



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 ceftiofur. However, the resistances to kanamycin, tetracycline and gentamicin 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.

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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 food-borne 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 five 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 to human from 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 encodes against penicillin-binding protein (PBP2a) which is less influenced by beta-lactam antibiotics. However this encoded gene is located at the staphylococcal cassette chromosome *mec*(SCC*mec*) 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 misuse 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; Blumental 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 I.U (P10); cefoxitin 30 μ g (FOX30); Oxacillin 1 μ g (OX1); gentamicin 10 μ g (CN10); Kanamycin 30 μ g (K30); Ciprofloxacin 5 μ g (CF⁵); clindamycin 2 μ g (DA²); Azithromycin 15 μ g (AZM15); erythromycin 15 μ g (E¹⁵); Chloramphenicol 30 μ g (C³⁰); trimethoprim/sulfamethoxazole 1.25-23.75 μ g (SXT), doxycycline 30 μ g (DO³⁰); Tetracycline 30 μ g (T³⁰), amoxicillin + clavulanic acid 20 + 10 μ g (AMC30); cefotaxime 30 μ g (CTX 30); vancomycin 30 μ g (VA³⁰); levofloxacin 5 μ g (Lev 5), ampicillin 10 μ g (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'GTAGAAATGACTGAACGTCCGA-TAA3'	310 bp	(Nowrouzian et al.,2013)
		5'CCAATTCCACATTGTTTCG-GTCTAA3'		
	mecC	5'GAAAAAAAGGCTTAGAACGCCTc3'	138 bp	(Stegger et al.,2012)
		5'GAAGATCTTTTCCGTTTTCAGC3'		
Enterotoxins	sea	5'GGTTATCAATGTGCGGGTGG3'	102 bp	(Betley and Mekalanos, 1988)
		5'CGGCACTTTTTTCTCTTCGG3'		
	seb	5'GTATGGTGGTGTAAGTGAAGC 3'	164 bp	(Jones and Khan, 1986)
		5'CCAAATAGTGACGAGTTAGG 3'		
	sec	5'AGATGAAGTAGTTGATGTGTATGG 3'	451 bp	(Bohach and Schlievert, 1987)
		5'CACTTTTAGAATCAACCG 3'		
	sed	5'CCAATAATAGGAGAAAATAAAAG 3'	278 bp	(Bayles and Iandolo , 1989)
		5'ATTGGTATTTTTTTTCGTTTC 3'		
	see	5'AGGTTTTTTTCACAGGTCATCC 3'	209 bp	Couch et al.,1988
		5'CTTTTTTTTCTTCGGTCAATC 3'		
Toxic shock syndrome	tsst-1	5'ACCCCTGTTCCCTTATCATC 3'	326 bp	(Blomster-Hautamaa et al.,1986)
		5'TTTTCAGTATTTGTAACGCC 3'		
Erythromycin resistance	erm(B)-1	5'CATTTAACGACGAAACTGGC 3'	425 bp	(Jensen et al.,1999)
	erm(B)-2	5'GGAACATCTGTGGTATGGCG 3'		
Tetracycline resistance	tetK	5'GTAGCGACAATAGGTAATAGT 3'	360 bp	(Strommenger et al.,2003)
		5'GTAGTGACAATAAACCTCCTA 3'		
Aminoglycoside resistance	Aac6-aph2	5'GAAGTACGCAGAAGAGA 3'	491 bp	(Choi et al.,2003)
		5'ACATGGCAAGCTCTAGGA 3'		
Vancomycin resistance	vanA	5'CATGACGTATCGGTAAAATC 3'	885 bp	(Patel et al., 1997)

-ce ranges.

Molecular Identification of *Staph 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.), 4.5µL 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-

tion system (Alpha Innotech, biometra).

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)

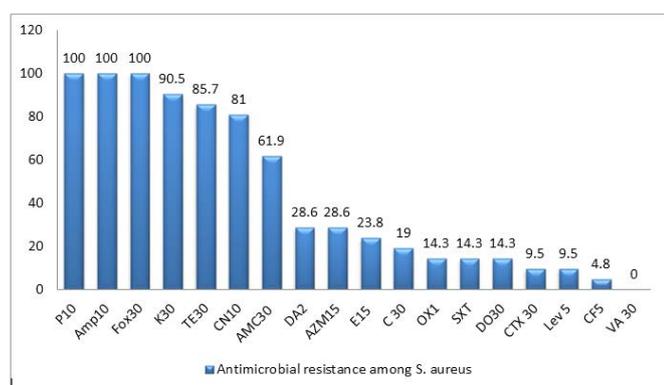


Figure 1: Antimicrobial resistance to 18 antimicrobials of *S. aureus* isolates from duck farms. The columns denote the percentages of resistant isolates for ducks. Penicillin-G (P¹⁰); ampicillin (AMP¹⁰); cefoxitin (FOX³⁰); Kanamycin (K³⁰); Tetracycline (T³⁰); gentamicin (CN¹⁰); Amoxicillin + clavulanic acid (AMC³⁰); Clindamycin (DA²); Azithromycin (AZM¹⁵); Erythromycin (E¹⁵); Chloramphenicol (C³⁰); Oxacillin (OX¹); Trimethoprim/sulfamethoxazole (SXT), Doxycycline (DO³⁰); cefotaxime (CTX³⁰); Levofloxacin (Lev⁵); Ciprofloxacin (CF⁵); vancomycin (VA³⁰)

10); Amoxicillin + clavulanic acid (AMC³⁰); Clindamycin (DA²); Azithromycin (AZM¹⁵); Erythromycin (E¹⁵); Chloramphenicol (C³⁰); Oxacillin (OX¹); Trimethoprim/sulfamethoxazole (SXT), Doxycycline (DO³⁰); cefotaxime (CTX³⁰); Levofloxacin (Lev⁵); Ciprofloxacin (CF⁵); vancomycin (VA³⁰)

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.

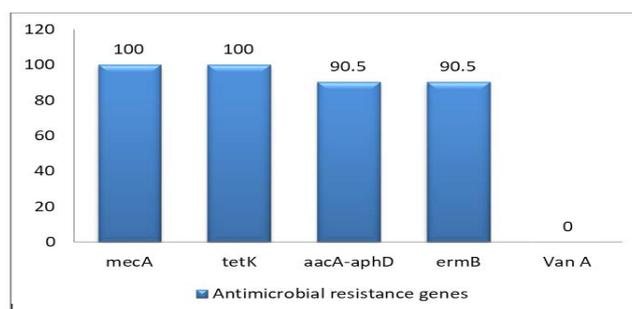


Figure 2: Antimicrobial resistance genes of *S. aureus* isolated from duck farms

Table 3: Correlation between phenotypic resistance and detection of resistance-associated genes in isolates

Antimicrobial classes	Target genes	Phenotypic resistance	Gene detection
Betalactam	<i>mecA</i>	21	21 (100%)
Gentamicin	<i>aacA-aphD</i>	17	19(90.5%)
Erythromycin	<i>ermB</i>	5	19 (90.5%)
Tetracycline	<i>tetK</i>	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	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, TE30, AMC30	<i>mecA, aacA-aphD, ermB, tetK</i>	
2	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, TE30, AMC30	<i>mecA, aacA-aphD, tetK, mecA</i>	
3	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, TE30, AMC30	<i>mecA, aacA-aphD, ermB, tetK</i>	
4	P ¹⁰ , AMP10, FOX30, OX1, CN ¹⁰ , K30, TE30, AMC30	<i>mecA, aacA-aphD, ermB, tetK</i>	
5	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, TE30, LEV5	<i>mecA, aacA-aphD, ermB, tetK</i>	sed
6	P ¹⁰ , AMP10, FOX30, DA ² , AZM ¹⁵ , E ¹⁵ , TE30	<i>mecA, ermB, tetK</i>	
7	P ¹⁰ , AMP10, FOX30, OX1, AMC30	<i>mecA, aacA-aphD, ermB, tetK</i>	
8	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, AZM ¹⁵ , C30, AMC30	<i>mecA, aacA-aphD, ermB, tetK</i>	sed
9	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, AZM ¹⁵ , TE30	<i>mecA, aacA-aphD, ermB, tetK</i>	
10	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, TE30, CTX30	<i>mecA, aacA-aphD, ermB, tetK</i>	
11	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, DA ² , E ¹⁵ , SXT, DO30, TE30, AMC30	<i>mecA, aacA-aphD, ermB, tetK</i>	
12	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, TE30, AMC30	<i>mecA, aacA-aphD, ermB, tetK</i>	
13	P ¹⁰ , AMP10, FOX30, OX1, CN ¹⁰ , K30, DA ² , E ¹⁵ , SXT, TE30	<i>mecA, aacA-aphD, ermB, tetK</i>	
14	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, C30, DO30, TE30	<i>mecA, aacA-aphD, ermB, tetK</i>	
15	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, TE30, AMC30	<i>mecA, aacA-aphD, ermB, tetK</i>	
16	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, DA ² , AZM ¹⁵ , E ¹⁵ , TE30	<i>mecA, aacA-aphD, tetK</i>	
17	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, TE30, AMC30	<i>mecA, ermB, tetK</i>	
18	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, DA ² , AZM ¹⁵ , C30, TE30, AMC30	<i>mecA, aacA-aphD, ermB, tetK</i>	sed
19	P ¹⁰ , AMP10, FOX30, CN ¹⁰ , K30, TE30	<i>mecA, aacA-aphD, ermB, tetK</i>	
20	P ¹⁰ , AMP10, FOX30, K30, CIP 5, DA ² , AZM ¹⁵ , E ¹⁵ , C30, SXT, DO30, TE30, AMC30, CTX30, LEV5	<i>mecA, aacA-aphD, ermB, tetK</i>	Sed
21	P ¹⁰ , AMP10, FOX30, K30	<i>mecA, aacA-aphD, ermB, tetK</i>	

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 methi-

cillin resistance in different poultry spp. such as chicken (Dweba et al., 2019), turkey (El-Adawy et al., 2016), and food products (Acheh 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

al., 2019).). 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 misuse 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 *S. aureus* isolates were resistant to erythromycin, while 14.3% were resistant to SXT. Thus, *S. 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 *S. 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 *S. 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)*

(Wendlandt et al. 2013; Schwarz et al., 1998). All MRSA isolates (100%) in this study also carry tetracycline resistance gene *tetK* as well as MRSA isolates of duck in China (Cao et al., 2016). On contrary, lower percentage of *tetK* gene from avian species (chicken, duck and wild birds in in South Africa (42.5%) was reported (Dweba et al., 2019).

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 due 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).

Although, it is well known that vancomycin is the first choice drug for treatment of MRSA in human (Saha et al., 2008), but in 2002, the CDC (Centers for Disease Control and Prevention) declared a first case of *S. aureus* resistant to methicillin and vancomycin (CDC, 2002). Therefore, a continuous monitoring for vancomycin resistance is required.

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., 2019).

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 *see* 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, but 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.

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