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Molecular characterization of some antibiotic resistant genes of *Staphylococcus aureus* isolated from different sources and human

Al-Abbou M.A[1,*], Ashraf A. Abd El-Tawab[1], Fatma I. El Hofy[1], Hend K. Sorour [2], Marwah H. Abd Ali [1], Hamouda,R.H [3]

[1] *Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Benha University, Egypt*

[2] *The Reference Lab. for Veterinary Quality Control on Poultry Production, Animal Health Institute – Dokki– Giza.*

[3] *Animal Reproduction Research Institute, Giza, Egypt*

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ABSTRACT

This work aims to study the genetic characteristics of *Staphylococcus aureus* (*S. aureus)* strains isolated from 200 samples collected from humans (mouth, pharynx and hand) and food as poultry, milk and human food (cocked Liver and burgers sandwich). Genetic characteristics were evaluated epeningon resistance genes against some antibiotics that may be used for treatment of infected cases. A total of 23 isolates of *S. aureus* were identified from the collected 200 samples in an incidence rate of 11.5%. The identified strains were screened for resistance against 11 different antimicrobial agents. The strains showed a high level of resistance about (85-90%) to methicillin, penicillin; tobramycin, trimethoprim, sulfamethoxazone-trimethoprim and ciprofloxacin. Furthermore, moderate resistance to gentamycin, levofloxacin and lomfloxacin about (40-60%), while clindamycin and tetracycline antimicrobial agents were had a very low resistance, reaching (5-10%). The isolated *S. aureus* strains were monitored for the most important resistant genes the incidence rate of *mec*A, *bla*Z and *tet*K were (100%), while the *aac(6'), aph(2")* and *nor*A were (45.5%) and (90.9%), respectively. This work revealed that about 70% of the isolated *S.aureus* strains were resistant to antibiotic drugs. Therefore, the miss, hub-hazard and uncontrolled use of antibiotics in veterinary medicine must be prevented.

1. INTRODUCTION

Staphylococcus aureus is a Gram-positive cocci, catalase positive, oxidase negative, immotile, non-spore forming, facultative anaerobes, aggregated in grapes like clusters, and sever toxinogenic bacterium, (Harris et al, 2002 and Jang et al, 2018) According to coagulase test, several coagulase positive and coagulase negative species of *S. aureus* were isolated from animal and human cases (Akindolire et al; 2015 and Alves et al, 2018). The *S. arueus* bacterium continues to be the main cause of threat to human and animal health. There is no vaccine for controlling and prevention *S. aureus* infections. It has resistant genes in its genetic structure against most antibiotic drugs used in the treatment of infected cases (Guest et al, 2016). The resistance pattern of *S. aureus* isolates is resistant to one or more antimicrobial agent. Also, some *S. aureus* isolates are multi-resistance, (Abo-Shama, 2014). The antibiotic resistance has been established for a variety of antimicrobial agents which include macrolides, aminoglycosides, fluoroquinolones, glycopeptides and tetracyclines (Akpaka et al, 2008). The methicillin-resistant *S. aureus* (MRSA) strains are expressed as to be the main cause of food-borne infection in hospitals. The pattern of antibiotic resistance and prevalence of antibiotic resistance genes of different biotypes of the MRSA strains isolated mainly from different types of hospital's food samples, (Dehkordi et al, 2017) and (Al Marjani, 2015). MRSA strains of both animal and human origins are believed to serve as important reservoirs of antimicrobial resistance genes which can transfer and integrate into the MRSA genome leading to the emergence of new and potentially more resistant strains. Reports showed that high presence of *mec*A, *aac*A-D and *tet*K antibiotic resistance genes in *S. aureus* strains isolated from foodstuffs caused severe occurrence of resistance against methicillin, aminoglycosides, and tetracyclines, respectively (Osman et al, 2016). Antibiogram assay showed that *S. aureus* isolates from poultry and human samples were identified as methicillin-resistant and owing also resistance for some types of antimicrobials including penicillin, tetracycline clindamycin. Application of PCR demonstrated that *S. aureus* isolates of poultry and human were harboring genes encoding for antimicrobial resistance *mec*A and *Bla*Z. (Mohamed et al, 2020). The resistance gene (*bla*Z) was detected in 13 (14.9%), of *S. aureus* isolates. The potential dissemination of multidrug-resistant *S. aureus* strains, Therefore the organism may rapidly spread through food and pose serious public health risk, (Pekana and Green, 2018). The antibiotic resistance genes play an important role in *S. aureus* resistance and including the macrolide resistance encoded by the *aph*A3 gene for kanamycin and streptomycin resistance and *acc*A-*aph*D and *tet*K genes for gentamicin, tobramycin and tetracycline resistance (DeLeo et al, 2009) and Que et al, 2015).The aim of this research

Correspondence to: mensoorali2016@gmail.com

is; determination of the genetic structure of isolated *S. aureus* strains with concerning to the encoded resistant genes specially genes that affecting the antibiotic used in the treatment of diseased cases.

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 200 samples of human samples (40) (swabs from mouth, pharynx and hand), (70) samples from poultry (fecal matter) and poultry byproducts (thighs, chest, gizzard and wings), (70) samples from milk (raw milk) and milk by products (cheese of different types, yoghurt and butter and ready to eat food (20) samples (cocked Liver and burgers sandwich), were collected aseptically using sterile scissor and forceps in separate sterile plastic bag for each sample. Each sample was subjected to bacteriological examination for *S. aureus* isolation (Monecke et al, 2012)

2.2. Preparation of samples (pre-enrichment)

The samples were collected from poultry and poultry byproducts, milk and milk byproducts, and ready to eat food. Ten grams of each sample were transferred into a flask containing 90 ml of 0.1% sterile peptone water to get a dilution of 10^{-1}, incubate at 37 ^{o}C for 24 hrs under aerobic condition (Sneath et al, 1986).

2.3. Bacterial Isolates and culture media

Isolated colonies were differentiated into coagulase positive and coagulase negative *Staphylococcus* isolates. The coagulase positive *Staphylococcus* isolates were identified by conventional methods, including morphological characters as Gram staining, blood hemolysis, catalase, coagulase and anaerobic fermentation of mannitol tests (Koneman et al, 2001). All isolated strains were stored on suitable maintenance media in the National Laboratory for further identification.

2.4. The antibiogram assays for determination the antimicrobial resistances genes of the isolated S. aureus (Koneman et al, 1979)

The antibiotic sensitivity tests were applied by the using of disk diffusion technique. Eleven types of antimicrobial agents as methicillin, penicillin, tobramycin, trimethoprim, sulfamethoxazon -trimethoprim, ciprofloxacin, gentamycin, levofloxacin, lomfloxacin, clindamycin and tetracycline was used. The interpretation of inhibition zone of tested culture was according to (Nccls, 2002).

2.5. DNA extraction and PCR

The stored isolated strains were re-cultivated on brain heart infusion agar plates and broth for an extraction of DNA to be used in PCR technique following *Riffon et al,* (*2001*). Five pairs of specific sequence primers were supplied from metabion (Germany) and Biobasic (Canada) as shown in Table (1). PCR amplification was performed with PTC-100 programmable thermal cycler (Peltier Effect cycling, MJ, Research, INC, UK) in a volume of 50 µl consisting of: 12.5µl of Emerald Amp GT PCR master mix (2x premix), 1 µl of 20 pmol of each primer for one sample, 6 µl of the DNA template and water, nuclease-free up to 25 µl in uniplex PCR. While, 25µl of Emerald Amp GT PCR master mix (2x premix), 1 µl of 20 pmol of each primer for one sample, 10µl of the DNA template and water, nuclease-free up to 50 µl in PCR.

Table (1): Specific oligonucleotide primer sequence used in this study

Gene	Primer sequence(5'-3')	Amplified product	Reference
MecA	F-GTA GAA ATG ACT GAA CGT CCG ATA A R-CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure et al., 2006
BlaZ	F-TACAACTGTAATATC-GGAGGG R-CATTACACTCTTGGC-GGTTTC	833 bp	Bagcigil et al. 2012
aac(6')aph (2")	F-GAAGTACGCAGAAG-AGA R-ACATGGCAAGCTCTA-GGA	491 bp	Duran et al., 2012
NorA	F-TTCACCAAGCCATCA-AAAAG R-CTTGCCTTTCTCCAG-CAATA	620 bp	Pourmand et al., 2014
TetK	F-GTAGCGACAATAGGT-AATAGT R-GTAGTGACAATAAAC-CTCCTA	360 bp	Duran et al., 2012

F: forward primer, R: reverse primer

3. RESULTS

3.1. Isolation of S. aureus from the examined samples

S. aureus was detected in 11.5% (23 from 200 total samples) poultry products positive samples were 10 % (7/70), human samples 17.5% (7/40), milk and milk byproducts 8% (6/70) and ready to eat food 15 % (3/20) samples) as presented in table (2). *S. aureus* isolates were Gram positive coccoi arranged in clusters. They had golden yellow colonies surrounded by yellow medium due to mannitol utilization on mannitol salt agar medium (figure 1). The isolates had black shiny convex colonies surrounded by a clear zone extended onto the opaque medium on baired parker agar medium (figure 2).

Table (2): The prevalence of S. *aureus* strains in the collected samples

Type of Sample	Number of Samples	*S. aureus* isolation	
		Positive No.	%
Poultry*	70	7	10%
Human	40	7	17.5%
Milk**	70	6	8%
Ready to eat food	20	3	15%
Total	200	23	11.5%

*poultry and poultry byproducts, ** milk and milk byproducts

3.2. The antibiogram assays for determination the antimicrobial resistances genes of the isolated S. aureus

The application of sensitivity tests on the isolated *S. aureus* strains for screening the ability of resistant genes against the used antibiotic discs. The recorded results showed a high level of resistance about (85-90%) to methicillin, penicillin; tobramycin, trimethoprim, sulfamethoxazone-trimethoprim and ciprofloxacin. Furthermore, moderate resistance to gentamycin, levofloxacin and lomfloxacin about (40-60%). While the clindamycin and tetracycline antimicrobial agents had a very low resistance, reaching (5-10%), as shown in figure (3).

3.3. Detection of resistant genes in the isolated coagulase positive S. aureus by using PCR

Using 5 different genes (*tet*K, *mec*A, *bla*Z, *nor*A and *aac(6') aph (2")*) for detection of resistant genes in isolated *S. aureus*. The genes (*tet*K, *mec*A and *bla*Z*)* were the most frequent resistant genes representing (100%), while the *nor*A gene was represented by (90%). On the other hand the *aac(6')aph(2")* gene was determined in (45%).

Fig (1): mannitol salt agar showed yellow colonies convex surrounded by yellow medium

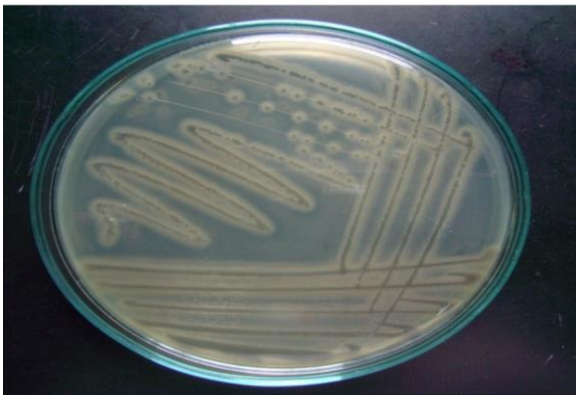

Fig (2): Baired parker agar showed black shiny colonies surrounded by a clear zone

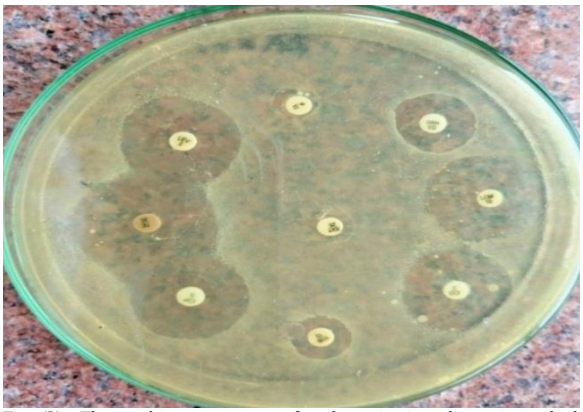

Fig (3): The antibiogram assayes for determination the antimicrobial resistances genes of the isolated *S. aureus*

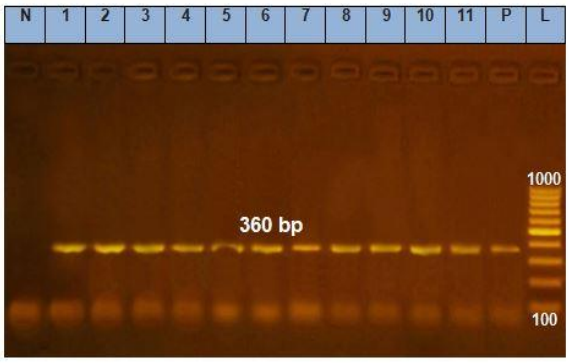

Fig.4. Agarose gel photo documentation for detection virulence factor encoding gene (*tet*K) of *S.aureus* as a genotyping identification of the isolates. Lane: 100-1000 bp DNA ladder. Pos: positive control (at 360 bp). Lanes 1,2,3,4,5,6,7,8,9,10 and11 *S.aureus* (*tetk) gene* positive

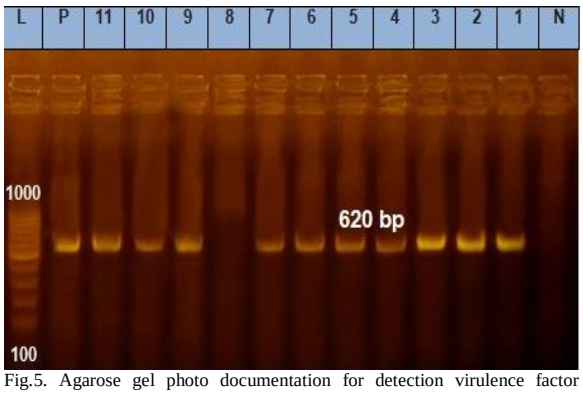

Fig.5. Agarose gel photo documentation for detection virulence factor encoding gene (*nor*A) of *S.aureus* as a genotyping identification of the isolates. Lane: 100-1000 bp DNA ladder. Pos: positive control (at 620 bp). Lanes 1,2,3,4,5,6,7,9,10 and11 *S.aureus* (*nor*A *) gene* positive

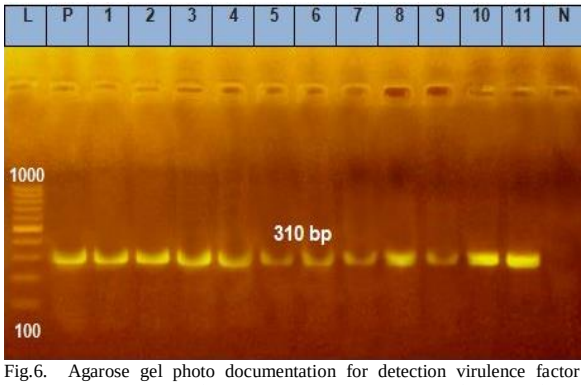

Fig.6. Agarose gel photo documentation for detection virulence factor encoding gene (*mec*A) of *S.aureus* as a genotyping identification of the isolates. Lane: 100-1000 bp DNA ladder. Pos: positive control (at 310 bp). Lanes 1,2,3,4,5,6,7,8,9,10 and11 *S.aureus* (*mec*A*) gene* positive

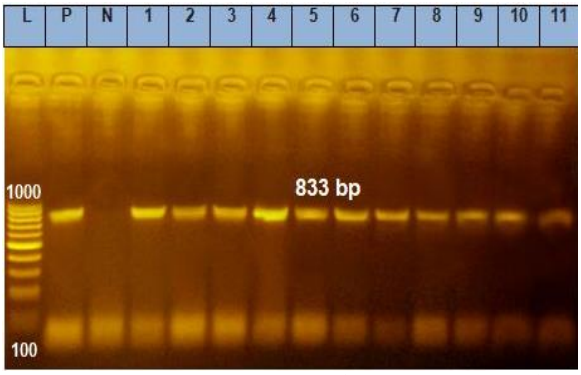

Fig.7. Agarose gel photo documentation for detection virulence factor encoding gene (*bla*Z *gene*) of *S.aureus* as a genotyping identification of the isolates. Lane: 100-1000 bp DNA ladder. Pos: positive control (at 833 bp). Lanes 1,2,3,4,5,6,7,8,9,10 and11 *S.aureus* (*bla*Z *)gene* positive

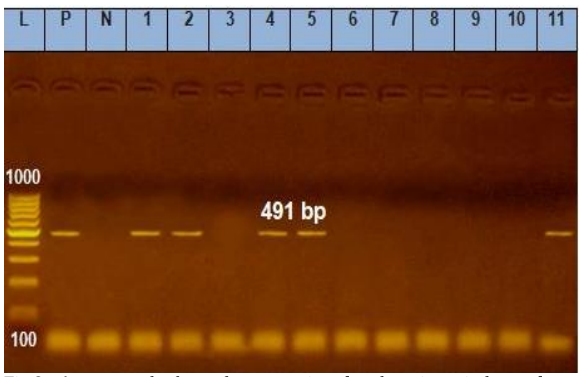

Fig.8. Agarose gel photo documentation for detection virulence factor encoding genes (*aac(6')aph (2'')*) of *S.aureus* as a genotyping identification of the isolates. Lane: 100-1000 bp DNA ladder. Pos: positive control (at 491 bp). Lanes 1,2 ,4,5and11 *S.aureus* (*aac(6')aph (2''))gense* positive

Table (3): Detection of resistant genes in the isolated coagulase positive *S.aureus* by using PCR

Sample	mecA	BlaZ	TetK	aac(6')aph (2")	norA
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	-	+
4	+	+	+	-	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	-	+
8	+	+	+	-	-
9	+	+	+	-	+
10	+	+	+	-	+
11	+	+	+	+	+

4. DISCUSSION

This work was carried out for explaining the prevalence of the most important resistant genes of *S. aureus* in 23 positive samples from the total 200 samples, represented by (11.5%). These resistant genes play an interesting role in the treatment and controlling of the infected cases with *S. aureus* strains. Inability to treat and cure the *S. aureus* infected and diseased animal and human cases, seemed to be due to the resistance of the M.O to many antimicrobial drugs Abo-Shama, (2014).The prevalence rate of *S. aureus* (11.5%) was in agreement with the result of Pekana and Green, (2018) which was (11.2%), from raw milk and meat, while Bendary et al, (2016) of a prevalence (19.1%) from human samples, this result did not agree with ABO-SHAMA, (2014) and Sonia et al, (2021) of (44.8%) from raw milk of (cattle buffalo, sheep and goat) and (54%) from frozen chicken meat respectively. Eleven isolates showed a positive result for the following resistant genes (*mecA, blaZ, and tetK*) with a percentage of (100%), this was resembling to Abd El- Tawab et al, (2017), with the results of (100%) for the genes (blaZ and mecA). But these results didn't match those corresponding to Ewelina et al, (2019), the *blaZ* (for ß-lactam) and *mecA* (for methicillin resistance) genes were in (58.3%) and (27.5%) respectively, and Duran et al, (2012) of (25%) for both resistant genes (*blaZ and mecA*). while Dehkordi et al, (2017) record that the titK gene at 72.97% nearly the same results were obtained by Argudín et al, (2012), where the results showed that the genes (blaZ and tetK)) were present by % 61.3 and % 3.2. On the other hand, Sonia et al, (2021) screening of methicillin resistance gene revealed that (43.5%).The gene (*norA*) was positive in most of the isolates by (90%), this high prevalence of this resistant gene may be due to the miss, un-controlling and hubhazzared use of antimicrobial drug in the treatment of the infected and diseased animal or human cases. The owing prevalence of the gene (*norA*), was represented in (90%). This high prevalence was elevated than that of Al Marjani, (2015) in (47%). As for the gene (*aac (6 ') aph (2'))*, the result was positive in (5 isolates) and at a rate of (45%) this corresponds to some extent with Azmiza et al, (2019) percentage (40%). The obtained result for the gene (*aac (6 ') aph (2'))* represented a very high incidence than that the results tabulated by Argudín et al, (2012) showed that the gene (*aac (6 ') aph (2'))* by (6.5 %) and Hamdi et al, (2018) aacA-aphD 6 was (18.18%). and Chen et al, (2021) in (11.4%). The main used antimicrobial drugs in this study were 11 antimicrobial drugs, including methicillin (MET-30), tobramycin (TOB-10), penicillin (P-10), trimethoprim (TRI-30), sulphamethoxazole-trimethoprim (STX-25), and ciprofloxacin (CIP-10), were had about (85-90%) resistance against the isolated *S.aureus* stains. The obtained results were accepted with that of Robert et al, (2013) and Richart, (2015). While this result did not be accepted with Abdeen et al, (2021). While gentamycin (CN-10), levofloxacin (LEV-5) and lomefloxacin (LOM-10) were of moderate resistance ranged from (40% to 60%), this was achieved with Abdeen et al, (2021). The clindamycin (CD-5) and tetracycline (TE-30) antimicrobial agents had a very low resistance, reaching (5-10%). This did not match with Mama et al, (2019). From this work about 70% of the isolated *S. aureus* strain were resistant against the most used antibiotic agents, so the use of antimicrobial agents must be under many restrictions and flowing up the rules of the used antimicrobial agents and consequently preventing the miss, hub-hazard and uncontrolled use of antibiotics in veterinary medicine.

5. CONCLUSION

The distribution of antibiotic resistance genes were related to *S. aureus* strains resulting in the presence of multi-drug resistance and also simultaneous presence of several antibiotic resistance genes that affecting the public health.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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