Int.J.Curr.Microbiol.App.Sci (2024) *13*(10): 213-222

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 13 Number 10 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article

https://doi.org/10.20546/ijcmas.2024.1310.026

Prevalence and Pattern of Antibiotic Resistance of *Staphylococcus aureus* Isolated from Poultry in Khartoum State, Sudan

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Keywords

Staphylococcus aureus, antibiotic resistance, farm workers

Article Info

Received:
18 August 2024
Accepted:
30 September 2024
Available Online:
10 October 2024

A B S T R A C T

This study investigates the prevalence of *Staphylococcus aureus* contamination and assesses antimicrobial resistance patterns in chicken meat, chicken swabs, and farm workers in Khartoum State, Central Sudan. Four hundred samples were collected from the poultry sector. *S. aureus* was found in 27.8% of the samples, with 18.9% from chicken meat, 34.7% from chicken swabs, and 23.5% from farm workers. The prevalence did not significantly differ between sources (P>0.05). Among the 111 isolates, 102 (91.9%) were mecA gene positive, classified as MRSA, while nine isolates were mecA negative but still methicillin-resistant. Resistance patterns against seven antibiotics were tested. For mecA positive isolates, resistance rates were 15.7% for Penicillin G, 86.3% for Oxacillin, 88.2% for Cefoxitin, 100% for Methicillin, 9.8% for Vancomycin, 100% for Erythromycin, and 97.1% for Tetracyclines. For mecA negative isolates, resistance rates were 11.1% for Penicillin G, 11.1% for Oxacillin, 33.3% for Cefoxitin, 100% for Methicillin, 11.1% for Vancomycin, 100% for Erythromycin, and 88.9% for Tetracyclines. Sensitivity to Penicillin and Vancomycin was observed in 84.3% and 90.1% of mecA positive isolates, and 88.9% of mecA negative isolates. MICs for Penicillin ranged from 45 to 0.005 µg/ml for mecA-positive isolates and from 11.25 to 0.01 µg/ml for mecA-negative isolates. MICs for Vancomycin ranged from 12.5 to 0.17 µg/ml for mecA-positive isolates and from 6.25 to 0.35 µg/ml for mecA-negative isolates. The reference strain ATCC25923 showed MICs of 2.8 µg/ml for Penicillin and 6.25 µg/ml for Vancomycin. The highest isolation rate of *S. aureus* was from chicken swabs, followed by chicken meat and farm workers. The highest incidence of MRSA was also from chicken swabs, indicating a potential source of antimicrobial-resistant *S. aureus* infection. Emphasizing food hygiene is essential to sustain public health.

Introduction

Staphylococcus aureus is a major pathogen alongside *S. intermedius* and *S. hyicus*, causing extensive animal infections including pneumonia, joint infections, osteomyelitis, and septicemia in poultry (Hermans *et al.*, 2004; McNamee and Smyth, 2000; Linares and Wigle, 2001). It's particularly significant in causing intramammary infections, leading to economic losses in cattle and poultry farming. *S. aureus* is found in healthy

chickens, slaughtered poultry, and poultry environments (Butterworth, 1999).

Antimicrobial resistance poses a critical global public health issue, driven by extensive antimicrobial use in both human and animal health, and as growth promoters in food animal production (Aestrup and Wegener, 1999; Aarestrup, et al., 2000; Barber *et al.,* 2003).

This extensive use promotes the spread of antimicrobial-resistant zoonotic bacteria, turning animals into reservoirs of resistant infections transmissible to humans through food (Wegener, 1999).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent nosocomial pathogen causing food poisoning, pneumonia, post-operative wound infections, and hospital-acquired infections (Karmi, 2013). MRSA's resistance complicates infection treatment, and its genetic diversity and ability to acquire genetic material enable it to adapt and increase resistance (Moellering, 2012).

The mecA gene, located on the staphylococcal cassette chromosome mec (SCCmec), is a mobile genetic element contributing to MRSA's adaptability (Rozgonyi *et al.,* 2007).

Poultry production is rapidly growing, with increasing consumer demand for safe, pathogen-free products (Kearney, 2010). The presence of *S. aureus* in poultry necessitates laboratory surveillance due to widespread antimicrobial use in treating poultry infections. However, there is limited information on the antimicrobial susceptibility of *S. aureus* in Sudan.

This study aims to characterize antibiotic resistance in *S. aureus* isolates from chicken meat, chicken's swabs, and farm workers in Khartoum State.

Materials and Methods

Sampling

A total of 400 samples were collected including 164 chicken meat samples from modern supermarkets, traditional markets, and retail stores in Khartoum State, 219 chicken swabs from various farms in Khartoum State, and 17 swabs from farm workers. Samples were stored in sterile polyethylene bags (Chicken meat) or universal containers (Swabs) and transported on ice to the Bacteriology Unit in the Central Lab.

Isolation and Detection of *S. aureus*

Chicken meat samples were shaken in sterile polyethylene bags with 100 ml of Phosphate-Buffered Saline (PBS) for 2 min., and 1 ml of the rinse was transferred to 9 ml of Brain Heart Infusion Broth (BHIB) (Jackson *et al.,* 2013). Swabs were placed in sterile universal containers with 10 ml of BHIB.

Cultured samples were incubated at 37ºC for 48 h., then streaked on Mannitol Salt Agar (MSA) and incubated for another 48 hours. Yellow colonies were purified through sub-culturing and identified using microscopic, biochemical (Barrow and Feltham, 1993 and Olutiola *et al.,* 2000) and genotypic methods, including Polymerase Chain Reaction (PCR). Instruction Manual for Genomic DNA Extraction (2012) and DNA sequencing.

PCR for the detection of 16S rRNA gene

To confirm isolate identification, PCR was performed using specific 16S rRNA primers for *Staphylococcus*:

Forward: 5'-AGTTTGATCCTGGCTCAG-3'
Reverse: 5'-AGGCCCGGGAACGTATTCAC-3'
(Neetu and Murugen, 2016)

PCR for the detection of mecA gene

Positive *S. aureus* DNA samples were tested for the mecA gene using primers:

Forward:5'-GTAGAAATGACTGAACGTCCGATAA-3'
Reverse:5'-CCAATTCCACATTGTTTCGGTCTAA-3'

PCR conditions followed the method by McClure *et al.,* (2006).

Antimicrobial Susceptibility test

Resistance to antimicrobial agents was assessed using the disk diffusion method on Mueller Hinton Agar (MHA) (Clinical and Laboratory Standards Institute (CLSI) guidelines) (2018).

Seven antibiotics were tested: Penicillin-G (P), Oxacillin (OX), Cefixime (CFM), Methicillin (MET), Vancomycin (VAC), Erythromycin (E), and Tetracycline (TE). *Staphylococcus aureus* ATCC25923 strain was used for quality control.

Minimum Inhibitory Concentration (MIC)

MIC for penicillin and vancomycin was determined using the broth dilution method of Clinical and Laboratory Standards Institute CLSI (2018). Serial dilutions method was prepared as follows: Stock solution of penicillin based on 90µg/ml and one ml from it was serially diluted in 1ml nutrient broth to prepare two-fold dilutions till 14th dilution as: 45, 22.5, 11.25, 5.6, 2.8, 1.4, 0.7, 0.35, 0.17, 0.09, 0.05, 0.02, 0.01,0.005 µg/ml. Stock solution of vancomycin based on 100µg/ml and one ml from it was serially diluted in 1ml nutrient broth to prepare two- fold dilutions till 9th dilution as: 50, 25, 125, 6.25, 3.1, 1.5, 0.7, 0.35, 0.17 mg/ml.

MIC was recorded as the lowest drug concentration with no visible growth. Bacterial suspension density was compared and adjusted according to the McFarland turbidity standard (Cheesbrough, 2006).

Statistical Analysis

Statistical analysis was performed using the Chi-square test to find the relationship between contaminated samples and sources of samples. The variations were regarded as significant at $p< 0.05$.

Results and Discussion

Out of the 400 collected samples, 111 (27.8%) were identified as *S. aureus* isolates, with 18.9% from 164 chicken meat samples, 34.7% from 219 chicken swab samples, and 23.5% from 17 farm worker samples (Table 1). The prevalence of *S. aureus* did not significantly differ between chicken meat, chicken swabs, and farm worker samples ($P > 0.05$). Out of the 111 *S. aureus* isolates, 102 (91.9%) were identified as carrying the mecA gene, with 93.6% from chicken meat samples, 90.8% from chicken swabs samples, and 100% from farm worker samples. The overall prevalence of MRSA in the 400 collected samples was 25.5% (Table 2), and no significant difference in MRSA prevalence was found between sample groups ($P > 0.05$).

Amplification of the mecA gene confirmed MRSA (Figure 1).

The 102 mecA gene-positive *S. aureus* isolates (29 from chicken meat, 69 from chicken swabs, and 4 from farm workers) were tested for antimicrobial resistance against seven different antibiotics: Penicillin-G, Oxacillin, Cefoxtin, Methicillin, Vancomycin, Erythromycin, and Tetracycline. Nine mecA-negative *S. aureus* isolates were also tested.

The antimicrobial resistances demonstrated by the isolates (MecA Positive *S.aureus*) from chicken meat were as follows: Vancomycin 4/10 (40%), Penicillin-G 6/16 (37.5%), Oxacillin 31/88 (35.2%), Cefixime 28/87 (32.2%), Methicillin 31/102 (30.4%), Erythromycin 31/102 (30.4% and Tetracycline 29/99 (29.3%). The antimicrobial resistances demonstrated by the isolates from chicken swabs were as follows: Tetracycline 66/99 (66.7%), Erythromycin 67/102 (65.7%), Methicillin 67/102 (65.7%), Cefixime 55/87 (63.2%), Oxacillin 53/88 (60.2%), Vancomycin 6/10 (60%) and Penicillin – G 6/16 (37.5%). The antimicrobial resistances demonstrated by the isolates from farm workers were as follows: Penicillin-G 4/16 (25%), Cefixime 4/87 (4.6%), Oxacillin 4/88 (4.5%), Tetracycline 4/99 (4%), Methicillin 4/102 (3.9%), Erythromycin 4/102 (3.9%) and Vancomycin 0/10 (0%) (Table 3).

The antimicrobial resistances demonstrated by the isolates (mecA Negative *S.aureus*) from chicken meat were as follows: Penicillin-G (100%, Oxacillin (100%), Vancomycin (100%), Cefixime (33.3%), Tetracycline (25%), Erythromycin (22.2%) and Methicillin (22.2%) (Table 4). The antimicrobial resistances demonstrated by the isolates (mecA Negative *S.aureus*) from chicken swabs were as follows: Methicillin (77.8%), Erythromycin (77.8%), Tetracycline (75%) and Cefixime (66.7%) (Table 4).

In this study, 86 isolates were sensitive to penicillin, while 16 isolates showed low resistance, significantly differing ($P < 0.05$) between sample sources. The other antibiotics (Oxacillin, Cefoxitin, Methicillin, Vancomycin, Erythromycin, and Tetracycline) showed no significant difference ($P > 0.05$) in resistance (Tables 3-4). All isolates resistant to methicillin in the sensitivity test were positive for the mecA gene and identified as MRSA, a known antimicrobial-resistant pathogen in poultry (Lee, 2003; Waters *et al.,* 2011; Karmi, 2013).

This study detected 100% resistance to methicillin and 50% resistance to other β-lactam antibiotics in chicken meat and swab samples, despite the absence of the mecA gene, possibly indicating the presence of the mecC gene. The MICs of penicillin-G varied from 45 to 0.005 µg/ml for sensitive mecA-positive *S. aureus* isolated from

chicken meat and swabs, while the reference strain ATCC25923 showed 2.8 µg/ml (Table 5). There were no significant differences among the diluents (P>0.05),

The MICs of vancomycin varied from 12.5 to 0.17 µg/ml for sensitive mecA-positive *S. aureus* from chicken meat, swabs, and farm workers, while the reference strain ATCC25923 showed 6.25 µg/ml (Table 6), with significant differences among diluents (P < 0.05)

In animals, *S. aureus* is one of the three major pathogenic staphylococcus species, together with *S. intermedius* and *S. hyicus* (Hermans *et al.,* 2004). In this study the contamination rate in chicken meat samples was 18.9%, slightly higher than the 17% found by Hanson *et al.,* (2011), but much lower than the 95.83% detected by Islam *et al.,* (2014), which poses a risk to human health if consumed without proper cooking.

The presence of *S. aureus* in chicken meat in this study is likely due to poor hygiene practices in poultry slaughter houses in Khartoum State, inadequate handling and transportation, and insufficient cooling and contamination in market and retail store refrigerators (Corner *et al.,* 2001; Malheiros *et al.,* 2010). The prevalence rate of 34.7% for *S. aureus* in chicken swab samples aligns with findings by Abdalrahman *et al.,* (2015) at 42.2% and Krupa *et al.,* (2014) at 38%. This prevalence is attributed to the breeding systems used in Sudan, where closed and semi-closed system farms suffer from poor ventilation and open system farms lack infrastructure, allowing dust and wild birds to enter and contaminate the barns, leading to *S. aureus* isolation from hen's nasal passages (McNamme and Smyth, 2000; Zong *et al.,* 2009; Liu *et al.,* 2012). Additionally, during sample collection, there were noticeable cases of bumble foot, osteomyelitis, and sudden death in chickens (Hassan *et al.,* 2012; McNamme and Smyth, 2000), only 17 samples were collected from farm workers due to their reluctance to provide nasal samples, resulting in a 23.5% presence rate of *S. aureus*.

This is consistent with studies isolating *S. aureus* from the hands and nasal passages of flight-catering staff, veterinarians, meat market, and farm workers (Hu *et al.,* 1995; Corner *et al.,* 2001; Kitai *et al.,* 2005). *S. aureus* isolates were tested for antimicrobial resistance against seven different antibiotics: Penicillin-G, Oxacillin, Cefoxtin, Methicillin, Vancomycin, Erythromycin, and Tetracycline. Nine mecA-negative *S. aureus* isolates were also tested. Because *S. aureus* is well known for the tendency to become resistant to some antimicrobials, so the study looked into the resistance pattern of *S. aureus* to some antimicrobial agents.

Antimicrobial resistance is an important public health concern worldwide. The extensive use of these antibiotics in animal husbandry contributes to the selection of drug-resistant strains. In this study, 86 isolates were sensitive to penicillin, while 16 isolates showed low resistance, significantly differing (P < 0.05) between sample sources. This contrasts with most studies reporting penicillin resistance in *S. aureus* (Lee, 2003; Febler *et al.,* 2011; Bhargava *et al.,* 2011; Hanson *et al.,* 2011; Nemeghaire *et al.,* 2013; Islam *et al.,* 2014; Abdalrahman *et al.,* 2015). This might be due to the absence of penicillin use in animal production for a long time or possible transmission from wildlife birds in contact with chickens (Monecke *et al.,* 2016). Tetracycline and erythromycin exhibited higher resistance percentages, consistent with previous studies (Lee, 2003; Febler *et al.,* 2011; Hanson *et al.,* 2011). This could be due to their extensive use in treating staphylococcal infections in poultry (White *et al.,* 2003) or as growth promoters (Lathers, 2001; Ishak *et al.,* 2017). Although vancomycin is usually considered sensitive, 10 isolates (4 from chicken meat and 6 from chicken swabs) showed no inhibition zone in sensitivity tests (Table 4), indicating possible vancomycin-resistant gene presence. Such strains pose a risk for antibiotic treatment as vancomycin is a last-resort antibiotic in humans and should not be used in animal treatment, aligning with Wijesekara *et al.,* (2017) findings that vancomycin-resistant genes evolved before bans on its use in livestock. The MICs of penicillin -G varied from 45 to 0.005 µg/ml for sensitive mecA-positive *S. aureus* isolated from chicken meat and swabs, while the reference strain ATCC25923 showed 2.8 µg/ml (Table 5). There were no significant differences among the diluents (P>0.05), contrasting with Losito *et al.,* (2005).

The MICs of penicillin -G varied from 45 to 0.005 µg/ml for sensitive mecA-positive *S. aureus* isolated from chicken meat and swabs, while the reference strain ATCC25923 showed 2.8 µg/ml. There were no significant differences among the diluents (P>0.05), contrasting with Losito *et al.,* (2005). This study detected 100% resistance to methicillin and 50% resistance to other β-lactam antibiotics in chicken meat and swab samples, despite the absence of the mecA gene, possibly indicating the presence of the mecC gene (Leonard and Markey, 2008; Shore *et al.,* 2011).

Table.1 Prevalence of *S. aureus* in chicken meat, chicken swabs and farm worker from Khartoum State, Central Sudan

Samples	No. of samples	Positive samples (%)	Prevalence (%)
Chicken meat	164	31	18.9
Chicken swabs	219	76	34.7
Farm workers	17	4	23.5
Total	400	111	27.75

Table.2 Prevalence of mecA gene in *S.aureus* isolated from different sources

Source of samples	Number of isolates	Number of contaminated isolates	Number of mecA +ve *S.aureus*	Frequency of mecA +ve *S.aureus*	Frequency of mecA +ve	Number of mecA –ve *S.aureus*	Frequency of mecA –ve *S.aureus*
Chicken meat	164	31	29	29/31 (93.6%)	29/164 (17.7%)	2	2/31 (6.5%)
Chicken swabs	219	76	69	69/76 (90.8%)	69/219 (31.5%)	7	7/76 (9.2%)
Farm workers	17	4	4	4/4 (100%)	4/17 (23.5%)	0	0
Total	400	111	102	102/111 (91.9%)	102/400 (25.5%)	9	9/111 (8.1%)

Table.3 Frequency of antimicrobial resistance of mecA-positive *S. aureus* (MRSA) isolated from different sources

Antibiotics	Samples	Number of resistant isolates	Number of tested isolates	Antibiotic resistant
Penicillin-G	Chicken meat	6	16	37.5%
	Chicken swabs	6	16	37.5%
	Farm workers	4	16	25%
Oxacillin	Chicken meat	31	88	35.2%
	Chicken swabs	53	88	60.2%
	Farm workers	4	88	4.5 %
Cefixime	Chicken meat	28	87	32.2%
	Chicken swabs	55	87	63.2%
	Farm workers	4	87	4.6%
Methicillin	Chicken meat	31	102	30.4%
	Chicken swabs	67	102	65.7%
	Farm workers	4	102	3.9%
Vancomycin	Chicken meat	4	10	40%
	Chicken swabs	6	10	60%
	Farm workers	0	10	0%
Erythromycin	Chicken meat	31	102	30.4%
	Chicken swabs	67	102	65.7%
	Farm workers	4	102	3.9%
Tetracycline	Chicken meat	29	99	29.3%
	Chicken swabs	66	99	66.7%
	Farm workers	4	99	4%

Table.4 The frequency of antimicrobial resistance of mecA negative *S. aureus* isolated from different samples.

Antibiotics	Samples	Number of resistant isolates	Number of tested isolates	Antibiotic-resistant
Penicillin-G	Chicken meat	1	1	100%
	Chicken swabs	0	1	0%
	Farm workers	0	0	0%
Oxacillin	Chicken meat	1	1	100%
	Chicken swabs	0	1	0%
	Farm workers	0	0	0%
Cefixime	Chicken meat	1	3	33.3%
	Chicken swabs	2	3	66.7%
	Farm workers	0	0	0%
Methicillin	Chicken meat	2	9	22.2%
	Chicken swabs	7	9	77.8%
	Farm workers	0	0	0%
Vancomycin	Chicken meat	1	1	100%
	Chicken swabs	0	0	0%
	Farm workers	0	0	0%
Erythromycin	Chicken meat	2	9	22.2%
	Chicken swabs	7	9	77.8%
	Farm workers	0	0	0%
Tetracycline	Chicken meat	2	8	25%
	Chicken swabs	6	8	75%
	Farm workers	0	0	0%

Table.5 Distribution of MICs of Penicillin-G for mecA positive *S.aureus* (MRSA) isolated from different samples.

Source of samples	Number and percentage of isolates with a MICs (µg /ml)													
	45	**22.5**	**11.25**	**5.6**	**2.8**	**1.4**	**0.7**	**0.35**	**0.17**	**0.09**	**0.05**	**0.02**	**0.01**	**0.005**
Chicken meat	0	2/5* 40%[a]	0	3/6* 50%[a]	3/7* 42.9%[a]	1/6* 16.7%[a]	3/9* 33.3%[a]	1/5* 20%[a]	1/3* 33.3%[a]	2/9* 22.2%[a]	1/3* 33.3%[a]	1/5* 20%[a]	1/7* 14.3%[a]	2/4* 50%[a]
Chicken swabs	3/3 100%[a]	3/5 60%[a]	3/3* 100%[a]	3/6 50%[a]	4/7 57.1%[a]	5/6 83.3%[a]	6/9 66.7%[a]	4/5 80%[a]	2/3 66.7%[a]	7/9 77.8%[a]	2/3 66.7%[a]	4/4 80%[a]	6/7 85.7%[a]	2/2 50%[a]
Farm workers	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total of isolates	**3**	**5**	**3**	**6**	**7**	**6**	**9**	**5**	**3**	**9**	**3**	**5**	**7**	**4**

*No. of isolates within each diluents/Total of isolates
*Percentages within columns are non-significantly different (P>0.05)

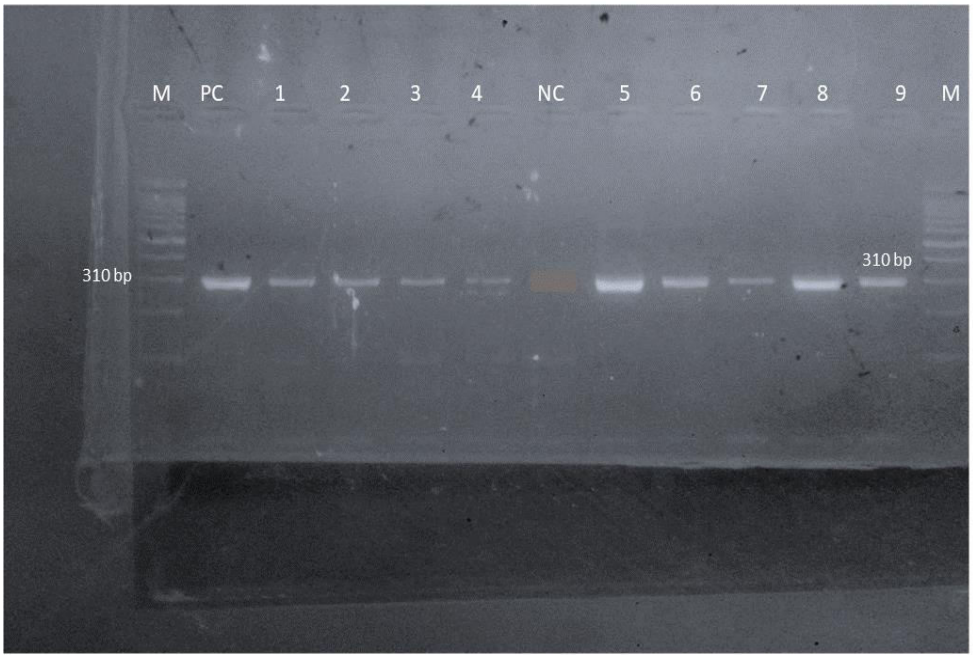
Table.6 Distribution of MICs of vancomycin for mecA positive *S.aureus* (MRSA) isolated from different samples

Source of samples	Number and percentage of isolates with a MICs (µg/ml)								
	50	**25**	**12.5**	**6.25**	**3.1**	**1.5**	**0.7**	**0.35**	**0.17**
Chicken meat	0	0	7/12* 58%[b]	3/6* 50%[b]	3/9* 33.3%[b]	2/14* 14.3%[b]	5/19* 26.3%[b]	2/11* 18.2%[b]	0
Chicken swabs	0	0	5/12 41.7%[b]	8/22 36.4%[b]	6/9 66.7%[b]	12/14 85.7%[b]	14/19 73.7%[b]	6/11 54.5%[b]	3/4 75%[b]
Farm workers	0	0	0	0	0	0	0	3/11 27.3%[b]	1/4 25%[b]
Total of isolates	**0**	**0**	**12**	**22**	**9**	**14**	**19**	**11**	**4**

* No. of isolates within each diluent / Total of isolates

[b] Percentages within columns are significantly different (P<0.05).

Figure.1 Amplification of 310 bp mecA gene on 2% agarose gel by PCR for the detection of MRSA isolated from different samples.



Lane M: DNA Marker (100 bp)
Lane NC negative control
Lane PC positive control
Lane 1,2,3,5,6,7,8,9 Positive samples
Lane 4 Negative samples.

The logic here was that if the origin of bacteria and drug resistance were the same, the prevalence of resistance should be similar among poultry samples. The MICs of vancomycin varied from 12.5 to 0.17 µg/ml for sensitive mecA-positive *S. aureus* from chicken meat, swabs, and farm workers, while the reference strain ATCC25923 showed 6.25 µg/ml (Table 6), with significant differences among diluents ($P < 0.05$) which agree with Lee (2003).

The highest isolation rate of *S. aureus* was found in chicken swabs samples, followed by chicken meat and farm workers. Similarly, the highest incidence of MRSA

was in chicken swabs samples, followed by chicken meat and farm workers. Molecular characterization of *S. aureus* and MRSA using PCR methods allows for rapid and specific identification of these isolates. The *S. aureus* isolates exhibited complete resistance to methicillin, tetracycline, and erythromycin. However, some isolates were highly sensitive to penicillin-G and vancomycin, suggesting the potential for reusing these antibiotics in the poultry sector in Khartoum State. Since vancomycin is a last-resort antibiotic, crucial for treating humans, pets, and livestock when other antibiotics fail, its efficacy must be preserved. Some countries have banned vancomycin analogues in animal feed, but this decision came late as vancomycin-resistant genes had already evolved before the bans were implemented. Vigilant monitoring of antibiotic resistance is essential to prevent such incidents.

Author Contributions

Nada Elsir Ahmed Fagir: Investigation, formal analysis, writing—original draft. Hanan MoawiaIbrahim: Validation, methodology, writing—reviewing. Hatil Hashim El-kamali:—Formal analysis, writing—review and editing. Osama Mohamed: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Nada Elsir Ahmed Fagir, Hanan Moawia Ibrahim, Hatil Hashim El-kamali and Osama Mohamed. 2024. Prevalence and Pattern of Antibiotic Resistance of *Staphylococcus aureus* Isolated from Poultry in Khartoum State / Sudan. *Int.J.Curr.Microbiol.App.Sci.* 13(10): 213-222. **doi:** https://doi.org/10.20546/ijcmas.2024.1310.026