Food-borne diseases caused by zoonotic *Salmonella enterica* species represent an important public health problem worldwide. In the United States, there are an estimated 1.4 million *Salmonella* infections per year, and approximately 600 are fatal (27). Among the more than 2,500 serotypes of the genus *Salmonella* described to date (30), two, Enteritidis and Typhimurium, are predominant in many developed countries. *S. enterica* serotype Typhimurium was the second most prevalent serotype (Enteritidis ranked first) in Europe during the period 1998 to 2003 (13). It has a large animal reservoir, including farm animals, pets, and wild animals. Although most *Salmonella* infections cause mild diseases (gastroenteritis), life-threatening infections (e.g., bacteremia) may occur, particularly in cases involving patients at the extremes of age or those who are immunocompromised. An appropriate antimicrobial drug therapy is necessary in these severe infections. Two antimicrobial classes are used for that purpose, ciprofloxacin and, when resistant to ESC due to plasmid-mediated class A extended-spectrum β-lactamase (ESBL) belonging to the TEM (TEM-3, TEM-52, and TEM-131), SHV (SHV-2, SHV-5, and SHV-9), CTX-M (CTX-M-2 to CTX-M-7 and CTX-M-15), or PER (PER-1 and PER-2) family have been reported throughout the world since 1988 (5, 21, 28, 42). More recently, serotype Typhimurium isolates producing the plasmidic class C cephaprinase CMY (CMY-2 and, to a lesser extent, CMY-7) have been described in various countries (28).

Emergence of serotype Typhimurium strains that are resistant to ESC due to plasmid-mediated class A extended-spectrum β-lactamase (ESBL) belonging to the TEM (TEM-3, TEM-52, and TEM-131), SHV (SHV-2, SHV-5, and SHV-9), CTX-M (CTX-M-2 to CTX-M-7 and CTX-M-15), or PER (PER-1 and PER-2) family have been reported throughout the world since 1988 (5, 21, 28, 42). More recently, serotype Typhimurium isolates producing the plasmidic class C cephaprinase CMY (CMY-2 and, to a lesser extent, CMY-7) have been described in various countries (28).

The objectives of this study were to determine the antimicrobial resistance profiles seen among a representative sample of 320 serotype Typhimurium strains isolated from humans in 2002 through the French National Reference Center for *Salmonella* (NRC-Salm) network and to describe the distribution of phage types and pulsed-field gel electrophoresis (PFGE) types. The molecular characterization of genes coding
for resistance to ampicillin and to quinolones and the detection of class 1 integrons by PCR were also performed. The trends in the evolution of antimicrobial resistance of serotype Typhimurium strains isolated from humans in France were reviewed by using NRC-Salm data from 1993, 1997, 2000, and 2003.

**MATERIALS AND METHODS**

**Bacterial strains.** In 2002, a total of 11,775 serotyped *S. enterica* isolates from humans were registered at the NRC-Salm (6,636 isolates received at NRC-Salm for serotyping and 5,139 laboratory-confirmed cases reported to the NRC-Salm by its network). The NRC-Salm network comprises approximately 1,500 voluntary hospital or private clinical laboratories representing approximately 30% of all French clinical laboratories.

The present study was conducted on a sample of 320 serotype Typhimurium isolates collected from humans and received at the NRC-Salm in 2002. The isolates (one per patient) were randomly selected from various geographic regions of France from January to December.

*Escherichia coli* ATCC 25922 was used as a control in the disk diffusion method and in MIC determinations. *S. enterica* serotype Braenderup H9812 was used as a molecular size marker.

**Serotyping.** Isolates were serotyped on the basis of somatic O and phase 1 and phase 2 H flagellar antigens by agglutination tests with antisera (Bio-Rad, Marnes la Coquette, France, and WHO Collaborative Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France) as specified by the White-Kauffmann-Le Minor scheme (30).

**Phage typing.** Phage typing of serotype Typhimurium isolates was done following a standardized methodology using 31 phage suspensions (1). Five additional typing phages (2, 3, 18, 10, and 10 variant) were also used. Phage suspensions and the interpretive guide were kindly provided by the Health Protection Agency, Colindale, United Kingdom.

**Pulsed-field gel electrophoresis.** PFGE using XbaI (Amersham Biosciences, Freiburg, Germany) was carried out with a CHEF-DR III system (Bio-Rad) as described previously (44). The running conditions were 6 V/cm at 14°C for 20 h with pulse times ramped from 2.2 to 63.8 s. XbaI-digested plugs of *S. enterica* serotype Braenderup H9812 were used as molecular size markers.

**Antimicrobial susceptibility testing.** Antibiotic susceptibility was determined by disk diffusion on Mueller-Hinton agar according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (35). The following antibiotics (Bio-Rad) were tested: amoxicillin (A), amoxicillin-clavulanic acid, ticarcillin, ticarcillin-clavulanic acid, pipercillin, pipercillin-tazobactam, cephalothin, cefamandole, cefoperazone, cefotaxim, ceftazidime, cefepime, aztreonam, moxalactam, imipenem, streptomycin (S), spectinomycin (Sp), kanamycin (K), tobramycin (To), netilmicin, gentamicin (G), amikacin, isepamicin, nalidixic acid (NaI), pefloxacin, ciprofloxacin (Cip), sulfonamides (Su), trimethoprim (Tmp), chloramphenicol (Chl), and tetracycline (T).

**Multidrug resistance.** Multidrug resistance was defined as isolates being resistant to ≥2 separate classes of antibiotics.

**PCR amplification and DNA sequencing.** Total DNA was extracted using the InstaGene matrix kit (Bio-Rad) in accordance with the manufacturer’s recommendations. Table 1 lists the oligonucleotide primers synthesized by MWG-Biotech (Ebersberg, Germany). Amplification of antibiotic resistance genes, the *bla*<sub>TEM</sub>, *bla*<sub>PSE-1</sub>, *bla*<sub>OXA-1</sub> group and *bla*<sub>SHV</sub>, was performed on all isolates resistant to amoxicillin. Amplifications of the regions (QRDR) of *gyrA* and the plasmid-mediated *qnrA* gene were performed on all isolates resistant to nal. Amplifications of the QRDR of *gyrB* and *parC* were performed on the isolate highly resistant to Cip. Amplifications of left and right junctions of SGI1 with the chromosome were performed on all isolates positive for the *bla*<sub>OXA-1</sub> group gene. Amplification of the gene cassette of the class 1 integron was performed by using 5′-CS and 3′-CS primers on all isolates.
resistant to Su. Mapping of class 1 integrons by using the A (5′-CS and OXA-1-R) and B (aadA1-F and 3′-CS) sets of primers was performed on all isolates positive for the blaOXA-5 gene. All amplifications were performed on 50-μl samples containing DNA (2.5 μl), primers (50 pmol each), deoxynucleoside triphosphate (200 μM), Taq DNA polymerase (1.25 U AmpliTaq Gold; Roche) and its buffer, MgCl₂ (2 mM), and dimethyl sulfoxide (10%). The cycling conditions included 10 min of denaturation at 94°C (1 cycle) and 30 s of denaturation at 94°C, 30 s of annealing at 50°C (for blaOXA, 54°C for qnrA, and 55°C for class 1 integrons and left and right junctions of SGI1), and 1 min (2 min for class 1 integrons) of polymerization at 72°C (35 cycles; 30 cycles for class 1 integrons), followed by 10 min of extension at 72°C.

Sequencing of purified amplicons was performed on both strands by Genome Express (Meylan, France) using an ABI 100 DNA sequencer (Applied Biosystems, Foster City, CA).

The nucleotide sequence was analyzed with Lasergene software (Dnastar, Madison, WI). The BLASTN program of NCBI (http://www.ncbi.nlm.nih.gov) was used for database searches.

RESULTS

S. enterica serotype Typhimurium isolates from humans, NRC-Salm, France, 1993 to 2003. A total of 168,034 Salmonella isolates from humans were registered at the NRC-Salm from 1993 to 2003. Serotypes Enteritidis and Typhimurium represented 35% (58,766 isolates) and 32.5% (54,551 isolates) of all the Salmonella isolates, respectively. During the periods 1993 to 1994 and 1998 to 2003, serotype Typhimurium was the second most prevalent serotype (after Enteritidis), whereas between 1995 and 1997, it ranked first (Fig. 1).

In 2002, of the 11,775 Salmonella isolates (belonging to 232 serotypes) registered at the NRC-Salm, 4,469 (38%) were serotype Enteritidis and 3,998 (34%) were serotype Typhimurium. Among the 3,998 registered isolates of serotype Typhimurium, 1,756 isolates were serotyped at the NRC-Salm and 2,242 were serotyped locally and reported to the NRC-Salm.

Antimicrobial susceptibility testing. The antibiotics against which the 320 serotype Typhimurium isolates tested demonstrated the highest levels of resistance in 2002 were tetracycline (71%), sulfonamides (68%), amoxicillin (64.7%), streptomycin (64.5%), spectinomycin (59%), and chloramphenicol (57%) (Table 2). A single isolate resistant to ESC (MIC of cefazidime, >256 mg/liter; MIC of ceftriaxone, 128 mg/liter) which exhibited an ESBL phenotype was detected. Another isolate (HRC) was highly resistant to Cip (MIC > 32 mg/liter). No isolates were resistant to amikacin or imipenem. The most common multiple antibiotic resistance pattern (R type) was resistance to amoxicillin, chloramphenicol, streptomycin and spectinomycin, sulfonamides, and tetracycline (R type ACSSpSuT), with 156 isolates (48.8%). The number of isolates that were pansusceptible was 69 (21.5%). Single resistance to tetracycline was found in 27 isolates (8.5%). R types ASSuT, ACSSpSuTn (with reduced susceptibility to ciprofloxacin; MIC range, 0.25 to 0.5 mg/liter), and ACSSpSuTTmp were found in 12 (3.8%), 12, and 10 (3%) isolates, respectively. Other R types found in less than 10 isolates are indicated in Table 3. Tables 2 and 4 show the percentages of resistance to individual antimicrobials and the distribution of R types commonly associated with the DT104 clone among samples of serotype Typhimurium isolates collected from humans by NRC-Salm in 1993, 1997, 2000, and 2003.

Phage typing. Of the 320 serotype Typhimurium isolates phage typed, 169 (52.8%) were DT104, 16 (5%) were DT120 or DT8, 13 (4.1%) were atypical DT104 (DT104at), 11 (3.4%) were DT12 or DT14, and 8 (2.5%) were RDNC (reacts but does not conform to the scheme) or untypeable; 28 other phage types (including two atypical phage types, U302at and 12var) were identified at low frequency (in less than 10 isolates) among the remaining 71 isolates (Table 3).

Among the 69 pansusceptible isolates, there were 20 different phage types (including atypical phage types, U302at and 12var) that were identified at low frequency (in less than 10 isolates) among the remaining 71 isolates (Table 3).

Among the 156 isolates that were R type ACSSpSuT, 135

FIG. 1. Distribution of Salmonella serotypes reported by the NRC-Salm in humans in France from 1993 to 2003.


<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>1993 (n = 280; N = 1,595)</th>
<th>1997 (n = 205; N = 2,801)</th>
<th>2000 (n = 320; N = 1,613)</th>
<th>2002 (n = 320; N = 1,756)</th>
<th>2003 (n = 100; N = 1,489)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>54.3 (48.5–60.1)</td>
<td>66.3 (59.8–72.8)</td>
<td>64.3 (59.1–69.5)</td>
<td>64.7 (59.5–69.9)</td>
<td>62.0 (52.5–71.5)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3 (0–0.9)</td>
<td>0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>53.9 (48.1–59.7)</td>
<td>65.9 (59.4–72.4)</td>
<td>71.8 (66.9–76.7)</td>
<td>64.5 (59.3–63.7)</td>
<td>57.0 (47.3–66.7)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>3.2 (1.1–5.3)</td>
<td>1.0 (0–2.4)</td>
<td>1.3 (0.1–2.5)</td>
<td>0.9 (0–1.9)</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.4 (0–1.1)</td>
<td>1.0 (0–2.4)</td>
<td>0.9 (0–1.9)</td>
<td>0.3 (0–0.9)</td>
<td>0</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>3.2 (1.1–5.3)</td>
<td>2.9 (0.6–5.2)</td>
<td>10.3 (7.0–13.6)</td>
<td>4.0 (1.9–6.1)</td>
<td>1.0 (0–3.0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3 (0–0.9)</td>
<td>0</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>58.2 (52.4–64.0)</td>
<td>68.8 (62.5–75.1)</td>
<td>69.6 (64.6–74.6)</td>
<td>68.0 (60–72)</td>
<td>64.0 (54.6–73.4)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>11.8 (8.0–15.6)</td>
<td>5.9 (2.7–9.1)</td>
<td>8.7 (5.6–11.8)</td>
<td>5.3 (2.8–7.8)</td>
<td>8.0 (2.7–13.3)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>43.6 (37.8–49.4)</td>
<td>60.0 (53.3–66.7)</td>
<td>59.0 (53.6–64.4)</td>
<td>57.0 (51.6–62.4)</td>
<td>46.0 (36.2–55.8)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>69.6 (64.2–75.0)</td>
<td>82.0 (76.7–85.3)</td>
<td>81.2 (76.9–85.5)</td>
<td>71.0 (66.0–76.0)</td>
<td>67.0 (57.8–76.2)</td>
</tr>
</tbody>
</table>

* n, number of Salmonella isolates studied; N, number of Salmonella isolates received at NRC-Salm.

"Downloaded from http://jcm.asm.org/ on August 29, 2017 by guest"
Among the 23 isolates that were R type ACSSpSuT with additional resistance to Nal (n = 12), to Tmp (n = 9), to K (n = 1), or to KToG (n = 1), 20 (87%) were DT104, 2 (8.7%) were DT120, 1 (4.3%) was DT12, and 1 other was RDNC.

Among the 169 DT104 isolates, 135 (79.9%) exhibited R type ACSSpSuT; 4 (2.4%) were pansusceptible; and 1 (2.3%) exhibited R type ACSSpKSuT. Of the 46 isolates of R type ACSSpSuT tested, profile X1 was found in 28 (61%); X8 in 10 (21.7%); X3 and X6 each in 2 (4.3%); and X2, X4, X7, and X12 in 1 (2.2%) isolate. Of the nine isolates tested that were R type ACSSpSuT with additional resistance to Nal, profile X1 was found in six (66.6%) and profiles X2, X6, X7, and X11 in 1 (11.1%) isolate. Among 30 susceptible isolates, profile X1 was found in 28 (93.3%); X8 in 3 (10%); X3 in 1 (3.3%); and X2, X4, X7, and X12 in 1 (3.3%) isolate.

(86.5%) were DT104, 8 (5.1%) were DT120, 7 (4.5%) were U302, 3 (1.9%) were untypeable, 2 (1.3%) were DT204c, 1 (0.6%) was DT41, and 1 other was RDNC.

Among the 51 DT104 isolates tested, profile X1 was found in 38 (74.5%); X8 in 9 (17.5%); and X2, X4, X7, and X12 in 1 (2.3%) isolates.

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<tr>
<th>Antimicrobial resistance profile</th>
<th>1993 (n = 280; N = 1,593)</th>
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<th>2003 (n = 100; N = 1,489)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS[Sp]SuT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.3 (28.7–39.9)</td>
<td>54.6 (47.8–61.4)</td>
<td>50.9 (45.4–56.4)</td>
<td>48.8 (43.3–53.3)</td>
<td>43.0 (33.3–52.7)</td>
</tr>
<tr>
<td>ACS[Sp]SuTNaT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>1.5 (0–3.2)</td>
<td>3.8 (1.7–5.9)</td>
<td>3.8 (1.7–5.9)</td>
<td>1 (0–3.0)</td>
</tr>
<tr>
<td>ACS[Sp]SuTmp&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 (0.7–4.3)</td>
<td>1.5 (0–3.2)</td>
<td>2.8 (1.0–4.6)</td>
<td>3.0 (1.1–4.9)</td>
<td>2 (0–4.7)</td>
</tr>
<tr>
<td>S[Sp]Su&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1 (0–0.3)</td>
<td>0.5 (0–1.5)</td>
<td>0.9 (0–1.9)</td>
<td>2.2 (0.6–3.8)</td>
<td>2 (0–4.7)</td>
</tr>
<tr>
<td>ASu</td>
<td>0.7 (0–1.7)</td>
<td>0</td>
<td>0.3 (0–0.9)</td>
<td>1.8 (0.6–3.3)</td>
<td>3 (0–6.3)</td>
</tr>
<tr>
<td>Total</td>
<td>38.6 (32.9–44.3)</td>
<td>58.0 (51.2–64.8)</td>
<td>58.8 (53.4–64.2)</td>
<td>59.6 (54.2–65.0)</td>
<td>51.0 (41.2–60.8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> n, number of Salmonella isolates studied; N, number of Salmonella isolates received at NRC-Salm.
<sup>b</sup> Spectinomycin was not tested before 2002.
DISCUSSION

Overall, the total number of nontyphoid salmonellae from humans registered at the NRC-Salm decreased from 1997 (19,174 isolates) to 2003 (10,472 isolates). The total number of serotype Typhimurium isolates also decreased during the same period from 6,755 isolates in 1997 to 3,222 in 2003. As no change in surveillance practice had occurred in France and as similar results have been observed in Europe and North America (12, 16), this trend is most likely correct. The present comprehensive study of serotype Typhimurium isolates collected from humans in France in 2002 through a representative network comprising both private and public clinical laboratories concluded that multidrug resistance remains common within the serotype (68.8% of the isolates were resistant to ≥2 separate classes of antimicrobials) and is due mainly to the DT104 clone (82.3% of the MDR isolates belonged to the DT104 complex, as defined below).

Multiresistant (R type ACSSuTe) DT104 strains of serotype Typhimurium were first identified in the United Kingdom in the early 1980s in gulls and exotic birds. Isolations in humans started in 1989, when the clone became epidemic in cattle throughout the United Kingdom. Afterward, the DT104 clone also became common in poultry, pigs, and sheep (36). The SG1 structure was first described in serotype Typhimurium DT104 and was well characterized (6). This structure is located between the chromosomal genes thdf and int2. The int2 gene, located upstream of the yidY gene, is part of a cryptic retrophage sequence reported to date only in serotype Typhimurium (6, 24). In other S. enterica serotypes (Agona, Albany, Cerro, Derby, Dusseldorf, Emek, Infantis, Kiambu, Meleagridis, Newport, and Paratyphi B biotype Java), SG1 is located between the Salmonella genes thdf and yidY (6, 10, 24). The multidrug resistance region is located at the 3’ end of SG1 in a 13-kb region corresponding to a large class 1 integron named In104 (6, 24). The resistance genes floR and tet(G) are bracketed by two class 1 integrons, one carrying an aadA2 cassette (1.0 kb) and the other a blaPSE-1 cassette (1.2 kb). Transduction by phages or self-transmission of the SG1 structure has been proposed to explain the insertion of SG1 in S. enterica strains (10, 11). Strains containing

FIG. 2. Dendrograms generated by BioNumerics showing the results of cluster analysis on the basis of XbaI-PFGE of S. enterica serotype Typhimurium isolates. Similarity analysis was performed using the Dice coefficient, and clustering was by the unweighted pair group method with arithmetic averages. The different PFGE profiles and corresponding numbers of isolates, the types of bla genes (if present), and the phage types are indicated.
SGI1 variants (classified as SGI1-A to SGI1-J), possibly generated by recombination between homologous regions of the MDR region and conferring different antibiotic resistance profiles, have recently been described in various serotypes of *Salmonella* enterica (6, 10, 24).

In serotype Typhimurium, the SGI1 resistance gene cluster has been detected mainly in multiresistant DT104 isolates and to a lesser extent in closely related phage type U302 or DT120 and -12 (22, 40). It has been suggested that for non-DT104, the phage typing results were due to changes in phage susceptibility in a small proportion of SGI1-containing DT104 strains, possibly after acquisition of new phages or plasmids, rather than to horizontal transfer of resistance genes (22).

After analyzing the results of phage typing, XbaI-PFGE, and antimicrobial susceptibility testing and characterization of *bla* genes and class 1 integrons in the present study, we found that 183 isolates among the 320 tested (57.2%) belonged to the DT104 complex (DT104 and closely related phage types harboring SGI1 or variants of SGI1) in France in 2002. The use of phage typing as a single subtyping method gave us 52.8% (169/320) DT104 isolates. This proportion of DT104 isolates was relatively high compared to the data from an international study (16). In that study, for the period 2000 and 2001, the percentage of DT104 among serotype Typhimurium isolates ranged from less than 1% in Oceania (Australia and New Zealand) to approximately 52% in Eastern Europe (35.5% for North America). In Europe, there were large differences in national trends: Spain, 18.3%; Nordic countries, 22.1%; Belgium, 27%; The Netherlands, 37.2%; England and Wales, 42.3%; Germany, 44%; Scotland, 56.3%; and Hungary, 57.7%.

The main resistance pattern observed in the isolates of the DT104 complex in the present study was the panresistant ACSSuTe R type (83.2%). This result is in accordance with other studies (16, 31, 37), confirming that the classical SGI1 is the most prevalent resistance mechanism among serotype Typhimurium DT104 isolates.

Analysis of the distribution of PFGE profiles among DT104 isolates of R type ACSSuTe confirmed the low discriminatory power of the PFGE method for clonal DT104 (six profiles were seen, with one highly prevalent at 65%). Due to the high prevalence of such DT104 isolates of R type ACSSuTe in France since 1997, it became necessary to use a method complementary or alternative to PFGE for investigation of outbreaks due to serotype Typhimurium. Consequently, we are evaluating the multilocus variable number of tandem repeats analysis developed by Lindstedt et al. (25).

In our study, it was noteworthy that 5.8% (9/156) of isolates with R type ACSSuT, 8.3% (1/12) with R type ACSSuTNal, and 10% (1/10) with R type ACSSuTTmp did not belong to the DT104 complex as defined above. They did not contain SGI1, as detected by PCR of right and left junctions, and they possessed the *bla* gene coding for ampicillin resistance (instead of *bla* for SGI1-positive isolates) located with *aadA1* (instead of *aadA2* for SGI1-positive isolates) within a class 1 integron of 2 kb (instead of two class 1 integrons of 1.0 and 1.2 kb for SGI1-positive isolates). Without performing the detection of resistance genes, such isolates, which belong mostly to DT120 (7/11, 63.6%), closely related to DT104, and which may contain SGI1 (22), would have been classified in the DT104 complex. Serotype Typhimurium isolates of R type ACSSuTe containing the plasmid-mediated *bla* gene (also located with *aadA1* within a class 1 integron of 2.0 kb) were identified in 2002 and 2003 in Portugal, and the authors suggested that pigs were the source of the isolates (2).

Isolates exhibiting the ACSSuTe R type with additional resistance to relevant drugs, such as Nal or Tmp, were found in 6% and 4.9% of the DT104 complex isolates. Our results are in accordance with the data from the international study, indicating that Nal resistance and Tmp resistance were found in 6% and 6.6% of the MDR serotype Typhimurium DT104 isolates tested in 2001, respectively (16). There was an exception for the United Kingdom, where serotype Typhimurium isolates of R types ACSSuTNal and ACSSuTTmp were more prevalent in humans. In the United Kingdom, Tmp resistance in MDR DT104 isolates began in 1992 (0.9%), peaked in 1995 (27.2%), and then decreased until 2000 (13%), while Nal resistance began in 1992 (0.1%), peaked in 1996 (13%), and then decreased until 2000 (9%) (37, 39).

Although quinolone resistance can be caused by different mechanisms of chromosomal origin, mutations within gyrA resulting in amino acid substitutions in the QRDR of the A subunit of DNA gyrase play a major role in Nal resistance in *Salmonella* (17). The mutations occur most frequently at codons Ser83 and Asp87 (17). In the present study, we found three different mutations among 12 isolates resistant to Nal (and with reduced susceptibility to Cip): Ser83 to Phe (25%), Asp87 to Asn (41.7%), and Asp87 to Tyr (33.3%). In serotype Typhimurium, Asp87 to Asn was the most commonly isolated mutation in human DT104 isolates in the United Kingdom between 1994 and 1997 (33, 38), and all three mutations were observed with a different distribution (Ser83 to Phe, 60%; Asp87 to Asn, 34.3%; and Asp87 to Tyr, 5.7%) among a panel of 40 veterinary isolates collected between 1997 and 2000 (12), also in the United Kingdom. The presence of these different mutations within the chromosomal *gyrA* gene may be used to confirm epidemiological relationships between Nal-resistant serotype Typhimurium isolates, particularly if they belong to the DT104 clone. High-level resistance to Cip in salmonellae is of great concern but has been found rarely in serotype Typhimurium isolates, particularly if they belong to the DT104 clone. High-level resistance to Cip in salmonellae is of great concern but has been found rarely in serotype Typhimurium isolates, particularly if they belong to the DT104 clone. High-level resistance to Cip in salmonellae is of great concern but has been found rarely in serotype Typhimurium isolates, particularly if they belong to the DT104 clone. High-level resistance to Cip in salmonellae is of great concern but has been found rarely in serotype Typhimurium isolates, particularly if they belong to the DT104 clone.
explained by different mechanisms: (i) the presence of a plasmid harboring a Tmp resistance gene (9, 38), (ii) the presence of an independent chromosomally located class 1 integron containing the dfrA1 gene (9), (iii) or the presence of a variant of SGI, SGI1-A, which comprises a dfrA10 gene between the two class I integrons of SGI1 (6). However, SGI1-A was identified in serotypes Agona, Klium, and Infantis but not yet in serotype Typhimurium (24). Other R types associated with variants of SGI1 were found in DT104 isolates in the present study; 3.8% (7/183) were of R type SSpSu (SGI1-C), and 3.3% (6/183) were of R type ASu (SGI1-A). These results also confirmed the observation by Threlfall et al. (38) that evolutionary changes have occurred within DT104 involving both loss (variants of SGI1) and acquisition of drug resistance genes (plasmid-located or chromosomally located due to a mutation[s] in the QDRD).

Interestingly, only 1.1% (2/183) of the DT104 isolates were pansusceptible. We found 13 isolates with R types or PFGE profiles different from those of the DT104 clone which could have been classified as DT104 on the basis of the 31 selected routine phage suspensions and the interpretive guide. However, there were unexpected susceptibilities to additional phages 2 and 3, and thus, they have been reclassified as DT104-atypical to avoid confusion with the MDR DT104 clone.

Analysis of previous NRC-Salm antimicrobial resistance surveys revealed that the DT104 clone emerged in France before 1993 (no susceptibility data were available before 1993). In 1993, 38.6% of the isolates displayed R types commonly associated with the DT104 clone, and a study conducted on 86 ampicillin-resistant strains isolated in 1994 through a hospital-based network found that 76.7% of the strains belonged to the DT104 clone (7). It became prevalent between 1993 and 1997 and was still the main cause of multidrug resistance in 2003 without significant decrease.

The prevalence of resistance to ESC was low in our study. A single isolate was detected in 2002 (prevalence, 0.3%), and no isolates were detected in 1993, 1997, 2002, and 2003. This isolate produced the plasmid-mediated TEM-52 ESBL. Three other TEM-52-producing S. enterica isolates of different serotypes were isolated from human cases (without prior treatment with ESC and without recent hospitalization) in France in 2002 and 2003. A common plasmid carrying blaTEM-52 was found in three of the four isolates (43). As TEM-52 has been identified increasingly in various serotypes of Salmonella in poultry in The Netherlands (14) and in Belgium (A. Cloeckaert, unpublished results) since 2001, we can hypothesize that poultry is the source for human contamination.

In conclusion, multidrug resistance is the rule for French serotype Typhimurium isolates collected from humans from 1993 until 2003. This is due mainly to the DT104 clone, which seems well established in France and which has undergone changes in antimicrobial resistance phenotypes. The emergence of serotype Typhimurium isolates resistant to ESC and Cip have been reported since 2002. These trends should be followed carefully by the use of adequate subtyping methods and by the detection of resistance genes and class 1 integrons.

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