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 shotgun 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 phylogenetic 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

INTRODUCTION

Resistance to antimicrobial agents is regarded as one of the world's main and increasingly global issues, especially among nosocomial pathogens. *Staphylococcus* 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*, particularly methicillin 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). *Staphylococcus* 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...
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 glycoïd-6-N-acetyltransferase/2-O-nucleotidytranferencease (van Den Braak et al., 1999; Landecker, 2016).

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 <i>S. aureus</i> was fusidic acid (Besier et al., 2003). <i>S. aureus</i> 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 <i>S. aureus</i> 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 <i>S. saprophyticus</i> (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 <i>STAPHYLOCOCCUS AUREUS</i>**

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 <i>S. aureus</i> 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 <i>S. aureus</i>, such as bacterial sensitivity tests. Multiplex PCR used to classify <i>S. aureus</i> 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 <i>S. aureus</i> isolates. Two separate types of coagulase testing were used, namely the slide coagulase test and the tube coagulase test (Field and Smith, 1995).

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DETECTION OF RESISTANT GENES

The classification of \textit{S. aureus} into methicillin-susceptible or resistant (MSSA or MRSA) was confirmed by using \textit{mecA} gene. Multiplex PCR was used to ensure the presence of \textit{mecA} gene, in addition to the \textit{mecC} gene, which was detected recently as a homolog for \textit{mecA} and the \textit{femB} gene used as a confirmatory gene for \textit{S. aureus} (Nakagawa et al., 2005; Paterson et al., 2014). For Detection of \textit{mecA}, \textit{blaZ}, \textit{aac(6')-aph(2'')}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{tet(K)}, \textit{tet(38)}, and \textit{fusB} genes. The genes were extracted from the \textit{S. aureus} genome obtained by shotgun sequencing (Sanger Institute, UK). \textit{mecA}, \textit{blaZ}, \textit{aac(6')-aph(2'')}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{tet(K)}, \textit{tet(38)}, and \textit{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, AY482528, M26832, AF330699, AB033763, AJ302698, AM292600, AP004832, AP003139, J01764, and FR821779).

WHOLE-GENOME SEQUENCING

The DNA of \textit{S. aureus} both resistant and susceptible strains (MRSA and MSSA) were extracted and the genomic DNA used to obtain the whole genome sequence of \textit{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 \textit{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 homologous 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 \textit{S. aureus} out of 140 milk samples were isolated from dairy cows and 31 \textit{S. aureus} out of 100 milk samples were isolated from she-camels. The result of the presence or absence of some resistance genes in \textit{S. aureus} isolated from cattle (A1, A2, A3, A4, A5, A6, A7, and A10). A1 isolate showed that there were 3 genes, \textit{aac(6')-aph(2'')}, \textit{mecA}, and \textit{tet(K)} were identically 100 % to its corresponding reference genes, but in the case of \textit{tet(38)} gene, it was the identically 99.85% (Table 2). The absence of \textit{blaZ}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, and \textit{fusB} genes (Table 1). A2 isolate had 3 genes, \textit{aac(6')-aph(2'')} and \textit{mecA} were identically 100 %, but in the case of \textit{tet(38)} genes the percent of identically 99.85% compared with reference genes and absence of \textit{blaZ}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{str}, \textit{tet(K)}, and \textit{fusB} genes (Table 1). A3 isolate, there were 6 genes \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{mecA}, \textit{blaZ}, \textit{fusB} and \textit{tet(K)} were identically 100 % and \textit{tet(38)} was identically by 99.85% (Table 2) and there were absent of \textit{aac(6')}, and \textit{aph(2'')}genes (Table 1). A4 isolate had 2 genes, \textit{mecA} and \textit{blaZ} were identically 100 % but, the \textit{tet(38)} genes identically were 99.85% compared with genes in Gene bank (Table 2), and there were absent of \textit{aph(2'')}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{tet(K)}, and \textit{fusB} genes (Table 1). A5 isolate had 2 genes \textit{aac(6')-aph(2'')} and \textit{mecA} were identically 100 % but, the \textit{tet(38)}, \textit{blaZ} and \textit{tet(K)} were identically 99.85%, 99.23% and 99.89% (Table 2), in addition to, absences of \textit{aph(3')-III}, \textit{ant(6')-Ia}, and \textit{fusB} genes (Table 1). A6 isolate had 3 genes \textit{aac(6')-aph(2'')} and \textit{mecA} were identically 100 % but in the case of \textit{tet(38)} and \textit{tet(K)} genes were 99.85% and 99.89% identical to its reference genes (Table 2), \textit{blaZ}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, and \textit{fusB} genes were absent (Table 1). A7 isolate had 3 genes \textit{aac(6')-aph(2'')} and \textit{mecA} and \textit{tet(K)} were identically 100 %, but in case of \textit{tet(38)} genes was identically 99.85% (Table 2), and there were absent of \textit{blaZ}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, and \textit{fusB} genes (Table 1). Strain A10 had only one gene \textit{blaZ} was identically 100 %, and \textit{tet(38)} genes was identically 98.97% (Table 2), and there were absence of \textit{mecA}, \textit{aac(6')}, \textit{aph(2'')}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{tet(K)}, and \textit{fusB} genes (Table 1). The result of the presence and absence of resistance genes in \textit{S. aureus} isolated in this study from she-camel included isolates A11, A12, A13, and A15. A11 isolate had only one gene \textit{blaZ} was identically 100 %, but the \textit{tet(38)} genes identically 99.97% (Table 2), and the absences of \textit{mecA}, \textit{aac(6')}, \textit{aph(2'')}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{tet(K)}, and \textit{fusB} genes (Table 1). The result of the presence and absence of resistance genes in \textit{S. aureus} was similar 99.79% to its reference gene (Table 2) in addition to the absence of \textit{mecA}, \textit{blaZ}, \textit{aac(6')}, \textit{aph(2'')}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{tet(K)}, and \textit{fusB} genes (Table 1). A12 and A13 isolates showed that there is one gene \textit{tet(38)} was identified 99.63 % (Table 2) in addition to the absence of \textit{mecA}, \textit{blaZ}, \textit{aac(6')}, \textit{aph(2'')}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{tet(K)}, and \textit{fusB} genes (Table 1). A15 isolate had one gene \textit{blaZ} was identically 100 %, but in case of the \textit{tet(38)} genes was similar 99.79% to its reference gene (Table 2) in addition to the of absence of \textit{mecA}, \textit{aac(6')}, \textit{aph(2'')}, \textit{aph(3')-III}, \textit{ant(6')-Ia}, \textit{str}, \textit{tet(K)}, and \textit{fusB} genes (Table 1). \textit{Str} gene (Aminoglycoside 6-adenyllytransferase) was absent from all isolates of \textit{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 |
|-----|----------|---------|---------|------------------------------------------|
| 1   | A1       | Cattle  | S. aureus | mecA | blaZ | aac(6’)-I | aph(2’)-III | ant(6’)-Ia | str | tet(K) | tet(38) | fusB |
|     |          |         |          | 100% | Nil  | 100% | 100% | *Nil | Nil | Nil | 100% | 99.85% | Nil |
| 2   | A2       | Cattle  | S. aureus | 100% | Nil  | 100% | 100% | Nil | Nil | Nil | 99.85% | Nil |
| 3   | A3       | Cattle  | S. aureus | 100% | 100% | Nil  | 100% | 100% | 100% | 100% | 99.85% | 100% |
| 4   | A4       | Cattle  | S. aureus | 100% | 100% | Nil  | 100% | Nil | Nil | Nil | 99.85% | Nil |
| 5   | A5       | Cattle  | S. aureus | 100% | 100% | 100% | 100% | Nil | Nil | 99.89% | 99.85% | Nil |
| 6   | A6       | Cattle  | S. aureus | 100% | Nil  | 100% | 100% | 100% | 100% | 99.89% | 99.85% | Nil |
| 7   | A7       | Cattle  | S. aureus | 100% | Nil  | 100% | 100% | Nil | 100% | 99.85% | Nil |
| 8   | A10      | Cattle  | S. aureus | Nil  | 100% | Nil  | 100% | 100% | Nil | 99.97% | Nil |
| 9   | A11      | Camel   | S. aureus | Nil  | 100% | Nil  | 100% | Nil | Nil | 98.97% | Nil |
| 10  | A12      | Camel   | S. aureus | Nil  | Nil  | Nil  | Nil | Nil | 99.63% | Nil |
| 11  | A13      | Camel   | S. aureus | Nil  | Nil  | Nil  | Nil | Nil | 99.63% | Nil |
| 12  | A15      | Camel   | S. aureus | Nil  | 99.88% | Nil | 100% | 99.96% | 99.56% | Nil |

*Nil=Nothing

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

| Iso-   | Resistance gene | Identity percent | Query/HSP length | Contig    | Position in contig | Predicted phenotype | Accession number |
|--------|-----------------|------------------|------------------|----------|-------------------|----------------------|-------------------|
| A1     | aac(6’)-aph(2’) | 100              | 1440 / 1440      | EM-BOSS_001 | 2651606..2653045 | Aminoglycoside resistance | M13771          |
|        | mecA            | 100              | 2010 / 2010      | EM-BOSS_001 | 2756089..2758098 | Beta-lactam resistance | AB512767        |
|        | tet(38)         | 99.85            | 1353 / 1353      | EM-BOSS_001 | 679533..680885 | Tetracycline resistance | AY825285        |
| A2     | aac(6’)-aph(2’) | 100              | 1440 / 1440      | EM-BOSS_001 | 2774658..2776097 | Aminoglycoside resistance | M13771          |
|        | mecA            | 100              | 2010 / 2010      | EM-BOSS_001 | 2763972..2765981 | Beta-lactam resistance | AB512767        |
|        | tet(38)         | 99.85            | 1353 / 1353      | EM-BOSS_001 | 849985..851337 | Tetracycline resistance | AY825285        |
| A3     | aph(3’)-III     | 100              | 795 / 795        | EM-BOSS_001 | 2714370..2715164 | Aminoglycoside resistance | M26832          |
|        | ant(6’)-Ia      | 100              | 909 / 909        | EM-BOSS_001 | 2715796..2716704 | Aminoglycoside resistance | AF330699        |
|        | mecA            | 100              | 2007 / 2007      | EM-BOSS_001 | 2728940..2730946 | Beta-lactam resistance | AB033763        |
|        | blaZ            | 100              | 846 / 846        | EM-BOSS_001 | 2740917..2741762 | Beta-lactam resistance | AJ302698        |
|        | fusB            | 100              | 642 / 642        | EM-BOSS_001 | 2860095..2860736 | Fusidic acid resistance | AM292600        |
|        | tet(38)         | 99.85            | 1353 / 1353      | EM-BOSS_001 | 2402116..2403468 | Tetracycline resistance | AY825285        |
|        | tet(K)          | 100              | 1380 / 1380      | EM-BOSS_001 | 2824205..2825584 | Tetracycline resistance | U38428          |
|   | Gene    | Identity (%) | Accession Number | Sequence Description          | Accession Number |
|---|---------|--------------|------------------|--------------------------------|------------------|
| A4 | mecA    | 100          | EM-BOSS_001      | Beta-lactam resistance        | AB033763         |
|   | blaZ    | 99.85        | EM-BOSS_001      | Beta-lactam resistance        | AP004832         |
|   | tet(38) | 99.85        | EM-BOSS_001      | Tetracycline resistance       | AY825285         |
| A5 | aac(6')-aph(2') | 100       | EM-BOSS_001      | Aminoglycoside resistance     | M13771           |
|   | blaZ    | 98.23        | EM-BOSS_001      | Beta-lactam resistance        | AP003139         |
|   | mecA    | 100          | EM-BOSS_001      | Beta-lactam resistance        | AB512767         |
|   | tet(38) | 99.85        | EM-BOSS_001      | Tetracycline resistance       | AY825285         |
|   | tet(K)  | 99.89        | EM-BOSS_001      | Tetracycline resistance       | J01764           |
| A6 | aac(6')-aph(2') | 100       | EM-BOSS_001      | Aminoglycoside resistance     | M13771           |
|   | mecA    | 100          | EM-BOSS_001      | Beta-lactam resistance        | AB512767         |
|   | tet(38) | 99.85        | EM-BOSS_001      | Tetracycline resistance       | AY825285         |
|   | tet(K)  | 99.89        | EM-BOSS_001      | Tetracycline resistance       | J01764           |
| A7 | aac(6')-aph(2') | 100       | EM-BOSS_001      | Aminoglycoside resistance     | M13771           |
|   | mecA    | 100          | EM-BOSS_001      | Beta-lactam resistance        | AB512767         |
|   | tet(38) | 99.85        | EM-BOSS_001      | Tetracycline resistance       | AY825285         |
|   | tet(K)  | 100          | EM-BOSS_001      | Tetracycline resistance       | U38428           |
| A10| blaZ    | 100          | EM-BOSS_001      | Beta-lactam resistance        | AP004832         |
|   | tet(38) | 98.97        | EM-BOSS_001      | Tetracycline resistance       | FR821779         |
| A11| blaZ    | 100          | EM-BOSS_001      | Beta-lactam resistance        | AP004832         |
|   | tet(38) | 98.97        | EM-BOSS_001      | Tetracycline resistance       | FR821779         |
| A12| tet(38) | 99.63        | EM-BOSS_001      | Tetracycline resistance       | FR821779         |
| A13| tet(38) | 99.63        | EM-BOSS_001      | Tetracycline resistance       | FR821779         |
| A15| blaZ    | 99.88        | EM-BOSS_001      | Beta-lactam resistance        | AP004832         |
|   | tet(38) | 99.56        | EM-BOSS_001      | Tetracycline resistance       | AY825285         |

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 measured by the number of substitutions per site.
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 found only 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 adenylyltransferases 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 re-
ference 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.

REFERENCES

- Ali AO (2016). Molecular Epidemiology Based on SPA Genotyping of Staphylococcus aureus Isolated from Cattle and Camels in Egypt. Alexandria J. Vet. Sci. 48:62-68.
- Ali AO (2015). Detection of MecA, MecC and femB genes by Multiplex Polymerase Chain Reaction. J. Vet. Adv. 5:1-7.
- Ali AO, El Shrief LMT (2016). Molecular Characterization of Tetracycline-Resistant Genes in Staphylococcus aureus Isolated From Dairy Cows And She-camels Suffering from Subclinical Mastitis. Alexandria Journal of Veterinary Sciences, 48:1-8.
- Ali AO, Mahmoud HYM, Sameh A, Zaky S, Adel Elsayed A (2015). Antibiotic Resistance Profile of Coagulase Positive Staphylococcal infection in Dairy Buffalo. World Vet J. 5:46-50. http://dx.doi.org/10.5455/wvj.20151055
- Ardic N, Ozyrut M, Sarayyupoglu B, Haznedaroglu T (2005). Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant staphylococci. Int. J. Antimicrob Agents. 26:213-218. https://doi.org/10.1016/j.ijantimicag.2005.06.013
- Besier S, Ludwig A, Brade V, Wichelhaus TA (2003). Molecular analysis of fusidic acid resistance in Staphylococcus aureus. Mol. Microbiol. 47:463-469. https://doi.org/10.1046/j.1365-2958.2003.03307.x
- Bismuth R, Zilhao R, Sakamoto H, Guesdon J L, Courvalin P (1990). Gene heterogeneity for tetracycline resistance in Staphylococcus spp. Antimicrob. Agents Chemother. 34:1611-4.
- Bougnom BP, Piddock LJ (2017). Wastewater for Urban Agriculture: A Significant Factor in Dissemination of Antibiotic Resistance. Environ. Sci. Technol. 51: 5863-864. https://doi.org/10.1021/acs.est.7b01852
- Emaneini M, Taherikalani M, Eslampour MA, Sedaghat H, Aligholi M, Jabalameli F, Shahsavan S, Sotoudeh N (2009). Phenotypic and genotypic evaluation of aminoglycoside resistance in clinical isolates of staphylococci in Tehran, Iran. Microb Drug Resist. 15:129 - 32. https://doi.org/10.1089/mdr.2009.0869
- Esposito S, Leone S, Petta E, Noviello S, Ianniello F (2009). Treatment options for skin and soft tissue infections caused by methicillin-resistant Staphylococcus aureus: oralvs. parenteral; home vs. hospital. Int. J. Antimicrob Agents. 34:30-35. https://doi.org/10.1016/s0924-8579(09)70547-3
- Everitt RG, Didelot X, Betty EM, Miller RR, Knox K, Young BC,Bowden R, Auton A, Votintseva A, Larner-Svensson H, Charlesworth J, Golubchik T, Camilla LC, Godwin H, Fung R, Pero TE, Walker AS, Crook DW, Wilson DJ (2014). Mobile elements drive recombination hotspots in the core genome of Staphylococcus aureus. Nat. Commun.. 5:3956. https://doi.org/10.1038/ncomms4956
- Fair RJ, Tor Y (2014). Antibiotics and bacterial resistance in the 21st century. Perspect. Medcin. Chem. 6:25-64. http://doi.org/10.4137/pmc.s14459
- Fatholahzadeh B, Emaneini M, Feizabadi MM, Sedaghat H, Aligholi M, Taherikalani M, Jabalameli F (2009). Characterisation of genes encoding aminoglycoside-modifying enzymes among meticillin-resistant Staphylococcus aureus isolated from two hospitals in Tehran, Iran. Int. J. Antimicrob Agents. 33:264-5. https://doi.org/10.1016/j.ijantimicag.2008.09.018
- Field HI, Smith W (1995). The coagulase test for staphylococci. J. Comp. Pathol. 55: 63–69. https://doi.org/10.1016/S0368-1742(45)80007-3
- Hall TA (1999). A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symposium Series 41, 95–98. https://doi.org/10.14610/Phytopathol_Mediterr-14998s1.29
- Ito T, Okuma K, Ma X, Yuzawa H, Hiramatsu K (2003). Insights on antibiotic resistance of Staphylococcus aureus from its whole genome: genomic island SCC. Drug Resist Updat. 6:41–52. https://doi.org/10.1016/s1368-7646(03)00003-7
- Kimura M (1980) A simple method for estimating
evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.

- Land M, Hauser L, Jun SR, Nookaew I, Leuze MR, Ahn TH, Karpinet T, Lund O, Kora G, Wassenaar T, Poudel S, Ussery DW (2015). Insights from 20 years of bacterial genome sequencing, Funct. Integr. Genomics. 15:141-161. https://doi.org/10.1007/s10142-015-0433-4

- Landecker H (2016). Antibiotic Resistance and the Biology of History. Body Soc. 22:19-52. https://doi.org/10.1177/1357034x14561341

- Lannergård J, T Norstrom, D Hughes (2009). Genetic determinants of resistance to fusidic acid among clinical bacteremia isolates of Staphylococcus aureus. Antimicrob. Agents Chemother. 53: 2059-2065. https://dx.doi.org/10.1128%2FAAC.00871-08

- Livermore DM (2000). Antibiotic resistance in staphylococci. Int.J, Antimicrob. Agents. 1:53-10. https://doi.org/10.1016/s0924-8579(00)00299-5

- Lowy FD (2003). Antimicrobial resistance: the example of Staphylococcus aureus. J. Clin. Invest.111:1265-73. https://doi.org/10.1172/jci18535

- Luepeh KH, Suda KJ, Boucher H, Russo RL, Bonney MW, Hunt TD, Mohr JF (2017). Past, Present, and Future of Antibacterial Economics: Increasing Bacterial Resistance, Limited Antibiotic Pipeline, and Societal Implications. Pharmacotherapy, 37:71-84. https://doi.org/10.1002/phar.1868

- Maranan MC, Moreira B, Boyle-Vavra S, Daum RS (1997). Tetracyclines in veterinary medicine and bacterial resistance to them. Vet. Med. Czech. 49: 79-100.

- Mojumdar M, Khan SA (1988). Characterization of the tetracycline resistance gene of plasmid pT181 of Staphylococcus aureus. Journal of Bacteriology 170: 5522–5528. https://doi.org/10.1128/jb.170.12.5522-5528.1988

- Ramirez MS, Tolmasey ME (2010). Aminoglycoside modifying enzymes. 2010. Drug Resist. Updat. 13:151-71. https://doi.org/10.1016/j.drup.2010.08.003

- Rutherford K, Parkhill J, Crook J, Horssell T, Rice P, Rajandream MA, Bart B (2000). Artemis: Sequence visualization and annotation. Bioinformatics. 10: 944–945. https://doi.org/10.1093/bioinformatics/16.10.944

- Saitou N, Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425. https://doi.org/10.1093/molbev/mol010454

- Schwarz S, Roberts MC, Werckenthin C, Pang Y J, Lange C (1998). Tetracycline resistance in Staphylococcus spp. from domestic animals. Vet. Microbiol. 63: 217–227.

- Stegger M, Andersen PS, Kearns A, Pichon B, Edwards G, Laurent F, Feale C, Skow R, Larsen AR (2012). Rapid detection, differentiation and typing of meC-resistant S. aureus harbouring either mecA or the new meCA homologue mecA(LGA251). Clin. Microbiol. Infect.18:395-400. https://doi.org/10.1111/j.1469-0691.2011.03715.x

- Strommenger B, Kettlitz C, Werner G, Witte W (2003). Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in Staphylococcus aureus, J. Clin. Microbiol. 41:4089–4094. https://dx.doi.org/10.1128%2FJCM.41.9.4089-4094.2003

- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30:2725–2729. https://doi.org/10.1093/molbev/mst194

- Neamn-Allah ANF, Hend M, El Damaty (2016). Strangles in Arabian horses in Egypt: Clinical, epidemiological, hematological, and biochemical aspects. VetWorld. 9:820–826. https://dx.doi.org/10.14202%2Fvetworld.2016.820-826

- Nesin M, Svec P, Lupski J R, Godson G N, Kreiswirth B, Korblum J, Projan SJ (1990). Cloning and nucleotide sequence of a chromosomally encoded tetracycline resistance determinant, tetA(M), from a pathogenic, mectinillin-resistant strain of Staphylococcus aureus. Antimicrob. Agents Chemotherap. 34: 2273–2276. https://doi.org/10.1128/ac.34.11.2273

- Norström T, J Lannergard, D Hughes (2007). Genetic and phenotypic identification of fusidic acid-resistant mutants with the small-colony-variant phenotype in Staphylococcus aureus. Antimicrob. Agents Chemother. 51: 4438-4446. https://doi.org/10.1128/aac.00328-07

- O’Neill AJ, Larsen RA, Henrikse AS, Chopra I (2004). A fusidic acid-resistant epidemic strain of Staphylococcus aureus carries the fusB determinant, whereas fuaA mutations are prevalent in other resistant isolates. Antimicrob. Agents Chemother.48:3594-3597. https://dx.doi.org/10.1128%2FAAC.48.3594-3597.2004

- O’Neill AJ, Chopra I (2006). Molecular basis of fusB-mediated resistance to fusidic acid in S. aureus. Mol. Microbiol. 59:664-676. https://doi.org/10.1111/j.1365-2958.2005.04971.x

- O’Neill AJ, McLaws F, Kalmeter G, Henrikse A S, Chopra I. (2007). Genetic basis of resistance to fusidic acid in staphylococci. Antimicrob. Agents Chemother.51:1737-1740. https://dx.doi.org/10.1128%2FAAC.01542-06

- Paterson GK, Ewan MH, Mark AH (2014). The emergence of mecC methicillin-resistant Staphylococcus aureus. Trends Microbiol. 22: 42–47. https://doi.org/10.1016/j.tim.2013.11.003

- Rajandream MA, Bart B (2000). Artemis: Sequence visualization and annotation. Bioinformatics. 10: 944–945. https://doi.org/10.1093/bioinformatics/16.10.944

- Saifou N, Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
van Den Braak N, van Belkum A, Kreft D, te Witt R, Verbrugh HA, Endtz HP (1999). The prevalence and clonal expansion of high-level gentamicin-resistant enterococci isolated from blood cultures in a Dutch university hospital. J. Antimicrob. Chemother. 44: 795-798. https://doi.org/10.1093/jac/44.6.795

Vanderhaeghen W, Hermans k, Haesebrouck F, Butaye P (2012). Methicillin-resistant Staphylococcus aureus (MRSA) in food production animals. Epidemiol. Infect. 138:606-625.

Woodford N (2005). Biological counterstrike: antibiotic resistance mechanisms of Gram-positive cocci. Clin. Microbiol. Infect. 11: 2-21

Zapun A, Contreras-Martel C, Vernet T (2008). Penicillin-binding proteins and beta-lactam resistance. FEMS Microbiol. Rev. 32:361–385. https://doi.org/10.1111/j.1574-6976.2007.00095.x