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One-year prevalence of antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* recovered from raw meat

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Abstract. Food with animal origins and especially meat may play an important role in transmission of methicillin-resistant strains of *Staphylococcus aureus* to humans. The present investigation was carried out to determine the prevalence of MRSA in various types of Iranian meat samples as well as study their antimicrobial resistance properties. Nine-hundred raw meat samples were collected during various months of the year. Samples were cultured and those that were MRSA-positive were subjected to the disk diffusion method to study the antibiotic resistance pattern. One-hundred and sixty out of 900 raw meat samples (17.7%) were positive for MRSA. Raw sheep meat samples had the highest (24.0%), while raw camel meat samples had the lowest (10%) prevalence of MRSA. Samples which were collected in June, July, August, September and June months had the highest prevalence of MRSA. Bacterial strains were also resistant to ampicillin (100%), penicillin G (100%), gatifloxacin (96.8%), ceftriaxone (80%) and oxacillin (76.2%) antibiotics. We found that only one isolate was resistant to all tested antimicrobial agents. Contaminated meat samples are potential risk factor for transmission of MRSA. Thoughtful antibiotics prescription, control the hygienic quality of meat inspections and increase the hygienic status of butchers and slaughterhouses can decrease the prevalence of MRSA in meat.

INTRODUCTION

High value of red meat as a complete source of minerals, vitamins, proteins and lipids (especially Saturated Fatty Acids (SFA)) increased its nutritional value in comparison to vegetables (Givens., 2010 and McAfee *et al.*, 2010). A lot of people use from red meat in their meals. Of the various types of meat, beef had the highest consumption rate in Iran, followed by sheep, goat and camel. However, the principles of meat hygiene and inspection are not observe in some Iranian slaughterhouses and butchers (Hemmatinezhad *et al.*, 2015; Momtaz *et al.*, 2013; Rahimi *et al.*, 2014). Therefore, there is a dangerous possibility of food poisoning due to consumption of contaminated meat.

Staphylococcus aureus is associated with nosocomial and community-acquired infections all- worldwide (Kadariya *et al.*, 2014). It is also an important foodborne pathogen involved in severe gastrointestinal disorders. It has been estimated that the

S. aureus is a causative agent for 241,000 foodborne illnesses per year in the United States (Scallan *et al*., 2011). Meat is one of the most important food stuffs related to the Staphylococcal foodborne diseases (Hanson *et al*., 2011; Lim *et al*., 2010; Pu *et al*., 2009; Weese *et al*., 2010). Staphylococcal nosocomial acquired infections are primarily related to the emergence of antibiotic resistance (De Boer *et al*., 2009; Hanson *et al*., 2011; Johnson, 2011; Pu *et al*., 2009; Weese *et al*., 2010). The bacterium has the highest levels of resistant against methicillin and other types of beta-lactams antimicrobial agents (De Boer *et al*., 2009). Epidemiological investigations have revealed that the prevalence of antibiotic resistance in the *S. aureus* strains of meat samples against commonly used groups of antibiotics including aminoglycosides, quinolones, cephalosporins, macrolides, sulfonamides, tetracycline and fluoroquinolones were 10 to 100%. It has been documented that about 50% of strains of this bacterium were methicillin-resistant *S. aureus* (MRSA) (Johnson, 2011; Shen *et al*., 2013). In a Klevens *et al.* (2007) showed that an annual prevalence of MRSA infections and also its mortality rate in the United States were 94,000 and 20%, respectively (Klevens *et al*., 2007). In addition, it has been shown that the methicillin-resistant strains of this bacterium had a higher prevalence of resistance against other types of antibiotics than methicillin-sensitive strains (Abdalrahman *et al*., 2015; Hasani *et al*., 2013).

Unfortunately, basic principles of good hygiene have not been observed in most Iranian slaughterhouses. In addition, the levels of good meat inspection are low in majority of Iranian slaughterhouses. During slaughtering of MRSA-positive animals, contamination of carcasses with MRSA may occur and consequently the meat of these animals may get contaminated. Therefore, from the microbiological, clinical and epidemiological prospective, it is important to know the exact hygienic conditions of raw meats especially for MRSA. The present study was carried out in order to study the antibiotic resistance pattern of MRSA strains recovered from Iranian beef, sheep, goat and camel meat samples collected from one-year period of time.

MATERIALS AND METHODS

Ethical consideration

The present research project was approved by the ethical committee of Young Researchers and Elites Club (Number YREC 174711). A legal consent was signed between the University and authors of the project. Verification of this research project and the licenses related to sampling process were also approved by Prof. Ebrahim Rahimi (Approval Ref Number Vet 3578914).

Samples collection and MRSA identification

Overall 900 raw meat samples including beef (n=250), sheep (n=225), goat (n=225) and camel (n=200) were purchased from farm butchers of several geographic regions of Iran, from January 2014 to January 2015. The carcasses were all clinically healthy and the meat samples showed normal physical characteristics. Before collecting meat samples, the external surfaces were disinfected with 70% alcohol to minimize surface contamination. Separate 10-g femur muscle samples were collected using sterile scissors and tissue forceps. Samples were collected under sterile hygienic conditions and were immediately transported to the laboratory at 4°C in a cooler with ice packs.

Twenty-five grams of meat samples was inoculated on Mueller–Hinton broth (MHB, Merck, Germany) supplemented with 6.5% NaCl and homogenized. The suspension was incubated for 16–20 h at 37°C. One milliliter of the enriched MHB media was added to 9 ml of phenol red mannitol broth containing ceftizoxime (5 µg/ml) and aztreonam (75 µg/ml) (PHMB) and incubated for 16–20 h at 37°C. The surface of the selective isolation medium MRSA ID was inoculated with a sterile loop. The plates were incubated for 24 h at 37°C (when the colonies were difficult to identify the incubation was protracted for another 24 h). Typical green colonies were primary known as MRSA. Five selected typical colonies per plate were subcultured

on Tryptone Soya Agar (TSA, Merck, Germany). Typical colonies were tested with the Staphytect Plus test (Oxoid), a latex agglutination test for the detection of clumping factor, Protein A and certain polysaccharides found in MRSA.

DNA extraction and *mecA* detection

All of the MRSA strains recovered from the culture-based technique have also been confirmed using the *mecA* gene amplification. PCR reactions (Klevens *et al.*, 2007) were performed in a final volume of 50 µL containing 5 µL 10 × buffer + $MgCl_2$, 2 mM dNTP, 2 unit Taq DNA polymerase, 100 ng genomic DNA as a template, and 25 picomole of each primer (*mecA*-F: 5'-TGGCTATCG TGTCACAATCG-3' and *mecA*-R: 5'-CTGG AACTTGTTGAGCAGAG-3'). PCR was performed using a thermal cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) under the following conditions: an initial denaturation for 1 minutes at 94°C and 30 cycles including 94°C for 30 s, 45°C for 30 s and 72°C 1 min, and a final extension at 72°C for 8 minutes. Fifteen microliters of PCR products were resolved on a 1.5% agarose gel containing 0.5 mg/ml of SYBR Green (Fermentas, Germany) in Tris–borate–EDTA buffer at 90 V for 20 min, also using suitable molecular weight markers. The products were examined under ultraviolet illumination. *S. aureus* ATCC 25923 and sterile distilled water (Merck, Germany) were used as a positive and negative controls in all PCR reactions.

Antimicrobial susceptibility testing of MRSA strains

Pattern of antimicrobial resistance was studied using the simple disk diffusion technique. The Mueller–Hinton agar (Merck, Germany) medium was used for this purpose. Antimicrobial resistance of the MRSA strains against 13 commonly used antibiotics was determined using the instruction of Clinical and Laboratory Standards Institute guidelines (Wikler., 2006). Susceptibility of MRSA isolates were tested against ampicillin (10 u/disk), ceftriaxone (30 µg/disk), amoxicillin-clavulanic acid (30 u/disk), lincomycin (2 µg/disk), gatifloxacin (5 µg/disk), tetracycline (30 µg/disk), minocycline (30 µg/disk), cotrimoxazole (30 µg/disk), clindamycin (2 µg/disk), penicillin G (10 u/disk), oxacillin (1 µg/disk), erythromycin (15 µg/disk) and azithromycin (15 µg/disk) antimicrobial agents (Oxoid, UK). The plates containing the discs were allowed to stand for at least 30 min before incubated at 35°C for 24 h. The diameter of the zone of inhibition produced by each antimicrobial disc was measured and interpreted using the CLSI zone diameter interpretative standards (Wikler., 2006). *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as quality control organism in antimicrobial susceptibility determination.

Statistical analysis

The results were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) for analysis. Statistical analysis was performed using SPSS/18.0 software (SPSS Inc., Chicago, IL) for significant relationship between the prevalence of bacteria and antimicrobial resistance pattern. The chi-square test and Fisher's exact test analysis were performed in this study. Statistical significance was regarded at a P value < 0.05.

RESULTS

Total distribution of MRSA in raw meat samples

The results of the present investigation revealed the considerable prevalence of MRSA in the raw meat samples. Table 1 represents the total distribution of MRSA in

Table 1. Total distribution of methicillin-resistant *Staphylococcus aureus* strains in various types of raw meat samples

Types of samples	No. samples collected	No. MRSA (%)
Beef	250	40 (16)
Sheep	225	54 (24)
Goat	225	46 (20.4)
Camel	200	20 (10)
Total	900	160 (17.7)

various types of raw meat samples. We found that 160 out of 900 raw meat samples (17.77%) were positive for MRSA. Raw sheep meat samples had the highest (24%), while raw camel meat samples had the lowest (10%) prevalence of MRSA. All of the MRSA had *mecA* gene in the PCR amplification (Figure 1). Significant statistical differences were seen for the prevalence of MRSA strains between raw sheep and camel (P=0.015) and also raw sheep and beef (P=0.026) meat samples.

Monthly distribution of MRSA

Figure 2 shows the monthly distribution of MRSA in various types of raw meat samples. Results revealed that the meat samples which were collected in July, August, September and June months had the highest prevalence of MRSA. Significant statistical differences were seen for the prevalence of MRSA strains between July and May (P=0.015) and July and October months (P=0.026). Statistically significant differences were seen for the prevalence of MRSA strains between cold and hot months of the year (P <0.05).

Antimicrobial resistance properties of MRSA

Table 2 represents the Antimicrobial resistance pattern of the MRSA strains isolates from various types of raw meat samples. MRSA strains of our research harbored the highest levels of resistance against ampicillin (100%), penicillin G (100%), gatifloxacin (96.87%), ceftriaxone (80%) and oxacillin (76.25%) antimicrobial

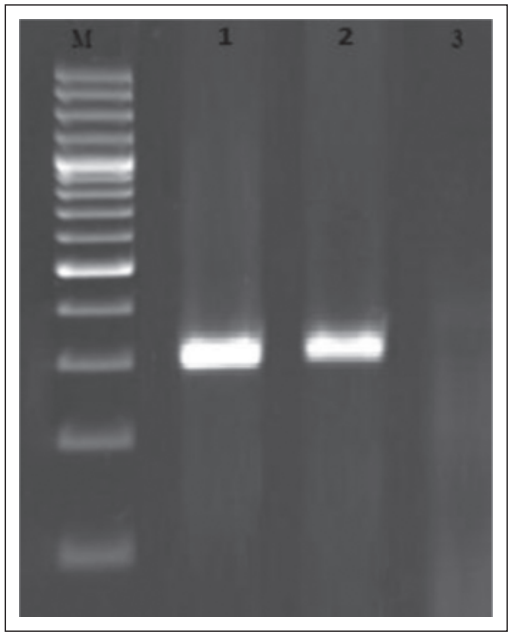

Figure 1. PCR gel electrophoresis of *mecA* gene of the MRSA strains of meat samples. M: 100 bp ladder, 1: Positive sample (310 bp), 2: Positive control and 3: Negative control.

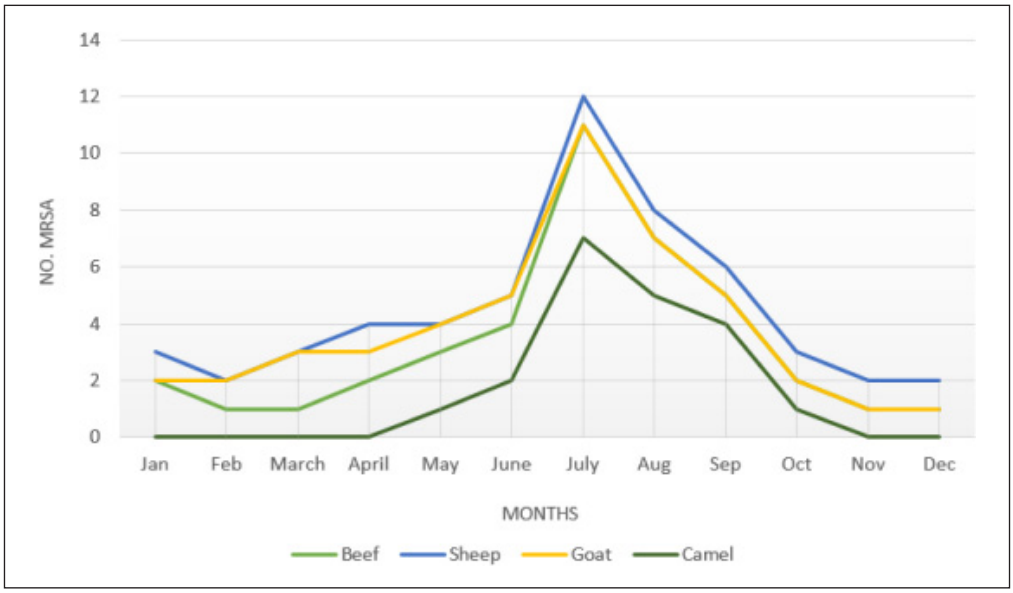

Figure 2. Monthly distribution of MRSA strains isolates from various types of raw meat samples.

Table 2. Prevalence of antibiotic resistance in the methicillin-resistant *Staphylococcus aureus* isolated from various types of raw meat samples

Antibiotic agents	Prevalence of resistance (%)				
	Beef (40*)	Sheep (54)	Goat (46)	Camel (20)	Total (160)
Ampicillin	40 (100)	54 (100)	46 (100)	20 (100)	160 (100)
Ceftriaxone	37 (92.5)	47 (87.0)	33 (71.7)	11 (55)	128 (80)
Amoxicillin-Clavulanic Acid	28 (70)	19 (35.1)	13 (28.2)	4 (20)	64 (50)
Lincomycin	22 (55)	37 (68.5)	30 (65.2)	9 (42.8)	98 (61.2)
Tetracycline	36 (90)	28 (51.8)	21 (45.6)	6 (30)	88 (55)
Gatifloxacin	40 (100)	54 (100)	45 (97.8)	16 (80)	155 (96.8)
Minocycline	35 (87.5)	26 (48.1)	19 (41.3)	5 (25)	82 (51.2)
Cotrimoxazole	34 (85)	25 (46.2)	18 (39.1)	3 (15)	73 (45.6)
Clindamycin	22 (55)	34 (62.9)	26 (48.1)	5 (25)	87 (54.3)
Azithromycin	36 (90)	26 (48.1)	18 (39.1)	4 (20)	77 (48.1)
Erythromycin	20 (50)	27 (50)	12 (26.0)	1 (5)	60 (37.5)
Oxacillin	36 (90)	45 (83.3)	31 (67.3)	10 (50)	122 (76.2)
Penicillin G	40 (100)	54 (100)	46 (100)	20 (100)	160 (100)

*Number of positive MRSA strains.

Table 3. Combined resistance pattern of methicillin-resistant *Staphylococcus aureus* strains isolated from various types of raw meat samples

Antimicrobial agents	Prevalence of combined resistance (%)				
	Beef (40*)	Sheep (54)	Goat (46)	Camel (20)	Total (160)
AMP	40 (100)	54 (100)	46 (100)	20 (100)	160 (100)
AMP+PG	39 (97.5)	51 (94.4)	44 (95.6)	18 (90)	152 (95)
AMP+PG+GFX	35 (87.5)	40 (74.0)	14 (30.4)	6 (30)	95 (59.3)
AMP+PG+GFX+CFX	33 (82.5)	40 (74.0)	7 (15.2)	4 (20)	84 (52.5)
AMP+PG+GFX+CFX+OX	32 (80)	17 (27.4)	9 (14.5)	4 (6.4)	62 (38.7)
AMP+PG+GFX+CFX+OX+LIN	21 (52.5)	16 (29.6)	9 (19.5)	4 (6.5)	50 (31.2)
AMP+PG+GFX+CFX+OX+LIN+TET	15 (37.5)	13 (24.0)	7 (15.2)	3 (15)	38 (23.7)
AMP+PG+GFX+CFX+OX+LIN+TET+CLN	12 (30)	6 (11.1)	3 (6.5)	1 (5)	22 (13.7)
AMP+PG+GFX+CFX+OX+LIN+TET+CLN+MIN	6 (15)	2 (3.7)	1 (2.1)	–	9 (5.6)
AMP+PG+GFX+CFX+OX+LIN+TET+CLN+MIN+AZT	3 (7.5)	1 (1.8)	–	–	4 (2.5)
AMP+PG+GFX+CFX+OX+LIN+TET+CLN+MIN+AZT+COT	2 (5)	–	–	–	2 (1.2)
AMP+PG+GFX+CFX+OX+LIN+TET+CLN+MIN+AZT+COT+AMCL	1 (2.5)	–	–	–	1 (0.6)
AMP+PG+GFX+CFX+OX+LIN+TET+CLN+MIN+AZT+COT+AMCL+ERT	1 (2.5)	–	–	–	1 (0.6)

*Number of positive MRSA strains.

agents. Strains which were recovered from the beef meat samples harbored the highest levels of resistance against all of the studied antimicrobial agents excluding lincomycin and clindamycin. Significant statistical differences were seen between the types of meat samples and for the prevalence of antibiotic resistance ($P < 0.05$).

Combined pattern resistance of MRSA

Table 3 shows the combined resistance pattern of the MRSA strains isolated from various types of meat samples. MRSA strains recovered from the beef meat samples had the highest levels of combined resistances, while those of camel had the lowest. All of the 160 MRSA strains were resistant to both

ampicillin and penicillin G. Only one isolate was resistant to all of the tested antimicrobial agents.

DISCUSSION

In the current investigation, we studied the prevalence of MRSA strain isolated from various types of raw meat samples as well as their antibiotic resistance pattern against commonly used antibiotics. MRSA contaminated a substantial proportion of samples from all types of meat samples (10-24%). Total prevalence of MRSA in beef, sheep, goat and camel meat samples were 16%, 24%, 20.44% and 10%, respectively. Low prevalence of MRSA in camel meat samples is may be due the high levels of hygiene in their slaughterhouses. The numbers of slaughtered camel are usually lower than those of beef, sheep and goat. Therefore, there is a lower risk for transmission of MRSA. Sheep and goat meat have the higher levels of pH (near to neutral pH) than beef (Sañudo., 2007). The survival of bacteria like *S. aureus* is, therefore, easier in sheep and goat meat samples. The results of Nnachi *et al.* (Nnachi *et al.*, 2014) revealed that meat handlers were the most effective carriers of MRSA. They showed that all of the goat meat handlers harbored the MRSA in their hands and half of them harbored the bacterium in their nasal canal. They reported that the prevalence of MRSA in the nasal and hand swabs of meat handlers were 42.9% and 41.7%, respectively. Our findings were different from those of United States (Pu *et al.*, 2009), Switzerland (Huber., 2010), Korea (Lim *et al.*, 2010) and Nigeria (Nnachi *et al.*, 2014). Bhargava *et al.* (2011) reported that the prevalence of MRSA in beef, chciken and turkey meat samples were 20.5%, 25.0% and 24.6%, respectively (Bhargava *et al.*, 2011). Findings of Febler *et al.* (2011) (Febler *et al.*, 2011) showed that MRSA strains was most prevalent in turkey (35.3%), followed by chicken (16.0%), veal (15.2%) pork (10.7%), and beef (10.6%) which was in contrast with our results. Variation in the results of different studies may be due to the fact that type of samples, number of samples collected, method of sampling and experiment and finally geographical and climate conditions of area of samples collections may be different in each investigation.

Significant monthly distribution was found for the prevalence of MRSA strains in raw meat samples. *S. aureus* has a higher growth and surveillance in warmer conditions. In addition, observation of the individual and public health is harder during hot months. Similar results have been reported by Leekha *et al.* (2012) (Leekha *et al.*, 2012) and Klein *et al.* (2013) (Kleinet *et al.*, 2013).

Another part of our investigation was focused on antimicrobial resistance pattern of MRSA isolated from various types of meat samples. Our data showed the high prevalence of resistance against ampicillin (100%), penicillin G (100%), gatifloxacin (96.87%), ceftriaxone (80%) and oxacillin (76.25%) antibiotics. High prevalence of antibiotic resistance with respect to the considerable incidence of combined resistance in MRSA strains of Iranian meat samples showed highly irregular and unauthorized prescription of antibiotics in veterinary and medical fields. Several investigations have been done in this field all-around the world. Gundogan *et al.* (2005) (Gundogan *et al.*, 2005) reported that the *S. aureus* strains of meat samples had the high levels of resistance against penicillin G (53.8%), while the levels of resistance against erythromycin was low (7.5%). They showed that all strains were susceptible to vancomycin, sulbactam–ampicillin, ciprofloxacin and cefaperazone–sulbactam which was in contrast with our results. In a study which was conducted on Iranian chicken meat samples (Momtaz *et al.*, 2013), the *S. aureus* strains harbored the highest levels of resistance against tetracycline (97.5%), methicillin (75.6), sulfamethoxazol (31.7%), trimethoprim (31.7%), streptomycin (31.7%), gentamicin (29.2%), enrofloxacin (28.0%), ampicillin (26.8%), chloramphenicol (20.7%), and cephalothin (17.0%). Another similar study (Udo *et al.*, 2009) revealed that the prevalence of resistance against

penicillin G, tetracycline, erythromycin, clindamycin, trimethoprim, kanamycin, streptomycin and ciprofloxacin were 82.0%, 19.0%, 2.5%, 2.0%, 7.5%, 2.5%, 1.5% and 1.5%, respectively. Study the prevalence of multidrug resistant *S. aureus* in the meat and poultry samples of United States (Waters *et al*., 2011) showed complete and intermediate resistance of isolates against tetracycline, penicillin, ampicillin, and erythromycin. Shahrzad *et al*. (2012) (Shahraz *et al*., 2012) revealed that resistance rate of *S. aureus* strains of meat products against meticillin, erythromycin, penicillin G, cefazolin, ciprofloxasin, vacomycin and amoxiclave were 89%, 20.3%, 18.7%, 15.6%, 14%, 26.6% and 12.5%, respectively. Ombui *et al*. (2000) (Ombui *et al*., 2000) reported the high frequency of *S. aureus* resistance against lincomycin (67.7%), penicillin (66.7%) and cotrimoxazole (51%). They showed that 76% of isolates were susceptible to minocycline followed by erythromycin (57.3%). Ombui *et al*. (2000) (Ombui *et al*., 2000) showed that most of isolates (80.2%) were multiply resistant to between two and six antibiotics which was lower than our results. We found that the minimum levels of combined resistance were seen for ampicillin+ penicillin G+gatifloxacin+ceftriaxone+ oxacillin+lincomycin+tetracycline+ clindamycine in beef (30%), sheep (11.1%), goat (6.5%) and camel (5%) meat samples. As far as we know, there were no previously published data about the combined antibiotic resistance of MRSA in various types of raw meat samples. Abdalrahman *et al*. (2015) (Ombui *et al*., 2000) which was conducted on chicken and turkey meat samples MRSA strains had the higher prevalence of resistance than methicillin-sensitive strains. They also showed that the prevalence of MRSA resistance against azithromycin, ciprofloxacin, gentamicin, oxacillin, cefoxitin, tetracycline, vancomycin, doxycycline, trimethoprim/sulfamethoxazole, clindamycin, penicillin, ampicillin, kanamycin, erythromycin, rifampin and chloramphenicol were 100%, 33.33%, 0%, 100%, 100%, 0%, 0%, 0%, 91.70%, 58.30%, 100%, 100%, 91.7%, 100%, 0% and 0%, respectively which was lower than our results.

In conclusions, this is the first and the most extensive report of the presence of MRSA in beef, sheep, goat and camel meat samples with respect to study the distribution of antimicrobial resistance pattern. High prevalence of MRSA in sheep, marked monthly distribution with higher prevalence of bacteria in July, high levels of resistance against ampicillin, penicillin G, gatifloxacin, ceftriaxone and oxacillin and finally high prevalence of combined resistance are the most important finding of our study. Contaminated meat samples play an important role in the ecology of antimicrobial resistance of *S. aureus* in Iran. Besides of the cautious use of antibiotics in human and animals, monitoring the hygienic conditions of slaughterhouses and butchers can reduce the risk of resistant strains of MRSA food poisonings. In the current situation in Iran, prescription of erythromycin, cotrimoxazole and azithromycin can be effective for treatment of the cases of MRSA infections.

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