Vol. 17(12), pp. 306-313, December 2023
DOI: 10.5897/AJMR2023.9728
Article Number: A5FD8A671642
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

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African Journal of Microbiology Research

Full Length Research Paper

Prevalence, antibiotic resistance of *Staphylococcus aureus* isolated from layer farms (eggs, droppings/litter) in the outskirts of the city of Ouagadougou, Burkina Faso

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Received 19 October, 2023; Accepted 1 December, 2023

Eggs are susceptible to microbiological contamination by various pathogens on farms, particularly those belonging to the genus *Staphylococcus*, which are zoonotic bacteria capable of contaminating humans, animals, and/or birds, posing a significant public health concern. In Burkina Faso, limited data exists on the prevalence of *Staphylococcus aureus*, prompting this study to investigate the prevalence and antibiotic resistance of *S. aureus* on layer farms. To achieve this, a socio-demographic survey was conducted, followed by egg sampling from different farms. A pool of six eggs constituted one sample for each farm, and microbiological analysis was performed using standard methods. The antibiotic susceptibility of the strains was also tested. The results indicated a prevalence of 51.78% from litter, 35.59% from shells, and 10.16% from egg contents. The strains exhibited the highest resistance levels to Tetracycline (98%), Erythromycin (84%), Trimethoprim/Sulfamethoxazole (77%), Colistin (56%), Oxacillin (55%), and Cefoxitin (38%). However, the strains showed a high sensitivity to gentamycin (81%), and 56% were sensitive to Chloramphenicol. These findings highlight the contamination of eggs by antibiotic-resistant *S. aureus*, emphasizing the need to enhance good practices in the egg production chain to prevent such contamination.

Key words: Prevalence, antibiotic resistance, *Staphylococcus aureus*, poultry, Ouagadougou.

INTRODUCTION

According to FAO (2019), 1.6 million households (56%) engage in poultry farming in Burkina Faso, with intensive farms predominantly located around major cities. The breeding of imported chicks, particularly laying hens, is flourishing due to the high demand for eggs in various cities. Table eggs serve as a vital protein source for

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vulnerable populations, and in some cases, they are utilized in the treatment and recovery of Malnourished children (Stark et al., 2021). Despite eggs being considered a complete food for growth and sustenance, they are susceptible to contamination by various microorganisms (Buriro et al., 2017).

Staphylococcus species are among the microflora found on the surface of table eggs, posing a risk of spoilage and infection for consumers (Salihu et al., 2015). Eggshells harbor numerous microorganisms, including *Staphylococcus* spp., *Salmonella* species, *Streptococcus* species, *Escherichia coli, Bacillus* species, and *Listeria monocytogenes* (Mahdavi et al., 2012). Common foodborne pathogens on eggshells include *E. coli and S. aureus* (Maktabi et al., 2018). *Staphylococcus* spp. are zoonotic pathogenic bacteria, capable of contaminating humans, animals, and birds, contributing to the emergence of zoonotic pathogens that infect domestic and wild animals (Dowd et al., 2013).

Methicillin-resistant *S. aureus* (MRSA) has been identified by the World Health Organization as a high-priority pathogen urgently requiring new antibiotics (WHO, 2017).

S. aureus infections range from superficial skin infections to life-threatening syndromes, including subcutaneous abscess, impetigo, osteomyelitis, infective endocarditis, pneumonia, septicemia, and septic shock syndrome (Gordon and Lowy, 2008). While studies in Burkina Faso on poultry have highlighted the presence of pathogenic bacteria with high antimicrobial resistance in poultry and poultry products (Kagambèga et al., 2018; Bouda et al., 2019), there is currently no available data on the prevalence of *S. aureus* in eggs. Consequently, monitoring and controlling the circulation of *S. aureus* in entities such as humans, animals, and the environment are imperative. The lack of data on the prevalence of *S. aureus* in eggs in Burkina Faso is a significant concern, prompting this study to assess the prevalence and antibiotic resistance of *S. aureus* in eggs and the associated risk practices in egg farming.

MATERIALS AND METHODS

Ethical clearance

The study received approval from the ethical committee of the Ministry of Health, Burkina Faso, under reference number 2020-9-186. Informed consent was obtained from each participant before they were interviewed, and consequently, all participants provided their consent to participate in the study.

Study areas and sampling

A cross-sectional study was conducted between February and May, 2022 in urban and peri-urban poultry farms in Ouagadougou, the most densely populated city with 2,415,266 inhabitants (INSD, 2022). Given the high demand for meat and eggs, poultry farmers have established their operations in and around the city to meet this demand.

Data collection and sampling

A socio-demographic survey was conducted through direct interviews with stakeholders on the farms. Following the compilation of survey data, sampling was performed on the selected farms. For litter, fifty-six samples were collected (from five different points to form one sample from each farm). In the case of egg sampling, a sample of ten eggs was collected from each farm. Once at the laboratory for analysis, six eggs were chosen from the ten to form a sample (pooling of six), resulting in a total of fifty-nine egg samples taken from an equal number of farms. All samples were labeled, placed in freezer bags, stored in coolers containing ice, and transported to the laboratory for microbiological analysis.

Microbiological analysis

Culture media were prepared following the manufacturer's instructions, and microbiological analysis was conducted on a sample of six eggs from each farm. The shell was examined for germs using shell rinsing water, utilizing the method described by Moats (1981). Each sample (a pool of 6 eggs) was washed in a sterile Stomacher bag with 100 ml buffered peptone water (Liofilchem, Italy). 10 µL of this solution was inoculated onto Baird Parker medium supplemented with egg yolk and tellurite. After rinsing, the eggs were disinfected with bleach, alcohol, and dried. The eggs were then prepared for evacuation of their contents according to the procedure outlined by APHA (2004). The eggs were cracked with a sterile rod, and the contents were poured into a Stomacher bag. The contents were mixed and agitated in the Stomacher bag. A quantity of 10 ml was taken and introduced into 90 ml of peptone-buffered water, and 10 µL of this solution was seeded onto Baird Parker medium.

For litter analysis, 10 g of each sample was weighed and dissolved in 90 ml of buffered peptone water, and 10 µL was inoculated into Baird Parker medium. The isolation and identification of coagulase-positive *S. aureus* were carried out following ISO (2003) standards. Germ identification involved microscopic observation, Gram staining, catalase, and coagulase positive tests of suspect colonies. Strains confirmed after identification (109) were tested for antibiotic susceptibility in accordance with EUCAST (CA-SFM /EUCAST, 2022), using *S. aureus* ATCC 29213 as a reference strain.

The antibiotics chosen were those commonly used locally. The disk diffusion method was employed for all antibiotics except colistin, for which the liquid dilution method was used. Furthermore, methicillin-resistant *S. aureus* (MRSA) strains were phenotypically identified when the diameter of the cefoxitin disc (30 µg) was ≤21 mm (CLSI, 2020). The most common antibiotics were selected to assess the susceptibility of the strains, including Chloramphenicol (C), Ciprofloxacin (CIP), Gentamycin (CN), Colistin (CT), Erythromycin (E), Cefoxitin (FOX), Oxacillin (OX), Trimethoprim-Sulfamethoxazole (SXT), Tetracycline (TE), and Vancomycin (VA).

RESULTS

The characteristics of those interviewed are shown in Table 1. The respondents comprised 74% male and 36% female participants. The predominant educational level was secondary school (46%), followed by university education (26%). A notable 19% of respondents had received no formal education. Only 40% of the participants had undergone training in livestock farming before commencing their activities. Regarding knowledge of microbiology, 79% indicated having little knowledge of bacteria.

Table 1. Socio-demographic characteristics.

Characteristics	Category	Effective (%)
Sex	Man	52 (74)
	Woman	18 (36)
Education level	No formal education	13 (19)
	Primary	7 (10)
	Secondary	32 (46)
	Academic	18 (26)
Training	Vocational training in animal husbandry	28 (40)
	No training related to breeding	42 (60)
Notion about bacteria	Yes	55 (79)
	No	15 (21)

Farm characteristics and practices

The poultry breeds with the highest numbers were Dutch Blue at 40%, Local Improved at 26% and Isa Brown at 23%. Chickens were primarily housed day and night (79%). Concerning hygiene on the farms, the cleaning methods applied included standard cleaning, involving the cleaning and removal of waste/droppings, along with the replacement of shavings. In terms of cleaning frequency, 46% of respondents cleaned monthly, 21% cleaned every two weeks, and 21% cleaned when droppings became heavy on the farm. Additionally, 56% used disinfectant or soapy water for cleaning. Finally, 94% did not observe a waiting period after drug administration before resuming normal production use. Table 2 shows the farm characteristics and practices.

Antibiotics use in farms

The antibiotics used on the farms in the 4 weeks prior to the study are as shown in Figure 1. The most used antibiotic family is Tetracycline (67.1%), followed by Macrolides (19.3%).

A total of four hundred and twenty-seven suspect colonies were isolated. After confirmatory tests, one hundred and nine colonies were confirmed as coagulase-positive *S. aureus* strains. The prevalences of *S. aureus* in the matrices analyzed are shown in Table 3. The highest prevalence was observed in the litter sample with 51.78% (29/56). Eggshell showed a prevalence of 35.56% (21/59), and the prevalence for egg contents was 10.16% (06/59).

Antibiotic resistance

S. aureus strains exhibited high resistance to antibiotics such as Tetracycline (98%), Erythromycin (84%), Trimethoprim/Sulfamethoxazole (77%), Vancomycin (59%), Colistin (56%), Cefoxitin, and Oxacillin (55%, 38%).

Conversely, these strains demonstrated sensitivity to Gentamicin (81%) and Chloramphenicol (56%). Additionally, 79% of strains showed intermediate resistance to Ciprofloxacin (Figure 2).

DISCUSSION

The study reveals that 94% of respondents do not adhere to waiting times after drug prescriptions, a rate higher than the 56.66% reported by Samandoulougou et al. (2016). This discrepancy may be attributed to the nature of the farms under consideration in this study (layers). Adhering to waiting times would involve classifying all eggs as non-saleable during this period, leading to financial losses for the farmers. Non-compliance with waiting periods poses a risk of food product contamination by drug residues, which could result in intoxication, allergies, and antibiotic resistance (Goff et al., 2017).

The survey indicated that the most common cleaning method involved the superficial removal of residues and feces every month without disinfectants. Regarding cleaning eggs after collection, only 19% used water for rinsing before sale, and 81% relied solely on their hands to clean the surface of the eggs. This might explain the presence of feces on the shell in 2% of cases. These hygiene deficiencies could contribute to the contamination and spread of pathogens.

The results demonstrate a high resistance to Tetracyclines (98%) among the study strains, potentially explained by their widespread use and affordable cost in animal health. This aligns with Figure 1, which indicates that 67.1% of respondents used Tetracyclines on their farms. The study suggests that antimicrobial resistance

Table 2. Farm characteristics and practices.

	Category	Effective (%)
Chicken breed	Dutch blue	28 (40)
	Improved local chicken	18 (26)
	Isa Brown	16 (23)
	Other	8 (11)
Chicken keeping	Housed day and night	55 (79)
	Free by day and housed by night	7 (10)
	Freedom day and night	8 (11)
Farm cleaning	Standard cleaning	58 (83)
	Copal replacement	12 (17)
Use of medication without professional advice	Drugs already used without professional advice	51 (73)
	Always after professional advice	19 (27)
Waiting time after drug administration	Not respecting waiting periods	66 (94)
	Respecting waiting times	4 (6)
Cleaning frequency	Every day	9 (13)
	Chips changed every two weeks	15 (21)
	Every month	32 (46)
	Every 3 months	4 (6)
	Whenever there are too many droppings	15 (21)
	Every 6 months	1 (1)
Use of disinfectants to clean farm	No disinfectants	31 (44)
	Veterinary disinfectant	25 (36)
	Bleach	12 (17)
	Soap	2 (3)
Hygiene appreciation	Satisfied	10 (14)
	Acceptable	27 (39)
	Insufficient*	33 (47)
Egg cleaning	Rinse with cloth or by hand	57 (81)
	Disinfectant solution (soapy water or bleach)	13 (19)

*In 2% of cases, pieces of feces and feathers were found on the shell.

(AMR) of livestock origin is increasing in low- and middle-income countries (LMICs), particularly in poultry and pigs (Van Boeckel et al., 2019). A more recent study has demonstrated that antibiotic use in poultry farms significantly increases resistance in bacterial strains present in the upper layer of soil around the poultry farm within at least a 25 m range (Kousar et al., 2021). The prevalence of *S. aureus* in egg content and eggshell was 8.75 and 34.46%, respectively, in the present study. The prevalences are lower than the 13.3 and 40% reported in egg content and eggshell, respectively, in Egypt by Sadek et al. (2016). The presence of microorganisms in eggs could be attributed to the fact that the egg leaves the hen's body through the same passage as feces are excreted. *S. aureus* contamination of eggs is a major concern, especially considering that eggs are consumed by immunocompromised individuals and malnourished children. The contamination of the eggshell and contents might be associated with the type of floor-laying farm where the eggs come into contact with droppings (Gunnarsson et al., 2020). Frequent cleaning of the farm could reduce contact between eggs and droppings and

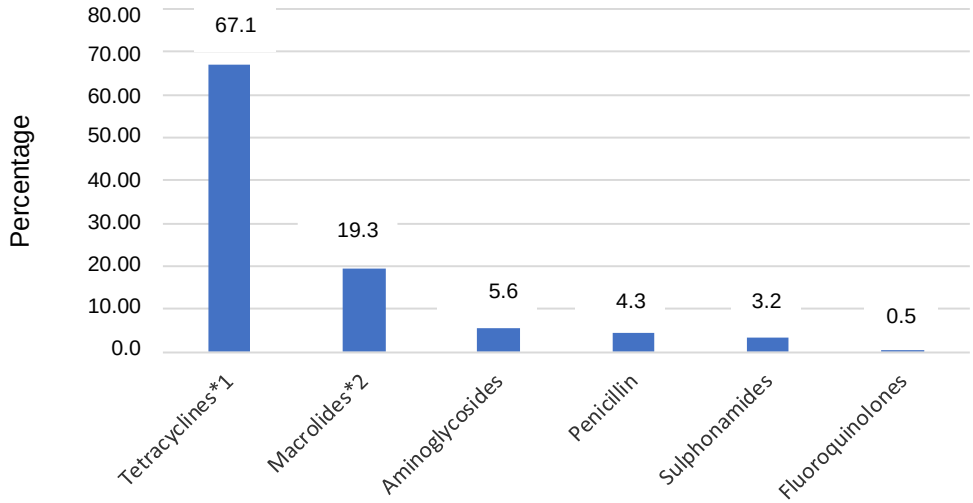

Figure 1. Antibiotics used in the last 4 weeks preceding survey. Tetracycline*1: Among the farmers who used tetracyclines (47/70), 85.1% (40/47) used them as a preventive measure. Macrolides *2: Among farmers who used macrolides (14/70), 92.85% (13/14) used them preventively.

Table 3. Prevalence of S. aureus by sampling.

Sample	Prevalence of *S. aureus*
Litter (n= 56)	29/56 (51.78%)
Shell (n= 59)	21/59 (35.59%)
Egg content (n= 59)	06/59 (10.16%)

n: Number of sample.

dust, thereby limiting egg contamination.

Microorganisms present inside an intact or uncracked egg may be due to the presence of a pathogen in the hen's ovary or oviduct before the shell forms around the yolk and albumen (USDA FSIS, 2011). Ansah et al. (2009) have reported that, as eggs are left for longer periods, their resistance decreases, allowing organisms to penetrate the egg contents. Warm weather could facilitate the penetration of *S. aureus* present on the egg surface by weakening the shell.

Storing eggs at room temperature without refrigeration is a factor that could permit the proliferation of germs in eggs (Momani et al., 2017), explaining the prevalence of *S. aureus* in eggs in this present study.

A study carried out on layers in Pakistan showed that daily cleaning of drinking troughs helped reduce the bacterial load in layers. This suggests that both the flock of birds and consumers of eggs and chicken meat are at risk of bacterial infection if strict farm hygiene is not ensured through regular monitoring (Folorunso et al., 2014).

According to a study on eggs by El-Toukhy et al. (2021) in Egypt, *S. aureus* exhibited 90% resistance to Ciprofloxacin, which is significantly higher compared to the resistance reported in this study (20%). Additionally, these authors reported 65% resistance to Erythromycin and 100% resistance to Trimethoprim-Sulfamethoxazole and Tetracycline. The resistance to Tetracycline is similar to that reported in the present study (98%). Tetracycline and Chloramphenicol resistance are also similar to that reported in poultry eggs by Buriro et al. (2017) (100%). These elevated levels of resistance could be attributed to the strains' exposure to antibiotics, as a previous study revealed the misuse of antibiotics in poultry farms (CDC, 2019). Antibiotic-resistant bacteria isolated from these samples can be transmitted to humans through handling and ingestion of raw eggs or egg products. Given that eggs are a vital protein source, especially for vulnerable populations, such as cases where eggs are used for the treatment and recovery of malnourished children (Stark et al., 2021); these strains pose a significant risk to consumers, especially the most vulnerable, such as children and immunocompromised individuals.

S. aureus strains isolated from the nasopharynxes of children in Burkina Faso showed over 85% resistance to Tetracycline and 96% resistance to Penicillin (Bonko et

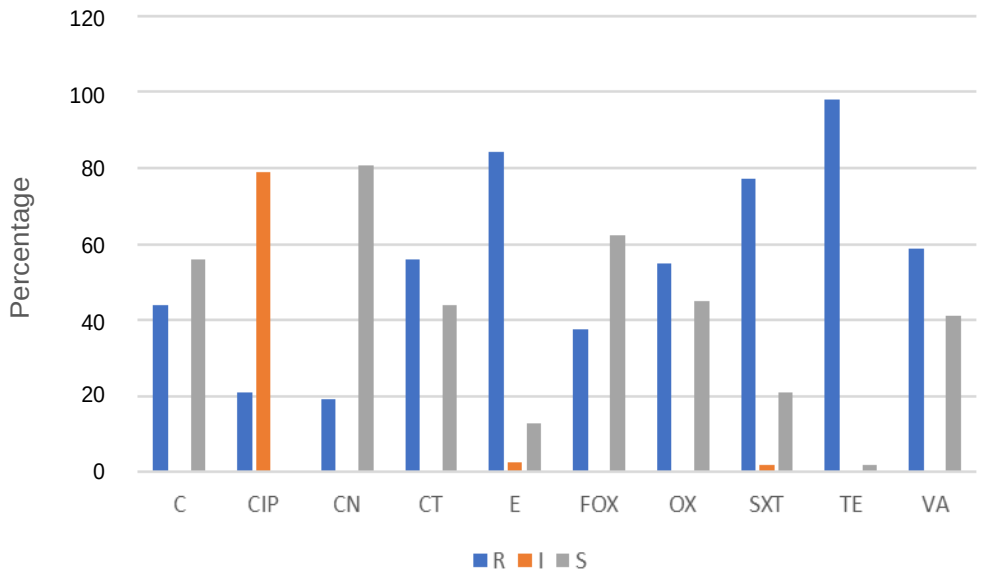

Figure 2. Susceptibility of *S. aureus* strains to antibiotics. R: Resistant; I: Intermediate; S: Sensible, C: Chloramphenicol; CIP: Ciprofloxacin; CN: Gentamycin; CT: Colistine, E: Erythromycin; FOX: Cefoxitine; OX: Oxacilline; SXT: Triméthoprime-Sulphametoxazole; TE: Tetracycline; VA: Vancomycin.

al., 2021). Other *S. aureus* strains isolated from nasal carriage in patients in Burkina Faso exhibited high rates of resistance, with amoxicillin at 90%, followed by tetracycline at 61%, and erythromycin at 54% (Ouedraogo et al., 2016), confirming the trend in *S. aureus* antibiotic resistance. Vancomycin resistance was detected in 59% of the tested isolates, a rate lower than the 70% reported by El-Toukhy et al. (2021). This represents a considerably high rate of resistance, raising concerns as Vancomycin is the drug of choice for treating MRSA. Resistance to Cefoxitin was 38%, indicating a phenotypic MRSA prevalence of 38%. Antibiotics are extensively used for preventive purposes on a large scale (Figure 1), contributing to increased antibiotic resistance in the relevant germs (WHO, 2021). The observed resistance to oxacillin (55%) is lower than that reported by Syed et al. (2019) (83.6%). Oxacillin is a molecule more commonly used in humans, so the relatively high resistance observed in poultry may be attributed to transfer from humans to the farm through various contacts, such as human feces discharged in nature or direct contact between humans and the farm (Syed et al., 2019). MRSA contamination poses significant risks to human health, particularly for individuals living and working on farms (da Silva et al., 2020). Infections caused by MRSA result in higher expenses in the public health sector and a greater morbidity and mortality rate compared to non-resistant strains (Guzmán-Blanco et al., 2009).

Poultry manure, often used as organic fertilizer in agriculture without prior treatment, contains antibiotic residues that persist in the environment for an extended period. These residues can eventually reach the human body through contaminated food or crops. According to the Food and Agriculture Organization (FAO), the persistence of antibiotics in the environment could contribute to the spread of antibiotic residues, likely leading to resistance or even allergies after the ingestion of contaminated food (FAO, 2008). Immersing eggs in disinfectants (H_2O_2 solution) could reduce the microbial load on eggs before commercialization (El-Toukhy et al., 2021). The use of an easily available natural product, baking soda, could provide a low-cost decontamination solution while protecting the environment from chemical pollution (Fong et al., 2011).

Conclusion

This study revealed a lack of hygiene in various stages of the egg production chain, potentially contributing to the presence of *S. aureus* in farms, on eggshells, and in egg contents. Among these bacterial strains, a high level of antibiotic resistance was observed against several antibiotics, and multi-resistant bacteria were also identified. The circulation of these resistant strains on farms poses a major health concern, particularly considering that eggs are consumed by even the most vulnerable populations. It is imperative to prioritize and adhere to hygienic measures in egg production. Additionally, there is a pressing need to regulate the use of antibiotics and implement measures to control the circulation of resistant bacteria on farms.

CONFLICT OF INTERESTS

The authors have not declared ant conflict of interests.

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