



Detection and molecular characterization of Methicillin resistant *staphylococcus aureus* (MRSA) in some meat products.

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ABSTRACT

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Food poisoning is one of the most important problems that can affect large part of population. *Staphylococcus aureus* (*S. aureus*) is one of the main causes of bacterial food poisoning. A hundred random samples of burger, chicken nuggets, luncheon and kofta (25 of each) obtained from retail outlets were examined bacteriologically for the occurrence of methicillin resistance *Staphylococcus aureus* (MRSA). A total of 25 *S.aureus* isolates was recovered from - burger 9/25 (36%), chicken nuggets 7/25 (28%), luncheon 6/25 (24 %) and kofta 3/25 (12%) Application of antibiotic sensitivity test on isolated *S.aureus* cleared that the highest resistance was exhibited against penicillin (96%) followed by flucloxacillin (92%), cefotaxime (68%), erythromycin (60%), clindamycin (61.54%), linezolid (36%) and vancomycin (28%) , using Polymerase chain reaction (PCR) on *S.aureus* to detect their virulence and resistance genes Hemolysin virulence gene (*hlg*) which was detected in (50%) of the examined isolates, while beta- lactam resistance gene (*blaZ*) and methicillin resistance gene (*mec A*) were found in (50%) and (100%) of tested *S.aureus*, respectively.

1.INTRODUCTION

Food safety is an important issue that affects everyone throughout the world. Unsafe food causes more than 200 diseases, ranging from diarrhea to cancers (1, 2).

S.aureus is a significant food pathogen in many nations , and it causes; Staphylococcal Food Poisoning (SFP) , which is characterized by vomiting , sepsis-related infections, pneumonia, and toxic shock syndrome (TSS) (3). Upon contamination of food by *S.*

aureus, it developed heat – stable staphylococcal enterotoxins (SE) which causes SFP (4)

S.aureus can potentially infect food when it is being prepared, processed, or stored under temperature-inappropriate circumstances that promote bacterial growth and the development of enterotoxins. *S. aureus* can thrive and exhibit virulence in a variety of environmental circumstances, including pH ranges from 4.5 to 9.0 and NaCl concentrations up to 9% (5).

Staphylococci are famous for rapidly evolving resistance to many antibiotics. MRSA was previously only known as a clinical infection, but as evidenced by its appearance in retail meat products, it can now be linked to food safety, creating a challenge for occupational employees in the food business. (6, 7, 8). Since food products contaminated with MRSA may not exhibit any visual spoilage appearance or bad smell, it is challenging for consumers to detect the contaminated foods.

Penicillin and cephalosporin are among the numerous medications known as β -lactams that MRSA is resistant to. Penicillin and other β -lactam antibiotics work to kill bacteria by preventing the manufacture of their cell walls. Recently, *S. aureus* strains expressing penicillinase (an enzyme that breaks down penicillin) were discovered, which was discovered not long after penicillin was initially used to treat human infections. It was estimated that more than 80% of *S. aureus* strains produce penicillinase. The *mecA* gene, which produces the low affinity penicillin binding protein (PBP) 2a or PBP2' with a poor affinity for β -lactam antibiotics, is what causes methicillin resistance ((9). It is well recognized that MRSA in humans is primarily acquired from meat and meat products (10)

Therefore, the present study was conducted to investigate the occurrence of coagulase-positive *S. aureus* and methicillin-resistant *S. aureus* (MRSA) strains in different popular meat products (Burger, chicken nuggets, luncheon and kofta) at Gharbia Governorate.

2. MATERIALS AND METHODS

2.1 Samples collection:

A total of 100 random samples of meat products burger, chicken nuggets, Luncheon and Kofta. (25 of each kind). was collected from various shops and supermarkets at Gharbia Governorate, Egypt. The collected samples were sent directly to the animal health laboratory under complete strict conditions in icebox within one hour and examined for bacteriological detection *S. aureus* and MRSA strains contamination.

2.2 Bacteriological examination (11, 12)

Samples were homogenized in 0.1% peptone water and maintained for 1 hr at 25 °C then the prepared samples were pre-enriched into nutrient broth and incubated at 37°C for 24 hrs. A loopful from incubated nutrient broth was streaked on Baird

parker agar plates (oxoid) and incubated at 37° C 24-48 hrs: where *S. aureus* grew (black shiny colonies with clear halo zone around colonies and opaque zone of precipitation). Also, a loopful from incubated nutrient broth was streaked on Mannitol salt agar (Oxoid) and incubated at 37 °C for 24 -48 hrs; Positive samples (showed yellow colonies and turned media to colorless). In order to do PCR testing and Biochemical identification on these colonies, Brain Heart infusion broth was used.

2.3 Morphological examination (13).

The suspected *S. aureus* isolates were stained with Gram stain for Morphological examination.

2.4 Biochemical identification (12, 14):

Indol test, Oxidase test, Coagulase production test and β – hemolysis test.

2.5 Antibiotic sensitivity test:

The collected bacterial isolates underwent in vitro testing to determine which antimicrobial discs they were susceptible to.: penicillin (P) 10 mcg, flucloxacillin (FL) 5 mcg, erythromycin (E) 15 mcg, vancomycin (VA) 30 ml , clindamycin(DA) 2 mcg, cefotaxime (CTX) 30 mcg and linezolid (LNZ) 30 mcg. According to (15) and the degree of sensitivity was interpreted according (16)

2.6 Molecular detection of MRSA:

DNA was extracted from the isolated *S. aureus* using QIAamp DNA mini kit. It was applied on 6 random isolates. PCR reaction Mix and cycling conditions of the primers during PCR was prepared according to Emerald Amp GT PCR master mix (Takara) kit. PCR was used for confirmation of *S. aureus* isolates by using primers specific to *S. aureus* , 23S rRNA . Also ,it used for detection of Hemolysin virulence gene (*hlg*) , beta -lactam resistance gene (*blaZ*) and methicillin resistance gene (*mecA*) Oligonucleotide primers sequence and amplicon size as were shown in (table1) . DNA samples were amplified in a total volume of 25 μ l as follows: 12.5 μ l of Emerald Amp GT PCR master mix, 1 μ l of each primer of 20 pmol concentrations, 4.5 μ l of water and 6 μ l of template DNA. The reaction was performed in a Biometra thermal cycler. Temperature and time

documentation system and the data was analyzed through computer software.

Conditions of the primers during PCR were applied (**table 2**). Aliquots of amplified PCR products were electrophoresed in 1.5 % agarose gel (ABgene) in 1x TBE buffer at room temperature. For gel analysis, 15 µl of PCR products were loaded in each gel slot. A 100 bp DNA ladder (QIAGEN Inc, Valencia, CA, USA) was used to determine the fragment sizes. The gel was photographed by a gel

Table (1): Sequence and amplicon size of the primers used for detection of *S. aureus*, virulence and resistance genes.

Target gene	Sequence	Amplified product	Reference
<i>S. aureus</i> 23S rRNA	F- AC GGAGTTACAAAGGACGAC	1250 bp	(17)
	R- AGCTCAGCCTTAACGAGTAC		
<i>mecA</i>	F-GTA GAA ATG ACT GAA CGT CCG ATA A	310 bp	(18)
	R- CCA ATT CCA CAT TGT TTC GGT CTA A		
<i>blaZ</i>	F-TACAACTGTAATATCGGAGGG	833 bp	(19)
	R-CATTACACTCTTGCGGTTTC		
<i>Hlg</i>	F-GCCAATCCGTTATTAGAAAATGC	937 bp	(20)
	R- CCATAGACGTAGCAACGGAT		

Table (2): Cycling conditions of the primers during cPCR:

Target gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
<i>S. aureus</i> 23S rRNA	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 12 min.	35	72°C 12 min.
<i>MecA</i>	94°C 5 min.	94°C 30 sec.	50°C 30 sec.	72°C 30 sec.	35	72°C 7 min.
<i>blaZ</i>	94°C 5 min.	94°C 30 sec.	50°C 40 sec.	72°C 50 sec.	35	72°C 10 min.
<i>Hlg</i>	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 50 sec.	35	72°C 10 min.

3.RESULTS:

3.1. Incidence of *S. aureus* isolated from examined samples.

1. According to phenotypic and biochemical identification. A total of 25 isolates of *S. aureus* were recovered from 100 Samples represented as 9/25 (36%) from burger, 7/25 (28%) Chicken nuggets, 6/25(24 %) luncheon and 3/25 (12%) from kofta, respectively. (Table 3)

Table (3): Incidence *S. aureus* isolated from examined samples:

Samples	No.of samples	No.of isolates.	%
Burger	25	9	36
Chicken nuggets	25	7	28
Luncheon	25	6	24
Kofta	25	3	12
Total	100	25	25

3.2.Morphological character and Colonies appearance.

The isolated strains were cultured, on Baird-Parker agar: showed black shiny colonies with clear halo zone around colonies and opaque zone of precipitation **figure (1)**, on Mannitol salt agar: showed (yellow colonies and turned media to colorless) **figure (2)**. Morphological

examination of suspected *S.aureus* colonies revealed non-motile Gram-positive Cocci (**figure3**).

Biochemically, Negative results were recorded on Indol test, Oxidase test while positive results were recorded on coagulase test (**fig 4**) and β – hemolysis test (**fig5**)

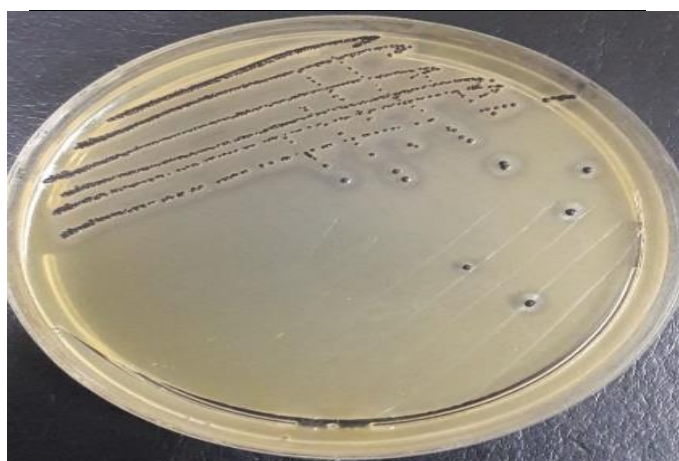


Fig (1) Colonies of *S.aureus* on baird parker agar media.

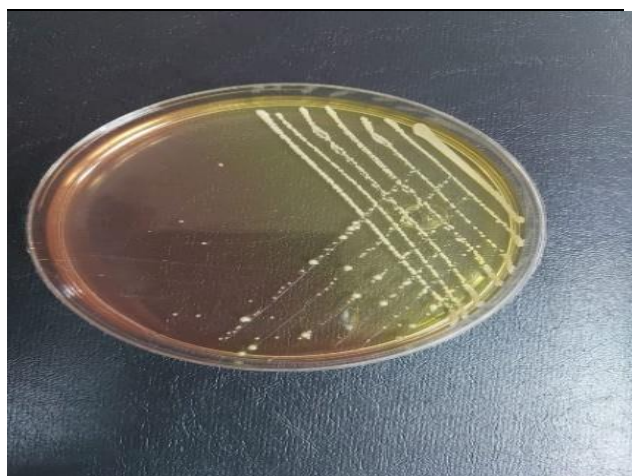


Fig. 2 showed. *S.aureus* on mannitol salt agar

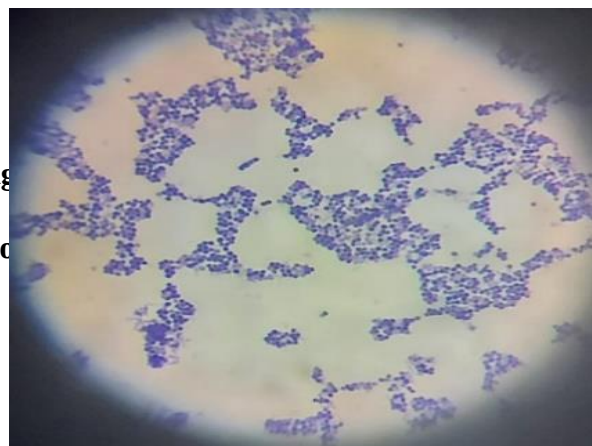


Fig
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Fig (3) *S.aureus* under microscope after Gram' s staining appear as grape like cluster.



Fig (4) Positive coagulase test show that clot form when bacterial cell are incubated with plasma.

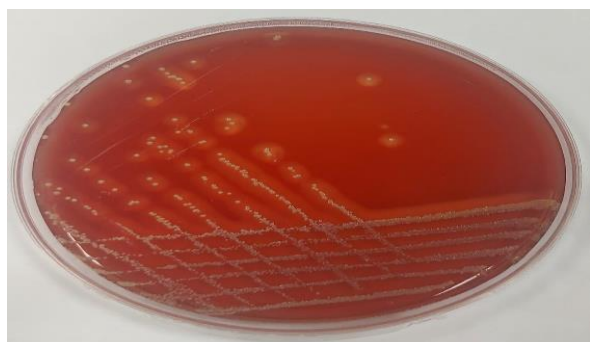


Fig (5). *S.aureus* showed β – hemolysis on blood agar media.

3.3. Antibiotic sensitivity test.

Application of antibiotic sensitivity test on 25 of *S. aureus* isolates recovered from meat products exhibited the highest resistance against penicillin (96 %) followed by flucloxacillin. (92

%) , cefotaxime (68%) , erythromycin (60 %) , clindamycin (61,54 %) , linezolid (36%)and vancomycin (28%) . Figure (6).



Fig (6). Antibiotic sensitivity test of *S.aureus*

3.4 Detection of virulence and resistant genes of *S.aureus* by PCR.

S.aureus isolates were examined for detection of staphylococcal virulence and resistance genes by uniplex PCR; the result revealed that 50% of the examined isolates harbored Hemolysin virulence gene (*hlg*), resistance

genes, *blaZ* and *mecA* resistant genes by uniplex PCR. The results revealed that all the tested isolates harbored 50%and 100% *blaZ* and *mecA* respectively

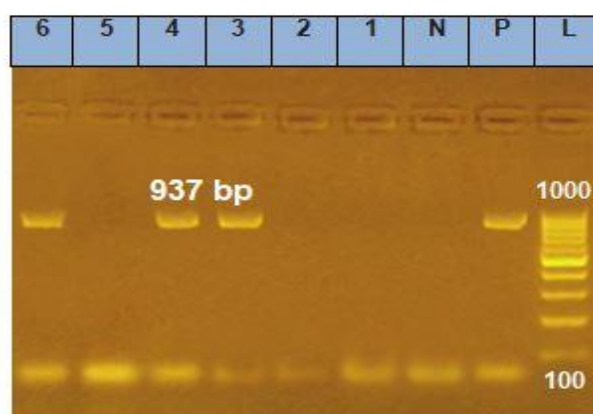


Fig (7): PCR pattern for the detection of staphylococcal hemolysin (*hlg*) genes at 937 bp on agarose gel electrophoresis.

L: Ladder from 100 bp to 1000 bp

Neg: Negative control: Field isolate that were tested and confirmed to be negative by PCR for the related genes in R.L.Q.P

Pos: Positive control: *S.aureus* ATCC 25923.

Lane 1, 2, 5: Negative amplification of staphylococcal hemolysin (*hlg*) gene.

Lane 3, 4, 6: Positive amplification of staphylococcal hemolysin (*hlg*) gene at 937 bp

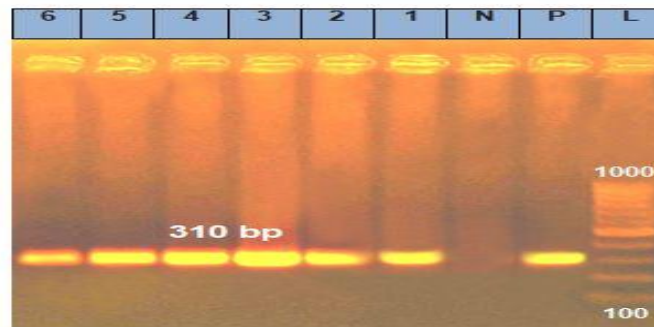


Fig. (8): PCR agarose gel electrophoresis pattern for *S. aureus mecA* gene identification at 310 bp.

L: Ladder from 100 bp to 1000 bp

Pos: Positive control: *S.aureus* ATCC 25923

Neg: Negative control: Field isolate that were tested and confirmed to be negative by PCR for the related genes in R.L.Q.P

Lane 1, 2, 3, 4, 5, 6: Positive amplification of *mecA* gene at 310 bp

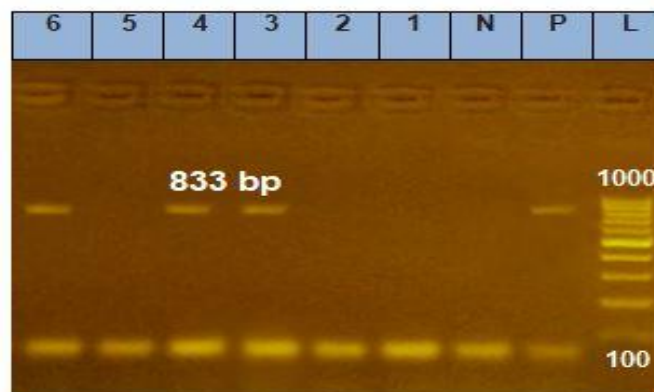


Fig. (9): PCR agarose gel electrophoresis pattern for *blaZ* gene of *S. aureus* detection at 833 bp.

L: Ladder from 100 bp to 1000 bp.

Pos: Positive control: *S.aureus* ATCC 25923.

Neg: Negative control: Field isolate that were tested and confirmed to be negative by PCR for the related genes in R.L.Q.P

Lane 3, 4, 6: Positive amplification of *blaZ* gene at 833 bp

Lane 1, 2, 5: Negative amplification of staphylococcal beta- lactam resistance (*blaZ*) gene.

4.DISCUSSION.

Incidence of foodborne illnesses has increased in recent years, creating a significant global public health issue. (21). Staphylococci pose a serious threat to public health since *S. aureus* is widely acknowledged as a significant food-borne pathogen due to its capacity to produce a variety of toxins and virulence factors, which often induce quick onset of nausea, vomiting, and abdominal cramps. (22) In the current study 25 isolates of *S. aureus* (25 %) were recovered from 100 samples which nearly agreed with the results of (23) and (24) who reported that *S. aureus* isolates in meat product samples were (23.81%) and (23.4%) respectively, But it disagreed with (25) who isolated *S.aureus* at incidence rate (50.8%), this high result was due to unhygienic conditions of food handlers and contamination of meat products during cutting and preparation.

S. aureus was isolated from burger samples at an incidence of (36%). Similar results were obtained by (26) and (27) who reported an incidence of (40%) and (33.3 %) respectively. Conversely, these results were considered low when compared to that of (23) and (28) who detected *S.aureus* at an incidence of (71.43%) and (56.6%) respectively The low incidence rate from burger may be attributed to the addition of some food additives that have antibacterial activity or due to the processing temperature during burger manufacture (29). While, the occurrence of isolation of *S. aureus* from chicken nuggets samples was(28%) which similar to (30) who reported *S. aureus* in chicken nuggets at (28%) and (31) who reported occurrence of *S. aureus* in chicken nuggets in a percentage of (23.3%). The obtained result in this study were lower than (28) who isolated *S.aureus* from chicken nuggets by (60%) which was attributed to poor hygiene of meat handlers as well as lack of sterilization of utensils.

While, the incidence of isolation of *S. aureus* from luncheon samples was (24%).

This result was similar to (32) and (33) who isolated *S. aureus* from luncheon (24%) and (25%) respectively. While higher results reported by (25) , Who isolated *S. aureus* from luncheon in a percentage of (56.6%), this may be attributed to excessive handling of luncheon with contaminated cutting knives and slicing machines. (34).

S. aureus was isolated from kofta samples with percentage 12% this result nearly similar to (35) and (36) who detected *S.aureus* at an incidence of (8%) and (12%) respectively . On the opposite side, (25) reported that occurrence of

S. aureus from kofta at percent 66.6 % this higher incidence may be due to microbial contamination during handling which indicate poor personal hygiene practices (37).

Antimicrobials are widely used in the veterinary field either as growth promoters or as prophylaxis. Antibiotic-resistant strains may emerge as a result of uncontrolled use of antibiotics or their administration at doses below therapeutic levels. (38)

All identified isolates in the current study were at least somewhat resistant to one or more of the employed antibiotics.

Twenty-five. *S. aureus* isolates were further examined for their susceptibility to antimicrobials. *S.aureus* were highly resistant for penicillin (96 %) followed by flucloxacillin. (92 %) , cefotaxime (68%) erythromycin(60 %) , clindamycin (61.54 %) , linezolid (36%) and vancomycin(28 %).

Nearly, similar results were obtained by (39) , who reported that *S.aureus* was 100% resistance to Penicillin , but lower result were detected by (40) , (83,6%).

Also, nearly similar result were obtained by (23) ; 57,5% (40); 52% and (34) 60% for erythromycin and 30% for vancomycin ; Moreover the susceptibility of the isolates to cefotaxime (68%) was close to that obtained by (41) and (25), who found it (58 .3%) and (55.7%) respectively; As well (31) recorded high resistance of *S.aureus* to clindamycin (81.5%) , Conversely, these results disagreed with that reported (42) for erythromycin (14.8%), (43) for vancomycin (5.9%), (44) for cefotaxime (10%) , (39) and (45)

for vancomycin (100%) and (0%) respectively ; (46) for penicillin (20.8%) and (47) who reported that none of the isolates were resistant to linezolid .

The low susceptibility of *S. aureus* to beta-lactam antibiotics observed in this study may be due to the production of beta-lactamase enzymes caused by modification of the drug target site (48) Polymerase chain reaction (PCR) has become, since its discovery in the 1980s, a powerful diagnostic tool for the analysis of microbial infections as well as for

the analysis of microorganisms in food samples (49).

Staphylococci have two primary resistance mechanisms to β -lactam antibiotics. One is the expression of β -lactamase enzymes, which destroy β -lactams by hydrolysis and are expressed by activation of the *blaZ* gene. The other is the expression of penicillin-binding protein 2a (PBP 2a), which is not susceptible to inhibition by beta-lactam antibiotics and are expressed by *mecA* gene that results in higher-level β -lactam resistance (MRSA) (3).

Staphylococci have virulence gene hemolysin (*hlg*); it could have an impact on virulence *S.aureus* is (50).

The findings of the present investigation showed that 50% the examined *S. aureus* isolates (3/6) harbored *blaZ* gene. which disagreed with (51) who detected *blaZ* gene in all the examined isolates (8/8) and (52) who detected *blaZ* gene in (24/25) of the examined isolates.

While, *mecA* gene was found in (6/6) of tested *S. aureus* isolates. This result agreed with (53) who detected it 6 out of 8 studied *S. aureus* strains, (54) found *mecA* gene in 68/82 of the examined isolates but our result disagreed with (55) who reported that 13 out of the 25 (52%) *S. aureus* isolates harbored *mecA* gene. Also, it disagreed with (52) who failed to detect *mecA* gene.

In this study, 3/6 (50%) of tested *S. aureus* isolates harbored *hlg* gene, disagreed with (56) who reported that 15 out of 20 (75%) *S. aureus* isolates from meat, luncheon, burger, minced meat and sausage harbored (*hlg*) gene.

CONCLUSION:

The results obtained from this study indicated that a high prevalence of *S. aureus* in examined meat products while MRSA was mainly isolated from burger (36%), chicken nuggets (28%), luncheon (24%) and kofta (12%), This bacterium species' presence in examined meat products may be a result of contamination during the products' production, shipment, storage, cutting, packaging, and retail sale. This subsequently raises consumer health risks, hence every precaution should be taken to maintain proper cleanliness during production, handling, and storage in order to manage these dangerous microorganisms and reach the highest level of consumer safety.

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