Serotyping of sub-Saharan Africa Salmonella Strains Isolated from Different Sources Using Multiplex PCR and Capillary Electrophoresis Analysis and whole Genome Sequencing

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Abstract

Background

Salmonella enterica remains a leading cause of food-borne diseases worldwide. Serotype information is important in food safety and public health activities to reduce the burden of salmonellosis. In the current study, two methods were used to determine serotypes of 225 clinical, environmental, food and veterinary isolates of Salmonella from Burkina Faso. First, Salmonella Multiplex Assay for Rapid Typing (SMART) Polymerase Chain Reaction (PCR) was used to determine the serovars of the S. enterica isolates. Second, serovar prediction based on whole genome sequencing (WGS) data was performed using SeqSero 2.0.

Results

Among the 225 Salmonella isolates, serotypes for 48 (21.33%) isolates were identified based on comparison to a panel of representative SMART codes previously determined for the 50 most common serovars in the United States. One hundred and seventy-six new SMART codes were developed for common and uncommon serotypes. Serotypes for a total of 205 (91.1%) isolates were identified using SeqSero 2.0 for serovar prediction based on WGS data.

Conclusion

We determined that SeqSero 2.0 was more comprehensive for identifying Salmonella serotypes from Burkina Faso than SMART PCR.

Background

The discovery of Salmonella was made by Theobald Smith in 1855 from the intestines of a pig suffering from swine fever (1; 2). Salmonella is a genus of gram-negative bacteria in the family of Enterobacteriaceae with two species: Salmonella bongori and Salmonella enterica. The species S. enterica includes more than 2,579 serovars and is a major cause of food-borne illness in humans (3; 4). The subspecies of S. enterica are enterica, salamae, arizonae, diarizonae, houtenae, and indica (5). Salmonella enterica subsp. enterica includes over 1400 serotypes and causes approximately 99% of Salmonella infections in humans and warm-blooded animals (6). Salmonella serovars Typhi, Paratyphi A, and B cause enteric fever: a systemic febrile illness that only occurs in humans. Non-typhoidal Salmonella (NTS) infect a variety of hosts including warm blood animals. NTS are one of the leading causes of bacterial diarrhea worldwide (7). Human disease can result from exposure to many sources such as infected animals, contaminated foodstuffs, contaminated water, and direct contact with infected environment or directly between humans.

In Sub-Saharan Africa, the socio-economic burden of NTS is difficult to quantify due to the lack of a standard method of assessment, which is compounded by under-reporting in many cases (8; 9; 10). NTS serotype identification is important for the control of foodborne disease incidence. The traditional method
for *Salmonella* serotyping is the slide and tube agglutination tests using the O and H antigen according to Kauffman-White scheme (11; 12). However, this method is not accessible in the developing world because the anti-sera used for agglutination is very expensive and some stocks are often not available. In many developing countries like Burkina Faso, only antisera for the agglutination of Vi antigen is available for the detection of *Salmonella* Typhi and Paratyphi A or B in hospitals. A few studies have investigated *Salmonella* serotyping using the Kauffman-White scheme in Burkina Faso by collaborating with laboratories in industrialized countries (13; 14; 15). To establish the true incidence of NTS diseases in developing countries, development of new, cheaper methods is needed for *Salmonella* serotyping.

Molecular methods are well suited for this because of their specificity is comparable with traditional Kauffman-White serotyping. However, many of these modern detection methods for *Salmonella* are still absent in some developing countries, particularly in Burkina Faso. Developing countries have limited financial resources and cannot implement well-established yet complex nucleic acid analysis systems or laboratory-developed tests through a network of centralized laboratories. These molecular methods required specific and complex equipment, sensitive reagents, dedicated infrastructure, and deep technical knowledge, which are not available in many low- and middle-income countries (LMICs). Therefore, it is an often necessity for researchers from LMICs to collaborate with high income countries and test available modern techniques for *Salmonella* serotyping to determine if the method can be adapted for their country. In this study, the high-throughput molecular determination of *Salmonella enterica* serovars by use of *Salmonella* Multiplex Assay for Rapid Typing (SMART) PCR using capillary electrophoresis and whole genome sequencing (WGS) were compared to determine their accuracy in identifying serotypes of NTS isolated from Burkina Faso. The SMART method was developed by Leader et al. (2009) for discrimination of most common serotypes that are reported in the United States based upon their genetic differences. The genotypic serotype prediction was done using SeqSero 1 and 2 (Zang et al., 2015;2019).

**Methods**

**Bacterial strains**

The 225 isolates used in this study were obtained from the Laboratoire de Biologie Moléculaire, d'épidémiologie et de surveillance des bactéries et virus transmissible par les aliments (LaBESTA)/Université Joseph KI-ZERBO, Burkina Faso. The strains were isolated from different sources and the serotype of each confirmed following the methodologies described in the International Organization for Standardization 6579 – 2017 (ISO 6579-1, 2017). The isolates were collected from clinical, veterinary, and food produce samples. Specifically, the breakdown was as follows; 28 from diarrheic patients; 105 from poultry feces, 22 from guinea fowl feces, 19 from poultry carcasses, 17 from eggs, 30 from fish and 4 were from sandwiches.

**High-throughput molecular determination of Salmonella enterica serovars**
We used the SMART method developed by Leader et al., 2009, with slight modification. Salmonella strains were streaked onto blood agar and incubated for 18–20 hrs at 36 °C. Then, one colony from each plate was cultured in 5 mL of Luria Bertani (LB) broth, (Difco™, Becton Dickinson and Company, Sparks, MD) and incubated for 18 hrs at 37 °C with shaking. The genomic DNA was then isolated from the overnight culture using the GenElute bacterial genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA) and following the kit instructions for use. Once extractions were completed, the DNA was analyzed on the Nanodrop 2000 for DNA quality measuring the 260/280 nm. All DNA were then stored at -20 °C until ready for PCR and library preparation.

**PCR amplification.**

Each PCR mixture contained 12.5 µL of Immolase DNA polymerase 2X master mix (Bioline, Inc., Randolph, MA, USA), 2.5 µL of 10X primer master mix, 3 mM MgCl2, and 1 µL of extracted DNA with addition of nuclease free water to a final reaction volume of 25 µL. The cycling conditions used in the thermal cycler were 94 °C for 10 min; 25 cycles of 94 °C for 30 s, 57 °C for 90 s, and 72 °C for 30 s; 72 °C for 5 min; 15 cycles of 94 °C for 30 s, 68 °C for 90 s, and 72 °C for 30 s; and 72 °C for 5 min. For each run, the negative control was sterile water and positive controls were genomic DNA from Salmonella Typhimurium LT2, S. Typhi CT18, S. Enteritidis strains 21027 and 98104. The primers describe by Leader et al. (2009) were used in this study. The amplicon samples were then diluted and analysed on an ABI XXXX using capillary electrophoresis.

Genemapper software v3.5 (Applied Biosystems, Foster City, CA, USA) was used to analyze the sizes of resulting PCR products according to the protocol developed by Leader et al. (2009). Scoring was based upon the presence of a PCR product that corresponded to the predicted amplicon size, as detected in control reactions with DNA from S. Typhimurium, S. Typhi, and S. Enteritidis. Each PCR product detected was given a number (1 through 16) according to the size of the amplicon (Leader et al., 2009). The amplicons detected for each isolate were combined to create a SMART code that corresponds to serotypes previously screened by this method.

**Whole genome sequencing of Salmonella strains**

Extracted DNA was quantified using the Qubit double-strandedDNA high-sensitivity assay kit according to the manufacturer’s instructions (Life Technologies Corp., Carlsbad, CA, USA). The Illumina libraries were prepared using the Nextera XT DNA library preparation kit and Nextera XT index primers (Illumina, san Diego, CA, USA). The library fragment size distribution was checked using the Bioanalyzer 2100 with an Agilent HS DNA kit (Agilent Technologies, Santa Clara, CA, USA) and quantified using a Qubit DNA HS assay kit in a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The generated libraries were then sequenced using a MiSeq version 2 reagent kit (Illumina) with 500 and 300 cycles. The paired-end read length of 2 × 250 bp was used for 500 cycles and 2 × 150 bp for 300 cycles on the MiSeq platform (Illumina). The quality metrics of the reads were performed by FastQC.
The sequences were then assembled using the A5-miseq assembler (Coli et al., 2015), and the genome sequence was annotated via the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016).

Serovar Prediction From Wgs Using In Silico Seqsero Tool

SeqSero version 2.0 was used to determine the serotype of the 225 *Salmonella* isolates (Zang et al., 2019).

Results

The SMART PCR generated some new codes different to those found in the United States

Two hundred and twenty-five samples were analyzed to determine the serotypes of *Salmonella* strains isolated from Burkina Faso using the SMART PCR database developed for the 50 most common *Salmonella* serovars found in the U.S. (Leader et al., 2009). Among the 225 *Salmonella* isolates, 48 (21.33%) serotypes were identified based on comparison to the panel of SMART codes. One hundred and seventy-six isolates were assigned new codes not included in the SMART PCR database (Table 1). The serovars Enteritidis, Agona, Virchow, Poona, and Liverpool did not generate any new codes. However, serovars Typhimurium, Duesseldorf, Tennessee, Gaminara, and Schwarzengrund developed at least one new code in addition to the original (Table 1). New SMART codes were determined for some uncommon serotype including Bredeney, Hato, Brancaster, Kaapstad, Amoutive, Kokomlemle, and others (Table 2).

SeqSero method for determination of *Salmonella* serovars using Whole Genome Sequencing data

We performed WGS on the 225 *Salmonella* isolates. Using SeqSero 2 we determined the serotype of 205 (91.1%) *Salmonella* isolates. Thirty three different serotypes were identified: Enteritidis, Typhimurium, Kentucky, Agona, Virchow, Anatum, Derby, Hato, Chester, Jedburgh, Schwarzengrund, Tennessee, Albany, Duesseldorf, Poona, Eastbourne, Gaminara, Drac, Kaapstad, Alexanderplatz, Brancaster, Bredeney, Amoutive, Telelkebir, Liverpool, Kalamu, Kalina, Kokomlemle, Muenster, Monschau, Takoradi, Bargny, Nima. Among the serotypes predicted, *S*. Derby was the most frequently identified serotype with 41 of the 205 isolates (20%), followed by Hato (18.5%), Tennessee (9.26%), Typhimurium (8.29%), and Kentucky (6.31%) (Table 1).

SMART PCR assigned some isolates with new codes without serotype predictions and SeqSero assigned them a serotype. For example, SMART PCR predicted a new code for one isolate and SeqSero predicted Takoradi or Bargny because both share the same antigenic profile “8:i:1,5”(table 1).
| Sample ID | Serogroup | SMART PCR code | Serotype by SMART | Antigenic Serotype profile | Serotype by SeqSero2 |
|-----------|-----------|----------------|------------------|---------------------------|----------------------|
| S1        | B         | 1-5-6-7-9-10-11-16 | new code         | 4:i,v:1,7                 | Bredeney             |
| S2        | B         | 1-2-5-6-11        | new code         | 4:g,m,s:-                 | Hato                 |
| S3        | B         | 1-5-6-7-11-13     | new code         | 4:i,v:1,7                 | Bredeney             |
| S4        | C2-C3     | 1-2-5-11-13-16    | new code         | 8:i:1,5                   | Takoradi or Bargny    |
| S5        | B         | 1-2-5-6-8-11-13   | new code         | 4:g,m,s:-                 | Hato                 |
| S6        | B         | 1-2-5-11-13       | Agona            | 4:f,g,s:-                 | Agona                |
| S7        | B         | 1-5-7-9-11-16     | new code         | 4:i:1,2                   | Typhimurium          |
| S8        | C2-C3     | 1-5-6-9-11-16     | Kentucky         | 8:i:z6                    | Kentucky             |
| S9        | B         | 1-2-5-11-13       | Agona            | 4:f,g:-                   | Derby                |
| S10       | C2-C3     | 1-2-4-5-6-10-11-13-16 | new code     | 8:i:z6                    | Kentucky             |
| S11       | E2-E3     | 1-5-6-7-10-11-16  | new code         | 3,10:e,h:1,5              | Muenster             |
| S12       | D1        | 1-2-3-5-6-11-14-15 | Enteritidis     | 9:g,m:-                   | Enteritidis          |
| S13       | B         | 1-2-5-11-13       | Agona            | 4:f,g,s:-                 | Agona                |
| S14       | C2-C3     | 1-2-5-11-16       | new code         | 8:i:z6                    | Kentucky             |
| S15       | C2-C3     | 1-2-5-11-16       | new code         | 8:i:z7                    | Kentucky             |
| S16       | B         | 1-2-5-11-13       | Agona            | 4:f,g,s:-                 | Agona                |
| S17       | B         | 1-2-5-11-13       | Agona            | 4:f,g,s:-                 | Agona                |
| S18       | B         | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium     | 4:i:1,2                   | Typhimurium          |
| S19       | D1        | 1-2-3-5-6-11-14-15 | Enteritidis     | 9:g,m:-                   | Enteritidis          |
| S20       | D1        | 1-2-3-5-6-11-14-15 | Enteritidis     | 9:g,m:-                   | Enteritidis          |
| Code | Subgroup | Date Range | Code Type | Code | Location |
|------|----------|------------|-----------|------|----------|
| S21  | B        | 1-5-6-11-16 | new code | 4:d:1,7 | Schwarzengrund |
| S22  | C2-C3    | 1-2-5-11-16 | new code | 8:i:z6 | Kentucky |
| S23  | C1       | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| S24  | C1       | 1-2-5-11-13 | new code | 8:i:z6 | Kentucky |
| S25  | G        | 1-5-6-7-11-16 | Teelkebir | 13:d:e,n,z15 | Teelkebir |
| S26  | B        | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S27  | G        | 1-5-6-7-9-11-16 | new code | 13:d:e,n,z15 | Teelkebir |
| S28  | D1       | 1-2-3-5-6-11-14-15 | Enteritidis | 9:g,m:- | Enteritidis |
| S29  | D1       | 1-2-3-5-6-11-14-15 | Enteritidis | 9:g,m:- | Enteritidis |
| S30  | B        | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S31  | B        | 1-2-5-11-13 | Agona | 4:f,g,s:- | Agona |
| S32  | B        | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S33  | B        | 1-2-5-6-11-13 | new code | 4:z29:- | Brancaster |
| S34  | C2-C3    | 1-2-5-10-11-13-16 | new code | 8:i:z6 | Kentucky |
| S35  | D1       | 1-2-3-5-11 | new code | 9:z29:- | N/A |
| S36  | E4       | 1-2-5-10-11-13-16 | new code | 8:i:z6 | Kentucky |
| S37  | B        | 1-5-6-7-11-16 | new code | 4:i:1,2 | Typhimurium |
| S38  | C2-C3    | 1-2-5-6-11 | Dusseldorf | 8:z4,z24:- | Albany or Duesseldorf |
| S39  | B        | 1-5-7-9-11-16 | new code | 4:e,h:e,n,x | Chester |
| S40  | E1       | 1-2-5-7-11-13 | new code | 3,10:z29:- | Jedburgh |
| S41  | E4       | 1-5-6-7-11-13 | new code | 1,3,19:b:- | N/A |
| S42  | E4       | 1-5-6-7-11-13 | new code | 1,3,19:b:- | N/A |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| S43 | B | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S44 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S45 | B | 1-2-5-6-11 | new code | 4:f,g:- | Derby |
| S46 | B | 1-2-5-6-7-8-11-12-13-14-16 | new code | 4:f,g:- | Derby |
| S47 | B | 1-2-5-6-10-11 | new code | 4:g,m,s:- | Hato |
| S48 | B | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S49 | B | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S50 | B | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S51 | B | 1-2-5-6-7-10-11 | new code | 4:z29:- | Brancaster |
| S52 | B | 1-5-7-9-11-16 | new code | 4:i:1,2 | Typhimurium |
| S53 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S54 | B | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S55 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S56 | B | 1-5-7-11-16 | new code | 4:e,h:1,7 | Kaapstad |
| S57 | B | 1-2-5-6-11-13 | new code | 4:z29:- | Brancaster |
| S58 | G | 1-5-6-7-11-16 | Teelkebir | 13:d:e,n,z15 | Teelkebir |
| S59 | B | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S60 | G | 1-5-6-7-9-11-16 | new code | 13:d:e,n,z15 | Teelkebir |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| S61 | D1 | 1-2-3-5-6-11-14-15 | Enteritidis | 9:g,m:- | Enteritidis |
| S62 | D1 | 1-2-3-5-6-11-14-15 | Enteritidis | 9:g,m:- | Enteritidis |
| S63 | B  | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S64 | B  | 1-2-5-11-13 | Agona | 4:f,g,s:- | Agona |
| S65 | B  | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S66 | E4 | 1-2-5-6-11-13-16 | new code | 1,3,19:f,g:1,5 | N/A |
| S67 | B  | 1-5-7-9-11-16 | new code | 4:e,h:e,n,x | Chester |
| S68 | B  | 1-2-5-6-10-11 | new code | 4:g,m,s:- | Hato |
| S69 | C2-C3 | 1-2-5-10-11-13-16 | new code | 8:i:z6 | Kentucky |
| S70 | B  | 1-2-5-6-11 | new code | 4:f,g:- | Derby |
| S71 | C1 | 1-5-7-11-13-16 | Virchow | 7:r:1,2 | Virchow |
| S72 | M  | 1-2-5-11-13-16 | new code | 28:d:1,5 | Amoutive |
| S73 | E1 | 1-2-5-7-11-13 | new code | 3,10:z29:- | Jedburgh |
| S74 | C2-C3 | 1-2-5-10-11-13-16 | new code | 8:i:z6 | Kentucky |
| S75 | B  | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S76 | B  | 1-5-7-11-16 | new code | 4:e,h:1,7 | Kaapstad |
| S77 | B  | 1-5-7-11-16 | new code | 4:e,h:1,7 | Kaapstad |
| S78 | E1 | 1-2-5-6-7-11-13 | new code | 4:e,h:1,7 | Kaapstad |
| S79 | B  | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S80 | B  | 1-2-5-6-10-11 | new code | 4:f,g:- | Derby |
| S81 | B  | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S82  | B   | 1-5-7-9-11-16 | new code | 4:e,h:e,n,x | Chester |
| S83  | B   | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | 4:i:1,2 | Typhimurium |
| S84  | B   | 1-2-5-6-11-13 | new code | 4:z29:- | Brancaster |
| S85  | C2-C3 | 1-2-5-6-11 | Dusseldorf | 8:z4,z24:- | Albany or Duesseldorf |
| S86  | B   | 1-2-5-6-7-10-11-13 | new code | 4:z29:- | Brancaster |
| S87  | M   | 1-6-10-11-16 | new code | 28:y:1,5 | Nima |
| S88  | C1  | 1-2-5-6-10-11-13 | Tennessee | 7:z29:- | Tennessee |
| S89  | PolyE | 1-2-5-11-16 | new code | 47:l,z13,z28:l,z13,z29 | N/A |
| S90  | I   | 1-5-6-10-11-16 | Gaminara | 16:d:1,7 | Gaminara |
| S91  | B   | 1-2-5-6-7-10-11-13 | new code | 16:d:1,8 | Derby |
| S92  | B   | 1-2-5-6-7-10-11-13 | new code | 4:f,g:- | Derby |
| S93  | B   | 1-5-6-7-11-16 | Schwarzengrund | 4:d:1,7 | Schwarzengrund |
| S94  | B   | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S95  | B   | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S96  | Poly D | 1-5-6-7-11-13-16 | new code | 43:z4,z23:1,2 | Farmingdale |
| S97  | B   | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S98  | PolyD | 1-2-4-5-10-11-13-16 | new code | 39:z10:e,n,z15 | N/A |
| S99  | E1  | 1-2-5-6-7-11-16 | new code | 3:10:e,h:1,6 | Anatum |
| S100 | N   | 1-2-5-6-10-11-14 | new code | 30:y:- | N/A |
| S101 | G  | 1-2-5-10-11-13-16 | new code | 13:i:z6 | N/A |
| S102 | B  | 1-5-6-7-10-11-16 | Bredeney | 4:l,v:1,7 | Bredeney |
| S103 | E4 | 1-5-7-11-16      | Liverpool | 4:f,g:-  | Liverpool |
| S104 | polyE | 1-2-5-6-9-11 | new code | 47:z38:- | Alexanderplatz |
| S105 | B  | 1-2-5-6-7-10-11-13 | new code | 4:f,g:-  | Derby |
| S106 | C2-C3 | 1-2-5-10-11-16 | new code | 8:i:z6 | Kentucky |
| S107 | B  | 1-5-6-7-10-11-16 | Bredeney | 4:l,v:1,7 | Bredeney |
| S108 | C1 | 1-2-5-11-13      | new code | 7:z29:- | Tennessee |
| S109 | B  | 1-2-5-6-7-10-11-13 | new code | 4:f,g:-  | Derby |
| S110 | D1 | 1-3-5-7-10-11-16 | new code | 9:e,h:1,5 | Eastbourne |
| S111 | C1 | 1-2-5-11-13      | new code | 7:z29:- | Tennessee |
| S112 | B  | 1-2-5-6-8-11-13 | new code | 4:g,m,s:- | Hato |
| S113 | E4 | 1-5-7-11-16      | Liverpool | 1,3,19:d:e,n,z15 | Liverpool |
| S114 | B  | 1-2-5-6-8-11-13 | new code | 4:g,m,s:- | Hato |
| S115 | B  | 1-2-5-6-11-13 | new code | 4:f,g:-  | Derby |
| S116 | C1 | 1-2-5-6-10-11-13 | Tennessee | 7:z29:- | Tennessee |
| S117 | C1 | 1-2-5-11-13      | new code | 7:z29:- | Tennessee |
| S118 | G  | 1-6-7-9-11-16    | Poona | 13:z:1,6 | Poona |
| S119 | C1 | 1-2-5-11-13      | new code | 7:z29:- | Tennessee |
| S120 | B  | 1-2-5-6-7-10-11-13 | new code | 4:f,g:-  | Derby |
| S121 | G  | 1-6-7-9-11-16    | Poona | 13:z:1,6 | Poona |
| S122 | C1 | 1-2-5-11-13      | new code | 7:z29:- | Tennessee |
| Code | Type | Pattern | Code | Pattern | Location |
|------|------|---------|------|---------|----------|
| S123 | B    | 1-5-6-7-10-11-16 | Bredeney | 4:l,v:1,7 | Bredeney |
| S124 | B    | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S125 | B    | 1-2-5-6-8-11-13 | new code | 4:g,m,s:- | Hato |
| S126 | C2-C3 | 1-2-5-10-11-16 | new code | 8:i:z6 | Kentucky |
| S127 | C1   | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| S128 | C1   | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| S129 | M    | 1-6-10-11 | new code | 28:y:1,5 | N/A |
| S130 | C1   | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| S131 | C1   | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| S132 | B    | 1-2-5-6-7-10-11-13 | new code | 4:f,g:- | Derby |
| S133 | B    | 1-2-5-6-7-10-11-13 | new code | 4:f,g:- | Derby |
| S134 | PolyD | 1-5-6-7-9-11-16 | new code | 39:l,v:e,n,x | Kokomlemle |
| S135 | G    | 1-2-5-10-11-13-16 | new code | 13:i:z6 | N/A |
| S136 | E4   | 1-5-7-11-16 | Liverpool | 1,3,19:d:e,n,z15 | Liverpool |
| S137 | C1   | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| S138 | M    | 1-6-10-11-16 | new code | 28:y:1,5 | N/A |
| S139 | C1   | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| S140 | PolyE | 1-2-5-6-9-11 | new code | 47:z38:- | Alexanderplatz |
| S141 | C1   | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| S142 | M    | 1-6-10-11-16 | new code | 28:y:1,5 | N/A |
| S143 | PolyE | 1-2-5-6-9-11 | new code | 47:z38:- | Alexanderplatz |
| S144 | PolyD | 1-2-4-5-10-11-13-16 | new code | 39:f,g:e,n,z15 | N/A |
| S145 | C1   | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| PID | Code | Values | Location | Notes |
|-----|------|--------|----------|-------|
| S146 | C1 | 1-2-5-6-10-11-13 | Tennessee | 7:z29:- | Tennessee |
| S147 | PolyE | 1-5-6-7-9-11-16 | new code | 47:l,v:e,n,x | Drac |
| S148 | E1 | 1-5-6-7-10-11-16 | new code | 3,10:e,h:1,5 | Muenster |
| S149 | E1 | 1-5-6-7-10-11-16 | new code | 3,10:e,h:1,6 | Muenster |
| S150 | E1 | 1-5-6-7-9-11-16 | Muenster | 3,10:e,h:1,5 | Muenster |
| S151 | E1 | 1-5-6-7-9-11-16 | Muenster | 3,10:e,h:1,6 | Muenster |
| S152 | D1 | 1-5-6-7-9-11-16 | Muenster | 3,10:e,h:1,5 | Muenster |
| S153 | E1 | 1-5-6-7-9-11-16 | Muenster | 3,10:e,h:1,5 | Muenster |
| S154 | E1 | 1-5-6-7-10-11-16 | new code | 3,10:e,h:1,5 | Muenster |
| S155 | G | 1-7-9-10-11-16 | new code | 39:f,g:e,n,z15 | N/A |
| S156 | G | 1-7-9-10-11-16 | new code | 13:z:1,6 | Poona |
| S157 | E4 | 1-5-6-7-11-13 | new code | 1,3,19:b:- | N/A |
| S158 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S159 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S160 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S161 | E4 | 1-5-6-7-11-13 | new code | 1,3,19:b:- | N/A |
| S162 | E4 | 1-5-6-7-11-13 | new code | 1,3,19:b:- | N/A |
| S163 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S164 | E4 | 1-5-6-7-11-13 | new code | 1,3,19:b:- | N/A |
| S165 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| Code | Type | Dates | Description | Location |
|------|------|-------|-------------|----------|
| S166 | B    | 1-2-5-6-11-13 | new code | Derby |
| S167 | E4   | 1-5-6-7-11-13 | new code | N/A |
| S168 | B    | 1-2-5-6-7-8-11-12-13-14-16 | Typhimurium | Typhimurium |
| S169 | B    | 1-2-5-6-7-10-11 | new code | Hato |
| S170 | B    | 1-2-5-6-11-13 | new code | Derby |
| S171 | B    | 1-2-5-6-10-11-13 | new code | Derby |
| S172 | C2-C3 | 1-2-5-10-11-13-16 | new code | Kentucky |
| S173 | B    | 1-2-5-6-11-13 | new code | Brancaster |
| S174 | B    | 1-2-5-6-11-13 | new code | Brancaster |
| S175 | B    | 1-2-5-6-11 | new code | Hato |
| S177 | B    | 1-2-5-6-11-13 | new code | Hato |
| S178 | B    | 1-2-5-10-11-13 | new code | Kalamu |
| S179 | E1   | 1-2-5-7-11-13-16 | new code | Kalina |
| S180 | B    | 1-2-5-6-11-13 | new code | Derby |
| S181 | O    | 1-2-4-5-6-11 | new code | Monschau |
| S182 | O    | 1-2-4-5-6-11 | new code | Monschau |
| S183 | B    | 1-2-5-6-11-13 | new code | Derby |
| S184 | B    | 1-2-5-6-11 | new code | Hato |
| S185 | B    | 1-2-5-6-11 | new code | Hato |
| S186 | B    | 1-2-5-6-11 | new code | Hato |
| S187 | B    | 1-2-4-5-6- | new code | Hato |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| S188 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S189 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S190 | E1 | 1-2-5-7-11-13-16 | new code | 3,10:b:1,2 | Kalina |
| S191 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S192 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S193 | B | 1-2-5-10-11-13 | new code | 4:z4,z24:- | Kalamu |
| S194 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S195 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S196 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S197 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S198 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S199 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S200 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S201 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S202 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S203 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S204 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S205 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S206 | B | 1-2-5-6-11-13 | new code | 4:f,g:- | Derby |
| S207 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S208 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S209 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S210 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S212 | B | 1-2-5-6-11 | new code | 4:g,m,s:- | Hato |
| S213 | O | 1-2-5-6-11-13 | new code | 35:m,t:- | Monschau
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| S214 | B | 1-2-5-6-11 | new code | 4:gm,s:- | Hato |
| S215 | B | 1-2-5-6-11 | new code | 4:gm,s:- | Hato |
| S216 | B | 1-2-5-6-11 | new code | 4:gm,s:- | Hato |
| S217 | B | 1-2-5-6-11 | new code | 4:gm,s:- | Hato |
| S219 | B | 1-2-5-6-11 | new code | 4:gm,s:- | Hato |
| S247 | B | 1-2-5-6-11 | new code | 4:fg:- | Derby |
| S248 | B | 1-2-5-6-10-11 | new code | 4:fg:- | Derby |
| S249 | B | 1-2-5-6-10-11 | new code | 4:fg:- | Derby |
| S250 | B | 1-2-5-6-11-13 | new code | 4:fg:- | Derby |
| S251 | B | 1-2-5-6-10-11-13 | new code | 4:fg:- | Derby |
| S252 | C1 | 1-2-5-11-13 | new code | 7:z29:- | Tennessee |
| S253 | E4 | 1-5-6-7-11-13 | new code | 1,3,19:b:- | N/A |
| S254 | B | 1-2-5-10-11-13-16 | new code | 8:i:z6 | Kentucky |
| S255 | C2-C3 | 1-2-5-10-11-13-16 | new code | 8:i:z6 | Kentucky |
| Serotypes       | New SMART codes | Existing SMART code from Leader et al., 2009 |
|-----------------|-----------------|--------------------------------------------|
| Bredeney        | 1-5-6-7-9-10-11-16 | -                                          |
|                 | 1-5-6-7-11-13    |                                            |
| Hato            | 1-2-5-6-11       | -                                          |
|                 | 1-2-5-6-8-11-13  |                                            |
|                 | 1-2-5-6-10-11    |                                            |
|                 | 1-2-5-6-7-10-11  |                                            |
|                 | 1-2-4-5-6-11     |                                            |
| Takoradi or Bargny | 1-2-5-11-13-16  | -                                          |
| Typhimurium     | 1-5-7-9-11-16    | 1-2-5-6-7-8-11-12-13-14-16                |
|                 | 1-5-6-7-11-16    |                                            |
|                 | 1-2-5-10-11-13-16|                                            |
| Kentucky        | 1-2-4-5-6-10-11-13-16 | 1-5-6-9-11                       |
|                 | 1-2-5-11-16      |                                            |
|                 | 1-2-5-11-13      |                                            |
|                 | 1-2-5-10-11-13-16|                                            |
|                 | 1-2-5-10-11-16   |                                            |
| Derby           | 1-2-5-6-11-13    | 1-2-5-6-7-11-14                          |
|                 | 1-2-5-6-11       |                                            |
|                 | 1-2-5-6-7-8-11-12-13-14-16 |                        |
|                 | 1-2-5-6-10-11    |                                            |
|                 | 1-2-5-6-7-10-11-13|                                          |
|                 | 1-2-5-6-11-13    |                                            |
|                 | 1-2-5-6-11       |                                            |
|                 | 1-2-5-6-10-11    |                                            |
|                 | 1-2-5-6-10-11-13 |                                            |
| Muenster        | 1-5-6-7-10-11-16 | 1-5-6-7-9-11                              |
| Location                | Date            | Date            |
|-------------------------|-----------------|-----------------|
| Schwarzengrund          | 1-5-6-11-16     | 1-5-6-7-11      |
| Tennessee               | 1-2-5-11-13     |                 |
| Telelkebir              | 1-5-6-7-9-11-16| 1-5-6-7-11      |
| Brancaster              | 1-2-5-6-11-13   |                 |
|                         | 1-2-5-6-7-10-11|                 |
|                         | 1-2-5-6-7-10-11-13|             |
|                         | 1-5-6-7-11-13-16|               |
| 9:z29:-                 | 1-2-3-5-11      |                 |
| 1,3,19:b:-              | 1-5-6-7-11-13   |                 |
| 1,3,19:f,g:1,5          | 1-2-5-6-11-13-16|               |
| 47:l,z13,z28:l,z13,z29 | 1-2-5-11-16     |                 |
| 39:z10:e,n,z15          | 1-2-4-5-10-11-13-16|         |
|                         | 1-7-9-10-11-16  |                 |
| 30:y:-                  | 1-2-5-6-10-11-14|                 |
| 13:i:z6                 | 1-2-5-10-11-13-16|               |
|                         | 1-6-10-11       |                 |
| 28:y:1,5                | 1-6-10-11-16    |                 |
| 13:i:z6                 | 1-2-5-10-11-13-16|               |
| 1,3,19:b:-              | 1-5-6-7-11-13   |                 |
| Albany or Duesseldorf   | 1-2-5-6-11      |                 |
| Chester                 | 1-5-7-9-11-16   | 1-5-7-11      |
| Jedburgh                | 1-2-5-7-11-13   |                 |
| Kaapstad                | 1-5-7-11-16     |                 |
|                         | 1-2-5-6-7-11-13|               |
| Amoutive                | 1-2-5-11-13-16  |                 |
| Nima                    | 1-6-10-11-16    |                 |
| Gaminara                | 1-2-5-6-7-10-11-13|           |
|                         | 1-2-5-6-11-13   |               |
| Anatum                  | 1-2-5-6-7-11-16 | 1-2-5-6-7-11-12|
Discussion

Serotyping is an important tool for monitoring for foodborne outbreaks and in understanding the diversity and distribution of serotypes within populations, flocks, and herds. However, serotyping by traditional methods remains inaccessible for many LMICs. In this study, we investigated two molecular serotyping methods to identify serotypes for 225 isolates from Burkina Faso. The goal was to serotype *Salmonella enterica* using rapid and accessible molecular methods as opposed to immunologic approaches to antigen characterization. The traditional method of serotyping using the Kauffman-White Scheme is expensive, time consuming, and training is needed to accurately read results. The SMART PCR is faster and cheaper than traditional serotyping and can be automated to read results. For example, it is possible to test two 96-well plates *Salmonella* strains in one SMART PCR run. The reagents required are also less expensive than antisera and available from many different vendors worldwide (Leader et al., 2009). Both factors would benefit outbreak control in the developing world. In the present study, 176 isolates were assigned new codes not previously included in the SMART PCR database. This result will benefit LMICs by extending the original SMART code database with serotypes more prevalent in other parts of the world, particularly from Sub-Saharan Africa. This could be used in future studies to analyze the diversity of *Salmonella* serotypes. Moreover, *Salmonella* infections can globally circulate and a serotype from another region can potentially emerge as a common serotype persistent in other places than from where first reported. For example, Wong et al. (2015) demonstrated that a Multidrug Resistant (MDR) S. Typhi H58 emerged in South Asia was propagated to many locations around the world, including countries in Southeast Asia, western Asia, and East Africa.

However, a limitation of SMART PCR is the identification of new codes without an assigned serotype, previously pulse field gel electrophoresis patterns were used to compliment SMART codes. The SMART assay was initially developed to identify the 50 most common serotypes from clinical found in the Northwestern USA (Leader et al., 2009). Our study identified many new SMART codes associated with uncommon serotypes and there is an urgent need to extend the database to include more *Salmonella*
serotypes as classified by the Kauffmann-White scheme (Kauffmann, 1971). This will greatly increase the usability of the SMART PCR around the world. The original SMART codes should be renewed every five years because *Salmonella* infections are in constant flux and any serotype can emerge as a top serotype at any time. Moreover, the capillary electrophoresis machine is very sensitive to power surges and needs to be protected appropriately, which is a challenge for laboratories in developing countries. Furthermore, the widespread application of NGS tools will at some point render capillary electrophoresis redundant.

### SeqSero 2

SeqSero 2.0 is a new software tool for *Salmonella* serotype determination from WGS data described by Zang et al., (2019). SeqSero 2.0 identifies *Salmonella* serotypes with more precision in comparison to the first edition of SeqSero (Zang et al., 2015). We initially used SeqSero 1.0 and found many strains but with unknown serotypes. When we used SeqSero 2.0 there was a significant improvement in serotyping results (data not shown). Therefore, we can say that SeqSero 2.0 is a very powerful tool for determining serotypes using WGS data. However, this tool must be constantly updated to consider new serotypes that are identified by the Kauffman-White scheme (Kauffmann, 1971). In the present study, SeqSero 2.0 was able to predict 91.1% of the serotypes from the submitted strains. This result agrees with the results found by Banerji et al. (2020); SeqSero 2.0 was able to accurately predict most serotypes but not all. As serotyping based on WGS becomes the new gold stand, it will be important to resolve serotypes to a single correct identification.

SeqSero 2.0 has many advantages for *Salmonella* serotyping and fewer limitations as compared to SMART PCR. SeqSero 2.0 uses assembled whole genomes and returns a result of an antigenic profile with the serotype name in a few minutes. In the present study, we were able to predict 205 *Salmonella* serotypes from 225 submitted (91.11%) using SeqSero 2.0. However, using SMART PCR, only 48 of 225 strains analyzed (21.33%) were assigned a serotype. These findings demonstrate the limitation of SMART PCR assays for *Salmonella* strains isolated outside the U.S. The original SMART codes were created using only clinical isolates from the North Western USA, in this current study there are differences not only in the geographic region but also in using predominantly veterinary and food related isolates which may have a bearing on the significant variation in SMART codes.

Whole genome sequencing is a powerful tool for understanding *Salmonella* epidemiology and distribution of disease. However, sequencing is very expensive, time consuming, and requires data storage capacities and staff with high technical and bioinformatic skills. SeqSero 2 analysis of some isolates provided two possible serotypes sharing the same antigenic profile (or formula) but with differing minor O antigenic factors or in other cases gave no serotype. These unpredicted serovars are either not included in the SeqSero database and could be shared as part of the iterative construction of the serotype database for use in the next version 3.0.
In this study we noticed that SeqSero and SMART PCR can be complementary for the determination of certain serotypes. For example, SMART PCR predicted some isolates as *S. Duesseldorf*, and these same isolates were predicted by SeqSero 2 as *S. Albany* or *Duesseldorf*.

**Conclusions**

*Salmonella* epidemiology is a worldwide public health problem. In this study, the results highlight the accuracy of modern molecular methods and in doing so also the need of less expensive methods for rapid serotyping of *Salmonella* in developing countries. SeqSero 2.0 is very accurate for *Salmonella* serotyping, but WGS is very expensive, especially for LMICs and a role for NGS may be as a shared resource with other needs such as antimicrobial drug resistance, a growing risk to public health with grave consequences. Therefore, researchers should continue developing less expensive and accurate methods of *Salmonella* serotyping that can be accessible worldwide. However, both methods require a clonal culture isolate and so while these molecular tools offer great accuracy, the need for classical microbiology cannot be overlooked in first culturing and identifying the pathogen from its sample matrix.

**Abbreviations**

**SMART**

*Salmonella* Multiplex Assay for Rapid Typing

**PCR**

Polymerase Chain Reaction

**WGS**

Whole Genome Sequencing

**LMICs**

low- and middle-income countries

**NTS**

Non-typhoidal *Salmonella*

**ISO**

International Organization for Standardization

**CDC**

Centers for Disease Control and Prevention

**NGS**

New Generation Sequencing

**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 carried out the strain’s characterization. HR, SC, SBH, TAW participated in phenotypic characterization. LH, AK, SP, SKG carried out the WGS analysis, AK drafted the manuscript. DSB, EAM, LH participated in manuscript writing. CRJ, HK, NB 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

Data provided in this paper including supplementary materials are sufficient to assess the findings of this paper. Additional data of this paper can be obtained upon request.

Consent for publication

Not applicable.

Ethical considerations and consent to participate

Not applicable
Competing interests

The authors declare that they have no competing interests.

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