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ORIGINAL ARTICLE

Comparative study of all *Salmonella enterica* serovar Enteritidis strains isolated from food and food animals in Greece from 2008 to 2010 with clinical isolates

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Abstract The aim of the present work was to study the epidemiology of *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) in Greece, comparing all the food and food animal isolates during a 3-year period with clinical isolates. Submission of the generated data to the PulseNet Europe database was carried out in order to study the population structure of this particular serovar and indicate possible connections with European strains. One hundred and sixty-eight (168) *S.* Enteritidis strains of human, animal, and food origin, isolated during the period 2008–2010 in Greece, were studied. Strains were characterized by phenotypic (antibiotic resistance) and molecular [pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)] methods. PFGE revealed 39 *Xba*I, 48 *Bln*I, and 80 *Xba*I–*Bln*I distinct pulsotypes, suggesting several clones circulating through the food chain and multiple sources of transmission. Submission to the PulseNet Europe database indicated that PFGE profile SENTXB.0001, the most common PFGE profile in Europe, was also predominant in Greece (33.3 %). MLST showed that all the strains studied shared the same sequence type (ST11), representing the most common ST in Europe. High rates of resistance to nalidixic acid were observed among human and poultry isolates (~25 %), indicating the potential fluoroquinolone treatment failure. Our data suggest that strains originating from multiple reservoirs circulated in Greece through the food chain during the study period. Predominant profiles in Greece were common to PulseNet Europe profiles, indicating similarities between the *S.* Enteritidis populations in Greece and Europe.

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Introduction

Non-typhoidal salmonellae (NTS) are commonly acquired from contaminated food products and are responsible for numerous foodborne infections worldwide. Although 2659 serovars have been identified worldwide [1], *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) is responsible for the majority of reported cases of salmonellosis [2]. Poultry and poultry products are the main reservoir and most of the strains are often isolated from broiler meat, laying hens, and breeders [2]. In Greece, *S.* Enteritidis ranked first during the period 2007–2012 among human isolates, while the notification rate in 2012 was among the lowest in 27 European Union member States (≤5 per 100,000) [2–4]. Most NTS infections result in gastroenteritis, but, sometimes, severe invasive infections require antibiotic treatment, such as fluoroquinolones (ciprofloxacin) and third-generation cephalosporins (the drug of choice for children) [5]. Resistance to these drugs have been widely reported for the past two decades [6, 7].

Several molecular methods, such as pulsed-field gel electrophoresis (PFGE) coupled with phage typing or multiple

locus variable number of tandem repeats analysis (MLVA), are often used where further strain discrimination is required [8–10], mainly during outbreaks investigations. PulseNet Europe has set up a standardized protocol to ensure that PFGE fingerprints from various laboratories are reproducible and comparable [11]. Additionally, multilocus sequence typing (MLST) is used for epidemiological purposes in order to study the population structure of *Salmonella* spp. [12].

The aim of the present work was to study the epidemiology of *S.* Enteritidis in Greece during a 3-year period. Strains isolated from patients, animals, and food products were characterized by (i) antimicrobial susceptibility testing and (ii) genotyping using PFGE. A subset of strains was studied using MLST.

Methods

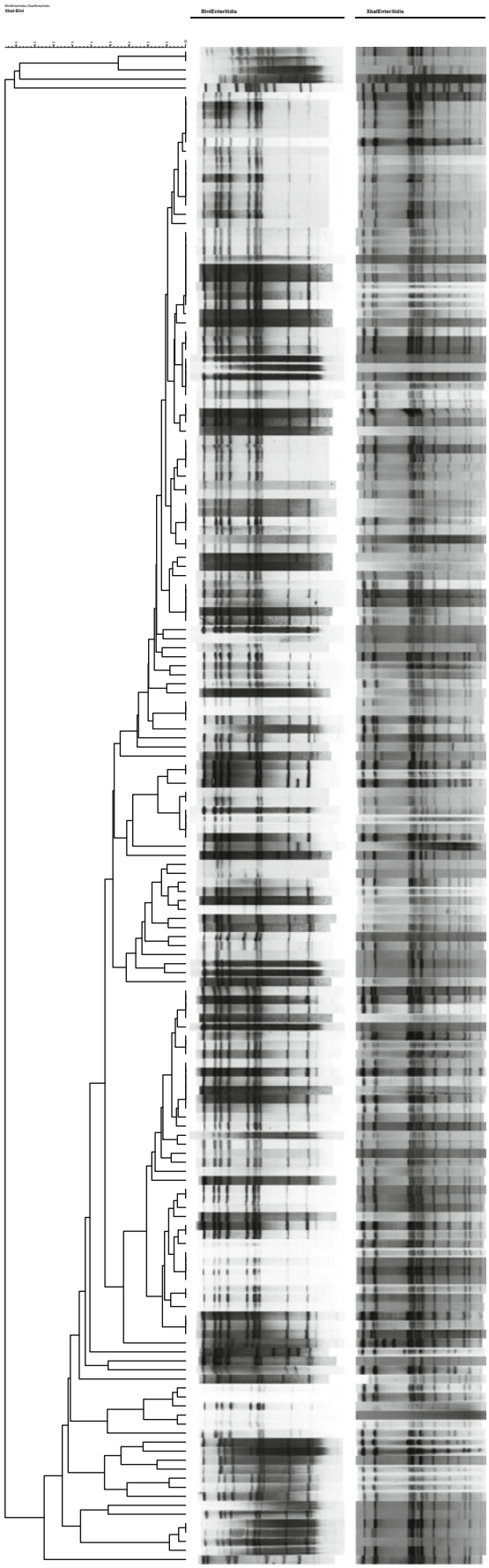
A total of 88 isolates isolated in Greece between 2008 and 2010 during the official control programs for *Salmonella* were serotyped as *S.* Enteritidis. Sixteen isolates were from food of Greek origin (poultry products), originating from six regions, and 72 isolates were from poultry, representing 57 farms and 17 regions. During the same period, 1039 human isolates were serotyped by the National Reference Centre for *Salmonella* (NRCS) as *S.* Enteritidis. Eighty of them were randomly selected for this study [3]. Identification of the *Salmonella* isolates was carried out at the NRCS by serotyping in accordance to the White–Kauffmann–Le Minor scheme [13, 14]. Susceptibility to a panel of 20 antimicrobials was determined using the agar dilution method in Iso-Sensitest agar (Oxoid, Basingstoke, UK). The final plate concentrations (mg/L) were: ampicillin (A) 8 and 128; chloramphenicol (C) 8; colomycin (Co) 8; gentamicin (G) 4; kanamycin (K) 16; neomycin (Ne) 8; streptomycin (S) 16 and 128; spectinomycin (Sp) 64; sulfonamide (Su) 64; tetracycline (T) 8 and 128; trimethoprim (Tm) 2; ciprofloxacin (Cp) 0.125 and 1; nalidixic acid (Nx) 16; furazolidone (Fu) 8; amikacin (Ak) 4; cephalexin (Cx) 16; cephradine (Cr) 16; cefuroxime (Cf) 16; ceftriaxone (Cn) 1; cefotaxime (Ct) 1. Multidrug resistance was defined as previously proposed [15]. PFGE was performed after the digestion of genomic DNA with *Xba*I and *Bln*I (Takara, Tokyo, Japan), according to the PulseNet protocol, as previously described [16], with *S. enterica* serovar Braenderup (H9812) as a DNA size marker. All profiles following *Xba*I digestion were submitted to the PulseNet Europe database for official PulseNet profile designation; *Bln*I and composite *Xba*I–*Bln*I profile designations were not official. MLST was performed by sequencing seven core genes, as previously described [12]. Data from MLST were submitted to the *Salmonella enterica* MLST Database (http://mlst.warwick.ac.uk/mlst/dbs/Senterica) in order to define the sequence types (STs). Statistical comparison between resistance rates among isolates was analyzed by the Chi-square test (Microsoft Excel 2007). Fingerprints were analyzed using BioNumerics v6.1 (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were constructed using the Dice similarity coefficient and the unweighted pair group method with arithmetic mean (UPGMA), optimization, and position tolerance set at 1.5%. The composite analysis was based on equal weighting of the *Xba*I and *Bln*I data and UPGMA clustering.

Results

Based on the antimicrobials tested, high resistance levels to nalidixic acid were observed among human and poultry isolates (25 %), and lower resistance levels ($p < 0.05$) among food isolates (6.25 %). Resistance to ampicillin was observed in two isolates, and resistance to streptomycin, trimethoprim, kanamycin, and spectinomycin was observed in one isolate each. Fifty-nine out of 80 (73,7 %) human isolates, 54 out of 72 (75 %) poultry isolates, and 15 out of 16 (93.7 %) food isolates were pan-susceptible to the 20 antimicrobials tested. Resistance to ciprofloxacin was not observed.

PFGE analysis identified 39 unique profiles using *Xba*I macrorestriction endonuclease. Submission to the PulseNet Europe database showed that 109 out of 168 isolates (64.9 %) were represented by the eight PulseNet-identified profiles SENTXB.0001, SENTXB.0002, SENTXB.0004, SENTXB.0005, SENTXB.0007, SENTXB.0023, SENTXB.0010, and SENTXB.0040 (Fig. 1). The remaining 32 profiles were represented by one to seven isolates, but did not correspond to PulseNet profiles. The most prevalent PFGE profile was SHADXB.0001 (55/168, 32.7 %). This profile was identified among human, poultry, and food isolates, and was present in every year from 2008 to 2010. The second highest occurring profile was SENTXB.0005 (29/168, 17.3 %), and was also identified during each year from all sample categories (Table 1). PFGE using *Bln*I restriction endonuclease revealed 48 distinct *Bln*I pulsotypes among the 168 strains. The predominant *Bln*I profile was *Bln*I-0007 and was present in 60 out of 168 (35.7 %) profiles (two from food, 26 from poultry, and 32 from humans). The second most dominant profile was *Bln*I-0010, with 21 (12.5 %) strains (four from foods, nine from poultry, and eight from humans). The remaining 46 *Bln*I profiles corresponded to one to nine isolates. The composite dataset divided strains into 80 distinct *Xba*I–*Bln*I profiles. The most common patterns were *Xba*I–*Bln*I-0028 (13.1 %), *Xba*I–*Bln*I-0026 (10.1 %), and *Xba*I–*Bln*I-0039 (7.1 %). All the remaining patterns were represented by one to ten isolates (Fig. 1).

Fig. 1 Dendrogram of *Xba*I, *Bln*I, and composite *Xba*I–*Bln*I pulsotypes and characteristics of the 168 *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) isolates examined in this study



	VRI id	CATEGORY	PLACE	YEAR	R-TYPE	PFGE type XbaI	PFGE type BlnI	composite
*	784	HUMAN	DODEKAN	2010	$	Unname00024	0004	Xba-Bln.0077
*	787	HUMAN	DODEKAN	2010	$	Unname00024	0004	Xba-Bln.0077
*	785	HUMAN	DODEKAN	2010	$	Unname00002	0004	Xba-Bln.0078
*	780	HUMAN	KORINTHIA	2010	$	Unname00004	0047	Xba-Bln.0076
*	759	HUMAN	FTHIOTIDA	2009	$	Unname00003	0005	Xba-Bln.0080
*	742	HUMAN	DODEKAN	2008	Nx	SENTXB0001	0012	Xba-Bln.0038
*	032	POULTRY	ILEIA	2008	Nx	SENTXB0001	0012	Xba-Bln.0038
*	036	POULTRY	RETHYMNO	2008	Nx	SENTXB0001	0012	Xba-Bln.0038
*	037	POULTRY	RETHYMNO	2008	Nx	SENTXB0001	0012	Xba-Bln.0038
*	048	FOOD	KERKYRA	2008	Nx	SENTXB0001	0012	Xba-Bln.0038
*	776	HUMAN	LEYKADA	2010	$	SENTXB0001	0012	Xba-Bln.0038
*	053	POULTRY	THESNIKI	2008	$	SENTXB0001	0012	Xba-Bln.0038
*	012	POULTRY	ATTIKI	2008	$	SENTXB0005	0012	Xba-Bln.0037
*	014	FOOD	GREECE	2008	$	SENTXB0005	0012	Xba-Bln.0037
*	019	FOOD	GREECE	2008	$	SENTXB0005	0012	Xba-Bln.0037
*	020	FOOD	GREECE	2008	$	SENTXB0005	0012	Xba-Bln.0037
*	034	POULTRY	THESNIKI	2008	$	SENTXB0005	0012	Xba-Bln.0037
*	055	POULTRY	THESNIKI	2008	$	SENTXB0005	0012	Xba-Bln.0037
*	033	POULTRY	DODEKAN	2008	$	Unname00005	0012	Xba-Bln.0039
*	056	POULTRY	THESNIKI	2008	$	SENTXB0001	0023	Xba-Bln.0041
*	224	POULTRY	GREECE	2009	Nx	SENTXB0005	0007	Xba-Bln.0032
*	229	POULTRY	ATTIKI	2009	$	SENTXB0005	0007	Xba-Bln.0032
*	483	POULTRY	GREECE	2010	$	SENTXB0005	0007	Xba-Bln.0032
*	738	HUMAN	VIOTIA	2008	$	SENTXB0005	0007	Xba-Bln.0032
*	042	FOOD	GREECE	2008	$	SENTXB0005	0007	Xba-Bln.0032
*	082	POULTRY	ILEIA	2008	$	SENTXB0005	0007	Xba-Bln.0032
*	769	HUMAN	VIOTIA	2009	$	SENTXB0005	0007	Xba-Bln.0032
*	775	HUMAN	DODEKAN	2010	$	SENTXB0005	0007	Xba-Bln.0032
*	777	HUMAN	DODEKAN	2010	$	SENTXB0005	0007	Xba-Bln.0032
*	062	POULTRY	PIERIA	2008	$	SENTXB0005	0007	Xba-Bln.0032
*	086	POULTRY	ARKADIA	2008	$	Unname00013	0007	Xba-Bln.0034
*	752	HUMAN	AITOLOAKAR	2008	$	SENTXB0001	0007	Xba-Bln.0026
*	757	HUMAN	FOKIDA	2009	$	SENTXB0001	0007	Xba-Bln.0025
*	756	HUMAN	DODEKAN	2009	$	SENTXB0001	0007	Xba-Bln.0025
*	155	POULTRY	KILKIS	2009	$	SENTXB0001	0007	Xba-Bln.0026
*	088	POULTRY	KILKIS	2008	Nx	SENTXB0001	0007	Xba-Bln.0026
*	095	POULTRY	IMATHIA	2008	$	SENTXB0001	0007	Xba-Bln.0026
*	221	POULTRY	THESNIKI	2009	$	SENTXB0001	0007	Xba-Bln.0026
*	743	HUMAN	DODEKAN	2008	$	SENTXB0001	0007	Xba-Bln.0026
*	712	HUMAN	DODEKAN	2008	$	SENTXB0001	0007	Xba-Bln.0026
*	079	POULTRY	IMATHIA	2008	$	SENTXB0001	0007	Xba-Bln.0026
*	798	HUMAN	DODEKAN	2010	$	SENTXB0001	0007	Xba-Bln.0026
*	074	POULTRY	KORINTHIA	2008	$	SENTXB0001	0007	Xba-Bln.0026
*	102	POULTRY	KILKIS	2009	Nx	SENTXB0001	0033	Xba-Bln.0049
*	187	POULTRY	IOANNINA	2009	$	SENTXB0001	0033	Xba-Bln.0049
*	214	POULTRY	ATTIKI	2009	Nx	SENTXB0001	0033	Xba-Bln.0049
*	216	POULTRY	RETHYMNO	2009	Nx	SENTXB0001	0033	Xba-Bln.0049
*	215	POULTRY	RETHYMNO	2009	Nx	Unname0005	0033	Xba-Bln.0048
*	406	POULTRY	GREECE	2010	$	SENTXB0005	0033	Xba-Bln.0050
*	099	POULTRY	ILEIA	2008	$	SENTXB0005	0033	Xba-Bln.0050
*	109	POULTRY	PIERIA	2009	Nx	SENTXB0001	0013	Xba-Bln.0051
*	110	POULTRY	PIERIA	2009	Nx	SENTXB0001	0013	Xba-Bln.0051
*	746	HUMAN	AITOLOAKAR	2008	$	SENTXB0001	0013	Xba-Bln.0051
*	710	HUMAN	AITOLOAKAR	2008	$	SENTXB0001	0013	Xba-Bln.0051
*	106	POULTRY	KILKIS	2009	$	SENTXB0005	0013	Xba-Bln.0052
*	479	POULTRY	GREECE	2010	$	SENTXB0005	0013	Xba-Bln.0052
*	069	FOOD	KAVALA	2008	$	Unname0005	0011	Xba-Bln.0043
*	071	POULTRY	PIERIA	2008	$	Unname0006	0007	Xba-Bln.0028
*	222	POULTRY	THESNIKI	2009	Nx	Unname0005	0013	Xba-Bln.0053
*	747	HUMAN	FOKIDA	2008	$	Unname0013	0007	Xba-Bln.0034
*	753	HUMAN	FTHIOTIDA	2008	$	Unname0013	0007	Xba-Bln.0034
*	754	HUMAN	FOKIDA	2008	$	Unname0013	0007	Xba-Bln.0034
*	068	POULTRY	HALKIDIKI	2008	$	Unname0013	0007	Xba-Bln.0034
*	522	POULTRY	GREECE	2010	$	Unname0013	0007	Xba-Bln.0034
*	054	POULTRY	THESNIKI	2008	$	Unname0012	0010	Xba-Bln.0044
*	052	POULTRY	THESNIKI	2008	$	Unname0030	0007	Xba-Bln.0046
*	031	POULTRY	ATTIKI	2008	$	Unname0026	0012	Xba-Bln.0058
*	731	HUMAN	KERKYRA	2008	$	SENTXB0001	0007	Xba-Bln.0025
*	736	HUMAN	KERKYRA	2008	Nx	Unname0019	0007	Xba-Bln.0023
*	737	HUMAN	DODEKAN	2008	Nx	Unname0017	0009	Xba-Bln.0024
*	762	HUMAN	FTHIOTIDA	2009	Nx	SENTXB0001	0019	Xba-Bln.0057
*	030	FOOD	IOANNINA	2008	$	SENTXB0001	0031	Xba-Bln.0055
*	218	POULTRY	ATTIKI	2009	$	Unname0018	0007	Xba-Bln.0022
*	219	POULTRY	ATTIKI	2009	$	Unname0018	0007	Xba-Bln.0022
*	760	HUMAN	AITOLOAKAR	2009	Nx	Unname0018	0007	Xba-Bln.0022
*	758	HUMAN	LESVOS	2009	$	SENTXB0005	0025	Xba-Bln.0059
*	761	HUMAN	FTHIOTIDA	2009	Nx	Unname0016	0038	Xba-Bln.0062
*	080	POULTRY	EVROS	2008	Nx S K	SENTXB0004	0033	Xba-Bln.0047
*	119	POULTRY	DODEKAN	2009	Nx	Unname0027	0020	Xba-Bln.0063
*	771	HUMAN	DODEKAN	2009	$	SENTXB0040	0035	Xba-Bln.0010
*	772	HUMAN	DODEKAN	2009	$	SENTXB0040	0035	Xba-Bln.0010
*	770	HUMAN	DODEKAN	2009	$	Unname0028	0036	Xba-Bln.0013
*	025	FOOD	IOANNINA	2008	$	SENTXB0010	0035	Xba-Bln.0010
*	028	FOOD	IOANNINA	2008	$	SENTXB0010	0035	Xba-Bln.0010
*	511	FOOD	GREECE	2010	$	SENTXB0040	0035	Xba-Bln.0010
*	773	HUMAN	DODEKAN	2009	A	SENTXB0010	0035	Xba-Bln.0010
*	233	FOOD	EVROS	2009	$	SENTXB0040	0035	Xba-Bln.0010
*	774	HUMAN	DODEKAN	2009	$	SENTXB0040	0035	Xba-Bln.0010
*	739	HUMAN	LAKONIA	2008	$	Unname0001	0041	Xba-Bln.0074
*	111	POULTRY	GREECE	2009	$	Unname0009	0046	Xba-Bln.0069
*	035	POULTRY	THESNIKI	2008	$	SENTXB0002	0035	Xba-Bln.0011
*	060	POULTRY	ARKADIA	2008	$	SENTXB0010	0033	Xba-Bln.0012
*	163	POULTRY	IOANNINA	2009	$	SENTXB0010	0029	Xba-Bln.0007
*	768	HUMAN	DODEKAN	2009	$	SENTXB0002	0029	Xba-Bln.0008
*	041	POULTRY	ARKADIA	2008	$	SENTXB0002	0026	Xba-Bln.0003
*	040	POULTRY	EVOIA	2008	$	Unname0029	0026	Xba-Bln.0004
*	124	POULTRY	IOANNINA	2009	$	SENTXB0002	0037	Xba-Bln.0009
*	123	POULTRY	IOANNINA	2009	$	SENTXB0010	0028	Xba-Bln.0006
*	729	HUMAN	LAKONIA	2008	$	SENTXB0010	0019	Xba-Bln.0001
*	307	POULTRY	KORINTHIA	2009	$	SENTXB0010	0022	Xba-Bln.0002
*	375	POULTRY	GREECE	2010	$	Unname0025	0028	Xba-Bln.0007
*	164	POULTRY	IOANNINA	2009	$	SENTXB0010	0039	Xba-Bln.0015
*	169	FOOD	EVOIA	2008	$	SENTXB0010	0043	Xba-Bln.0016
*	117	POULTRY	DODEKAN	2009	$	SENTXB0002	0027	Xba-Bln.0005
*	744	HUMAN	FTHIOTIDA	2008	Nx	SENTXB0005	0007	Xba-Bln.0032
*	767	HUMAN	LAKONIA	2009	$	SENTXB0005	0007	Xba-Bln.0032
*	750	HUMAN	KERKYRA	2008	$	SENTXB0005	0007	Xba-Bln.0032
*	045	POULTRY	GREECE	2008	$	SENTXB0005	0007	Xba-Bln.0032
*	195	POULTRY	LAKONIA	2009	$	SENTXB0005	0010	Xba-Bln.0045
*	740	HUMAN	LAKONIA	2008	$	Unname0018	0007	Xba-Bln.0022
*	482	POULTRY	GREECE	2010	$	Unname0018	0007	Xba-Bln.0022
*	741	HUMAN	LESVOS	2008	$	Unname0018	0007	Xba-Bln.0033
*	333	FOOD	GREECE	2010	$	SENTXB0001	0007	Xba-Bln.0026
*	766	HUMAN	MESSINIA	2009	$	SENTXB0001	0007	Xba-Bln.0026
*	745	HUMAN	DODEKAN	2008	$	SENTXB0001	0007	Xba-Bln.0026
*	044	POULTRY	THESNIKI	2008	Nx	SENTXB0001	0007	Xba-Bln.0026
*	763	HUMAN	KERKYRA	2009	$	SENTXB0001	0007	Xba-Bln.0026
*	765	HUMAN	KERKYRA	2009	$	SENTXB0001	0007	Xba-Bln.0026
*	308	POULTRY	EVOIA	2009	Nx	Unname0014	0007	Xba-Bln.0035
*	728	HUMAN	DODEKAN	2008	Nx	SENTXB0001	0020	Xba-Bln.0031
*	223	POULTRY	GREECE	2009	$	Unname0013	0007	Xba-Bln.0034
*	503	POULTRY	KORINTHIA	2009	$	Unname0005	0007	Xba-Bln.0042
*	290	POULTRY	KORINTHIA	2009	$	Unname0030	0007	Xba-Bln.0046
*	481	POULTRY	GREECE	2010	$	Unname0015	0007	Xba-Bln.0036
*	087	POULTRY	ARKADIA	2008	Nx Sp Tm	SENTXB0001	0034	Xba-Bln.0049
*	413	POULTRY	GREECE	2010	$	SENTXB0001	0014	Xba-Bln.0054
*	717	HUMAN	KERKYRA	2008	$	SENTXB0001	0007	Xba-Bln.0026
*	718	HUMAN	LAKONIA	2008	Nx	SENTXB0001	0007	Xba-Bln.0026
*	719	HUMAN	LAKONIA	2008	Nx	SENTXB0001	0017	Xba-Bln.0030
*	125	FOOD	THESNIKI	2009	$	SENTXB0001	0013	Xba-Bln.0051
*	764	HUMAN	KERKYRA	2009	$	SENTXB0001	0007	Xba-Bln.0026
*	720	HUMAN	FOKIDA	2008	Nx	SENTXB0001	0007	Xba-Bln.0026
*	722	HUMAN	DODEKAN	2008	$	Unname0024	0007	Xba-Bln.0027
*	709	HUMAN	AITOLOAKAR	2008	$	SENTXB0001	0012	Xba-Bln.0038
*	715	HUMAN	AITOLOAKAR	2008	$	SENTXB0001	0012	Xba-Bln.0038
*	711	HUMAN	FOKIDA	2008	$	SENTXB0001	0012	Xba-Bln.0038
*	713	HUMAN	KERKYRA	2008	$	SENTXB0001	0012	Xba-Bln.0038
*	723	HUMAN	DODEKAN	2008	Nx	SENTXB0001	0015	Xba-Bln.0029
*	725	HUMAN	KERKYRA	2008	Nx	SENTXB0001	0015	Xba-Bln.0029
*	721	HUMAN	DODEKAN	2008	Nx	SENTXB0007	0016	Xba-Bln.0060
*	751	HUMAN	DODEKAN	2008	Nx	SENTXB0005	0007	Xba-Bln.0032
*	521	POULTRY	GREECE	2010	$	SENTXB0005	0007	Xba-Bln.0032
*	735	HUMAN	KERKYRA	2008	Nx	SENTXB0005	0007	Xba-Bln.0032
*	749	HUMAN	LESVOS	2008	$	Unname0031	0018	Xba-Bln.0061
*	796	HUMAN	LAKONIA	2010	$	Unname0023	0045	Xba-Bln.0073
*	136	FOOD	THESNIKI	2009	$	Unname0007	0044	Xba-Bln.0070
*	748	HUMAN	LESVOS	2008	$	Unname0021	0036	Xba-Bln.0072
*	797	HUMAN	IRAKLEIO	2010	$	Unname0032	0040	Xba-Bln.0019
*	755	HUMAN	KERKYRA	2009	Nx	Unname0016	0006	Xba-Bln.0021
*	781	HUMAN	FTHIOTIDA	2010	$	Unname0018	0006	Xba-Bln.0022
*	733	HUMAN	DODEKAN	2008	Nx	Unname0008	0006	Xba-Bln.0020
*	730	HUMAN	KERKYRA	2008	$	Unname0011	0012	Xba-Bln.0040
*	732	HUMAN	KYKLADES	2008	$	SENTXB0001	0012	Xba-Bln.0038
*	778	HUMAN	AXAIA	2010	$	Unname0021	0036	Xba-Bln.0071
*	779	HUMAN	FTHIOTIDA	2010	$	SENTXB0002	0042	Xba-Bln.0075
*	783	HUMAN	DODEKAN	2010	$	SENTXB0001	0013	Xba-Bln.0056
*	782	HUMAN	FTHIOTIDA	2010	$	Unname0007	0032	Xba-Bln.0017
*	788	HUMAN	DODEKAN	2010	$	SENTXB0010	0030	Xba-Bln.0018
*	786	HUMAN	DODEKAN	2010	$	SENTXB0001	0013	Xba-Bln.0056
*	789	HUMAN	LAKONIA	2010	$	Unname0010	0008	Xba-Bln.0064
*	716	HUMAN	AITOLOAKAR	2008	A Nx	SENTXB0040	0008	Xba-Bln.0065
*	091	POULTRY	IMATHIA	2008	$	Unname0026	0021	Xba-Bln.0068
*	170	FOOD	EVOIA	2008	$	SENTXB0010	0029	Xba-Bln.0014
*	192	POULTRY	RETHYMNO	2009	Nx	Unname0020	0021	Xba-Bln.0066
*	200	POULTRY	THESNIKI	2009	$	Unname0020	0021	Xba-Bln.0066
*	196	POULTRY	LAKONIA	2009	$	Unname0022	0021	Xba-Bln.0067
*	188	POULTRY	IOANNINA	2009	$	Unname0022	0021	Xba-Bln.0067
*	727	HUMAN	KERKYRA	2008	Nx	SENTXB0001	0001	Xba-Bln.0079

Table 1 Origin, year, and proportion of PulseNet Europe profiles in *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) isolates, Greece, 2008–2010 ($n = 168$)

PulseNet Europe profile	No. (%)	Year	Origin
SENTXB.0001	55 (32.7)	2008: 33; 2009: 16; 2010: 6	32 H, 19 P, 4 F
SENTXB.0005	29 (17.3)	2007: –; 2008: 16; 2009: 7; 2010: 6	10 H, 15 P, 4 F
SENTXB.0010	9 (5.3)	2008: 5; 2009: 4	1 H, 4 P, 4 F
SENTXB.0002	6 (3.6)	2007: –; 2008: 2; 2009: 4; 2010: 2	2 H, 4 P
SENTXB.0040	6 (3.6)	2007: –; 2008: 1; 2009: 4; 2010: 1	4 H, 2 F
SENTXB.0023	2 (1.2)	2008: 1; 2010: 1	2 H
SENTXB.0004	1 (0.6)	2008: 1	1 A
SENTXB.0007	1 (0.6)	2008: 1	1 H
Other	59 (35.1)	2008: 24; 2009: 20; 2010: 15	27 H, 29 P, 3 F

Due to rounding, the sum of the percentages in a given category does not always equal 100

H human; *P* poultry; *F* food

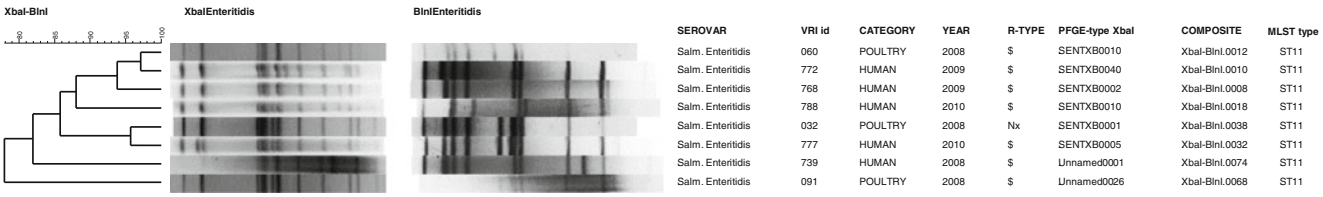
A subset of eight strains were chosen for MLST; five of them had been isolated from humans and three from poultry. These belonged to eight different *Xba*I pulsotypes, with similarity values ranging from 77 to 95 %. Five of them were identical to five PulseNet Europe profiles and three did not correspond to any of them. All eight isolates belonged to ST11 (allelic profile 5-2-3-7-6-6-11). ST11 includes the majority of *S.* Enteritidis isolates uploaded to the *Salmonella enterica* MLST Database (Fig. 2).

Discussion

To our knowledge, this is the first study that describes the population structure of *S.* Enteritidis based on PFGE types submitted to the PulseNet Europe database and drug resistance surveillance of this serovar in Greece. Even though the isolates cover the period 2008–2010, we believe that the general condition has not changed substantially during the last several years and the main conclusions are still an ongoing issue.

In general, *S.* Enteritidis is a serovar with low resistance rates and, thus, multidrug resistance is rarely observed [17]. Our data showed resistance to nalidixic acid mainly among human and poultry isolates (~25 %). There were variations in resistance to nalidixic acid among human isolates observed in European countries for 2012. High rates were reported from Spain (60.3 %), Romania (37 %), and France (31.7 %), whilst low rates were reported in Germany (1 %) and Hungary (2.3 %) [18]. Poultry population data from 2012 in the European Union reported resistance to nalidixic acid ranging from 0 % in Austria to 39.3 % in Poland and 92.6 % in Portugal [18]. Resistance to nalidixic acid in salmonellae is of great concern, as it has been reported to be an indicator for reduced susceptibility to the drug of choice, ciprofloxacin, resulting in possible treatment failure [19]. Consequently, the 25 % of nalidixic acid-resistant *S.* Enteritidis in this study must be considered as possibly having reduced susceptibility to ciprofloxacin, and, thus, potentially treatment-resistant, even though our results indicated no resistance/partial resistance to ciprofloxacin. The lack of or partial resistance to ciprofloxacin in this study could be explained as resistance to ciprofloxacin was studied for the breakpoints of 1 and 0.125 mg/L and not 0.064 mg/L, which is the current European Union clinical breakpoint [20]. This observation differed in some other European Union countries, where most *S.* Enteritidis strains, especially PT4, PT1, and PT6, were susceptible to nalidixic acid and other antimicrobial agents. It must be stressed that, in 2012, Greece had the highest levels of antimicrobial consumption in primary care in Europe [21]; this could explain the 25 % of resistance to nalidixic acid among humans in our study.

SENTXB.0001 was, according to our results, the most common PFGE profile in Greece, comprising 32.7 % of the isolates. This profile is also dominant in Europe; 62.5 %



SEROVAR	VRI id	CATEGORY	YEAR	R-TYPE	PFGE-type XbaI	COMPOSITE	MLST type
Salm. Enteritidis	060	POULTRY	2008	$	SENTXB0010	XbaI-BlnI.0012	ST11
Salm. Enteritidis	772	HUMAN	2009	$	SENTXB0040	XbaI-BlnI.0010	ST11
Salm. Enteritidis	768	HUMAN	2009	$	SENTXB0002	XbaI-BlnI.0008	ST11
Salm. Enteritidis	788	HUMAN	2010	$	SENTXB0010	XbaI-BlnI.0018	ST11
Salm. Enteritidis	032	POULTRY	2008	Nx	SENTXB0001	XbaI-BlnI.0038	ST11
Salm. Enteritidis	777	HUMAN	2010	$	SENTXB0005	XbaI-BlnI.0032	ST11
Salm. Enteritidis	739	HUMAN	2008	$	Unnamed0001	XbaI-BlnI.0074	ST11
Salm. Enteritidis	091	POULTRY	2008	$	Unnamed0026	XbaI-BlnI.0068	ST11

Fig. 2 PulseNet Europe pulsed-field gel electrophoresis (PFGE) types, *Xba*I–*Bln*I PFGE types, and corresponding multilocus sequence typing (MLST) types of *S.* Enteritidis isolates

($n=2210$) in England and Wales during 2001–2012 and 56.4 % ($n=6734$) of human isolates from other European countries (Austria, Denmark, Spain, Finland, Scotland, England and Wales, Italy, Netherlands, and Germany) (data not shown). The second most prevalent profile, SENTXB.0005 (17.3 %), is also common in Europe; 3.75 % ($n=133$) in the UK and 9.3 % ($n=1111$) in Europe. An interesting observation is that the third most common profile, SENTXB.0002, in the UK with 13 % incidence ($n=459$) and also in Europe with 15.7 % incidence ($n=1880$), is relatively rare in Greece, where the rate is only 3.6 %. It is also to be noted that SENTXB.00014, the fourth highest occurring profile in the UK and in Europe (~2.7 %), was absent in Greece, and profile SENTXB.0007 was rare; 0.6 % ($n=1$) in Greece, 2 % ($n=71$) in the UK, and 1.7 % ($n=205$) in Europe. In general, our data suggest mainly similarities but also some differences among Greek and European populations of *S.* Enteritidis. This diversity may suggest that strains originated from multiple reservoirs and, also, non-appearance of point-source outbreaks. The absence of some human *S.* Enteritidis PFGE patterns, in particular from food and food animals, suggests that sources other than food animals, such as fresh foods or vegetables, may play a role in clinical illness. Furthermore, some differences among occurring profiles could also be explained by the PulseNet database structure, which is biased towards isolates from outbreak investigations and, consequently, not accurately representative. However, as a strong correlation between SENTXB.0001 and PT4, PT1, and PT6 exists [10], future work involving phage typing could be undertaken in order to further define *S.* Enteritidis populations in Greece.

Due to the clonal nature of *S.* Enteritidis [22], the use of two to three additional restriction enzymes for PFGE or combinations of multiple methods like phage typing and MLVA have been proposed when enhanced strain discrimination is needed [9, 10, 23]. *Bln*I is mainly recommended as the second enzyme for discriminating epidemiologically unrelated strains, as well as partly discriminating *Xba*I-indistinguishable profiles [8, 24, 25]. This last observation has also been noticed in our study, with 48 different *Bln*I pulsotypes being generated. The predominant *Bln*I pulsotype was SENTBL.0007, which was seen in 35.7 % of all isolates. The composite dataset using *Xba*I and *Bln*I revealed that the SENTXB.0001–SENTBL.0007 clone was predominant, with an occurrence rate of 12 % ($n=21$). Seventeen out of these 21 strains shared the same R-type (nalidixic acid) and were isolated during each year from humans, animals, and foods. This clone was present in Greece throughout the study period and had spread through the food chain among eight different regions in Greece.

MLST has been suggested to be a good discriminatory tool for typing *S.* Enteritidis [26]. Our results do not support this suggestion, as all the isolates belonged to ST11, which is the most common sequence type for *S.* Enteritidis [12, 26]. The conserved DNA sequence of the internal fragments of the seven housekeeping genes tested in this study explains the lack of diversity among the strains studied.

The last few years has seen a revolution in the application of whole genome sequencing (WGS) for routine microbiology, as it is replacing current conventional phenotypic and molecular methods [27, 28]. WGS is a promising method that represents the opportunity for a step-change in diagnostic microbiological practice, as it can be used not only for research, but also as an alternative typing method for public health surveillance and outbreak investigation in routine practice [29]. Newer, higher resolution typing methods based on single nucleotide polymorphisms (SNPs) can be/have been developed to discriminate this closely related *S.* Enteritidis serovar [30]. One of the drawbacks of the current study was that, due to the lack of resources, we were not able to apply WGS methods to conduct the surveillance, but the PFGE and susceptibility data generated showed enough diversity to ensure that future *S.* Enteritis surveillance should involve both newer WGS-based typing methods and good epidemiological and clinical data to detect sources of infections. This, in turn, will allow the evaluation of control measures to be carried out to reduce resistance in *Salmonella*, specifically targeting the prudent use of antimicrobial agents by farmers and veterinarians.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research did not involve human participants and/or animals.

Informed consent Not applicable.

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