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 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. 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, 28 (17.61%) were found to be contaminated with Salmonella. Among the isolated Salmonella strains, 6 different serotypes, Nima, Liverpool, Kokomlemle, Gaminara, Derby, and Tennessee, were found using SeqSero2. S. Tennessee was the predominant serotype. All the isolates possessed at least one resistance gene. The aac6-laa and aac6-ly conferring resistance to aminoglycosides was the most prevalent gene found in the strains. The gene fosA7 was detected in two. All the S. Nima isolates were of Multilocus Sequence Type (MLST) 2258, Gaminara was ST 5197; Liverpool was ST 1959; Derby was ST 3997; Kokomlemle was ST 2696. The serotype Tennessee isolates gave many different STs such as ST 3763; 3997; 3135.

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.

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 Celsius) 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 (1). 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 (2; 3). These pathogenic bacteria can contaminate ready to eat fish product through cross-contamination during fish processing (4). Salmonella has been implicated in fish outbreaks worldwide (5; 6).
Nowadays, 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 (7). Many studies have shown widespread transmission of antibiotic resistant bacteria of the aquatic or fish to human trough environment and/or fish consumption (8, 9).

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 U.S (10). The World Health Organization (WHO) report on the burden of food-borne disease clearly shows that this burden is similar to the burden of malaria, tuberculosis and even HIV AIDS (11). The report also shows that the burden of food-borne disease is disproportionately borne by the least developed countries and by children. 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 placed in sterile polypropylene bag, placed in polystyrene box containing crushed ice and the temperatures was 4°C during transportation. The samples were transported to the laboratory and examined 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 (12). The fish samples were gently removed from coolers and processed in aseptic condition. The gills, intestines parts and skin parts were removed using sterile knifes. About 10 g of samples (fish gills, intestines parts and skin) were placed into a stomacher bag containing 90 mL of buffered peptone water (Liofilchem, Teramo, Italy) and homogenized using a stomacher (400 Circulator, Seward, London,UK) for 1 min and 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, Bacterial Epidemiology and Antimicrobial Resistance Research Unit for future analysis.
Antimicrobial susceptibility testing

The isolates were streaked onto Blood agar plates and incubated for 24 h at 37°C, and one colony from each plate was streaked onto new Blood agar plate for another 24h 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, with plate code GN4F (Thermo, Fisher Scientific, USA), according to manufacturer’s directions. MICs of the isolates for the 24 antimicrobials were determined using colonies from the last 24h Blood agar 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) [13]. Antimicrobials used breakpoints were as follows: Amikacin (≥ 64 µg mL-1); Piperacillin/tazobactam (≥ 128/4 µg mL-1); Tigecycline (≥ 1 µg mL-1); Ticarcillin/clavulanic acid (≥ 128/2 µg mL-1); Levofloxacin (≥ 2 µg mL-1); Nitrofurantoin (≥ 128 µg mL-1); Tetracycline (≥ 16 µg mL-1); Doripenem (≥ 4 µg mL-1); Minocycline (≥ 16 µg mL-1); Ertapenem (≥ 2 µg mL-1); Trimethoprim/sulfamethoxazole (≥ 4/76 µg mL-1); Imipenem (≥ 4 µg mL-1); Piperacillin (≥ 128 µg mL-1); Meropenem (≥ 4 µg mL-1); Gentamicin (≥ 16 µg mL-1); Cefazolin (≥ 8 µg mL-1); Tobramycin (≥ 16 µg mL-1); Ceftazidime (≥ 16 µg mL-1); Ampicillin/sulbactam (≥ 32/16 µg mL-1); Aztreonam (≥ 16 µg mL-1); Ampicillin (≥ 32 µg mL-1); Cefepime (≥ 32 µg mL-1); Ciprofloxacin (≥ 4 µg mL-1); Ceftriaxone (≥ 4 µg mL-1). For the analysis, isolates identified as intermediate were considered susceptible to the drug. *E. 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 x 10⁵ 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 DNAkit (Sigma-Aldrich, St. Louis, MO, USA) following instructions for Gram-negative bacteria, from 5 mL of overnight cultures grown in Luria-Bertani Broth, Miller (Difco™, Becton Dickinson and Company, Sparks, MD) 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 and 300 cycles. The paired-end read length of 2 X 250 bp was used for 500 cycles and 2 X 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 (14), and the genome sequence was annotated via the NCBI Prokaryotic Genome Annotation Pipeline (15).

The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession XXXXXX0000000000. The version described in this paper is version XXXXXX010000000.

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

Antibiotic resistance genes and chromosomal mutations were identified using ResFinder 3.2 (16). SeqSero 2 was used to determine the serotypes of salmonella strains from genome assembly data. MLST sequence type was identified using MLST database from the center of genomic epidemiology (17). The PlasmidFinder were used to detect plasmid from the strains (18).

Results

Out of the 159 fish samples analysed, 28 (17.61%) were found to be contaminated with Salmonella. Among the isolated Salmonella strains, 6 different serotypes such as Nima, Liverpool, Kokomlemle, Gaminara, Derby and Tennessee were found using SeqSero2 with some being N/A. S. Tennessee was the predominant serotype. All the isolates possessed at least one resistance gene. The non-functional aac6-Iaa and aac6-Iy conferring resistance to aminoglycosides was the most prevalent gene found in the strains. The gene fosA7 conferring resistance to Fosfomycin was detected in two strains. The isolates were susceptible to all drugs tested. Four S. Nima isolates were identified from the 28 isolates and were all MLST Sequence Type (ST) 2258. One S. Gaminara isolate was identified and was ST 5197; two S. Liverpool isolates were detected and were identified as MSLT ST 1959; one S. Derby isolate was found and was ST 3997; One S. Kokomlemle was detected and was MLST ST 2696. Thirteen S. Tennessee isolates were found with different sequence types including ST 3763, ST 3997, ST 3135, and ten were unknown. Two strains possessed plasmid replicons: one IncFII(S) and one IncFII(pCRY) (Table 1). The amino acid substitution Thr57Ser and Ser80Ile in ParC were detected in 25 isolates. Point mutations in the quinolone resistance-determining regions (QRDRs) were detected in 25 (89.28%) isolates at positions 57 (Thr57Ser) and 80 (Ser80Ile) for ParC (Table 1).
| Sample ID | Serotype   | Resistances genes | Plasmid replicon | QRDR point mutation | MLST sequence Type (ST) | Accession Numbers |
|-----------|------------|-------------------|-------------------|---------------------|------------------------|-------------------|
| 87        | Nima       | aac(6')-laa       | -                 | Thr57Ser            | 2258                   | SAMN18249072      |
| 88        | Tennessee  | aac(6')-laa; fosA7| -                 | Thr57Ser            | 3763                   | SAMN18249073      |
| 89        | N/A        | aac(6')-laa       | IncFII(pCRY)      | 0                   | 530                    | SAMN18249074      |
| 95        | Gaminara   | Aac6-ly           | -                 | Thr57Ser            | 5197                   | SAMN18249075      |
| 98        | N/A        | Aac6-ly           | -                 | Thr57Ser            | 565                    | SAMN18249076      |
| 100       | N/A        | aac(6')-laa       | -                 | Thr57Ser            | Unknown                | SAMN18249077      |
| 101       | N/A        | Aac6-ly           | -                 | 0                   | 3531                   | SAMN18249078      |
| 103       | Derby      | aac(6')-laa       | -                 | Thr57Ser            | 3997                   | SAMN18249079      |
| 108       | Tennessee  | Aac6-ly           | -                 | Thr57Ser            | 3997                   | SAMN18249080      |
| 113       | Liverpool  | aac(6')-laa       | -                 | Thr57Ser            | 1959                   | SAMN18249081      |
| 116       | Tennessee  | fosA7; aac(6')-laa| -                 | Thr57Ser            | Unknown                | SAMN18249082      |
| 117       | Tennessee  | Aac6-ly           | -                 | Thr57Ser            | 3135                   | SAMN18249083      |
| 119       | Tennessee  | aac(6')-laa       | -                 | Thr57Ser            | Unknown                | SAMN18249084      |
| 122       | Tennessee  | aac(6')-laa       | -                 | Thr57Ser            | Unknown                | SAMN18249085      |
| 127       | Tennessee  | aac(6')-laa       | -                 | Thr57Ser            | Unknown                | SAMN18249086      |
| 128       | Tennessee  | aac(6')-laa       | -                 | Thr57Ser            | Unknown                | SAMN18249087      |
| 129       | Nima       | aac(6')-laa       | -                 | Thr57Ser            | 2258                   | SAMN18249088      |
| 130       | Tennessee  | aac(6')-laa       | -                 | Thr57Ser            | Unknown                | SAMN18249089      |
| 131       | Tennessee  | aac(6')-laa       | -                 | Thr57Ser            | Unknown                | SAMN18249090      |
| 134       | Kokomlemle | aac(6')-laa       | IncFII(S)         | Thr57Ser            | 2696                   | SAMN18249091      |
| 135       | N/A        | aac(6')-laa       | -                 | 0                   | Unknown                | SAMN18249092      |
| 136       | Liverpool  | aac(6')-laa       | -                 | Thr57Ser            | 1959                   | SAMN18249093      |
| 137       | Tennessee  | aac(6')-laa       | -                 | Thr57Ser            | Unknown                | SAMN18249094      |

QRDR: quinolone resistance-determining region; MLST: Multilocus Sequence Typing
Table 1: Summary of selected resistance genes and plasmid replicons of Salmonella strains from imported and local fish consumed in Ouagadougou, Burkina Faso.

| Sample ID | Serotype | Resistances genes | Plasmid replicon | QRDR point mutation | MLST sequence Type (ST) | Accession Numbers |
|-----------|----------|-------------------|------------------|---------------------|-------------------------|-------------------|
| 138       | Nima     | aac(6')-Iaa       | -                | Thr57Ser            | 2258                    | SAMN18249095      |
| 139       | Tennessee | aac(6')-Iaa      | -                | Thr57Ser            | Unknown                 | SAMN18249096      |
| 141       | Tennessee | aac(6')-Iaa      | -                | Thr57Ser            | Unknown                 | SAMN18249097      |
| 142       | Nima     | aac(6')-Iaa       | -                | Thr57Ser            | 2258                    | SAMN18249098      |
| 144       | N/A      | aac(6')-Iaa; aac(6')-Iaa | - | Thr57Ser            | Unknown                 | SAMN18249099      |

QRDR: quinolone resistance-determining region; MLST: Multilocus Sequence Typing

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 17.41% from imported fish. This result could be explained by the fact that imported fish are exposed to several stages of handling including packaging at the farm in the origin country, 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 1 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 possibility of cross-contamination due to a lack of training and information on the causes and consequences of foodborne diseases (19). The prevalence of *Salmonella* in fish in this study is higher than those reported by Broughton and Walker, (20) from fish in China (5%) and by Heinitz et al. (21) 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 program still missing (22; 23; 24).

*Salmonella* Tennessee was the most prevalent serotypes 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 (25). *S. Tennessee* has also been implicated in outbreaks in the United States.
due to contaminated peanut butter; powdered milk products and infant formula (26; 27). These facts show us that the Tennessee serotype is not necessarily linked to a specific food or environment. Depending on the handling of food, this serotype can contaminate humans through any contaminated food.

We also have the presence of S. Liverpool, which is a pathogenic serotype and has not been identified in our previous studies carried out in Burkina Faso in diarrheal patients, chickens, the environment, or animals (25; 28; 29). S. Derby identified was the most dominant in our previous studies in chickens and slaughter animals (Kagambèga et al., 2013). S. Nima and S. Kokomlemle also have been isolated in chicken and beef previously in Burkina Faso (30). S. Gaminara has been identified in a patient suffering from diarrhoea in Burkina Faso (28).

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.

Twelve different MLST sequence type were find from the strains. A diversity of MSLT type was 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. Tenesese population structure has changed during evolution.

All the Salmonella strains found in this study possessed aminoglycoside resistance genes, encoding acetyltransferases (aac(6’)-Iaa; aac(6’)-Iy). While these genes were not functional in this study and are commonly non-functional in Salmonella, mutations in the promoter of the gene can lead to expression and phenotypic resistance (31). Rather et al. (32) demonstrated that aminoglycoside resistance in Salmonella strains is usually secondary to increased gene expression following regulatory mutations.

Point mutations in the quinolone resistance-determining regions (QRDRs) were detected in 25 (89.28%) isolates at positions 57 (Thr57Ser) and 80 (Ser80Ile) for ParC with known acquired antibiotic resistance as Nalidixic acid and Ciprofloxacin. However, all isolates were susceptible to both nalidixic acid and ciprofloxacin.

In this study, two Salmonella strains Kokomlemle and one unknown serotype possessed IncFII-type plasmids, which have been important in spreading resistance genes such as blaNDM−1 and blaCTX−M−15 (Xavier et al., 2016). Both strains did not harbour any beta-lactamase resistance genes. 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 as whole genome sequencing is a technique that is not yet available in the developing country. An urgent action is needed by decision-makers in Burkina Faso, other developing countries, and those around the world for collaboration in the regulation and monitoring of foodborne pathogens.

**Abbreviations**

WGS  
Whole Genome Sequencing

NTS  
Non-typhoidal *Salmonella*

CDC  
Centers for Disease Control and Prevention

MLST  
Multilocus sequence typing

MDR  
Multidrug Resistant

LaBESTA  
Laboratoire de Biologie Moléculaire, d’épidémiologie et de surveillance des bactéries et virus transmissible par les aliments

U.S  
United States

USA  
United States of America

**Declarations**

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**Authors’ contributions**
AK, SB, SKD carried out the strain’s isolation and characterization. LH, AK, SP, SKG, HR, EAM carried out
the WGS analysis, AK drafted the manuscript. EAM, LH participated in manuscript writing. NB, CRJ, and
JGF supervised the WGS and participated in writing the manuscript. All authors read, commented on, and
approved of the final manuscript.

Availability of data and materials

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession
XXXXXXXX000000000. The version described in this paper is version XXXXXX010000000.

Consent for publication

Not applicable.

Ethical considerations and consent to participate

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

1. Ministry of economic and sustainable development, Burkina Faso. Statistique douanieres. (2015).
2. Yan H, Li L, Alam MJ, Shinoda S, Miyoshi S, Shi L. Prevalence and antimicrobial resistance of
Salmonella in retail foods in northern China. International Journal of Food Microbiology. 2010.
143(3): 230-234.
3. Nwiyi P, Onyeabor A. Occurrence of Salmonella spp. From fresh fish (Tilapia nilotica Linn) using
improved isolation methods. Journal of Animal and Feed Research. 2012. 2(6):475-478.
4. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and
cardiovascular disease: AHA scientific statement. Circulation. 2002. 106(21):2747-57
5. Barrett KA, Nakao JH, Taylor E, Eggers C, Gould LH, (2017). Fish-Associated Foodborne Disease
Outbreaks: United States, 1998–2015. Foodborne Pathogens and Disease. 2017. 14(9). DOI:
10.1089/fpd.2017.2286
6. Amagliani G, Brandi G, Schiavano GF. Incidence and role of Salmonella in seafood safety. Food
Research International. 2012 45(2): 780-788.
7. Miranda CD, Godoy FA, Lee MR. Current Status of the Use of Antibiotics, and the Antimicrobial
Resistance in the Chilean Salmon Farms. Frontiers in microbiology. 2018. 9:1284. doi:
10.3389/fmicb.2018.01284
8. Gonzalez-Escalona N, Cachicas V, Acevedo C, Rioseco ML, Vergara JA, Cabello F, Romero J, Espejo
RT. Vibrio parahaemolyticus diarrhea, Chile, 1998 and 2004. Emerging Infectious Diseases. 2005.
9. Sørum H. Antimicrobial drug resistance in fish pathogens. In Antimicrobial Resistance in Bacteria of Animal Origin. Aarestrup, F.M., (ed.). Washington, DC, USA: American Society for Microbiology. 2006. Press pp. 213–238 (Chapter, 13).

10. Center for Disease Control and Prevention (CDC). Antibiotic Resistance: A Global Threat. 2019. https://www.cdc.gov/features/antibiotic-resistance-global/index.html

11. World Health Organization (WHO). Estimates of the global burden of foodborne diseases. 2015. http://www.who.int/foodsafety/areas_work/foodborne-diseases/ferg/en/

12. International Organization for Standardization (ISO) 6579-1. Microbiology of the food chain— Horizontal method for the detection, enumeration and serotyping of Salmonella. 2017. Part 1: Detection of Salmonella spp

13. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 2016. 26th ed. CLSI Supplement M100S. Wayne (PA): CLSI.

14. Coil D, Jospin G, Darling AE. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics. 2015. 31 (4):587–589. https://doi.org/10.1093/bioinformatics/btu661.

15. Tatusova T, DiCuccio M, Badretdin A, Chetverin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Research. 2016. 44(14):6614–6624. https://doi.org/10.1093/nar/gkw569.

16. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. Identification of acquired antimicrobial resistance genes. Journal of antimicrobial chemotherapy. 2012. 67(11):2640-4.

17. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. Multilocus Sequence Typing of Total Genome Sequenced Bacteria. Journal of Clinical Micobiology. 2012. 52(5): 1501–1510.

18. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Aarestrup FM, Hasman H. PlasmidFinder and pMLST: in silico detection and typing of plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. 2014. Antimicrobial Agents Chemotherapy; 58:3895-3903.

19. Barro N, Gamene AA, Itsiembou Y, Savadogo A, Nikiema AP, Ouattara CAT, De Souza CA, Traoré AS. Street-vended foods improvement: Contamination mechanisms and application of food safety objective strategy: Critical review. Pakistan Journal of Nutrition. 2007. 6: (1) :1-10

20. Broughton El, Walker DG. Prevalence of Antibiotic-Resistant Salmonella in Fish in Guangdong, China. 2009. Foodborne Pathogens and Disease 6(4):519-21.

21. Heinitz ML, Ruble RD, Wagner DE, Tatini SR. Incidence of Salmonella in fish and seafood. Journal of Food Protection. 2000. 63(5):579-92.

22. Barro N, Tahita MC, Traore O, Sangare L, De Souza CA, Traore AS. Risks associated with practices, processes, and environment of ready-to-eat and street-vended foods that lead to contamination by
common foodborne viruses. In: Hygiene and Its Role in Health. 2008. Ed. P. L. Aderson and J.P. Lachan: pp: 129-153.

23. Kagambèga A, Haukka K, Siitonen A, Traoré AS, Barro N. Prevalence of *Salmonella enterica* and the hygienic indicator *Escherichia coli* in raw meat at markets in Ouagadougou, Burkina Faso. Journal of Food Protection. 2011. 74(9):1547-51.

24. Kagambèga A, Barro N, Traoré AS, Siitonen A, Haukka K. Characterization of *Salmonella enterica* and detection of the virulence genes specific to diarrheagenic *Escherichia coli* from poultry carcasses in Ouagadougou, Burkina Faso. Foodborne pathogens and disease. 2012. 9(7):589-93.

25. Kagambèga A, Bouda SC, Bako E, Cissé H, Barro N, Haukka K. Diversity and antimicrobial resistance of *Salmonella* strains isolated from different sources in Burkina Faso. African Journal of Microbiology Research. 2017. 11(40): 1495-1504.

26. Center for Disease Control and Prevention (CDC). Multistate Outbreak of *Salmonella* Serotype Tennessee Infections Associated with Peanut Butter — United States, 2006—2007. 2007. Morbidity and Mortality Weekly Report; 56: 521-524.

27. Center for Disease Control and Prevention (CDC). Salmonella serotype Tennessee in powdered milk products and infant formula—Canada and the United States. 1993. Morbidity and Mortality Weekly Report; 42:501-19.

28. Bonkoungou IJO, Haukka K, Österblad M, Hakanen AJ, Traoré AS, Barro N, Siitonen A. Bacterial and viral etiology of childhood diarrhea in Ouagadougou, Burkina Faso. 2013. BMC Pediatrics; 13:36 http://www.biomedcentral.com/1471-2431/13/36.

29. Traoré O, Nyholm O, Siitonen A, Bonkoungou IJO, Traoré AS, Barro N, Haukka K. Prevalence and diversity of *Salmonella enterica* in water, fish and lettuce in Ouagadougou, Burkina Faso. BMC Microbiology. 2015. 15:151, doi: 10.1186/s12866-015-0484-7.

30. Kagambèga A, Lienemann T, Aulu L, Traoré AS, Barro N, Siitonen A, Haukka K. Prevalence and characterization of *Salmonella enterica* from the intestines of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human Salmonella isolate. Salmonella. BMC Microbiology. 2013. 13:253, doi: 10.1186/1471-2180-13-253.

31. Magnet S, Courvalin P, Lambert T. Activation of the Cryptic aac(6’)-IyAminoglycoside Resistance Gene of Salmonella by a Chromosomal Deletion Generating a Transcriptional Fusion. Journal of Bacteriology. 1999 181 (21) 6650-6655; DOI: 10.1128/JB.181.21.6650-6655.1999

32. Rather PN, Orosz E, Hare RS, Miller G, Shaw KJ. Characterization and transcriptional regulation of the 29-N-acetyltransferase gene of Providencia stuartii. Journal of Bacteriology. 1993. 175(20): 6492–6498.

33. Xavier BB, Lammens C, BUTAYE P, Goossens H, Malhotra-Kumar S. Complete sequence of an IncFII plasmid harbouring the colistin resistance gene mcr-1 isolated from Belgian pig farms. Journal of Antimicrobial Chemotherapy. 2016. 71(8): 2342–2344.