Bacteriological and molecular studies on antibiotic resistant *Staphylococcus aureus* isolated from meat and its products in Qaliobaya , Egypt

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A B S T R A C T

The present study was performed on 125 random samples of fresh meat and meat products *viz:* Minced meat, beef burger, sausage and Kofta (25 for each), were collected from different shops at Kaliobia Governorate for detection the prevalence of *S. aureus* in these samples, beside the phenotypic characterization and detection of some virulence and antibiotic resistant genes in them. Bacteriological examination of the collected samples resulted in, isolation of 24(19.2%) isolates of *S. aureus* from 125 samples that were isolated mostly from kofta samples (7= 28.0%) followed by minced meat (6= 24.0%), sausage and fresh meat (4=16.0% for each) and lastly beef burger samples (3=12.0%). The antibiotic sensitivity tests for the isolated *S. aureus* showed that, they were highly resistant for methicillin followed by oxacillin; ampicillin and Nalidixic acid then, oxytetracycline; amoxicillin; streptomycin and cefotaxime. Meanwhile, they were highly sensitive to enrofloxacin followed by gentamycin; vancomycin and ciprofloxacin. PCR results cleared that, *mec*A and *tet*K virulanc genes were detected in all four studied strains, meanwhile, *ica*A and *van*A were detected in only of them.

Keywords: Meat products, bacteriological evaluation, S. aureus, antibacterial resistant

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1. INTRODUCTION

Meat and meat products are the most palatable of highly nutritious and highly desirable foods for human being but they are considered as serious sources of food borne pathogens and have been linked to major outbreaks of food poisoning, illness and death all over the world (Datta *et al.*, 2012 and Zafar *et al*., 2016). *Staphylococci* are Gram-positive *cocci*, form grape-like clusters on Gram's stain, non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. *Staphylococcus aureus* is considered to be one of the causes of most important foodborne diseases worldwide due to its ability to produce wide variety of toxins (Argudin *et al*., 2010). The Staphylococcal enterotoxins (SEs) are responsible for the symptoms that associated with Staphylococcal food poisoning (Llewelyn and Cohen, 2002). The disease is characterized by symptoms including nausea, vomiting, abdominal cramps and diarrhea lasting from 24 to 48 h

and the complete recovery usually occurs within 1-3 days.

Antibacterial are often used for therapy of infected humans and animals as well as for prophylaxis and growth promotion of food producing animals. Many findings suggest that inadequate selection and abuse of antimicrobials may lead to resistance in various bacteria and make the treatment of bacterial infections more difficult (Kolár *et al*., 2001; Shea, 2004 and Kukanich *et al*., 2005). Antimicrobial resistance may be intrinsic or acquired. intrinsic resistances are generally chromosomally encoded, and are typically responsible for observed differences in resistance observed between genera, species and strains of bacteria. It can be associated with differences in cell wall structures, the ability to pump antimicrobial compounds out of the bacterial cell using efflux pumps, or the production of enzymes capable of inactivating antimicrobial compounds within the bacterial cell (Russell, 2001 and Gilbert and McBain, 2003). While acquired resistance, in which a previously sensitive bacterium becomes resistant, can arise as a result of a mutation in chromosomal DNA of a house-keeping structural or regulatory gene of the organisms (Courvalin and Trieu-Cuot, 2001 and Martinez and Baquero, 2002) or through the acquisition of one or more antimicrobial resistance genes as a result of horizontal gene transfer within and between bacterial species, which can occur in the environment (*in vitro*) or during infection (*in vivo*), include conjugation, transduction and transformation, and can involve one or more defined genetic elements including: bacteriophages, plasmids, conjugative transposons and integrons (Carattoli, 2001 and D'Costa *et al*., 2006). The emergence of antimicrobial resistance among *S. aureus* strains of meat and its products has important public health implications. The antibiotic resistant *S. aureus* is known to be one of the most prevalent nosocomial pathogens throughout the world and is capable of causing a wide range of food poisoning, pneumonia, post-operative wound infections and nosocomial infections (McMahon *et al*., 2007 and Moroney *et al*., 2007). Beside the antimicrobial resistance among some food borne pathogens, the pathogenicity of them could be attributed to the virulence factors the bacteria produce. the virulence of *S. aureus* could be attributed to intracellular adhesion (*ica*A); clumping factors A(*clf*A); toxins (enterotoxins, toxic shock syndrome toxin-1, Panton-Valentine Leukocidin); haemolysin; coagulase, thus clot blood; protease; hyaluronidase, and staphylokinase (Dinges *et al*.,2000; Lin and Peterson, 2010 and Abdalrahman *et al.*, 2015). *S. aureus* produce disease when the bacteria contaminate food, produce some enzymes which are implicated with *Staphylococcus* invasiveness and many extracellular substances some of which are heat stable enterotoxins that renders the food dangerous even though it appears normal and extensive cooking can be killed the bacteria but the toxins may not be destroyed because most of them are gene based i.e. they can be carried on the plasmid (Prescott *et al.*, 2005). As food-borne bacteria especially antimicrobial resistant ones constitutes serious problems for consumers, so, the present study was conducted to detect /estimate the prevalence of *S. aureus* in meat and common meat products (minced meat;

beef burger; sausage and kofta) in Qaliobaya governorate, beside the phenotypic characterization of the isolate determination of virulence and antibacterial resistant genes in them.

2. MATERIAL AND METHODS

2.1. Samples:

A total of 125 random samples of fresh meat and meat products *viz*: Minced meat, beef burger, sausage and Kofta (25 for each), were collected from different shops at Kaliobia Governorate (Qaliob, Benha).

2.2. Bacteriological examination:

A total of 25 grams of each sample under examination were prepared for bacteriological examination following (APHA, 2001).

Isolation and identification of *S. aureus* from different samples according to Quinn *et al.* (2002) and Arora (2003) as follow: One ml of prepared sample was inoculated into nutrient broth and incubated aerobically at 37ºC for 12 hours. A loopful from incubated nutrient broth was streaked on 7% salted nutrient agar and incubated for 24 hours at 37°C. Then the following tests (Oxidase test, Catalase test and Coagulase test) were performed on yellow convex colonies. The colonies that gave (Oxidase -ve, Catalase +ve, KOH 3% -ve and Coagulase +ve & -ve) were taken and cultivated on the following media: Baird Parker agar; Mannitol salt agar; Milk salted agar and Blood agar and incubated for another 48 hours at 37ºC, suspected *S. aureus* colonies (black colonies with yellow halo around them on Baird-Parker agar; yellow colonies surrounded by halo zone on Mannitol salt agar; yellow colonies and turned media to colorless on 7% salted milk agar and white or yellow, smooth round and shiny colonies on blood agar) were picked up and kept in Semi-solid agar. The purified isolates of *S. aureus* were morphologically identified by Gram stain and biochemical tests.

2.3. In-Vitro anti-microbial sensitivity test:

The isolated *S. aureus* strains were subjected to the sensitivity test against different antibiotics (table3) using the disc and agar diffusion method (Koneman *et al.*, 1997) and interpretation of results were carried out according to NCCLS (2007).

2.4. Detection of Virulence and antibiotic resistant genes of S. aureus by PCR

PCR was applied by using 4 sets of primers for detection of 4 virulence and antibiotic resistant genes that may play a role in virulence of *S. aureus*. These genes were intracellular adhesion gene (*ica*A); methicillin resistant gene (*mec*A); tetracycline resistant K gene (*tetK)* and vancomycin resistant gene (*van*A).It was applied on 4 +ve isolates of *S. aureus* following QIAamp® DNA Mini Kit instructions (Catalogue no. 51304), Emerald Amp GT PCR mastermix (Takara) with Code No. RR310A and 1.5% agarose gel electrophoreses (Sambrook *et al.*, 1989) using the Primers sequences, target genes, amplicons sizes and cycling conditions showed in Table (1).

Table (1): Oligonucleotide primers sequences source Metabion (Germany)

Target M.O.	Gene	Primer sequence (5'-3')	Length of amplified product	Reference
S. aureus	*mec*A	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure *et al*., 2006
	*tet*K	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360 bp	Duran *et al*., 2012
	*ica*A	CCT AAC TAA CGA AAG GTA G AAG ATA TAG CGA TAA GTG C	1315 bp	Ciftci *et al*., 2009
	*van*A	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC GMYTGGTTGCGRATGGT	885 bp	Patel *et al*., 1997

3. RESULTS

The results of bacteriological examination of examined samples (Table, 2) revealed a total of 24(19.2%) isolates of *S. aureus* were recovered from 125 samples and were isolated mostly from kofta samples (7= 28.0%) followed by minced meat (6= 24.0%), sausage and fresh meat (4=16.0% for each) and beef burger samples (3=12.0%).

The recovered isolates are Gram-positive cocci, arranged in irregular clusters (bunches of grapes) and non-motile. They grow well on different media and showed yellow convex colonies on 7% salted nutrient agar; yellow colonies (with fermentation of Mannitol) surrounded by halo zone on Mannitol salt agar medium; while on Baird-Parker agar media showed black shiny colonies (due to tellurite reduction) with yellow halos around them on the surface of the medium and some strains also produce a smaller, clear zone around the colonies due to proteolytic activity. The same isolates on blood agar showed a white or yellow, smooth round and shiny colonies with beta and alpha hemolysis and on 7% salted milk agar they gave yellow colonies and turned media to colorless due to lipase enzyme. In addition, all of them were coagulase positive *S. aureus strains*.

The results of in-vitro sensitivity test (Table 4) revealed that, the *S. aureus* isolates were highly resistant for methicillin (87.5%) followed by oxacillin (83.3%); ampicillin and Nalidixic acid (75.0% for each); oxytetracycline (70.8%); amoxicillin and streptomycin (62.5% for each) and cefotaxime (58.3%). Meanwhile, they were highly sensitive to enrofloxacin (83.3%) followed by gentamycin and vancomycin (79.2% for each) and ciprofloxacin (66.7%). Moreover, they were intermediate sensitive to trimethoprim/ sulphamethoxazol (62.5%) and erythromycin (54.2%).

PCR results showed that *mec*A and *tet*K genes were detected in all four studied strains, meanwhile, *ica*A and *van*A were detected in only 3of them. Moreover, the *ica* A gene was amplified in 3 out of 4 *S. aureus* strains giving product of 1315 bp; The *mec*A gene was amplified in all 4 of the studied *S. aureus* strains giving product of 310 bp; the *tet*K gene was amplified in all 4 of the

studied *S. aureus* strains giving product of 360 bp and the *van*A gene was amplified in 3 out of 4 of the *S. aureus* strains giving product of 885 bp as shown in Fig. (1-4).

Table (2): Prevalence of *S. aureus* strains isolated from examined samples (n=25).

Samples	*S. aureus* strains	
	NO.	%
Fresh meat	4	16.0
Minced meat	6	24.0
Kofta	7	28.0
Beef Burger	3	12.0
Sausage	4	16.0
TOTAL	24	19.2

* Percentage in relation to total No. of each examined sample (25 & 125 for total).

Table (3): Biochemical reaction of *S. aureus* isolates

Biochemical test	Reaction
1. Indole test	Negative
2. Methyl red test	Positive (production of bright red color)
3. Voges-Proskauer test (VP)	Positive (Bright pink coloration after 15 minutes)
4. Coagulase test	Positive
5. β- haemolysis	Positive
6. Triple sugar slant	Positive with yellow colour of slant and bottom.
7. Catalase test	Positive
8. Oxidase test	Negative
9. Sugar fermentation test	Glucose; Lactose; Mannitol Positive while most of isolates gave positive results for Sucrose.
10. Pigment production	Positive

Table (4): *In-Vitro* anti-microbial Sensitivity test for isolated *S. aureus* strains

Antimicrobial agents	Disk concentrations	Sensitive		Intermediate		Resistant		AA
		No.	%	No.	%	No.	%	
Amoxicillin	25µg	3	12.5	6	25.0	15	62.5	R
Ampicillin	20 µg	2	8.3	4	16.7	18	75.0	R
Cefotaxime	30 µg	4	16.7	6	25.0	14	58.3	R
Ciprofloxacin	5 µg	16	66.7	3	12.5	5	20.8	S
Enrofloxacin	5 µg	20	83.3	1	4.2	3	12.5	S
Erythromycin	15 µg	5	20.8	13	54.2	6	25.0	IS
Gentamicin	10 µg	19	79.2	3	12.5	2	8.3	S
Methicillin	5 µg	0	0.0	3	12.5	21	87.5	R
Nalidixic acid	30 µg	2	8.3	4	16.7	18	75.0	R
Oxacillin	1 µg	1	4.2	3	12.5	20	83.3	R
Oxytetracycline	30 µg	3	12.5	4	16.7	17	70.8	R
Streptomycin	S/10	3	12.5	6	25.0	15	62.5	R
Trimethoprim/ Sulphamethoxazol	(1.25/23.75) mcg	3	12.5	15	62.5	6	25.0	IS
Vancomycin	30 µg	19	79.2	0	0.0	5	20.8	S

No.: Number of isolates. %: Percentage in relation to total number of isolates (24). AA: Antibiogram activity.

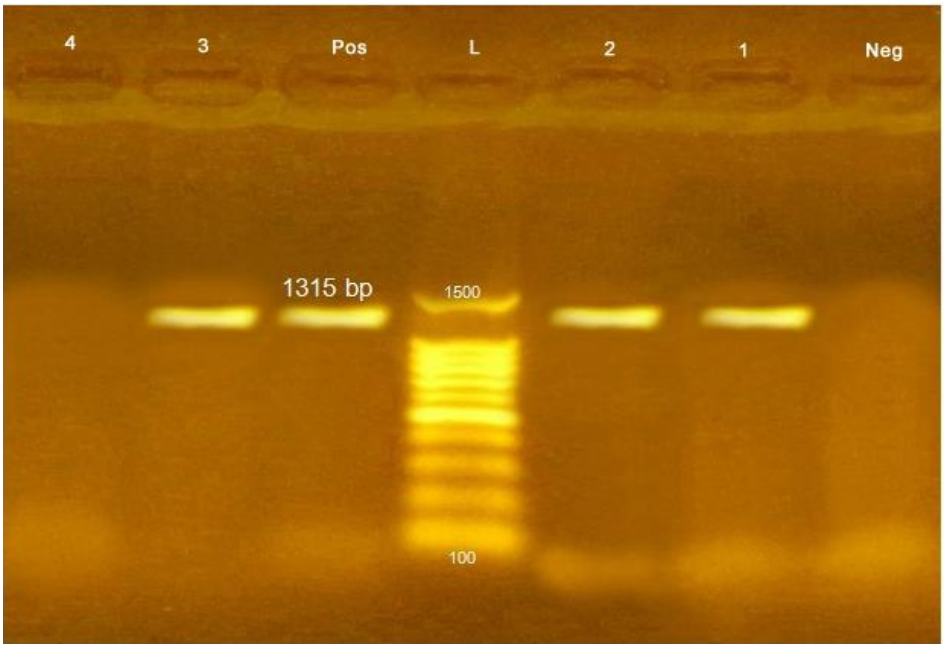

Fig. (1): Gel electrophoresis of intracellular adhesion gene (*icaA*),
Lane L: 100-1500 bp DNA Ladder, Neg.: Negative control,
Pos.: Positive control (at 1315 bp), Lane 1 -3: *S.aureus* (Positive),
Lane4: *S. aureus* (Negative).

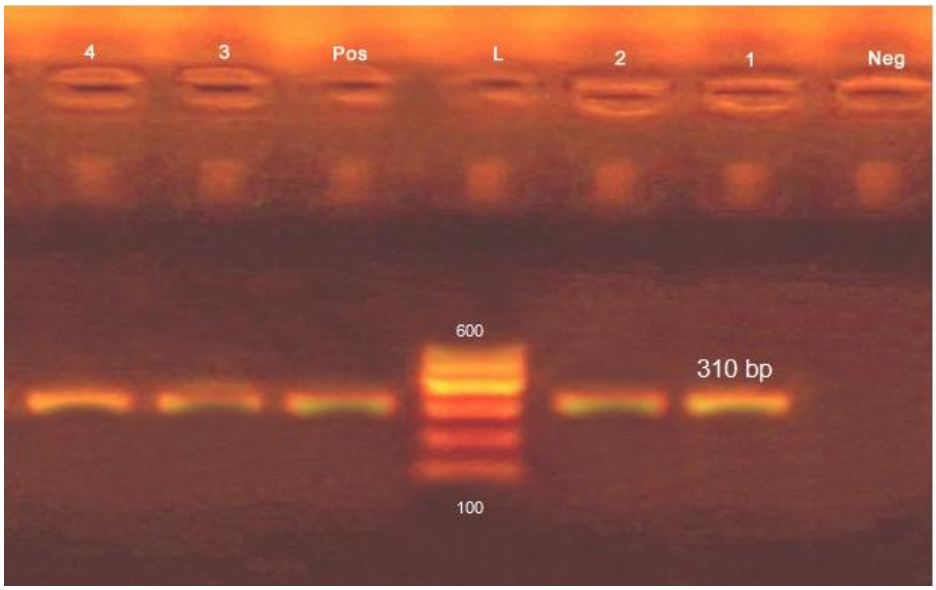

Fig. (2): Gel electrophoresis of methicillin resistant gene (*mec*A),
Lane L: 100-600 bp DNA Ladder. Neg.: Negative control.
Pos.: Positive control (at 310 bp) Lane 1 - 4:*S.aureus* (Positive).

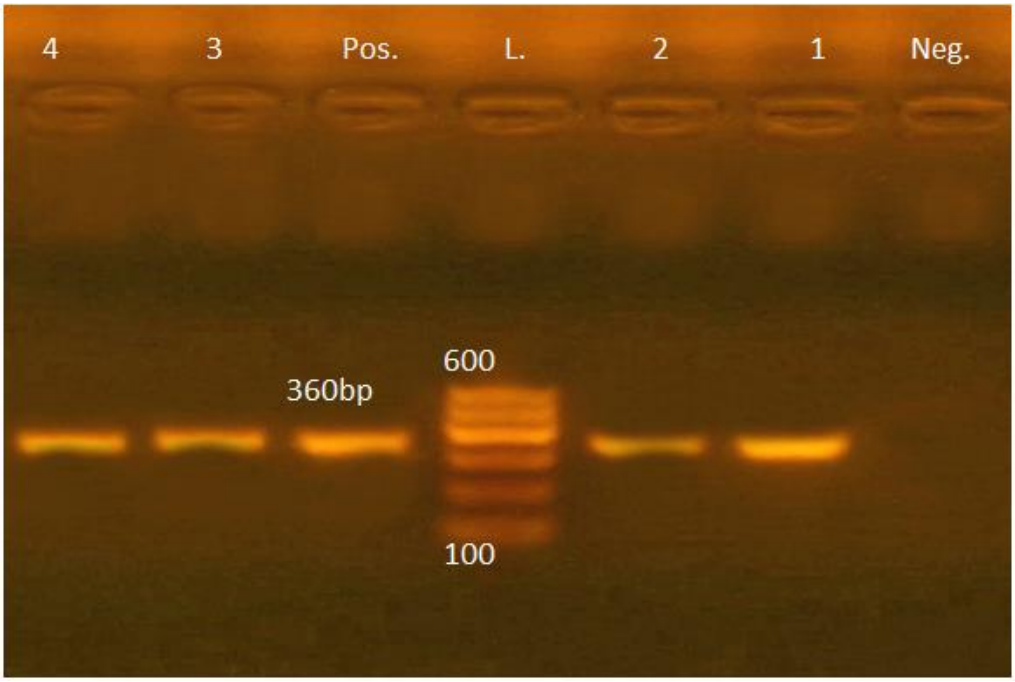

Fig. (3): Gel electrophoresis of tetracycline resistant gene resistant gene (*tetK*)
Lane L: 100-600 bp DNA Ladder. Neg.: Negative control.
Pos.: Positive control (at 360 bp). Lane 1 - 4: *S.aureus* (Positive).

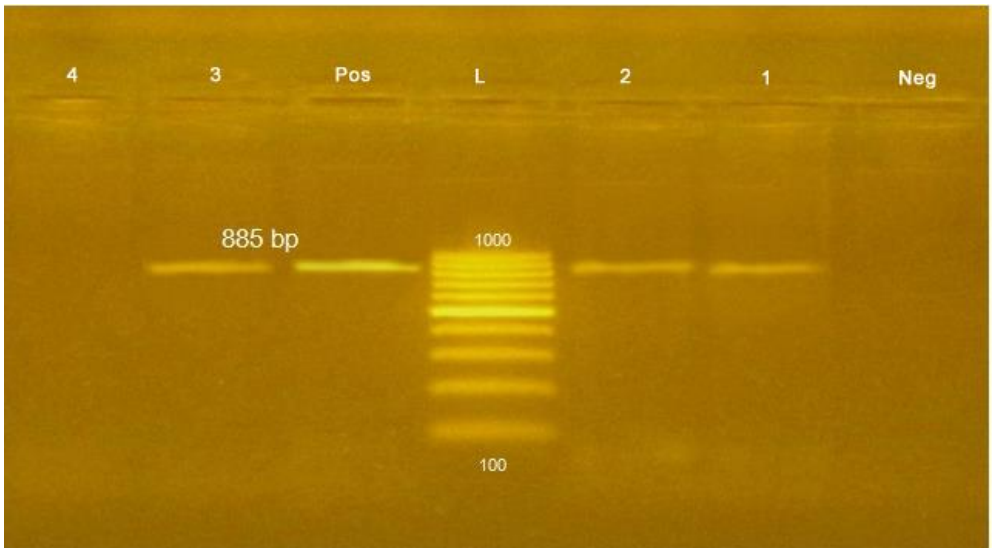

Fig. (4): Gel electrophoresis of vancomycin resistant gene (*van*A)

Lane L: 100-1000 bp DNA Ladder. Neg.: Negative control.

Pos.: Positive control (at 885 bp). Lane 1 -3: *S.aureus* (Positive).

Lane4: *S. aureus* (Negative)**.**

4. DISCUSSION

Staphylococcus aureus is one of the most important bacterial pathogens in meat and its products that are responsible for food-borne infections, especially antimicrobial resistant ones (Ghenghesh et al., 2008 and Hamed et al., 2015). So, the present study was conducted for detection the prevalence of S. aureus in meat and common meat products (minced meat; beef burger; sausage and kofta), beside the phenotypic characterization and detection of some virulence and antibiotic resistant genes in them.

The results of *S. aureus* isolation from examined samples (Table 2) revealed that a total of 24 (19.2%) isolates of *S. aureus* recovered from 125 samples and were isolated mostly from kofta samples (7= 28.0%) followed by minced meat (6= 24.0%), sausage and fresh meat (4=16.0% for each) and beef burger samples (3=12.0%). These results came in accordance with those obtained by Maarouf and Nassif-Marionette (2008); Abdel-Raouf *et al.* (2014); Ezzat *et al.* (2014); Abd El-Tawab *et al.* (2015) and Armany *et al.* (2016). Meanwhile, these results disagreed with those of Abdaslam *et al.* (2014); Mousa *et al.* (2014); Adwan *et al.* (2015) and Tarabees *et al.* (2015) who isolated *S. aureus* from fresh meat and meat products with high incidence. Also, disagreed with those recorded by Wehab and Hegazy (2007) and Kalantari *et al.* (2012) who did not isolate *S. aureus* from beef burger and beef sausage samples. The presence of *S. aureus* in meat and its products indicates poor hygiene of meat handlers as well as

lack of sterilization of utensils and they grow without pronounced change in odor or taste in the products and producing heat stable enterotoxins which lead to food poisoning with severe diarrhea and gastroenteritis among consumers (Protocarrero *et al*., 2002 and Plaatjies *et al.*, 2004).

The colonial appearance and the biochemical profile of *S. aureus* isolated was similar to those previously reported such as the fermentation of certain sugars or enzymatic reaction as lipase; extracellular pigmentation production (Staphyloxathine) and Staphylocoagulase (Quinn *et al.*, 2002; Ezzat *et al.*, 2014 and Abd El-Tawab *et al*., 2015).

The irrational widespread use of antimicrobial has undoubtedly accelerated the virulence of *S. aureus*, by acquiring multiple resistance genes, has become able to survive almost all antibiotic families. The *in- vitro* sensitivity tests for the isolated *S. aureus* (Table,4) showed that, the isolated *S. aureus* were highly resistant to methicillin followed by oxacillin ; ampicillin and Nalidixic acid then, oxytetracycline; amoxicillin; streptomycin and cefotaxime . Meanwhile, they were highly sensitive to enrofloxacin followed by gentamycin; vancomycin and ciprofloxacin. While, they were intermediate sensitive to trimethoprim/ sulphamethoxazol and erythromycin. These results were agreed with those reported by Kelman *et al*. (2011); Abd El-Tawab *et al*. (2015); Rahimi and Karimi (2015) and Mohamed- Hadeer (2017). The resistance to methicillin occurred mainly because of the presence of *mec*A gene on *S. aureus* chromosome that responsible for the production of Penicillin binding protein PBP2a. (Ito *et al*., 2003).

The PCR technique gives the capability of identifying the pathogenic *S. aureus* isolates. based on the fact that virulent and antibiotic resistant genes varies not only among different species but also among strains of the same species. Thus, numerous studies have been conducted to identify virulence factors and antibiotic-resistant genes of isolated *S. aureus* strains (Eftekhar and Dadaei, 2011; Podkowik *et al.*,2012 and Safarpoor–Dehkordi *et al.*, 2017). So, the present study was directed mainly to recognize some virulence genes that may play a role in virulence of these isolates by using one of the recent developments molecular biological techniques (PCR). These genes were intracellular adhesion (*ica*A) gene, that enhance the adherence of *S. aureus* to host cells and induction of the inflammation process, and antibiotic resistant genes, methicillin resistant (*mec*A); tetracycline resistant K (*tet*K*)* and vancomycin resistant (*van*A) genes. The results of PCR cleared that, *mec*A and *tetK* virulence genes were detected in all the four studied strains, meanwhile, *ica*A and *van*A were detected in three out of them.

Regarding to the intracellular adhesion (*ica*A) gene, the results of PCR amplification of *ica* A gene in *S. aureus* isolates (Fig. 1) showed that, the *ica*A gene was amplified in three out of four *S. aureus* strains giving product of 1315 bp. Similar findings were recorded by Park *et al.*, (2008); Ciftci *et al*., (2009); Plata *et al*.,

(2009); Eftekhar and Dadaei (2011) and FeBler *et al.* (2011). Meanwhile, for methicillin resistant gene (*mec*A), the results of PCR amplification of *mec*A gene in *S. aureus* isolates (Fig., 2) showed that, the *mec*A gene was amplified in all of the four studied *S. aureus* strains giving product of 310 bp. Similar detection of *mec*A gene in *S. aureus* strains (MRSA) were recorded by Plata *et al*.(2009); Podkowik *et al.* (2012); Bunnoeng *et al.* (2013); Chen *et al.* (2013); Mohammed and Nigatu (2015); Khoramrooz *et al.* (2017); Khosravi *et al.* (2017) and Safarpoor – Dehkordi *et al.* (2017). Meanwhile, Abdalrahman *et al.* (2015) have not detect *mec*A gene in *S. aureus* strains and said that, this might be due to over production of β-lactamase enzymes or the presence of a variant *mec*A gene that does not amplify with the available PCR primers. Regarding tetracycline resistant K gene (*tet*K), the results of PCR amplification of *tet*K gene in *S. aureus* isolates (Fig., 3) revealed that, the *tet*K gene was amplified in all of the four studied *S. aureus* strains giving product of 360 bp.Parallel detection of *tet*K gene in *S. aureus* strains were recorded by Podkowik *et al.* (2012); Khoramrooz *et al.* (2017) and Safarpoor – Dehkordi *et al.* (2017). With respect of vancomycin resistant gene (*van*A), the results of PCR amplification of *van*A gene in *S. aureus* isolates (Fig. 4) cleared that, the *van*A gene was amplified in only three out of four *S. aureus* strains giving product of 885 bp. Similar detection of *van*A gene in *S. aureus* strains was recorded by Chambers and Deleo (2009) and Podkowik *et al.* (2012).

Finally, the present results proved that multiple antibiotic resistances are widely spread among isolated strains of *S. aureus* and decided the fact of Shalini and Rameshwar (2005) that the food chain can be considered as the main route of transmission of antibiotic- resistant bacteria between the animal and human populations. Moreover, the recorded results showed a considerable (19%)bacterial load of *S. aureus*, this may be due to mishandling and the negligence of hygienic aspects. Therefore, it was concluded that coagulase positive *S. aureus* especially antibiotic resistant ones are meat-borne pathogens of public health important.

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