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Phenotypic and Molecular Characterization of *Staphylococcaceae* and *Enterobacteriaceae* Species Isolated from Smoked, Dried, and Braised Fish Marketed in Ouagadougou

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Abstract

Background: To preserve its nutritional properties, fish must have good sanitary quality. The objective of this study was to investigate some pathogens contaminating smoked, dried, and braised fish marketed in Ouagadougou. **Methodology:** Potential pathogens of *Enterobacteriaceae* and *Staphylococcus* were screened in eight (8) species of processed fish. The investigation of the germs was carried out following the normative methods of microbiology. The identities of the strains were determined by API 20 E (BioMerieux S.A., France) and API STAPH (BioMerieux S.A., France) kits for *Enterobacteriaceae* and *Staphylococcus* species respectively. The *uidA* gene profile in *Escherichia coli* isolates was determined by simplex PCR. The identity of *Staphylococcus aureus* was confirmed by amplification of specific 23S rDNA regions and *nuc* gene profile with PCR. **Results:** A total of 235 fish samples were analyzed. A diversity of *Enterobacteriaceae* and *Staphylococcus* was detected. Twenty species of *Enterobacteriaceae* were identified among which, the most frequent were *Escherichia coli*, *Salmonella* sp, *Raoultella ornithinolytica* and *Serratia odorifera*, respectively in 22.6%, 4.3%, 28.9%, 17.4% of the samples analyzed. However, eleven species of *Staphylococcus* were identified among

which, *Staphylococcus xylosus*, *Staphylococcus aureus*, *Staphylococcus lugdunensis*, and *Staphylococcus sciuri* were the most frequent with respective percentages of 47.7%, 23.4%, 12.8% and 10.6% of samples. For all the samples, the species frequently isolated were: *Raoultella ornithinolytica*, *Escherichia coli*, *Serratia odorifera*, *Staphylococcus aureus*, *Staphylococcus xylosus*, and *Staphylococcus lugdunensis*. The *uidA* gene specific to *Escherichia coli* was detected in 82.85% of strains (29/35). Amplification of the specific 23S rDNA region using *staur* primers was observed in 98% (49/50) of the isolated *Staphylococcus aureus* strains and the *nuc* gene was detected in 86% of *Staphylococcus aureus* strains. **Conclusion:** The isolated bacteria are potential pathogens involved in foodborne illnesses and intoxications. Effective sanitary safety systems must be implemented to guarantee the sanitary quality of fish supplied to consumers.

Keywords

Fish Contamination, *Enterobacteriaceae*, *Staphylococcus*, Phenotypic and Molecular Characterization, Ouagadougou

1. Introduction

Fish intake presents beneficial interests for human being. Indeed, fish is one of the healthiest foodstuffs on the earth and has the least negative impact on the natural environment [1] [2]. It is also a nutritious food that adds variety to the diet. The consumption of fish is in line with nutritional recommendations to restrict the consumption of fat and saturated fatty acids in the diet. Despite its well-appreciated nutritional qualities, the benefits of fish consumption are influenced by its sanitary quality. Therefore, post-capture, fish is a perishable food [3] [4] [5]. To reduce post-capture losses, people use processing techniques including smoking, drying, and salting [6] [7] [8]. These techniques improve the stability of fish and extend their shelf life [9] [10]. However, even smoked, dried, or salted, fish can spoil or be contaminated with chemicals or microbiological pathogens [11].

In addition, the sales conditions (environment, equipment, labor, storage, and preservative) of fish are a potential source of contaminants in fish [12] [13] [14]. Foodborne diseases (FBDs) are frequently reported throughout the world, especially in developing countries. Food poisoning can occur through the ingestion of food contaminated either by a pathogen (viruses, parasites, and bacteria) [15] or by the toxins it produces [16] [17]. These foods borne diseases are one of the main public health problems [18]. According to FAO, several cases of FBDs are reported worldwide with about 420,000 deaths. Food contaminated by microorganisms accounts for 70% of foodborne illnesses. The economic cost of these foodborne diseases is very significant [19] [20]. *Enterobacteriaceae* such as *Escherichia coli* 0157 H 7, *Salmonella* as well as *Staphylococcus aureus* are among the pathogens responsible for foodborne illness [21] [22] [23]. These pa-

thogens are frequently detected in foods [24] [25] [26] [27] especially in fish [28] [29] [30] [31] [32] as well as in patients with gastroenteritis [33] [34]. These bacteria can contaminate fish from the origin as well as at each step of the processing [35].

Braised, dried, and smoked fish have an important part in the diet of the population of Ouagadougou. A survey conducted by our laboratory team in Ouagadougou between February and July 2019 revealed microbiological contamination risks related to the conditions under which fish are sold [36]. Therefore, it is essential for us to monitor the sanitary quality of fish provided to consumers. The objective of this study was to investigate certain pathogens that contaminate smoked, dried, and braised fish marketed in Ouagadougou. Specifically, the aim was to isolate and identify potential pathogenic *Enterobacteriaceae* and *Staphylococcus* strains from smoked, dried, and braised fish.

2. Material and Methods

2.1. Study Setting

The isolation of the germs was achieved at the Laboratory of Biochemistry and Applied Immunology (LABIA) of Joseph KI-ZERBO University (Ouagadougou/Burkina Faso).

Phenotypic identification of the strains was conducted at the Laboratoire National Vétérinaire (LANAVET) annex of Douala (Cameroon) and molecular identification of the strains was conducted at the Molecular and Cell Biology Laboratory, University of Buea (Cameroon).

2.2. Sampling

Sampling was conducted from July 2019 to February 2020 in the markets (50) identified in our previous survey in Ouagadougou [36]. The most consumed types of fish (smoked, dried, braised) were purchased, packaged in sterile bags. Each sample was labeled and placed in a cooler containing ice boxes and transported to the laboratory for analysis. Sampling was done in a randomized fashion.

2.3. Isolation of *Enterobacteriaceae* and *Staphylococcus* Strains from Fish Samples

2.3.1. Preparation of Stock Solution and Dilutions

A quantity of 10 g of fish flesh was aseptically collected and introduced into a sterile bottle containing 90 ml of sterile peptone water. The mixture was homogenized for 1 to 2 minutes. The supernatant was collected in a sterile vial to form the 10^{-1} dilution stock solution. Dilutions were made from the stock solution by taking 1 ml each time added to 9 ml of sterile peptone water contained in a test tube to constitute successively, 10^{-2}, 10^{-3}, and 10^{-4} dilutions.

2.3.2. Research of *Enterobacteriaceae*

1) Enumeration, Isolation of Thermotolerant Coliforms and *Escherichia*

coli

The detection of these germs was done according to the international standard ISO 4832 (2006)/NF V08-017; 1980. Inoculation was done in Violet Red Bile Agar (VRBL agar). A volume of 15 ml of VRBL previously prepared and brought to 45˚C was poured into Petri dishes. The inoculum and VRBL were homogenized by rotating the Petri dish and dried. After solidification, a second layer of 4 ml VRBL was poured to prevent the growth of any surface contamination flora. The plates were incubated in an incubator at 44˚C for 24 h ± 2 h. Typical bright red to pinkish or purple colonies with a diameter of more than 0.5 mm were enumerated according to AFNOR ISO 7218 (2007). After enumeration, some characteristic Escherichia coli colonies were plated on EMB agar and incubated at 37˚C for 24 h. After the 24-h incubation period, the strains were stored at −20˚C in cryotubes containing 20% glycerol brain broth for subsequent identification.

2) Research of *Salmonella*

Salmonella was detected on Xylose Lysine Desoxycholate (XLD) and Salmonella-Shigella (SS) agar media according to NF ISO 6579: 2002. The following procedure described by Edel and Kampelmacher [37] was used:

- Preenrichment: 25 g of sample (fish flesh) were aseptically collected and put in 225 ml of sterile peptone water and incubated at 37˚C for 24 h;
- Enrichment: 10 µl of pre-enriched peptone water for each sample was pipetted into a tube containing 10 ml of Rappaport Vassiliadis broth (Oxoid, Basingtoke, England) and incubated at 37˚C for 24 h;
- Isolation: 10 µl of the enrichment broth was pipetted and inoculated onto XLD and SS agar plates by the streak method using a sterile Pasteur pipette. The dishes were incubated at 37˚C for 24 h. After incubation, suspect colonies were isolated on XLD for biochemical characterization;
- Identification: suspect colonies that are red with a black center on XLD and colorless with or without a black center on SS agar were characterized using biochemical tests [38]: Gram stain, urease, indole production, glucose, lactose and mannitol fermentation, H_2S and gas production, motility test.

Each strain was scraped and stored at −20˚C in cryotubes containing 20% glycerol brain broth for subsequent identification.

3) Phenotypic Identification of *Escherichia coli* and Other *Enterobacteriaceae* Strains by API 20 E Gallery

Enterobacteriaceae strains were identified using an API 20 E kit (BioMerieux S.A., Marcy l'Etoile, France). The isolates were submitted to 20 biochemical tests in microtubes containing dehydrated substrates. A bacterial suspension of McFarland turbidity equal to 0.5 was prepared and introduced into the gallery tubes. For CIT, VP, and GEL tests, the tubes and cups were filled. For the other tests, only the tubes were filled (not the cups). For the tests, ADH, LDC, ODC, H_2S, URE anaerobiosis was created by filling their cups with paraffin oil (Glentham Life Sciences, Euro. Grade). The incubation box was then closed and incubated at 37˚C for 24 h.

Revelation tests requiring the addition of reagents after incubation was performed: addition of a drop of TDA, JAMES, and VP 1 + VP 2 reagents in TDA, IND, and VP microtubes respectively. The reading of the galleries was done according to the manufacturer's Reading Table. Strains were identified on the basis of numerical profiles using the Apiweb™ software. Only isolates with a compatibility percentage higher than 80% were selected.

2.3.3. Pathogenic *Staphylococcus* Detection

1) Isolation and Enumeration of Pathogenic *Staphylococcus*

The search and isolation of *Staphylococcus* were performed on Mannitol Salt Agar (ISO 6888; 2003). A volume of 0.1 ml of two successive dilutions was spread on the surface of the agar in Petri dishes and incubated at 37˚C for 24 h. After incubation, all Staphylococci were enumerated according to AFNOR ISO 7218 (2007). The results allowed us to discriminate *Staphylococci* based on their ability to ferment or not mannitol. Mannitol fermentation induces acidification that leads to a yellow, the medium in the presence of phenol red (pH indicator). The strains were subjected to additional standard biochemical tests: catalase and DNAse tests. Suspect *Staphylococcus aureus* and other colonies were subcultured onto Mannitol Salt Agar and the bacterial mass of each strain was scraped off and stored in cryotubes containing 20% glycerol brain broth for subsequent identification.

2) Phenotypic Identification of *Staphylococcus* Strains by the API STAPH Gallery

Staphylococcus strains were identified using API Staph kit (BioMerieux S. A., Marcy l'Etoile, France). Isolates were subjected to 20 biochemical tests in microtubes containing dehydrated substrates. A bacterial suspension of 0.5 McFarland turbidity was prepared and introduced into the gallery tubes. For all tests, the microtubes were filled without exceeding the level of the tubes. For the ADH and URE tests, anaerobiosis was created by filling their cups with paraffin oil. The incubation box was then closed and incubated at 37˚C for 24 h. After incubation, the reagents NIT1, NIT2, ZYM-A, ZYM-B, VP1, and VP2, were added for revelation tests in the NIT, PAL, and VP tubes respectively. The reading of the galleries was done according to the manufacturer's Reading Table. Strains were identified on the basis of numerical profiles using Apiweb ™ software.

Isolates with a staphylococcal compatibility percentage higher than 80% were retained.

2.4. Molecular Characterization of *Escherichia coli*, and *Staphylococcus aureus* Isolates

2.4.1. DNA Extraction

The total genomic DNA extraction was performed by a thermal method. For this purpose, one to three colonies of each isolate (24 h) on Muller Hinton agar plates, were picked using a sterile Pasteur pipette, then suspended in 200 µl ste-

rile1X PBS in an Eppendorf tube and vortexed to homogenize the mixture. The cells were washed by centrifugation at 20,000 ×g for 10 min. The supernatant was removed and the pellet was used for total genomic DNA extraction. For cell lysis, the pellet was resuspended in 20 µl of nuclease-free water, frozen for 15 min, and 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. The resulting supernatant was collected and stored at −20˚C in Eppendorf tubes for future use.

2.4.2. Detection of *uidA* Gene in *Escherichia coli* Isolates

The molecular identification of *Escherichia coli* was done by detecting the *uidA* gene encoding the production of the *β*-D-glucuronidase (GUD) enzyme. The *uidA* gene detection was done using the primers *uidA*-F 5' ATGCCAGTCCAGCGTTTTTGC 3' and *uidA*-R 5' AAAGTGTGTGGGTCAATAATCAGGAAGTG 3'. The primer oligonucleotide sequences were obtained from Inquaba Biotec West Africa Ltd., Africa's Genomics Company.

PCR was performed with a thermal cycler (2720 Thermal Cycler, Applied Biosystem). The reaction mixture was made in 25 µl according to the OneTaq master mix indication as follows: 12.5 µl of OneTaq® Quick-Laord® 2× 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 amplification conditions were: initial denaturation at 94˚C for 90 s, and 30 cycles of (denaturation at 94˚C for 30 s, annealing at 58˚C for 25 s, and extension at 72˚C for 30 s), final extension at 72˚C for 5 min.

The amplified products were run on a 1.5% agarose gel (Agarose CSL-AG500, LE Multi-Purpose Agarose, Cleaver Scientific, UK) containing Safeview™ Classic Cat ≠ G108, Canada (5 µl in 100 ml of agarose gel), in 1× TAE (Tris-Acetate-EDTA) buffer for 20 min at 100 V. Ten microliters (10 µl) of each amplicon were dropped into the wells of the agarose gel. PCR fragments were visualized with UV Transilluminator (UVP Transilluminator, Analytikjena, US) and photographed with Gel Doc (Gel DocTM XR+ with Image Lab™ Software, Molecular Imager®). Amplicon sizes were assessed by reference to a 100 bp molecular size ladder (Gel Laoding Dye Purple (6X), SDS B7025S, 100 bp DNA Ladder N3231L, New England Biolabs®). The presence of the *uidA* gene is identified by the occurrence of a 1487 bp band.

2.4.3. Molecular Characterization of *Staphylococcus aureus* Strains by Amplification of the 23S rDNA Gene and the *nuc* Gene

The primers *Staur*4 5'ACG GAG TTACAAAGG ACGAC 3' and *Staur*6 5'AGCTCAGCCTTA ACGAGTAC 3' were used for the amplification of specific coding regions of the 23S ribosomal rDNA of *Staphylococcus aureus* species as described by [39].

The specific sense primer *nuc*F 5'GCGATTGATGGTGATACGGT 3' and antisense primer *nuc*R 5'AGCCAAGCCTTGACGAACTAAAGC 3' (Inquaba Biotec West Africa Ltd, Africa's Genomics Company) were used to amplify the *nuc* gene encoding the coagulase-positive *Staphylococcus* thermostable endonuclease.

The reaction mixture was prepared in of 25 µl according to the OneTaq master mix: 12.5 µl of OneTaq® Quick-Laord® 2× 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 was performed in a thermal cycler (2720 Thermal Cycler, Applied Biosystem). The program used in the case of the *Staur* primers was: predenaturation 94˚C/5min, 35 cycles of (94˚C/30sec; 55˚C/40seconds; 72˚C/1.2minutes) and final extension 72˚C/5minutes. In the case of the nuc gene, the following steps were applied: initial denaturation 95˚C for 5 min, 30 cycles of (denaturation 94˚C for 60 sec, annealing 55˚C for 30 sec, extension 72˚C for 90 sec), final extension 72˚C for 5 min. Amplicons were stored at +4˚C.

For visualization of the amplified PCR fragments, 10 µl of each amplicon was loaded into agarose wells (Agarose CSL-AG500, LE Multi-Purpose Agarose, Cleaver Scientific, UK) stained with Safeview™ Classic Cat ≠ G108, Canada (5 µl in 100 ml of agarose). Migration was done in 1× TAE (Tris-Acetate-EDTA) buffer for 20 min at 100 V to separate the fragments by electrophoresis. The bands of the amplicons were visualized under UV light with UV Transilluminator (UVP Transilluminator, Analytikjena, US) and with Gel Doc (Gel Doc™ XR+ with Image LabTM Software, Molecular Imager®). Amplicon sizes were determined using a 100 bp molecular weight marker (Gel Loading Dye Purple (6×), SDS B7025S, 100 bp DNA Ladder N3231L, New England Biolabs®). The characteristic bands are 1250 bp with the *Staur* primer and the amplified *nuc* gene fragment shows a 280 bp band.

2.5. Statistical Analysis

The data were analyzed using IBM SPSS 25.0 and Sphinx Plus2 software to calculate percentages and develop tables of characteristics to highlight the most frequently reported strains and the most specific strains in regard to the sample types.

3. Results

3.1. Distribution of the Types of Fish Analyzed

Figure 1 illustrates the distribution of the different categories of fish analyzed. A total of 235 fish samples were analyzed in this study. Twelve (11) kinds of fish were purchased according to their abundance on Ouagadougou markets and the most consumed by the population.

Figure 2 illustrates the pictures of some fish samples analyzed.

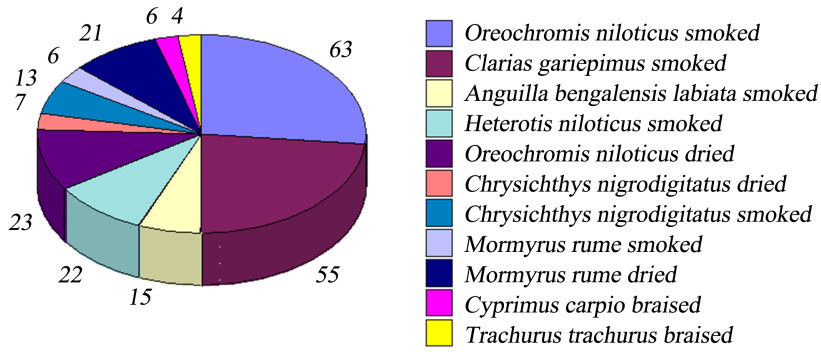

Figure 1. Proportion des échantillons sources des souches isolées.

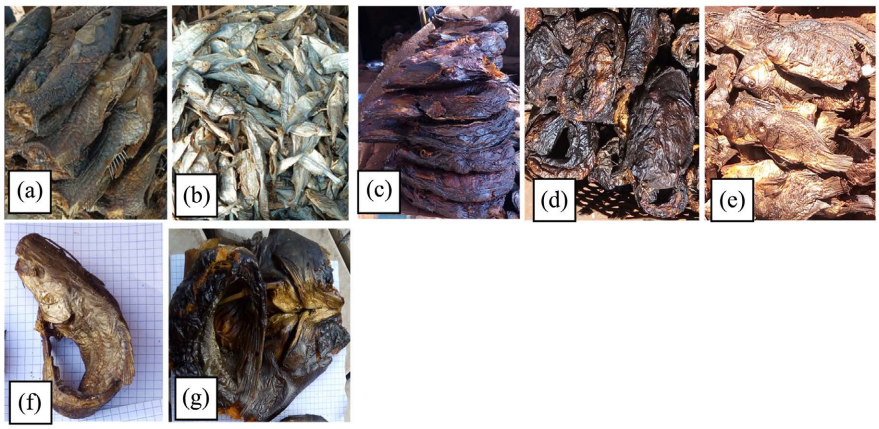

Figure 2. Picture of some fish samples analyzed: (a) *Heterotis niloticus* smoked, (b) *Mormyrus rume* dried, (c) *Clarias gariepinus* smoked, (d) *Anguilla bengalensis labiata* smoked, (e) *Oreochromis niloticus* smoked, (f) *Chrysichthys nigrodigitatus* smoked, (g) *Chrysichthys nigrodigitatus* dried.

3.2. Phenotypic Identification of Isolated Strains

3.2.1. *Enterobacteriaceae* Isolated from Fish Samples

The *Enterobacteriaceae* detected in the fish samples are presented in **Table 1**. The results of the biochemical tests revealed a diversity of *Enterobacteriaceae* species. The total coliforms enumerated averaged $(4.92 \pm 5.66) \times 10^8$ CFU/ml and fecal coliforms, $(0.84 \pm 2.27) \times 10^3$ CFU/ml. **Figure 3** shows various colonies of *Enterobacteriaceae* isolated on specific culture media.

In total, 20 species of *Enterobacteriaceae* were identified. Among these potentially pathogenic strains, *Escherichia coli* was detected in 22.6% of samples, *Salmonella* sp at 4.3%, *Raoultella ornithinolytica* at 28.9%, and *Serratia odorifera* at 17.4%. The percentages of the strains correspond to the number of samples in which the strain was detected compared to the total number of samples analyzed. In addition, about 17% of the samples did not contain *Enterobacteriaceae*.

The difference with the reference distribution was significant ($1 - p \geq 99.99\%$).

The distribution of the identified strains in relation to the analyzed samples is significant with $1 - p \geq 99.99\%$ (**Table 2**). Indeed, as shown in **Table 2**, 29.3%

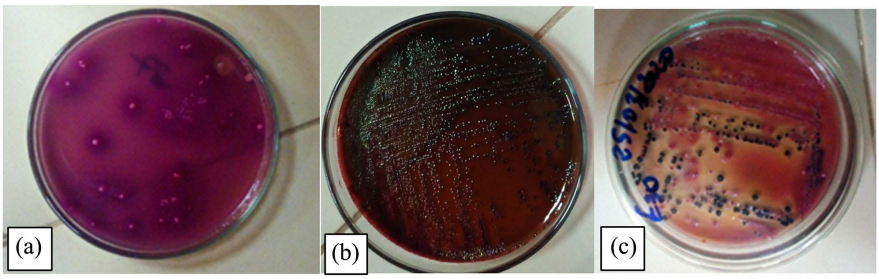

Figure 3. Bacterial strains isolated from fish (a) Colonies of thermotolerant coliforms on VRBL, (b) Presumptive *Escherichia coli* re-isolated on EMB agar, (c) Presumptive *Salmonella* on SS agar.

Table 1. *Enterobacteriaceae* strains identified in fish samples.

Enterobacteriaceae **strains identified**	**Number of contaminated samples**	**Percentage**
Cedecea davisae	29	12.3%
Raoultella ornithinolytica	68	**28.9%**
Salmonella enterica ssp arizonae	15	6.4%
Proteus mirabilis	17	7.2%
Serratia odorifera	41	**17.4%**
Salmonella spp	10	4.3%
Enterobacter cloacae	6	2.6%
Proteus vulgaris group	18	7.7%
Citrobacter braakii	7	3.0%
Serratia marcescens	13	5.5%
Serratia liquefaciens	8	3.4%
Klebsiella pneumoniae ssp pneumoniae	6	2.6%
Serratia ficaria	6	2.6%
Enterobacter gergoviae	9	3.8%
Enterobacter aerogenes	9	3.8%
Escherichia coli	53	**22.6%**
Kluyvera spp	6	2.6%
Cronobacter sakazakii	11	4.7%
Acinetobacter baumannii	6	2.6%
Acinetobacter calcoaceticus	6	2.6%
None	40	17.0%

and 31.7% of *Serratia odorifera* species were found in *Oreochromis niloticus* (dried) and *Mormyrus rume* (dried) respectively, 80.0% of *Salmonella* spp in *Mormyrus rume* (dried), 33.3% and 27.8% *Proteus vulgaris* group in *Anguilla*

Table 2. Distribution of *Enterobacteriaceae* identified by fish category.

Types of fish / *Enterobacteriaceae* identified	*ONS*	*CGS*	*ABS*	*HNS*	OND	*CND*	*CND*	*MRS*	*MRD*	*CCB*	*TTB*
Cedecea davisae	31.0	27.6	13.8	13.8	0.0	0.0	13.8	0.0	0.0	0.0	0.0
Raoultella ornithinolytica	47.1	16.2	8.8	0.0	10.3	1.5	16.2	0.0	0.0	0.0	0.0
Salmonella enterica ssp arizonae	6.7	46.7	0.0	0.0	0.0	0.0	6.7	0.0	40.0	0.0	0.0
Proteus mirabilis	47.1	47.1	0.0	0.0	0.0	0.0	0,0	0.0	0.0	0.0	5.9
Serratia odorifera	1.5	17.1	0.0	0.0	**29.3**	0.0	0.0	0.0	**31.7**	2.4	0.0
Salmonella spp	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	**80.0**	0.0	0.0
Enterobacter cloacae	0.0	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proteus vulgaris group	33.3	0.0	**33.3**	0.0	0.0	**27.8**	5.6	0.0	0.0	0.0	0.0
Citrobacter braakii	0.0	14.3	0.0	**85.7**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Serratia marcescens	0.0	0.0	0,0	0.0	0.0	7.7	**46.2**	0.0	38.5	0.0	7.7
Serratia liquefaciens	87.5	12.5	0,0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Klebsiella pneumoniae ssp pneumoniae	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Serratia ficaria	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Enterobacter gergoviae	88.9	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	0.0	0.0
Enterobacter aerogenes	0.0	11.1	0.0	11.1	0.0	11.1	0.0	0.0	**55.6**	0.0	11.1
Escherichia coli	43.4	34.0	9.4	3.8	0.0	0.0	1.9	0.0	3.8	3.8	0.0
Kluyvera spp	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cronobacter sakazakii	9.1	**90.9**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0,0
Acinetobacter baumannii	0.0	0.0	0.0	0.0	**100**	00	0.0	0.0	0.0	0.0	0,0
Acinetobacter calcoaceticus	0.0	0.0	0.0	0.0	**100**	0.0	0.0	0.0	0.0	0.0	0.0
None	5.0	20.0	0.0	**37.5**	12.5	0.0	0.0	**15.0**	0.0	7.5	2.5
TOTAL	26.8	23.4	6.4	9.4	9.8	3.0	5.5	2.6	8.9	2.6	1.7

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).

bengalensis labiata (smoked) and *Chrysichthys nigrodigitatus* (dried) respectively, 85.7% *Citrobacter braakii* in *Heterotis niloticus* (smoked), 46.2% of *Serratia marcescens* in *Chrysichthys nigrodigitatus* (smoked), 55.6% of *Enterobacter aerogenes* in *Mormyrus rume* (dried), 90.9% *Cronobacter sakazakii* in *Clarias gariepinus* (smoked). *Acinetobacter baumannii* and *Acinetobacter calcoaceticus* were only detected in *Oreochromis niloticus* (dried). *Oreochromis niloticus* (smoked) was the type of fish in which most germs were detected (26.8%) followed by smoked *Clarias gariepinus* (23.4%). *Mormyrus rume* (dried) contained most *Salmonella* spp (80%) and smoked *Clarias gariepinus* most *Salmonella enterica ssp arizonae* (46.7%).

Figure 4 shows the profile of some *Enterobacteriaceae* strains on API 20 E galleries.

3.2.2. *Staphylococcus* Isolated from Fish Samples

Table 3 shows *Staphylococcus* strains isolated from fish samples analyzed. The *Staphylococcus* enumerated were on average $(0.68 \pm 1.17) \times 10^6$ CFU/ml. Figure 5 shows different strains of *Staphylococcus* spotted on Mannitol Salt Agar. Among eleven (11) species of *Staphylococcus* identified, *Staphylococcus xylosus*, *Staphylococcus aureus*, *Staphylococcus lugdunensis*, and *Staphylococcus sciuri* were the most representative with respective percentages of 47.7%, 23.4%, 12.8% and 10.6% of samples. On the other hand, no *Staphylococcus* was detected in 12.8% of the samples analyzed. The distribution of the identified strains in comparison to the analyzed samples was highly significant with $1 - p \geq 99.99\%$ (Table 4).

Figure 4. API 20 E profiles of some *Enterobacteriaceae* strains identified: (a) *Raoultella ornithinolytica* at 90%, (b) *Escherichia coli* at 99.5%, (c) *Salmonella* spp presumed.

Figure 5. Various strains of *Staphylococcus* sp spiked on Mannitol Salt Agar detected in fish.

Table 3. Distribution of *Staphylococcus* strains identified in fish samples.

***Staphylococcus* strains identified**	**Number of contaminated samples**	**Percentage**
Staphylococcus xylosus	112	**47.7%**
Staphylococcus aureus	55	23.4%
Staphylococcus lugdunensis	30	12.8%
Staphylococcus sciuri	25	10.6%
Staphylococcus intermedius	3	1.3%
Staphylococcus lentus	16	6.8%
Staphylococcus hominis	20	8.5%
Staphylococcus simulans	2	0.9%
Staphylococcus saprophyticus	7	3.0%
Staphylococcus haemolyticus	10	4.3%
Staphylococcus capitis	6	2.6%
None	30	12.8%

Table 4. Distribution of identified *Staphylococcus* strains according to fish species.

Types of fish / ***Staphylococcus* identified**	**ONS**	**CGS**	**ABLS**	**HNS**	**OND**	**CND**	**CND**	**MRS**	**MRD**	**CCB**	**TTB**
Staphylococcus xylosus	30.4	22.3	**0.0**	11.6	4.5	5.4	**11.6**	5.4	5.4	2.7	0.9
Staphylococcus aureus	49.1	29.1	14.5	0.0	0.0	0.0	0.0	0.0	3.6	1.8	1.8
Staphylococcus lugdunensis	26.7	23.3	23.3	10.0	16.7	0.0	0.0	0.0	0.0	0.0	0.0
Staphylococcus sciuri	**72.0**	0.0	24.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0
Staphylococcus intermedius	0.0	33.3	0.0	0.0	0.0	0.0	0.0	**33.3**	33.3	0.0	0.0
Staphylococcus lentus	37.5	0.0	31.3	**31.3**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Staphylococcus hominis	0.0	0.0	**30.0**	5.0	**35.0**	5.0	0.0	0.0	**25.0**	0.0	0.0
Staphylococcus simulans	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0
Staphylococcus saprophyticus	14.3	28.6	0.0	0.0	14.3	0.0	0.0	0.0	0.0	0.0	**42.9**
Staphylococcus haemolyticus	10.0	20.0	0.0	**70.0**	0.0	0.0	0.0	0.0	0,0	0.0	0.0
Staphylococcus capitis	0.0	**100**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
None	3.3	**50.0**	3.3	0.0	16.7	0.0	0.0	0.0	**20.0**	6.7	0.0
TOTAL	26.8	23.4	6.4	9.4	9.8	3.0	5.5	2.6	8.9	2.6	1.7

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).

The difference with the reference distribution is highly significant $1 - p \geq 99.99\%$. Cells with bold text are those for which the effective population is significantly higher or lower than the theoretical population.

Figure 6 illustrates the profiles of some *Staphylococcus* strains identified by the API STAPH gallery.

Table 5 illustrates the most frequently occurring strains of *Enterobacteriaceae* and *Staphylococcus* in each kind of fish. Percentages are calculated individually based on the total number of samples. For all samples, *Staphylococcus xylosus*, *Staphylococcus aureus*, and *Staphylococcus lugdunensis* were the most frequently mentioned *Staphylococcus*. *Raoultella ornithinolytica*, *Escherichia coli*, and *Serratia odorifera* are the *Enterobacteriaceae* frequently found in the samples.

Table 5 gives the modalities that are significantly different (frequency ratio). Only the most remarkable modalities are displayed (at the threshold of 1.20).

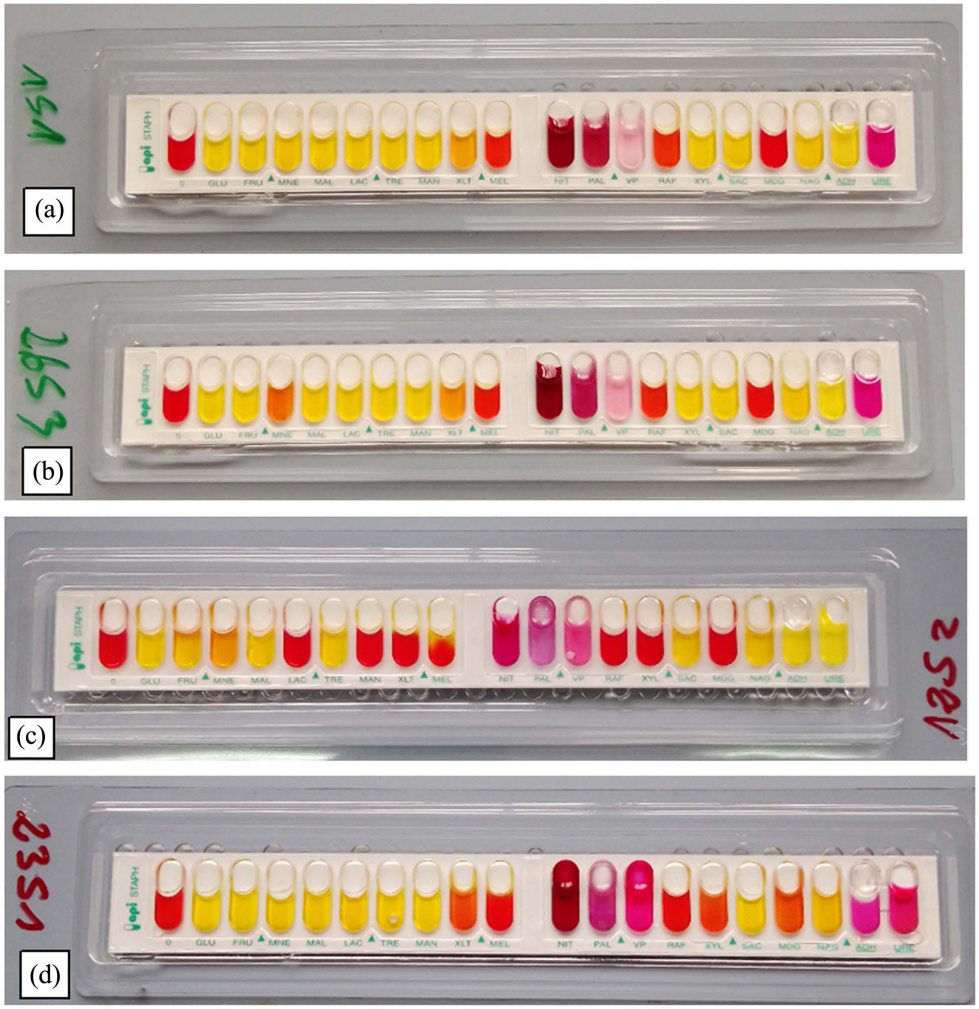

Figure 6. API STAPH profiles of some identified *Staphylococcus* strains: (a) and (b) *Staphylococcus xylosus* 99.9%, (c) *Staphylococcus lugdunensis* 90%, (d) *Staphylococcus aureus* 97.7%.

Table 5. Table of characteristics of the sample in relation to the most frequently identified strains (*Staphylococcus* and *Enterobacteriaceae*).

Types of fish	*Staphylococcus*	*Enterobacteriaceae*
Oreochromis niloticus smoked (63)	*Staphylococcus xylosus* (4.0%) *Staphylococcus aureus* (42.9%) *Staphylococcus sciuri* (28.6%)	*Raoultella ornithinolytica* (50.8%) *Escherichia coli* (36.5%) *Cedecea davisae* (14.3%)
Clarias gariepinus smoked (55)	*Staphylococcus xylosus* (45.5%) *Staphylococcus aureus* (29.1%) None (27.3%)	*Escherichia coli* (32.7%) *Raoultella ornithinolytica* (20.0%) *Cronobacter sakazakii* (18.2%)
Anguilla bengalensis labiata smoked (15)	*Staphylococcus aureus* (53.3%) *Staphylococcus lugdunensis* (46.7%) *Staphylococcus sciuri* (40.0%)	*Raoultella ornithinolytica* (40.0%) *Proteus vulgaris group* (40.0%) *Escherichia coli* (33.3%)
Heterotis niloticus smoked (22)	*Staphylococcus xylosus* (59.1%) *Staphylococcus haemolyticus* (31.8%) *Staphylococcus lentus* (22.7%)	None (68.2%) *Citrobacter braakii* (27.3%) *Cedecea davisae* (18.2%)
Oreochromis niloticus dried (23)	*Staphylococcus hominis* (30.4%) *Staphylococcus xylosus* (21.7%) *Staphylococcus lugdunensis* (21.7%)	*Serratia odorifera* (52.2%) *Raoultella ornithinolytica* (30.4%) *Acinetobacter baumannii* (26.1%)
Chrysichthys nigrodigitatus dried (7)	*Staphylococcus xylosus* (85.7%) *Staphylococcus hominis* (14.3%)	*Proteus vulgaris group* (71.4%) *Raoultella ornithinolytica* (14.3%) *Serratia marcescens* (14.3%)
Chrysichthys nigrodigitatus smoked (13)	*Staphylococcus xylosus* (100.0%)	*Raoultella ornithinolytica* (84.6%) *Serratia marcescens* (46.2%) *Cedecea davisae* (30.8%)
Mormyrus rume smoked (6)	*Staphylococcus xylosus* (100.0%) *Staphylococcus intermedius* (16.7%)	None (100.0%)
Mormyrus rume dried (21)	*Staphylococcus xylosus* (28.6%) None (28.6%) *Staphylococcus hominis* (23.8%)	*Serratia odorifera* (61.9%) *Salmonella spp* (38.1%) *Salmonella enterica ssp arizonae* (28.6%)
Cyprinus carpio braised (6)	*Staphylococcus xylosus* (50.0%) None (33.3%) *Staphylococcus aureus* (16.7%)	None (50.0%) *Escherichia coli* (33.3%) *Serratia odorifera* (16.7%)
Trachurus trachurus braised (4)	*Staphylococcus saprophyticus* (75.0%) *Staphylococcus xylosus* (25.0%) *Staphylococcus aureus* (25.0%)	*Proteus mirabilis* (25.0%) *Serratia marcescens* (25.0%) *Enterobacter aerogenes* (25.0%)
Combined (235)	*Staphylococcus xylosus* *Staphylococcus aureus Staphylococcus lugdunensis*	*Raoultella ornithinolytica* *Escherichia coli* *Serratia odorifera*

3.3. Confirmation of Isolated Strains by PCR

3.3.1. Profile of *Escherichia coli* Strains Confirmed by *uidA* Gene Detection

Thirty-five (35) strains of *Escherichia coli* identified by the API 20 E gallery were used for molecular characterization. The *uidA* gene characterizing specifically *Escherichia coli* was detected in 82.85% of the strains (29/35). Among the tested strains, six (6) did not present the gene of interest. **Figure 7** shows the agarose

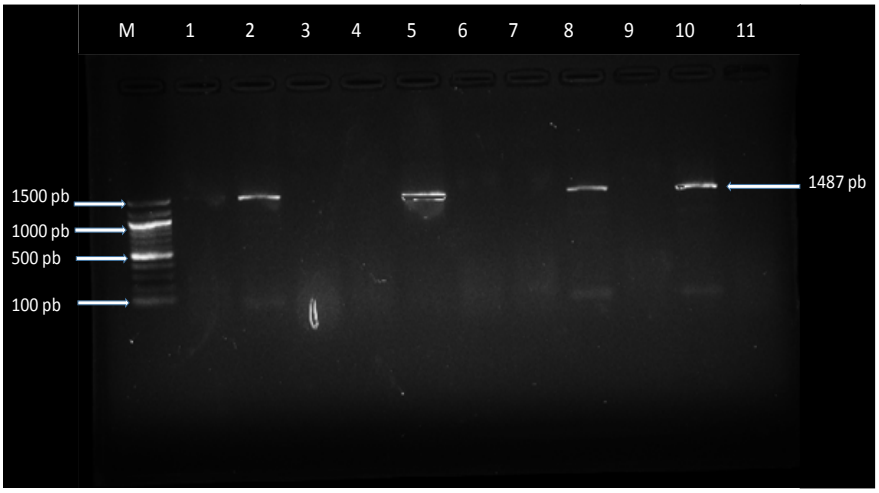

Figure 7. Photograph of an agarose gel showing *uidA* gene detected in *Escherichia coli* strains isolated from fish: M = DNA molecular weight marker; 2, 5, 8, 10 = *uidA* positive isolates; 1, 3, 4, 6, 7, 9 = *uidA* negative isolates; 11 = negative control.

gel picture of the *uidA* gene electrophoresis detected in some of the tested strains.

3.3.2. Confirmation of *Staphylococcus aureus* Strains by Detection of the Specific 23S rDNA Gene and the *nuc* Gene

Amplification of the 23S rDNA specific gene (**Figure 8**) using the *staur* primers was observed in 49/50 (98%) of the isolated *Staphylococcus aureus* strains. The *nuc* gene (**Figure 9**) was detected in 86% of the strains.

4. Discussion

Fish contributes to the improvement of the nutritional level of the population. This food can compromise the health of consumers if basic hygiene quality is not ensured. To maintain its nutritional properties, fish must have good sanitary quality. Eight (8) species of processed fish sold in the Ouagadougou markets were analyzed in this study: smoked *Oreochromis niloticus*, smoked *Clarias gariepinus*, smoked *Anguilla bengalensis labiata*, smoked *Heterotis niloticus*, dried *Oreochromis niloticus*, dried Chrysichthys nigrodigitatus, dried *Chrysichthys nigrodigitatus*, smoked *Mormyrus rume*, dried *Mormyrus rume*, braised *Cyprinus carpio*, braised *Trachurus trachurus*. In this study, a diversity of *Enterobacteriaceae* and *Staphylococcus* was detected. The study conducted by Agbeko *et al.* [40], Kyule *et al.* [41], and Guetarni & Labdi [42] also revealed a diversity of bacteria isolated from fish in Kenya, Ghana, and Algeria, respectively. Proper processing methods are expected to considerably reduce pathogens [11] [43] [44]. The presence of pathogenic microorganisms detected in fish is due to several factors. In some cases, the fish may already be infected with germs (in its gastrointestinal tract) since the source (in fish farms) [45] [46] [47]. The sanitary quality of fish after capture may be due to contamination related to poor storage conditions that favor microorganism proliferation [7].

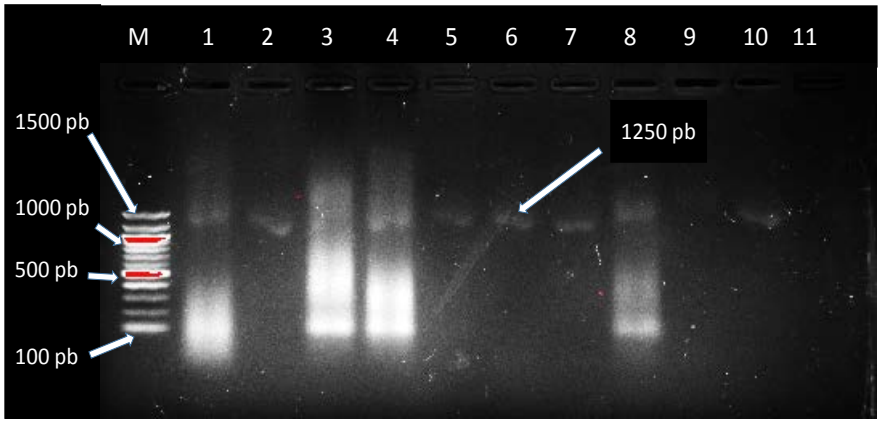

Figure 8. Photograph of the agarose gel showing the 23S rDNA gene detected with the *Staur* primer in *Staphylococcus* strains isolated from fish: M = DNA molecular weight marker; 1 - 9 and 10 = positive isolates; 9 = negative isolates; 11 = negative control.

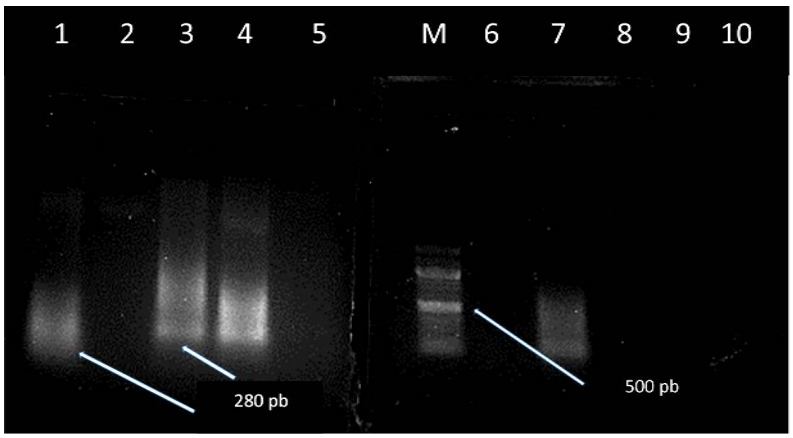

Figure 9. Agarose gel picture of *nuc* gene detected in *Staphylococcus aureus* strains isolated from fish: 1, 3, 4 = positive isolates; M = DNA molecular weight marker; 2, 5, 6, 7, 8, 9 = negative isolates; 10 = negative control.

The isolates of *Enterobacteriaceae* and *Staphylococcus* species are not specific to one type of processed fish, but their load differs from one type of fish to another. This variability could be explained by the fact that the samples come from different origins and from different processors, and sellers [36].

The isolates of *Enterobacteriaceae* were mainly represented in order by *Raoultella ornithinolytica*, *Escherichia coli*, and *Serratia odorifera*. The contamination of fish by *Enterobacteriaceae* is frequently mentioned in many previous studies [42] [48] [49]. Compared to the study conducted by Anihouvi *et al.* [50] on smoked fish in Benin, high contamination of smoked fish by *Enterobacteriaceae* (63.9% of the samples) and *Escherichia coli* detected in 27.8% of the samples was higher than the values found in this study (22.6%).

Furthermore, Elsherief *et al.* [51] also found high contamination (94%) of fish in their study with a significant proportion of *Serratia* spp, *Salmonella* spp., and *E. coli*. The detection of these germs indicates poor hygiene conditions during processing, storage, or sale. It is known that processing, transport, and storage

conditions significantly affect the hygienic quality of fish [52] [53] [54] [55] [56].

Escherichia coli is a commensal bacterium found in the colon of humans and also warm-blooded animals and used as an indicator of fecal contamination in fish [57]. It is a pathogen with multiple pathovars that cause gastroenteritis disorders [58] [59] [60] [61].

Raoultella ornithinolytica and *Serratia odorifera* are ubiquitous in both water and soil [62] [63] [64] [65]. Therefore, their occurrence in samples could be due to contamination through soil or equipment, especially on display tables during the sale [66], but also due to lack of hygiene, because some strains have been isolated from patients suffering from diarrhea [67]. They are opportunistic pathogens. *Raoultella ornithinolytica* is an emerging pathogen able to cause poisoning by the production of histamine from histidine [68] [69] [70] and several hospital infections [51] [71] [72]. *Serratia odorifera* is rarely implicated in clinical infections but is often associated with invasive human infections [73]. Some strains that have been isolated in hospitals have shown multidrug resistance to antibiotics [74]. Their presence in fish thus constitutes a significant health risk because they can be transmitted to the consumer through contaminated food [75].

In this study, *Proteus vulgaris*, *P. mirabilis*, *Enterobacter aerogenes*, *E. cloacae*, *S. liquefaciens*, and *C. braakii* were also isolated in this study. Contamination of fish by these germs has been reported in several studies [76] and also has the ability to produce histamine [77] [78] [79]. *Cronobacter sakazakii* is an opportunistic pathogen involved in food poisoning [80] often isolated from fish [81] [82].

Cedecea davisae was detected in this study. To our knowledge, *Cedecea davisae* has not yet been detected in fish. The presence of this germ in the samples is particularly unusual. Some studies report that this bacterium has been isolated from patient blood and is involved in human bacteremia [83]. Thus, the presence of *Cedecea davisae* in fish could be due to human contamination (handlers).

The results of gallery identification revealed the presence of *Salmonella enterica ssp arizonae* as well as Salmonella spp. However, confirmation of these strains still requires additional serological tests. Some presumptive *Salmonella* strains obtained on the XLD culture medium were identified as *Proteus vulgaris* or *P. mirabilis*. The presumption of Salmonella in the samples requires special attention. Ava *et al.* [29] found high contamination of fish by *Salmonella* (57.8%) in their study.

Several species of *Staphylococcus* were identified in the analyzed fish samples. Bacteria of the genus *Staphylococcus* are ubiquitous [84]. Their presence in fish may be due to contamination related to handling with the hands of sellers who carry these germs during the sale [85]. It is one of the most dangerous pathogens responsible for foodborne diseases around the world [86]. The bacteria can be destroyed by cooking processes; however, it can produce various heat-stable enterotoxins in food that cause vomiting and diarrhea [87] [88] [89] [90] [91]. In

addition to food poisoning related to its enterotoxins, *Staphylococcus aureus* also causes toxic shock syndrome, and pneumonia [92] [93].

Staphylococcus xylosus and *Staphylococcus lugdunensis* are also predominant species identified in this study. They are commensal bacteria of human skin [94] [95] and are also found in the environment [96] [97] [98]. *Staphylococcus xylosus* occurs naturally in foods and is used as a starter to ferment certain foods such as fermented sausages, and fish [99] [100] [101] [102]. Furthermore, *Staphylococcus xylosus* has been reported to inhibit the growth of spoilage microorganisms [103] [104] and degrade biogenic nitrites and amines in fermented sausages [105]. However, studies have reported that *Staphylococcus xylosus* can cause animal and human infections [106] [107] [108]. *Staphylococcus lugdunensis* is also known to be a potential pathogen for humans [75] [109] [110]. The presence of these germs in fish could be due to hand contamination during sales [36].

The suspected *Escherichia coli* isolates were confirmed by checking for the *uidA* gene. The results of this study indicate that 82.85% of the isolates contain the *uidA* gene. Sina *et al.* [67] detected the *uidA* gene in 88.89% of *Escherichia coli* strains tested. This variation in values could be due to mutations in the structure of the *uidA* gene [111]. Taha & Yassin [27] also detected the *uidA* gene in all strains isolated from food in Iraq. The *uidA* gene is generally used to specifically identify *E. coli* [112] [113] [114].

The use of *staur* primers allowed the amplification of 23S rDNA specific regions in most tested strains, thus confirming that they are *Staphylococcus aureus* species. This primer has been used in several studies for the identification of *Staphylococcus aureus* isolates [115] [116]. However, the *nuc* gene was detected in 86% of the *Staphylococcus aureus* strains tested. The *nuc* gene is used to identify specific strains of *Staphylococcus aureus* [117] [118]. Pinto *et al.* [119] confirmed 99% of *Staphylococcus aureus* strains are involved in food poisoning cases by detecting the *nuc* gene. These values are higher than those obtained in this study. This can be attributed to PCR inhibitors during amplification [120] [121] [122].

5. Conclusions

The isolation and identification of microorganisms that contaminate fish carried out in this study revealed a diversity of *Enterobacteriaceae* and *Staphylococcus* species. The most important species isolated were the following: *Raoultella ornithinolytica*, *Escherichia coli*, *Serratia odorifera*, *Staphylococcus aureus*, *Staphylococcus xylosus*, and *Staphylococcus lugdunensis*. The isolated *Enterobacteriaceae* and *Staphylococcus* species are not specific to one type of processed fish, but their quantity varies from one species to another. The presence of these bacteria in the fish indicates poor handling conditions of the fish at the time of sale. These isolated bacteria are potential pathogens involved in foodborne diseases and intoxications. Therefore, it would be necessary for the population to be

careful in the consumption of these products. Furthermore, traders must improve the hygienic conditions of their sales and storage environments. The government services of Burkina Faso responsible for public health must pay particular attention to the monitoring of fish throughout the production and supply chain by establishing effective sanitary safety systems. This will ensure the sanitary quality of the fish supplied to consumers.

The detection of these bacteria, potentially dangerous to the health of the consumer, reveals the need to confirm the pathogenicity of these by molecular analysis by investigating the pathogenicity genes of the isolated strains with PCR.

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Authors' Contributions

OA performed the collection of the fish samples, and carried out the isolation and identification of the strains as well as writing the manuscript. SMG and TF supervised the work in the laboratory and the writing of the manuscript. TR, OGA and SNS and CH read and corrected the manuscript. ZC and SA read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

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