - 2 min 72 ^{0C} —— - 1 min 1 cycle: 72 ^{0C} —— - 8 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 5 µL DNA template
etA	F: CTAGTGCATTTGTTATTCAA R: TGCATTGACACCATAGTACT	119		
etB	F: ACGGCTATATACATTCAATT R: TCCATCGATAATATACCTAA	200		

Molecular typing

DNA samples were amplified by PCR for the repetitive element sequence using the primer ERIC-PCR: ERIC1R: 5'-ATGTAAGCTCCTGGGGATTCAC-3', ERIC2: 5'-AAGTAAGTGACTGGGGTGAGC-3'[67, 68]. Electrophoretic patterns were analyzed either visually or by using the Bionumerics software (Applied Maths, Sint-Martems-Latem, Belgium). The BioNumerics analysis was performed using the Dice coefficient and the unweighted pair group method of averages (UPGMA) with a 1% tolerance limit and 1% optimization. Isolates that clustered with ≥ 80% similarity were considered to belong to the same ERIC type, respectively.

Statistical analysis

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

Declarations

Ethics approval and consent to participate

The research was extracted from the Ph.D thesis in the field of Microbiology and was ethically approved by the Council of Research of the Faculty of Basic Science, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran (Consent Ref Number IR.IAU.SHK.REC.1398.051). Verification of this research project and the licenses related to sampling process were approved by the Prof. Hassan Momtaz (Approval Ref Number MIC201946).

Consent for publication

Not applicable.

Availability of data and materials

All data analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HM, and SK carried out the molecular genetic studies, participated in the primers sequence alignment and drafted the manuscript. NF and SK carried out the sampling and culture method. HM and NF participated in the design of the study, performed the statistical analysis and writing the manuscript. All authors read and approved the final manuscript.

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Figures

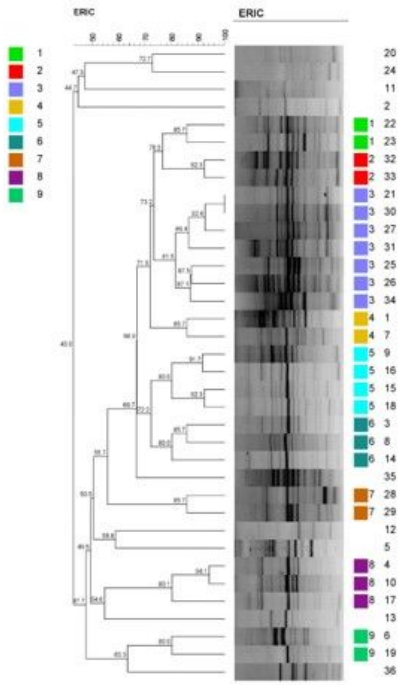

Figure 1

ERIC-based molecular typing of S. aureus strains isolated from chicken meat samples.