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**Title: Prevalence and characterization of extended-spectrum-betalactamases-producing *Salmonella enterica* isolates in Saragossa, Spain (2001-2008)**

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**ABSTRACT**

We analysed the prevalence of resistance to extended-spectrum cephalosporins (ESC) among clinical strains of *Salmonella enterica* collected by the Laboratory of Clinical Microbiology in the University Clinical Hospital Lozano Blesa in the region of Aragon (Spain), for which very few epidemiological information exists. A total of 2,092 strains of *S. enterica* were identified in stool samples from patients with gastroenteritis. Five isolates showed an ESBL phenotype: four isolates of *S. enterica* serotype Virchow harboured the extended-spectrum beta lactamase (ESBL) encoding *bla*<sub>CTX-M-9</sub> gene and an isolate of serotype Enteritidis carried a *bla*<sub>CTX-M-1</sub> gene that, to the best of our

knowledge, is being described here for the first time in this serotype of *S. enterica*. The five ESC-resistant isolates were also resistant to spectinomycin, streptomycin, kanamycin, sulfonamides, tetracycline, and trimethoprim as well as to nalidixic acid. The ESBL isolate of serotype Enteritidis though remained susceptible to kanamycin and nalidixic acid. A class 1 integron of 1.5 kb was detected for the four serotype Virchow isolates with the gene cassette *dfrA16—aadA2*. The *bla*<sub>CTX-M-9</sub> gene was carried by a ~300 kb IncHI2 conjugative plasmid in the case of the *S. enterica* serotype Virchow isolates. The *bla*<sub>CTX-M-1</sub> gene was carried by a ~100 kb IncI1-N conjugative plasmid for the serotype Enteritidis ESC-resistant isolate. All the four ESC-resistant strains of *S. enterica* serotype Virchow clustered together in a *Xba*I-pulsed-field gel electrophoresis, which also revealed a strong similarity between them and some pulsotypes of *S. enterica* serotype Virchow from France.

## 1 INTRODUCTION

2 Nontyphoidal salmonellosis is one of the most prevalent food borne bacterial  
3 infections in Europe and in North America. In the United States, the burden of this  
4 infection has been estimated annually to be 1.4 million of cases resulting in 400 deaths  
5 <sup>37</sup>. In Europe, 181,876 cases were reported in 2005, which meant a ratio of 39/100,000  
6 inhabitants <sup>11</sup>. Most infections are self-limited and do not require antimicrobial  
7 treatment. However, severe, life-threatening bacteraemia sometimes occur, particularly  
8 in children and immunocompromised hosts and in these cases an antimicrobial therapy  
9 is recommended <sup>35</sup>. Appropriate drugs for *Salmonella* infections include ampicillin,  
10 extended-spectrum cephalosporins (ESC), fluoroquinolones, and trimethoprim-  
11 sulfamethoxazole. However, rising rates of resistance to ampicillin and trimethoprim-  
12 sulfamethoxazole have significantly reduced their efficacy and fluoroquinolones are not  
13 approved for the use in children. Consequently, ESC have become the current drugs of  
14 choice for the treatment for invasive infections in children <sup>4</sup>. The number of cases of  
15 salmonellosis caused by isolates resistant to these ESC is in continuous increase since  
16 the very first case was detected in the early 1980s <sup>1</sup>. Nonetheless, they are still rare over  
17 the total of *Salmonella* foodborne infections, just reaching a 0.2 % in Europe in 2004 <sup>25</sup>.  
18 Resistance to these drugs is mainly mediated by the bacterial production of beta-  
19 lactamases that degrade ESC. Two main classes of plasmid beta-lactamases that  
20 inactivate ESC have been identified in *Salmonella*: the Ambler class A extended-  
21 spectrum beta-lactamases (ESBLs), the most prevalent class in this genus, and the  
22 Ambler class C cephamycinases. Most ESBLs belong to three families, TEM, SHV and  
23 CTX-M. Over the last decade, CTX-Ms have become the most prevalent family of  
24 ESBLs in the genus *Salmonella* in Europe <sup>1</sup>.

25 In Spain, the first ESBL-producing *Salmonella enterica* isolate was described in  
26 1996 <sup>26</sup>. It was a *S. enterica* serotype Othmarschen producing TEM-27 causing a  
27 nosocomial outbreak in Madrid. Thereafter, *S. enterica* isolates producing ESBLs CTX-  
28 M-9, CTX-M-27, TEM-52 or cephamycinase CMY-2 were reported sporadically in  
29 humans or in animals <sup>4, 13, 34</sup>. A recent study on randomly selected strains from different  
30 hospitals in Spain identified 27 (0.26 %) human isolates of *S. enterica* producing  
31 ESBLs or cephamycinases between 2001 and 2005 <sup>16</sup> and in a specific study about *S.*  
32 *enterica* serotype Virchow, 79 out of 504 (15%) isolates recovered from 14 of the 17  
33 provinces in Spain were ESBL producers, 48 of them carrying a *bla*<sub>CTX-M-9</sub> gene <sup>18</sup>.

34 In 2000, in Aragón, a northwestern region of Spain (1,326,918 inhabitants), 129  
35 foodborne outbreaks that affected 2,030 people (including 1,464 in Saragossa), resulting  
36 in 103 hospitalizations were reported. *S. enterica* was found to be the causative agent in  
37 62% of the cases. In 2005, a foodborne outbreak was reported with 179 cases of  
38 salmonellosis from the whole region (109 occurring in Saragossa), 19 patients needed  
39 hospitalization. This outbreak was epidemiologically related to the consumption of  
40 locally farmed chicken (<http://www.aragon.es>). Very few studies have been published  
41 about the epidemiological situation of *S. enterica* or about the profile of antimicrobial  
42 resistances shown by them, both for the whole region of Aragon and for the Sanitary  
43 Area Saragossa 3. The last clinical study showed a continuous increase of isolations of  
44 *S. enterica*, from 1994 (118 isolates) to 2000 (287 isolates) <sup>29</sup>.

45 In this study, we assessed the frequency of different serotypes and the  
46 prevalence of ESC resistance among *S. enterica* isolates obtained in Saragossa during  
47 an eight-year period (2001-2008). The characterization of the beta-lactamase genes and  
48 their genetic support, and class 1 integron cassettes was done on ESC-resistant *S.*  
49 *enterica* isolates.

## 51 MATERIALS AND METHODS

### 52 *Strains*

53 *S. enterica* isolates were recovered from stool samples at the Laboratory of  
54 Clinical Microbiology of the University Hospital H.C.U. “Lozano Blesa” between 2001  
55 and 2008. The Hospital “Lozano Blesa” is a 900-bed teaching hospital located in  
56 Saragossa, the capital city of Aragón. It is the reference hospital of the Sanitary Area  
57 Saragossa 3 (268,624 inhabitants in 2005). During the study period, the Laboratory of  
58 Clinical Microbiology received 59,977 stool samples, and a pathogen was found in  
59 roughly 10% of the samples. *S. enterica* was the main agent with 2,092 isolates (only  
60 one isolate per patient per month was considered).

61 *S. enterica* strains were identified using the WIDER system (Soria Melguizo,  
62 Madrid, Spain)<sup>36</sup> and serotyped on the basis of somatic O, and both phase 1 and phase  
63 2 flagellar antigens by agglutination tests with antisera (Bio-Rad, Marnes la Coquette,  
64 France) as specified by the White-Kauffmann-Le Minor scheme<sup>17</sup>.

65

### 66 *Antimicrobial susceptibility*

67 Antimicrobial susceptibility was first carried out for all the isolates using the  
68 WIDER system according to the recommendations of the Clinical and Laboratory  
69 Standards Institute guidelines<sup>9</sup>. For ESC, the antibiotic concentration range was 0.12 to  
70 8 µg/ml for cefotaxime and 0.5 to 16 µg/ml for ceftazidime. All strains of *Salmonella*  
71 showing a decreased susceptibility to one or both of these antibiotics, meaning a  
72 minimal inhibitory concentration (MIC)  $\geq 1$  µg/mL but remaining susceptible to  
73 cephamycins and to the association with clavulanic acid were selected for further  
74 analysis, according with the classical definition of ESBL<sup>31</sup>.

75 ESBL phenotype was detected by the double-disk synergy method <sup>20</sup> and  
76 measuring the MIC for ceftriaxone (CRO) ceftazidime (CAZ) and cefotaxime (CTX)  
77 with and without clavulanic acid, using the ESBL detection Etest strips (AB Biodisk,  
78 Solna, Sweden).

79 Additional testing was carried out by the disk-diffusion method with 32  
80 antimicrobial drugs (Bio-Rad), as previously described <sup>38</sup>.

81

### 82 *PCR amplification of antimicrobial resistance genes and sequence analysis*

83 Total DNA was extracted using the InstaGene matrix kit (Bio-Rad) according to  
84 the manufacturer's recommendations. The resistance genes, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA-1</sub>  
85 group, *bla*<sub>CTX-M</sub>, and class 1 integron gene cassettes were amplified by PCR as  
86 described previously <sup>38</sup>. Also *qnr*, *qepA* and *aac(6')-Ib* genes were investigated when  
87 resistance to NAL was observed, as previously described <sup>30,33</sup>.

88 Sequencing was performed at the "Plateforme de Génotypage des Pathogènes et  
89 Santé Publique, PF8" (Institut Pasteur). The nucleotide sequences and the deduced  
90 protein sequences were analyzed with EditSeq and Megalign software (Dnastar,  
91 Madison, WI). The BLASTN program of NCBI (National Center for Biotechnology  
92 Information, US National Library of Medicine, Bethesda, MD, U.S.A.) was used for  
93 database searches.

94

### 95 *Resistance transfer determination*

96 A resistance transfer experiment was carried out on solid media, using either  
97 *Escherichia coli* K-12 BM14 resistant to sodium azide or *E. coli* C1a resistant to  
98 nalidixic acid (NAL), as the recipient strain <sup>12</sup>. Transconjugants were selected on  
99 Drigalski agar (Bio-Rad) supplemented with CRO (20 µg/mL) and sodium azide (500

100  $\mu\text{g/mL}$ ) or NAL (64  $\mu\text{g/mL}$ ). Three *E. coli* transconjugants were arbitrarily selected for  
101 each experiment.

102

### 103 *Plasmid analysis*

104 Plasmid DNA from parental and transconjugant strains was analyzed by  
105 pulsed field gel electrophoresis (PFGE) after linearization with the S1 nuclease enzyme,  
106 as described previously <sup>12</sup>. PCR-based replicon typing analysis was performed, as  
107 described by Carattoli et al. <sup>7</sup>. The 18 primer pairs targeting FIA, FIB, FIC, HI1, HI2,  
108 II-I $\gamma$ , L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FII replicons were used in separate  
109 PCR reactions.

110

### 111 *PFGE typing*

112 The genetic diversity of the four *S. enterica* serotype Virchow isolates resistant  
113 to ESC was assessed by PFGE of genomic DNA digested with *Xba*I (Roche,  
114 Mannheim, Germany), as described previously <sup>38</sup>. The running conditions and the  
115 molecular size marker were as described in the standardized PulseNet protocol  
116 (<http://www.cdc.gov/pulsenet/>). BioNumerics 4.0 (Applied Maths, Saint-Martens-  
117 Latem, Belgium) was used for image normalization and construction of similarity  
118 matrices. Bands were assigned manually. Clustering was carried out by the unweighted  
119 pair-group method with arithmetic averages (UPGMA) based on the Dice similarity  
120 index, using a 1% optimization parameter and 1% band-position tolerance. The results  
121 were compared to PFGE profiles of *S. enterica* from the French National Reference  
122 Centre for *Salmonella* (FNRC-Salm) database.

123

124



125 **RESULTS**

126           Between 2001 and 2008, 2,092 *S. enterica* isolates were identified (one per  
127 patient within a period of 30 days), with a continuous tendency to decrease from 2002  
128 (394 isolates) to 2008 (138 isolates). The distribution of serotypes is presented in TABLE  
129 1. Enteritidis was the predominant serotype, accounting for 52% of all the isolates.

130           The results of susceptibility testing are shown in TABLES 2 AND 3. Of the 2,092  
131 isolates, 387 (18.5 %) were susceptible to all antimicrobial agents tested. The most  
132 frequent types of resistance observed concerned ampicillin (increasing from 33.5 % in  
133 2001 to 59.4 % in 2008), NAL (47.2 % in 2005 but decreasing since then to a 21.7 % in  
134 2008) and trimethoprim/sulfamethoxazole (peaking at 15 % in 2004). Although up to 17  
135 isolates had a MIC for ceftriaxone of  $\geq 1$   $\mu\text{g}/\text{mL}$ , only five isolates (0.24 %) of  
136 serotypes Enteritidis (isolate 06-424914) and Virchow (isolates 02-236146, 02-214992,  
137 03-1672608 and 04-1831083), showed an ESBL phenotype determined by double-disk  
138 diffusion test and ESBL-Etest® (TABLE 3). All five isolates were susceptible to  
139 cephamycins, association with clavulanic acid and carbapenems. The mechanisms of  
140 resistance to beta-lactam antibiotics of these five isolates were investigated and are  
141 shown below. The other 12 isolates with a MIC for ceftriaxone of  $\geq 1$   $\mu\text{g}/\text{mL}$  were  
142 resistant to cefoxitin and gave a negative double disk synergy test (despite the use of  
143 several ESC disks placed at distances of both 15 and 20 mm from the clavulanic acid  
144 disk), therefore they were considered as cephamycinase-producing isolates. No further  
145 molecular characterization of these isolates has been possible, as they were accidentally  
146 discarded as no-ESBL-producers.

147           The serotype Enteritidis isolate 06-424914 was also resistant to aminoglycosides  
148 (streptomycin, spectinomycin), trimethoprim-sulfamethoxazole, and tetracycline  
149 (TABLE 3). The serotype Virchow isolates 02-236146, 02-214992, 03-1672608, 04-

150 1831083 had a similar susceptibility profile, except an additional resistance to  
151 kanamycin and NAL with a decreased susceptibility to ciprofloxacin (MIC of 0.25 to  
152 0.5 mg/L).

153 PCR and sequence analysis detected in the five isolates both the penicillinase  
154 *bla*<sub>TEM-1</sub> gene and an ESBL *bla*<sub>CTX-M</sub> gene. Serotype Enteritidis isolate 06-424914  
155 contained the *bla*<sub>CTX-M-1</sub> gene, whereas serotype Virchow isolates contained the *bla*<sub>CTX-</sub>  
156 <sub>M-9</sub> gene. The serotype Virchow isolates also harboured a 1.5 kb class 1 integron  
157 containing a *dfrA16-aadA2* gene cassette known to confer resistances to trimethoprim  
158 and streptomycin and spectinomycin respectively. No class 1 integrons were found for  
159 the Enteritidis isolate.

160 In serotype Enteritidis isolate 06-424914, the *bla*<sub>CTX-M-1</sub> gene was carried by a  
161 ≈100 kb conjugative IncI1-IncN multireplicon plasmid. Other resistance determinants,  
162 affecting streptomycin, spectinomycin, sulfamides, trimethoprim-sulfamethoxazole, and  
163 tetracycline were cotransferred to transconjugants with ceftriaxone resistance.

164 In serotype Virchow isolates, the *bla*<sub>CTX-M-9</sub> gene was carried by a ≈300 kb  
165 conjugative plasmid of replicon IncHI2. The same resistance determinants as listed for  
166 the serotype Enteritidis isolate 06-424914, were cotransferred to transconjugants with  
167 ceftriaxone resistance. The resistance to NAL was due to a chromosomal mutation on  
168 the QRDR region of the *gyrA* leading to a substitution of a serine in position 83 by a  
169 phenylalanine, as described previously<sup>22</sup>.

170 In this study, all the CTX-M-9-producing *S. enterica* serotype Virchow isolates  
171 clustered together independently to their geographic area of origin, might it be Spain or  
172 France, as it was shown in a database comparison between the isolates from Saragossa  
173 and some pulsotypes of the FNRC-Salm (FIGURE 1).

174

175 **DISCUSSION**

176 Since the first *bla*<sub>CTX-M</sub> genes were described in the early 1990s, *S. enterica* has  
177 been one of the first species of *Enterobacteriaceae* to be identified harbouring this kind  
178 of resistances<sup>1</sup>. CTX-M-9 was first reported in Spain in 1996, produced by an *E. coli*  
179 human isolate and *S. enterica* serotype Virchow carrying a *bla*<sub>CTX-M-9</sub> appeared just a  
180 few years later<sup>34</sup>. Retrospective studies in the United Kingdom have found strains that  
181 were isolated in the 1990s, from patients with a history of travelling abroad<sup>19</sup>. Strains  
182 isolated from both humans and poultry were reported in France, suggesting an  
183 interspecies transfer, which affected several serotypes (Virchow and Enteritidis among  
184 them) and different ESBL enzymes (CTX-M-2, TEM-52, CTX-M-9)<sup>38</sup>. This is  
185 supported as well by the latest studies on poultry in Spain<sup>5</sup>.

186 The ESBL gene *bla*<sub>CTX-M-1</sub> was first reported in Germany in 1996, harboured by  
187 an *E. coli* strain<sup>2</sup> and the first case of *S. enterica* producing CTX-M-1 was a strain of  
188 serotype Typhimurium isolated in France<sup>21</sup>. Recent studies found *S. enterica* serotypes  
189 Enteritidis, carrying the *bla*<sub>CTX-M-32</sub> gene, and Litchfield carrying the *bla*<sub>CTX-M-1</sub> gene in  
190 Spain, in relation with conjugative plasmids of IncN and IncI1, respectively<sup>16</sup>. The  
191 plasmid carrying the *bla*<sub>CTX-M-1</sub> gene had also a similar size (110 kb) to the plasmid  
192 found for the Enteritidis isolate of our study, and both share a similar multi-drug  
193 resistance profile and the lack of a class 1 integron. *E. coli* strains carrying this ESBL  
194 gene on a IncI1 plasmid have been reported Italy and France from both humans and  
195 animals (poultry and dogs)<sup>14,15</sup>. Another study on *E. coli* strains recovered from human  
196 samples in France showed that the *bla*<sub>CTX-M-1</sub> gene was carried by either IncI1 or IncN  
197 plasmids<sup>24</sup>. Multireplicon plasmids do often occur<sup>6</sup>, but an IncI1-N has not been  
198 described yet. Previous findings in animals, mainly poultry, of both ESBL *bla*<sub>CTX-M-1</sub>

199 and *bla*<sub>CTX-M-9</sub> harboured in IncI or IncHI2 plasmids suggest that poultry might play an  
200 important role as a reservoir for these bacteria <sup>16, 38</sup>.

201 The first ESBL *S. enterica* strains were detected in our laboratory in 2002 <sup>8</sup>.  
202 During this eight-year prospective study we have found a total of four isolates of  
203 serotype Virchow harbouring a *bla*<sub>CTX-M-9</sub> gene and one *S. enterica* serotype Enteritidis  
204 with the *bla*<sub>CTX-M-1</sub> gene, all of them during the period 2002-2006. ESBL strains were  
205 no longer recovered after 2006. Although the final rate of ESBL among the total figures  
206 was rather low (0.24 %), we call out the fact that those four isolates of serotype  
207 Virchow producing a CTX-M-9 occurred in 26.6 % of all isolates of that serotype  
208 during the time period of this study. The four strains of serotype Virchow appear to  
209 carry the same IncHI2 conjugative plasmid previously described in CTX-M-9  
210 producing strains from Spain (in the region of La Rioja, adjacent to Aragon <sup>32</sup>, in  
211 Barcelona <sup>10</sup> and the city of Madrid <sup>28</sup>). These works describe plasmids of the same size  
212 and belonging to the same incompatibility group IncHI2, carrying the same gene  
213 cassette *dfrA16—aadA2* within the complex class 1 integron In60, altogether with the  
214 *bla*<sub>CTX-M-9</sub> gene <sup>3, 10, 27, 32</sup>. This has finally become one of the most predominant  
215 combinations of *S. enterica* serotype and ESBL in Spain and Portugal <sup>23</sup>.

216 Clonal transmission of multi-drug-resistance has been proven in isolates from  
217 poultry and humans <sup>32, 38</sup>. The PFGE pulsotypes, with more than 96 % of similarity  
218 among the four ESBL Virchow isolates and the phenotype, gene cassettes and plasmids  
219 found suggest that they could be associated to the clonal spread of *S. enterica* serotype  
220 Virchow PT19 previously described in Spain and France <sup>18, 38</sup>.

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## REFERENCES

1. **Arlet, G., T.J. Barrett, P. Butaye, A. Cloeckert, M.R. Mulvey, and D.G. White.** 2006. *Salmonella* resistant to extended-spectrum cephalosporins: prevalence and epidemiology. *Microbes Infect* **8**:1945-54.
2. **Bauernfeind, A., I. Stemplinger, R. Jungwirth, S. Ernst, and J.M. Casellas.** 1996. Sequences of beta-lactamase genes encoding CTX-M-1 (MEN-1) and CTX-M-2 and relationship of their amino acid sequences with those of other beta-lactamases. *Antimicrob Agents Chemother* **40**:509-13.
3. **Bonnet, R.** 2004. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* **48**:1-14.
4. **Cantón, R., A. Novais, A. Valverde, E. Machado, L. Peixe, F. Baquero, and T.M. Coque.** 2008. Prevalence and spread of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* **14 Suppl 1**:144-53.
5. **Cápita, R., C. Alonso-Calleja, and M. Prieto.** 2007. Prevalence of *Salmonella enterica* serovars and genovars from chicken carcasses in slaughterhouses in Spain. *J Appl Microbiol* **103**:1366-75.
6. **Carattoli, A.** 2009. Resistance plasmid families in *Enterobacteriaceae*. *Antimicrob Agents Chemother* **53**:2227-38.
7. **Carattoli, A., A. Bertini, L. Villa, V. Falbo, K.L. Hopkins, and E.J. Threlfall.** 2005. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* **63**:219-28.
8. **Castillo García, F.J., C. Seral García, M. Pardos De la Gándara, M.I. Millán Lou, and C. Pitart Ferré.** 2007. Prevalence of fecal carriage of ESBL-producing *Enterobacteriaceae* in hospitalized and ambulatory patients during two non-outbreak periods. *Eur J Clin Microbiol Infect Dis* **26**:77-8.
9. **Clinical and Laboratory Standards Institute/NCCLS. Clinical and Laboratory Standards Institute/NCCLS.** 2009. Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement. M100-S19. CLSI, Wayne, PA.
10. **Diestra, K., C. Juan, T. Curiao, B. Moya, E. Miró, J. Oteo, T.M. Coque, M. Pérez-Vázquez, J. Campos, R. Cantón, A. Oliver, and F. Navarro.** 2009. Characterization of plasmids encoding *bla*ESBL and surrounding genes in Spanish clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *J Antimicrob Chemother* **63**:60-6.

11. **European Centre for Disease Prevention and Control (ECDC).** Salmonellosis (non-typhi, non-paratyphi) - Epidemiological situation. 2008. European Centre for Disease Prevention and Control (ECDC). Available at [ecdc.europa.eu/en/publications](http://ecdc.europa.eu/en/publications). (Online.)
12. **Fabre, L., A. Delauné, E. Espié, K. Nygard, M. Pardos, L. Polomack, F. Guesnier, M. Galimand, J. Lassen, and F.X. Weill.** 2009. Chromosomal integration of the extended-spectrum beta-lactamase gene *bla*CTX-M-15 in *Salmonella enterica* serotype Concord isolates from internationally adopted children. *Antimicrob Agents Chemother* **53**:1808-16.
13. **Fernández Vázquez, M., J.L. Muñoz Bellido, M.I. García García, and J.A. García-Rodríguez.** 2006. *Salmonella enterica* serovar Enteritidis producing a TEM-52 beta-lactamase: first report in Spain. *Diagn Microbiol Infect Dis* **55**:245-6.
14. **García-Fernández, A., G. Chiaretto, A. Bertini, L. Villa, D. Fortini, A. Ricci, and A. Carattoli.** 2008. Multilocus sequence typing of IncII plasmids carrying extended-spectrum beta-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. *J Antimicrob Chemother* **61**:1229-33.
15. **Girlich, D., L. Poirel, A. Carattoli, I. Kempf, M.F. Lartigue, A. Bertini, and P. Nordmann.** 2007. Extended-spectrum beta-lactamase CTX-M-1 in *Escherichia coli* isolates from healthy poultry in France. *Appl Environ Microbiol* **73**:4681-5.
16. **González-Sánchez, R., S. Herrera-León, M. de la Fuente, M. Arroyo, and M.A. Echeíta.** 2009. Emergence of extended-spectrum beta-lactamases and AmpC-type beta-lactamases in human *Salmonella* isolated in Spain from 2001 to 2005. *J Antimicrob Chemother* **64**:1181-6.
17. **Grimont, F., and F.X. Weill.** 2007. Antigenic Formulae of the *Salmonella* serovars. WHO-Institut Pasteur. Available at [www.pasteur.fr/sante/clre/cadrecnr/salmoms-index.html](http://www.pasteur.fr/sante/clre/cadrecnr/salmoms-index.html). (Online.)
18. **Herrera-León, S., R. González-Sanz, I. Rodríguez, M.R. Rodicio, and M.A. Echeíta.** 2010. Spread of a multiresistant CTX-M-9-producing *Salmonella enterica* serotype Virchow phage type 19 in Spain. *Eur J Clin Microbiol Infect Dis* **29**:901-5.
19. **Hopkins, K.L., A. Deheer-Graham, E.J. Threlfall, M.J. Batchelor, and E. Liébana.** 2006. Novel plasmid-mediated CTX-M-8 subgroup extended-spectrum beta-lactamase (CTX-M-40) isolated in the UK. *Int J Antimicrob Agents* **27**:572-5.
20. **Jarlier, V., M.H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-



- lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis* **10**:867-78.
21. **Lartigue, M.F., L. Poirel, J.W. Decousser, and P. Nordmann.** 2005. Multidrug-resistant *Shigella sonnei* and *Salmonella enterica* serotype Typhimurium isolates producing CTX-M beta-lactamases as causes of community-acquired infection in France. *Clin Infect Dis* **40**:1069-70.
  22. **Le, T.A., L. Fabre, P. Roumagnac, P.A. Grimont, M.R. Scavizzi, and F.X. Weill.** 2007. Clonal expansion and microevolution of quinolone-resistant *Salmonella enterica* serotype typhi in Vietnam from 1996 to 2004. *J Clin Microbiol* **45**:3485-92.
  23. **Livermore, D.M., R. Cantón, M. Gniadkowski, P. Nordmann, G.M. Rossolini, G. Arlet, J. Ayala, T.M. Coque, I. Kern-Zdanowicz, F. Luzzaro, L. Poirel, and N. Woodford.** 2007. CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* **59**:165-74.
  24. **Marcadé, G., C. Deschamps, A. Boyd, V. Gautier, B. Picard, C. Branger, E. Denamur, and G. Arlet.** 2009. Replicon typing of plasmids in *Escherichia coli* producing extended-spectrum beta-lactamases. *J Antimicrob Chemother* **63**:67-71.
  25. **Meakins, S., I.S. Fisher, C. Berghold, P. Gerner-Smidt, H. Tschape, M. Cormican, I. Luzzi, F. Schneider, W. Wannett, J. Coia, A. Echeíta, and E.J. Threlfall.** 2008. Antimicrobial drug resistance in human nontyphoidal *Salmonella* isolates in Europe 2000-2004: a report from the Enter-net International Surveillance Network. *Microb Drug Resist* **14**:31-5.
  26. **Morosini, M.I., J. Blázquez, M.C. Negri, R. Cantón, E. Loza, and F. Baquero.** 1996. Characterization of a nosocomial outbreak involving an epidemic plasmid encoding for TEM-27 in *Salmonella enterica* subspecies *enterica* serotype Othmarschen. *J Infect Dis* **174**:1015-20.
  27. **Novais, A., R. Canton, R. Moreira, L. Peixe, F. Baquero, and T.M. Coque.** 2007. Emergence and dissemination of Enterobacteriaceae isolates producing CTX-M-1-like enzymes in Spain are associated with IncFII (CTX-M-15) and broad-host-range (CTX-M-1, -3, and -32) plasmids. *Antimicrob Agents Chemother* **51**:796-9.
  28. **Novais, A., R. Cantón, A. Valverde, E. Machado, J.C. Galán, L. Peixe, A. Carattoli, F. Baquero, and T.M. Coque.** 2006. Dissemination and persistence of *bla*CTX-M-9 are linked to class 1 integrons containing CR1 associated with defective transposon derivatives from Tn402 located in early antibiotic resistance plasmids of IncHI2, IncP1-alpha, and IncFI groups. *Antimicrob Agents Chemother* **50**:2741-50.

29. **Olivera, S., F.J. Castillo, M.T. Llorente, A. Clavel, M. Varea, C. Seral, and M.C. Rubio.** 2002. [Antimicrobial resistance of clinical strains of *Salmonella enterica* isolated in Zaragoza]. *Rev Esp Quimioter* **15**:152-7.
30. **Perichon, B., P. Courvalin, and M. Galimand.** 2007. Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic fluoroquinolones by QepA-mediated efflux in *Escherichia coli*. *Antimicrob Agents Chemother* **51**:2464-9.
31. **Philippon, A., R. Labia, and G. Jacoby.** 1989. Extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* **33**:1131-6.
32. **Riaño, I., M. García-Campello, Y. Sáenz, P. Álvarez, L. Vinué, M. Lantero, M.A. Moreno, M. Zarazaga, and C. Torres.** 2009. Occurrence of extended-spectrum beta-lactamase-producing *Salmonella enterica* in northern Spain with evidence of CTX-M-9 clonal spread among animals and humans. *Clin Microbiol Infect* **15**:292-5.
33. **Richter, S.N., I. Frasson, C. Bergo, R. Manganelli, A. Cavallaro, and G. Palu.** 2010. Characterisation of qnr plasmid-mediated quinolone resistance in *Enterobacteriaceae* from Italy: association of the *qnrB19* allele with the integron element ISCR1 in *Escherichia coli*. *Int J Antimicrob Agents* **35**:578-83.
34. **Simarro, E., F. Navarro, J. Ruiz, E. Miró, J. Gómez, and B. Mirelis.** 2000. *Salmonella enterica* serovar virchow with CTX-M-like beta-lactamase in Spain. *J Clin Microbiol* **38**:4676-8.
35. **Threlfall, E.J.** 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. *FEMS Microbiol Rev* **26**:141-8.
36. **Valverde, A., T.M. Coque, L. García-San Miguel, F. Baquero, and R. Cantón.** 2008. Complex molecular epidemiology of extended-spectrum beta-lactamases in *Klebsiella pneumoniae*: a long-term perspective from a single institution in Madrid. *J Antimicrob Chemother* **61**:64-72.
37. **Voetsch, A.C., T.J. Van Gilder, F.J. Angulo, M.M. Farley, S. Shallow, R. Marcus, P.R. Cieslak, V.C. Deneen, and R.V. Tauxe.** 2004. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. *Clin Infect Dis* **38 Suppl 3**:S127-34.
38. **Weill, F.X., R. Lailier, K. Praud, A. Kerouanton, L. Fabre, A. Brisabois, P.A. Grimont, and A. Cloeckaert.** 2004. Emergence of extended-spectrum-beta-lactamase (CTX-M-9)-producing multiresistant strains of *Salmonella enterica* serotype Virchow in poultry and humans in France. *J Clin Microbiol* **42**:5767-73.

TABLES AND FIGURE LEGENDS

FIGURE 1. CLONAL RELATEDNESS OF *Xba*I-PGFE PROFILES OBTAINED FROM *S. ENTERICA* SEROTYPE VIRCHOW ISOLATES FROM SARAGOSSA AND FRANCE

Dice (Opt: 1-20%) (Tel: 1.2%-1.2%) (H>: 0% S>: 0%) (p: 0%-100.0%)  
 PFGE-XbaI PFGE-XbaI

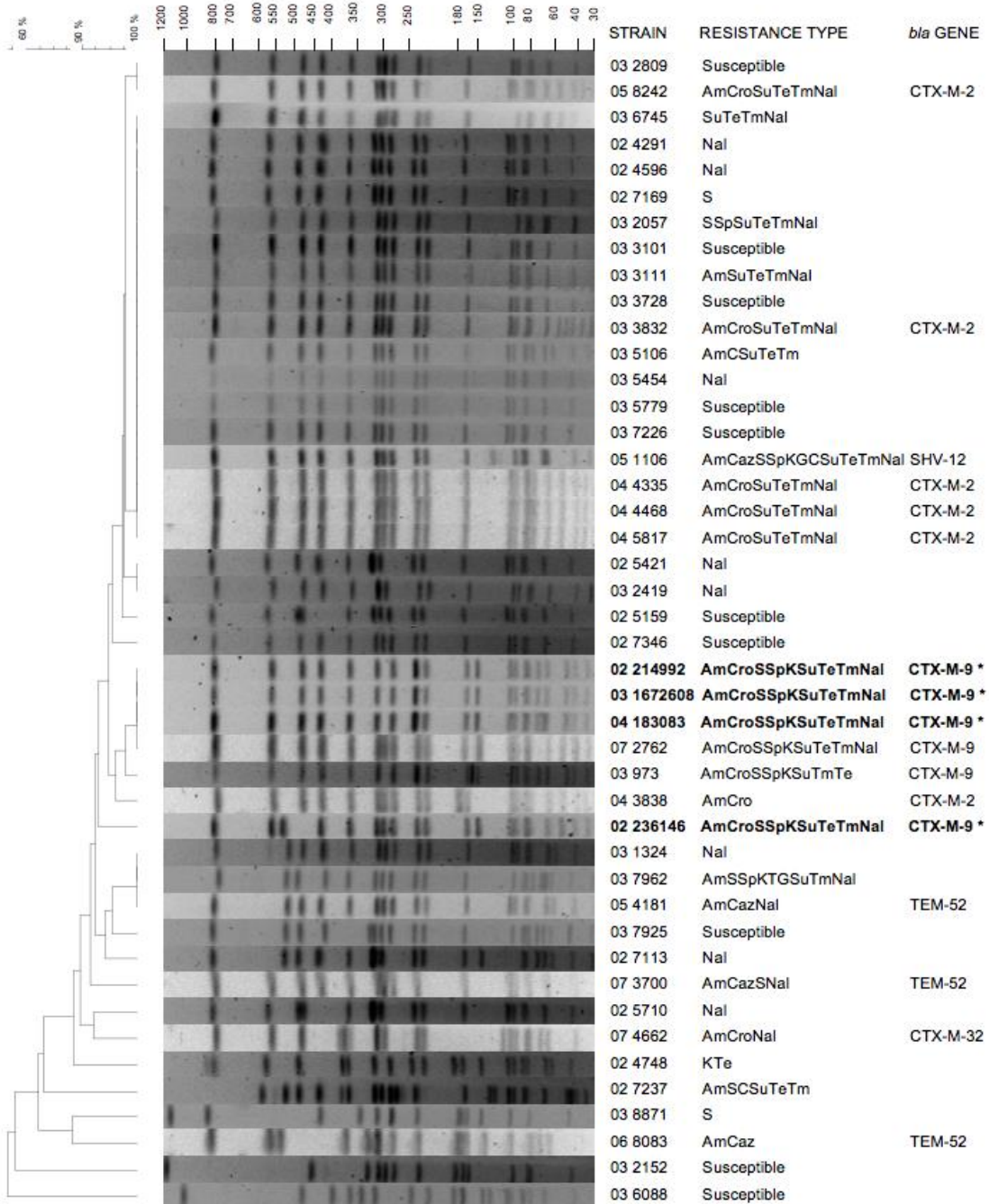


FIGURE 1. The two first numbers indicate the year of isolation of the strain. The strains from Saragossa are outlined in bold and with asterisks (\*). The remaining strains belong to the database of the French National Reference Centre for *Salmonella*. Am: amoxicillin, Cro: ceftriaxone, Caz: ceftazidime, S: streptomycin, Sp: spectinomycin, K: kanamycin, G: gentamicin, Su: sulfonamide, Te: tetracycline, Tm: trimethoprim, Nal: nalidixic acid.

TABLE 1. DISTRIBUTION OF SALMONELLA ENTERICA SEROTYPES IN THE CLINICAL MICROBIOLOGY LABORATORY OF H.C.U. LOZANO BLESA IN ARAGON, SPAIN, DURING THE PERIOD 2001-2008

<i>Salmonella</i> serotypes	2001 (n= 304)	2002 (n= 394)	2003 (n= 334)	2004 (n= 346)	2005 (n=197)	2006 (n= 207)	2007 (n= 172)	2008 (n= 138)	<i>total</i> (n= 2092)
O:9 (formerly D1 group)	183	233	219	197	97	83	59	32	1103
<b>Enteritidis</b>	<b>181</b>	<b>233</b>	<b>216</b>	<b>195</b>	<b>97</b>	<b>81</b>	<b>57</b>	<b>28</b>	<b>1088</b>
Others	2	0	3	2	0	2	2	4	15
O:4 (formerly B group)	72	115	67	89	61	72	65	72	613
<b>Typhimurium</b>	<b>68</b>	<b>107</b>	<b>66</b>	<b>89</b>	<b>58</b>	<b>68</b>	<b>59</b>	<b>67</b>	<b>582</b>
Others	4	8	1	0	3	4	6	5	31
O:6,7 (formerly C1 group)	13	22	21	14	18	11	9	9	117
<b>Virchow</b>	<b>0</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>15</b>
Others	13	19	18	12	15	10	8	7	102
O:6,8 (formerly C2 group)	21	17	5	8	17	13	4	11	96
Other groups	15	7	22	38	2	23	33	12	152
Non typable	0	0	0	0	2	5	2	2	11

TABLE 2. PERCENTAGE OF RESISTANCE TO SPECIFIC ANTIBIOTICS IN *S. ENTERICA*  
IN ARAGÓN, SPAIN FROM 2001 TO 2008

<i>Antibiotics</i>	<i>% of isolates resistant</i>								<i>total</i> (n=2092)
	2001 (n=304)	2002 (n=394)	2003 (n=334)	2004 (n=346)	2005 (n=197)	2006 (n=207)	2007 (n=172)	2008 (n=138)	
Ampicillin	33.5	38.8	24.0	35.2	43.8	36.2	34.8	59.4	36.3
Cefotaxime	0	0.2	0.9	0.3	1	1.9	1.1	2.9	0.8
Gentamicin	4.3	4	1.2	1.7	1	1.9	1.7	10.8	3
Nalidixic acid	28.6	27	31	31	47.2	33.3	30.8	21.7	31
Ciprofloxacin	0.3	0.7	0.2	0.3	0	0.9	0	0.7	0.4
Co-trimoxazole	7.8	12.6	7.8	15	12	10.6	12.7	5	10.8

TABLE 3. CHARACTERISTICS OF THE S. ENTERICA STRAINS AND THE TRANSCONJUGANTS IN THIS STUDY

<i>strain</i>	<i>serotype</i>	<i>year</i>	<i>coresistance phenotype</i>	<i>ESBL</i>	<i>CTX</i>	<i>CTL</i>	<i>CAZ</i>	<i>CZL</i>	<i>CPM</i>	<i>CPL</i>	<i>CRO</i>	<i>CIP</i>	<i>Inc</i>	<i>plasmid size</i>
<b>02-236146</b>	Virchow	2002	SSpKSuTeTmNal	CTX-M-9	>16	0.047	<0.5	0.19	1	0.064	15	28	HI2	290 kb
236146-TC2			SSpKSuTeTm	CTX-M-9	2	0.032	<0.5	0.094	0.5	0.064	17	35	HI2	290 kb
<b>02-214992</b>	Virchow	2002	SSpKSuTeTmNal	CTX-M-9	>16	0.064	<0.5	0.25	2	0.064	16	26	HI2	320 kb
214992-TC1			SSpKSuTeTm	CTX-M-9	2	0.032	<0.5	0.125	0.38	<0.064	20	35	HI2	320 kb
<b>03-1672608</b>	Virchow	2003	SSpKSuTeTmNal	CTX-M-9	16	0.047	0.75	0.19	1	<0.064	16	26	HI2	320 kb
1672608-TC			SSpKSuTeTm	CTX-M-9	2	0.032	<0.5	0.125	0.38	<0.064	16	35	HI2	320 kb
<b>04-1831083</b>	Virchow	2004	SSpKSuTeTmNal	CTX-M-9	16	0.047	0.75	0.125	1	0.064	16	26	HI2	320 kb
1831083-TC			SSpKSuTeTm	CTX-M-9	12	0.032	0.5	0.125	0.5	<0.064	16	35	HI2	320 kb
<b>06-424914</b>	Enteritidis	2006	SSpSuTeTm	CTX-M-1	>16	0.032	1.5	0.19	3	<0.064	15	35	I1-N	100 kb
424914-TC1			SSpSuTeTm	CTX-M-1	>16	0.023	1	0.094	2	<0.064	15	35	I1-N	145 kb

TC: E. coli transconjugant; S: streptomycin; Sp: spectinomycin; K: kanamycin; Su: sulfonamides; Te: tetracyclin; Tm: trimethoprim; Nal: nalidixic acid; CTX: cefotaxime; CTL: cefotaxime/clavulanic acid; CAZ: ceftazidime; CZL: ceftazidime/clavulanic acid; CPM: cefepime; CPL: cefepime/clavulanic acid; CRO: ceftriaxone; CIP: ciprofloxacin. Inc: plasmid incompatibility group. All measurements are E-test MICs ( $\mu\text{g/ml}$ ) except for CRO and CIP when figures represent diameters on disk diffusion test (mm).