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Molecular identification of vancomycin and methicillin resistant *Staphylococcus aureus* in baby chicks with omphalitis in Egypt

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ABSTRACT

Vancomycin and methicillin-resistant *Staphylococcus aureus* (VMRSA) is one of the important emerging pathogens worldwide that pose a challenge to a wide range of diseases in humans and animals. It is responsible for causing nosocomial and community infections, but it has also been reported to cause livestock infections. The aim of the study was to investigate the occurrence of vancomycin- and methicillin-resistant *S. aureus* in baby chicks with omphalitis in Egypt. Out of a total of 200 chicks, 10 isolates were recognized as *S. aureus* by microbiological and molecular methods. All *S. aureus* isolates were subjected to in vitro antimicrobial susceptibility testing against 12 antimicrobial agents by the disc diffusion method. Isolates revealed 100% resistance to clindamycin, methicillin, and amoxicillin, followed by azithromycin and quinupristin/dalfopristin (81.8%). Whereas, perceptible resistance was observed against sulfamethoxazole/trimethoprim, erythromycin, Linezolid, and tetracycline, with a percentage of 72.7%. Conversely, there is a higher sensitivity to gentamycin and ciprofloxacin, with percentages of 27% for each. The MIC results of vancomycin did not display inhibition of the growth of the tested isolates. Vancomycin had high MIC values of 64 µg/ml against all tested *S. aureus* isolates, which exhibited MIC values of 32 µg/ml. All isolates were found to be multidrug-resistant (MDR), indicating that they were resistant to at least three different classes of antibiotics. Molecular detection for the antibiotic resistance gene revealed that the *mecA* gene was detected in (10/11) isolates of *S. aureus* with a percentage of 90.9% which is responsible for methicillin resistance. None of the isolates contained *mecC* gene. Amplification of *Spa* virulence gene by using PCR showed that all isolates were positive for *spa* gene with a percentage of (100%). Furthermore, van A gene was detected in (6/11) isolates (54.4%), and van B was detected in 5/11 isolates with a percentage of 45.5%. The findings of this study confirm the presence of multi-drug resistant organisms in chicks. It significantly points to the great need to evaluate and monitor the incidence rate of multi-drug resistant organisms. The presence of VMRSA in poultry meat is of great concern for public health. Strict supervision and enforcement of laws to control the antibiotic usage in the food chain within established safe levels must be followed.

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INTRODUCTION

Egypt's poultry sector faced a variety of issues. One of them was early chick mortality, which caused significant economic losses, and many poultry experts and men have requested that it be investigated. In chickens, staphylococci, particularly *S. aureus*, are known to cause a variety of illnesses ranging from acute septicemia to chronic osteomyelitis. *S. aureus* is attracting widespread attention as a result of multi-resistant strains, which reduce the utility of antibiotics in human and animal medicine and so limit therapeutic alternatives. Staphylococcal infections were a widespread problem in chickens and turkeys, resulting in economic losses due to reduced egg production and weight gain (Adayel, 2005).

S. aureus can be found in the natural microbiota of chicken skin, feathers, respiratory tracts, and digestive tracts, just like in humans and other animals (Olayinka et al. 2010). *S. aureus* is associated with a number of clinical signs, including tenosynovitis, omphalitis, femoral head necrosis, infected hock and stifle joints caused by coccidiosis, and "bumblefoot" (Suleiman et al. 2013). It also causes skin and food-borne illnesses in animals, birds, and humans (El-Ghany, 2021). It is well recognized that *S. aureus* has zoonotic potential and poses a considerable foodborne danger to global public health (Zaman et al. 2020). However, this pathogen has been linked to a number of clinical conditions, including dermatitis, arthritis, osteomyelitis, synovitis, and omphalitis (El-Tawab et al. 2017).

Omphalitis can be defined as an inflammation of the navel. The term "navel ill" is widely used to describe incorrect navel closure followed by bacterial infection (mushy chick illness). It occurs within the first few days of life. It is spread from filthy hatchery equipment to newly hatched birds with unhealed navels. Bacteria linked with omphalitis have been shown to deteriorate and decompose critical residual yolk sac (RYS) nutrients that should have been used as a source of energy during the post-hatch period (Khan et al. 2004). Bacterial infection of the navel area is one of the leading causes of mortality in chicks within the

first week of hatching (Pattison et al. 2008). Several microbes, including *E. coli*, *Salmonella* spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* species, *Streptococcus* species, *Clostridium* spp., *Bacillus cereus* and Enterococcus were identified from bird yolk sac infections (Cortes et al. 2004).

The clinically most important MDR bacteria include extended spectrum beta-lactamase (ESBL) Enterobacteriaceae, methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant Enterococci (VRE). These three types of resistant bacteria are seen not just in hospitals, but also in farm animals and meat (Holzel et al. 2010). At present, about 70% of pathogenic microorganisms leading to nosocomial infections are resistant to at least one antimicrobial drug that were previously effective (Fair and Tor, 2014).

The term "MRSA" represents resistance to at least one antibiotic in three or more categories. Resistance to oxacillin or cefoxitin suggests non susceptibility to most β lactam antibiotics (Magiorakos et al. 2012). Waters et al. (2011) found that around 96% of *S. aureus* strains are resistant to at least one antibiotic with 32% resistant to multiple antimicrobial classes.

MRSA had its first appearance in 1961 in England (Fluit et al. 2013).

It is a common human and animal pathogen that has been detected in numerous clinical trials (Persoons et al. 2009).

MRSA is currently known as a superbug since it is resistant to practically every antibiotic available for treating *Staphylococcus* spp. (Stapleton & Taylor, 2007). Vancomycin is the recommended antibiotic for MRSA infections' was isolated for the first time more than two decades ago (Chambers, 2001). After this, vancomycin-resistant *S. aureus* (VRSA) has been isolated from more countries in North America, Europe, Asia, Africa, and South America (Chesneau et al. 2000).

The use of antibiotics in poultry and livestock production is favorable to farmers and the economy as well because it has generally improved poultry performance effectively and economically, but at the same time, the likely dissemination of antibiotic resistant strains of pathogenic and non-pathogenic organisms into the environment and their further transmission to humans via the food chain could also lead to serious consequences on public health (WHO, 2014). In addition to antibiotic resistance increasing from natural selection, bacteria can receive genetic material through the process of Horizontal Gene Transfer (HGT). HGT conferring resistance to many classes of antimicrobials has resulted in a worldwide epidemic of nosocomial and community infections caused by multidrug-resistant microorganisms, leading to suggestions that mankind in effect is returning to the pre-antibiotic era (Warnes et al. 2012).

Regulatory measures such as restriction or banning the use of antimicrobial compounds for specific purposes, or in specific animal species, have also been established. Keeping these concerns of multidrug resistance in consideration, this study is undertaken for isolation and molecular identification of vancomycin and Methicillin-Resistant *S. aureus* in baby chicks with omphalitis in Egypt.

2. MATERIALS and METHODS

2.1. Samples

Between November 2022 and August 2023, 200 organ samples (liver, heart, intestine and yolk sac) were collected from 1-day-old broiler chicks with omphalitis from different governorates in Egypt. The broiler chicks ranged in age from 1 to 7 days. The samples were transported in sterile plastic bags under aseptic conditions to the Reference Laboratory for Veterinary Quality Control on Poultry Production, Dokki, Egypt (RLQP), in cool boxes with ice packs to isolate *Staphylococcus aureus*.

2.2. Isolation and identification of *Staphylococcus aureus*

S. aureus was isolated according to Shokry et al. (2018) by inoculating 10 g of

samples into 90 ml of buffer peptone water (Oxoid Limited, Thermo Fisher Scientific Inc., UK) and incubating at 37 °C for 24 h. A loopful of enriched samples was then streaked on Baird Parker agar (Oxoid Limited, Thermo Fisher Scientific Inc., UK) and Mannitol Salt Agar plates (Oxoid Limited, Thermo Fisher Scientific Inc., UK), and incubated at 37 °C for 48 h. The plates were examined for the presence of black colonies without clear or opaque zones on Baird Parker and yellow or red colonies on Mannitol Salt agar. The suspected *S. aureus* colonies were identified according to Holt et al. (1994) using different biochemical tests such as coagulase, catalase, sugar fermentation (mannose, sucrose, mannitol, trehalose, maltose, lactose, and raffinose), urea, Voges-Proskauer, ONPG, and novobiocin sensitivity.

2.3. Antimicrobial susceptibility testing of isolated *S. aureus* isolates using disk diffusion

The phenotypic antimicrobial profile of the isolated *S. aureus* was assessed by disk diffusion technique using 12 commercial disks (Oxoid Limited, Thermo Fisher Scientific Inc., UK) following Clinical and Laboratory Standard Institute guidelines (CLSI 2020). The tobramycin and methicillin breakpoints for the disk diffusion test were interpreted according to CLSI (2017). The antibiotic disks and corresponding concentrations used in this study were clindamycin (2 µg), erythromycin (15 µg), gentamycin (10 µg), tetracycline (30 µg), azithromycin (15 µg), methicillin (5 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), trimethoprim-sulphamethoxazole (25 µg), amoxicillin (25 µg), linezolid (30 µg), and quinupristin/dalfopristin (15 µg). Isolates shown to be resistant to at least three different classes of agents were classified as multidrug resistant (MDR) (Kiratisin et al. 2008).

2.4. Determination of multi-drug resistance index (MDRI)

Multi-drug resistance index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was exposed (Chandran et al. 2008). Isolates with MDRI values of more than 0.2 were considered highly resistant.

2.5. Determination of minimum inhibitory concentration (MIC) for vancomycin using the broth macrodilution method

The MIC test was used to determine the susceptibility of *S.-aureus* isolates to vancomycin, as the disk diffusion test cannot distinguish between vancomycin-susceptible, intermediate, and resistant isolates. All of these give inhibition-zones with similar diameter sizes (CLSI 2020). The microdilution broth technique was used to determine the minimum inhibitory concentration of vancomycin against 25 isolated *S.-aureus* following the Clinical and Laboratory Standards Institute (CLSI 2017; CLSIM07-A9, 2012). Vancomycin was obtained from Sigma-Aldrich (St. Louis, USA). The MIC breakpoints of vancomycin against *Staphylococcus* species range from 4 to 32 mg/mL (CLSI 2020). To obtain the different concentrations of vancomycin, a twofold serial dilution was carried out from the working solution (1 mg/mL). For the preparation of bacterial inoculums, pure overnight yellow or red colonies cultured on mannitol salt agar medium were suspended in Mueller–Hinton broth (MHB) medium (Oxoid Limited, Thermo Fisher Scientific Inc., UK) and adjusted to 5×10^5 CFU/mL as described by CLSIM07-A9 (2012). In the dilution series, 100 µl of each bacterial inoculum was added to 2 ml of MHB medium containing vancomycin. The tubes were incubated for 16 to 20 h at 35 ± 2 °C. The last two tubes contained a positive control for each *Staphylococcus* species isolate and a negative control for MHB. The MIC was considered the lowest vancomycin concentration, which produced no visible growth (no turbidity was recorded).

2.6. Genotypic characterization by PCR:

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's rec-

ommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide primers used in PCR have specific sequences and amplify specific products, as shown in Table 1. Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an Applied Biosystems 2720 thermal cycler.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm (Sambrook et al. 1989). For gel analysis, 15 µl of the products were loaded into each gel slot. Agene ruler 100 bp ladder (Fermentas, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software. (Automatic Image Capture Software, Protein Simple, Formerly Cell Bioscience, USA)

RESULTS

Incidence of *S. aureus* in chicks

In this study, out of 200 organ samples, 10 (5%) were positive for *S. aureus* by phenotypic isolation and PCR technique.

Antimicrobial sensitivity test:

Antimicrobial susceptibility testing against 12 antimicrobial agents was done by the disc diffusion method. *S. aureus* revealed 100% resistance to clindamycin, methicillin, and amoxicillin, followed by azithromycin and quinupristin/ dalfopristin with a percentage of 81.8%. Whereas perceptible resistance was observed against sulfamethoxazole/ trimethoprim, erythromycin, Linezolid, and tetracycline, with 72.7% of the isolates being resistant (for each). Conversely, there is a higher sensitivity to gentamycin and ciprofloxacin, with percentages of 27% for each. Table 2. The MIC results of vancomycin did not display inhibition of the growth of the tested iso-

lates. Vancomycin had high MIC values of 64 $\mu\text{g ml}^{-1}$ against all tested *S. aureus* isolates, which exhibited MIC values of 32 $\mu\text{g ml}^{-1}$. All isolates were found to be multidrug-resistant (MDR), indicating they were resistant to at least three different classes of antibiotics. (Table3).

Identification of MRSA and VRSA producers

All isolates that were resistant to methicillin are considered MRSA. The strains of *S. aureus* that were found to be resistant to vancomycin were screened as VRSA with a percentage of 81.8%. Table 2.

Molecular characterization of resistant and resistant genes in *S. aureus*:

Molecular detection for the antibiotic resistance gene revealed *mecA* was detected in

10/11 of isolated *S. aureus* with a percentage of 90.9%, which is responsible for methicillin resistance in *S. aureus* (Fig. 1). None of the isolates contained the *mec C* gene (Fig. 2). Furthermore, the *vanA* gene was detected in 6/11 isolates (54.4%), *vanB* was detected in 5/11 isolates with a percentage of 45.5%, and *van C1* was detected only in two isolates with a percentage of 18.2%, as shown in Figs. 4 and 6.

Molecular characterization of virulence genes of *S. aureus* revealed that the *Spa gene* was present in all isolates with a percentage of 100% (Fig 3).

Table 1. Oligonucleotide primers for PCR amplification of antibiotic resistance and virulence genes of isolated *S. aureus*

Microbial agent	Gene	Primer sequence (5'-3')	Amplified segment (bp)	Reference
<i>S. aureus</i>	<i>mecC</i>	GCTCCTAATGCTAATGCA TAAGCAATAATGACTACC	304 bp	Cuny et al.2011
	<i>mecA</i>	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure et al.2006
	<i>vanA</i>	CATGACGTATCGGTAATAATC ACCGGGCAGRGTATTGAC	885 bp	Patel et al.1997
	<i>Spa</i>	TCA ACA AAG AAC AAC AAA ATG C GCT TTC GGT GCT TGA GAT TC	226 bp	Wada et al.2010
	<i>vanB</i>	GTGACAAACCGGAGGCGAGGA CCGCCATCCTCCTGCAAAAAA	433 bp	Kariyama et al.2000
	<i>VanC1</i>	GGTATCAAGGAAACCTC CTTCCGCCATCATAGCT	822 bp	

Table 2. Antimicrobial susceptibility of the isolated *S. aureus* using the disk diffusion test

Antibiotic	Resistant number (%)	isolates	Intermediate isolates number (%)	iso-	Sensitive isolates number (%)
Clindamycin (CD)	11(100%)		0		0
Erythromycin (E)	8(72.7%)		3(27%)		0
Trimethoprim -sulphamethoxazole (SXT)	8(72.7%)		1(9%)		2(18%)
Gentamycin (G)	4(36%)		4(36%)		3(27%)
Tetracycline (T)	8(72.7%)		3(27%)		0
Azithromycin (AZI)	9(81.8%)		2(18%)		0
Methicillin (M)	11(100%)		0		0
Ciprofloxacin (CIP)	2(18%)		6(54.5%)		3(27%)
Chloramphenicol (C)	6(54.5%)		4(36%)		1(9%)
Amoxicillin (AMX)	11(100%)		0		0
Linezolid (LNZ)	8(72.7%)		0		3(27%)
Quinupristin/dalfopristin (QDA)	9(81.8%)		2(18.2%)		0
Vancomycin	9(81.8%)		(18.2%)		

The percentage of sensitive, intermediate, and resistant *S. aureus* isolates were calculated according to total number of isolates for *Staphylococcus aureus* (11), breakpoints for disk diffusion were according to CLSI 2017 and 2020.

Table 3. Drug resistance patterns and molecular characterization of the 11 strains of *S. aureus*.

Isolate	Resistant Antibiotic	MDR index	MDR	<i>mec A</i>	<i>mec C</i>	<i>vanA</i>	<i>van B</i>	<i>van C</i>	<i>spa</i>
1.	CD-SXT-G-V-M-AMX	0.5	+	+	-	+	-	-	+
2.	CD-E-SXT-AZ-T-C-L-AMX-Q-V-M	0.9	+	+	-	+	-	-	+
3.	CD-E-G-AZ-C-CIP-L-T-AMX-M-Q-V	1	+	+	-	-	+	+	+
4.	CD-E-SXT-AZ-C-T-M-CIP-L-AMX-Q-V	1	+	+	-	-	+	-	+
5.	CD-E-AZ-G-C-T-M-L-AMX-Q	0.8	+	+	-	+	-	-	+
6.	CD-E-SXT-AZ-AMX-Q-V-M-T-L	0.8	+	+	-	+	-	-	+
7.	CD-SXT-AZ-V-M-AMX-L	0.6	+	+	-	+	-	-	+
8.	CD-Q-V-M-SXT	0.4	+	-	-	+	-	-	+
9.	CD-E-G-AZ-T-Q-M-AMX	0.7	+	+	-	-	+	+	+
10.	CD-E-SXT-AZ-T-C-L-AMX-Q-V-M	0.9	+	+	-	-	+	-	+
11.	CD-E-SXT-AZ-T-C-L-AMX-Q-V-M	0.9	+	+	-	-	+	-	+
			11/11	10/11	0/11	6/11	5/11	2/11	11/11

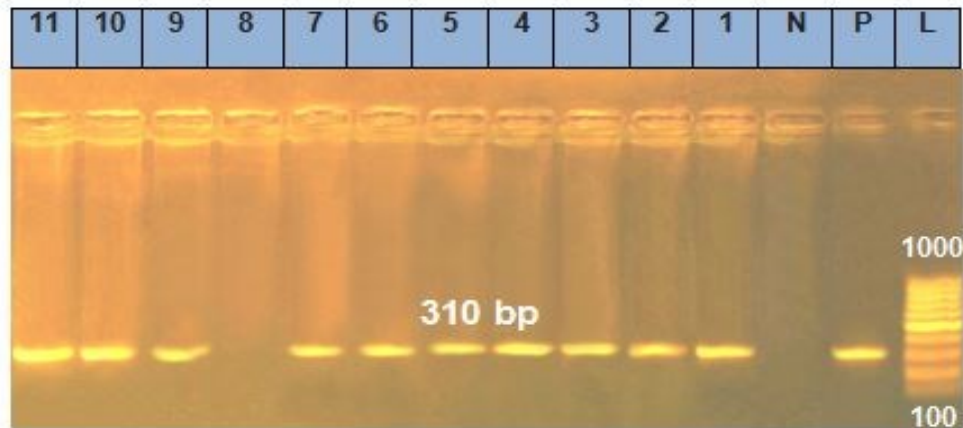


Fig 1. Agarose gel electrophoresis of PCR for detection of *mecA* gene in *S. aureus*. Showing amplification of 310 bp. in 10/11 examined samples. L (Ladder): DNA ladder (100-1000 bp). All samples are positive except one sample Lane (8). P: Positive control; N: Negative control

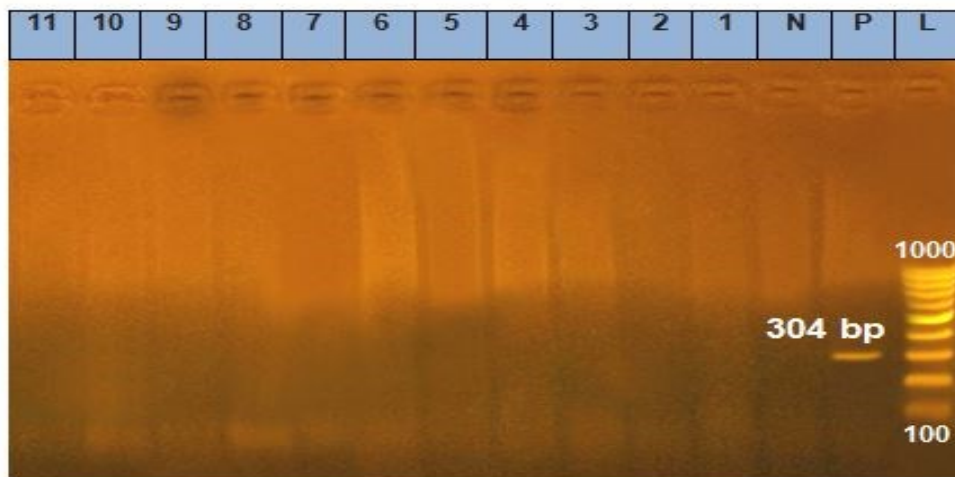


Fig 2. Agarose gel electrophoresis of PCR for detection of *mecC* gene in *S. aureus*. Showing no amplification of 304 bp. in 11 examined samples. L (Ladder): DNA ladder (100-1000bp).. P: Positive control; N: Negative control.

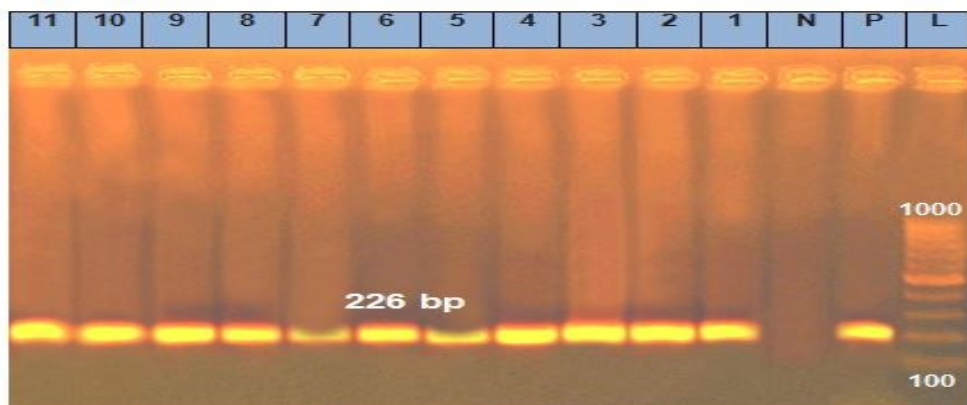


Fig3. Agarose gel electrophoresis of PCR for detection of *Spa* gene in *S. aureus*. Showing amplification of 226 bp. in 11 examined samples. L (Ladder): DNA ladder (100-1000bp). P: Positive control; N: Negative control

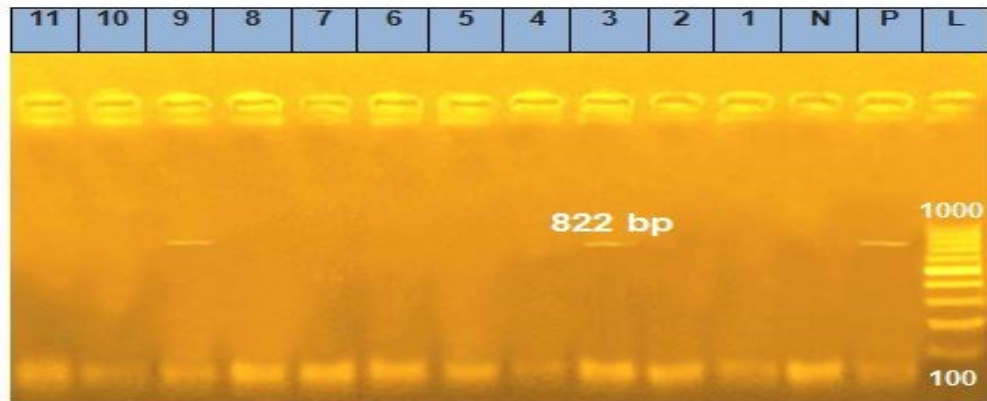


Fig 4. Agarose gel electrophoresis of PCR for detection of Van C1 gene in *S. aureus* in 2/11 examined samples. L (Ladder): DNA ladder (100-1000bp). All samples are negative except two samples lanes (3&9) is positive. P: Positive control; N: Negative control

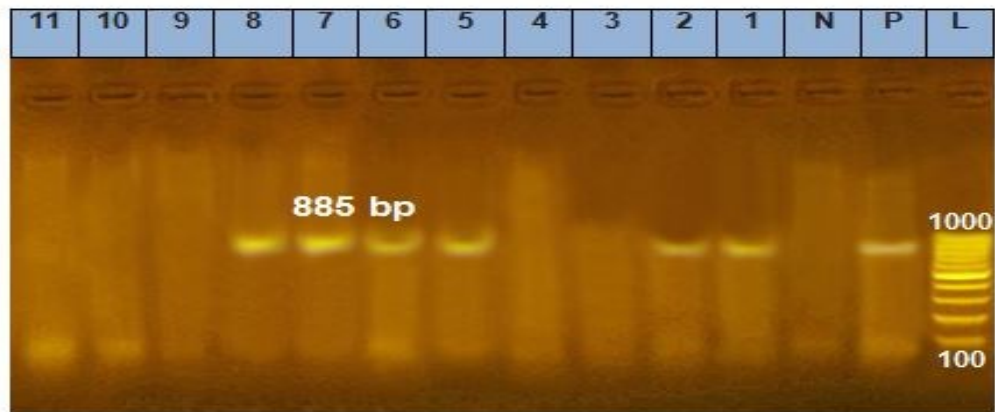


Fig 5. Agarose gel electrophoresis of PCR for detection of Van A gene in *S. aureus* in 6/11 examined samples. L (Ladder): DNA ladder (100-1000bp). six samples are positive lanes (1,2,5,6,7&8). P: Positive control; N: Negative control.

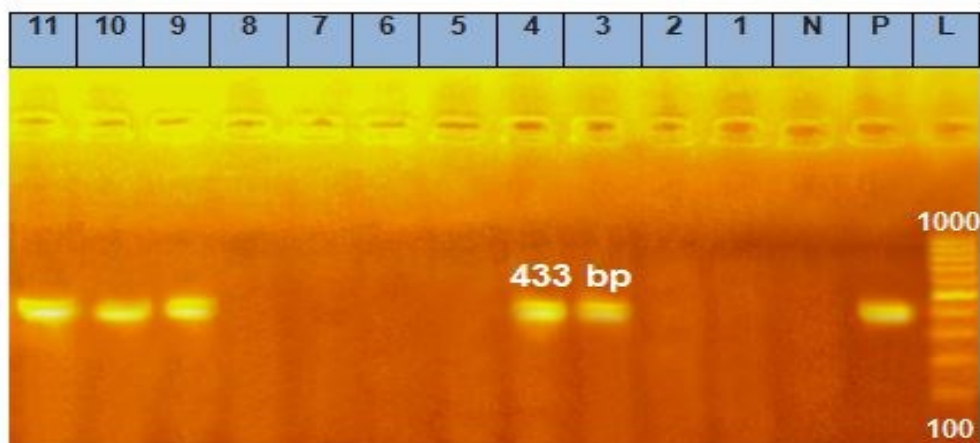


Fig 6. Agarose gel electrophoresis of PCR for detection of Van B gene in *S. aureus* in 5/11 examined samples. L (Ladder): DNA ladder (100-1000bp). five samples are positive lanes (3,4,9,10&11). P: Positive control; N: Negative control

DISCUSSION

S. aureus is a bacterium of significant importance because of its ability to cause a wide range of diseases and its capacity to adapt to diverse environmental forms (Waldvogel, 2000). In poultry, *S. aureus* is a ubiquitous pathogen found in feedstuffs as well as on equipment and utensils present in farms. It is considered an important cause of omphalitis, gangrenous dermatitis, and localized abscesses. (Abou-Zahr et al. 2018). Many studies recorded that *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. were identified as the major bacterial species responsible for omphalitis in broiler and layer chicks examined. These bacterial agents were also found to be associated with omphalitis in chicks earlier by Sato et al. (1961) and Ijaz et al. (1994).

The prevalence of Staphylococci with omphalitis cases ranked at 5% in this study, which disagrees with Nasrin et al. (2012), who recorded 24% in 1-3-day-old chicks and 28.6% in 4-7-day-old chicks, but a previous study (Iqbal et al. 2006) recorded a 0.5% prevalence of Staphylococci. It is therefore suggested that chicks be obtained from hatcheries that adopt strict hygienic measures during the whole hatching process. Moreover, a hygienic environment should be provided to the young chicks during brooding, and special attention should be paid to the humidity in the brooding house.

The low incidence of *S. aureus* in this study agreed with Wladyka et al. (2011), who stated that the incidence rate of staphylococcosis in chickens ranges from 0.5 to 20%. On the other hand, the current result disagreed with Rasheed (2011), who isolated a higher percent (50.98%) of *S. aureus* from broiler chickens. The difference in percentage of isolation could be attributed to many factors, some of which are related to birds, such as age, immune virulence, and resistance gene detection. Other factors might be related to differences in hygienic measures inside farms.

Extensive use and misuse of antimicrobial drugs in the poultry industry is the main cause of resistance to commonly used antibiotics and

the rise of multi-drug-resistant strains (Sharada et al. 2009). In the present study, the antibiotic resistance patterns of *S. aureus* in poultry show resistance to various antibiotics commonly used in veterinary medicine. High percentages of resistance were detected for clindamycin and amoxicillin (100% resistant isolates). Contrary to the findings of Benrabria et al. (2020) and Silva et al. (2022), who reported resistance to clindamycin in a percentage of 68.6%, 97.5%, and 97.3%, respectively, the level of resistance in this study was higher.

The resistance to quinupristin/dalfopristin in this study was higher than that obtained by Malik et al. (2023), who revealed resistance in a percentage of 13%. It was reported that the azithromycin resistance in this study was 81.8%, lower than those obtained by Malik et al. (2023), who reported resistance in a percentage of 84%. Considering this study, 11 isolates (100%) were methicillin-resistant, which was higher than those obtained by Malik et al. (2023), of whom only 4% were resistant to methicillin. The reason behind the high prevalence of MRSA might be due to the high common use of methicillin for treatment and growth purposes, as well as to avoid the widespread use of penicillin. MRSA is frequently associated with other antibiotic resistance, particularly aminoglycoside resistance, fluoroquinolones, lincosamides, and macrolides. In addition, vancomycin and linezolid (Shlaes & Projan, 2009).

Increase the prevalence of MRSA in the current study. Go ahead with many studies. Previously, El-Tawab et al. (2017) detected 66.6% MRSA from chicken samples in Giza, Egypt. Recently, Olayinka et al. (2010) also reported 39.8% MRSA from poultry farm isolates in Zaria, Nigeria. Benrabria et al. (2020) reported 33.5% MRSA in laying hens. But the dissimilar results of MRSA reported in chicken meat were found to be 20.0% in Bangladesh (Ali et al. 2017) and 25.0% from fresh chicken in Germany (Feßler et al. 2011). 15.9% MRSA in broilers in Algeria. Earlier,

Moreover, an increase in the prevalence of vancomycin-resistant *S. aureus* (VRSA, 80.8%) was observed in the present study. This is in

line with other documented percentages of vancomycin resistance (9%–46%) among *S. aureus* isolates in animals and food on the African continent, as reported by **Lozano et al. (2016)**. This study's findings on vancomycin resistance contrast with the findings reported by **Naeim et al. (2023)**, who found 100% susceptibility to vancomycin. Also, **Osman et al. (2016)** found vancomycin- and methicillin-resistant poultry in proportions of 27.8% and 52.8%, respectively. Recently, **El-Ghany et al. (2021)** reported 54% VRSA from poultry in northwest Algeria. Vancomycin is not commonly used for the treatment of poultry infections. But various degrees of vancomycin resistance were found during antibiotic susceptibility testing of poultry samples. For decades, vancomycin has been frequently used where MRSA is found (**Cong et al. 2019**).

The antimicrobial susceptibility testing revealed the occurrence of multidrug-resistant *S. aureus* in poultry. Similar high resistance to beta-lactams and erythromycin has been equally reported among coagulase-positive *S. aureus* isolated from smallholder flocks in Maiduguri, northeastern Nigeria (**EFSA, 2011**) and in Zaria, north-central Nigeria (**Otalu et al. 2011**).

the overuse of antibiotics in animal husbandry and for other veterinary purposes encourages resistant microbes to emerge via selective pressure (**Enemor et al. 2015**). The high resistance of *S. aureus* to beta lactams may be due to the actions of β -lactamase and/or cephalosporins, which destroy the β -lactam antibiotics (**Ogundare & Ekundayo, 2016**), or the production of PBP2a, an alternative target that is resistant to inhibition by the penicillins (**Akagha et al. 2015**).

It was found that most isolates were highly sensitive to ciprofloxacin and gentamicin; similar findings were reported by **Ciocîrlan (2008)**, contradictory to those reported by **Jamali et al. (2015)**, who stated that *S. aureus* is resistant to gentamicin and ciprofloxacin. The differences in resistance patterns are widely due to factors that include differences in geographical locations, particular bacteria species involved, animal production systems, the extent to which antibiotics are used, sampling

techniques, and the period of sampling (**Adzitey et al. 2015**).

All 11 (100%) *S. aureus* isolates detected proved to be MDR. Similar results of MDR were reported in previous investigations on chicken meat and were found to be 75.0% in Bangladesh (**Ali et al. 2017**) and 18.2% in the United States (**Hanson et al. 2011**).

Molecular detection of the antibiotic resistance gene for *S. aureus* revealed that all examined 10/11 isolates (90.9%) of *S. aureus* were positive for the *mecA* gene. The *mecA* gene is responsible for the resistance to methicillin, which codes for the penicillin-binding protein PBP 2A (**Wielders et al. 2002**). This was also reported by **Pyzik et al. (2014)**, who detected the *mecA* gene in two *S. aureus*-like strains isolated from table eggs. **Zarfel et al. (2010)** detected 26 ESBL-producing *E. coli*, five *mecA* genes harboring Staphylococci (but no MRSA), and four VRE in chicken meat samples of Austrian origin.

In the current study, the van A gene was detected in 6/11 isolates (54.4%), and van B was detected in 5/11 isolates with a percentage of 45.5%. The high detection rate of the van A phenotype in the poultry is consistent with the findings by other researchers (**Radu et al. 2001**). Only two isolates harbored van C1. A previous study by **Radu et al. (2001)** reported that van A and vanC2/C3 genotypes were isolated from poultry in Malaysia but not van B. Considering VRE isolated from poultry and other animal sources, Van A-type resistance is predominant (**Chen et al. 2002**).

S. aureus attaches to the surface of the host cell to begin the colonization process via adhesins that the bacteria have on their surface (**Yılmaz et al. 2022**). The majority of the adhesins present in *S. aureus* are protein A proteins in the X-region and Lg G-binding regions that are found in cell peptidoglycan (*spa*) (**Sharma et al. 2016**). Due to its ability to attach to molecules and agglutinate bacteria against particular bacterial antigens, protein A is utilized as a crucial reagent in immunology and diagnostic laboratory technology because it can attach to molecules and agglutinate bacteria against a

particular antigen (Atalla, 2010). The *spa* gene produces protein A as a result of this process. The investigation indicated that all isolates were positive for the presence of the *spa* (X-region) gene.

In this study 11 isolates were screened for virulence genes using PCR. The results of this investigation revealed 100 % (11/11) of the isolates carrying the *spa* gene. This result disagrees with the result recorded by Momtaz et al. (2013), who reported a *spa* with a percentage of 26.82%, respectively.

CONCLUSION

MRSA found in poultry and poultry farm personnel is a major health issue that indicates the zoonotic significance of MRSA. In this study, we found MRSA and VRSA in broiler chicks's samples, which reveal the direct threat to the consumer's health. The findings underscore the necessity of mitigating actions based on the farm-to-fork principle at all points in the poultry production chain to prevent or eradicate MRSA transmission in humans, poultry, and farms. The implementation of continued surveillance and monitoring schemes for the *S. aureus* continuum to monitor their emergence, spread, and significance to human health. Additionally, measures such as reducing the use of antibiotics as growth promoters.

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