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

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INTRODUCTION

Nontyphoidal salmonellosis is one of the most prevalent food borne bacterial infections in Europe and in North America. In the United States, the burden of this infection has been estimated annually to be 1.4 million of cases resulting in 400 deaths [37]. In Europe, 181,876 cases were reported in 2005, which meant a ratio of 39/100,000 inhabitants [11]. Most infections are self-limited and do not require antimicrobial treatment. However, severe, life-threatening bacteraemia sometimes occur, particularly in children and immunocompromised hosts and in these cases an antimicrobial therapy is recommended [35]. Appropriate drugs for *Salmonella* infections include ampicillin, extended-spectrum cephalosporins (ESC), fluoroquinolones, and trimethoprim-sulfamethoxazole. However, rising rates of resistance to ampicillin and trimethoprim-sulfamethoxazole have significantly reduced their efficacy and fluoroquinolones are not approved for the use in children. Consequently, ESC have become the current drugs of choice for the treatment for invasive infections in children [4]. The number of cases of salmonellosis caused by isolates resistant to these ESC is in continuous increase since the very first case was detected in the early 1980s [1]. Nonetheless, they are still rare over the total of *Salmonella* foodborne infections, just reaching a 0.2 % in Europe in 2004 [25]. Resistance to these drugs is mainly mediated by the bacterial production of beta-lactamases that degrade ESC. Two main classes of plasmid beta-lactamases that inactivate ESC have been identified in *Salmonella*: the Ambler class A extended-spectrum beta-lactamases (ESBLs), the most

prevalent class in this genus, and the Ambler class C cephamycinases. Most ESBLs belong to three families, TEM, SHV and CTX-M. Over the last decade, CTX-Ms have become the most prevalent family of ESBLs in the genus *Salmonella* in Europe [1].

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

In 2000, in Aragón, a northwestern region of Spain (1,326,918 inhabitants), 129 foodborne outbreaks that affected 2,030 people (including 1,464 in Saragossa), resulting in 103 hospitalizations were reported. *S. enterica* was found to be the causative agent in 62% of the cases. In 2005, a foodborne outbreak was reported with 179 cases of salmonellosis from the whole region (109 occuring in Saragossa), 19 patients needed hospitalization. This outbreak was epidemiologically related to the consumption of locally farmed chicken (http://www.aragon.es). Very few studies have been published about the epidemiological situation of *S. enterica* or about the profile of antimicrobial

47 resistances shown by them, both for the whole region of Aragon and for the
48 Sanitary Area Saragossa 3. The last clinical study showed a continuous
49 increase of isolations of *S. enterica*, from 1994 (118 isolates) to 2000 (287
50 isolates) [29].

51 In this study, we assessed the frequency of different serotypes and the
52 prevalence of ESC resistance among *S. enterica* isolates obtained in
53 Saragossa during an eight-year period (2001-2008). The characterization of the
54 beta-lactamase genes and their genetic support, and class 1 integron cassettes
55 was done on ESC-resistant *S. enterica* isolates.
56

MATERIALS AND METHODS

Strains

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

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

Antimicrobial susceptibility

Antimicrobial susceptibility was first carried out for all the isolates using the WIDER system according to the recommendations of the Clinical and Laboratory Standards Institute guidelines [9]. For ESC, the antibiotic concentration range was 0.12 to 8 µg/ml for cefotaxime and 0.5 to 16 µg/ml for ceftazidime. All strains of *Salmonella* showing a decreased susceptibility to one

80 or both of these antibiotics, meaning a minimal inhibitory concentration (MIC) ≥
81 1 µg/mL but remaining susceptible to cephamycins and to the association with
82 clavulanic acid were selected for further analysis, according with the classical
83 definition of ESBL [31].

84 ESBL phenotype was detected by the double-disk synergy method [20] and
85 measuring the MIC for ceftriaxone (CRO) ceftazidime (CAZ) and cefotaxime
86 (CTX) with and without clavulanic acid, using the ESBL detection Etest strips
87 (AB Biodisk, Solna, Sweden).

88 Additional testing was carried out by the disk-diffusion method with 32
89 antimicrobial drugs (Bio-Rad), as previously described [38].

90

91 *PCR amplification of antimicrobial resistance genes and sequence analysis*

92 Total DNA was extracted using the InstaGene matrix kit (Bio-Rad)
93 according to the manufacturer's recommendations. The resistance genes,
94 bla_{TEM}, bla_{SHV}, bla_{OXA-1} group, bla_{CTX-M}, and class 1 integron gene cassettes
95 were amplified by PCR as described previously [38]. Also *qnr, qepA* and *aac(6')-*
96 *Ib* genes were investigated when resistance to NAL was observed, as
97 previously described [30, 33].

98 Sequencing was performed at the "Plateforme de Génotypage des
99 Pathogènes et Santé Publique, PF8" (Institut Pasteur). The nucleotide
100 sequences and the deduced protein sequences were analyzed with EditSeq
101 and Megalign software (Dnastar, Madison, WI). The BLASTN program of NCBI

(National Center for Biotechnology Information, US National Library of Medicine, Bethseda, MD, U.S.A.) was used for database searches.

Resistance transfer determination

A resistance transfer experiment was carried out on solid media, using either *Escherichia coli* K-12 BM14 resistant to sodium azide or *E. coli* C1a resistant to nalidixic acid (NAL), as the recipient strain [12]. Transconjugants were selected on Drigalski agar (Bio-Rad) supplemented with CRO (20 μg/mL) and sodium azide (500 μg/mL) or NAL (64 μg/mL). Three *E. coli* transconjugants were arbitrarily selected for each experiment.

Plasmid analysis

Plasmid DNA from parental and transconconjugant strains was analyzed by pulsed field gel electrophoresis (PFGE) after linearization with the S1 nuclease enzyme, as described previously [12]. PCR-based replicon typing analysis was performed, as described by Carattoli et al. [7]. The 18 primer pairs targeting FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FII replicons were used in separate PCR reactions.

PFGE typing

The genetic diversity of the four *S. enterica* serotype Virchow isolates resistant to ESC was assessed by PFGE of genomic DNA digested with *Xba*I (Roche, Mannheim, Germany), as described previously [38]. The running

125 conditions and the molecular size marker were as described in the standardized
126 PulseNet protocol (http://www.cdc.gov/pulsenet/). BioNumerics 4.0 (Applied
127 Maths, Saint-Martens-Latem, Belgium) was used for image normalization and
128 construction of similarity matrices. Bands were assigned manually. Clustering
129 was carried out by the unweighted pair-group method with arithmetic averages
130 (UPGMA) based on the Dice similarity index, using a 1% optimization
131 parameter and 1% band-position tolerance. The results were compared to
132 PFGE profiles of *S. enterica* from the French National Reference Centre for
133 *Salmonella* (FNRC-Salm) database.
134
135

RESULTS

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

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

these isolates has been possible, as they were accidentally discarded as no-ESBL-producers.

The serotype Enteritidis isolate 06-424914 was also resistant to aminoglycosides (streptomycin, spectinomycin), trimethoprim-sulfamethoxazole, and tetracycline (TABLE 3). The serotype Virchow isolates 02-236146, 02-214992, 03-1672608, 04-1831083 had a similar susceptibility profile, except an additional resistance to kanamycin and NAL with a decreased susceptibility to ciprofloxacin (MIC of 0.25 to 0.5 mg/L).

PCR and sequence analysis detected in the five isolates both the penicillinase *bla*$_{\text{TEM-1}}$ gene and an ESBL *bla*$_{\text{CTX-M}}$ gene. Serotype Enteritidis isolate 06-424914 contained the *bla*$_{\text{CTX-M-1}}$ gene, whereas serotype Virchow isolates contained the *bla*$_{\text{CTX-M-9}}$ gene. The serotype Virchow isolates also harboured a 1.5 kb class 1 integron containing a *dfrA16-aadA2* gene cassette known to confer resistances to trimethoprim and streptomycin and spectinomycin respectively. No class 1 integrons were found for the Enteritidis isolate.

In serotype Enteritidis isolate 06-424914, the *bla*$_{\text{CTX-M-1}}$ gene was carried by a ≈100 kb conjugative IncI1-IncN multireplicon plasmid. Other resistance determinants, affecting streptomycin, spectinomycin, sulfamides, trimethoprim-sulfamethoxazole, and tetracycline were cotransfered to transconjugants with ceftriaxone resistance.

In serotype Virchow isolates, the *bla*$_{\text{CTX-M-9}}$ gene was carried by a ≈300 kb conjugative plasmid of replicon IncHI2. The same resistance determinants as

182 listed for the serotype Enteritidis isolate 06-424914, were cotransfered to
183 transconjugants with ceftriaxone resistance. The resistance to NAL was due to
184 a chromosomal mutation on the QRDR region of the *gyr*A leading to a
185 substitution of a serine in position 83 by a phenylalanine, as described
186 previously [22].

187 In this study, all the CTX-M-9-producing *S. enterica* serotype Virchow
188 isolates clustered together independently to their geographic area of origin,
189 might it be Spain or France, as it was shown in a database comparison between
190 the isolates from Saragossa and some pulsotypes of the FNRC-Salm (FIGURE
191 1).
192

DISCUSSION

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

The ESBL gene *bla*$_{\text{CTX-M-1}}$ was first reported in Germany in 1996, harboured by an *E. coli* strain [2] and the first case of *S. enterica* producing CTX-M-1 was a strain of serotype Typhimurium isolated in France [21]. Recent studies found *S. enterica* serotypes Enteritidis, carrying the *bla*$_{\text{CTX-M-32}}$ gene, and Litchfield carrying the *bla*$_{\text{CTX-M-1}}$ gene in Spain, in relation with conjugative plasmids of IncN and IncI1, respectively [16]. The plasmid carrying the *bla*$_{\text{CTX-M-1}}$ gene had also a similar size (110 kb) to the plasmid found for the Enteritidis isolate of our study, and both share a similar multi-drug resistance profile and the lack of a class 1 integron. *E. coli* strains carrying this ESBL gene on a IncI1 plasmid have been reported Italy and France from both humans and animals (poultry and dogs) [14, 15]. Another study on *E. coli* strains recovered from human

samples in France showed that the *bla*$_{\text{CTX-M-1}}$ gene was carried by either IncI1 or IncN plasmids [24]. Multireplicon plasmids do often occur [6], but an IncI1-N has not been described yet. Previous findings in animals, mainly poultry, of both ESBL *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CTX-M-9}}$ harboured in IncI or IncHI2 plasmids suggest that poultry might play an important role as a reservoir for these bacteria [16, 38].

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

Clonal transmission of multi-drug-resistance has been proven in isolates from poultry and humans [32, 38]. The PFGE pulsotypes, with more than 96 % of

239 similarity among the four ESBL Virchow isolates and the phenotype, gene
240 cassettes and plasmids found suggest that they could be associated to the
241 clonal spread of *S. enterica* serotype Virchow PT19 previously described in
242 Spain and France [18, 38].

ACKNOWLEDGEMENTS

We would like to acknowledge Dr. Joseph Dragavon for the careful reading of this manuscript.

María Pardos de la Gándara was the recipient of the following grants during this work:

1. Grant for Short Term Research in Clinical Centres Abroad by the S.E.I.M.C. (2007)
2. Grant “Project Europe XXI” of the CAI-Social Action and the Government of Aragón for Short Term Research Abroad (2008)
3. Postdoctoral / Post-Specialization Fellowship for Research in Universities or Centres Abroad from the “Alfonso Martín Escudero” Foundation, Spain (2009)
4. Student Travel Fellowship from the SEIMC (Spanish Society of Infectious Diseases and Clinical Microbiology) for the Meeting in Seville, Spain (2009)

AUTHOR DISCLOSURE STATEMENT

The authors declare no commercial associations that might create a conflict of interest in connection with the submitted manuscript:

- María PARDOS DE LA GÁNDARA: has received research funding from the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (Beca Martín Luengo 2007), from the CAI-Gobierno de Aragón (Proyecto Europa XXI 2007-2008) and from the Fundación Alfonso Martín Escudero (Becas para estudios en el extranjero 2008-2009).

- Cristina SERAL GARCÍA: absence of any relationship or any degree of conflicting or dual interest, financial or of any other nature, that may affect professional judgment in relation to the submitted article.

- Francisco Javier CASTILLO GARCÍA: absence of any relationship or any degree of conflicting or dual interest, financial or of any other nature, that may affect professional judgment in relation to the submitted article.

- Carmen RUBIO CALVO: absence of any relationship or any degree of conflicting or dual interest, financial or of any other nature, that may affect professional judgment in relation to the submitted article.

- François Xavier WEILL: absence of any relationship or any degree of conflicting or dual interest, financial or of any other nature, that may affect professional judgment in relation to the submitted article.

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TABLES AND FIGURE LEGENDS

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.

Running title: **ESBL producing *Salmonella enterica* in Saragossa, Spain**

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 $bla_{\text{CTX-M-9}}$ gene and an isolate of serotype Enteritidis carried a $bla_{\text{CTX-M-1}}$ 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 $bla_{\text{CTX-M-9}}$ gene was carried by a ~300 kb IncHI2 conjugative plasmid in the case of the *S. enterica* serotype Virchow isolates. The $bla_{\text{CTX-M-1}}$ 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.

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

Salmonella 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)	*total* (n= 2092)
O:9 (formerly D1 group)	183	233	219	197	97	83	59	32	1103
Enteritidis	**181**	**233**	**216**	**195**	**97**	**81**	**57**	**28**	**1088**
Others	2	0	3	2	0	2	2	4	15
O:4 (formerly B group)	72	115	67	89	61	72	65	72	613
Typhimurium	**68**	**107**	**66**	**89**	**58**	**68**	**59**	**67**	**582**
Others	4	8	1	0	3	4	6	5	31
O:6,7 (formerly C1 grou	13	22	21	14	18	11	9	9	117
Virchow	**0**	**3**	**3**	**2**	**3**	**1**	**1**	**2**	**15**
Others	13	19	18	12	15	10	8	7	102
O:6,8 (formerly C2 grou	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

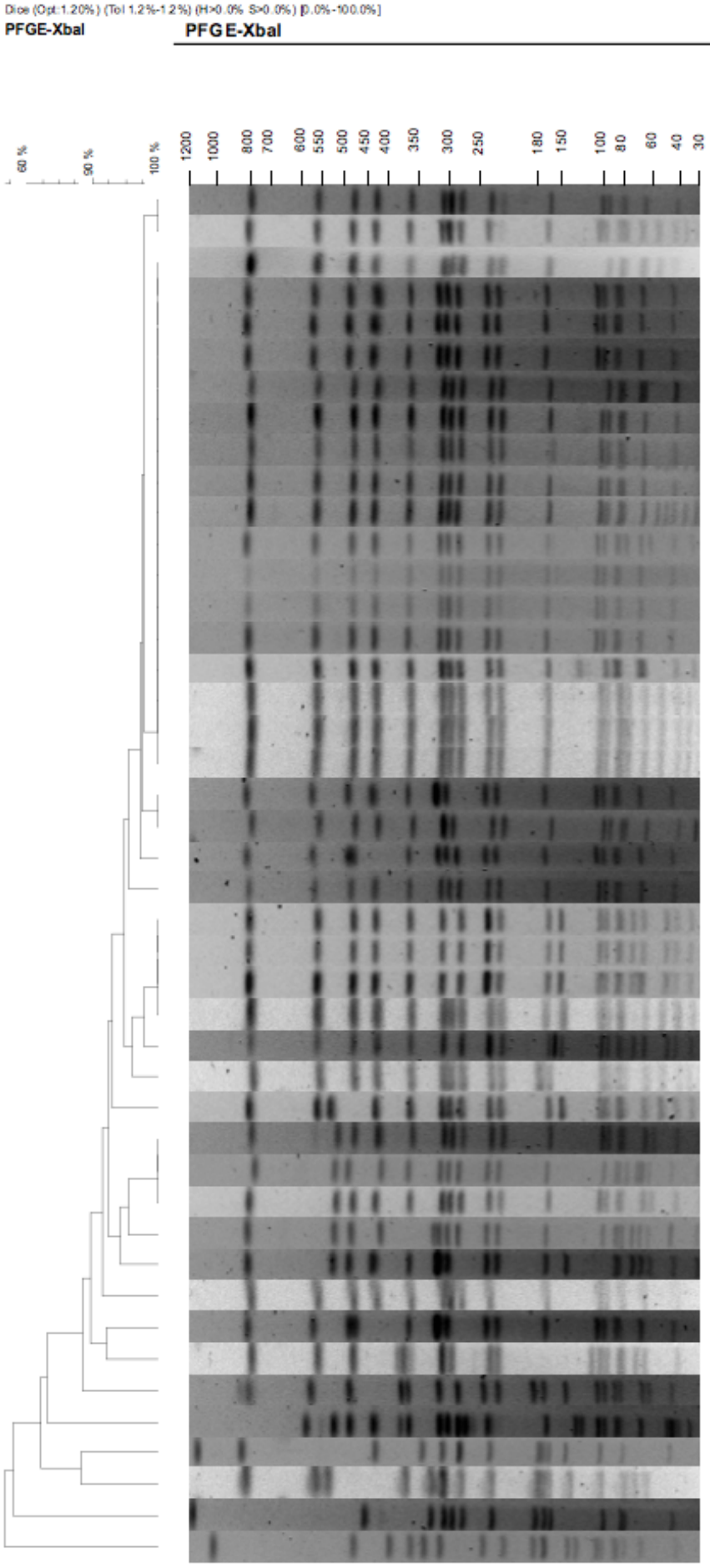
Antibiotics	*% of isolates resistant*								*total*
	2001 (n= 304)	2002 (n= 394)	2003 (n= 334)	2004 (n= 346)	2005 (n=197)	2006 (n= 207)	2007 (n= 172)	2008 (n= 138)	(n= 2092)
Ampicillin	33.5	38.8	24.0	35.2	43.8	36.2	34.8	59.4	36.3
Cefotaxime	0	0.2	0.9	0.3	1	1.9	1.1	2.9	0.8
Gentamicin	4.3	4	1.2	1.7	1	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

strain	*serotype*	*year*	*presistance phenotyp*	*ESBL*	*CTX*	*CTL*	*CAZ*	*CZL*	*CPM*	*CPL*	*CRO*	*CIP*	*Inc*	*plasmid size*
02-236146	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
02-214992	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
03-1672608	Virchow	2003	SSpKSuTeTmNal	CTX-M-9	16	0.047	0.75	0.19	1	<0.064	16	26	HI2	320 kb
1672608-TC2			SSpKSuTeTm	CTX-M-9	2	0.032	<0.5	0.125	0.38	<0.064	16	35	HI2	320 kb
04-1831083	Virchow	2004	SSpKSuTeTmNal	CTX-M-9	16	0.047	0.75	0.125	1	0.064	16	26	HI2	320 kb
1831083-TC3			SSpKSuTeTm	CTX-M-9	12	0.032	0.5	0.125	0.5	<0.064	16	35	HI2	320 kb
06-424914	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 (µg/ml) expcept for CRO and CIP, when figures represent diameters on disk diffusion test (mm).

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



STRAIN	RESISTANCE TYPE	*bla* GENE
03 2809	Susceptible	
05 8242	AmCroSuTeTmNal	CTX-M-2
03 6745	SuTeTmNal	
02 4291	Nal	
02 4596	Nal	
02 7169	S	
03 2057	SSpSuTeTmNal	
03 3101	Susceptible	
03 3111	AmSuTeTmNal	
03 3728	Susceptible	
03 3832	AmCroSuTeTmNal	CTX-M-2
03 5106	AmCSuTeTm	
03 5454	Nal	
03 5779	Susceptible	
03 7226	Susceptible	
05 1106	AmCazSSpKGCSuTeTmNal	SHV-12
04 4335	AmCroSuTeTmNal	CTX-M-2
04 4468	AmCroSuTeTmNal	CTX-M-2
04 5817	AmCroSuTeTmNal	CTX-M-2
02 5421	Nal	
03 2419	Nal	
02 5159	Susceptible	
02 7346	Susceptible	
02 214992	**AmCroSSpKSuTeTmNal**	**CTX-M-9 ***
03 1672608	**AmCroSSpKSuTeTmNal**	**CTX-M-9 ***
04 183083	**AmCroSSpKSuTeTmNal**	**CTX-M-9 ***
07 2762	AmCroSSpKSuTeTmNal	CTX-M-9
03 973	AmCroSSpKSuTmTe	CTX-M-9
04 3838	AmCro	CTX-M-2
02 236146	**AmCroSSpKSuTeTmNal**	**CTX-M-9 ***
03 1324	Nal	
03 7962	AmSSpKTGSuTmNal	
05 4181	AmCazNal	TEM-52
03 7925	Susceptible	
02 7113	Nal	
07 3700	AmCazSNal	TEM-52
02 5710	Nal	
07 4662	AmCroNal	CTX-M-32
02 4748	KTe	
02 7237	AmSCSuTeTm	
03 8871	S	
06 8083	AmCaz	TEM-52
03 2152	Susceptible	
03 6088	Susceptible	