**UNIVERSITÀ DEGLI STUDI DI MILANO**

## ORIGINAL ARTICLE

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# Genome analysis of *Salmonella* strains isolated from imported frozen fish in Burkina Faso

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## Abstract

**Purpose:** Fish is an excellent source of protein and vitamins for humans, but improperly handled, fish can expose consumers to pathogenic bacteria. This study was aimed to isolate and characterize the genomes of *Salmonella* strains isolated from imported fish sold in the open market in Ouagadougou.

**Methods:** One hundred and fifty-nine fish were collected from open markets and were cultured for *Salmonella*. Antimicrobial susceptibility was determined by broth microdilution. Whole-genome sequencing was done to further study antibiotic resistance genes, plasmid replicons, and MSLT types. Serotyping was done using SeqSero 2.

**Result:** Out of the 159 fish samples analyzed, 30 (18.9%) were found to be contaminated with *Salmonella*. Among the isolated *Salmonella* strains, six different serotypes, Nima, Liverpool, Kokomlemle, Teshie, Derby, and Tennessee, were found using SeqSero2. *Salmonella* Tennessee was the predominant serotype. All the isolates possessed at least one resistance gene. The aac6-Iaa aminoglycoside resistance gene was the most prevalent gene found in the strains. The gene fosA7 was detected in three strains. All the *S.* Nima isolates were of Multilocus Sequence Type (MLST) 8086, *S.* Teshie isolate was ST 530; Liverpool was ST 1959; Derby was ST 7880; Kokomlemle was ST 2696. The Tennessee isolates gave two different STs including ST 8395 and 8398.

**Conclusion:** The presented results highlight the prevalence of *Salmonella* on imported fish purchased from the open markets. More attention should be paid regarding fish selling conditions in the country to prevent the potential health risk for consumers.

**Keywords:** *Salmonella*, Antimicrobial resistance, Serotype, Fish

## Introduction

Burkina Faso is a landlocked tropical country located in sub-Saharan Africa. This country is characterized by a dry season from October-May with hot temperature (35-45 °C) and a short rainy season (June-September). In recent years, fish consumption has increased exponentially in this country with more than 96% of commercially sold fish imported from another country (Ministry of economic and sustainable development, Burkina Faso. Statistique douanieres 2015). Fish is an important source of essential amino acids and good fatty acids for humans, but fish can be contaminated by pathogenic bacteria that pose a high risk for consumer's health (Yan et al. 2010; Nwiyi and Onyeabor 2012). These pathogenic bacteria can contaminate ready to eat fish product through cross-contamination during fish processing (Kris-Etherton et al. 2002).

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*Salmonella* is a gram-negative foodborne pathogen and has been implicated in fish outbreaks worldwide (Barrett et al. 2017; Amagliani et al. 2012). More than 2500 serotypes of *Salmonella* have been designated based on the O and H surface antigens (Issenhuth-Jeanjean et al. 2014). Several serotypes, including Enteritidis and Typhimurium, have been implicated in outbreaks associated with seafood sources, though data from Africa is limited (Amagliani et al. 2012).

Currently, the use of antibiotics in aquaculture practices as growth promoters or for treatment and prevention of fish diseases is increasing the risk of development of antibiotic resistant bacteria among the microbiome of fish gut and/or fishing water (Miranda et al. 2018). Many studies have shown widespread transmission of antibiotic-resistant bacteria of the fish or aquatic environment to humans (Gonzalez-Escalona et al. 2005; Sørum 2006).

According to the Centers for Disease Control and Prevention (CDC), antibiotic-resistant infection is responsible for 25,000 annual deaths in the European Union and 23,000 annual deaths in the USA (Center for Disease Control and Prevention (CDC) 2019). The World Health Organization (WHO) reported that the burden of food-borne disease is similar to the burden of malaria, tuberculosis, and even HIV/AIDS and is disproportionately borne by the least developed countries and by children (World Health Organization (WHO) 2015). Since imported fish is widely consumed in Burkina Faso, it is important to know the microbiological quality of these fish. Therefore, the present study aims to understand the epidemiology and antibiotic resistance of *Salmonella* strains isolated from fish using whole-genome sequencing and phenotypic methods.

## Materials and methods
### Sampling
Imported fish samples were purchased from different open markets. All fish samples during collection were first placed in sterile polypropylene bags, then placed in polystyrene boxes containing crushed ice and stored at 4 °C during transportation. The samples were transported to the laboratory and processed on the same day for the presence of *Salmonella* spp.

### Bacteriological analysis
*Salmonella* strains were isolated from fish samples following the methodologies described in the International Organization for Standardization 6579-2017 (International Organization for Standardization (ISO) 6579-1 2017). The fish samples were gently removed from coolers and processed using aseptic conditions. The gills, intestines, and skin were removed using sterile knives. About 10 g of samples (fish gills, intestines, and skin) were placed into a stomacher bag containing 90 mL of buffered peptone water (Liofilchem, Teramo, Italy) and homogenized (400 Circulator, Seward, London, UK) for 1 min, then incubated for 24 h at 37 °C. From this non-selective pre-enrichment, 0.1 mL were transferred into 10 mL of Rappaport-Vassiliadis broth (Oxoid, Basingstoke, England) and incubated for 24 h at 42 °C. A loopful from the selective enrichment broth was streaked onto XLD (Oxoid, Basingstoke, England) agar and incubated for 24 h at 37 °C. Suspected colonies on selective agar plates were purified and bio-typed by using biochemical tests and API 20E strips (BioMerieux, Marcy l'Etoile, France).

Confirmed colonies were sent to the United States Department of Agriculture, Agricultural Research Service, Bacterial Epidemiology and Antimicrobial Resistance Research Unit for future analysis.

### Antimicrobial susceptibility testing
The isolates were streaked onto Tryptic Soy Agar (TSA) with 5% Sheep blood (BA), (Fisher Scientific, USA) plates and incubated for 24 h at 37 °C, and one colony from each plate was streaked onto a second BA2 plate for another 24 h at 37 °C. Minimum inhibitory concentrations (MIC, μg/mL) of all *Salmonella* isolates were determined by broth-microdilution using the Sensititre™ semi-automated antimicrobial susceptibility system (TREK Diagnostic Systems Inc., Cleveland, OH, USA) and the Sensititre™ Gram-Negative plate format, GN4F, (ThermoFisher Scientific, USA), according to manufacturer's directions. MICs of the isolates for 24 antimicrobials were determined using colonies from the BA2 plates, and each isolate was classified as resistant, intermediate, or susceptible to the antimicrobials tested using the breakpoints set by Clinical and Laboratory Standards Institute (CLSI) (2016). Antimicrobials used breakpoints were as follows: Amikacin ($\geq$ 64 μg mL$^{-1}$); Piperacillin/tazobactam ($\geq$ 128/4 μg mL$^{-1}$); Tigecycline ($\geq$ 1 μg mL$^{-1}$); Ticarcillin/clavulanic acid ($\geq$ 128/2 μg mL$^{-1}$); Levofloxacin ($\geq$ 2 μg mL$^{-1}$); Nitrofurantoin ($\geq$ 128 μg mL$^{-1}$); Tetracycline ($\geq$ 16 μg mL$^{-1}$); Doripenem ($\geq$ 4 μg mL$^{-1}$); Minocycline ($\geq$ 16 μg mL$^{-1}$); Ertapenem ($\geq$ 2 μg mL$^{-1}$); Trimethoprim/sulfamethoxazole ($\geq$ 4/76 μg mL$^{-1}$); Imipenem ($\geq$ 4 μg mL$^{-1}$); Piperacillin ($\geq$ 128 μg mL$^{-1}$); Meropenem ($\geq$ 4 μg mL$^{-1}$); Gentamicin ($\geq$ 16 μg mL$^{-1}$); Cefazolin ($\geq$ 8 μg mL$^{-1}$); Tobramycin ($\geq$ 16 μg mL$^{-1}$); Ceftazidime ($\geq$ 16 μg mL$^{-1}$); Ampicillin/sulbactam ($\geq$ 32/16 μg mL$^{-1}$); Aztreonam ($\geq$ 16 μg mL$^{-1}$); Ampicillin ($\geq$ 32 μg mL$^{-1}$); Cefepime ($\geq$ 32 μg mL$^{-1}$); Ciprofloxacin ($\geq$ 4 μg mL$^{-1}$); Ceftriaxone ($\geq$ 4 μg mL$^{-1}$). For the analysis, isolates identified as intermediate were considered susceptible to the drug. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus*

aureus ATCC 29213 were controls for determination of MIC.

For each isolate, a final inoculum of $5 \times 10^5$ CFU/ml was targeted. The panels were read after 18 h of incubation at 35 °C.

### Whole-genome sequencing

Genomic DNA was isolated using the GenElute bacterial genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA) following instructions for Gram-negative bacteria. Cultures were grown in 5 mL of Luria-Bertani Broth, Miller (Difco™, Becton Dickinson and Company, Sparks, MD) overnight at 37 °C with shaking. The extracted DNA quality was read using NanoDrop 2000c spectrophotometer (Thermo, Fisher Scientific, USA). DNA was stored at −20 °C prior to library preparation.

Extracted DNA was quantified using the Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit according to the manufacturer's instructions (Life Technologies, Inc., USA). The Illumina libraries were prepared using the Nextera XT DNA library preparation kit and Nextera XT index primers (Illumina, USA). The library fragment size distribution was checked using the Bioanalyzer 2100 with an Agilent HS DNA kit (Agilent Technologies, USA) and quantified using a Qubit DNA HS assay kit in a Qubit fluorometer (Thermo, Fisher Scientific, USA). The generated libraries were then sequenced using a MiSeq version 2 reagent kit with 500 or 300 cycles, depending on fragment size. The paired-end read length of $2 \times 250$ bp was used for 500 cycles and $2 \times 150$ bp for 300 cycles on the Illumina MiSeq platform. The quality metrics of the reads were performed by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The sequence data were assembled using the A5-miseq assembler (Coil et al. 2015), and the genome sequence was annotated via the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016).

### Identification of antibiotic resistance genes, chromosomal mutations, serotypes, MLST, and plasmid from total genome sequence

SeqSero 2 was used to determine the serotypes of *Salmonella* strains from genome assembly data. Using the Center for Genomic Epidemiology web tools, antibiotic resistance genes were identified using ResFinder 4.1 (Zankari et al. 2012). MLST sequence type was identified using MLST 2.0 (Larsen et al. 2012), and plasmids were detected using PlasmidFinder 2.0 (Carattoli et al. 2014).

### Results

Out of the 159 fish samples analyzed, 30 (18.9%) were found to be contaminated with *Salmonella*. From these 30 isolates, whole-genome sequences were generated and the assembly statistics are shown in Table 1. Among the isolated *Salmonella* strains, six different named serotypes, including Nima, Liverpool, Kokomlemle, Teshie, Derby, and Tennessee were found, with five having no name and only antigenic formulas. *Salmonella* Tennessee was the predominant serotype. All the isolates possessed at least one resistance gene. The non-functional *aac*6-Iaa conferring resistance to aminoglycosides was the most prevalent gene found in the strains. The gene fosA7 conferring resistance to Fosfomycin was detected in three strains. The isolates were susceptible to all drugs tested using phenotypic method. Four *S.* Nima isolates were identified from the 30 isolates and were all MLST Sequence Type (ST) 8086. One *S.* Teshie isolate was identified and was ST 530; three *S.* Liverpool isolates were detected and were identified as ST 1959; one *S.* Derby isolate was found and was ST 7880; one *S.* Kokomlemle was detected and was ST 2696, 15 *S.* Tennessee isolates were found, 14 have one of two different sequence types, ST 8395 and 8398, and one was unknown. Plasmid replicon typing results showed six isolates possessing plasmids: IncFIB and IncFII in the Teshie isolate, Col(pHAD28) and Col4401 in three Liverpool isolates, Col (MG828) in one Tennessee isolate, and IncFII(S) in one Kokomlemle (Table 2).

The whole-genome shotgun project has been deposited at GenBank under BioProject number PRJNA713376 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA713376), and the accession numbers are listed in Table 2. The versions described in this paper are the first versions.

### Discussion

The present study was initiated to determine the microbiological quality of imported and local fish consumed in the city of Ouagadougou, Burkina Faso. The prevalence of *Salmonella* strains was 18.9% from imported fish. This result could be explained by the fact that imported fish are exposed to several stages of handling and packaging at the farm in the country of origin, transport to Burkina Faso, reception at wholesalers, delivery to semi-wholesalers, and delivery to different retailers. All these steps undoubtedly favor the contamination by bacteria like *Salmonella*. However, the consumption of imported fish is very high in Burkina Faso because it is very accessible and inexpensive in all the localities of the country. In these localities, the imported fish is cut into small pieces by small traders and sold at a minimum price of 50 FCFA (about one cent of dollar). This necessitates permanent monitoring of the prevalence of germs that can affect the health of consumers as well as chemicals. The population of Burkina Faso is over 80% illiterate, which will undoubtedly lead to an increase in contamination of raw fish and the

**Table 1** Assembly statistics of Salmonella strains

| Isolate ID | Contigs | Scaffolds | Genome size | Longest scaffold | N50 | Raw reads | EC reads | % reads passing EC | Raw nt | EC nt | % nt passing EC | Raw cov | EC cov | Median cov | 10th percentile cov | Bases ≥ Q40 | % GC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 87 | 61 | 61 | 4621725 | 703602 | 292745 | 2075760 | 2055651 | 99.03 | $3.9 \times 10^8$ | $3.6 \times 10^8$ | 93.77 | 83.55 | 78.35 | 82 | 59 | 4617943 | 52.2 |
| 88 | 61 | 61 | 4654102 | 433362 | 231906 | 2480308 | 2461694 | 99.25 | $3.5 \times 10^8$ | $3.4 \times 10^8$ | 97.2 | 74.39 | 72.31 | 73 | 54 | 4651947 | 52.2 |
| 89 | 180 | 180 | 5345366 | 265739 | 89976 | 1585778 | 1571781 | 99.12 | $2.1 \times 10^8$ | $2 \times 10^8$ | 96.96 | 39.18 | 37.99 | 37 | 24 | 5332372 | 51.8 |
| 95 | 40 | 40 | 4680005 | 668083 | 504361 | 2410566 | 2379641 | 98.72 | $4.7 \times 10^8$ | $4.4 \times 10^8$ | 92.32 | 100.7 | 93.01 | 97 | 72 | 4679387 | 52.2 |
| 98 | 63 | 63 | 4918496 | 775042 | 398010 | 1718264 | 1687773 | 98.23 | $3.3 \times 10^8$ | $3.1 \times 10^8$ | 92.42 | 67.53 | 62.41 | 65 | 46 | 4916559 | 51.9 |
| 100 | 80 | 80 | 4963160 | 748091 | 287769 | 2265232 | 2202986 | 97.25 | $4.4 \times 10^8$ | $3.9 \times 10^8$ | 88.29 | 89.07 | 78.65 | 85 | 62 | 4961107 | 52 |
| 101 | 52 | 52 | 4902134 | 944301 | 501799 | 1868462 | 1821376 | 97.48 | $3.8 \times 10^8$ | $3.4 \times 10^8$ | 90.4 | 77.46 | 70.02 | 74 | 54 | 4900597 | 51.8 |
| 103 | 49 | 49 | 4749239 | 1115741 | 340547 | 2323594 | 2296420 | 98.83 | $4.5 \times 10^8$ | $4.2 \times 10^8$ | 93.23 | 93.97 | 87.61 | 87 | 63 | 4748210 | 52.2 |
| 108 | 44 | 44 | 4738594 | 568197 | 405801 | 2091224 | 2069633 | 98.97 | $3.6 \times 10^8$ | $3.5 \times 10^8$ | 95.15 | 76.54 | 72.83 | 74 | 55 | 4737525 | 52.2 |
| 111 | 47 | 47 | 4741632 | 568232 | 454680 | 2514870 | 2487391 | 98.91 | $4.9 \times 10^8$ | $4.5 \times 10^8$ | 92.81 | 102.4 | 95.07 | 99 | 76 | 4740854 | 52.2 |
| 113 | 49 | 49 | 4749008 | 1444467 | 340548 | 2281594 | 2260595 | 99.08 | $4.6 \times 10^8$ | $4.3 \times 10^8$ | 93.21 | 97.31 | 90.7 | 92 | 69 | 4748045 | 52.2 |
| 116 | 38 | 38 | 4661074 | 871623 | 424548 | 1877226 | 1855294 | 98.83 | $3.6 \times 10^8$ | $3.3 \times 10^8$ | 92.87 | 76.86 | 71.37 | 74 | 54 | 4659622 | 52.2 |
| 117 | 44 | 44 | 4739093 | 568232 | 427385 | 2101626 | 2085634 | 99.24 | $3.6 \times 10^8$ | $3.5 \times 10^8$ | 95.73 | 76.7 | 73.42 | 74 | 55 | 4738049 | 52.2 |
| 119 | 51 | 51 | 4730026 | 508935 | 323835 | 1916062 | 1891245 | 98.7 | $2.5 \times 10^8$ | $2.4 \times 10^8$ | 97.36 | 52 | 50.62 | 50 | 35 | 4728141 | 52.2 |
| 122 | 64 | 64 | 4732251 | 461578 | 226770 | 2578950 | 2550077 | 98.88 | $3.4 \times 10^8$ | $3.3 \times 10^8$ | 96.81 | 72.58 | 70.26 | 71 | 49 | 4730117 | 52.2 |
| 127 | 46 | 46 | 4740433 | 568232 | 394001 | 1464044 | 1447638 | 98.88 | $2.6 \times 10^8$ | $2.4 \times 10^8$ | 95.1 | 53.95 | 51.31 | 52 | 36 | 4738090 | 52.2 |
| 128 | 52 | 52 | 4729542 | 454349 | 323835 | 1476660 | 1454883 | 98.53 | $1.9 \times 10^8$ | $1.8 \times 10^8$ | 97.27 | 39.56 | 38.48 | 38 | 26 | 4722471 | 52.2 |
| 129 | 43 | 43 | 4617569 | 720868 | 300407 | 1313142 | 1295563 | 98.66 | $2.4 \times 10^8$ | $2.2 \times 10^8$ | 93.89 | 51.58 | 48.43 | 50 | 34 | 4614768 | 52.2 |
| 130 | 43 | 43 | 4740056 | 568232 | 400228 | 3575342 | 3520930 | 98.48 | $6.6 \times 10^8$ | $6 \times 10^8$ | 91.2 | 138.7 | 126.5 | 133 | 101 | 4739679 | 52.2 |
| 131 | 53 | 53 | 4741832 | 568232 | 394156 | 1566306 | 1545820 | 98.69 | $2.9 \times 10^8$ | $2.7 \times 10^8$ | 91.49 | 62.2 | 56.91 | 60 | 42 | 4739118 | 52.2 |
| 134 | 34 | 34 | 4797111 | 1850190 | 750647 | 2710124 | 2671347 | 98.57 | $5.3 \times 10^8$ | $4.8 \times 10^8$ | 91.32 | 110.4 | 100.8 | 106 | 78 | 4796675 | 52.2 |
| 135 | 54 | 54 | 4903282 | 944125 | 424401 | 2061468 | 1996430 | 96.85 | $3.7 \times 10^8$ | $3.3 \times 10^8$ | 87.78 | 75.59 | 66.35 | 71 | 50 | 4901400 | 51.8 |
| 136 | 46 | 46 | 4747148 | 857353 | 340487 | 1895802 | 1849828 | 97.57 | $3.3 \times 10^8$ | $3 \times 10^8$ | 90 | 69.25 | 62.33 | 63 | 43 | 4745627 | 52.2 |
| 137 | 44 | 44 | 4739464 | 514012 | 394016 | 2219520 | 2160947 | 97.36 | $4.1 \times 10^8$ | $3.6 \times 10^8$ | 88.01 | 87.08 | 76.64 | 83 | 60 | 4738438 | 52.2 |
| 138 | 42 | 42 | 4617148 | 720868 | 331382 | 1581084 | 1563497 | 98.89 | $3.1 \times 10^8$ | $2.8 \times 10^8$ | 90.95 | 67.5 | 61.39 | 65 | 46 | 4615739 | 52.2 |
| 139 | 45 | 45 | 4740410 | 514012 | 394637 | 2120290 | 2100494 | 99.07 | $3.8 \times 10^8$ | $3.6 \times 10^8$ | 94.39 | 80.57 | 76.06 | 78 | 56 | 4739354 | 52.2 |
| 141 | 71 | 71 | 4730418 | 434837 | 164778 | 1976130 | 1952830 | 98.82 | $2.7 \times 10^8$ | $2.6 \times 10^8$ | 96.44 | 56.5 | 54.49 | 55 | 39 | 4726407 | 52.2 |
| 142 | 45 | 45 | 4620013 | 720868 | 387756 | 2282854 | 2264502 | 99.2 | $4.4 \times 10^8$ | $4.1 \times 10^8$ | 93.92 | 95.63 | 89.81 | 93 | 68 | 4619149 | 52.2 |
| 144 | 688 | 688 | 5699254 | 126378 | 26527 | 1655604 | 1640692 | 99.1 | $3.3 \times 10^8$ | $3.1 \times 10^8$ | 93.14 | 58.29 | 54.29 | 58 | 27 | 5652021 | 51.6 |
| 146 | 385 | 385 | 5031347 | 222688 | 74906 | 2781576 | 2757394 | 99.13 | $3.7 \times 10^8$ | $3.6 \times 10^8$ | 97.48 | 72.79 | 70.96 | 73 | 46 | 4998376 | 52.1 |

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**Table 2** Distribution of plasmid replicons and MLSTs of Salmonella from fish

| Sample ID | Serotype | Antimicrobial resistances genes | Plasmid replicon | MLST (ST) | ParC mutation | Accession numbers |
|---|---|---|---|---|---|---|
| 87 | Nima | aac(6')-Iaa | 0 | 8086 | Thr57Ser | SAMN18249072 |
| 88 | Tennessee | aac(6')-Iaa; fosA7 | 0 | 8395 | Thr57Ser | SAMN18249073 |
| 89 | Teshie | aac(6')-Iaa | IncFIB, IncFII | 530 | - | SAMN18249074 |
| 95 | Derby | aac(6')-Iaa | 0 | 7880 | Thr57Ser | SAMN18249075 |
| 98 | I 39:z10:e,n,z15 | aac(6')-Iaa | 0 | 8401 | Thr57Ser | SAMN18249076 |
| 100 | I 30:y:- | aac(6')-Iaa | 0 | 8397 | Thr57Ser | SAMN18249077 |
| 101 | I 13:i:z6 | aac(6')-Iaa | 0 | 8396 | - | SAMN18249078 |
| 103 | Liverpool | aac(6')-Iaa | Col(pHAD28), Col4401 | 1959[a] | Thr57Ser | SAMN18249079 |
| 108 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249080 |
| 111 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN16842897 |
| 113 | Liverpool | aac(6')-Iaa | Col(pHAD28), Col4401 | 1959 | Thr57Ser | SAMN18249081 |
| 116 | Tennessee | aac(6')-Iaa; fosA7 | 0 | 8395 | Thr57Ser | SAMN18249082 |
| 117 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249083 |
| 119 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249084 |
| 122 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249085 |
| 127 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249086 |
| 128 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249087 |
| 129 | Nima | aac(6')-Iaa | 0 | 8086 | Thr57Ser | SAMN18249088 |
| 130 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249089 |
| 131 | Tennessee | aac(6')-Iaa | Col(MG828) | 8398 | Thr57Ser | SAMN18249090 |
| 134 | Kokomlemle | aac(6')-Iaa | IncFII(S) | 2696 | Thr57Ser | SAMN18249091 |
| 135 | I 13:i:z6 | aac(6')-Iaa | 0 | 8396 | - | SAMN18249092 |
| 136 | Liverpool | aac(6')-Iaa | Col(pHAD28), Col4401 | 1959 | Thr57Ser | SAMN18249093 |
| 137 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249094 |
| 138 | Nima | aac(6')-Iaa | 0 | 8086 | Thr57Ser | SAMN18249095 |
| 139 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249096 |
| 141 | Tennessee | aac(6')-Iaa | 0 | 8398 | Thr57Ser | SAMN18249097 |
| 142 | Nima | aac(6')-Iaa | 0 | 8086 | Thr57Ser | SAMN18249098 |
| 144 | I 39:f,g:e,n,z15 | aac(6')-Iaa | 0 | [b] Unknown | Thr57Ser | SAMN18249099 |
| 146 | Tennessee | aac(6')-Iaa, fosA7 | 0 | [c] Unknown | Thr57Ser | SAMN16842913 |

[a]Less than 100% identity
[b]Nearest STs: 384, 696, 1859, 2320, 4924, 5805, 6540, 6730, 6732, 6734
[c]Nearest ST: 8398

possibility of cross-contamination due to a lack of training and information on the causes and consequences of foodborne diseases (Barro et al. 2007). The prevalence of *Salmonella* in fish in this study is higher than those reported by Broughton and Walker (2009), from fish in China (5%) and by Heinitz et al. (2000) in U.S.-imported raw seafood from several Asian countries (10%). These variations in prevalence can be explained by differences in farming methods, and in the food safety regulations of each country. For example, in Burkina Faso, many researchers demonstrated that good hygienic practices are not respected yet by food sellers and domestic food safety regulation and/or training programs are still missing (Barro et al. 2008; Kagambèga et al. 2011; Kagambèga et al. 2012).

*Salmonella* Tennessee was the most prevalent serotype among fish samples. This serotype of *Salmonella* was detected in different types of samples and in the stools of patients with diarrhea in other studies from Burkina Faso (Kagambèga et al. 2017). *Salmonella* Tennessee has also been implicated in outbreaks in the USA due to contaminated peanut butter, powdered milk products, and infant formula (Center for Disease Control and Prevention (CDC) 2007; Center for Disease Control and Prevention (CDC) 1993). These facts show us that the Tennessee serotype is not necessarily linked to a specific

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food or environment but can infect humans through many contaminated foods.

We also have the presence of S. Liverpool and S. Teshie, which are pathogenic serotype and have not been identified in our previous studies carried out in Burkina Faso in diarrheal patients, chickens, the environment, or animals (Kagambèga et al. 2017; Bonkoungou et al. 2013; Traoré et al. 2015). Salmonella Derby was identified as the most dominant in our previous studies in chickens and slaughter animals (Kagambèga et al. 2013). Salmonella Nima and S. Kokomlemle also have been isolated in chicken and beef previously in Burkina Faso (Kagambèga et al. 2013).

The presence of these serotypes in fish shows that chicken, slaughter animals, the environment, and humans share the same pathogens that circulate in our country.

Ten different MLST sequence types were found in this study, and two strains possessed Unknown ST. Two MSLT types were detected in our study with S. Tennessee. This may show that S. Tennessee has genetic diversity within its population. We can say that the other serotype with a unique MSLT type retained their genetic characteristic during their evolution while keeping the same type of MLST. On the other hand, S. Tennessee population structure has changed during evolution.

All the Salmonella strains found in this study possessed the aminoglycoside resistance gene encoding acetyltransferases, aac(6′)-Iaa. While this gene was not functional in the present study and are commonly non-functional in Salmonella, mutations in the promoter of the gene can lead to expression and phenotypic resistance (Magnet et al. 1999). Rather et al. (1993) demonstrated that aminoglycoside resistance in Salmonella strains is usually secondary to increased gene expression following regulatory mutations.

The fosA7 gene conferring resistance to fosfomycin was detected in three antibiotic susceptible strains. Since this antibiotic was not tested in this study, we cannot conclude that this gene is functional or not. Rehman et al. (2017) demonstrated that the gene fosA7 is responsible for fosfomycin resistance.

Point mutations in the quinolone resistance-determining regions (QRDRs) were detected in 27 (90%) isolates at positions 57 (Thr57Ser), which can confer resistance to quinolones. However, all isolates were susceptible to both nalidixic acid and ciprofloxacin. Consistent with the results presented here, other Salmonella isolates containing this mutation also exhibited susceptibility to nalidixic acid or ciprofloxacin; thus, this mutation is thought to not confer resistance in all serotypes of Salmonella (Baucheron et al. 2005).

In this study, two Salmonella Kokomlemle and one S. Teshie possessed IncFII-type plasmids, which have been important in spreading resistance genes such as bla $_{NDM-1}$ and bla $_{CTX-M-15}$ (Xavier et al. 2016). Both strains did not harbor any beta-lactamase resistance genes. All the Salmonella Liverpool contained Col(pHAD28), Col4401, and one S. Tennessee possessed Col(MG828) plasmid types. These plasmid types have been associated with quinolone resistances in Salmonella strains (Fiegen et al. 2017). More investigation into these plasmid sequences are needed to determine any benefit they provide the strains.

## Conclusion
This study has shown that widely consumed fish in Burkina Faso are contaminated with pathogenic bacteria of the genus Salmonella. The microbiological quality of fish sold in Burkina Faso must be improved to reduce the risks of contamination to consumers. Improved food safety will lead to reduced losses, better access to markets, and hence better incomes. The modern molecular biology technique used in this study, whole-genome sequencing, is a technique that is not yet available in the developing countries. An urgent action is needed by decision-makers in Burkina Faso, other developing countries, and countries around the world to collaborate in the regulation and monitoring of foodborne pathogens.

**Availability of data and materials**
The whole-genome shotgun project has been deposited at GenBank under BioProject number PRJNA713376 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA713376).

## Declarations

**Ethics approval and consent to participate**
Not applicable.

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