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Original Research Article

METHICILLIN RESISTANT *Staphylococcus aureus* (MRSA) IN CAMEL MEAT: PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY

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Abstract: This study is an initiative study intended to investigate the prevalence and antibiotic susceptibility of *Staphylococcus aureus* and MRSA obtained from fresh camel meat retailed in Al-Hasa, Saudi Arabia. The survey has been conducted for five months where fresh camel meat were microbiologically analyzed to isolate *S. aureus* using culture media and VITEK2 technique. The Identification of *S. aureus* was done by DNA expression of gene specific 16S rRNA, while mecA gene expression was recognized in the identified MRSA isolates using PCR. The recorded prevalence of *S. aureus* was 10.7 % (20/187) from raw camel meat. Three isolates out of twenty *S. aureus* isolates were confirmed to be MRSA. Susceptibility to 10 antimicrobials was estimated using the disc diffusion method. These results suggested that MRSA is found in camel meat retailed at Al-Hasa, Saudi Arabia. We recommend that, surveillance protocol should be embraced in meat safety and public health programs.

Key words: camel meat, *S. aureus*, MRSA, antimicrobial resistance

Introduction

Staphylococcus aureus is an important pathogen causing food-borne illness worldwide and causes serious diseases in human being (1). The main sources of infection could be the inadequate personnel hygiene, unhygienic handling and storage of food infected with staphylococci. Frequently associated foods are meat, dairy products, cream-filled bakery items and salads (2). Methicillin-resistant *S. aureus* (MRSA) appeared as a dangerous agent for different patients and especially in those with impaired immune system. MRSA strains are actually widespread human isolate but are unusual animal isolates (3). MRSA firstly recognized as nosocomial infection. Later in the community and livestock. It has a continuous and dynamic epidemiology. Human may acquire MRSA infection via contact with contaminated environment or contaminated marketed meat (4, 5). Resistance in MRSA is mediated by the gene mecA, which located on the chromosome in staphylococcal cassette chromosome mec (SCCmec), and this encodes penicillin-binding protein (PBP) 2a with a low affinity for beta-lactams (6).Transmission of MRSA from food

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to people causes a serious problem especially for the immunocompromised people. *S. aureus* was identified in 42 out of 176 raw meat (beef, poultry and pork) over a one-year survey (7) and they did not find any evidence indicating vancomycin, or methicillin-resistance. *S. aureus* also isolated from the wild animal carcasses (2.0% prevalence) and authors found no methicillin-resistance (8). Several studies in Saudi Arabia were conducted to investigate the prevalence, risk factors and genetic distribution of MRSA isolated from healthy and clinical human and animal cases (9-14). However, rare reports were recorded with regard to the prevalence of MRSA in food especially camel meat. To the best of our knowledge, few studies were conducted to isolate and identify MRSA from camel meat allover Arabian countries (15, 16).This study was done to investigate the MRSA prevalence in camel meat samples retailed to the public at Al-Hasa, Saudi Arabia. mec-A gene expression was carried out in identified *S. aureus* isolates using PCR. Antimicrobial resistance profile for the *S. aureus* isolates were further analyzed. This study is one of the very few studies regarding the prevalence and genetic characters of MRSA from camel meat in Saudi Arabia.

Materials and methods

All experiments followed the ethical and scientific principles adopted by King Faisal University, Saudi Arabia.

Collection of samples

A total of 187 minced camel meat samples were collected randomly from supermarkets in Al-Hasa province, Saudi Arabia in a 5 months period. The collected samples were taken directly in sterile polyethylene bags (icebox) to the Meat Hygiene laboratory at the Veterinary Public Health and Animal Husbandry Department, College of Veterinary Medicine, King Faisal University for the subsequent microbial culturing and identification.

Isolation and identification of coagulase positive S. aureus

For investigation of coagulase positive *S. aureus*, after sample digestion in a Stomacher® 400 Circulator, an inoculum of 0.1 ml of serial dilution 10^{-1}&10^{-2} was evenly surface distributed on Baird Parker agar base supported with egg yolk tellurite emulsion Agar (Oxoid CM0275) according to (17). After incubation at 37°C for 48 hours, counting of all typical colonies using colony counter was performed. For confirmation, five typical colonies were selected and transferred to brain heart infusion broth tubes (BHIB) (Oxoid CM1135) for subsequent culture and preservation. Presumptive colonies were transferred and subcultured on Mannitol Salt Agar (Oxoid CM0085) and then incubated for 24 hrs at 37°C. Gram stain, catalase, mannitol fermentation, DNAs and coagulase tests were applied on suspected colonies to identify coagulase *S. aureus* (18). In addition, VITEK 2-compact was used to identify and differentiate staphylococci species (BioMérieux, Rev 03, 2004).

Screening for MRSA

Screening of All *S. aureus* isolates for their methicillin resistance was done by the standard disc diffusion procedure described by Clinical and Laboratory Standards Institute (19) using cefoxitin (30 μg).

DNA Extraction

Staphylococcus aureus isolates were grown in BHIB at 37º C for 18 hours. The cells were harvested by centrifugation at 8000 rpm for 20 minutes at 4º C and washed twice by phosphate-buffered saline. The pellet was suspended in 1 ml of 10 mMTris-HCl (pH 8) containing lysozyme (2.5 mg/ml) and incubate at 37º C for 2 hours. Genomic DNA was extracted using QIAamp DNA Mini Kit (QIAGEN) according to the instructions from manufacturer.

DNA amplification by Polymerase Chain Reaction (PCR):

Detection of Staphylococcus 16S rRNA

PCR was used for detection of Staphylococcus 16S rRNA using primers in (Table 1), according to (20).The PCR was performed in a 25ul volume, where 2 ul of the extracted DNA (100-ng) was added to 12.5 ul of oasig ™2X

qPCR Master mix , 1 ul (0.5 mM concentration) of each primer set and 8.5 ul PCR grade H2O.PCR amplifications were performed in a MyGenie 32 Thermal Block (BIONEER as follows: Initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 2 min and ended with final extension for 10 min at 72°C and finally maintained at 4°C.

Detection of mecA-gene

The polymerase chain reaction (PCR) was used for detection of mecA-gene in antibiotic resistance strains using primers in (Table 1), according to (21). In a final volume of 50 ul, three ul of DNA template was added to 25 ul of oasig ™2X qPCR Mastermix; 1 ul (0.25 mM concentration) of each primer set and 20 ul PCR grade H2O. DNA amplifications were carried out in a MyGenie 32 Thermal Block (BIONEER as follows: Initial denaturation at 94°C for 5 min followed by 36 cycles of denaturation of 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min with a final extension at 72°C for 5 min. Strain MF01 (GenBank, KY647024.1) was used as a positive control for mecA gene.Ten microliters of PCR products (16S rRNA and mecA- genes) was analyzed by 1% agarose gel electrophoresis. A 100 bp molecular weight DNA ladder (Gel Pilot Plus, QIAGEN) was used for the validation of length of the amplified products.

Antimicrobial susceptibility

All isolates were tested using 10 antimicrobial agents: Penicillin G (10U), ampicillin (10 µg), tetracycline (30 µg), amoxicillin-clavulanic acid (20/10 µg), ciprofloxacin (5 µg), cefoxitin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), Antimicrobial sensitivity was monitored with the standard disk diffusion assay .The zone of inhibition was interpreted according to CLSI guidelines (19). *S. aureus* ATCC 25923 was used as reference strain.

Results

Primers for both staphylococcus 16S rRNA and mecA-gene were tabulated in table (1). Identification of *S. aureus* was done by DNA expression of gene specific 16S rRNA gene (figure 1).

Figure (2) clarified that, *S. aureus* was present in 20 out of the 187 samples (10.7%). Of the 20 *S. aureus* isolates, three (15 %) were found to be MRSA, therefore, Figure 2 shows the prevalence of MRSA strains among total samples was only three (3/187) (1.6%).

Identification of MRSA was done by DNA expression of gene specific mec-A gene (figure 3).

Table (2) showed different patterns of Antimicrobial susceptibility of *S. aureus* isolates. 35% were resistant to β-lactam, 50% were resistant to penicillin, 65% were sensitive to ciprofloxacin, 90% were sensitive to Erythromycin and Clindamycin, 85% were sensitive to Cefoxitin, 80% sensitive to Gentamicin, 60% were resistant to Penicillin G, 75% were sensitive to Trimethoprim and finally 75% were sensitive to tetracycline.

Figure (4) declared the percentages of *S. aureus* resistant strains among the identified isolates using the disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. Results showed a higher resistant level for both penicillin and Ampicillin (60% and 50%, respectively). As shown in table (3), only two isolates (10%) out of all analyzed strains exhibited single resistance to penicillin. MRSA isolates presented antimicrobial multi resistance.

Table 1: Primers for both Staphylococcus 16S rRNA and mecA-gene

DNA target	Primer pair	Size (bp)	Reference
Staphylococcus 16S rRNA	5'GTT ATT AGG GAA GAA CAT ATG TG-3' 5'CCA CCT TCC TCC GGT TTG TCA CC-3'	750	Jaffe *et al.*, 2000
mecA-gene	5' AAAATCGATGGTAAAGGTTGGC-3' 5' AG TTCTGCAGTACCGGATTTGC-3'	530	Murakami *et al.*, 1991

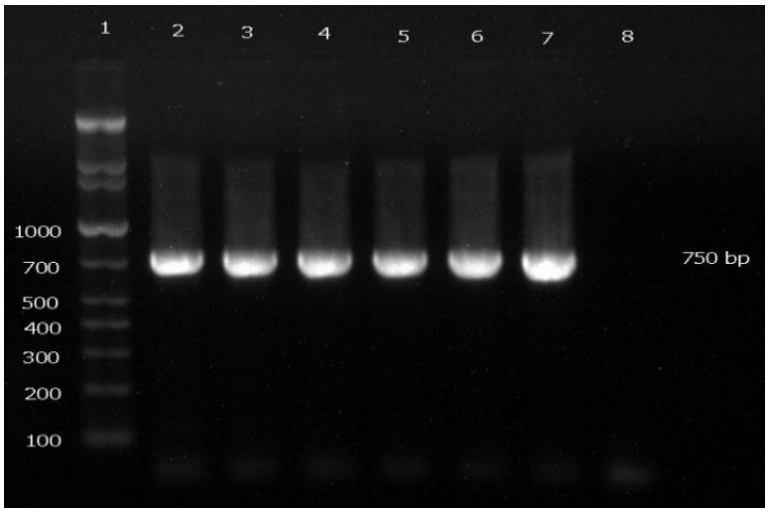

Figure 1: Agarose gel electrophoresis of amplified 750-bp DNA fragment. Lanes: 1 DNA ladder molecular weight marker (GelPilot), 2-6 staphylococcus isolates, 7 ATCC 12600

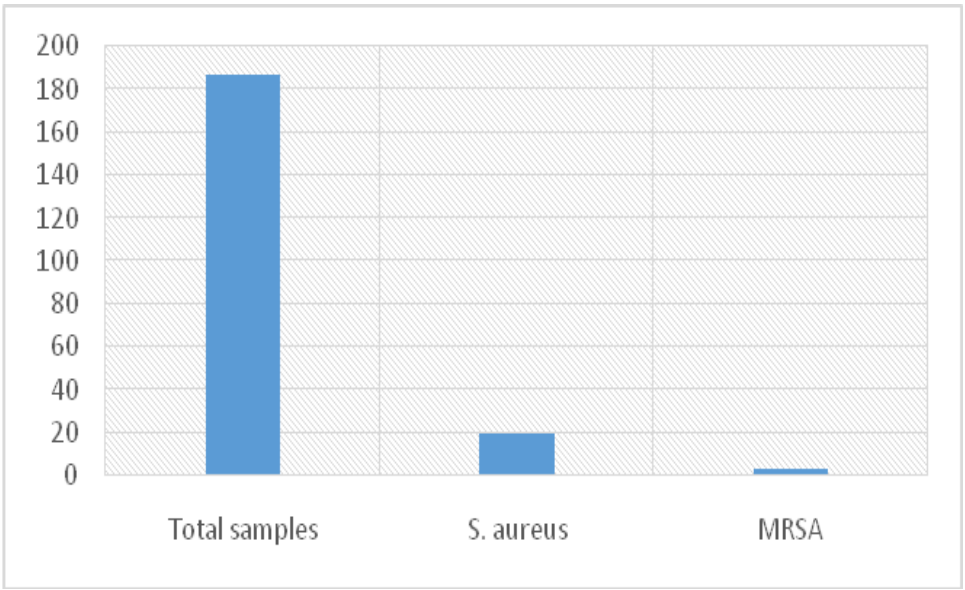

Figure 2: Prevalence (%) of *S. aureus* and MRSA in camel meat samples

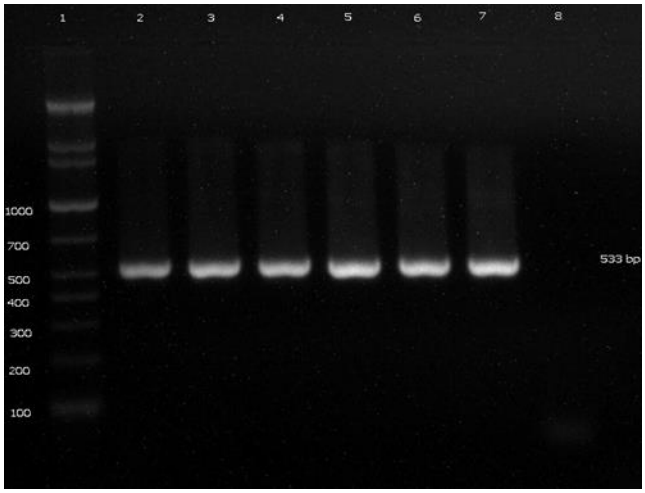

Figure 3: Agarose gel electrophoresis of amplified 533-bp DNA fragment. Lanes: 1 DNA ladder molecular weight marker (GelPilot), 2-6 MRSA isolates, 7 Strain MF01, 8 Negative control.

Table 2: Antimicrobial susceptibility of *S. aureus* isolates (n= 20):

% S	% I	% R	Antibiotic class	Antimicrobial agent
65	0	35	β-lactam+Inhibitors	Amoxicillin/Clavulanic acid (AMC)
50	0	50	Penicillins	Ampicillin (AMP)
65	5	30	Quinolones	Ciprofloxacin (CIP)
90	0	10	Lincosamides	Clindamycin (CLI)
90	0	10	Macrolides	Erythromycin (ERY)
85	0	15	Cephems	Cefoxitin (FOX)
80	0	20	Aminoglycosides	Gentamicin (GEN)
40	0	60	Penicillins	Penicillin G (PEN)
75	0	25	Folate pathway inhibitors	Trimethoprim/Sulfamethoxazole (SXT)
75	0	25	Tetracyclines	Tetracycline (TCY)

R= Resistant, T=Intermediate, S=Sensitive

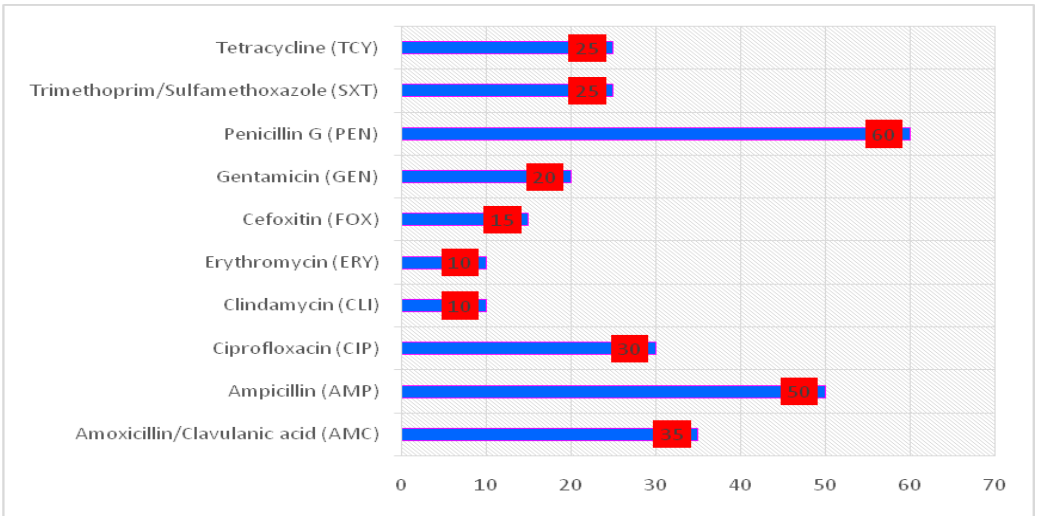

Figure 4: Percentages of *S. aureus* resistant strains among the identified isolates using the disk diffusion method according to Clinical and Laboratory Standards Institute guidelines

Table 3: Antibiotic resistance profile (number of S. aureus isolates= 20)

%	N. of isolates	Resistance profile									
40	8										
10	2	PEN									I
5	1	PEN	AMP		GEN						II
5	1	PEN	AMP	CIP							III
5	1	PEN	AMP	CIP	GEN	TCY					IV
10	2	PEN	AMC	AMP							V
5	1	PEN	AMC	AMP	CIP	SXT					VI
5	1	PEN	AMC	AMP	CIP	CLI	GEN	SXT	TCY		VII
5	1	FOX	PEN	AMC	AMP	CIP	GEN	SXT	TCY		VIII
5	1	FOX	PEN	AMC	AMP	ERY	CIP	SXT	TCY		IX
5	1	FOX	PEN	AMC	AMP	ERY	CIP	CLI	SXT	TCY	X

Discussion

As a major meat species marketed in Saudi Arabia, camel meat was used for determination of the prevalence of *S. aureus*& MRSA and antimicrobial susceptibility profile of isolates. In a survey conducted for 5 months, 187 raw camel meat samples (retailed in Al-Hasa, Saudi Arabia) were analyzed. Identification of *S. aureus* was done by DNA expression of gene specific 16S rRNA (figure 1).

Figure (2) clarifies that, *S. aureus* was present in 20 out of the 187 samples (10.7%). The prevalence of *S. aureus* in different foodstuffs and meats varied in the previous studies. They were reported 20.5% *S. aureus* from beef at USA (22).

MRSA has been already isolated and identified from retail meat worldwide, and the possible human transmission existed (23-26 and 22). The reported prevalence varied which indicated that contamination by MRSA in different types of meat varied in the different localities. Of the 20 *S. aureus* isolates, three (15 %) were found to be MRSA, therefore, Figure 2 shows the prevalence of MRSA strains among total samples was only three (3/187) (1.6%) and this result is very low in comparison with that obtained by (16) who found that the prevalence of MRSA in camel meat retailed in Riyadh was (20%) while the prevalence of MSSA was (28%). This difference may be due to variations in hygienic levels, preparation and handling of meat. While our result was nearly similar to (22) and (27) who reported that 1.3% and 1.2%, respectively were positive for MRSA from meat retailed in USA.

Meat play a significant role in transmission of antimicrobial resistance from livestock and food animals to human being, and antibiotic resistance usually has a great concern in hospital infection (nosocomial infections). Antimicrobial resistance may transfer via 3 ways: Through antimicrobial residues in food and meat, or consumption of resistant parts of original food microflora and transfer resistance to pathogenic microorganisms (28-31).

The antimicrobial resistance was higher for Penicillin G and Ampicillin (60 and 50%, respectively). In addition, 15% of tested *S. aureus* strains were methicillin-resistant while 85% were sensitive to cefoxitin (table 2). Another study showed antibiogram sensitivity differences in *S. aureus* isolated from food (7).

It was clear that MRSA isolates were resistant to all antimicrobials used.

Figure 4 showed a higher resistant level for both penicillin and Ampicillin and this result is similar to that stated by (32) in chicken giblets. Only two isolates (10%) out of all analyzed strains exhibited single resistance to penicillin. MRSA isolates presented antimicrobial multi resistance (7 and 32); similar result was found in our study (table 3).

Conclusion

From the above, it could be concluded that 20 *S. aureus* positive isolates were identified out of 187 camel meat samples (10.7% prevalence). Three were resistant to methicillin (1.6 % prevalence), 35% of *S. aureus* isolates were resistant to β-lactam antibiotic. MRSA isolates were resistant to all antimicrobials tested, and 15 % of tested *S. aureus* strains were methicillin-resistant. Our results indicated low MRSA prevalence in camel meat, which pointed to the limited risk of transmission via meat. However, attention should be paid to the safety along food chain.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

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