Characterization of Toxin Gene Profiles and Antibiotic Resistance Genes of Methicillin Resistant *Staphylococcus aureus* Isolated from Ducks

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**Abstract** | Although Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most widespread Bacteria causing infection over the world for poultry sector, MRSA in duck farms hasn’t yet been investigated seriously. Thus, this study intensively investigates the prevalence of MRSA through 100 duck farms. *S. aureus* has been isolated from 21 farms. The classical enterotoxins (A–E) production plus toxic shock syndrome toxin (TSST-1) were screened by PCR, only four strains harbored *sed* gene. The antimicrobial resistance profiles were tested by agar diffusion assays showed that 90.5% of isolates exhibit multidrug resistance (MDR). All the tested isolates revealed 100% resistance towards penicillin G, ampicillin and cefoxitin. However, the resistances to kanamycin, tetracycline and gentamycin were 90.5%, 85.7% and 81% respectively. Additionally, Methicillin-resistant *S. aureus* (MRSA) isolates were identified by the presence of *mecA* gene, but all isolates were negative for *mecC* gene. The isolates were tested for the presence of four antimicrobial resistance genes including tetracycline resistance gene *tetK* that was detected in all isolates. Secondly, 90.5% of isolates carried erythromycin resistant gene *ermB* as well as gentamicin resistant gene, *aacA-aphD*. Finally, none of those isolates has carried *vanA* gene for vancomycin resistance. Consequently, continuous tracking the presence of MRSA in duck farms is very important to avoid developing a reservoir for antimicrobial resistances.

**Keywords** | *Staphylococcus aureus*, *tetK*, Duck, *mecA*, Enterotoxins.

**INTRODUCTION**

*S. aureus* is one of the most common bacterial diseases in poultry which causes drop in egg production, body weight loss, and lameness which is leading to carcass condemnation. Therefore, *S. aureus* has negative impact on the economy based on farming business alongside the foodborne illness causes (Andreasen, 2013). Moreover, *S. aureus* influences on the human health and causes food poisoning regarding to their enterotoxins productions (Han et al., 2013).

Although diagnosing, detecting and genotyping of *S. aureus* are routinely processed among sick poultry (Andreasen, 2013), alongside pigs, cows and goats, (Wang et al., 2017), the detection of *S. aureus* in ducks is too rare. However, in Egypt, the ducks are taking the second place of the poultry meat production (Radwan et al., 2019). On the other hand,
the *S. aureus* is considered as one of the bacteria causing diseases in ducks and is responsible for suppurative dermatitis, suppurative arthritis, and septicemic lesions (Smyth and McNamee, 2008). This is in a similar way to chicken but it has different immunity response (Andreasen, 2013; Eid et al., 2019). Moreover, the *S. aureus* causes diseases in both human and animals as consequences of virulence factors production also (Marek et al., 2018). These virulence factors are such as the enterotoxins which are heat stable, having 23 types including main fives types namely SEA, SEB, SEC, SED, and SEE. Additionally toxic shock syndrome of toxin-1 (*TSST*-1) (Wang et al., 2017; Ono et al., 2015) is one of virulence factors as well.

Methicillin-resistant *S. aureus* (MRSA) has the ability to be transmitted from human to livestock and vice-versa (Marek et al., 2018), so that it causes a severe hazardous effect on the public health. MRSA carries variable antibiotic resistance patterns leading to the treatment failure and also causes nosocomial infection in some cases (Han et al., 2013; Ben Zakour et al., 2008; Achek et al., 2018). Evermore, Methicillin-resistant *S. aureus* (MRSA) is characterized by harboring *mecA* gene which is responsible for methicillin resistance. Therefore, the gene encrypts against methicillin resistance. Therefore, the gene encrypts against beta-lactam antibiotics. However this encoded gene is located at the staphylococcal cassette chromosome mec(SCCmec) part, which is a genomic island ubiquitously disseminated among staphylococci (Han et al., 2013; García-Garrote et al., 2014). Recently, in Germany, a new gene has been discovered and known as *mecC* gene but its similarity to *mecA* gene is about 70% of identical nucleotide (Stegger et al., 2012), noting that both animals and humans are carrying *mecC* gene (García-Garrote et al., 2014).

Thus, MRSA has been categorized as one of a superbug (Dweba et al., 2019; WHO, 2020), because of its wide spread alongside to the misusage of antibiotics treating *S. aureus* infections which both are developing the antibiotic resistance. Throughout livestock and food producing animals the *S. aureus* infection can be easily transmitted to human via the food chain (Wang et al., 2017; WHO, 2020; Rodríguez-Lázaro et al., 2017; Grema et al., 2015). The resultant, several cases had been reported as *S. aureus* infections in human (Rodríguez-Lázaro et al., 2017; Blumenthal et al., 2013).

Erythromycin is one of the antibiotics used in the treatment of Gram-positive bacteria as *S. aureus* (Khan et al., 2002). However, there are about 17 genes responsible for erythromycin resistance in *S. aureus* (Nawaz et al., 2000), where the most common genes are *ermA*, *ermB* and *ermC*. These genes are targeting and responsible for ribosomal manipulation in macrolides, lincosamides and type B streptogramins (Achek et al., 2018). Additionally, both *tet(K)* and *tet(L)* genes are rising up the resistance for tetracyclines at staphylococci (Wendlandt et al., 2013).

Therefore, the aim of this study was MRSA detection from apparently healthy duck farms with their phenotypic and genotypic antimicrobial resistance characters as well as enterotoxin genes.

**MATERIALS AND METHODS**

**SAMPLES**
The Samples were taken from 100 duck farms, where 3 freshly dead ducks were transferred in ice boxes to reference laboratory for veterinary quality control on poultry production within 24 h for examination and testing. All samples were collected from ducks joint while the stab swabs were gathered from internal organs such as lung and liver.

**BACTERIAL ISOLATION**
The swabs from lung and liver organs were inoculated into 5% sheep blood agar and incubated for 24 h at 37°C. After incubation, the *S. aureus* isolates are β-hemolytic. Heavily contaminated material was inoculated into a selective medium inhibitory for gram-negative bacteria, such as mannitol-salt agar (Andreasen, 2013). After that, the typical Staphylococcus spp. colonies were investigated and examined by gram staining, slide catalase test, oxidase test, and tube coagulase test (Quinn et al., 2002).

**ANTIMICROBIAL SUSCEPTIBILITY TESTS**
Antimicrobial susceptibility testing for 21 *S. aureus* isolates throughout 18 antimicrobials agents was performed by using the disc diffusion method. The test was performed according to the Clinical and Laboratory Standards Institute (CLSI, 2015). The 18 antibiotics used in the disc diffusion method are namely: (Oxoid®) Penicillin-G 10 IU (P10); cefoxitin 30 μg (FOX30); Oxacillin 1μg (OX1); gentamicin 10 μg (CN10); Kanamycin 30 μg (K30); Ciprofloxacin 5 μg (CF5); clindamycin 2 μg (DA2); Azithromycin 15 μg (AZM15); erythromycin 15 μg (E15); Chloramphenicol 30 μg (C30); trimethoprim/sulfamethoxazole 1.25-23.75 μg (SXT), doxycycline 30μg (DO30); Tetracycline 30 μg(T30); amoxicillin + clavulanic acid 20 + 10 μg (AMC30); cefotaxime 30μg (CTX 30); vancomycin 30 μg(VA 30); levofoxacin 5μg(Lev 5), ampicillin 10 μg (AMP10).

According to CLSI guidelines, after aerobic incubation at 37°C for 18–24 h, the susceptibilities of *S. aureus* isolates to the individual antimicrobial agents were determined and interpreted. The Test results were considered valid only, when the diameters of the inhibition zones for the control *S. aureus* (ATCC 25922) strain were within the performan-
**Table 1:** PCR primers used in this study and sizes of PCR products of methicillin resistance, enterotoxins (A-E), TSST-1, and antibiotic resistance genes.

| Target | Gene | Primer Sequence 5′-3′ | Amplified fragment | References |
|--------|------|------------------------|--------------------|------------|
| Methicillin resistance | mecA | 5′GTAGAAATGACTGAAACGTCCGA-TA3′ | 310 bp | (Nowrouzian et al., 2013) |
| | | 5′CCAATTCCAATGTTTCTG-GTCTAA3′ | | |
| | mecC | 5′GAAGATCTTTTCCGTTTTACG3′ | 138 bp | (Stegger et al., 2012) |
| Enerotoxins | sea | 5′GTTATCAATGTGCCGGGTG3′ | 102 bp | (Betley and Mekalanos, 1988) |
| | | 5′CGGCACATTTTCTCTCTG3′ | | |
| | seb | 5′GTATGTGGTGTAACTGAGC 3′ | 164 bp | (Jones and Khan, 1986) |
| | | 5′CCAAATAGTGGAGTTAGG 3′ | | |
| | sec | 5′AGATGAATAGTGTGATGTATGG 3′ | 451 bp | (Bohach and Schlievert, 1987) |
| | | 5′CACACTTTTGAATCAACCG 3′ | | |
| | sed | 5′CCAATAATAAGGAGAAAATAAAG 3′ | 278 bp | (Bayles and Iandolo, 1989) |
| | | 5′ATTGGATT TTTCCTGCTC 3′ | | |
| | see | 5′AGGTTTTTTTCACAGGTCATCC 3′ | 209 bp | (Couch et al., 1988) |
| | | 5′CTTTTTTTTTTTTCCGTTAATC 3′ | | |
| Toxic shock syndrome | tsst-1 | 5′ACCCCTGTCTCTCTATCATC 3′ | 326 bp | (Blomster-Hautamaa et al., 1986) |
| Erythromycin resistance | erm(B)-1 | 5′CATTTAACGACGAAACTGGC 3′ | 425 bp | (Jensen et al., 1999) |
| | | 5′GGAACATCTTGATGTGACGC 3′ | | |
| Tetracycline resistance | tetK | 5′GTAGCCGCAATAGGTGTAATAGT 3′ | 360 bp | (Strommenger et al., 2003) |
| | | 5′GTAGTGACATTTTTCCTTCACT 3′ | | |
| Aminoglycoside resistance | Aac6-aph2 | 5′GAAGTACGCAGAAGAGA 3′ | 491 bp | (Choi et al., 2003) |
| | | 5′ACATGGCAAGCTCTAGGA 3′ | | |
| Vancomycin resistance | vanA | 5′CATGACGTATCGTAAAATC 3′ | 885 bp | (Patel et al., 1997) |

- ce ranges.

**Molecular Identification of *Staphylococcus Aureus* Isolates Using Polymerase Chain Reaction Assay:** DNA extraction was fulfilled using QIAamp DNA mini kit (Qiagen, Germany, GmbH) according to manufacturer’s instruction.

The used Oligonucleotide primers were supplied from Metabion (Germany), as mentioned in Table (1).

PCR amplification was employed using 25 μL PCR reaction which was containing 12.5 μL of Emerald Amp Max PCR Master Mix (Emerald, Japan), 1 μL of each primer (20 pmol conc.), 4.5 μL of PCR grade water and 6 μL of a template using a Biometra T3 thermal cycler. The PCR products were separated by the agarose gel electrophoresis using 1.5% agarose gel which stained with Ethidium bromide. The gel was photographed using a gel documenta-

-The efficiency of the amplification was verified for positive field samples that might have mecA, aacA-aphD, tetK, mecA, sea, seb, sec, see, sed and tsst-1 genes which were previously examined in veterinary quality control reference laboratory for poultry production -Animal health research.

**RESULTS**

**Prevalence of *S. aureus***

There were 100 farms under examination for existence of *S. aureus*, only 21 farms (21%) were confirmed by *S. aureus* isolates. The 21 isolates were affirmed *S. aureus* as positive from the results of the slide catalase test, tube coagulase test and negative oxidase test.
### Table 2: Antimicrobial susceptibility of S. aureus isolated from duck farms

| Antimicrobial | Resistance % | Intermediate % | Sensitive % |
|--------------|--------------|----------------|-------------|
| Penicillin   | 100          | 0              | 0           |
| Ampicillin   | 100          | 0              | 0           |
| Cefoxitin    | 100          | 0              | 4           |
| Oxacillin    | 14.3         | 9.5            | 76.2        |
| Gentamycin   | 81           | 0              | 19          |
| Kanamycin    | 90.5         | 0              | 9.5         |
| Ciprofloxacin| 4.8          | 19             | 76.2        |
| Clindamycin  | 28.6         | 23.8           | 47.6        |
| Azithromycin | 28.6         | 23.8           | 47.6        |
| Erythromycin | 23.8         | 66.7           | 9.5         |
| Chloramphenicol | 19   | 0            | 81          |
| SXT          | 14.3         | 0              | 85.7        |
| Doxycycline  | 14.3         | 28.6           | 57.1        |
| Tetracycline | 85.7         | 4.8            | 9.5         |
| AMC          | 61.9         | 0              | 38.1        |
| CTX 30       | 9.5          | 57.1           | 33.4        |
| Vancomycin   | 0            | 0              | 21          |
| Levofloxacin | 9.5          | 0              | 90.5        |

**Antimicrobial Susceptibility Testing**

All the 21 *S. aureus* isolates were tested against 18 antibiotics agents. The antimicrobial resistance profiles of the isolates to antimicrobial agents are shown in (Table 2). All MRSA isolates were resistant to penicillin, ampicillin and cefoxitin, in addition to high resistance rate to Kanamycin (90.5%), Tetracycline (85.7%) and Gentamycin (81%), while Ciprofloxacin showed the lowest resistance rate of 4.8%. In this work, the majority of isolates are classified as MDR-SA ones, as 19 isolates (90.5%) are resistant to three or more antimicrobial classes among different classes are well known as multidrug resistant (MDR), as shown in Figure (1), Table (2) and Table (4).

**Molecular Characteristics of *S. aureus* Isolates**

**Detection of presence of mecA and mecC genes:**

PCR has confirmed the presence of mecA gene in all *S. aureus* isolates (as 100%) as well as the absence of mecC gene.

**Detection of Virulence-Associated Genes**

Additionally, the PCR results for five classical enterotoxins (A–E) and toxic shock syndrome toxin 1 (tsst-1) have revealed the MRSA isolates don't own sea, seb, sec, and see genes and. On contrary for sed gene, only four isolates have had it.
### Table 3: Correlation between phenotypic resistance and detection of resistance-associated genes in isolates

| Antimicrobial classes | Target genes | Phenotypic resistance | Gene detection |
|-----------------------|--------------|-----------------------|----------------|
| Betalactam            | meca         | 21                    | 21 (100%)      |
| Gentamicin            | aacA-aphD    | 17                    | 19 (90.5%)     |
| Erythromycin          | ermA         | 5                     | 19 (90.5%)     |
| Tetracycline          | tetK         | 18                    | 21 (100%)      |
| Vancomycin            | VanA         | 0                     | 0              |

Table 4: Relationship between phenotypic antimicrobial resistance and detection of resistance genes and enterotoxin genes in MRSA isolated from ducks

| Strain | Phenotypic antibiotic resistance | Genotypic antibiotic resistance | Type of se gene |
|--------|----------------------------------|---------------------------------|-----------------|
| 1      | P10, AMP10, FOX30, CN10, K30, TE30, AMC30 | meca, aacA-aphD, ermB, tetK   |                |
| 2      | P10, AMP10, FOX30, CN10, K30, TE30, AMC30 | meca, aacA-aphD, tetK, mecA   |                |
| 3      | P10, AMP10, FOX30, CN10, K30, TE30, AMC30 | meca, aacA-aphD, ermB, tetK   |                |
| 4      | P10, AMP10, FOX30, OX1, CN10, K30, TE30, AMC30 | meca, aacA-aphD, ermB, tetK   |                |
| 5      | P10, AMP10, FOX30, CN10, K30, TE30, LEV5 | meca, aacA-aphD, ermB, tetK, sed |               |
| 6      | P10, AMP10, FOX30, DA2, AZM15, E15, TE30 | meca, ermB, tetK               |                |
| 7      | P10, AMP10, FOX30, OX1, AMC30       | meca, aacA-aphD, ermB, tetK   |                |
| 8      | P10, AMP10, FOX30, CN10, K30, AZM15, C30, AMC30 | meca, aacA-aphD, ermB, tetK   |                |
| 9      | P10, AMP10, FOX30, CN10, K30, AZM15, TE30 | meca, aacA-aphD, ermB, tetK   |                |
| 10     | P10, AMP10, FOX30, CN10, K30, CTX30 | meca, aacA-aphD, ermB, tetK   |                |
| 11     | P10, AMP10, FOX30, CN10, K30, DA2, E15, SXT, DO30, , TE30, AMC30 | meca, aacA-aphD, ermB, tetK   |                |
| 12     | P10, AMP10, FOX30, CN10, K30, TE30, AMC30 | meca, aacA-aphD, ermB, tetK   |                |
| 13     | P10, AMP10, FOX30, OX1, CN10, K30, DA2, E15, SXT, , TE30 | meca, aacA-aphD, ermB, tetK   |                |
| 14     | P10, AMP10, FOX30, CN10, K30, C30, DO30, TE30 | meca, aacA-aphD, ermB, tetK   |                |
| 15     | P10, AMP10, FOX30, CN10, K30, TE30, AMC30 | meca, aacA-aphD, ermB, tetK   |                |
| 16     | P10, AMP10, FOX30, CN10, K30, DA2, AZM15, E15, TE30 | meca, aacA-aphD, tetK       |                |
| 17     | P10, AMP10, FOX30, CN10, K30, TE30, AMC30 | meca, ermB, tetK               |                |
| 18     | P10, AMP10, FOX30, CN10, K30, DA2, AZM15, C30, TE30, AMC30 | meca, aacA-aphD, ermB, tetK   |                |
| 19     | P10, AMP10, FOX30, CN10, K30, TE30 | meca, aacA-aphD, ermB, tetK   |                |
| 20     | P10, AMP10, FOX30, K30, CIP 5, DA2, AZM15, E15, C30, SXT, DO30, TE30, AMC30, CTX30, LEV5 | meca, aacA-aphD, ermB, tetK, Sed |               |
| 21     | P10, AMP10, FOX30, K30 | meca, aacA-aphD, ermB, tetK   |                |

### Determination of antimicrobial Resistance Genes

The prevalence of 5 antimicrobial resistance genes testing was done for the isolates. The highest resistant gene were meca and tetK (100%) to betalactam and tetracycline, whereas aacA-aphD and ermB genes were (90.5%) only. However, the VanA gene was negative as shown in Figure (2), Table (3) and Table (4).

### DISCUSSION

Many infections are caused basically by S. aureus whatever its zoonotic importance (Voss et al.,2005). Therefore, there are many reports about incidences of S. aureus and methicillin resistance in different poultry spp, such as chicken (Dweba et al., 2019), turkey (El-Adawy et al., 2016), and food products (Achek et al., 2018). There is lack or report about the incidence of S. aureus and methicillin resistance in ducks, especially in Egypt (Eid et al., 2019).

In this study, the prevalence of S. aureus results in ducks was (21%). In comparison to previous studies, this prevalence of S. aureus is higher than prevalence rates for 100 ducks in Egypt (12.2%) (Eid et al., 2019), Dutch duck farms (10%) (Van Duijkeren et al., 2016) and retail duck (7.2%) (Wang et al., 2017). However, the prevalence of S. aureus for this study is still lower than reported results for ducks in South African livestock (40%) (Dweba et
The incidence of MRSA in duck farms represents a great concern to protect consumers. These results encourage us to assess the risk of any health hazards which may happen and become more curious to know the its possibility to induce infections, so this study showed important information about MRSA from duck farms based on phenotypic and genotyping characterization and detection of enterotoxin genes and some antimicrobial resistance determinants.

Moreover, the antimicrobial resistance is one of the most global threats to human causing severe public health diseases (Wang et al., 2017). Moreover, WHO is doing its supreme effort to support health project of control antibiotic resistance in humans and veterinary sector by cooperation with other organizations as the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) (WHO, 2020). Especially for diseases have ability to be transmitted from food producing animals throughout food chain. Uncontrolled usage and intake abuse of antibiotics for either human or animals without physician or veterinarian advice will have been bad consequence in dissemination of antibiotic resistance (WHO, 2020). Hence, Staphylococcal infections are usually treated by excessive usage of penicillin and tetracycline. The misusage of those antibiotics leads to increase antimicrobial resistance (Nemati et al., 2008), as similar as to our antimicrobial resistance data in this current study.

It is found that the Cefoxitin is more accurate and is better than oxacillin in detection and identification of methicillin resistance. This regarding to Cefoxitin has high sensitivity, specificity and a higher effect on penicillin-binding protein 2a (PBP2a) (Marek et al., 2018) rather than oxacillin (CLSI, 2015).

In the present work, all MRSA isolates are having resistance to penicillin, ampicillin. There is agreement with previous reports for isolated MRSA from ducks in Egypt (Eid et al., 2019). Moreover, all resistant isolates to penicillin and ampicillin has recorded meca gene which is the principle inducer for methicillin resistance (Marek et al., 2018).

In this study, 23.8% of Staphylococcus aureus isolates were resistant to erythromycin, while 14.3% were resistant to SXT. Thus, Staphylococcus aureus isolates were less resistant to SXT than erythromycin, but both are pretty less than reported for ducks in Egypt before (80% for each one) (Eid et al., 2019).

On other hand, the resistance of Staphylococcus aureus against gentamycin (81%) was higher than what was reported previously in Egyptian duck farms (20%) (Eid et al., 2019), ducklings (26.7%) (Farghaly et al., 2015), and from duck and turkey farms in Netherlands (52.5%) (Van Duijkeren et al., 2016).

The isolated MRSA from ducks has shown 100% susceptibility to vancomycin in good agreement with previous results from Netherlands farms (Van Duijkeren et al., 2016).

Additionally, the antimicrobial resistance rates in this study for clindamycin and erythromycin (28.6%, & 23.8%) as shown in Table (2) respectively were lower than those of MRSA isolates from duck and turkey that reported in Netherlands farms (60% each). Nevertheless, the ciprofloxacin has higher resistance rate (52.5%) than reported rates in our work (4.8%) (Van Duijkeren et al., 2016).

In this study, 90.5% of Staphylococcus aureus isolates exhibited multi-drug resistance as stated by others who are relating to poultry isolates (Dweba et al., 2019; El-Adawy et al., 2016). These MDR isolates are serious threats to human who is with direct contact with ducks in the infected farms (Van Duijkeren et al., 2016). Furthermore, the majority of MRSA isolates exhibit MDR and have high percentage of resistance to tetracycline and gentamycin as previously mentioned (Marek et al., 2018; Lyon and Skurray, 1987). Moreover as shown in Table (4), all of these isolates are resistant to penicillin which is mediated by penicillinase and all of them having meca gene (Leonard and Markey, 2008).

For these reasons, the mecc and meca genes are responsible for detection of beta-lactam resistance (El-Adawy et al., 2016). The mecc gene in MRSA is proved by numerous studies to be of zoonotic importance in livestock animals (Dweba et al., 2019). In this study, All MRSA isolates do not harbor mecc gene although meca gene was detected in all isolates which is in agreement with previous study (Van Duijkeren et al., 2016).

Moreover, resistance of staphylococci to aminoglycoside (such as gentamicin, kanamycin and tobramycin) is mediated by aacA-aphD gene (Wendlandt et al., 2013; Achek et al., 2018); ). Thus, several studies have mentioned the prevalence of aminoglycoside and methicillin resistance relationship (Choi et al., 2003), with good agreement to our study as well. This relation can be explained as a result of neighboring positions of meca gene and aminoglycoside resistance genes (Choi et al., 2003).

Although, all MRSA isolated from duck in China harbored aminoglycoside resistance gene aacA-aphD (Cao et al., 2016), whereas in this study only 90.5% of ducks MRSA isolated could be detected.

Moreover, MRSA from different origins as pigs, cattle, chickens and ducks commonly carried tet(K) and/or tet(L)
However, there are several genes responsible for the resistance to macrolides, **ermB** is one of those genes. The **ermB** gene was found in 90.5% of isolates too. In spite of the resistance to **ermB** hasn’t been studied in ducks before, the resistance of **ermB** due to its occurrence in coagulase positive and coagulase-negative staph strains in poultry is 8.3% in USA farms (Nawaz et al., 2000), while it was 50% in turkey (El-Adawy et al., 2016).

Moreover, **VanA** gene associated with vancomycin resistance wasn’t detected in **S. aureus** isolates in this work. This result was in good agreement with the result reported by Ahmed et al. (Ahmed et al., 2020). Recently, a new report has identified a first record of **VanA** gene where its detection came from camels’ meat in Egypt (Al-Amery et al., 2018).

The heat stable staphylococcal enterotoxins (SEs) are the main reason of food poisoning over the worldwide (Le Loir et al., 2003). Thus, (Chao et al., 2015) reported that **sea**, **seb**, **sec**, and **sed** genes were not found except one strain harbored **sec** gene in ducks, whereas the presence of **sed** gene was only found in four isolates in this work. Furthermore, all other classical enterotoxin genes and (tst-1) weren’t detected in the current work which is similar to results recorded in Whiteface whistling ducks in Germany (Feßler et al., 2018).

### CONCLUSION

The study of Methicillin-resistant **S. aureus** (MRSA) from ducks was conducted to acquire more information for hidden problem solving in duck farms in Egypt. Although **S. aureus** has low significance as a problem in ducks, it is a real source for antimicrobial resistance spreading in surroundings and in vicinity of duck farms. MRSA has proved influences on public health of human and on the environment causing infection through livestock animals, so we need more investigation and surveillance studies were needed on the dissemination of MRSA.

### CONFLICT OF INTEREST

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

### AUTHORS' CONTRIBUTIONS

Abdel Rahman: Methodology (Bacterial Isolation, Antimicrobial Susceptibility Tests), writing–reviewing and editing of manuscript. Amer: Methodology (Molecular Identification of **S. aureus** Isolates Using Polymerase Chain Reaction Assay) and approved the final manuscript.

### REFERENCES

- Achek R, Hotzel H, Cantekin Z, Nabi I, Hamdi TM, Neubauer H, El-Adawy H (2018). Emerging of antimicrobial resistance in staphylococci isolated from clinical and food samples in Algeria. BMC Res. Notes.;11(1):663.http://doi: 10.1186/s13104-018-3762-2.
- Ahmed W, Neubauer H, Tomaso H, El Hofy FI, Monecke S, Abdelwab AA, Hotzel H (2020). Characterization of Staphylococci and Streptococci Isolated from Milk of Bovides with Mastitis in Egypt. Pathogens. 9:381-398. http://doi:10.3390/pathogens9050381.
- Al-Amery K, Elhariri M, Elsayed A, El-Moghazy G, Elhelw R, El-Mahallawy H, El Hairi M, Hamza D (2019). Vancomycin-resistant **S. aureus** isolated from camel meat and slaughterhouse workers in Egypt. Antimicrob. Resist. Infect. Control. 8:129. http://doi:10.1186/s13756-019-0585-4.
- Andreasen CB (2013). Staphylococcosis. In: Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, Nair V. (13th ed) Diseases of Poultry, 13th edn. Wiley-Blackwell Publishing, Ames, IA, pp 971–977.
- Bayles KW, Iandolo JJ (1989). Genetic and molecular analyses of the gene encoding staphylococcal enterotoxin D. J. Bacteriol. 171:4799-806. http://doi:10.1128/jb.171.9.4799-4806.1989
- Ben Zakour NL, Sturdevant DE, Even S, Guinane CM, Barbe C, Alves PD, Cochet MF, Gautier M, Otto M, Fitzgerald JR, Le Loir Y (2008). Genome-wide analysis of ruminant Staphylococcus aureus reveals diversification of the core genome. J. Bacteriol. 190(19):6302-17. http://doi: 10.1128/jb.01984-07
- Betley MJ, Mekalanos JJ (1988). Nucleotide sequence of the type A staphylococcal enterotoxin gene. J. Bacteriol. 170:34-41. http://doi:10.1128/jb.170.1.34-41.1988
- Blomster-Hautamaa DA, Kreiswirth BN, Kornblum JS, Novick RP, Schlievert PM (1986). The nucleotide and partial amino acid sequence of toxic shock syndrome toxin-1. J. Biol. Chem. 261:15783-6. PMID: 3782090
- Blumenthal S, Deplano A, Jourdain S, De Mendonça R, Hallin M, Nonhoff C, Rottiers S, Vergison A, Denis O (2013). Dynamic pattern and genotypic diversity of S. nasopharyngeal carriage in healthy pre-school children. J. Antimicrob. Chemother. 68(7):1517-23. http://
Bohach GA, Schlievert PM (1987). Nucleotide sequence of the staphylococcal enterotoxin C1 gene and relatedness to other pyrogenic toxins. Mol. Gen. Genet. 209: 15-20. http://doi:10.1007/BF00329830.

Cao Y, Wang J, Wang R, Zhang X, Chao G, Wu Y (2016). Prevalence and characterization of resistance genes with genetic clones among S. aureus isolates obtained from various sources in China. J. Med. Microbiol. 65:569-571. http://doi:10.1099/jmm.0.002250.

CDC (2002). Centers for Disease Control and Prevention, S. aureus resistant to vancomycin--United States, MMWR Morb Mortal Wkly Rep. 51: 565-567. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5126a1.htm.

Chao G, Bao G, Cao Y, Yan W, Wang Y, Zhang X, Zhou L, Wu Y (2015). Prevalence and diversity of enterotoxin genes with genetic background of S. aureus isolates from different origins in China. Int. J. Food Microbiol. 211:142-7. http://doi:10.1016/j.ijfoodmicro.2015.07.018.

Choi SM, Kim SH, Kim HJ, Lee DG, Choi JH, Yoo JH, Kang JH, Shin WS, Kang MW (2003). Multiplex PCR for the detection of genes encoding aminoglycoside modifying enzymes and methicillin resistance among Staphylococcus species. J. Korean Med. Sci. 18: 631-6. http://doi:10.3346/jkms.2003.18.5.631.

CLSI (2015). Performance Standards for Antimicrobial Susceptibility Testing, 25th ed. Supplement M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA. pp 64–71.

Couch JL, Soltis MT, Betley MJ (1988). Cloning and nucleotide sequence of the type E staphylococcal enterotoxin gene. J. Bacteriol. 170:2954-60. http://doi:10.1128/jb.170.7.2954-2960.1988.

Dweca CC, Zishiri OT, El Zowalaty ME (2019). Isolation and Characterization of Methicillin-Resistant S. aureus Isolated from Hatched Imported Poultry. Suez Canal Vet. Med. J. SCVMJ. 20: 317-330. http://doi:10.21608/SCVMJ.2015.64643

Eid HM, Alghamal AM, Elrefi WK, Youssef FM, Harb SM, Abd-Allah EM (2019). Prevalence, molecular typing, and antimicrobial resistance of bacterial pathogens isolated from ducks. Vet. World. 12:5:677-683. http://doi:10.14202/vetworld.2019.677-683.

El-Adawy H, Ahmed M, Hotzel H, Monecke S, Schulz J, Hartung J, Ehrich R, Neubauer H, Hafez HM (2016). Characterization of Methicillin–Resistant S. aureus Isolated from Healthy Turkeys and Broilers Using DNA Microarrays. Front Microbiol. 7:2019. http://doi:10.3389/fmicb.2016.02019.

Farghaly E, Shalaby A, Badr H (2015). Identification and Molecular Characterization of S. aureus from Newly Hatched Imported Poultry, Suez Canal Vet. Med. J. SCVMJ. 20: 317-330. http://doi:10.21608/SCVMJ.2015.64643.

Feßler AT, Thomas P, Mühldorfer K, Grobels M, Brombach J, Eichhorn I, Monecke S, Ehrlich R, Schwarz S (2018). Phenotypic and genotypic characteristics of S. aureus isolates from zoo and wild animals. Vet. Microbiol. 218:98-103. http://doi:10.1016/j.vetmic.2018.03.020.

García-Garrote F, Cercenedo E, Marín M, Bal M, Trincado P, Corredorja J, Ballesteros C, Pita J, Alonso P, Vindel A (2014). Methicillin-resistant S. aureus carrying the meC gene: emergence in Spain and report of a fatal case of bacteraemia. J. Antimicrob. Chemother. 69(1):45-50.

Grema HA, Geidam YA, Gadzama GB, Ameh JA, Suleiman A (2015). Methicillin resistant S. aureus (MRSA): a review. Adv. Anim. Vet. Sci. 3: 79–98. http://dx.doi.org/10.14737/journal.aavs/2015/3.2.79.98.

Han JE, Hwang SY, Kim JH, Shin SP, Jun JW, Chai JY, Park YH, Park SC (2013). CPR Methicillin resistant coagulase-negative staphylococci isolated from South Korean ducks exhibiting tremor. Acta Vet. Scand. 55:88. http://doi:10.1186/1751-0147-55-88.

Jensen LB, Frimodt-Møller N, Aarestrup FM (1999). Presence of erm gene classes in gram-positive bacteria of animal and human origin in Denmark. FEMS Microbiol. Lett. 170:151-8. http://doi:10.1111/j.1574-6968.1999.tb13368.x.

Jones CL, Khan SA (1986). Nucleotide sequence of the enterotoxin B gene from S. aureus. J. Bacteriol.166:29–33. http://doi:10.1128/jb.166.1.29-33.1986.

Khan AA, Nawaz MS, Khan SA, Steele R (2002). Detection and characterization of erythromycin-resistant methylese genes in Gram–positive bacteria isolated from poultry litter. Appl. Microb. Biotechnol. 59:377-81. http://doi:10.1007/s00253-001-1013-9.

Le Loir Y, Baron F, Gautier M (2003). S. aureus and food poisoning. Genet. Mol. Res. 2: 63–76.

Leonard FC, Markey BK (2008). Methicillin-resistant S. aureus in animals: a review, Vet. J.175: 27–36. http://doi:10.1016/j.tvjl.2006.11.008.

Lyon BR, Skurray R (1987) Antimicrobial resistance of S.aureus: genetic basis. Microbiol. Rev. 51: 88–134.

Marek A, Pyzik E, Stepieni-Pysniak D, Urban-Chmiel R, Jarosz LS (2018). Association Between the Methicillin Resistance of S. aureus Isolated from Slaughter Poultry, Their Toxin Gene Profiles and Prophage Patterns. Curr. Microbiol. 75(10):1256-1266. http://doi:10.1007/s00284-018-1518-9.

Nawaz MS, Khan SA, Khan AA, Khambaty FM, Cerniglia CE (2000). Comparative molecular analysis of erythromycin-resistance determinants in staphylococcal isolates of poultry and human origin. Mol. Cell Probes. 14:311-9. http://doi:10.1006/mcpp.2000.0320.

Nemati M, Hermans K, Lipinska U, Denis O, Deplano A, Struelens M, Devirese LA, Pasmans F, Haesebrock F (2008). Antimicrobial resistance of old and recent Staphylococcus aureus isolates from poultry: first detection of livestock-associated methicillin-resistant strain ST398. Antimicrob. Agents Chemother. 52: 3817–9. http://doi:10.1128/AAC.00613-08.

Nowrouzian FL, Karami N, Welinder-Olsson C, Ahrens C (2013). Virulence gene typing of methicillin-resistant S. aureus as a complement in epidemiological typing. J Microb. Methods. 93:173-6. http://doi:10.1016/j.mimet.2013.03.020.

Ono HK, Satoo Y, Narita K, Naito I, Hirose S, Hisatsune J, Asano K, Hu DL, Omoe K, Sugai M, Nakane A (2015). Identification and Characterization of a Novel Staphylococcal Emetic Toxin. Appl. Environ. Microbiol. 81(20):7034–40. http://doi:10.1128/AEM.01873-15.

Patel R, Uhl JR, Kohner P, Hopkins MK, Cockerill FR (1997). Multiplex PCR detection of vanA, vanB, vanC-1, and vanC-2/3 genes in enterococci. J. Clin. Microbiol. 35:703-7. http://doi:10.1128/JCM.35.3.703-707.1997.

Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FE (2002). Veterinary microbiology and microbial disease, first ed., Iowa: Blackwell Publishing Professional, pp 461–464.
• Radwan L, Madian H, Mahrous M, Badaw, Y, Zein El-Dein A (2019). Comparative studies on some productive traits and genetic diversity of two local strains of ducks: Sudani and Domyati. Arab Universities J. Agri. Sci. 27: 869-875. http://doi:10.21608/ajs.2019.43837
• Rodríguez-Lázaro D, Oniciuc EA, García PG, Gallego D, Fernández-Natal I, Dominguez-Gil M, Eiros-Bouza JM, Wagner M, Nicolau AI, Hernández M (2017). Detection and Characterization of S. aureus and Methicillin-Resistant S. aureus in Foods Confiscated in EU Borders. Front Microbiol. 8:1344. http://doi: 10.3389/fmicb.2017.01344
• Saha B, Singh AK, Ghosh A, Bal M (2008). Identification and characterization of a vancomycin-resistant S. aureus isolated from Kolkata (South Asia). J. Med. Microb. 57:72-79. http://doi:10.1099/jmm.0.47144-0
• Schwarz S, Roberts MC, Werckenthin C, Pang Y, Lange C (1998). Tetracycline resistance in Staphylococcus spp. from domestic animals. Vet. Microb. 1998;63:217-27. http://doi:10.1006/s1378-1135(98)00234-x
• Smyth JA, McNamee PT (2008). Staphylococci, streptococci and enterococci, in: Pattison M, McMullin P, Bradbury JM (6th ed) Poultry Diseases, 6th edn. Elsevier Health Sciences, Philadelphia, PA, pp 191-200
• Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR (2012). Rapid detection, differentiation and typing of methicillin-resistant S. aureus harbouring either mecA or the new mecA homologue mecA(LGA251). Clin. Microb. Infect. 18(4):395-400. http://doi:10.1111/j.1469-0691.2011.03715.x
• Strommenger B, Kettlitz C, Werner G, Witte W. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in S. aureus(2003). J. Clin. Microb. 41:4089-94. http://doi:10.1128/jcm.41.9.4089-4094.2003.
• Van Duijkeren E, Hengeveld P, Zomer TP, Landman F, Bosch T, Haenen A, van de Giessen A (2016). Transmission of MRSA between humans and animals on duck and turkey farms. J. Antimicrob. Chemother. 71:58-62. http://doi: 10.1093/jac/dkv313
• Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M (2005). Methicillin-resistant S. aureus in pig farming. Emerg. Infect. Dis. 11:1965-6. http://doi:10.3201/eid1112.050428.
• Wang W, Baloch Z, Jiang T, Zhang C, Peng Z, Li F, Fanning S, Ma A, Xu J (2017). Enterotoxigenicity and Antimicrobial Resistance of S. aureus Isolated from Retail Food in China. Front Microb. 8:2256. http://doi:10.3389/fmicb.2017.02256.
• Wendlandt S, Feßler AT, Monecke S, Ehricht R, Schwarz S, Kadlec K (2013). The diversity of antimicrobial resistance genes among staphylococci of animal origin. Int. J. Med. Microbiol. 303:338-49. http://doi:10.1016/j.ijmm.2013.02.006
• WHO (2020). World Health Organization Antibiotic Resistance. Geneva, Switzerland. Available online: who.int/news-room/fact-sheets/detail/antibiotic-resistance. Accessed 2020.