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Prevalence and Molecular Characterization of Enterotoxin- and Antibiotic Resistance-Encoding Genes in the Methicillin-resistant *Staphylococcus aureus* Recovered From Poultry Meat

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ENTEROTOXIGENIC methicillin-resistant *Staphylococcus aureus* (MRSA) is considered as one of the common foodborne pathogens. The existing research was performed to investigate the distribution of enterotoxigenic and antibiotic resistance genes of MRSA bacteria that are isolated from raw poultry meat samples. Two-hundred and sixty poultry meat samples were cultured and MRSA bacteria were recognized using cefoxitin and oxacillin susceptibility test. Antibiotic resistance and enterotoxigenic gene profiles were studied using PCR test. Prevalence of MRSA amongst poultry samples was 5%. Chicken (12%) had the highest prevalence rate, while ostrich (1.66%) had the lowest. Total distribution of *sea*, *seb*, *sec*, *sed* and *see* enterotoxin encoding genes were 50%, 25%, 8.33%, 75% and 8.33%, respectively. *BlaZ* (100%), *aacA-D* (58.33%), *tetK* (58.33%), *msrA* (58.33%) and *dfrA1* (50%) were the most regularly detected antibiotic resistance-encoding genes. Concurrent presence of enterotoxins and antibiotic resistance-encoding genes in MRSA bacteria stipulates important public health matters regarding the consumption of contaminated chicken, turkey, quail and ostrich meat.

Keywords : Methicillin-resistant *Staphylococcus aureus*, Enterotoxigenic gene profile, Antibiotic resistance genes, Raw poultry meat.

Introduction

Poultry meat is very good source of proteins, numerous of vitamins (especially B12) and minerals. Human involvement in the poultry meat inspection and purchase augmented the potential of microbial existence and foodborne diseases [1-5]. There were numerous imperative kinds of foodborne bacteria responsible for diverse foodborne disorders [2, 3, 5-18].

Staphylococcus aureus (*S. aureus*) is an imperative bacterium resident in the skin and respiratory system [4, 19-21]. The bacterium is accountable for clinical infections and foodborne diseases globally [4, 19-21]. Foodborne diseases related to *S. aureus* are mostly familiar with weakness, nausea, abdominal cramps, vomiting, and rarely diarrhea with the occurrence of toxic shock syndrome (TSS) in some cases [4, 19-21]. The presence of multiple enterotoxins is the exact reason for foodborne disorders by this bacterium [22, 23]. Staphylococcal enterotoxins (SEs) are low-molecular-weight and single-chain proteins with boost resistance against high temperature and stomach enzymes [22, 23]. They are classified to twenty-three kinds. SEA, SEB, SEC, SED and SEE are the most imperative types with advanced clinical impacts [22, 23].

Boost prevalence of antibiotic resistance of *S. aureus* bacteria is an emerging issue both in hospital environment and food systems. Currently, methicillin-resistant *S. aureus* (MRSA) has developed a considerate issue in foodstuff and healthcare [4, 19-21]. MRSA bacteria are characteristically resistant against numerous kinds of antibiotics [4, 19-21]. Reports exhibited

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the boost presence of *rpoB*, *gyrA*, *blaZ*, *aacA-D*, and *mecA*, *grlA tetK*, *dfrA*, *tetM*, *msrA*, *ermA* and *cat1* antibiotic resistance genes in MRSA bacteria [20, 24]. Mentioned genes are responsible for the occurrence of resistance against specific types of antibiotics.

Rendering MRSA's high importance, the current survey was performed to assess the prevalence and distribution of enterotoxin and antibiotic resistance-encoding genes amongst MRSA bacteria recovered from poultry meat.

Materials and Methods

Samples

From May to August 2018, two-hundred and forty numerous kinds of raw poultry meat samples including chicken (n= 50), turkey (n= 60), quail (n= 70) and ostrich (n= 60) were arbitrarily collected from the shopping centers of the Tehran province, Iran. Samples (100 g of each from the femur muscle) were closely transferred to the laboratory using refrigerator. To diminish surface contamination of samples, the external surface of samples were cleaned with alcohol (70%, Merck, Germany).

Bacterial isolation

Isolation and identification of *S. aureus* was performed using the technique designated beforehand [19, 20]. Firstly, 225 ml of buffered peptone water (Merck, Germany) was added to 25 g of samples and homogenized in a Stomacher Bagmixer 400W (Interscience, Saint-Nom, France) for two min. Trypticase Soy Broth (TSB, Merck, Germany) supplemented with 10% NaCl and 1% sodium pyruvate and Baird-Parker agar supplemented with egg yolk tellurite emulsion (Merck, Germany) were then applied. The samples were enriched in the broth and incubated at 37°C for 18h then the enrichment was plated on Baird-Parker agar and incubated for 24h. The bacteria were identified using biochemical tests such as catalase, mannitol, DNAse, coagulase and hemolysis [19, 20].

MRSA identification

MRSA bacteria were recognized using cefoxitin (30 µg) and oxacillin (1 µg) susceptibility test [25] and *mecA* detection [20]. Principles of Clinical and Laboratory Standards Institute (CLSI) was applied [25]. Furthermore, presence of the *mecA* gene was examined in MRSA bacteria identified in previous stage [19]. Overnight culture of MRSA bacteria in Tryptic Soy Broth media (TSB, Merck, Germany) was used for DNA extraction according to the guidelines of kit (Thermo Fisher Scientific, Germany). Purity of extracted DNA was examined using NanoDrop (NanoDrop, Thermo Scientific, Waltham, MA, USA). Eppendorf Mastercycler (Hamburg, Germany) device was applied in PCR.

PCR-based detection of enterotoxigenic and antibiotic resistance genes

Table 1 displays the primers and PCR circumstances applied for amplification of enterotoxigenic and antibiotic resistance genes [23]. All runs included a negative DNA control of sterile PCR grade water (Thermo Fisher Scientific, Germany) and positive DNA control consisting of positive DNA of each target gene. Ten microliters of PCR products were examined by electrophoresis in a 2% agarose gel in 1× TBE buffer at 90 V for 30-40 min, stained with SYBR Green (Thermo Fisher Scientific, Germany). MRSA (BAA 2313) and PCR-grade water (Thermo Fisher Scientific, Germany) were used as positive and negative controls, respectively.

Statistical analysis

Data were examined by the SPSS 25.0 software (Chicago, USA) with application of Chi-square test and Fisher's exact tests. P value <0.05 was considered as statistically significant level.

Results

Prevalence of MRSA

Table 2 displays the prevalence of MRSA amongst examined samples. Prevalence of MRSA amongst poultry samples was 5%. Chicken (12%) had the highest prevalence rate, while ostrich (1.66%) had the lowest. Arithmetically noteworthy variance was perceived between kind of samples and prevalence of MRSA bacteria (P <0.05).

Distribution of enterotoxin-encoding genes

Table 3 displays the distribution of enterotoxin encoding genes amongst MRSA bacteria that are isolated from the numerous kinds of raw poultry meat samples. We found that the total distribution of *sea*, *seb*, *sec*, *sed* and *see* enterotoxin encoding genes were 50%, 25%, 8.33%, 75% and 8.33%, respectively. MRSA bacteria isolated from chicken meat samples harbored the highest distribution of enterotoxin encoding genes, while those of ostrich had the minimum. MRSA bacteria isolated from raw ostrich meat samples were only positive for *sea* and *sed* enterotoxin encoding genes. Arithmetically noteworthy variance was perceived in the prevalence of enterotoxin encoding gene between diverse kinds of raw poultry meat samples (P <0.05).

TABLE 1. Target genes, oligonucleotide primers and PCR conditions applied for detection of antibiotic resistance and enterotoxin encoding genes in the MRSA bacteria isolated from poultry meat samples [23, 24].

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
AacA-D	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227	1 cycle: 0C ---------- 5 min. 94 25 cycle: 0C ---------- 60 s 94 0C ---------- 70 s 55 0C ---------- 60 s 72 1 cycle: 0C ---------- 10 min 72	5 µL PCR buffer 10X 1.5 mM Mgcl, 200 µM dNTP 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase 2.5 µL DNA template
ermA	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190		
tetK	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360		
tetM	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	1 cycle: 0C ---------- 6 min. 94 34 cycle: 0C ---------- 50 s 95 0C ---------- 70 s 55 0C ---------- 60 s 72 1 cycle: 0C ---------- 8 min 72	5 µL PCR buffer 10X 2 mM Mgcl, 200 µM dNTP 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase 5 µL DNA template
msrA	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	1 cycle: 0C ---------- 6 min. 94 34 cycle: 0C ---------- 60 s 95 0C ---------- 70 s 50 0C ---------- 70 s 72 1 cycle: 0C ---------- 8 min 72	5 µL PCR buffer 10X 2 mM Mgcl, 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template
blaZ	F: ACTTCAACACCTGCTGCTTTC R: TGACCACTTTTATCA CAACC	490	1 cycle: 0C ---------- 5 min. 94 30 cycle: 0C ---------- 20 s 94 0C ---------- 30 s 60 0C ---------- 90 s 72 1 cycle: 0C ---------- 5 min 72	5 µL PCR buffer 10X 2 mM Mgcl, 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
cat1	F: AGTTGCTCAATGTACCTATAACC R: TTGTAATTCATTAAGCATTCTGCC	547	1 cycle: 0C ---------- 8 min. 94 32 cycle: 0C ---------- 60 s 95 0C ---------- 70 s 55 0C ---------- 2 min 72 1 cycle: 0C ---------- 8 min 72	5 µL PCR buffer 10X 2 mM Mgcl, 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template

TABLE 1. Cont.

gyrA	F: AATGAACAAGGTATGACACC R: TACGCGCTTCAGTATAACGC	223	1 cycle: 0C ---------- 10 min. 94 25 cycle: 0C ---------- 20 s 94 0C ---------- 20 s 52 0C ---------- 50 s 72 1 cycle: 0C ---------- 5 min 72	5 µL PCR buffer 10X 2 mM Mgcl, 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
grlA	F: ACTTGAAGATGTTTTAGGTGAT R: TTAGG AAATCTTGATGGCAA	459		
dfrA	F: CTCACGATAAACAAAGAGTCA R: CAATCATTGCTTCGTATAACG	201	1 cycle: 0C ---------- 2 min. 94 30 cycle: 0C ---------- 60 s 94 0C ---------- 60 s 50 0C ---------- 60 s 72 1 cycle: 0C ---------- 5 min 72	5 µL PCR buffer 10X 2 mM Mgcl, 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
rpoB	F: ACCGTCGTTTACGTTCTGTA R: TCAGTGATAGCATGTGTATC	460	1 cycle: 0C ---------- 5 min. 94 32 cycle: 0C ---------- 60 s 94 0C ---------- 45 s 56 0C ---------- 60 s 72 1 cycle: 0C ---------- 10 min 72	5 µL PCR buffer 10X 2 mM Mgcl, 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
Sea	F: TTGGAAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	120	1 cycle: 0C ---------- 2 min. 94 30 cycles: 0C ---------- 120 s 94 0C ---------- 120 s 55 0C ---------- 60 s 72 1 cycle: 0C ---------- 8 min 72	5 µL PCR buffer 10X 2 mM Mgcl, 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
Seb	F: TCGCATCAAACTGACAAACG R: GCAGGTACTCTATAAGTGCC	478		
Sec	F: GACATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCC	257		
Sed	F: CTAGTTTGGTAATATCTCCT R: TAATGCTATATCTTATAGGG	317		
See	F: AGGTTTTTTCACAGGTCATCC R: CTTTTTTTTCTTCGGTCAATC	209	1 cycle: 0C ---------- 2 min. 94 35 cycles: 0C ---------- 120 s 94 0C ---------- 120 s 57 0C ---------- 60 s 72 1 cycle: 0C ---------- 8 min 72	5 µL PCR buffer 10X 2 mM Mgcl, 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template

TABLE 2. Prevalence of MRSA of poultry meat samples.

Samples of meat	Numbers of collected Samples	MRSA positive (%)
Chicken	50	6 (12)
Turkey	60	3 (5)
Quail	70	2 (2.85)
Ostrich	60	1 (1.66)
Total	240	12 (5)

TABLE 3. Distribution of enterotoxin-encoding genes amongst the MRSA isolates.

Samples (N of MRSA)	N (%) isolates harbor each enterotoxin gene				
	sea	***seb***	***sec***	***sed***	***see***
Chicken (6)	3 (50)	2 (33.33)	1 (16.66)	4 (66.66)	1 (16.66)
Turkey (3)	1 (33.33)	1 (33.33)	-	2 (66.66)	-
Quail (2)	1 (50)	-	-	2 (100)	-
Ostrich (1)	1 (100)	-	-	1 (100)	-
Total (12)	6 (50)	3 (25)	1 (8.33)	9 (75)	1 (8.33)

Antibiotic resistance-encoding genes

Table 4 displays the distribution of antibiotic resistance-encoding genes amongst the MRSA isolates. The gene encode resistance against penicillins (*blaZ*) (100%) had the maximum prevalence amongst the MRSA strain isolated from numerous kinds of raw poultry meat samples, while that of fluoroquinolones (*grlA*) (16.66%) had the minimum. Prevalence of *aacA-D* (58.33%), *tetK* (58.33%), *msrA* (58.33%) and *dfrA1* (50%) were also considerable. Prevalence of *rpoB* and *cat1* antibiotic resistance genes were 33.33% and 25%, respectively. Arithmetically noteworthy variance was perceived in the prevalence of antibiotic encoding gene between diverse kinds of raw poultry meat samples ($P < 0.05$).

Discussion

S. aureus is an imperative pathogen involved in gastrointestinal, foodborne and nosocomial infections resist to diverse antibiotic agents [26, 27]. Currently, enterotoxigenic MRSA has become a thoughtful issue not only in hospitals but also in food products [28-30].

The existing investigation was performed to examine the distribution of enterotoxigenic and antibiotic resistance genes of MRSA bacteria isolated from raw chicken, turkey, quail, and ostrich meat samples. MRSA›s total incidence was 5% (12 out of 240 samples), which was substantial. Moreover, our findings demonstrated a significant correlation between specific kinds of raw poultry meat and MRSA incidence. In other newly described investigations, a lower incidence of MRSA was reported in foodstuffs from Japan [31], Korea [32], Italy [33], and Netherlands [34]. Incidence of MRSA in samples examined in Brazil [35], Turkey [36], Egypt [37], Germany [38] and Denmark [39] was 21.72%, 30%, 40.80%, 71.50% and 52.00%, respectively. Similar records were also reported from Australia [40], United Kingdom [41], and United States [42].

We also perceived that majority of MRSA bacteria harbored some kinds of enterotoxigenic genes. Concurrent presence of two or more kinds of enterotoxigenic genes is supplementary imperative outcome of the existing research. *Sea* and *sed* were the most regularly detected enterotoxigenic genes amongst the MRSA bacteria isolated from raw poultry meat samples. Boost prevalence of *sea* enterotoxin gene displayed the considerable ability of MRSA bacteria to cause staphylococcal food poisoning. Otherwise, the gene for enterotoxin A was prevalent (50.00%) among bacteria, pointing to a boost risk of these isolates causing foodborne diseases. Besides the presence of the enterotoxin A gene, prevalence of the genes encodes enterotoxin D (*sed*) was 75%. *Sed* is measured as specific enterotoxin of the poultries. Its boost prevalence was also reported from Iran [22], Brazil [43] and China [44].

TABLE 4. Distribution of antibiotic resistance-encoding genes amongst the MRSA isolates.

Samples (N of MRSA)	Antibiotic resistance-encoding genes (%)											
	Aminoglycosides	Tetracyclines		Macrolides		Penicillins	Folate inhibitors	Fluoroquinolones		Ansamycins	Phenicols
	aacA-D	***tetK***	***tetM***	***msrA***	***ermA***	***blaZ***	***dfrA***	***gyrA***	***grlA***	***rpoB***	***cat1***
Chicken (6)	4 (66.66)	3 (50)	1 (16.66)	3 (50)	2 (33.33)	6 (100)	3 (50)	2 (33.33)	1 (16.66)	2 (33.33)	1 (16.66)
Turkey (3)	2 (66.66)	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	3 (100)	2 (66.66)	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)
Quail (2)	1 (50)	1 (50)	-	1 (50)	1 (50)	2 (100)	1 (50)	1 (50)	-	1 (50)	1 (50)
Ostrich (1)	-	1 (100)	-	1 (100)	-	1 (100)	-	-	-	-	-
Total (12)	7 (58.33)	7 (58.33)	9 (75)	7 (58.33)	4 (33.33)	12 (100)	6 (50)	4 (33.33)	2 (16.66)	4 (33.33)	3 (25)

The ending portion of the existing investigation attentive on distribution of antibiotic resistance-encoding genes. Significant prevalence of *msrA*, *blaZ*, *tetK*, *grlA*), aminoglycosides (*aacA-D*, and *dfrA1* was described in bacteria. Boost prevalence of these genes was also reported beforehand from food samples and also human clinical infections investigated in Iran [20, 24], Algeria [28], China [45] and Africa [46]. Significant prevalence of *blaZ*, *tetK* and *mecA* was recorded in poultries examined in South Africa [47]. Significant prevalence of *grlA*, *gyrA* and *mecA* was recorded in poultries examined in Egypt [48]. Simeoni *et al.* (2008) [49] recorded the significant prevalence of *erm*, *tet*, *bla* and *mec* genes (0-100%) in MRSA. Johler *et al.* (2011) [50] and Podkowik *et al.* (2012) [51] recorder significant prevalence of tetracycline-resistance and macrolide-resistance genes (4-60%) amongst diverse food samples. MRSA and other foodborne bacteria have high importance as foodborne pathogens amongst diverse kinds of food samples globally [52-72]. The present survey was limited to the low numbers of isolated bacteria in examined samples and also lack of the studying the distribution of virulence factors. However, high numbers of examined samples and their variety and also using multiple antibiotic resistance encoding genes are strength point of the survey.

Conclusion

We document the boost prevalence of the genes encode resistance toward the antibiotics and also enterotoxins amongst the MRSA isolated from raw poultry meat samples. Raw chicken meat samples had the maximum prevalence of MRSA bacteria. *Sed* and *sea* had the maximum distribution among tested enterotoxin genes. *BlaZ*, *aacA-D*, *tetK*, *msrA*, and *dfrA* had the highest prevalence. Some MRSA bacteria exhibited concurrent presence of multiple enterotoxin and antibiotic resistance encoding genes which had also boost clinical and epidemiological standing. The large portion of the control of antibiotic prescription for the cases of MRSA in food animals should be considered. Rendering the high prevalence of antibiotic resistance-encoding and enterotoxin-encoding genes, a comprehensive survey should address to understand other impacts of MRSA in poultry meat samples.

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Conflict of interest

The authors declared that no conflict of interest.

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