The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain

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While the spread of *Salmonella enterica* serotype Kentucky resistant to ciprofloxacin across Africa and the Middle-East has been described recently, the presence of this strain in humans, food, various animal species (livestock, pets, and wildlife) and in the environment is suspected in other countries of different continents. Here, we report results of an in-depth molecular epidemiological study on a global human and non-human collection of *S. Kentucky* (*n* = 70). We performed XbaI-pulsed field gel electrophoresis and multilocus sequence typing, assessed mutations in the quinolone resistance-determining regions, detected β-lactam resistance mechanisms, and screened the presence of the *Salmonella* genomic island 1 (SGI1). In this study, we highlight the rapid and extensive worldwide dissemination of the ciprofloxacin-resistant *S. Kentucky* ST198-X1-SGI1 strain since the mid-2000s in an increasingly large number of contaminated sources, including the environment. This strain has accumulated an increasing number of chromosomal and plasmid resistance determinants and has been identified in the Indian subcontinent, Southeast Asia and Europe since 2010. The second substitution at position 87 in GyrA (replacing the amino acid Asp) appeared helpful for epidemiological studies to track the origin of contamination. This global study provides evidence leading to the conclusion that high-level resistance to ciprofloxacin in *S. Kentucky* is a simple microbiological trait that facilitates the identification of the epidemic clone of interest, ST198-X1-SGI1. Taking this into account is essential in order to detect and monitor it easily and to take rapid measures in livestock to ensure control of this infection.

**Keywords:** S. Kentucky, ST198, SGI1, QRDR, MDR *Salmonella* dissemination, poultry

**INTRODUCTION**

Despite the substantial progress made in preventing foodborne diseases, new pathogens have emerged, some of which have spread worldwide decade after decade (Tauxe, 1997). These pathogens include strains of multi-drug resistant (MDR) *Salmonella* (Arlet et al., 2006; Walsh and Fanning, 2008). Their treatment in both animals and humans has become more difficult and the number of reports of foodborne infections and outbreaks of MDR *Salmonella* has increased (Angulo et al., 2000; Mølbak, 2005). The global spread of an MDR *Salmonella enterica* serotype Typhimurium phage type DT104 in animals and humans since the 1990s (Threlfall, 2000; Mather et al., 2013) is a good example.
While the spread of DT104 may have been facilitated by the use of antimicrobials, the national and international trade of infected animals is thought to have played a major role in its spread across borders (Ribot et al., 2002; Weill et al., 2006a).

More recently, an emerging S. Kentucky strain has been described (Weill et al., 2006b; Le Hello et al., 2011) and belonged to the ST198-X1 subtype. It has accumulated various chromosomal resistance determinants since the mid-1990s with the integration of the Salmonella genomic island 1 (SGI1), a 43-kilobase genomic island initially described in DT104 (Boyd et al., 2001), encoding resistance to multiple antimicrobials including amoxicillin, gentamicin, and sulfonamides (Doublet et al., 2008), followed by cumulative mutations in the gyrA and parC genes, leading to resistance to nalidixic acid and then to ciprofloxacin in 2002 (S. Kentucky CIP-R). This population was mostly detected in Egypt before 2005, but has now rapidly spread throughout Africa and the Middle East (Le Hello et al., 2011). Another matter of concern is the expanding livestock reservoir of this S. Kentucky CIP-R strain. It was initially identified in autochthonous poultry but was then found in various animals and foods (contaminated spices in France and the United States of America (US), turkey flocks in Germany and Poland, wild animals, etc) (Le Hello et al., 2011; Beutlich et al., 2012; Münch et al., 2012; Wasyl and Hoszowski, 2012).

Several isolates give rise to considerable concern, as they have become producers of various carbapenemases and/or cephalosporinase and/or extended spectrum β-lactamases (ESBL) (Le Hello et al., 2013).

Since these studies, several reports have mentioned S. Kentucky CIP-R isolates that have been identified from different sources (animals, food, the environment and humans) and geographic locations, in particular in several new countries in the Indian sub-continent and Southeast Asia. The purpose of this study was to examine whether the S. Kentucky strains isolated around the world from different ecosystems belong to this expanding ST198-X1-SGI1 strain.

**MATERIALS AND METHODS**

**COLLECTION OF STRAINS**

Following the publication of the first studies on S. Kentucky ST198-X1-SGI1 CIP-R surveillance (Weill et al., 2006b; Le Hello et al., 2011, 2013), public health agencies in several different regions have notified the same isolation of Salmonella Kentucky from around the world (four continents, 28 countries) covering a long time-span (including preantibiotic era isolates, from 1937 to 2013), various livestock species (swine, turkey, layer, and broiler poultry farms), humans, food (soya bean, meat, seafood, and spices), wild and domestic animals (reptiles, horses, camels, birds, dogs) and the environment (compost and rivers).

**MICROBIOLOGICAL INVESTIGATIONS**

Serotyping was performed on the basis of the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). We carried out antimicrobial susceptibility testing (AST) on all S. Kentucky isolates with the disk diffusion method, for a panel of 32 antimicrobial drugs (Bio-Rad, Marnes-La-Coquette, France). Using Etests (bioMérieux, Marcy l’Etoile, France), we determined the minimal inhibitory concentration (MIC) of ciprofloxacin, as previously described (Le Hello et al., 2013). The results were then interpreted using the breakpoints of the Antibiotic Committee of the French Society for Microbiology (CA-SFM) which implements the EUCAST breakpoints. Resistance to ciprofloxacin is defined as having an MIC of more than 1 mg/L and being susceptible at an MIC of 0.5 mg/L or less, irrespective of isolate source (i.e., intestinal or extraintestinal).

For molecular typing, we performed multilocus sequence typing (MLST) on all S. Kentucky isolates and PulseNet standard pulsed-field gel electrophoresis (PFGE) of Xbal-digested chromosomal DNA on a selection of isolates (n = 50) (Le Hello et al., 2011; Achtman et al., 2012).

To determine the resistance mechanisms, we assessed the presence of β-lactam resistance genes (blaTEM, blaSHV, blaPSE, blaOXA-1–19 group, blaCMY, blaCTX-M, blaOXA-48, blaVIM, blaNDM, and blaKPC), plasmid-mediated quinolone resistance genes (known PMQR genes, qnrA, qnrB, qnrS, qnrD, aac(6)’-Ib-cr, qepA and the recent qepX and qepB), macrolide resistance genes (mphA) and Salmonella genomic island 1 (SGI1) by PCR, as described previously (Le Hello et al., 2013; Li et al., 2013). Variants SGI1-K, P, and Q and J were differentiated in some strains (n = 28) by selected primers, as described in Table 2 and also described previously (Doublet et al., 2008; Le Hello et al., 2012).

The quinolone resistance-determining region (QRDR) of gyrA, gyrB, parC, and parE (encoding subunits of the DNA gyrase and the topoisomerase IV) was sequenced in all S. Kentucky strains as described previously (Le Hello et al., 2013). The nucleotide and deduced amino acid sequences were analyzed and compared with sequences available from the National Center for Biotechnology Information.

**RESULTS**

The Table 1 presents the characteristics of serotype Kentucky isolates and summarizes the results of antimicrobial resistance and genomic typing.
Table 1 | Characteristics of the *S. enterica* serotype Kentucky isolates that constitute the representative Kentucky ST198 collection in this study.

| Numbering | Year of isolation | Country of contamination | Sources | AST<sup>a</sup> | *bla* genes<sup>b</sup> | CIP MIC mg/L | PFGE-type | MLST | SGI<sup>c</sup> | References<sup>d</sup> |
|-----------|-------------------|--------------------------|---------|----------------|---------------------|-------------|-----------|------|--------|----------------------|
| **HISTORICAL STRAINS (FROM 1937 TO 1999)**<br>98K | 1937 | USA | Chicken | Susceptible | – | No | 0.008 | X4 | 198 | Absence | Edwards, 1938<br>1–61 | 1961 | Senegal | Human | Susceptible | – | No | 0.016 | X1d | 198 | Absence | Le Hello et al., 2011<br>1–66 | 1966 | Vietnam | Human | R2 | TEM | No | 0.016 | X18 | 198 | Absence | id<br>1–68 | 1968 | Senegal | Human | SSu | – | No | 0.016 | X1b | 727 | Absence | id<br>2–75 | 1975 | Senegal | Human | Susceptible | – | No | 0.023 | X1b | 198 | Absence | id<br>5–76 | 1976 | France | Soya bean | Susceptible | – | No | 0.008 | X4 | 198 | Absence | id<br>19–85 | 1985 | Egypt | meat | SSpSuC | – | No | 0.016 | X2b | 198 | J6 | Le Hello et al., 2011<br>93–6429 | 1993 | Indonesia | Human | Su | – | No | 0.016 | X2c | 198 | J4 | Le Hello et al., 2012<br>96–11313 | 1996 | Egypt | Human | SSpGSuTe | TEM | No | 0.008 | X1a | 198 | Ks | Le Hello et al., 2011<br>97–6819 | 1997 | Egypt | Human | ASSpSuC | TEM | No | 0.008 | X1a | 198 | Ks | id<br>97–1473 | 1997 | Egypt | Human | R1-Nal | TEM | No | 0.125 | X1k | 198 | Ks | id<br>99–2998 | 1999 | Egypt | Human | HR1-Nal | CMY-2 | Asn | 0.125 | X1t | 198 | Absence | id<br>**RECENT STRAINS (SINCE 2000)**<br>HUMAN<br>00-1059 | 2000 | Egypt | Human | R2-Nal | TEM | No<sup>1</sup> | 0.125 | X1a | 198 | P1 | id<br>01-2100 | 2001 | Egypt | Human | R1-Nal | TEM | No<sup>1</sup> | 0.125 | X1a | 198 | K1 | id<br>02-9666 | 2002 | Egypt | Human | R1-NalCip | TEM | Asn<sup>2</sup> | 8 | X1a | 198 | Ks | id<br>04-4567 | 2004 | Egypt | Human | R1-KTmCNA1Cip | TEM | Gly<sup>2</sup> | 4 | X1g | 198 | K1 | id<br>05-1016 | 2005 | Kenya | Human | R3 | – | Tyr<sup>2</sup> | 4 | X1a | 198 | Q2 | id<br>05-4680 | 2005 | Sudan | Human | SSpGSuTrmNalCip | – | Gly<sup>2</sup> | 4 | X1l | 198 | K4 | id<br>07-1511 | 2007 | Morocco | Human | R2-NalCip | TEM | Asn<sup>2</sup> | 16 | X1a | 198 | Ps | id<br>07-7991 | 2007 | Tunisia | Human | R2-NalCip | TEM | Asn<sup>2</sup> | 12 | X1b | 198 | + | id<br>08-4705 | 2008 | Iran | Human | R3 | – | Asn<sup>2</sup> | 12 | X1a | 198 | + | id<br>08-5707 | 2008 | Tanzania | Human | R1-NalCip | TEM | Tyr<sup>2</sup> | 16 | X1c | 198 | + | id<br>09-8391 | 2009 | Morocco | Human | HR2-NalCip | CMY-2 | Asn<sup>2</sup> | 32 | X1e | 198 | + | Le Hello et al., 2013<br>09-9322 | 2009 | Egypt | Human | HR3-NalCipAzi | TEM-1 + CMY-2 | Gly<sup>2</sup> | 12 | X1w | 198 | + | id<br>2010/00305 | 2010 | Egypt | Human | KCTmPNalCip | – | Gly<sup>2</sup> | 12 | X1w | 198 | + | id<br>2010/00720 | 2010 | Turkey | Human | HR4-NalCip | TEM-1 + CTX-M-1 | Asn<sup>2</sup> | 16 | X1b | 198 | + | id<br>2010/01922 | 2010 | Morocco | Human | HR5-NalCip | TEM-1 + VIM-2 | Gly<sup>2</sup> | 12 | X1m | 198 | + | id<br>2010/05456 | 2010 | Algeria | Human | HR6-NalCipAzi | CTX-M-15 | Asn<sup>2</sup> | 12 | X1a | 198 | Qs | id<br>2010/07071 | 2010 | Cote d’Ivoire | Human | R1-NalCip | TEM | Tyr<sup>2</sup> | 12 | 198 | + | This study<br>2010/07297 | 2010 | Unknown | Human | ASul | PSE-1 | No | 0.016 | 198 | + | This study<br>2010/07503 | 2010 | India | Human | R1-NalCip | TEM | Tyr<sup>2</sup> | 8 | X1b | 198 | Ks | This study

(Continued)
## Table 1 | Continued

| Numbering | Year of isolation | Country of contamination | Sources | AST<sup>a</sup> | blα genes<sup>b</sup> | CIP MIC mg/L | PFGE-type | MLST | SGI<sup>c</sup> | References<sup>d</sup> |
|-----------|-------------------|--------------------------|---------|-----------------|-----------------|-------------|-----------|------|-------------|--------------------------|
| 201/08553 | 2010              | Senegal                  | Human   | R1-NalCip       | TEM             | 12          |           |      | +           | This study               |
| 201/09778 | 2010              | Libya                    | Human   | R1-NalCip       | TEM             | 24          |           |      | +           | This study               |
| 2011/00664| 2011              | Egypt                    | Human   | R2-NalCipAz   | OXA-48          | 8           | X1w       | 198   | +           | Le Hello et al., 2013    |
| 201/01683 | 2011              | India                    | Human   | R1-NalCip       | TEM             | Tyr²       | >32       |      | +           | This study               |
| 2011/01801| 2011              | Mali                     | Human   | HR2            | CMY2            | No          | 0.016     | X3    | 1679        | Absence                  |
| 2011/06349| 2011              | India                    | Human   | R1-NalCip       | TEM             | Tyr²       | 8         |      | +           | This study               |
| 2011/11973| 2011              | Cameroon                 | Human   | R3             |                 | 12          |           |      | +           | This study               |
| 80-11-2275139 | 2011              | India                  | Human   | R1-NalCip       | TEM             | Tyr²       | 6         |      | +           | This study               |
| 80-11-252-4482 | 2011              | Iraq                    | Human   | ASgSulCpTeNalCip | TEM             | Gly²       | 8         |      | +           | This study               |
| 80-11-309-2385 | 2011              | Cambodia                | Human   | ASuTeNalCip    | TEM             | Asn²       | 8         |      | +           | This study               |
| 2012/03/105 | 2012              | Indonesia               | Human   | R1-NalCip       | TEM             | Asn²       | 32        | X1a   | 198         | Ks                       |
| 2012/05/363 | 2012              | Kuwait                  | Human   | ASSpGTeNalCip  | TEM             | Gly²       | 16        | X1l   | 198         | Ks                       |
| 2012/07/374 | 2012              | Vietnam                 | Human   | ASuTeNalCip    | TEM             | Asn²       | 32        | X1c   | 198         | Ks                       |
| 2013/01/062 | 2013              | Algeria                 | Human   | HR7-NalCipAz   | TEM-1 + OXA-48  | Asn²       | 8         | X1b   | 198         | Ks                       |

**RECENT STRAINS (SINCE 2000) HUMAN**

**NON HUMAN**

| Numbering | Year of isolation | Country of contamination | Sources | AST<sup>a</sup> | blα genes<sup>b</sup> | CIP MIC mg/L | PFGE-type | MLST | SGI<sup>c</sup> | References<sup>d</sup> |
|-----------|-------------------|--------------------------|---------|-----------------|-----------------|-------------|-----------|------|-------------|--------------------------|
| BfR 05-04/625 | 2005              | Ethiopia                | Swine   | R3             |                 | 8           | X1a       | 198   | +           | This study               |
| 07AF4403  | 2006              | Ethiopia                | Chicken | R1-NalCip       | TEM             | Gly²       | 12        | X1a   | 198         | Ks                       |
| Em 06/02/339 | 2006              | United Arab Emirates   | Camel   | R1-KNalCip      | TEM             | Gly²       | 8         |      | +           | Münch et al., 2012       |
| Em 07-04654 | 2007              | United Arab Emirates   | Houbara | ASpKTSuTeNalCip | TEM             | Gly²       | 12        |      | +           | id                       |
| 08-KS6  | 2008              | Nigeria                 | Chicken | R1-NalCip       | TEM             | Gly²       | 12        | X1a   | 198         | Ks                       |
| 09-015  | 2009              | Morocco                 | Seafood | R1-TNalCip      | Nd              | Asn²       | 16        | X1d   | 198         | Ks                       |
| 09-8745 | 2009              | Togo                    | Chicken | R1-NalCip       | TEM             | Gly²       | 12        |      | +           | This study               |
| K-50  | 2009              | Bangladesh              | Layer poultry farms | R1-NalCip       | TEM             | Tyr²       | 8         | X1e   | 198         | +                        |
| K-26  | 2009              | Bangladesh              | Layer poultry farms | R1-NalCip       | TEM             | Tyr²       | 16        | X1e   | 198         | +                        |
| BfR 10-02/164 | 2010              | Germany                 | Turkey meat | R1-NalCip      | TEM             | Tyr²       | 8         | X1b   | 198         | Ks                       |
| BfR 10-02/979 | 2010              | Germany                 | Reptile organs | R1-NalCip      | TEM             | Tyr²       | 12        | X1n   | 198         | Ks                       |
| 1090/10 | 2010              | Poland                  | Turkey meat | R2-NalCip      | TEM             | Tyr²       | 16        | X1b   | 198         | +                        |
| 10CEB962 | 2010              | France                  | Compost | R2-NalCip       | TEM             | Asn²       | 12        |      | +           | This study               |
| 10CEB8465 | 2010              | France                  | Côte d’Ivoire | R1-NalCip      | TEM             | Tyr²       | 8         |      | +           | This study               |
| 10CEB748 | 2010              | France                  | Horse placenta | R1-TmpNalCip   | TEM             | Asn²       | 16        |      | +           | This study               |
| 10CEB766 | 2010              | France                  | river    | SSpGSulTeNalCip | –              | Asn²       | 12        |      | +           | This study               |

(Continued)
Table 1 | Continued

| Numbering | Year of isolation | Country of contamination | Sources | AST<sup>a</sup> | bla genes<sup>b</sup> | Asp87 substitution in GyRA | CIP MIC mg/L | PFGE-type | MLST | SGI<sup>c</sup> | References<sup>d</sup> |
|-----------|------------------|--------------------------|---------|---------------|----------------|--------------------------|--------------|-----------|------|----------|----------------|
| NON HUMAN |                  |                          |         |               |                 |                          |              |           |      |          |                |
| B-81      | 2010             | Bangladesh               | Broiler poultry farms | R1-NalCip | TEM            | Tyr<sup>2</sup> | 8           | X1a | 198 | +        | Barua et al., 2013 |
| B-11      | 2010             | Bangladesh               | Broiler poultry farms | R1-TTmpNalCip | TEM        | Tyr<sup>2</sup> | 8           | X1e | 198 | +        | id |
| K-78      | 2010             | Bangladesh               | Layer poultry farms | R1-NalCip | TEM            | Tyr<sup>2</sup> | 8           | X1i | 198 | +        | Barua et al., 2012 |
| 2189/11   | 2011             | Poland                   | Reptile farms       | R2-NalCip | TEM            | Gly<sup>2</sup> | 16          | X1a | 198 | +        | Zając et al., 2013 |
| 11CEB3342 | 2011             | France                   | Spice               | R2-NalCip | TEM-1          | Asn<sup>2</sup> | 12          |     | 198 | +        | This study |
| 11CEB4816 | 2011             | France                   | Marinated turkey meat | R1-NalCip | TEM-1          | Asn<sup>2</sup> | 12          |     | 198 | +        | This study |
| 12CEB716  | 2012             | France                   | Dog                 | R2-NalCip | TEM            | Asn<sup>2</sup> | 8           |     | 198 | +        | This study |
| 12CEB4452 | 2012             | France                   | Turkey farms        | R1-NalCip | TEM-1          | Asn<sup>2</sup> | 16          | X1f | 198 | Ks       | Guillon et al., 2013 |
| 13CEB2160 | 2013             | Poland                   | Turkey meat          | R1-NalCip | TEM            | Tyr<sup>2</sup> | 8           | X1x | 198 | Ks       | This study |

<sup>a</sup>R1: resistance to amoxicillin, A; streptomycin, S; spectinomycin, Sp; gentamicin, G; sulfamethoxazole, Su; and tetracycline, Te.
R2: resistance to A.
R3: no resistance associated with NalCip.

ceftiraxone, Cro; cefazidime, Caz; cefoxitin, Fox; trimethoprim, Tmp; chloramphenicol, C; azithromycin, Azi; imipenem, Imp; kanamycin, K; tobramycin, T; netilmicin, N; amikacin, A; isepamicin, I.

HR1: ACroCazFoxSSuTmpCTe.
HR2: ACroCazFox.
HR3: ACroCazFoxSpKTNCSuTmp.
HR4: ACroSpGSuTe.
HR5: ACroCazFoxImpSpKTNGASuTe.
HR6: ACroCazSpKTNGASuTmp.
HR7: AlmpSpGSuTmpTe.

Nd, not done; --, any bla gene found.

<sup>1</sup>Associated with the gyrA Ser83Phe substitution.
<sup>2</sup>Associated with the gyrA Ser83Phe substitution and parC Ser80Ile substitution.
<sup>3</sup>+ positive for SGI1; Js, Ks, Ps, and Qs, variants of SGI1.
<sup>4</sup>Id, idem, same reference as above.
Molecular Typing

All but one of the 70 S. Kentucky isolates belonged to the e-burst group 56: ST198 and two single locus variants (SLV), ST727 and ST1680. The remaining isolate belonged to a new ST, ST1679, sharing only three loci out of seven (this isolate was susceptible to quinolones and belonged to an X3 PFGE type). We distinguished diverse PFGE types among the ST198 and SLV isolates. The major one, X1 and its many variants (X1a–X1x), representing almost 90% of the ST198 isolates (n = 44), has been associated with strains isolated over the last 50 years, whereas X2 (n = 2/N = 50) has been linked to isolates from Asia isolated before the 2000s and several other patterns for older strains isolated before the 1980s (X4 for the reference strain 98K and strain 5–76 isolated from soya bean, and X18 for a Vietnamese strain, isolated in 1937, 1976, and 1966, respectively). Most common representative PFGE subtypes are shown in Figure 1.

The SGI1-K variants were firstly identified in Kentucky ST198 isolates from Egypt in 1996. Then, the SGI1-Ks and its derivative variants -Ps or -Qs were present in all the ST198 (or its SLV) isolates. As shown here, all the SGI1 variants were independently distributed between periods, countries and sources (Table 1).

Antimicrobial Susceptibility Testing and Resistance Genes

Among the S. Kentucky isolates studied, increased resistance to fluoroquinolones has been observed since the isolation of the first ciprofloxacin-resistant isolate in 2002. As shown in Table 1 ciprofloxacin MICs seem to increase over time, from 0.008 mg/L to 0.125 mg/L during the 1990s, 4 mg/L to 12 mg/L between 2002 and 2006 and since 2007 with the emergence of highly ciprofloxacin resistant strains (16 to 50 mg/L). Apart from quinolone resistance, additional resistance was observed in some S. Kentucky CIP-R isolates (Table 1). The most prevalent drug resistance patterns were R1 (n = 25, 46%), which included resistance to amoxicillin, streptomycin, spectinomycin, gentamicin, sulfamethoxazole, and tetracycline; R2 (n = 8, 15%), which included resistance to amoxicillin; and R3 (n = 4, 7%), which included resistance only to nalidixic acid and ciprofloxacin. Before 2002, these resistance patterns were also described with decreased susceptibility to ciprofloxacin (CIP-DS; MIC, 0.125 mg/L). These patterns and other less frequent ones were all associated with the presence of SGI1-Ks (for R1) and -Ps (for R2), both carrying the blaTEM gene, and -Qs (for R3 which do not carry any drug resistance genes).

More recently, additional resistance to third generation cephalosporins (C3G) and/or carbapenems was observed in CIP-R S. Kentucky X1-ST198-SGI1 isolates. These isolates contained the cephemase blacMY (n = 3), the ESBLs blacCTX–M–1 (n = 1) and blacCTX–M–15 (n = 1), and the carbapenemases blavIM–1 (n = 1) and blaoXA–48 (n = 2). High-level resistance to azithromycin (32 mg/L to 128 mg/L) was found in four of them which carried the phosphotransferase mphA gene known to inactivate macrolide antimicrobial drugs.

In addition to the ST198-X1 SGI1-Ks, -Ps, and -Qs isolates, we observed other drug-resistant S. Kentucky ST198 populations in our collection. Initially, we identified ST198-X2, which carried SGI-Js and was isolated in Asia before the 2000s (see Le Hello et al., 2012); secondly, we identified here for the first time a putative SGI1-B variant carrying S. Kentucky ST198 isolate (no. 2010/07297) which contains a blapse–1 gene found occasionally in other serotypes such as Typhimurium DT104 and Paratyphi B d-tartrate fermenting (Boyd et al., 2002; Weill et al., 2005). Lastly, an SGI1 free isolate susceptible to nalidixic acid and producing the CMY-2 cephamycinase was acquired in Mali in 2011 (no. 2011/01801).
TEMPORAL AND GEOGRAPHIC DISTRIBUTION OF S. KENTUCKY ISOLATES WITH QRDR MUTATIONS

As shown in Table 1, ciprofloxacin resistance in all the 54 CIP-R S. Kentucky isolates was related to GyrA and ParC substitutions (Table 1). All contained double substitutions in GyrA (at codons Ser83 and Asp87) and a single ParC substitution (Ser80 encoding an isoleucine residue). None of the isolates contained GyrB or ParE modifications. In GyrA, all the isolates contained phenylalanine at codon Ser83, whereas mutations in codon Asp87 resulted in different substitutions to asparagine (Asn), tyrosine (Tyr), or glycine (Gly) residues depending on the geographic origin of the isolates (Figure 2). All the Egyptian isolates presented three possible mutations in codon Asp87 (n = 6), whereas the isolates from North Africa (Morocco, Algeria, Tunisia, Libya) had a modification that resulted in Asp87Asn (n = 7). An exception was the VIM-2 producing Kentucky 2010/01922 isolate from Morocco which had Asp87Tyr. Those from the Middle East (Iraq, United Arab Emirates, Kuwait, Turkey, and Iran) presented both Asp87Gly and Asp87Asn, apart from two nonhuman Ethiopian isolates and one Sudanese human isolate in 2005 that had an Asp87Gly substitution; those from East Africa, India and Bangladesh had an Asp87Tyr modification (n = 12). Apart from the two strains from the Ivory Coast which presented Asp87Tyr, those from West Africa presented Asp87Gly (n = 3). All three isolates from Southeast Asia (Cambodia, Indonesia and Vietnam) had an Asp87Asn amino acid change.

Isolates from food, animal feed and the environment isolated in Europe presented an intermediate situation with isolates mostly having an Asp87Tyr residue in Germany and Poland and an Asp87Asn residue in France.

No PMQR genes, such as qnr, aac(6′)-Ib-cr, qepA and oqxAB were detected in S. Kentucky CIP-R isolates of this study.

NONHUMAN SOURCES OF THE S. KENTUCKY ST198-X1-SGI1 STRAIN

The nonhuman isolates of S. Kentucky ST198-X1-SGI1 have mainly been found in poultry farms (chicken, layer and turkey, n = 14) since its first description in a chicken from Ethiopia in 2006 (Le Hello et al., 2011). This strain has further been described in poultry flocks in East, West and North Africa, the Indian subcontinent and Europe. It has also been described in food products (seafood, meat, or spice), domestic animals (dog, horse, camel, or pet reptiles) and wild animals (houbara or reptiles) and various environments (river or compost) (see Table 1).

DISCUSSION

By gathering and studying this global collection, we confirmed that one strain has disseminated throughout the developing countries in both human and nonhuman sources. Strain ST198-X1 displays high-level resistance to ciprofloxacin and harbors SGI1-Ks or its derivative variants -Ps or -Qs. The high-diversity of antibiotic resistance patterns could be related to genetic rearrangements mediated by various insertion sequences (in particular IS26) and transposons in SGI1-Ks, -Ps, and -Qs (Doublet et al., 2008). In parallel, contrary to the relatively few PFGE patterns associated with S. Typhimurium DT104, S. Kentucky ST198 displayed high-diversity in X1 subtypes, suggesting frequent events of genomic rearrangements present in SGI1 or the acquisition/loss of various plasmids. Several other ST198 strains belonging to other PFGE subtypes and/or containing different antibiotic resistance mechanisms have been isolated sporadically or have no longer been isolated since the 2000s (Le Hello et al.,...
The odyssey of the S. Kentucky ST198-X1 isolate has lasted since it was found in Egypt in the mid-1990s. It has since then accumulated various chromosomal resistance determinants, with the integration of SG1 (encoding resistance to multiple antimicrobial drugs), followed by cumulative mutations in the gyra and parC genes, leading to resistance to nalidixic acid, and then to ciprofloxacin in 2002. Since 2002, this strain has spread rapidly throughout Africa and the Middle East (Le Hello et al., 2011) and, in 2009, it was identified in India in travelers and in Bangladesh in poultry flocks (Barua et al., 2012, 2013). Furthermore, the first description of SG1 variant K was identified in a Salmonella serotype Kentucky strain isolated in 2001 from spices imported into Australia from India (Levings et al., 2007). Since 2011, a pattern of propagation across Asia is also suggested by the recent recovery of ciprofloxacin-resistant S. Kentucky isolates from stool samples of patients in France and Australia with a history of recent travel to Vietnam, Cambodia and Indonesia. This global collection also makes it possible to confirm the recent increase of S. Kentucky ST198-X1-SG1 CIP-R clinical strains that have acquired additional genes, making them resistant to extended spectrum cephalosporins and/or carbapenems, in particular in the Mediterranean basin (Collard et al., 2007; Le Hello et al., 2013). High-level resistance to azithromycin due to the acquisition of the mphpA gene among these isolates is of concern as this antimicrobial agent is presented as a good alternative treatment for severe Salmonella infections (Hill and Beeching, 2010) and it completes the variety of enteric bacteria already described producing this phosphotransferase (Boumghar-Bourtchai et al., 2008). Last but not least, as shown in this study, ciprofloxacin MIC has increased decade after decade in S. Kentucky isolates. The recent increase in MIC is not due to additional mutations in QRDR, nor to the presence of PMQR. However, the increase could be due to the overexpression of an efflux system such as AcrAB-ToLC (Baucheron et al., 2013).

Another matter of concern is the expanding livestock reservoir of this S. Kentucky ST198-X1-SG1 CIP-R strain, initially identified in African autochthonous poultry but subsequently found in various animals and food (Le Hello et al., 2011; Barua et al., 2012, 2013; Beutlich et al., 2012; Münch et al., 2012; Wasyl and Hoszowski, 2012). Poultry flocks have contributed to the global dissemination of this clone in developing countries since 2005. Hence this strain was isolated in Ethiopia in 2006 (chicken), Nigeria (chicken), and Morocco (turkey) in 2008 (Le Hello et al., 2011), in Togo (chicken) in 2009, and from layers in 2009, from broilers in 2010 in Bangladesh (Barua et al., 2012, 2013), and in developed countries since 2010 (Poland, Wasyl and Hoszowski, 2012; Germany, Beutlich et al., 2012; and France, Guillon et al., 2013). Interestingly, the S. Kentucky ST198-X1-SG1 CIP-R identified in Europe was exclusively associated with turkeys. An investigation following the epidemics in Poland (Wasyl and Hoszowski, 2012) has established that at least one of the infected flocks was hatched from eggs imported from the Middle East. The diversity of nonhuman sources described in this study is another reason of concern. In particular, the description of S. Kentucky ST198-X1-SG1 CIP-R in the environment (rivers and compost) and animals such as reptiles, indicates its potential long-term presence, suggesting its capacity to produce biofilm, as was previously identified in Tunisian isolates (Turki et al., 2012). This intrinsic characteristic could possibly facilitate secondary contaminations and persistence into a novel host/source but this remains to be investigated.

Regarding its recent establishment in Bangladesh (18% of 500 farms were S. Kentucky positive during the period 2009–2010), we do not have any information on how S. Kentucky ST198-X1-SG1 CIP-R was introduced in poultry, although locally produced fish meal used as a protein source has been associated with the presence of Salmonella in flocks (Barua et al., 2012, 2013). Small poultry farmers purchase this raw ingredient from the local market to produce low-cost feed by mixing it with other ingredients. Meat and bone meal imported from different countries are also used for preparing poultry and fish feed as a source of protein. Regarding broiler chicks, they are supplied from commercial breeding farms. Further investigations are necessary to obtain information on locally produced poultry feed. The microbiological characteristics of Kentucky ST198-X1-SG1 CIP-R isolated from poultry farms in Bangladesh indicated that the amino acid substitution in codon 87 of Gyra was exclusively a tyrosine residue like that found in Kentucky for India, East Africa, Egypt and in some Middle East countries. We do not know whether humans play any role in the introduction of this strain in various flocks. Regarding this, it is noteworthy that several million emigrants from Bangladesh work in the Middle East. The human role in the contamination of livestock by S. Kentucky ST198-X1-SG1CIP-R has been pinpointed recently in local turkey flocks in France. The epidemiological investigation performed highlighted the introduction of this strain following the return of farmers from Morocco, a country where this strain is endemic, and suffering from diarrhea (Guillon et al., 2013). This hypothesis may be further reinforced as this isolate (12CEB4452) presented Gyra Asp87Asn substitution which is clearly associated with the North African S. Kentucky ST198-X1-SG1 CIP-R, while isolate 13CEB2160, found in turkey meat sold in France but imported from Poland, presented tyrosine substitution, a characteristic described more frequently in Polish turkey flocks.

In the era of globalized food supply, livestock, and international human travel, the ciprofloxacin-resistant S. Kentucky ST198-X1-SG1 strain is not restricted to one country, rendering measures to subject livestock to controls more difficult. National and international health, food, and agricultural authorities should include it among the strains targeted in national programs to control Salmonella spp in poultry. Based on different studies, including this one, the S. Kentucky ST198-X1-SG1 epidemic clone of interest can nowadays easily be identified by simple serotyping (Kentucky) and by testing its susceptibility in vitro to ciprofloxacin (disk diffusion or MIC). It is essential to consider this strain at both national and international level, in order to take preventive measures as soon as possible to limit its worldwide propagation. Like this, we suggest including this emerging Salmonella in the European Union’s list of target serotypes for mandatory monitoring (EU Commission regulations No.200/2012 of 8 March 2012).
AUTHOR CONTRIBUTIONS

Simon Le Hello and François-Xavier Weill conceived and designed the experiments, analyzed the data, and wrote the report. Amany Bekhit, Lucile Sontag, Laetitia Fabre, Martine Garnier, and Véronique Guibert performed the experiments. The other Kentucky working group authors participated in the continuous monitoring of Kentucky strains, sent materials, participated in the discussion and reviewed the manuscript.

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REFERENCES

Achtman, M., Wain, J., Weill, F.-X., Nair, S., Zhou, Z., Sanga1, V., et al. (2012). Multilocus sequence typing as a replacement for serotyping in Salmonella enterica. PLoS Pathog. 8:e1002776. doi: 10.1371/journal.ppat.1002776

Angulo, F. J., Johnson, K. R., Tauxe, R. V., and Cohen, M. L. (2000). Origins and consequences of antimicrobial-resistant nontyphoidal Salmonella: implications for the use of fluoroquinolones in food animals. Microbiol. Drug Resist. 6, 77–83. doi: 10.1089/mdr.2000.6.77

Arlet, G., Barret, T. J., Butaye, P., Cloeckaert, A., Mulvey, M. R., and White, D. G. (2006). Salmonella resistant to extended-spectrum cephalosporins: prevalence and epidemiology. Microbes Infect. 8, 1945–1954. doi: 10.1016/j.micinf.2005.12.029

Barua, H., Biswas, P. K., Olsen, K. E. P., and Christensen, J. P. (2012). Prevalence and characterization of motile Salmonella in commercial layer poultry farms in Bangladesh. PLoS ONE 7:e32914. doi: 10.1371/journal.pone.0032914

Barua, H., Biswas, P. K., Olsen, K. E. P., Shil, S. K., and Christensen, J. P. (2013). Molecular characterization of motile serovars of Salmonella enterica from breeder and commercial broiler poultry farms in Bangladesh. PLoS ONE 8:e57811. doi: 10.1371/journal.pone.0057811

Baucherou, S., Le Hello, S., Doublet, B., Giraud, E., Weill, F.-X., and Cloeckaert, A. (2013). ram1 mutations affecting fluoroquinolone susceptibility in epidemic multidrug-resistant Salmonella enterica serovar Kentucky ST198. Front. Microbiol. 4:213. doi: 10.3389/fmicb.2013.00213

Beutlich, J., Guerra, B., Schroeter, A., Arvand, M., Szabo, I., and Helmuth, R. (2012). [Highly ciprofloxacin resistant Salmonella enterica serovar Kentucky isolated in turkey meat and a human patient]. Berl. Munch. Tierarztl. Wochenchr. 125, 89–95. doi: 10.2336/007-9366-125-89

Boumghar-Bourtchai, L., Mariani-Kurkdjian, P., Bingen, E., Filliol, I., Dhalluin, A., and Guibert, V. (2008). The French National Reference Center for Salmonella enterica serotype Kentucky monitored in commercial layer poultry farms in Southeast Asia harbor Salmonella genomic island 1-J variants with a novel insertion sequence. Antimicrob. Agents Chemother. 56, 5096–5102. doi: 10.1128/AAC.00732-12

Le Hello, S., Weill, F.-X., Guibert, V., Pradl, K., Cloeckaert, A., and Doublet, B. (2012). Early strains of multidrug-resistant Salmonella enterica serovar Kentucky sequence type 198 from Southeast Asia harbor Salmonella genomic island 1-J. Antimicrob. Agents Chemother. 53, 317–323. doi: 10.1128/AAC.01229-06

Le Hello, S., Weill, F.-X., Guibert, V., Pradl, K., Cloeckaert, A., and Doublet, B. (2012). Prevalence, serovars, phage types, and antibiotic susceptibilities of Salmonella enterica serotype Kentucky ST198 resistant to ciprofloxacin. J. Infect. Dis. 204, 675–684. doi: 10.1093/infdis/jir409

Le Hello, S., Hendriksen, R. S., Doublet, B., Fisher, I., Nielsen, E. M., Whichard, J. M., et al. (2011). International spread of an epidemic population of Salmonella enterica serotype Kentucky ST198 resistant to ciprofloxacin. J. Infect. Dis. 204, 675–684. doi: 10.1093/infdis/jir409

Le Hello, S., Hendriksen, R. S., Doublet, B., Fisher, I., Nielsen, E. M., Whichard, J. M., et al. (2011). International spread of an epidemic population of Salmonella enterica serotype Kentucky ST198 resistant to ciprofloxacin. J. Infect. Dis. 204, 675–684. doi: 10.1093/infdis/jir409

Baucheron, S., Le Hello, S., Doublet, B., Giraud, E., Weill, F.-X., and Cloeckaert, A. (2013). ram1 mutations affecting fluoroquinolone susceptibility in epidemic multidrug-resistant Salmonella enterica serovar Kentucky ST198. Front. Microbiol. 4:213. doi: 10.3389/fmicb.2013.00213

Beutlich, J., Guerra, B., Schroeter, A., Arvand, M., Szabo, I., and Helmuth, R. (2012). [Highly ciprofloxacin resistant Salmonella enterica serovar Kentucky isolated in turkey meat and a human patient]. Berl. Munch. Tierarztl. Wochenchr. 125, 89–95. doi: 10.2336/007-9366-125-89

Mather, A. E., Reid, S. W., Maskell, D. J., Parkhill, J., Fookes, M. C., Harris, S. R., et al. (2013). Distinguishable epidemics of multidrug-resistant Salmonella Typhimurium DT104 in different hosts. Science 341, 1514–1517. doi: 10.1126/science.1240578

Molbak, K. (2005). Human health consequences of antimicrobial drug-resistant Salmonella and other foodborne pathogens. Clin. Infect. Dis. 41, 1613–1620. doi: 10.1086/467599

Münch, S., Braun, P., Wernery, U., Kinne, J., Pees, M., Flieger, A., et al. (2008). Multilocus sequence typing as a replacement for serotyping in Salmonella enterica serovar Agona. J. Antimicrob. Chemother. 61, 1613–1620. doi: 10.1111/j.1365-2958.2007.05469.x

München, S., Braun, P., Wernery, U., Kinne, J., Pees, M., Flieger, A., et al. (2012). Prevalence, serovars, phage types, and antibiotic susceptibilities of Salmonella strains isolated from animals in the United Arab Emirates from 1996 to 2009. Trop. Anim. Health Prod. 44, 1725–1738. doi: 10.1007/s11250-012-0130-4

Ribot, E. M., Wierzba, R. K., Angulo, F. J., and Barrett, T. J. (2002). Salmonella enterica serotype Typhimurium DT104 isolated from humans, United States, 1985, 1990, and 1995. Emerging Infect. Dis. 8, 387–391. doi: 10.3201/eid0804.010202

Tauxe, R. V. (1997). Emerging foodborne diseases: an evolving public health challenge. Emerg. Infect. Dis. 3, 425–434. doi: 10.3201/eid0304.970403

Threlfall, E. J. (2000). Epidemic Salmonella typhimurium DT 104–a truly international multiresistant clone. J. Antimicrob. Chemother. 46, 7–10. doi: 10.1093/jac/dkf046.17

Turki, Y., Ouzari, H., Merhi, L., Ben Aissa, R. and Hassen, A. (2012). Biofilm formation, virulence gene and multi-drug resistance in Salmonella Kentucky isolated in Tunisia. Food Res. Int. 45, 940–946. doi: 10.1016/j.foodres.2011.05.031

Walsh, C., and Fanning, S. (2008). Antimicrobial Resistance in Foodborne Pathogens – A Cause for Concern? Curr. Drug Targets. 9, 808–815. doi: 10.2174/138945008785747761

Vasyl, D., and Hozowski, A. (2012). First isolation of ESBL-producing Salmonella and emergence of multiresistant Salmonella Kentucky in turkey in Poland. Food Res. Int. 45, 958–961. doi: 10.1016/j.foodres.2011.07.024

Weill, F.-X., Guibert, V., Weill, F.-X., Guibert, V., Timimouni, M., Demartin, M., Polomack, L., et al. (2006a). Multidrug resistance in Salmonella enterica serotype
Typhimurium from humans in France (1993 to 2003). J. Clin. Microbiol. 44, 700–708. doi: 10.1128/JCM.44.3.700-708.2006

Weill, F.-X., Bertrand, S., Guesnier, F., Baucheron, S., Cloeckaert, A., and Grimont, P. A. (2006b). Ciprofloxacin-resistant Salmonella Kentucky in travelers. Emerg. Infect. Dis. 12, 1611–1612. doi: 10.3201/eid1210.060698

Weill, F.-X., Fabre, L., Grandry, B., Grimont, P. A. D., and Casin, I. (2005). Multiple-antibiotic resistance in Salmonella enterica serotype Paratyphi B isolates collected in France between 2000 and 2003 is due mainly to strains harboring Salmonella genomic islands 1, 1-B, and 1-C. Antimicrob. Agents Chemother. 49, 2793–2801. doi: 10.1128/AAC.49.7.2793-2801.2005

Zająć, M., Wasyl, D., Hoszowski, A., Le Hello, S., and Szulowski, K. (2013). Genetic lineages of Salmonella enterica serovar Kentucky spreading in pet reptiles. Vet. Microbiol. 166, 686–689. doi: 10.1016/j.vetmic.2013.07.023

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