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Microbial Quality and Molecular Identification of Enterotoxigenic Staphylococcus Strains Isolated from Dried, Smoked, and Braised Fish Sold in Ouagadougou Markets

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Abstract

Background: The investigation of toxin genes in strains involved in staphylococcal food poisoning contributes to food safety. The aim of this study was to isolate and identify enterotoxigenic *Staphylococcus* strains from dried, smoked, and braised fish sold in Ouagadougou markets. **Methodology:** Staphylococci were isolated using standard microbiology methods. *Staphylococcus* strains were identified using API Staph kit (Reference # 20500, BioMerieux S.A., Marcy l'Etoile, France). The molecular identification of isolated *Staphylococcus aureus* strains was specifically confirmed by PCR using the Staur4 and Staur6 primers. The genes encoding enterotoxins, enterotoxin-like toxins, exfoliative toxins, and TSST-1 toxin were detected by multiplex PCR using specific primers from Inquaba Biotec West Africa Ltd, Africa's Genomics Company. **Results:** The results of the microbiological quality assessment indicated that most of the samples analyzed were found to be of unsatisfactory microbiological quality according to the *Staphylococcus aureus* microbiological criteria (m = 102). Overall, only 12.55% of samples were satisfactory, while 97.45% were unsatisfactory. The STAPH API gallery allowed the identification of the following species: *Staphylococcus aureus*, *Staphylococcus xylosus*, *Staphylococcus lugdunensis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus lentus*, *Staphylococcus sciuri* and *Staphylococcus capitis*. Of the 108 *Staphylococcus* isolates, 81 (75%) showed at least one (1) toxin gene. Among the 21 toxin genes tested in this study, 20 genes were detected in all strains analyzed. The staphylococcal toxin genes detected were present in both *Staphylococcus aureus* and the other coagulase-negative

strains isolated in this study. In addition, these genes are found individually or in association in certain strains. The most frequent genes detected in toxin gene-positive strains were: the tsst-1 gene in 45 isolated strains (41.7%), *sei* (16/14.8%), *seg* (13/12%), *ser* (7/6.5%) *sec* (6/5.5%), and *sea* (5/4.6%) for staphylococcal enterotoxins, *seln* (14/12.9%), *selq* (8/7.4%), for enterotoxin-like toxin gene and *eta* (3/2.7%) for exfoliative toxin genes. **Conclusion**: This study highlighted the pathogenicity of *Staphylococcus* strains isolated from dried, smoked, and braised fish sold in Ouagadougou markets. Monitoring toxin-producing strains of *Staphylococcus* is invaluable for better prevention of food poisoning.

Keywords

Fish, *Stapholocuccus*, Toxin Genes, Ouagadougou

1. Introduction

Fish is one of the fishery products of interest in the human diet as a source of essential nutrients and micronutrients for healthy and varied diets [1]. Given its perishable nature, fish is often smoked, dried, or salted [2] [3] [4]. These techniques improve fish stability and extend its shelf life [5] [6]. Despite these processes, fish can spoil or be contaminated by pathogenic microorganisms if proper storage and sale conditions are not ensured.

Food safety is a major concern worldwide. Staphylococcal food poisoning (SPF) is one of the major public health problems [7] [8]. These foodborne illnesses can be caused by the ingestion of food contaminated with pathogens (viruses, parasites, and bacteria) [9], or by toxins [10] [11]. *Staphylococcus aureus* is one of the most common pathogens involved both in infection and intoxication through fish and certain seafood products [12]. Drying and smoking reduce the activity of *S. aureus* in dried fish, thus slowing down spoilage [13] [14] [15]. However, these techniques do not eliminate the bacterium, which is still capable of surviving and producing toxins [16]. *S. aureus* is often detected in dried, smoked and braised fish at high loads [17]. This is sometimes due to contamination during sale carried out in improper packaging, storage or display conditions [16]. The pathogenicity of the bacteria is based on its ability to cause infection or produce toxins after ingestion of contaminated food.

Food can be contaminated by hand contact or by the airway secretions of food handlers who carry enterotoxin-producing *S. aureus* in their hands during preparation and processing. Air, dust, and food contact surfaces are also potential pathways for the transfer of *S. aureus* into food.

S. aureus is able to grow over a wide range of temperatures, pH, and high sodium chloride concentrations (up to 15% NaCl), as well as at low water activity levels (0.86 water activity) [18]. Mood *et al.* [16] reported that *S. aureus* grows better at 10˚C than at 25˚C and 30˚C. These characteristics enable the bacteria to

grow in a wide variety of foods. Some strains of *Staphylococcus* (enterotoxigenic strains) are also able to produce staphylococcal enterotoxins (SEs), responsible for staphylococcal food poisoning (SPF).

These toxins are classified into several serotypes, consisting of superantigens (SAgs) that cause typical food poisoning symptoms such as vomiting and diarrhea, and other staphylococcal superantigens (staphylococcal superantigen-like SSL) without emetic properties. There are more than 23 serotypes of staphylococcal SAgs toxins described, in particular the toxic shock syndrome toxin (TSST-1), staphylococcal enterotoxins (classic: SEA to SEE, new: SEG to SEJ, SEL to SEQ and SER to SET) [19], and staphylococcal superantigen-like (SEIK to SEIQ, SEIU to SEIX). Among these identified staphylococcal enterotoxins (SE), SEA is highly thermostable, and the most frequent cause of staphylococcal food poisoning worldwide [20]. Staphylococcal enterotoxins are classified as bacterial superantigens (SAgs). The action mechanism of these superantigens consists of binding MHC class II of antigen presenting cell receptors to T cells. This lead to the activation of T cell. SAgs stimulate helper T cells to produce cytokins such as interleukins (IL), gamma interferon, and tumor necrosis factor [21]. Among cytokines produced, IL-2 is responsible for many of the symptoms of staphylococcal gastroenteritis.

Given their low molecular weight, staphylococcal toxins are resistant to heat treatment and proteolytic enzyme activity and are active in small quantities (20 ng). Therefore, the occurrence of *Staphylococcus* poses a threat to food safety and wholesomeness. Consequently, the investigation of food samples, particularly fish, for the presence of these pathogens is important for the development and implementation of preventive measures and programs to ensure food safety.

The aim of this study was to isolate and identify strains of enterotoxigenic *Staphylococcus* species from dried, smoked, and braised fish sold in Ouagadougou markets.

2. Material and Methods

2.1. Enumeration and Isolation of Pathogenic Staphylococcus

Staphylococci were detected and isolated on Mannitol Salt Agar (ISO 6888; 2003). A 0.1 ml volume of two successive dilutions was used to spread on the surface of the agar poured into Petri dishes, and incubated at 37˚C for 24 hours. After incubation, all Staphylococci were enumerated according to AFNOR ISO 7218 (2007). Based on their ability to ferment mannitol or not, Staphylococci can be differentiated. Mannitol fermentation induces acidification, leading to a yellow coloration of the medium in the presence of phenol red (pH indicator). The strains were subjected to complementary standard biochemical tests: catalase, DNAse, and coagulase tests. Suspect *Staphylococcus aureus* colonies were subcultured on Mannitol Salt Agar and the bacterial mass of each strain on the agar was scraped off and stored in cryotubes containing brain broth at 20% glycerol for subsequent identification.

Bacterial load calculation formula

Petri dishes containing 15 to 300 colonies were used to calculate the "N" number of microorganisms. Equation (1) is the formula used to calculate the number of microorganisms:

$$N = \frac{\sum C}{(n_1 + 0.1n_2)V \times d}$$

where:

N = Number of microorganisms (cfu/g of product)

ΣC: Sum of colonies counted on plates retained from two successive dilutions

n_1: Number of plates retained from first dilution

n_2: Number of plates retained for the second dilution

d: dilution factor corresponding to the low dilution (first dilution)

V: inoculum volume.

2.2. Assessment of Microbiological Quality of Fish Analyzed

Microbiological results were interpreted using the European regulation N˚ 2073/2005 three-class plan.

m = official microbiological criterion: all results less than or equal to this number are satisfactory.

M = threshold limit of acceptability (10 m): above which results are no longer considered satisfactory, without the product being toxic.

- A result is satisfactory if the value obtained is less than or equal to 3 m;
- A result is unsatisfactory if the value obtained is higher than M;
- A result is acceptable if the value obtained is between 3 m and M.

Specifically, for the interpretation of *Salmonella* results, a two-class plan was used (presence or absence).

2.3. Phenotypic Identification of *Staphylococcus* Strains

Staphylococcus strains were identified using the API Staph kit (Reference # 20500, BioMerieux S.A., Marcy l'Etoile, France). For this purpose, the micro-tubes of each gallery were inoculated with a bacterial suspension of turbidity equal to 0.5 McFarland prepared from each isolate. Tests and gallery readings were carried out according to the manufacturer's instructions. Strain identity was obtained on the basis of digital profiles using Apiweb TM software. Isolates with a staphylococcal compatibility percentage higher than 80% were retained.

2.3. Molecular Analysis of *Staphylococcus* Isolates

2.3.1. DNA Extraction

Total genomic DNA was extracted using the heat shock method. For this, one to three colonies of each isolate (24 h) on Muller Hinton agar plates were picked using a sterile Pasteur pipette, then introduced into an Eppendorf tube containing 200 µl sterile 1X PBS and the mixture was homogenized by vortexing. Cells were washed by centrifugation at 20,000 × g. The supernatant was discarded and

the pellet was used for total genomic DNA extraction. For cell lysis, the pellet was resuspended in 20 µl of nuclease-free water, then frozen for 15 min, and then boiled in a water bath for 10 min.The lysate was then centrifuged at 12,000 rpm in a microcentrifuge (Biofuge fresco, Thermo Scientific) for 10 min [22]. The resulting supernatant was collected and stored at −20˚C in Eppendorf tubes for further analysis.

2.3.2. Identification of *Staphylococcus aureus* Strains by PCR

Molecular identification of isolated *Staphylococcus aureus* strains was performed using Staur4 5'ACGGAG TTACAAAGGACGAC 3' and Staur6 5'AGCTCAGCCTTAACGAGTAC 3' primers to amplify specific regions of the 23S rDNA of the *Staphylococcus aureus* species as described by Straub *et al.* 1999. The specific sense primer nucF 5'GCGATTGATGGTGATACGGT 3' and antisense primer nucR 5'AGCCAAGCCTTGACGAACTAAAGC 3' (Inquaba Biotec West Africa Ltd, Africa's Genomics Company) were used to amplify the segment of the nuc gene encoding the thermostable endonuclease of coagulase-positive *Staphylococcus*.

The reaction mixture was prepared in 25 µl according to the OneTaq master mix as follows: 12.5 µl of OneTaq® Quick-Laord® 2X Master Mix with Standard Buffer (New England Biolabs®), 0.5 µl of sense primer (10 µM), 0.5 µl of reverse primer (10 µM), 2.5 µl of DNA extract and 9 µl of Nuclease free water (DNA/DNAse/RNAse free Sterile, PCR Inhibitor free, Bioconcepts).

PCR reactions were performed in a thermal cycler (2720 Thermal Cycler, Applied Biosystem). The PCR program used for Staur primers was: predenaturation 94˚C/5min, 35 cycles (94˚C/30sec; 55˚C/40 seconds; 72˚C/1.2minutes), and final elongation at 72˚C/5minutes. For the nuc gene, the following steps were applied: initial denaturation 95˚C for 5 min, 30 cycles of (denaturation 94˚C for 60 sec, hybridization 55˚C for 30 sec, elongation 72˚C for 90 sec), final extension at 72˚C for 5 min. Amplicons were stored at +4˚C.

The amplified PCR fragments were visualized by dropping 10 µl of each amplicon into agarose wells (Agarose CSL-AG500, LE Multi-Purpose Agarose, Cleaver Scientific, UK) stained with SafeviewTM Classic Cat≠ G108, Canada (5 µl in 100 ml agarose). Migration was performed in TAE 1X (Tris-Acetate-EDTA) buffer for 20 min at 100 V to separate fragments by electrophoresis.

2.3.3. Detection of Genes Encoding Staphylococcal Toxins in *Staphylococcus* Isolates

The genes encoding enterotoxins, enterotoxin-like toxins, exfoliative toxins, and TSST-1 were detected using specific primers presented in Table 1. Primers were supplied by Inquaba Biotec West Africa Ltd, Africa's Genomics Company. Detection was performed by multiplex PCR (Fijalkowski *et al.*, 2016; Chajecka-Wierzchowska, 2020).

The PCR mixture was prepared with Nuclease-free water (DNA/DNAse/RNAse free Sterile, PCR Inhibitor free, Bioconcepts) with a final concentration of the individual components as follows: One Taq® Quick-Laord® 1X Master Mix with

Table 1. Sequences of primers used to detect gene fragments encoding staphylococcal enterotoxins (SEs), staphylococcal toxic shock toxin (TSST-1), and exfoliative toxins in isolated *Staphylococcus* strains.

Genes		Primers	Nucleotides sequence	Fragments size (bp) 5' to 3'	References
23S rRNA		Staur4 Staur6	ACG GAG TTA CAA AGG ACG AC AGC TCA GCC TTA ACG AGT AC	1250	Straub *et al.*, 1999
nuc		*nucF* *nucR*	GCGATTGATGGTGATACGGT AGCCAAGCCTTGACGAACTAAAGC	280	Brakstad *et al.*, 1992
Multiplex I	*sea*	sea-1 sea-2	GAA AAA AGT CTG AAT TGC AGG GAA CA CAA ATA AAT CGT AAT TAA CCG AAG GTT C	560	Jarraud *et al.*, 2002
	seh	seh-1 she-2	CAA TCA CAT CAT ATG CGA AAG CAG CAT CTA CCC AAA CAT TAG CAC C	376	Jarraud *et al.*, 2002
	sec	sec-1 sec-2	CTT GTA TGT ATG GAG GAA TAA CAA AAC ATG CAT ATC ATA CCA AAA AGT ATT GCC GT	275	Jarraud *et al.*, 2002
	tst	tst-1 tst-2	TTC ACT ATT TGT AAA AGT GTC AGA CCC ACT TAC TAA TGA ATT TTT TTA TCG TAA GCC CTT	180	Jarraud *et al.*, 2002
Multiplex II	*sed*	sed-1 sed-2	GAA TTA AGT AGT ACC GCG CTA AAT AAT ATG GCT GTA TTT TTC CTC CGA GAG T	492	Jarraud *et al.*, 2002
	etd	etd-1 etd-2	CAA ACT ATC ATG TAT CAA GGA TGG CCA GAA TTT CCC GAC TCA G	358	Zhang *et al.*, 1998
	eta	eta-1 eta-2	ACT GTA GGA GCT AGT GCA TTT GT TGG ATA CTT TTG TCT ATC TTT TTC ATC AAC	190	Jarraud *et al.*, 2002
	selk	sek-1 sek-2 sek-3	ATG CCA GCG CTC AAG GC AGA TTC ATT TGA AAA TTG TAG TTG ATT AGC T TGC CAG CGC TCA AGG TG	134	Holtfreter *et al.*, 2007
Multiplex III	*see*	see-1 see-2	CAA AGA AAT GCT TTA AGC AAT CTT AGG C CAC CTT ACC GCC AAA GCT G	482	Jarraud *et al.*, 2002
	seb	seb-1 seb-2	ATT CTA TTA AGG ACA CTA AGT TAG GGA ATC CCG TTT CAT AAG GCG AGT	404	Jarraud *et al.*, 2002
	selm	sem-1 sem-2	CTA TTA ATC TTT GGG TTA ATG GAG AAC TTC AGT TTC GAC AGT TTT GTT GTC AT	326	Jarraud *et al.*, 2002
	sell	sel-1 sel-2	GCG ATG TAG GTC CAG GAA AC CAT ATA TAG TAC GAG AGT TAG AAC CAT A	234	Holtfreter *et al.*, 2007
	selo	seo-1 seo-2	AGT TTG TGT AAG AAG TCA AGT GTA GA ATC TTT AAA TTC AGC AGA TAT TCC ATC TAA C	180	Jarraud *et al.*, 2002
Multiplex IV	*seln*	sen-1 sen-2	CGT GGC AAT TAG ACG AGT C GAT TGA TYT TGA TGA TTA TKA G	474	Holtfreter *et al.*, 2007
	seg	seg-1 seg-2	TCT CCA CCT GTT GAA GG AAG TGA TTG TCT ATT GTC G	323	Holtfreter *et al.*, 2007
	selq	seq-1 seq-2	ACC TGA AAA GCT TCA AGG A CGC CAA CGT AAT TCC AC	204	Holtfreter *et al.*, 2007
	selj	sej-1 sej-2	TCA GAA CTG TTG TTC CGC TAG GAA TTT TAC CAY CAA AGG TAC	138	Holtfreter *et al.*, 2007

Continued

Multiplex V	*sei*	sei-1 sei-2	CTY GAA TTT TCA ACM GGT AC AGG CAG TCC ATC TCC TG	461	Holtfreter *et al.*, 2007
	ser	ser-1 ser-2	AGC GGT AAT AGC AGA AAA TG TCT TGT ACC GTA ACC GTT TT	363	Holtfreter *et al.*, 2007
	selu	seu-1 seu-2	AAT GGC TCT AAA ATT GAT GG ATT TGA TTT CCA TCA TGC TC	215	Holtfreter *et al.*, 2007
	selp	sep-1 sep-2	GAA TTG CAG GGA ACT GCT GGC GGT GTC TTT TGA AC	182	Holtfreter *et al.*, 2007

Standard Buffer (New England Biolabs®), 0.2 - 0.4 µM of each primer and 20 - 50 ng of DNA.

PCR was performed in a thermal cycler (2720 Thermal Cycler, Applied Biosystem). The PCR program used was: predenaturation 95˚C for 10 min for initial DNA denaturation, 35 cycles (denaturation at 95˚C for 30 s, annealing of the primers at 55˚C for 45 s, extension at 72˚C for 60 s), and final elongation 72˚C for 7 min. Amplicons were stored at +4˚C.

PCR fragments were visualized by depositing 10 µl of each amplicon in agarose gel (1.5%) wells (Agarose CSL-AG500, LE Multi-Purpose Agarose, Cleaver Scientific, UK) stained with SafeviewTM Classic Cat≠ G108, Canada (5 µl in 100 ml agarose). Migration was performed in TAE 1X (Tris-Acetate-EDTA) buffer for 20 min at 100 V to separate fragments by electrophoresis. Amplicon bands were visualized under UV light with UV Transilluminator (UVP Transilluminator, Analytikjena, US) and Gel Doc (Gel DocTM XR+ with Image LabTM Software, Molecular Imager®). Amplicon sizes were determined using a 100 bp molecular weight marker (Gel Loading Dye Purple (6X), SDS B7025S, 100 bp DNA Ladder N3231L, New England Biolabs®).

A negative control (reaction mixture without DNA extract) to verify any contamination of the DNA extract.

3. Results

3.1. Microbiological Quality of Fish Analyzed

The average load of presumed pathogenic staphylococci in the samples analyzed is shown in **Table 2**.

The average loads of presumed pathogenic staphylococci ranged from (1.27 ± 1.42) × 10^5 CFU/g to (2.11 ± 0.11) × 10^6 CFU/g respectively. Most of the samples analyzed were of unsatisfactory microbiological quality according to the microbiological criterion for *Staphylococcus aureus* (m = 10^2): 100% for samples of smoked *Oreochromis niloticus*, smoked *Clarias gariepinus*, smoked *Anguilla bengalensis labiata*, smoked *Heterotis niloticus*, dried *Chrysichthys nigrodigitatus*, smoked *Chrysichthys nigrodigitatus*, smoked *Mormyrus rume*, dried *Mormyrus rume*, braised *Cyprinus carpio* and 78.26% and 75% respectively for dried

Table 2. Average *Staphylococcus* load (CFU/g) and assessment of microbiological quality of fish analyzed.

Fish	N	Average load	Microbiological quality		
			Satisfactory	Acceptable	Unsatisfactory
ONF	63	$(1.52 \pm 1.80) \times 10^6$	0 (0%)	0 (0%)	63 (100%)
CGF	55	$(1.60 \pm 0.73) \times 10^5$	0 (0%)	0 (0%)	55(100%)
ONS	23	$(1.27 \pm 1.42) \times 10^5$	5 (21.74%)	0 (0%)	18 (78.26%)
CNF	13	$(1.78 \pm 1.79) \times 10^5$	0 (0%)	0 (0%)	13 (100%)
MRS	21	$(1.48 \pm 1.04) \times 10^5$	0 (0%)	0 (0%)	21 (100%)
HNF	22	$(9.11 \pm 9.65) \times 10^5$	0 (0%)	0 (0%)	22 (100%)
CNS	7	$(0.95 \pm 1.18) \times 10^6$	0 (0%)	0 (0%)	7 (100%)
ABLF	15	$(2.11 \pm 0.11) \times 10^6$	0 (0%)	0 (0%)	15 (100%)
MRF	6	$(1.14 \pm 0.95) \times 10^6$	0 (0%)	0 (0%)	6 (100%)
CCB	6	$(1.03 \pm 1.95) \times 10^6$	0 (0%)	0 (0%)	6 (100%)
TTB	4	$(1.36 \pm 1.56) \times 10^5$	1 (25%)	0 (0%)	3 (75%)
			Total (Percentage)		
$p = 2.73 \times 10^{-13}$			6 (2.55%)	0 (0%)	229 (97.45%)
			Criterion: $m = 10^2$		

N: number of samples; ONF: *Oreochromis niloticus* (smoked); CGF: *Clarias gariepinus* (smoked); ABLF: *Anguilla bengalensis labiata* (smoked); HNF: *Heterotis niloticus* (smoked); ONS: *Oreochromis niloticus* (dried); CNS: *Chrysichthys nigrodigitatus*, dried; CNF: *Chrysichthys nigrodigitatus* (smoked); MRF: *Mormyrus rume* (smoked); MRS: *Mormyrus rume* (dried); CCB: *Cyprinus carpio* (braised); TTB: *Trachurus trachurus* (braised). S: Satisfactory; A: Acceptable; NS: Not Satisfactory.

Oreochromis niloticus and braised *Trachurus trachurus*. Of the total, only 2.55% of samples were of satisfactory quality, and 97.45% were of unsatisfactory quality.

The API STAPH gallery allowed the identification and conservation of 108 strains of Staphylococcus. Among the strains identified, 45 were *Staphylococcus aureus*, 50 *Staphylococcus xylosus*, 3 *Staphylococcus lugdunensis*, 2 *Staphylococcus hominis*, 3 *Staphylococcus haemolyticus*, 2 *Staphylococcus lentus*, 1 *Staphylococcus sciuri* and 1 *Staphylococcus capitis*.

3.1. Prevalence and Distribution of Staphylococcal Superantigens (SAgs)

A total of 81 (75%) strains of 108 *Staphylococcus* isolates were positive for at least one (1) toxin gene (Table 3). The most frequent genes detected in toxin gene-positive strains were: *tsst*-1 gene in 45 isolated strains (41.7%), *sei* (16/14.8%), *seg* (13/12%), *ser* (7/6.5%), *sec* (6/5.5%), and *sea* (5/4.6%) for enterotoxins, *seln* (14/12.9%), *selq* (8/7.4%) for enterotoxin-like proteins and *eta*

Table 3. Distribution of staphylococcal toxin genes detected in isolated *Staphylococcus* strains (Number/%).

Toxin genes	*S. aureus* (45)	*S. xylosus* (50)	*S. lugdunensis* (3)	*S hominis* (2)	*S. haemolyticus* (3)	*S. lentus* (3)	*S. sciuri* (1)	*S. capitis* (1)	*Number (%) of strains positive for each gene*
sea	3 (6.7)			1 (50)	1 (33.3)				5 (4.6)
seb	3 (6.7)								3 (2.7)
sec	5 (11.1)				1 (33.3)				6 (5.5)
sed	3 (6.7)								3 (2.7)
see									0 (0)
seg	12 (26.7)					1 (33.3)			13 (12)
seh	2 (4.4)								2 (1.8)
sei	9 (20)	6 (12)				1 (33.3)			16 (14.8)
selj	1 (2.2)								1 (0.9)
selk	2 (4.4)								2 (1.8)
sell	2 (4.4)								2 (1.8)
selm	1 (2.2)								1 (0.9)
seln	9 (20)	5 (10)							14 (12.9)
selo	1 (2.2)								1 (0.9)
selp	1 (2.2)	3 (6)							4 (3.7)
selq	3 (6.7)	5 (10)							8 (7.4)
selu		1 (2)							1 (0.9)
ser	3 (6.7)	4 (8)							7 (6.5)
eta	3 (6.7)								3 (2.7)
etd			1 (33.3)						1 (0.9)
tsst-1	18 (40)	24 (48)	1 (33.3)				1 (100)	1 (100)	45 (41.7)
Total toxin gene positive strains	39 (86.6)	33 (66)	2 (66.7)	1 (50)	2 (66.7)	2 (66.7)	1 (100)	1 (100)	81/108 (75)

(3/2.7%) for exfoliative toxin genes. However, *see* was not detected in all strains.

In particular, the *sea* gene was detected in *Staphylococcus aureus* (3/6.7%), *Staphylococcus hominis* (1/50%) and *Staphylococcus haemolyticus* (1/33.3%). The *eta* gene was only detected in *Staphylococcus aureus* (3/6.7%). The *tsst*-1 gene was present in *Staphylococcus aureus* (18/40%), *Staphylococcus xylosus* (24/48%) *Staphylococcus*, *lugdunensis* (1/33.3%), *Staphylococcus sciuri* (1/100%) *Staphylococcus capitis* (1/100%).

A high percentage of each isolated species had SAgs toxin genes (**Table 3**): *Staphylococcus aureus* (86.6%), *Staphylococcus xylosus* (66%), *Staphylococcus lugdunensis* (66.7%), *Staphylococcus hominis* (50%), *Staphylococcus haemoly-*

ticus (66.7%), *Staphylococcus lentus* (66.7%), *Staphylococcus sciuri* (100%), *Staphylococcus capitis* (100%).

Considering the distribution of *Staphylococcus* strains harboring toxin genes by type of sample analyzed (Table 4), all strains isolated from braised *Trachurus trachurus* possessed at least one toxin gene (100%). Large numbers of toxin gene-positive *Staphylococcus* were also observed in smoked *Oreochromis niloticus* (84.2%), smoked *Clarias gariepinus* (79.2%), smoked *Heterotis niloticus* (71.4%), smoked *Anguilla bengalensis labiata* (80%), and smoked *Cyprinus carpio* (75%).

The different combinations of toxin genes (genotypes) in each *Staphylococcus* species isolated from fish are presented in Table 5. Seventeen (17) toxin gene combinations were obtained in *Staphylococcus aureus* strains. Eleven (11/24.4%) *Staphylococcus aureus* strains presented only the *tsst*-1 gene. The most frequent gene combination was *seg*, *sei*, *seln*, found in 8 (17.8%) *Staphylococcus aureus* strains. Ten (10) distinct combinations of toxin genes were observed in *Staphylococcus xylosus* strains. The presence of *tsst*-1 alone was observed in 18 (36%) *Staphylococcus xylosus* strains. The combination *sei*, *seln* was the most frequent, found in 3 (6%) *Staphylococcus xylosus* strains.

Table 4. Distribution of toxin gene-positive strains in the different types of fish analyzed.

Types of fish	N	*S. aureus* (45)		*S. xylosus* (50)		*S. lugdunensis* (3)		*S. hominis* (2)		*S. haemolyticus* (3)		*S. lentus* (3)		*S. sciuri* (1)		*S. capitis* (1)		Total toxin gene positive strains
		n	PS	*n*	PS	*n*	PS	*n*	PS	*n*	PS	*n*	PS	*n*	PS	*n*	PS	
ONF	63	19	17	15	11	1	1	0	0	1	1	1	1	1	1	0	0	32/38 (84.2)
CGF	55	11	9	10	7	1	1	0	0	1	0	0	0	0	0	1	1	19/24 (79.2)
ONS	23	0	0	3	1	0	0	1	0	0	0	0	0	0	0	0	0	1/3 (33.3)
CNF	13	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	2/3 (66.7)
MRS	21	4	3	3	1	0	0	0	0	0	0	0	0	0	0	0	0	4/7 (57.1)
HNF	22	0	0	5	4	0	0	0	0	1	1	1	0	0	0	0	0	5/7 (71.4)
CNS	7	0	0	5	3	0	0	0	0	0	0	0	0	0	0	0	0	3/5 (60)
ABLF	15	7	6	0	0	1	0	1	1	0	0	1	1	0	0	0	0	8/10 (80)
MRF	6	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	2/3 (66.7)
CCB	6	2	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	3/4 (75)
TTB	4	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	3/3 (100)
TOTAL	235	45	39	50	33	3	2	2	1	3	2	3	2	1	1	1	1	81/108

Legend: ONS: *Oreochromis niloticus* (Smoked), CGS: *Clarias gariepinus* (Smoked), ABLS: *Anguilla bengalensis labiata* (Smoked), HNS: *Heterotis niloticus* (Smoked), OND: *Oreochromis niloticus* (Dried), CND: *Chrysichthys nigrodigitatus* (Dried), CNS: *Chrysichthys nigrodigitatus* (Smoked), MRS: *Mormyrus rume* (Smoked), MRD: *Mormyrus rume* (Dried), CCB: *Cyprinus carpio* (Braised), TTB: *Trachurus trachurus* (Braised). N: Number of sample; PS: number of positive strains.

Table 5. Combinations of enterotoxin genes in each Staphylococcus species isolated from fish.

Staphylococcus species	Gene combinations	Number (%) of strains
S. aureus (45)	*sea*	2 (4.4)
	seb	2 (4.4)
	eta	1 (2.2)
	tsst-1	11 (24.4)
	sea, sell	1 (2.2)
	seb, selk	1 (2.2)
	sec, seg	2 (4.4)
	sed, selp	1 (2.2)
	seh, selq	1 (2.2)
	tsst-1, *eta*	2 (4.4)
	tsst-1, *selq*	1 (2.2)
	sed, seg, ser	2 (4.4)
	seg, sei, seln	8 (17.8)
	tsst-1, *sec, sell*	1 (2.2)
	tsst-1, *seh, selk, selq*	1 (2.2)
	tsst-1, *sec, sei, selo, seln*	1 (2.2)
	tsst-1, *sec, selj, selm, ser*	1 (2.2)
	None	6
S. xylosus (50)	*sei*	2 (4)
	tsst -1	18 (36)
	selp	2 (4)
	selq	2 (4)
	sei, seln	3 (6)
	tsst -1, *selq*	2 (4)
	tsst -1, *selq, ser*	1 (2)
	tsst -1, *selr, selu*	1 (2)
	tsst-1, *seln, ser*	1 (2)
	tsst -1, *sei, seln, selp, ser*	1 (2)
	None	17 (34)
S. lugdunensis (3)	*etd, tst*-1	1 (66.7)
	None	1 (33.3)
S. hominis (2)	*sea*	1 (50)
	None	1 (50)

Continued

S. haemolyticus (3)	*sea*, *sed*	2 (66.7)
	None	1 (33.3)
S. lentus (3)	*seg*	1(33.3)
	sei	1 (33.3)
	None	1 (33.3)
S. sciuri (1)	*tsst* -1	1 (100)
	None	0
S. capitis (1)	*tsst*-1	1 (100)
	None	0

4. Discussion

In this study, we isolated and identified strains of *Staphylococcus* from dried, smoked, and braised fish. The results of the microbiological quality assessment indicated that most of the samples analyzed were found to be of unsatisfactory microbiological quality according to the microbiological criterion on *Staphylococcus aureus* (m = 10^2). Indeed, only 12.55% of samples were of satisfactory quality, while 97.45% were of unsatisfactory quality. This could be explained by the multiple cross-contaminations at the sales sites [23].

Many species of *Staphylococcus* were identified: *Staphylococcus aureus*, *Staphylococcus xylosus*, *Staphylococcus lugdunensis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus lentus*, *Staphylococcus sciuri* and *Staphylococcus capitis*. These species are frequently isolated from foods [24] [25] [26] [27]. *Staphylococcus aureus* (45/108) and *Staphylococcus xylosus* (50/108) were the predominant species isolated. Food handlers and food-contact surfaces are a source of staphylococcal contamination [28] [29] [30].

Many studies investigated the presence of toxin genes in strains implicated in staphylococcal food poisoning. Staphylococcal toxin genes were found in the strains isolated from fish analyzed. Among the 21 toxin genes tested, 20 genes were detected in all strains analyzed excepted the *see* gene. The genes detected included staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *seg*, *seh*, *sei*, *ser*), enterotoxin-like toxin genes (*selj-selq* and *selu*), exfoliative toxin genes (*eta*) and toxic shock syndrome toxin-1 (*tsst*-1). The most frequent staphylococcal enterotoxin genes detected in positive strains involved *sei* (16/14.8%), *seg* (13/12%), *sec* (6/5.5%) and *sea* (5/4.6%). The staphylococcal enterotoxin genes *sea-sei* are frequently isolated from strains involved in staphylococcal food poisoning cases [31] [32] [28] [25]. SEA is the toxin most implicated in these intoxications [33].

The *eta* gene was only detected in *Staphylococcus aureus* (3/6.7%). Exfoliative toxins can be implicated in diseases such as skin syndrome in children and also in some infections of the blood, urinary tract etc. [34] [35]. In addition, the *tsst*-1 gene was the most frequently detected in all strains isolated (41.7%). This

gene was found individually or in combination in isolates of *Staphylococcus aureus* (18/40%), *Staphylococcus xylosus* (24/48%), *Staphylococcus lugdunensis* (1/33.3%), *Staphylococcus sciuri* (1/100%), *Staphylococcus capitis* (1/100%). Vitale *et al.* [36] also detected the *tsst*-1 gene in the majority (42%) of strains isolated from foods implicated in food poisoning. However, Fijałkowski *et al.* [25] did not detect *tsst*-1 gene in their study. The *tsst*-1 gene is located on different pathogenicity islands such as SaPI1, SaPI2 and SaPIbov1 and encodes the protein toxic shock syndrome toxin 1 (TSST-1) with a size of 22 kDa [37]. This toxin is implicated in vaginal toxic shock syndrome [38]. Some studies have shown that this toxin purified can induce fever, mucosal suffusion, renal failure, liver damage, hypocalcemia, lymphocytopenia, and hypotension in animals [39].

Enterotoxin-like toxin genes were also detected among the strains analyzed, and the most frequent were *seln* (14/12.9%) and *selq* (8/7.4%). These toxin genes are present in both *Staphylococcus aureus* and the other coagulase-negative strains isolated in this study (*Staphylococcus xylosus, Staphylococcus lugdunensis, Staphylococcus hominis, Staphylococcus haemolyticus, Staphylococcus lentus, Staphylococcus sciuri and Staphylococcus capitis*). These strains had at least one toxin gene. Some strains, such as *Staphylococcus xylosus*, are known for their non-pathogenicity and are commonly used in food fermentation processes [40] [41].

However, the identification of toxin genes in these species in recent studies is increasingly clarifying their potential implication in food poisoning [42] [43], the toxigenic ability of coagulase-negative *Staphylococcus* should not be ignored and should also be investigated in food on an ongoing basis.

Staphylococcus aureus and *Staphylococcus xylosus* are the strains that simultaneously harbor several toxin genes. The most frequent combinations of toxin genes found were seg, sei and seln. Although the presence of toxin genes does not automatically mean production of toxins by strains, the detection of these genes requires special attention [44]. Expression of these genes can lead to the production of toxins implicated in cases of staphylococcal food poisoning.

The distribution of *Staphylococcus* strains harboring toxin genes by type of sample analyzed showed that enterotoxinogenic *Staphylococcus* strains are present in all types of fish analyzed, with a high prevalence in braised *Trachurus trachurus* (100%), smoked *Oreochromis niloticus* (84.2%), smoked *Clarias gariepinus* (79.2%), smoked *Heterotis niloticus* (71.4%), smoked *Anguilla bengalensis labiata* (80%), and smoked *Cyprinus carpio* (75%). Given that food handlers are a source of staphylococcal contamination, enterotoxigenic *Staphylococcus* would be introduced into fish by sellers through manual contact or respiratory secretions during sale [28] [30]. To this end, many studies have demonstrated the presence of enterotoxinogenic strains in fish samples and workers [45] [46] [47] [48].

5. Conclusion

In this study, we isolated and identified strains of *Staphylococcus* contaminating

dried, smoked and braised fish. The results of the microbiological quality assessment indicated that most of the samples analyzed were of unsatisfactory microbiological quality. The strains isolated from the fish analyzed were found to harbor staphylococcal toxin genes. Of the 21 toxin genes examined in this study, 20 were detected in all the strains tested. This demonstrates the pathogenicity of *Staphylococcus* strains isolated from fish collected in Ouagadougou markets. The staphylococcal toxin genes detected were present in both *Staphylococcus aureus* and the other coagulase-negative strains isolated in this study. The results of this study provide an important database that will enable people to control the consumption of smoked, dried, and braised fish to avoid staphylococcal food poisoning.

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Authors' Contributions

OA performed the collection of the fish samples, carried out the analyses and wrote the manuscript. TF supervised the work in the laboratory. OGA and OHS read and corrected the manuscript. ZC and SA read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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