



Prevalence and Antibiogram Assessment of *Staphylococcus aureus* in Chicken Carcass Rinse Water at Artisanal Slaughterhouses in Abidjan District, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author GGB designed the study, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Authors KMPL and BS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the current study was to determine the antibiotic resistance profile of *Staphylococcus aureus* strains isolated from rinse water of chicken carcass in artisanal slaughterhouses in Abidjan.

Place and Duration of Study: Chicken's rinse water samples were collected between January and March 2020 in three areas of Abidjan district.

Methodology: A total of 75 rinse water samples were collected from three markets of Abidjan district. Enumeration and isolation of *S. aureus* were carried out on Baird Parker agar supplemented with egg yolk tellurite emulsion followed by morphological and biochemical identification. Antibiotics resistance profiles were performed by using disks diffusion methods.

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Results: Out of 75 samples, 21 (28%) were contaminated with *S. aureus*. Among the isolates, 21 (one by positive sample) were tested for antimicrobial resistance against 14 most commonly used antibiotics. All strains were resistant to two antibiotics (minocyclin and fusidic acid). However, some drugs such as gentamycin, norfloxacin, and Tigecyclin showed great activity on tested isolates.

Conclusion: Results of this study suggest that rinse water could consist of a major critical point of chicken carcass contamination by *S. aureus* with high drugs resistance capacity. Therefore periodic control is need to good hygiene practice and improving the poultry meat sanitary quality produce from these slaughterhouses.

Keywords: Hygiene practice; resistance to antibiotics; rinse water; *S. aureus*; slaughterhouses.

1. INTRODUCTION

In Côte d'Ivoire, the poultry farming is a key sector in the animal production system [1] with constant increasing of consumption of poultry meat and eggs. Moreover, according to the initiatives of the Côte d'Ivoire government, poultry meat could be the main source of animal protein for the population from 2020. However, poor hygiene conditions during farming, in artisanal and modern slaughterhouses and during cooking could increase the fresh and cooked meat risk contamination by many pathogenic bacteria. Moreover, overuse of antibiotics in animal husbandry is also permanent danger for the Ivorian consumer. Indeed, previous studies performed in poultry farm in Abidjan district indicated low implementation level of biossecurity measures and overuse of antibiotics. In present study, we are interested in the slaughter conditions of poultry in artisanal slaughterhouses in the markets of the district of Abidjan in order to identify the critical points of contamination of the carcass by bacteria potentially pathogenic to consumers. In general, the digestive tract of poultry is one of the reservoirs of many pathogenic bacteria [2,3,4] such as *S. aureus* [5].

Staphylococcus genus is a Gram-positive bacterium including more than 40 species and subspecies well identified to date [6]. However, *S. aureus* is the most pathogenic species of this genus [7,8].

Indeed, this bacterium that can be as a commensal flora, but might be considered as a major cause of some human illnesses [9,10].

Pathogenicity of *S. aureus* is essentially due to their ability to toxins production. Ingestion of staphylococcal enterotoxins produced in food by *S. aureus* enterotoxigenic strains will result in staphylococcal food poisoning that can be considered as one of the most common foodborne diseases.

Staphylococci are ubiquitous bacteria present on the skin, mucous membranes and the nasopharyngeal sphere and digestive tract in warm-blooded animals (mammals, birds) and particularly in humans. They can spread easily in the environment and can thus contaminate diverse foods. Moreover, *S. aureus* may lead to severe health problems due to its wide antibiotic resistance [11]. According to WHO data, people infected with MRSA, generally associated with nosocomial infections, have a 64% higher mortality risk than people with a non-resistant form. The emergence of antibiotic-resistant bacteria including *S. aureus* is therefore a major challenge in human medicine and the widespread contamination of poultry meat in particular requires an assessment in terms of risk to the consumer. Thus, the aim of this study is to determine the antibiotic resistance profile of *S. aureus* strains isolated from rinse water of chicken carcass in artisanal slaughterhouses in Abidjan.

2. MATERIALS AND METHODS

2.1 Samples Collection

A total of 75 chicken carcass rinse water samples were collected in the artisanal slaughterhouses of Yopougon, Adjamé and Abobo, three areas of the Abidjan district. Each sample was collected immediately after rinsing the carcasses in sterile container was marked, identified and transported at 4°C using ice box to the laboratory.

2.2 Isolation of *S. aureus*

All collected samples were analyzed for isolation of *S. aureus* according to the method described by International Organization for Standardization (ISO: 6888-1) [12]. Thus, for each sample, 10 ml of the rinse water was transferred to 90 mL of Buffered Peptone Water (Bio-Rad Marne-la-Coquette-France). The suspension was then

homogenized before a series of dilutions in salt buffer. Then, 0.1 mL of each dilution was spread onto Baird-Parker (BP) agar medium (CM 275, Oxoid, UK) supplemented with egg yolk tellurite emulsion (SR 54, Oxoid, UK).

Inoculated media were incubated at 37°C for 24-48 h and typical black colonies surrounded by opaque halo on Baird Parker agar were considered as presumptive *S. aureus* and were enumerated. Five (5) presumptive colonies from each agar plate were identified by morphological and biochemical tests including Gram stain, DNase, catalase and coagulase [13]. After identification, each typical colony was confirmed as *S. aureus* and streaked on BP agar and incubated at 37°C for 24 h. Then, each strain was harvested and stored using a sterile plastic loop in Eppendorf tube containing nutrient broth 30% (v/v) glycerol (BDH Chemicals Ltd. Pool, England) at -20°C for further investigation.

2.3 Antimicrobial Susceptibility

The determination of antimicrobial resistance patterns of *S. aureus* isolates was done using the agar disk diffusion on MH agar method and according to the CASFM/EUCAST criteria [14]. A total of fourteen (14) antibiotics with different classes and commonly used were tested including: penicillin (P-1 IU), tobramycin (TM- 10 µg), gentamicin (GM-10 µg), minocyclin (MNO-30 µg), tigecyclin (TGC- 15 µg), erythromycin (E-15 µg), clindamycin (CMN-2 µg), trimethoprim + sulfamethoxazole (SXT- 1.25+23.75 µg), fusidic acid (FAD-10 µg), vancomycin (VA-30 µg) and rifampicin (RA- 5 µg), norfloxacin (NOR-10 µg), fosfomycin (FOS-200 µg), and cefoxitin (FOX-30 µg). All antibiotics used were obtained from Oxoid, (Hampshire, England). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as reference strains.

3. RESULTS AND DISCUSSION

3.1 Prevalence of *S. aureus* in Chicken Carcass Rinse Water

Of the rinse water samples tested, 21 (28%) were positive for *S. aureus* and the mean contamination load was 2.70 log₁₀ CFU/g. Moreover, prevalence by commune was 24%, 40% and 20% for Yopougon, Adjamé and Abobo, respectively. The bacterial load ranged from 1.7-3.5 log₁₀ CFU/g for Yopougon, 1.3-3.3 log₁₀ CFU/g for Adjamé and 1.92-2.95 log₁₀ CFU/g for

Abobo. Variation of *S. aureus* load in rinse water might be due to different levels of hygiene practice during the chicken slaughter process.

The detection of *S. aureus* in the rinse water could be due to contamination of human origin or even from the chicken itself [15]. However, because of high level of bacterial load observed and slaughter conditions of the poultry in the slaughterhouses visited, these strains could be predominantly of avian origin.

According to the observations made during sampling, intestinal contents are most often released during evisceration contaminating not only poultry slaughterer hands but also the equipment (knives, cutting tables), the carcass and the rinse water of the chickens. In general, the presence of *S. aureus* has rarely been reported in chicken rinse water.

However, much data has been reported frequently on presence of *S. aureus* in poultry meat with variable prevalence: 6.42% in Iran [16], 18.18% in Thailand [17], 43.3% in Turkey [18] and 28.6% in Italy [19], 17.8 – 45% in USA [20,21,22] and 31% in South Africa [23].

As rinsing is the last step in the slaughter process, *S. aureus* present in this rinsing water may contaminate the carcass ready for delivery. Indeed, in the slaughterhouses visited, the plucking of the poultry is carried out on the floor. No disinfection of the evisceration equipment and the hands of the slaughterer before and after the slaughter of a batch and during rinsing is carried out. Finally, a large number of carcasses are rinsed in the same tank. Such practices can promote contamination of equipment and the environment by *S. aureus* present in the animal's digestive tract and ultimately be a source of contamination of all carcasses at the end of the slaughter process. In addition, most of the chickens slaughtered in these artisanal slaughterhouses are destined for fast-food and street restaurants, which generally do not follow recommended hygiene measures.

The presence of this pathogen is of primary concern, as it is able to produce heat-stable enterotoxins, and subsequently may cause food poisoning in humans [24]. In this context, the presence of *S. aureus* in the rinsing water, contaminating the carcasses, constitutes a potential hazard for the consumer even though the toxin production capacity of these strains must first be demonstrated.

3.2 Antibiogram Results

The frequency of antibiotic resistance for strains isolated from the rinse water of chicken carcasses after slaughter is shown in Fig. 1. In this study, intermediated resistance strains has also been considered as resistant. Results show that the antibiotics with the lowest activity on the isolated strains are fosfomicin, minocyclin, rifampicin and fusidic acid, (76 to 100% resistance), followed by erythromycin and penicillin (50 to 65% resistance). While, Vancomycin, cefoxitin, tobramycin and trimethoprim + sulfamethoxazole showed relatively moderate activity on the tested strains. On the other hand, norfloxacin, tigecyclin, clindamycin and gentamicin were active on more than 90% to 95% of strains tested.

In another studies, Naas et al. [5] showed that six (6) *S. aureus* isolates were resistant to Clindamycin (100%), tobramycin (50%), Erythromycin (50%), Gentamicin (50%), Penicillin (50%) and tetracyclin (66%) while no resistant strains were detected with trimethoprim + sulfamethoxazole (0%) and Vancomycin (0%). Tassew et al. [25] and Waters et al. [26] reported

high resistance to erythromycin, tetracycline, penicillin in a ratio ranged from 66% to 85%.

Staphylococcus aureus is well known to express the highest resistance to penicillin among the beta-lactam antibiotic class [27]. Moreover, the resistance to minocyclin is not surprising because cyclin family is one of the most commonly used antibiotics in Abidjan livestock production systems. In addition, resistance to Vancomycin seems to be low in many reported studies [5]. The difference in the percentage of antibiotic susceptibility may be due to the type of antibiotic used in farming sector or due to local *S. aureus* strains to each country [5,28].

In this study, the multiple drugs resistance (MDR) corresponding to resistance to three or more families of antimicrobial agents [29] was detected in 66.66% of tested isolates. This resistance concerned 3 to 7 families of antibiotics (Table 1). Moreover, cross-resistance was observed with beta-lactam antibiotics (FOX and P) and cyclin (NRM and TGC) in 19.04% and 4.76% of the tested strains respectively. The cross and multiple resistance profiles obtained in this study are presented in Table 1.

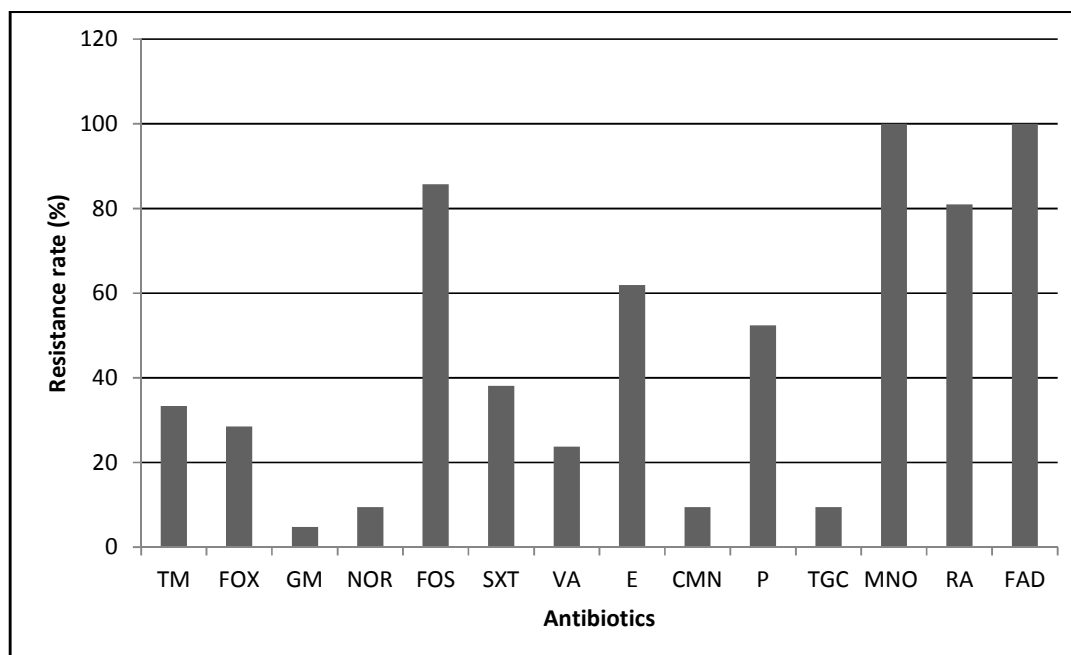


Fig. 1. Frequency of antibiotic resistance for *S. aureus* strains isolated from the rinse water of chicken carcasses after slaughter

*TM: tobramycin; FOX: cefoxitin; FOS: fosfomicin; GM: gentamicin; NOR: norfloxacin; SXT: Trimethoprim+sulfamethoxazole; VA: vancomycin; E: erythromycin; CMN: clindamycin; P: penicillin; TGC: tigecyclin; MNO: minocyclin; RA: rifampicin; FAD: fusidic acid

Table 1. Phenotypes of cross and multiple resistance of *S. aureus* strains isolated from carcass chicken rinse water in Abidjan

Profiles	Number of strains (%)	Numbers of drugs	Numbers of drugs families
Cross-resistance			
FOX/P	4 (19.04)	2	1
MNO/TGC	1 (4.76)	2	1
GM/TM	1 (4.76)	2	1
MDR			
TGC/MNO/FAD	1 (4.76)	3	3
NOR/MNO/FAD	2 (9.5)	3	3
CMN/MNO/FAD	2 (9.5)	3	3
VA/MNO/FAD	1 (4.76)	3	3
VA/E/MNO/FAD	1 (4.76)	4	4
VA/FOX/P/FOS/MNO/FAD	1 (4.76)	6	5
E/P/FOS/MNO/FAD	1 (4.76)	5	5
E/FOX/P/FOS/MNO/FAD	1 (4.76)	6	5
SXT/E/FOS/P/MNO/FAD	1 (4.76)	6	6
VA/E/FOS/TGC/MNO/RA/FAD	1 (4.76)	7	6
TM/SXT/E/FOX/FOS/P/MNO/RA/FAD	1 (4.76)	9	7
TM/GM/E/FOX/FOS/P/MNO/RA/FAD	1 (4.76)	9	7

*MDR: Multi drugs resistance

In general, the increase of antibiotic resistance rate in bacteria flora is due either to selection pressure for drugs resistant strains or to mutations in the target genes as a result of the massive and uncontrolled use of these antibiotics in human and animal medicine [30,31]. Indeed, the regular use of low-dose antibiotics in factory farming makes the animal an ideal breeding ground for resistant bacteria. A phenomenon that produces bacteria resistant to 3, 4, 5 or even 9 different antibiotics, leaving very few therapeutic options in case of human infection. In any case, *S. aureus* is considered as a pathogen involved in various human pathologies. In this context, the antibiotics tested being the molecules used in cases of *S. aureus* infection in human; the existence of these resistances could constitute a major public health problem given the risks of therapeutic failure.

4. CONCLUSION

Our results showed high contamination level of rinse water of chicken carcasses by *S. aureus*. This contamination could be attributed to poor hygienic practice in poultry slaughters in Abidjan. Moreover, the isolates tested for their antibiotic sensitivity profiles showed high rate of resistance to different drugs and different degrees of multi-resistance phenotype. As *S. aureus* detected in rinse water could finally contaminate all carcasses at the end of slaughter process, good

hygienic practice and improving the poultry meat quality is needed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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