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Highly drug-resistant *Salmonella* Kentucky ST198-X1 in the Mediterranean basin: a microbiological study

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SUMMARY (253)

Background

Salmonella is a major food-borne pathogen found worldwide, which can cause life-threatening infections. Ciprofloxacin and extended-spectrum cephalosporins (ESCs) are the drugs of choice for severe *Salmonella* infections. We previously reported a ciprofloxacin-resistant *S. enterica* serotype Kentucky strain (*Salmonella* Kentucky ST198-X1 CIP^R) that emerged in Egypt and spread throughout Africa and the Middle East from 2002 to 2008.

Methods

Data for *Salmonella* Kentucky collected by the French national *Salmonella* laboratory surveillance system from 2000 to 2011 and by two sites in Casablanca, Morocco, from 2003 to 2011 were analysed. Isolates displaying resistance to ESCs and/or with decreased susceptibility to carbapenems were studied by *Xba*I pulsed-field gel electrophoresis and by multilocus sequence typing. The mechanisms of resistance to antimicrobial drugs were identified.

Findings

Isolations of *Salmonella* Kentucky ST198-X1 CIP^R have recently increased in frequency (376 isolates for 2009-2011 versus 200 for 2000-2008) in France, and the geographic area in which infections occur has expanded to include the Indian subcontinent and South-East Asia. We have observed multiple acquisitions of extended-spectrum β-lactamase (CTX-M-1, CTX-M-15), plasmid-encoded cephalosporinase (CMY-2), or carbapenemase (OXA-48, VIM-2) genes by *Salmonella* Kentucky ST198-X1 CIP^R isolates from the Mediterranean area since 2009. Many of these highly drug-resistant *Salmonella* isolates are also resistant to most aminoglycosides (*armA* gene) and to azithromycin (*mph(A)* gene).

Panel: Research in context

Systematic review

We searched PubMed for articles published up to January 30, 2013, with the search terms "Salmonella" and "carbapenemases" or "carbapenems" or "NDM-1". No language restrictions were used. We identified only five studies describing various sporadic *Salmonella* spp. isolates resistant to carbapenems. None of these isolates was also resistant to fluoroquinolones. Two of these studies concerned isolates resistant to carbapenems due to mechanisms other than carbapenemase production: two clinical isolates of serotype Wien, which had lost a porin and produced cephamycinase CMY-4, in Tunisia in 2001, and one clinical isolate of serotype Typhimurium, which had lost two porins and produced cephamycinase CMY-2, in Taiwan in 2010. [20,21] The first carbapenemase producer was a clinical isolate of serotype Cubana, which produced carbapenemase KPC-2 and was obtained in the US in 1998.[22,23] In 2011 and 2012, the first two clinical isolates of NDM-1-producing *Salmonella* of serotypes Senftenberg and Westhampton were reported, isolated from patients returning from India.[24,25] In 2012, the first carbapenemase (VIM-1)-producing *S. enterica* isolates were isolated from food animals in Europe.[26]

Interpretation

This report confirms the emergence of highly drug-resistant *Salmonella* Kentucky, a potential risk to Public Health, in the Mediterranean basin. This ciprofloxacin-resistant *Salmonella* Kentucky ST198-X1 strain, which is increasingly frequently isolated, has recently acquired β-lactamases (CTX-M ESBLs, CMY-2 AmpC, and VIM-2 and OXA-48 carbapenemases) encoding resistance to extended-spectrum cephalosporins and carbapenems. Further efforts are required from national and international health, food and agricultural authorities, to

control the spread of this highly drug-resistant strain in humans and food animals. We propose the inclusion of ciprofloxacin-resistant *Salmonella* Kentucky as a new target strain, in national programmes for the control of *Salmonella* in poultry.

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Box

On October 14th 2009, a 69-year-old woman living in western France was hospitalised for an upper respiratory tract infection, fever and diarrhoea. The symptoms began during a holiday in Egypt (September 19th to October 2nd, 2009). The patient's clinical history included a high-grade follicular lymphoma in 2003, treated by chemotherapy and allogeneic transplantation, in remission since 2005. The patient had had repetitive respiratory infections due to sequellar hypogammaglobulinaemia. Her last hospital admission was for right hemicolectomy surgery in 2007. One day after admission, a *S. enterica* serotype Saintpaul isolate that was resistant to ampicillin, susceptible to ESCs and had intermediate resistance to imipemem (MIC 3 mg/L) was obtained from blood and stool cultures (Tables 2 & 3). Treatment with 1 g/day ciprofloxacin was administered for 10 days. The patient was given a blood transfusion (two units) and an intravenous polyclonal immunoglobulin perfusion and rapidly recovered. One month later, the patient presented a new episode of febrile bronchial and diarrhoeal infection, which was treated with 1 g/d ceftriaxone for five days. No bacteriological testing was performed and the patient recovered slowly, with persistent digestive disorders. A new stool culture was performed on December 16th 2009, and was positive for *Salmonella* Kentucky CIP^R, resistant to ESCs, cotrimoxazole and azithromycin, but susceptible to imipenem (Tables 2 & 3). No antimicrobial agents were given, but a series of stool samples was collected over time and cultured, to follow the elimination of the *Salmonella* strains. *Salmonella* Kentucky CIP^R was isolated in January 2010 and January 2011(Tables 2 & 3); additional stool cultures for *Salmonella* in March and April 2011 were also positive (isolates not sent to FNRC-Salm), despite the patient being free from digestive disorders.

INTRODUCTION

Antimicrobial drug-resistant bacteria are a serious challenge for the clinical care of patients and for Public Health in the 21st century.[1] The Gram-negative "superbugs", such as those resistant to extended-spectrum cephalosporins (ESCs) due to the production of either extended-spectrum β-lactamases (ESBLs) or cephamycinases (AmpC), seem to have now eclipsed Gram-positive "superbugs" (i.e., methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* spp). Furthermore, the recent emergence of Enterobacteriaeceae resistant to all β-lactam antibiotics, including ESCs and carbapenems, is of particular concern, because carbapenems are, in many cases the last option available for treating serious infection with ESC-resistant Gram-negative bacteria. Indeed, the development pipeline for new antimicrobial drugs with bactericidal activity against Gram-negative bacteria has now run dry.[2,3]

In a 2004 report entitled "Bad Bugs, No Drugs", the Infectious Diseases Society of America (IDSA) imagined a catastrophic scenario with an explosive epidemic of 220,000 cases and 1,730 deaths caused by a multidrug-resistant non-typhoidal *Salmonella*, resistant, in particular, to both ESCs and fluoroquinolones. This choice was based on the following observations (i) *Salmonella* is a prevalent zoonotic agent causing an estimated 1.7 million infections, resulting in 2,800 deaths per year in high-income regions of North America in 2006,[4] (ii) *Salmonella* can cause major food-borne outbreaks, such as that in the US in 1994 associated with manufactured ice cream contaminated with *Salmonella enterica* serotype Enteritidis, which caused sickness in an estimated 224,000 people,[5] (iii) fluoroquinolones, including ciprofloxacin, and ESCs are the drugs of choice for treating severe *Salmonella* infections and for people at risk of such infections (infants, the elderly and

134 immunocompromised patients), and (iv) infections with drug-resistant *Salmonella* are
135 associated with higher morbidity and mortality.[6]
136
137 We previously reported the international emergence of a multidrug-resistant *S. enterica*
138 serotype Kentucky (*Salmonella* Kentucky) strain, identified as being multilocus sequence
139 type (MLST) ST198 and as belonging to *Xba*I pulsed-field gel electrophoresis (PFGE) cluster
140 X1.[7] *Salmonella* Kentucky ST198-X1 isolates were resistant to several antimicrobial drugs,
141 including ciprofloxacin (minimal inhibitory concentration [MIC] $\geq$ 4 mg/L), which is a very
142 unusual resistance trait in *Salmonella*.[8,9] The first ciprofloxacin-resistant *Salmonella*
143 Kentucky (*Salmonella* Kentucky CIP^R) to be identified was isolated from a French tourist
144 who visited Egypt in 2002. From then until 2008, the *Salmonella* surveillance systems in
145 France, England, and Denmark detected 489 cases of infection with this strain in people who
146 had travelled to or stayed in Africa or the Middle East.[7] Hospitalisation was more frequent
147 among patients infected with CIP^R Kentucky (mean age, 36 years) than among those infected
148 with Kentucky strains susceptible to ciprofloxacin.[7]
149
150 All *Salmonella* Kentucky CIP^R isolates in our survey were susceptible to ESCs. However, one
151 case report described a Belgian traveller infected with *Salmonella* Kentucky CIP^R during a
152 trip to Libya in 2005, who required treatment with meropenem due to ESC resistance (CTX-
153 M-1 ESBL production) after multiple treatment failures for a severe infection.[10]
154 The aim of this study was to monitor recent trends in the global epidemiology and
155 antimicrobial resistance of the *Salmonella* Kentucky ST198-X1 CIP^R strain. The work was
156 conducted in parallel in France, where this infection occurred mostly in travellers or migrants,
157 and in Morocco, where most of the French travellers or migrants had acquired the infection.

158 This study identified highly drug-resistant (HDR) isolates, present in both France and
159 Morocco since 2009. These CIP^R isolates acquired in the Mediterranean area produce various
160 carbapenemases, cephamycinase, or ESBLs. This report indicates that *Salmonella* has taken a
161 major step towards panresistance and suggests that the catastrophic scenario imagined by the
162 IDSA might become all too real in the near future.
163

MATERIALS & METHODS

Data for human *Salmonella* infections

France

We used data from the French National Reference Centre for *Salmonella* (FNRC-Salm), established since 1947. During the 2000s, the FNRC-Salm network included a stable number of approximately 1,400 hospital and private clinical laboratories. In 2008, an unpublished survey of all French clinical laboratories (*n*=3,375, response rate of 95%) estimated that about 65% of all human *Salmonella* isolates in France were reported to the FNRC-Salm. Basic epidemiological data (date and site of isolation, sex and age of the patient, and international travel) were recorded for each isolate. From 2000 to 2011, 128,836 serotyped *Salmonella* isolates were registered at the FNRC-Salm, including 954 non-repeated *Salmonella* Kentucky isolates (0.7% of all *Salmonella* isolates).

Morocco

We used 2003-2011 data from two sites in Casablanca, the largest city in Morocco. The first site was the Microbiology Laboratory of the University Hospital Centre Ibn Rochd (UHCIR), Casablanca, a 1,700-bed teaching hospital. The second site was the Pasteur Institute of Morocco (PIM), which receive clinical strains of *Salmonella* for serotyping from private laboratories. Between 2003 and 2011, 226 *Salmonella* isolates were obtained and serotyped, including 30 non-repeated *Salmonella* Kentucky isolates (12.8% of all *Salmonella* isolates).

Microbiological investigations

Bacterial isolates

All but two (which could not be subcultured) of the 954 *Salmonella* Kentucky isolates obtained from humans between 2000 and 2011 in France were included in this study. Thirty (26 from UHCIR and 4 from PIM) *Salmonella* Kentucky isolates collected from humans in Casablanca, Morocco between 2003 and 2011 were also studied. One additional isolate from the FNRC-Salm was studied: one of serotype Saintpaul isolated from a patient co-infected with *Salmonella* Kentucky in 2009.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed on all *Salmonella* isolates, by the disk diffusion method with a panel of 32 antimicrobial agents (Bio-Rad, Marnes-La-Coquette, France).[7] The MICs of ceftriaxone, ceftazidime, imipenem, ertapenem, meropenem, ciprofloxacin, azithromycin, colistin, and tigecycline were determined by Etests (AB Biodisk, Solna, Sweden). Results were interpreted with the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (www.sfm-microbiologie.org/) breakpoints. In particular, susceptible isolates were defined as having a MIC $\leq$ 0.5 mg/L for ciprofloxacin, and resistant isolates were defined as having a MIC > 1 mg/L for ciprofloxacin, regardless of isolate source (i.e., intestinal or extraintestinal). Isolates were defined as highly drug-resistant if they were resistant to at least four antibiotic classes, including both fluoroquinolones (i.e., ciprofloxacin) and ESCs (i.e., ceftriaxone and/or ceftazidime).

Molecular typing

PulseNet standard pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested chromosomal DNA and multilocus sequence typing (MLST) were performed as previously described.[7]

Determination of resistance mechanisms

The presence of beta-lactam resistance genes (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{OXA-1}$ group, *bla*$_{CMY}$, *bla*$_{CTX-M}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$, *bla*$_{NDM}$, and *bla*$_{KPC}$), plasmid-mediated quinolone resistance genes, (*qnrA*, *qnrB*, *qnrS*, *qnrD*, *aacA4*-cr (also known as *aac(6')-Ib-cr*) and *qepA)*, macrolide resistance genes (*ermA*, *ermB*, *ermC, mph*(A), *ereA*, *ereB*, *mrsA*, *mrsB*, *mefA*, and *mefE)*, aminoglycoside resistance genes (*armA, rmtA, rmtB, rmtC, rmtD*, and *npmA),* class 1 integron gene cassettes and *Salmonella* genomic island 1 (SGI1) was assessed by PCR, as previously described.[7,11-14]

The quinolone resistance-determining region (QRDR) of *gyrA*, *gyrB*, *parC* and *parE* (encoding subunits of the DNA gyrase and the topoisomerase IV) was sequenced, as previously described.[7] The nucleotide and deduced amino-acid sequences were analysed and compared with sequences available from the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

We assessed resistance transfer by mating, with ESBL, cephamycinase and carbapenemase producers, using liquid and solid media, with *E. coli* K-12 BM14 resistant to sodium azide as the recipient strain. Transconjugants were selected on Drigalski agar (Bio-Rad) supplemented with ceftriaxone (4 mg/L), ceftazidime (16 mg/L), or imipenem (3 mg/L) plus sodium azide (500 mg/L). Three *E. coli* transconjugants were arbitrarily selected in each experiment. We used S1 nuclease treatment and PFGE to determine the sizes of bacterial plasmids accurately, and PCR-based replicon typing analysis was performed, as previously described.[15]

RESULTS

Occurrence of *Salmonella* Kentucky CIP^R in humans

France

Of the 497 isolates of *Salmonella* Kentucky obtained in France between 2000 and 2008, 200 (40.2%) were resistant to ciprofloxacin (previously reported in reference 7). For the period 2009-2011, 376 (82.6%) of the 455 *Salmonella* Kentucky tested were CIP^R (Figure). This near doubling of the number of *Salmonella* Kentucky CIP^R isolates obtained, in a third of the time, with a stable network of laboratories, against a backdrop of a general decrease in the number of isolations of *Salmonella* (≈11,000 clinical isolates received per year during the period 2000-2008 vs ≈10,000 during the period 2009-2011), indicates that this *Salmonella* Kentucky CIP^R strain continued to circulate and spread.

Travel information was available for 371 patients (64.5%) infected with CIP^R Kentucky during the period 2000-2011 (Table 1). Of these 371 patients, 338 (91.1%) had travelled internationally in the 15 days before the onset of illness, whereas the remaining 33 patients had not. Most of the patients seen between 2002 and 2005 had travelled to North-East or East Africa. Since 2006, patients have been reporting travel to North-East and East Africa, North Africa, West Africa, and the Middle East. Since 2009, the area of infection has extended to include India.

Morocco

Of the 30 clinical isolates of *Salmonella* Kentucky obtained in Casablanca between 2003 and 2011, 19 (63.3%) were CIP^R. The first *Salmonella* Kentucky CIP^R isolate was obtained in 2006 and the annual number of isolates obtained has since fluctuated between one and eight (2007, *n*=1; 2008, *n*=5; 2009, *n*=2; 2010, *n*=8; 2011, *n*=2).

Recent trends in the antimicrobial resistance of *Salmonella* Kentucky

Emergence of CIP^R-ESC^R Salmonella *Kentucky in the Mediterranean area since 2009*

Based on the FNRC-Salm 2000-2011 data, the first *Salmonella* Kentucky isolate resistant to ESCs (ESC^R) was isolated in 2009 (Figure). From 2009 to 2011, 10 *Salmonella* Kentucky ESC^R isolates (2.2% of all *Salmonella* Kentucky during this period) in total were identified: six were susceptible to ciprofloxacin and had a cephamycinase-like profile and four were resistant to both ciprofloxacin and ESCs. The four CIP^R-ESC^R isolates were acquired in Algeria, Morocco, Egypt, and Turkey (Tables 2 & 3). They produced the cephamycinase CMY-2 (*n*=2) or the ESBLs CTX-M-1 (*n*=1) or CTX-M-15 (*n*=1), encoded by 90 to 200 kb plasmids from the IncI1, IncL/M or IncA/C incompatibility groups.

Two Salmonella *strains producing carbapenemase OXA-48 in a traveller returning from Egypt in 2009*

One of the four *Salmonella* Kentucky CIP^R-ESC^R isolates detected since 2009, #09-9322 (see previous section), was isolated from a patient co-infected with another serotype of *Salmonella*, Saintpaul, which produced a carbapenamase not present in #09-9322, but subsequently found in one of the three sequential *Salmonella* Kentucky isolates from the same patient (box and Tables 2 & 3). The serotype Saintpaul isolate was found to contain the $bla_{\text{OXA-48}}$ carbapenemase gene on an IncL/M plasmid of about 70 kb. The three sequential

Salmonella Kentucky CIP^R isolates belonged to the ST198-X1 strain, and carried the *gyrA* and *parC* mutations previously encountered in Kentucky isolates from Egypt and West Africa.[7] The three isolates also contained the phosphotransferase *mph(A)* gene conferring high-level resistance to azithromycin. The three isolates presented different resistance profiles, due to the acquisition/loss of various R plasmids and also, probably, due to IS*26* rearrangements of the SGI1.[7] The first isolate was resistant to ESCs due to the presence of the bla_{CMY-2} gene, whereas the most recent isolate, collected one year later, contained the bla_{OXA-48} carbapenemase gene. All four isolates were susceptible to colistin and tigecycline.

Highly drug-resistant Salmonella *Kentucky isolates producing VIM-2 in Morocco in 2010*

Five of the 30 *Salmonella* Kentucky isolates obtained in Casablanca, Morocco, between 2003 and 2011 (16.6%) were ESC^R. These five *Salmonella* Kentucky ST198-X1 ESC^R-CIP^R isolates had decreased susceptibility to imipenem (MIC range, 1-3 mg/L). They all contained the bla_{VIM-2} gene within In*58*,[16] itself carried on a 30-kb plasmid. Three isolates originated from patients hospitalised in three different reanimation wards (one blood culture, two urine cultures) of the UHCIR during January 2010, the other two isolates being obtained from stool cultures performed at the PIM in January and August 2010.

DISCUSSION

We report a new step towards pan-antimicrobial resistance in *Salmonella*, a major foodborne pathogen found worldwide, which can cause life-threatening infections. The *Salmonella* Kentucky ST198-X1 isolates reported here are resistant to both fluoroquinolones and ESCs (except for the OXA-48-producing strain), and some also display full or intermediate resistance to carbapenems. Many are also resistant to most aminoglycosides (*armA* gene) and to azithromycin (*mph(A)* gene). *Salmonella* Kentucky ST198-X1 is a particularly successful strain that has accumulated various chromosomal resistance determinants since the mid-1990s, with the integration of the *Salmonella* genomic island 1 (encoding resistance to multiple antimicrobial drugs, including amoxicillin, gentamicin, and sulfonamides), followed by cumulative mutations in the *gyrA* and *parC* genes, leading to resistance to nalidixic acid, and then to ciprofloxacin, in 2002. This strain was mostly identified in Egypt before 2005,[7] but has since spread rapidly throughout Africa and the Middle East. The slight decrease in isolation rates for this strain in 2011 probably resulted from the "Arab Spring", which may have discouraged travel to the area in which this strain is endemic. Thus, 150 *Salmonella* Kentucky CIP^R isolates were obtained at the FNRC-Salm in 2012 (data not shown), a number similar to that obtained in 2010. The *Salmonella* Kentucky CIP^R strain was first identified in the Indian subcontinent in 2009, and a pattern of current spread across Asia is also suggested by the isolation of two *Salmonella* Kentucky CIP^R isolates from French patients reporting travel to Vietnam and Indonesia in 2012 (data not shown). As the geographic spread of this strain has been predicted by a French surveillance system, it may be partially biased by the preferred destinations of French travellers and particular migrant populations with historical links to France. Where possible, these data should be confirmed by local studies.

This epidemic was previously associated with a livestock (autochthonous poultry) reservoir of this *Salmonella* Kentucky CIP^R strain in Africa. It was suggested that the common use of fluoroquinolones in poultry and the lack of both laboratory-based surveillance of infections and control measures in the countries in which this strain circulates played a role in the rapid spread of this strain after 2002.[7] A survey performed on 92 poultry farms in Sudan, East Africa, in 2008 revealed that enrofloxacin, a fluoroquinolone, was commonly added to the drinking water on 14% of the farms surveyed.[17] Both here and in our previous study, ~11% of the patients reported no history of travel outside Europe, suggesting that these infections may have resulted from the consumption of contaminated foods or secondary contamination in Europe. Indeed, contaminated spices from North Africa have previously been identified in France and the US.[7] This strain also seems to have become established in some European flocks, another major source of concern. In 2010, *Salmonella* Kentucky CIP^R isolates were found in turkey meat products in Germany and in turkey meat or flocks in Poland.[18,19] One of the *Salmonella* Kentucky CIP^R isolates recovered from a flock in Poland in 2010 was also resistant to ESCs, due to the production of a CTX-M ESBL.[19]

The diversity of the recently acquired β-lactamases (CTX-M ESBLs, CMY-2 AmpC, and VIM-2 and OXA-48 carbapenemases) suggests that the increasingly common *Salmonella* Kentucky ST198-X1 CIP^R strain has been "collecting" genes for resistance to ESCs and carbapenems. Resistance to carbapenems is otherwise extremely rare in *Salmonella* spp. (panel). The simultaneous presence of ESCs and carbapenem determinants (CMY-2 and OXA-48) was even documented in this study in *Salmonella* Kentucky ST198-X1 CIP^R isolated from a single patient.

A similar scenario, but without the acquisition of carbapenemase, occurred in Taiwan for *S. enterica* serotype Choleraesuis (*Salmonella* Choleraesuis), a serotype acquired from pigs and associated with extraintestinal infection in humans.[8] The first CIP^R isolates appeared in 2000 and, in the third quarter of 2001, 60% of the *Salmonella* Choleraesuis isolates from humans were CIP^R.[8] This trait was attributed to the use of enrofloxacin in pigs. Additional resistance to ESCs mediated by the cephamycinase CMY-2 has appeared since 2002.[26] This enzyme is frequent in many *Salmonella* serotypes, including Newport in the US, where its emergence has been associated with the use of ceftiofur, an ESC licensed for use in cattle, pigs, and other food animals.[27] Unlike *Salmonella* Choleraesuis CIP^R and Newport ESC^R, *Salmonella* Kentucky ST198-X1 CIP^R is not restricted to a single country or region, rendering control measures in livestock more difficult.

We found that *Salmonella* Kentucky ST198-X1 had a broad geographic distribution, overlapping with that of certain plasmid-borne carbapenemases, such as OXA-48 and VIM.[12,16] This makes it likely that carbapenemase-producing *Salmonella* will become more frequent in the Mediterranean area in the near future, particularly if such carbapenemase producers become established in livestock, as previously observed for ESBL- and cephamycinase-producing *Salmonella* in industrialised countries.[27-29] Indeed, isolation of the VIM-1-producing *S. enterica* serotype Infantis from two pig farms and one poultry farm in Germany was reported in 2012.[25]

Another issue is the difficulty of phenotypic detection for several carbapenemase producers.[12] This problem is particularly difficult for OXA-48, which weakly hydrolyses carbapenems but not ESCs in the absence of additional ESBL and/or cephamycinase and permeability defects. Indeed, carbapenem MICs were found to be low for the carbapenemase producers. Two

isolates, one OXA-48-positive and one VIM-2-positive Kentucky isolate, were even classified as susceptible to the three carbapenems tested, on the basis of the CLSI or CA-SFM breakpoints. The use of rapid diagnostic tests, such as the recently developed Carba NP test, would facilitate the early detection of carbapenemase producers.[30]

In conclusion, this report highlights the recent emergence of HDR *Salmonella* and the need to screen *Salmonella* isolated either from humans or food-producing animals for carbapenemase producers. The main types of carbapenemase (KPC, OXA-48, NDM, VIM) have now been identified in *Salmonella*, and half of these enzymes have been found in the *Salmonella* Kentucky ST198-X1 strain. National and international health, food and agricultural authorities need to recognise rapidly the potential risk to Public Health posed by *Salmonella* Kentucky ST198-X1 CIP^R, so that *Salmonella* Kentucky CIP^R can be included, as a new targeted strain, in current national programmes for the control of *Salmonella* in poultry.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: SLH and FXW. Performed the experiments: DH, LS, DE, VG. Contributed reagents/materials/analysis tools: BB, KZ. Analysed the data: SLH and FXW. Wrote the paper: SLH and FXW. Reviewed, critiqued and offered comments on the text: DH, BB, KZ.

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CONFLICTS OF INTEREST

All authors declare that they have no competing interests or conflicts of interest.

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Table 1: Countries visited by patients infected with *S. enterica* serotype Kentucky resistant to ciprofloxacin in the 15 days before the onset of illness (data from the French National Reference Centre for *Salmonella*)

Country	2002-2005	2006-2008	2009-2011	Total
Africa				
Not specified		3	2	**5**
Algeria		9	60	**69**
Cameroon		2	1	**3**
Djibouti		1	2	**3**
Egypt	11	11	7	**29**
Ethiopia			2	**2**
Ivory Coast			6	**6**
Kenya	2	1		**3**
Libya		2	1	**3**
Mauritania			2	**2**
Morocco		69	78	**147**
Senegal			7	**7**
Sudan	1			**1**
Tanzania	1	2		**3**
Tunisia		5	21	**26**
Middle East				
Iran		1		**1**
Iraq			1	**1**
Israel			1	**1**
Lebanon		2	2	**4**
Saudi Arabia		1		**1**
Syria			1	**1**
Turkey		2	2	**4**
Asia				
India			8	**8**
North America				
Canada			1	**1**
Europe				
France[†]		6	27	**33**
Croatia			1	**1**
Greece			1	**1**
Spain			5	**5**
Total	15	117	239	**371**

[†]France is indicated as the country of infection in cases of notification of an absence of international travel for up to 2 months before the onset of symptoms

Table 2: Antimicrobial drug-resistant *Salmonella* isolates included in this study

Isolate	Serotype	Date of isolation	Source[a]	Country of infection	Antimicrobial resistance profiles[b]	MLST/ PFGE	SGI1	MIC (mg/L)[c]								
								Cro	**Caz**	**IMP**	**ETP**	**MEM**	**CIP**	**Azi**	**CST**	**TGC**
Isolates recovered through the French national *Salmonella* surveillance system																
09-8391	Kentucky	02 Nov 09	F, 65y, stool, H	Morocco	A Cro Caz Fox Nal CIP	ST198/X1e	+	64	48	0.38	0.064	0.064	32	8	0.25	0.5
09-9322	Kentucky	16 Dec 09	F, 70y, stool, N	Egypt	A Cro Caz Fox S Sp K T N Chl Sul Tmp Nal CIP Azi	ST198/X1w	+	24	64	0.5	0.023	0.047	12	128	0.19	0.38
10-0720	Kentucky	31 Jan 10	F, 25y, stool, H	Turkey	A Cro S Sp G Sul Te Nal CIP	ST198/X1b	+	64	4	0.25	0.016	0.064	16	4	0.25	1
10-5456	Kentucky	13 Aug 10	F, 7y, stool, H	Algeria	A Cro Caz S Sp K T N G Ak Chl Sul Tmp Nal Cip Azi	ST198/X1a	+	>256	>256	0.38	0.023	0.047	12	32	0.25	0.5
Isolates recovered through the survey in Casablanca, Morocco																
10-1923	Kentucky	04 Jan 10	M, 25y, urine, H	Morocco	A Cro Caz Fox IMP* S Sp K T G Sul Te Nal CIP	ST198/X1j	+	256	192	3	1.5	0.25	12	4	0.25	1
10-1922	Kentucky	07 Jan 10	M, 20y, blood, H	Morocco	A Cro Caz Fox IMP* S Sp K T N G Ak Is Sul Te Nal CIP	ST198/X1m	+	24	24	3	0.5	0.25	12	6	0.25	1
10-1924	Kentucky	21 Jan 10	M, 92y, urine, H	Morocco	A Cro Caz Fox IMP* S Sp K T N G Sul Te Nal CIP	ST198/X1j	+	256	96	3	3	1	16	6	0.25	1
10-1925	Kentucky	24 Jan 10	M, >18y, stool, N	Morocco	A Cro Caz Fox S Sp K T N G Ak Is Sul Te Nal CIP	ST198/X1j	+	32	24	1	0.19	0.25	8	4	0.25	0.75
10-1926	Kentucky	25 Aug 10	M, >18y, stool, N	Morocco	A Cro Caz Fox IMP* S Sp K T G Sul Te Nal CIP	ST198/X1j	+	>256	48	2	0.75	1	8	4	0.5	1
Isolates recovered from a single patient[¶]																
09-7981	Saintpaul	16 Oct 09	F, 69y, blood, H	Egypt	A IMP*	ST1670	-	1.5	2	3	1	1.5	0.023	2	0.25	0.38
10-0305	Kentucky	07 Jan 10	F, 70y, stool, N	Egypt	K Chl Tmp Nal CIP Azi	ST198/X1w	+	0.094	0.50	0.38	0.008	0.023	12	48	0.19	0.5
11-0664	Kentucky	28 Jan 11	F, 71y, stool, N	Egypt	A Nal CIP Azi	ST198/X1w	+	0.125	0.25	0.75	0.5	0.19	8	32	0.50	0.50

[a]F, female; M, male; y, years (age); H, hospitalised; N, not hospitalised

[b]A, amoxicillin; Cro, ceftriaxone; Caz; ceftazidime; Fox, cefoxitin; IMP, imipenem (*, intermediate resistance according to CA-SFM, resistance according to CLSI); ETP, ertapenem; MEM, meropenem; S, streptomycin; Sp, spectinomycin; K, kanamycin; T, tobramycin; N, netilmicin; G, gentamicin; Ak, amikacin; Is, isepamicin; Chl, chloramphenicol; Sul, sulfamethoxazole; Tmp, trimethoprim; Nal, nalidixic acid; Cip, ciprofloxacin, Azi, azithromycin; CST, colistin; TGC, tigecycline

[c]CA-SFM and CLSI (M100 S22) breakpoints for carbapenems: IMP and MEM (CA-SFM, S ≤ 2 mg/L, R > 8 mg/L; CLSI, S ≤ 1 mg/L, R ≥ 4 mg/L); ETP (CA-SFM, S ≤ 0.5 mg/L, R > 1 mg/L; CLSI, S ≤ 0.5 mg/L, R ≥ 2 mg/L). For categorisation, Etest MICs between standard dilutions were rounded up to the next two-fold dilution

[¶]Isolate 09-9322 recovered by the French national surveillance system was also isolated from this single patient who had travelled to Egypt

Table 3: Mechanisms of resistance to antimicrobial drugs in the antimicrobial drug-resistant *Salmonella* isolates included in this study

Isolate	Serotype	Main determinants of resistance to: (incompatibility group, plasmid size)							Class 1 integrons
		ESCs	Carbapenems	Ciprofloxacin			Azi	Aminoglycosides	
				GyrA	ParC	PMQR			
Isolates recovered through the French national *Salmonella* surveillance system									
09-8391	Kentucky	bla_{CMY-2} (IncI1, 90 kb)	-	Ser83Phe, Asp87Asn	Ser80Ile			-	-
09-9322	Kentucky	bla_{CMY-2} (IncI1, 90 kb; IncA/C, 200 kb)	-	Ser83Phe, Asp87Gly	Ser80Ile		*mph*(A) (NT)		1.8 kb (*dfrA12, aadA2)*
10-0720	Kentucky	$bla_{CTX-M-1}$ (IncI1, 90 kb)	-	Ser83Phe, Asp87Asn	Ser80Ile		-		1.5 kb (*aacA5, aadA7)*
10-5456	Kentucky	$bla_{CTX-M-15}$ (IncL/M, 90 kb)	-	Ser83Phe, Asp87Asn	Ser80Ile		*mph*(A) (NT)	*armA* (IncL/M, 90 kb)	1.8 kb (*dfrA12, aadA2)*
Isolates recovered through the survey in Casablanca, Morocco									
10-1923	Kentucky	-	bla_{VIM-2} (UT, 30 kb)	Ser83Phe, Asp87Gly	Ser80Ile		-		1.5 kb (*aacA5, aadA7)*, 3 kb (*aacA7*, bla_{VIM-2}, *aacC1*, *aacA4*)
10-1922	Kentucky	-	bla_{VIM-2} (IncW, 30 kb)	Ser83Phe, Asp87Gly	Ser80Ile		-		1.5 kb (*aacA5, aadA7)*, 3 kb (*aacA7*, bla_{VIM-2}, *aacC1*, *aacA4*)
10-1924	Kentucky	-	bla_{VIM-2} (IncW)	Ser83Phe, Asp87Gly	Ser80Ile		-		1.5 kb (*aacA5, aadA7)*, 3 kb (*aacA7*, bla_{VIM-2}, *aacC1*, *aacA4*)
10-1925	Kentucky	-	bla_{VIM-2} (IncW)	Ser83Phe, Asp87Gly	Ser80Ile		-		1.5 kb (*aacA5, aadA7)*, 3 kb (*aacA7*, bla_{VIM-2}, *aacC1*, *aacA4*)
10-1926	Kentucky	-	bla_{VIM-2} (IncW)	Ser83Phe, Asp87Gly	Ser80Ile		-		1.5 kb (*aacA5, aadA7)*, 3 kb (*aacA7*, bla_{VIM-2}, *aacC1*, *aacA4*)
Isolates recovered from a single patient[¶]									
09-7981	Saintpaul	-	bla_{OXA-48} (IncL/M, 70 kb)	WT	WT			-	-
10-0305	Kentucky	-	-	Ser83Phe, Asp87Gly	Ser80Ile		*mph*(A) (NT)		1.8 kb (*dfrA12, aadA2)*
11-0664	Kentucky	-	bla_{OXA-48} (NT)	Ser83Phe, Asp87Gly	Ser80Ile		*mph*(A) (NT)	-	-

NT, not transferable; UT, untypeable

[¶]Isolate 09-9322 recovered by the French national surveillance system was also isolated from this single patient who had travelled to Egypt.

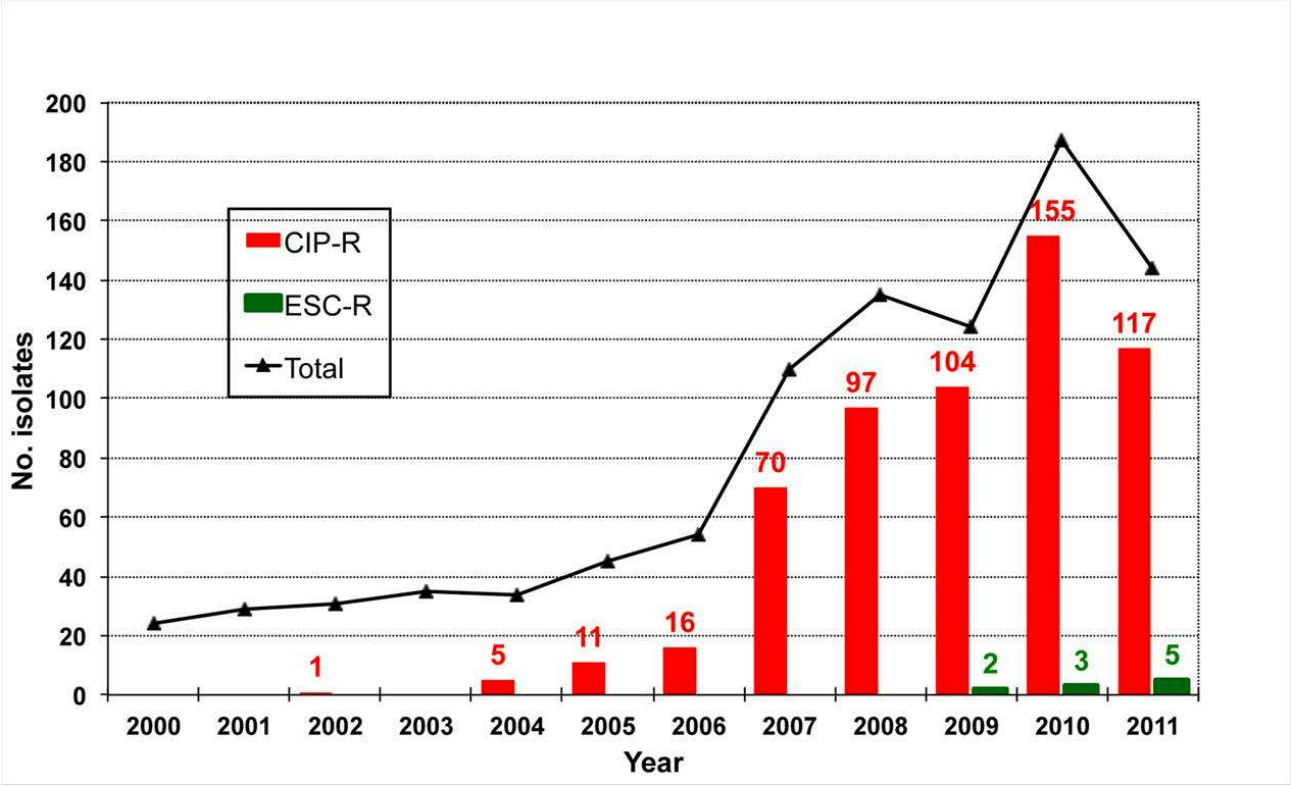

Figure 1. Human *S. enterica* serotype Kentucky isolates identified at the French National Reference Centre for *Salmonella* between 2000 and 2011

The total annual number of *S. enterica* serotype Kentucky isolates is indicated by a black triangle. The annual number of these isolates resistant to ciprofloxacin (CIP-R) is indicated in red, and that of isolates resistant to extended-spectrum cephalosporins (ESC-R) is shown in green. During this period, the total number of *Salmonella* spp. registered at the French National Reference Centre was 128,836 (2000, *n*=12,883; 2001, *n*=12,601; 2002, *n*=11,775; 2003, *n*=10,472; 2004, *n*=10,589; 2005, *n*=11,439; 2006, *n*=10,154; 2007, *n*=8,124; 2008, *n*=10,378; 2009, *n*=9,947; 2010, *n*=9,405; 2011, *n*=11,069).