Research Article

Molecular Comparison Between Resistance Genes in *Staphylococcus aureus* Clinically Isolated from Cattle and Camels in Southern Egypt

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Abstract | *S. aureus* is one of the most common organisms associated with infections among dairy animals with frequent and rapid development of antibiotic resistance. The presence of genes encoding aminoglycoside resistance tetracycline resistance, and beta-lactam resistance were detected by whole genome sequencing. Seventy-nine *S. aureus* clinical isolates were isolated from milk samples of dairy cattle and she-camels in Southern Egypt. The sequences of resistant genes extracted from the whole genome sequences of the bacterial chromosome that was done through shot-gun sequencing, through phylogenetic analysis, all resistant genes sequences were arranged into two main clusters. The first clusters contain *mecA* genes, aminoglycosides, *fusB* gene as well as *blaz* gene except A11, and two reference genes (AJ302698 and AP003139). The second cluster contains mainly the *tet(k)* and *tet(38)* genes which are more similar to each other, in addition to *mecA* reference gene (AB512767) and two *blaz* reference genes (AJ302698 & AP003139). The phylogenic tree revealed that all resistant genes are more or less stable genes such as *mecA*, aminoglycosides, *tet(k)*, *tet(38)*, and *fusB* except *blaz* gene showing more variations. Molecular detection continues to be increased to establish antimicrobial resistance trends for *S. aureus* found in subclinical mastitis of dairy animals.

Keywords | Antibiotic resistance, Camel, Cattle, *S. aureus*, Egypt

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INTRODUCTION

Resistance to antimicrobial agents is regarded as one of the world's main and increasingly global issues, especially among nosocomial pathogens. *Staphylococci* have been one of the most common causes of nosocomial infections. Multidrug-resistant staphylococci pose a rising alarm for public health. The rise of drug-resistant virulent strains of *S. aureus*, particularlymethacillin resistant *S. aureus* (MRSA) is a severe problem in the treatment and control of staphylococcal infections (Livermore, 2000; Zapun et al., 2008; Neamat-Allah and Hend, 2016). *Staphylococci* resistance to methicillin (MRSA) makes infections difficult to treat since they are resistant to most antibiotics such as beta-lactam, aminoglycosides, and macrolides. The recent series of numerous *S. aureus* gene and bioinformatics are highly promising in recognizing and characterizing target genes (Land et al., 2015; Everitt et al., 2014). It may also be inferred from the pathways by which genes have antibiotic resistance. These genes may also be used for a greater understanding of pathogenesis and pathogen; therefore, it is of interest to predict its mechanism by identifying the unique antibiotic-resistant genes from the genomes of different *S. aureus* strains (Everitt et al., 2014).

MecA, mecR1, and mecI are the main methicillin resistances

proteins that have been identified and expressed by most strains. The genes were well studied as analyses in most *S. aureus* strains, including MRSA (Ito et al., 2003; Monecke et al., 2010; Stegger et al., 2012), with antibiotic-resistant genes. The *mecR1* analysis of antibiotic-resistant genes plays a role in penicillin interactive as a potency anti-represent for methicillin resistance. Lowy has explored the various pathways by which these genes contribute to antibiotic resistance (Lowy, 2003). *MecI* and *mecR1* of antibiotic-resistant genes regulate *mecA* reaction to β-lactam antibiotics in a method like the regulation of *blaZ* by *blaR1* and *blaI* genes in penicillin sensitivity (Lowy, 2003; Stegger et al., 2012). The main mechanism for penicillin resistance is triggered by the development of an exogenous *mecA* gene, which encodes an extra penicillin-resistant β-lactam protein called PBP-2.

Aminoglycoside resistance mechanisms include decreased absorption, reduced cell permeability, ribosomal binding site change by mutation expression of rRNA methylases, and aminoglycoside modification enzyme production. The *aacA-aphD* gene resistance to all aminoglycosides, except streptomycin and neomycin; it is clinically used to encode the bifunctional enzyme amino glycoid-6-N-acetyltransferase/2-O-nucleotidytranferencease (van Den Braak et al., 1999; Landecker, 2016).

Aminoglycosides are broad range antibiotics that are used to treat *S. aureus* in combination with other antibiotics, such as ß- lactams (Ramirez and Tolmasky, 2010). The most common cause of aminoglycoside resistance is the non-activation of aminoglycoside antibiotics by aminoglycoside modified enzymes (such as aminoglycoside phosphotransferase, acetyltransferases, nuclear transferase enzymes). *Aac(6')-Ie-aph(2), «aph(3')-IIIa and ant(4')-Ia*, which can be found on plasmid or chromosomes and often contain transposable components, are the typical aminoglycoside-modifying enzymes that encode *S. aureus* (Ramirez and Tolmasky, 2010; Fatholahzadeh, 2009; Emaneini et al., 2009).

Tetracyclines have been used to treat and prevent bacterial infection with a broad spectrum of antibiotics. (Ardic et al., 2005) Many tetracycline-resistant bacteria have developed tetracycline resistance genes. Staphylococcus aureus has two major resistance mechanisms: aggressive efflux arising from the acquisition of *tetK*- and *tetL* genes found in plasmid and ribosomal protection by elongation factors of *tetM*- or *tetO* (Esposito et al., 2009; McCallum et al., 2010). The topical agent for infection of the skin and certain systemic infections caused by *S. aureus* was fusidic acid (Besier et al., 2003). *S. aureus* has two main fusidic acid resistance mechanisms reported: altering the target drug caused by *fusA* (Nagaev et al., 2001; O'Neill et al., 2004; Norström et al., 2007) or *rplF* (Lannergård et al., 2009) mutations and defending the drug target site with *fusB*, *fusC*, and *fusD* (Ardic et al., 2005; Ramirez and Tolmasky, 2010). *FusB*, *FusC* and *FusD* were found in *S. aureus* and coagulase-negative staphylococci (McLaws et al., 2008; O'Neill and Chopra, 2006; O'Neill et al., 2007) and *fusD* was an intrinsic factor causing fusidic acid resistance in *S. saprophyticus* (O'Neill et al., 2006).

MATERIALS AND METHODS

A total 240 milk samples included (140 milk samples from dairy cattle and 100 milk samples from she-camels) were collected in sterile plastic tubes from the Red Sea and Qena governorates, Egypt. All animals were apparently healthy and they did not show any signs of clinical mastitis. The milk samples were directly transferred in icebox to the laboratory of infectious diseases, department of animal medicine, Faculty of Veterinary Medicine, South Valley University. Animals have been tested concerning the presence of subclinical mastitis. Clinical parameters including body temperature, heart rate, and respiratory rate were recorded. Animals with no local or systemic infection were considered healthy.

Isolation and culturing of *Staphylococcus aureus*

The obtained milk samples (10 µL) were inoculated onto 5% sheep blood agar (Oxoid) incubated at 37°C for 24 hours. The suspected colonies of *S. aureus* were subculture on Baired-Parker media (Oxoid). A few standard colonies (black colonies with clear zone around) were collected and picked up by a bacteriological loop, then immersed in nutrient glycerol broth stock and preserved for further identification at -80 ° C.

Microbiological data

All isolates were tested for different bacteriological assays to detect the most properties of S. aureus, such as bacterial sensitivity tests. Multiplex PCR used to classify *S. aureus* by using femB, mecA, and mecC genes into MRSA and MSSA. The data was published previously (Ali, 2015; Ali et al., 2015; Ali, 2016; Ali and El Sherief, 2016).

Biochemical tests

Gram staining, characteristic features of colonies on Baired-Parker media (Oxoid), hemolysis patterns on blood agar enriched with 5% (v/v) sheep blood, catalase and coagulase tests were used to identify *S. aureus* isolates. Two separate types of coagulase testing were used, namely the slide coagulase test and the tube coagulase test (Field and Smith, 1995).

Detection of resistant genes

The classification of *S. aureus* into methicillin-susceptible or resistant (MSSA or MRSA) was confirmed by using *mecA* gene. Multiplex PCR was used to ensure the presence of *mecA* gene, in addition to the *mecC* gene, which was detected recently as a homolog for *mecA* and the *femB* gene used as a confirmatory gene for *S. aureus* (Nakagawa et al., 2005; Paterson et al., 2014). For Detection of *mecA, blaZ, aac(6')- aph(2"), aph(3')-III, ant(6)-Ia, tet(K), tet(38), and fusB* genes. The genes were extracted from the *S. aureus* genome obtained by shotgun sequencing (Sanger Institute, UK). *mecA, blaZ, aac(6')- aph(2"), aph(3')-III, ant(6)-Ia,, tet(K), tet(38)*, and *fusB* genes were extracted from the bacterial genome of local bacterial isolates (A1, A2, A3, A4, A5, A6, A7, A10, A11, A12, A13, A15). The following resistant genes (9 sequences) were retrieved from the NCBI GENEBANK and their accession numbers (M13771, AB512767, U38428, AY825285, M26832, AF330699, AB033763, AJ302698, AM292600, AP004832, AP003139, J01764, and FR821779).

Whole-genome sequencing

The DNA of The *S. aureus* both resistant and susceptible strains (MRSA and MSSA) were extracted and the genomic DNA used to obtain the whole genome sequence of *S. aureus* by shotgun sequencing (Sanger Institute, UK) which divided into several contigs and by using several bioinformatics tools, the bacterial chromosome sequence can be obtained, aligned and evaluated.

Bioinformatic analysis

The genome sequence of *S. aureus* was manipulated by using Artemis. This is a free genome browser and annotation tool that allows the visualization of sequence characteristics, next-generation details, and study findings within the sequence (Ali, 2016; Rutherford et al., 2000). Using BIOEDIT sequence alignments, translations, and comparisons were performed. (Hall, 1999). Gene Bank databases search for homologoue sequences and the BLAST algorithm was used. Based on genetic distances, neighbor-joining trees were constructed and calculated by the two-parameter method using MEGA6 (Kimura, 1980; Saitou and Nei, 1987; Tamura et al., 2013). Five hundred bootstrap confidence values estimated the reliability of the trees.

RESULTS

48 *S. aureus* out of 140 milk samples were isolated from dairy cows and 31 *S.aureus* out of 100 milk samples were isolated from she- camels. The result of the presence or absence of some resistance genes in *S. aureus* isolated from cattle (A1, A2, A3, A4, A5, A6, A7, and A10). A1 isolate showed that there were 3 genes, *aac(6')-aph(2")*, *mecA*, and *tet(K)* were identically 100 % to its corresponding reference genes, but in the case of tet(38) gene, it was the identically 99.85% (Table 2). The absence of *blaZ, aph(3')-III, ant(6)-Ia*, and *fusB* genes (Table 1). A2 isolate had 3 genes, *aac(6')-aph(2")* and *mecA* were identically 100 %, but in the case of *tet*(*38*) genes the percent of identically 99.85% compared with reference genes and absences of *blaZ*, *aph(3')-III, ant(6)-Ia, str, tet(K),* and *fusB* genes (Table 1). A3 isolate, there were 6 genes *aph(3')-III, ant(6)-Ia, mecA, blaZ, fusB and tet(K)* were identically 100 % and *tet(38)* was identically by 99.85% (Table 2) and there were absent of *aac(6'), and aph(2")*genes (Table 1). A4 isolate had 2 genes, *mecA and blaZ* were identically100 % but, the *tet(38)* genes identically were 99.85% compared with genes in Gene bank (Table 2), and there were absent of *aph(2"), aph(3')-III, ant(6)-Ia, tet(K), and fusB* genes (Table 1). A5 isolate had 2 genes *aac(6')-aph(2") and mecA* were identically 100 % but, the *tet(38), blaZ and tet(K)* were identically 99.85%, 99.23% and 99.89% (Table 2), in addition to, absences of *aph(3')-III, ant(6)-Ia, and fusB* genes (Table 1). A6 isolate had 3 genes *aac(6')-aph(2")* and *mecA* were identically100 % but in the case of *tet(38)* and *tet(K)* genes were 99.85% and 99.89% identical to its reference genes (Table 2), *blaZ, aph(3')-III, ant(6)-Ia, and fusB* genes were absent (Table 1). A7 isolate had 3 genes *aac(6')-aph(2"), mecA and tet(K)* were identically100 %, but in case of *tet(38)* genes was identically 99.85% (Table 2), and there were absent of *blaZ, aph(3')-III, ant(6)-Ia, and fusB genes* (Table 1). Strain A10 had only one gene *blaZ* was identically 100 %, and *tet(38)* genes was identically 98.97% (Table 2), and there were absence of *mecA, aac(6'), aph(2"), aph(3')-III, ant(6)-Ia, tet(K),and fusB* genes (Table 1). The result of the presence and absence of resistance genes in *S. aureus* isolated in this study from she-camel included isolates A11, A12, A13, and A15. A11 isolate had only one genes *blaZ* was identically 100 %, but the *tet(38)* genes identically 99.97% (Table 2), and the absences of *mecA, aac(6'), aph(2"), aph(3')-III, ant(6)-Ia, tet(K), and fusB* genes (Table 1). A12 andA13 isolates showed that there is one gene *tet(38)* was identically 99.63 % (Table 2) in addition to the absence of *mecA, blaZ, aac(6'), aph(2"), aph(3')-III, ant(6)-Ia, tet(K), and fusB* genes (Table 1). A15 isolate had one gene *blaZ* was identically 100 %, but in case of the *tet(38)* genes was similar 99.97% to its reference gene (Table 2) in addition to the of absence of *mecA, aac(6'), aph(2"), aph(3')-III, ant(6)-Ia, str, tet(K), and fusB* genes (Table 1). *Str* gene (Aminoglycoside 6-adenylyltransferase) was absent from all isolates of *S. aureus* originated from cattle and she camels (Table 1).

Evolutionary analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maxi

Table 1: Presence and absence of resistance genes in different *Staphylococci aureus* isolate from cattle and camels

No.	Isolates	Animals	Species	Presence and absence of resistance genes									
				mecA	*blaZ*	*aac(6')*	*aph(2")*	*aph(3')-III*	*ant(6)-Ia*	*str*	*tet(K)*	*tet(38)*	*fusB*
1	A1	Cattle	*S. aureus*	100%	Nil	100%	100%	*Nil	Nil	Nil	100%	99.85%	Nil
2	A2	Cattle	*S. aureus*	100%	Nil	100%	100%	Nil	Nil	Nil	Nil	99.85%	Nil
3	A3	Cattle	*S. aureus*	100%	100%	Nil	Nil	100%	100%	Nil	100%	99.85%	100%
4	A4	Cattle	*S. aureus*	100%	100%	Nil	Nil	Nil	Nil	Nil	Nil	99.85%	Nil
5	A5	Cattle	*S. aureus*	100%	100%	100%	100%	Nil	Nil	Nil	99.89%	99.85%	Nil
6	A6	Cattle	*S. aureus*	100%	Nil	100%	100%	Nil	Nil	Nil	99.89%	99.85%	Nil
7	A7	Cattle	*S. aureus*	100%	Nil	100%	100%	Nil	Nil	Nil	100%	99.85%	Nil
8	A10	Cattle	*S. aureus*	Nil	100%	Nil	Nil	Nil	Nil	Nil	Nil	98.97%	Nil
9	A11	Camel	*S. aureus*	Nil	100%	Nil	Nil	Nil	Nil	Nil	Nil	98.97%	Nil
10	A12	Camel	*S. aureus*	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	99.63%	Nil
11	A13	Camel	*S. aureus*	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	99.63%	Nil
12	A15	Camel	*S. aureus*	Nil	99.88%	Nil	Nil	Nil	Nil	Nil	Nil	99.56%	**Nil**

*Nil=Nothing

Table 2: Identity percent of resistance genes in different *Staphylococci aureus* isolate

Isolates	Resistance gene	Identity percent	Query/HSP length	Contig	Position in contig	Predicted phenotype	Accession number
A1	*aac(6')-aph(2")*	100	1440 / 1440	EMBOSS_001	2651606..2653045	Aminoglycoside resistance	M13771
	mecA	100	2010 / 2010	EMBOSS_001	2756089..2758098	Beta-lactam resistance	AB512767
	tet(K)	100	1380 / 1380	EMBOSS_001	2762824..2764203	Tetracycline resistance	U38428
	tet(38)	99.85	1353 / 1353	EMBOSS_001	679533..680885	Tetracycline resistance	AY825285
A2	*aac(6')-aph(2")*	100	1440 / 1440	EMBOSS_001	2774658..2776097	Aminoglycoside resistance	M13771
	mecA	100	2010 / 2010	EMBOSS_001	2763972..2765981	Beta-lactam resistance	AB512767
	tet(38)	99.85	1353 / 1353	EMBOSS_001	849985..851337	Tetracycline resistance	AY825285
A3	*aph(3')-III*	100	795 / 795	EMBOSS_001	2714370..2715164	Aminoglycoside resistance	M26832
	ant(6)-Ia	100	909 / 909	EMBOSS_001	2715796..2716704	Aminoglycoside resistance	AF330699
	mecA	100	2007 / 2007	EMBOSS_001	2728940..2730946	Beta-lactam resistance	AB033763
	blaZ	100	846 / 846	EMBOSS_001	2740917..2741762	Beta-lactam resistance	AJ302698
	fusB	100	642 / 642	EMBOSS_001	2860095..2860736	Fusidic acid resistance	AM292600
	tet(38)	99.85	1353 / 1353	EMBOSS_001	2402116..2403468	Tetracycline resistance	AY825285
	tet(K)	100	1380 / 1380	EMBOSS_001	2824205..2825584	Tetracycline resistance	U38428

A4	*mecA*	100	2007 / 2007	EM-BOSS_001	1645898..1647904	Beta-lactam resistance	AB033763
	blaZ	100	846 / 846	EM-BOSS_001	2734106..2734951	Beta-lactam resistance	AP004832
	tet(38)	99.85	1353 / 1353	EM-BOSS_001	2683841..2685193	Tetracycline resistance	AY825285
A5	*aac(6')-aph(2")*	100	1440 / 1440	EM-BOSS_001	2086295..2087734	Aminoglycoside resistance	M13771
	blaZ	98.23	846 / 846	EM-BOSS_001	2647711..2648556	Beta-lactam resistance	AP003139
	mecA	100	2010 / 2010	EM-BOSS_001	2677031..2679040	Beta-lactam resistance	AB512767
	tet(38)	99.85	1353 / 1353	EM-BOSS_001	2011056..2012408	Tetracycline resistance	AY825285
	tet(K)	99.89	888 / 888	EM-BOSS_001	2779341..2780227	Tetracycline resistance	J01764
A6	*aac(6')-aph(2")*	100	1440 / 1440	EM-BOSS_001	2768296..2769735	Aminoglycoside resistance	M13771
	mecA	100	2010 / 2010	EM-BOSS_001	2719292..2721301	Beta-lactam resistance	AB512767
	tet(38)	99.85	1353 / 1353	EM-BOSS_001	2089322..2090674	Tetracycline resistance	AY825285
	tet(K)	99.89	888 / 888	EM-BOSS_001	2761281..2762167	Tetracycline resistance	J01764
A7	*aac(6')-aph(2")*	100	1440 / 1440	EM-BOSS_001	2622102..2623541	Aminoglycoside resistance	M13771
	mecA	100	2010 / 2010	EM-BOSS_001	2758893..2760902	Beta-lactam resistance	AB512767
	tet(38)	99.85	1353 / 1353	EM-BOSS_001	1743374..1744726	Tetracycline resistance	AY825285
	tet(K)	100	1380 / 1380	EM-BOSS_001	2765067..2766446	Tetracycline resistance	U38428
A10	*blaZ*	100	846 / 846	EM-BOSS_001	552101..552946	Beta-lactam resistance	AP004832
	tet(38)	98.97	1353 / 1353	EM-BOSS_001	2461719..2463071	Tetracycline resistance	FR821779
A11	*blaZ*	100	846 / 846	EM-BOSS_001	552101..552946	Beta-lactam resistance	AP004832
	tet(38)	98.97	1353 / 1353	EM-BOSS_001	2461719..2463071	Tetracycline resistance	FR821779
A12	*tet(38)*	99.63	1353 / 1353	EM-BOSS_001	680449..681801	Tetracycline resistance	FR821779
A13	*tet(38)*	99.63	1353 / 1353	EM-BOSS_001	236737..238089	Tetracycline resistance	FR821779
A15	*blaZ*	99.88	846 / 846	EM-BOSS_001	2775234..2776078	Beta-lactam resistance	AP004832
	tet(38)	99.56	1353 / 1353	EM-BOSS_001	1399661..1401013	Tetracycline resistance	AY825285

mum Likelihood method and Tamura-Nei model (Tamura et al., 2013). The tree with the highest log likelihood (-8731.04) is shown. The initial tree for the heuristic search's initial tree was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths meas-

ured in the number of substitutions per site. This analysis involved 51 nucleotide sequences. There was a total of 678 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kimura, 1980; Saitou and Nei, 1987; Tamura et al., 2013). All resistant genes sequences were arranged into 2 main clusters. The first clusters contain *mecA* genes, aminoglycosides, *fusB* gene, and *blaz* gene except for A11 and 2 reference genes (AJ302698 & AP003139). The second cluster contains mainly the *tet(k)* and *tet(38)* genes which are more similar to each other, except some genes that related to the first cluster such as *mecA* reference gene (AB512767) and 2 *blaz* reference genes (AJ302698 & AP003139). The tree revealed that all resistant genes are more or less stable genes such as *mecA*, aminoglycosides, *tet*(k), *tet*(38), and *fusB* except *blaz* gene showing more variations (Figure 1).

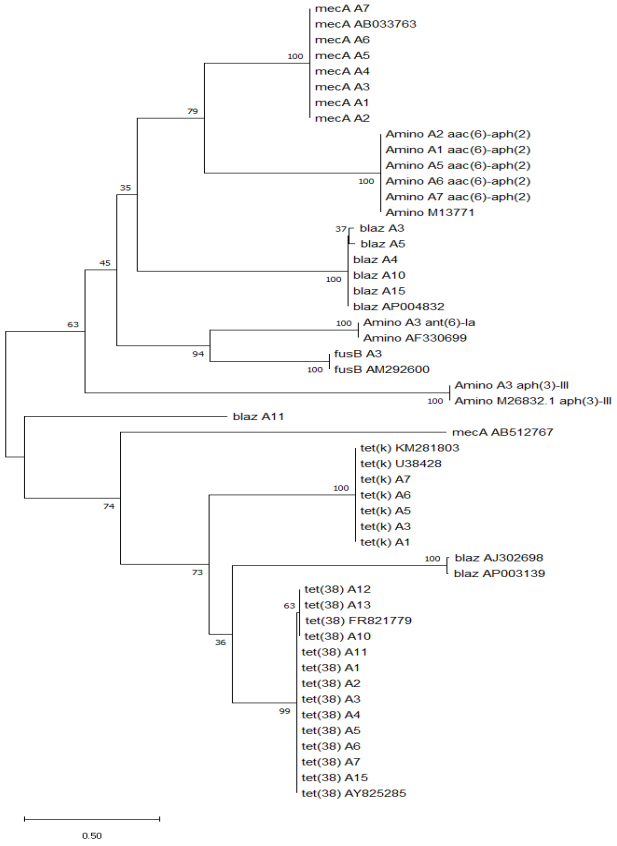

Figure 1: Molecular phylogenetic analysis by maximum likelihood method of resistance genes in different *Staphylococci aureus* isolates

DISCUSSION

The resistance of many widely used antimicrobial medications is one of the problems facing the management of infection with *S. aureus* (Bougnom and Piddock, 2017; Luepke et al., 2017). When *S. aureus* was first discovered, it was simple to treat using available antibiotics. Few years after the use of penicillin in 1940 to treat *S. aureus*; there were strains of different pathogens resistant to these antibiotics. After that the methicillin was developed and introduced to treat penicillin-resistant *S. aureus* strains in 1961 (Landecker, 2016). The mode of action of penicillin, and methicillin is very similar and involves inhibiting cell wall synthesis by blocking the peptidoglycan formation. In less than a year after methicillin; strains of *S. aureus* have been reported to be resistant to methicillin, and those strains have steadily spread globally (Vanderhaeghen et al., 2012; Fair and Tor, 2014).

Conversely, *S. aureus* strains carrying *tetK* gene only have been described as resistant to tetracycline, but susceptible to minocycline (Bismuth et al., 1990; Schwarz et al., 1998). The *tetM* gene is supposed to resist all drugs available in the group, including tetracycline and minocycline. Most isolates with *TetM*-positive gene and MRSA are typically *tetM* or *tetKM*. The *tetL* gene was only found in isolates already containing *tetM* gene from *S. aureus* (Bismuth et al., 1990), however, in vitro *S. aureus* induces both drug efflux and ribosomal protection (Mojumdar and Khan, 1988; Nesin et al., 1990).

In 1944, aminoglycosides were added, and by the 1950s, strains of *S. aureus* were aminoglycoside resistant. Through energy-dependent binding to the cell wall and energy-dependent transport through the cytoplasmic membrane, these drugs penetrate bacterial cells, eventually binding to one or more ribosomal sites, thus inhibiting protein synthesis (Maranan et al., 1997). Moreover, it is reported that Staphylococci resistance were resulted from: (1) a chromosomal defect leading to ribosome-binding altered aminoglycosides, (2) inadequate delivery of aminoglycosides into the bacterial cell, causing poor cross-resistance to most aminoglycosides, and, most generally, and (3) aminoglycoside enzyme modulation (Lowy, 2003). In the last case, resistant strains coded for aminoglycoside acetyltransferases, phosphotransferases, and adenyltransferases have the aminoglycoside-modifying genes acc, aph and ant (Maranan et al., 1997; Woodford, 2005). The acetylated, phosphorylated, or adenylated aminoglycosides do not bind to ribosomes and do not inhibit protein synthesis (Woodford, 2005). *S. aureus* is one of the most common organisms associated with infections among dairy animals with frequent and rapid development of antibiotic resistance. Tetracycline resistance genes have been identified; the main mechanisms conferring resistance to tetracycline to bacteria are active efflux proteins, ribosomal protection proteins, and enzymatic inactivation (Michalova et al., 2004). The aac *(6')-Ie-aph(2")* gene that inactivates a broad range of the majority of aminoglycosides has also been detected in these isolates in our study. In this study, all resistant genes sequences were arranged into 2 main clusters. The first clusters contain *mecA* genes, *aminoglycosides*, *fusB* gene, and *blaz* gene except for A11 and 2 ref-

erence genes (AJ302698 & AP003139). The second cluster contains mainly the *tet(k)* and *tet(38)* genes which are more similar to each other, in addition to *mecA* reference gene (AB512767) and 2 *blaz* reference genes (AJ302698 & AP003139). The tree revealed that all resistant genes are more or less stable genes such as *mecA*, aminoglycosides, *tet(k), tet(38)* and *fusB* except *blaz* gene showing more variations. In Egypt, this work is considered the first record on genetic characterizations based on whole genome sequences of *S. aureus* isolates, as there was no previous data published on antibiotic resistance. The purpose of this research was to determine the molecular detection of antibiotic genes and the evolutionary phylogenetic relationship of their sequence and clustering.

CONCLUSION

Techniques of molecular detection continue to be increased to establish antimicrobial resistance trends for *S. aureus* clinical screening test.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest for this publication.

AUTHORS CONTRIBUTIONS

All authors contributed equally to study design, sampling, methodology, interpretation of results, and writing of the manuscript

ETHICS STATEMENT

All animals were handled by the regulation of the Animal Ethics Committee at the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt, with good animal practice following the guidelines of Research Code of Ethics (RCOE-SVU) at the South Valley University.

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