ORIGINAL ARTICLE

Regional dissemination of *Salmonella enterica* serovar Enteritidis is season dependent

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Objective To carry out epidemiological typing of clinical isolates of *Salmonella enterica* serovar Enteritidis by pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) and analysis of their antibiotic resistance.

Methods Over a 12-month period, 44 *Salmonella* Enteritidis isolates, recovered from 40 patients admitted to the University Hospital Center of Amiens, France and from three outpatients, were characterized by the analysis of phenotypic and genotypic traits and clinical data from medical reports.

Results Forty nontyphoidal salmonellosis episodes were diagnosed in hospitalized patients (34 episodes of gastroenteritis, two episodes of bacteremia not affecting other organs, one episodes of bacteremia plus urinary infection, one episodes of bacteremia plus gastroenteritis, one episodes of chronic colitis plus gastroenteritis and one episode of peritonitis), and three carriers were observed in outpatients. By means of PFGE, RAPD and antibiotic susceptibility patterns 44 isolates were subdivided into 16 clonally related groups. Two of them were predominantly implicated in the course of these infections, being responsible for two successive waves of infection, while the others were encountered sporadically.

Keywords *Salmonella* Enteritidis, PFGE, RAPD, Nontyphoidal salmonellosis

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INTRODUCTION

Salmonella enterica serovar Enteritidis has become the predominant serovar responsible for human nontyphoidal salmonellosis in many countries [1–3]. The frequency of *S.* Enteritidis clinical isolates obtained in the Bacteriology and Hygiene Laboratory of the University Hospital Center of Amiens has risen steadily since 1988 to become higher than that of *S.* Typhimurium from 1991 until now [4]. This change was encountered at the national level since 1991 [5,7]. The trend continued and the following values were observed in this study: 51.4% (in 1997), 54.4% (in 1998), and 55% (in 1999) for *S.* Enteritidis, compared to 24.2% (in 1997), 20% (in 1998) and 27.5% (in 1999) for *S.* Typhimurium (in relation to all cases of *Salmonella* spp. isolated during these years). It is clear that serovar Enteritidis currently ranks as the most common *Salmonella* serotype isolated from humans and constitutes a major public-health problem. A number of studies have shown that the increasing incidence of gastrointestinal infections caused by *S.* Enteritidis may be related to the ingestion of raw, undercooked or contaminated eggs or egg products [8,9]. Epidemiological studies of serovar Enteritidis infections are hampered by a lack of adequate procedures. Standard methods, including phenotyping procedures, may not be discriminative, but use of genotyping techniques such as ribotyping [10], random amplified polymorphic DNA [11] and pulsed-field gel electrophoresis (PFGE) [10] have proved useful for discriminating isolates of *Salmonella* spp. The aim of this study was to evaluate nontyphoidal

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salmonellosis cases treated in Amiens University Hospital Center in 1999 and to determine the epidemiological relatedness between these *Salmonella* Enteritidis isolates. The study was conducted by analyzing the phenotypic and genotypic traits of isolates and the clinical and other features of the patients.

MATERIALS AND METHODS

Study patients

Forty-three patients, of whom 22 (51.2%) were female and 21 (48.8%) were male, presented with nontyphoidal salmonellosis owing to *S. enterica* serovar Enteritidis. Forty cases were inpatients and three cases were from an outpatients clinic. Data from medical reports, including age, sex and underlying diseases, were recorded. All the isolates were sporadic strains isolated throughout 1999 from patients admitted to the different hospital units included in this study. Details of the study population are given in Table 1. During the study period, there was no suspicion of *S.* Enteritidis outbreaks and no investigation of common vehicles of infection or other possible link among the patients was recorded.

Bacterial isolates and *Salmonella* Enteritidis characterization

Forty-four nonduplicate *S.* Enteritidis isolates, obtained from January 1999 to December 1999 were studied. Thirty-four were isolated from stools, four from blood, one from urine, one from biopsy of the colon and one from a lymph gland biopsy, from patients with clinically suspected bacteremia and/or focal infections admitted to the University Hospital Center, and three from stools of the patients of the outpatients clinic. The biochemical profile (biotype) was ascertained by ID32E system (Bio-Mérieux, Marcy l'Etoile, France), using a mini-API instrument reader (Bio-Mérieux). All strains were serotyped by the Kauffmann–White method [12] using agglutination with specific antisera (Biorad, Marne La Coquette, France).

Antimicrobial susceptibility test

Isolates were tested by the agar diffusion method on Mueller-Hinton II agar (Bio-Mérieux), against the following antimicrobial agents: ampicillin (AM: 10 μg), ticarcillin (TIC: 75 μg), piperacillin (PIP: 75 μg), amoxycillin plus clavulanic acid (75 + 10 μg), cephalothin (CF: 30 μg), cefoxitin (FOX: 30 μg), cefotaxime (CTX: 30 μg), ceftazidime (CAZ: 30 μg), aztreonam (ATM: 30 μg), gentamicin (GN: 15 μg), tobramycin (TM: 10 μg), netilmicin (NT: 30 μg), amikacin (AN: 30 μg), ofloxacin (OFX: 5 μg), trimethoprim plus sulfamethoxazole (SXT: 1.25 + 25.75 μg) (Biorad). Zone diameter of inhibition and equivalent minimal inhibitory concentrations (MIC) were read using the antibiogram reader I2a 'SIRSCAN' (Montpellier, France). The results were expressed as susceptible (S), intermediate (I), and resistant (R) according to the critical diameter of inhibition and MIC breakpoints established by the Antibiogram Committee of the French Society for Microbiology [13].

DNA fingerprint preparation and PFGE

DNA for PFGE analysis was prepared using the GenePath Reagents Kit Group 1 (Bio-Rad Laboratories, Ivry sur Seine, France) according to the manufacturer's instructions.

Selection of restriction enzymes was based on the recognition site of the enzymes and the G + C content of 50–54% previously reported for *Salmonella* spp. [14]. *SpeI* (5′-ACTAGT-3′ Promega France, Charbonnières, France) was used as the restriction endonuclease.

PFGE was performed in the contour-clamped homogeneous electric fields device (CHEF-DRII) (Bio-Rad Laboratories, Richmond, USA). Genomic DNA in 1% agarose plugs (low-melting agarose, Bio-Rad Laboratories) was digested overnight with 9 UI *SpeI* per plug with 300 μL of the *SpeI* buffer and with the reaction conditions recommended by the manufacturer. Fragments were separated on 1% molecular biology certified agarose and running was performed according to the instructions included in the GenePath gel kit (Bio-Rad Laboratories): 6 V/cm for 18.5 h at 14 °C with an initial switch time of 5 s and a final switch time of 40 s at an angle of 120 °C. The lambda ladder (Bio-Rad Laboratories) was used as the molecular weight marker. After the run was completed, gels were stained with an ethidium bromide solution for 15 min, followed by 60 min of destaining, and then photographed with UV transilluminator Quantity-one/Gel DOC 2000^{TM}/Molecular analyst finger printing (Bio-Rad Laboratories, France). The restriction endonuclease fragment patterns

Table 1 Date of isolation, origins, phenotypic and genotypic polymorphisms among Salmonella Enteritidis isolated in University Hospital Centre of Amiens in 1999

				Patient characteristics			Phenotyping	Genotyping			
								PFGE patterns	RAPD patterns		
Date of isolation (mo/day/yr)	isolates n°	Care units	Sample type	Sex	Age (years)	Clinical presentations	Antibiotic susceptible profile	Spel	AQ12	Q11	Genomic group
01/04/99	1	Dermatology	blood	M	71	Bacteremia	a	A	H	K	1
01/04/99	2	Geriatrics	urine	F	82	Urinary tract infection and bacteremia	d	A	H	K	1
01/04/99	3	Geriatrics	blood	F	82	Urinary tract infection and bacteremia	d	A	H	K	1
01/16/99	4	Pediatrics	stool	M	11	Gastroenteritis	d	A	H	K	1
02/19/99	5	Internal Medicine	stool	F	59	Gastroenteritis	d	A	H	K_1	1a
04/20/99	6	Pediatrics	stool	M	8	Gastroenteritis	c	B	H_1	L	2
04/20/99	7	Pediatrics	stool	F	9	Gastroenteritis	b	A	H	K	1
05/02/99	8	Outpatients Clinic	stool	F	3	Medical check up	d	A	H	K	1
05/06/99	9	Outpatients Clinic	stool	M	28	Medical check up	d	A	H	M	3
05/10/99	10	Geriatrics	stool	F	98	Gastroenteritis	a	C	H	K	4
05/14/99	11	Pediatrics	stool	M	11	Gastroenteritis	d	A	H	K	1
05/17/99	12	Cardiology Intensive Care Unit	stool	M	74	Gastroenteritis	a	A_1	H	K_2	1b
05/17/99	13	Blood Diseases	stool	M	36	Leukemia and Gastroenteritis	a	A	H	K_2	1c
05/18/99	14	Hepatogastroenterology	stool	F	28	Gastroenteritis	a	A	H	K_2	1c
05/19/99	15	Internal Medicine	stool	F	50	Gastroenteritis	b	A	H	K_2	1c
05/20/99	16	Outpatients Clinic	stool	F	29	Medical check up	d	A	H	K_2	1c
06/15/99	17	Internal Medicine	stool	F	27	Gastroenteritis	d	A	H	K_3	1d
06/18/99	18	Internal Medicine	stool	M	17	Gastroenteritis	d	A	H	K_4	1e
06/27/99	19	Nephrology	blood	M	55	Bacteremia	d	A	H	K_2	1c
07/06/99	20	Pediatrics	stool	M	3	Gastroenteritis	d	D	H	K_5	5
07/14/99	21	Rhumatology	stool	F	30	Gastroenteritis	d	E	I	k_3	6
07/14/99	22	Internal Medicine	stool	F	62	Gastroenteritis	d	E	I	N	7
07/14/99	23	Pediatrics	stool	M	9	Gastroenteritis	d	F	H	O	8
07/15/99	24	Internal Medicine	stool	F	89	Gastroenteritis	d	A	H	O	9
07/15/99	25	General visceral surgery	stool	F	26	Gastroenteritis	d	A	H	O	9

07/30/99	26	Pediatrics	stool	F	9	Gastroenteritis	d	A	H_2	O	9a
07/30/99	27	Pediatrics	stool	F	2	Gastroenteritis	a	A	J	P	10
07/30/99	28	Internal Medicine	stool	M	37	Gastroenteritis	d	A	H_2	O	9a
08/05/99	29	General visceral surgery	ganglionary biopsy	F	42	Peritonitis	d	A	H_2	O	9a
08/16/99	30	Cardiac Surgery	stool	M	78	Gastroenteritis	a	A	H_3	O	9b
08/17/99	31	General visceral surgery	stool	M	25	Gastroenteritis	d	G	H_2	O	11
08/18/99	32	Pediatrics	stool	F	2	Gastroenteritis	d	A_2	H_2	O	9c
08/19/99	33	Pediatric Surgery	stool	F	6	Gastroenteritis	a	A_1	H_2	O	9d
08/20/99	34	Geriatrics	stool + blood	M	85	Bacteremia and Gastroenteritis	a	A	H_2	O_1	9e
08/24/99	35	Internal Medicine	stool	F	33	Gastroenteritis	d	A_3	H_2	O_1	9f
08/29/99	36	Pediatrics	stool	M	11	Gastroenteritis	d	A	H_3	O_1	9g
08/29/99	37	Pediatrics	stool	M	9	Gastroenteritis	d	A	H_3	O_1	9g
10/13/99	38	Hepatogastroenterology	Biopsy of colon +stool	F	23	Chronic colitis + Gastroenteritis	d	A	H_2	O_1	9e
11/04/99	39	Pediatrics	stool	M	10	Gastroenteritis	d	A	H_2	O_1	9e
11/05/99	40	Pediatrics	stool	M	6	Gastroenteritis	d	A	H_2	O_1	9e
11/05/99	41	Pediatrics	stool	M	4	Gastroenteritis	d	A	H_3	O_2	9h
11/18/99	42	Pediatrics	stool	F	2	Gastroenteritis	d	A	H_2	O_3	9i
11/25/99	43	General visceral surgery	stool	F	49	Gastroenteritis	d	A	H_3	O_3	9j
12/11/99	44	Polyvalent pediatric intensive care unit	stool	M	2	Gastroenteritis	d	A	H_2	O	9a

were interpreted in accordance with the standards described previously [15,16].

Preparation and purification of whole-cell DNA for RAPD

A single colony of each isolate from a blood agar plate was picked and resuspended in lysis buffer supplied in the QIAamp DNA Kit (Qiagen S.A., Courtaboeuf, France). The suspension was vigorously stirred, digested with 20 μL of proteinase K solution (20 mg/mL), and incubated at 56 °C for 3 h (vortexing 3 times per hour during the whole incubation). After incubation, 200 μL of new lysis buffer was added, mixed and incubated at 70 °C for 10 min. After this incubation, 200 μL of ethanol (96–100%) was added, and mixed by pulse-vortexing for 15 s. The mixture was carefully applied to the QIAamp spin column in a 2-mL collection tube and centrifuged at 6000 × *g* for 1 min. The QIAamp spin column was then placed in a clean 2-mL collection tube; 500 μL of AW1 buffer (QIAGEN reagents) was then applied to the QIAamp spin column and centrifuged at 6000 × *g* for 1 min. A second wash of the QIAamp spin column was carried out with 500 μL of AW2 buffer (QIAGEN reagents), and centrifuged at 20 000 × *g* for 3 min. The QIAamp spin column was placed in a clean 1.5 mL microcentrifuge tube, then 150 μL of TE buffer (10 mM Tris-HCl [pH 9], 0.5 mM EDTA) was added, incubated at room temperature for 1 min and subsequently centrifuged at 6000 × *g* for 1 min (this step was repeated once). The genomic eluate was stored at – 20 °C until required.

Random amplification of polymorphic DNA

The primers used to discriminate the *S.* Enteritidis isolates were: AQ12 (5′-CAG-CTC-CTG-T-3′ [G+C: 60%]), and Q11 (5′-TCT-CCG-CAA-C-3′ [G +C: 60%]) synthesized by DNA technologies Eurogentec (Eurogentec, Seraing, Belgium). Amplification reactions were in a 20-μL solution containing PCR buffer 1 × (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 200 μM (each) deoxynucleotide triphosphate (ATP, CTP, GTP, and TTP), 1 μL (each) the 2 primers (40 μM), 0.2 μL (5 U/μL) of AmpliTaq DNA polymerase and 1.6 mM $MgCl_2$ solution (Perkin Elmer, Roche Molecular Systems, Branchburg, New Jersey, USA). Approximately 20 ng of DNA was added to the mixture. Amplification was carried out in a Perkin-Elmer thermal Cycler 60 biomed (Roche Diagnostics Systems) at 94 °C for 7 min, then 36 °C for 5 min, followed by 37 cycles (each) at 72 °C for 1 min, 94 °C for 1 min and 36 °C for 1 min, and a final extension of 72 °C for 5 min, and after that 4 °C for 5 min. Immediately before starting the program, the sample tray loaded with strips of capped tubes was rapidly transferred to the Gene Amp PCR systems 2400 block Perkin-Elmer (Roche Diagnostics Systems, Meylan, France) at 97 °C.

An aliquot of 1 μL of each amplification product was loaded on a 1.5% agarose gel with 1 × Tris-acetate-EDTA buffer (Carlo Erba Reagent, Val de Reuil, France) and was electrophoresed at 100 V for approximately 2.5 h (GNA 100 horizontal electrophoresis system, Amersham Pharmacia Biotech, Orsay, France). The molecular weight marker VI (Boehringer Mannheim, Meylan, France) whose sizes ranged from 154 to 2176 basepairs (bp) was run in parallel with the PCR fingerprint samples. Gels were stained, destained and analyzed as described in DNA fingerprint preparation for PFGE.

RESULTS

Patient characteristics

The investigation was carried out on 40 hospitalized patients who had totalled 330 patient days with a mean length of stay of 7.6 days (range 1–51 days). The mean age of these patients was 31.2 years (range 2–93 years) at the time of hospitalization. There were 44 *S.* Enteritidis infections, out of which 41 (93.2%) were in inpatients and three (6.8%) were in outpatients with normal stools who were considered to be *S.* Enteritidis carriers. The most relevant epidemiological and clinical features are summarized in Table 1. The pediatric patients had a mean age of 6.5 years (range 2–11 years). They presented with diarrhea plus fever but had no underlying disease. The young adults group had a mean age of 30.7 years (range 17–49 years). In this group, 11 gastroenteritis episodes and one episode of primary peritonitis were diagnosed, and six patients had predisposing conditions: cholecystectomy (patients 25, 31 and 43), polyarthritis (patient 21), leukemia (patient 13), and chronic colitis (patient 38). The elderly patients group had a mean age of 72.5 years (range 50–93 years). In this group, four gastroenteritis episodes with bacteremia were diagnosed in patients with (patients 19 and 23) or without

(patients 1 and 34) predisposing conditions. Two patients of this group (patients 10 and 30) had an underlying disease. The spectrum of the underlying disease was wide, but only two patients had a rapidly fatal prognosis; patient 10 with neoplasia and previous gastroenteritis and patient 13 with acute leukemia and previous gastroenteritis. Two patients with bacteremia, one with a urinary tract infection and another with primary peritonitis, were considered as suffering from serious underlying diseases. The remaining episodes were categorized as having nonfatal prognoses.

Genotyping analysis

Digestion with *SpeI* of chromosomal DNA from the 44 *S.* Enteritidis isolates studied produced seven different patterns of 16–24 fragments ranging in size from 48.5 to 485 Kbp arbitrarily designated PFGE patterns (PFGEps) A to G. PFGEp A was predominant (88.6% of total number of isolates), and included 37 isolates (1–5, 7–9, 11–19, 24–30, 32–44) out of which 33 were genetically indistinguishable, with the same size and number of fragments (18 in all). This pattern showed some heterogeneity with patterns displaying two or three fragment differences. They were considered to be variant subtypes of the same clone and were designated A_1, A_2 and A_3. Six PFGEps (B, C, D, E, F and G) with more than three differences were considered as unrelated types. Each PFGEp corresponded to one isolate (6, 10, 20, 23 and 31, respectively) except for E which had two isolates (21 and 22) and corresponded to sporadic cases. The PFGEps found are shown in Figure 1 and Table 1.

By using the primer AQ12 only, the amplified series were differentiated into three distinct RAPD types named H, I and J located in the region between 517 and 2176 bp. RAPD type H included 42 isolates: 23 (1–5, 7–21 and 23–25) were genetically indistinguishable in number (six in all) and size of bands. The subtypes, H_1, H_2 and H_3, were closely related to type H, and were considered as variants of the same pattern. RAPD patterns I and J

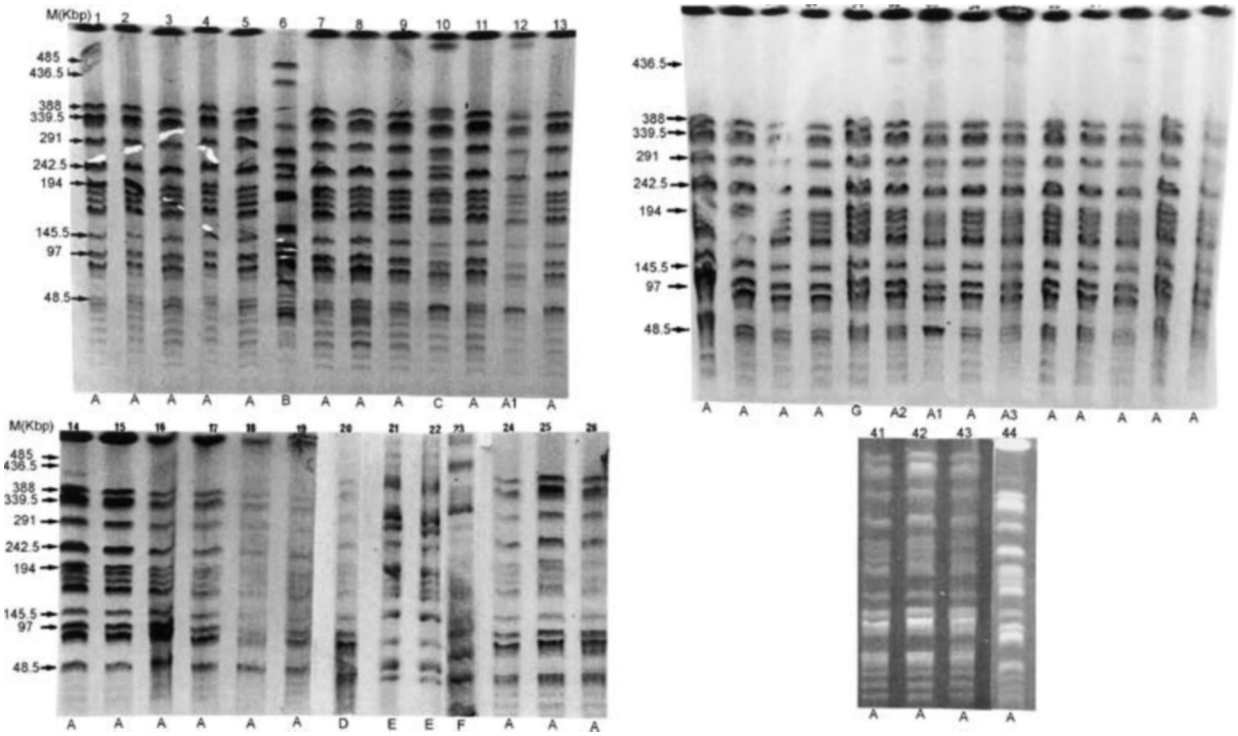

Figure 1 PFGE pattern after complete digestion of the *S. enterica* serovar Enteritidis Strains using *SpeI* restriction enzyme. *S. enteritidis* isolates numbered 1 to 44. Lane M contains the molecular size marker bacteriophage Lambda ladder. The separation size range is from 50 Kbp to 500 Kbp. Isolates 1–5, 7–9, 11, 13–19, 24–30, 34, 36–44 appear to be the same strain (strain A) with isolates 12, 32, 33 and 35 being related strains (strain A1 for isolates 11 and 33; strain A2 for isolate 32 and A3 for isolate 35). Isolates 6, 10, 20–23 and 31 do not appear related to any of others isolates and represent 6 unrelated strains (strains B, C, D, E, F and G respectively).

with more than three differences, including isolates 22 and 27, respectively, were considered to be unrelated types (Figure 2 and Table 1).

With the primer Q_{11}, six different patterns were identified (K, L, M, N, O and P) with between two and 12 bands located in the region between 394 and 2176 bp. RAPD-K and RAPD-O consisted of 19 and 21 isolates, respectively, among which eight isolates of K (1–4, 7, 8, 10 and 11) were genetically identical and 11 isolates of O (23–26, 28–33 and 44) also were identical. These patterns also showed some heterogeneity with patterns exhibiting few fragment differences. They were considered to be variant subtypes of patterns K (K_1, K_2, K_3, K_4 and K_5) and O (O_1, O_2 and O_3). The other four patterns (L, M, N and P) corresponding to isolates, 6, 9, 22 and 27, respectively, were considered to be unrelated types (Figure 3 and Table 1).

By using both primers (AQ12 and Q11), six distinct patterns (HK, H_1L, HM, IN, HO and JP) were distinguished. RAPD patterns HK (43.2%) and HO (47.7%) included 19 and 21 isolates, respectively. The other patterns (H_1L, HM, IN and JP) each corresponded to one isolate and were considered to be unrelated.

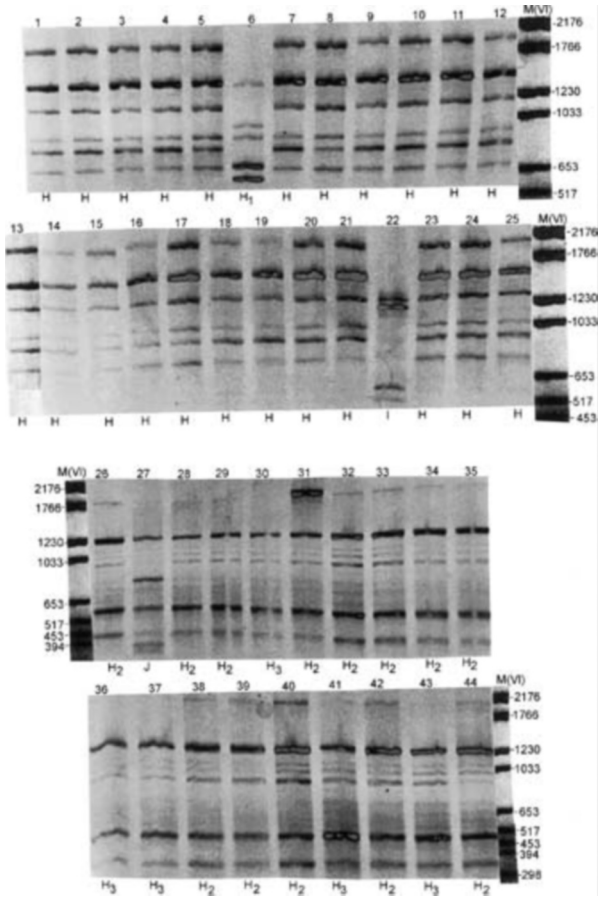

Figure 2 RAPD profiles obtained from amplification of *Salmonella enterica* serover Enteritidis genomic DNA using AQ12 as primer synthetized by DNA Technologies Eurogentec – Belgium. *S. Enteritidis* isolates numbered 1 to 44 were differentiated into three distinct RAPD types named H, I and J. RAPD type H included 42 isolates of which 23 (1–5, 7–21 and 23–25) were genetically indistinguishable. M, size marker VI: number of basepairs, 154 to 276 (Boehringer Mannheim, France).

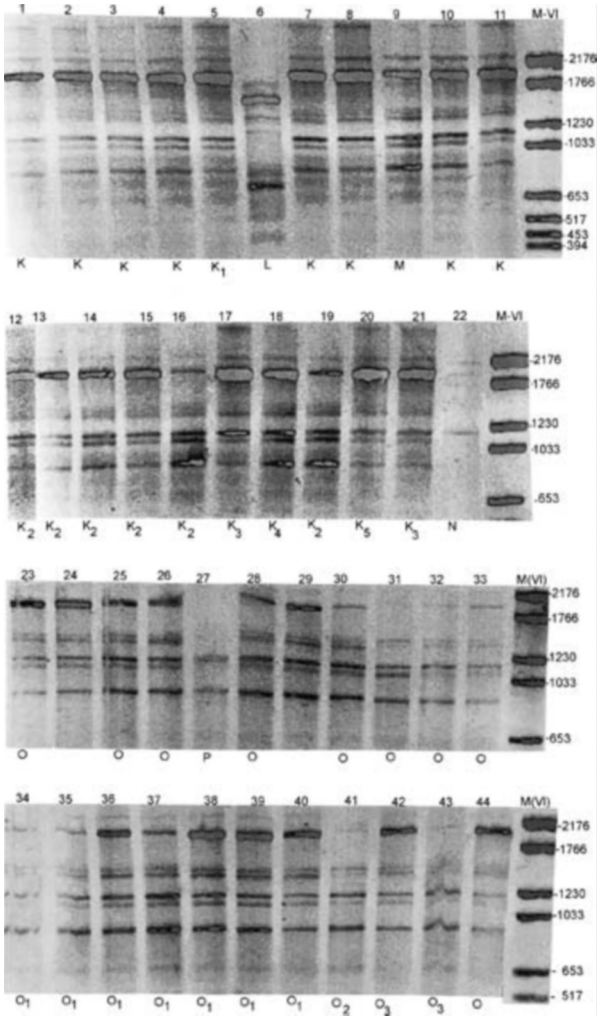

Figure 3 RAPD profiles obtained from amplification of *Salmonella enterica* serovar Enteritidis genomic DNA using Q11 as primer synthetized by DNA Technologies Eurogentec – Belgium. *S. Enteritidis* isolates numbered 1 to 44 were differentiated into six different patterns: K, L, M, N, O and P. RAPD type K and O consisted of 19 and 21 isolates respectively among which 8 isolates of K (1–4, 7, 8, 10 and 11) were genitically identical and 11 isolates of O (23–26, 28–33 and 44) also were identical. M, size marker VI: number of basepairs, 154 to 276 (Boehringer Mannheim, France).

In total, 11 different macrorestriction patterns were generated among the 44 clinical isolates by both PFGE and RAPD. Sixteen isolates (1–5, 7, 8 and 11–19) gave an identical pattern (AHK). They were observed during the first 6 months of the study (January to June) and 19 other isolates, giving the pattern AHO (24–26, 28–30, 32–44) were detected during the second period of study (July to December) at different times and were considered as characteristic of the epidemic isolates. Both these patterns presented some heterogeneity which corresponded to variant subtypes of the same clones (AHK_1, A_1HK_2, AHK_3, AHK_4, AHK_5 and AH_2O, AH_3O, A_2H_2O, A_1H_2O, AH_2O_1, $A_3H_2O_1$, AH_3O_1, AH_3O_2, AH_2O_3, AH_3O_3). Nine isolates 6, 9, 10, 20–23, 27 and 31 recovered from different patients gave different patterns (BH_1L, AHM, CHK, DHK_5, EHK_3, EIN, FHO, AJP and GH_2O, respectively) and were epidemiologically unrelated (Table 1).

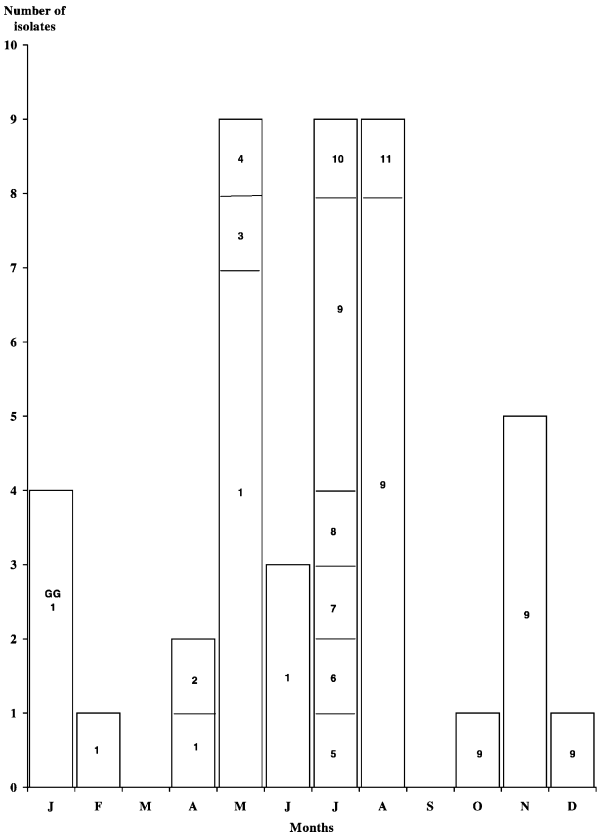

Figure 4 The monthly repartition of the isolates with their genomic groups (1-11). From January to June, the predominant GG1 plus the GG2,3 and 4 are observed. From July to December we noted a heterogeneity of genomic groups with GG9 as predominant.

Antimicrobial susceptibility

The susceptibility of *S.* Enteritidis isolates to different antibiotics was as follows: 12 (27.3%) isolates were resistant to ampicillin (MIC > 128 mg/L), and ticarcillin (MIC > 512 mg/L), 6 (13.6%) isolates to piperacillin (MIC range, 64–256 mg/L) and one (2.2%) isolate to trimethoprim-sulfamethoxazole (MIC = 16 mg/L). Four (9%) isolates, two (4.5%) isolates and three isolates (6.8%) were intermediate to piperacillin (MIC range, 16–32 mg/L), the combination amoxycillin plus clavulanic acid (MIC > 4 mg/L) and cephalothin (MIC > 8 mg/L), respectively. All isolates were susceptible to: cefoxitin [MIC range, 0.50–8 mg/L], cefotaxime [MIC range 0.03–0.50 mg/L], ceftazidime [MIC range, 0.03–2 mgL], aztreonam [MIC range, 0.06–0.50 mg/L], gentamicin [MIC range, 0.06–0.50 mg/L], tobramycin [MIC range, 0.06–1 mg/L], netilmicin [MIC range, 0.06–0.50 mg/L], amikacin [MIC range, 0.02–4 mg/L]), ofloxacin [MIC range, 0.06–0.25 mg/L].

All isolates were divided into three antimicrobial resistance patterns (designated R patterns a to c): AM^R TIC^R $PIP^{I/R}$ (a), AM^R TIC^R PIP^R AMC^I CF^I (b), AM^R TIC^R PIP^I SXT^R (c) and the phenotype (d) corresponded to isolates which showed full susceptibility to all antibiotics tested. Within pattern type AHK, three isolates (1, 13, 14) had phenotype (a), three (7, 12, 15) had phenotype (b) and 10 (2–5, 8, 11, 16–19) had phenotype (d). Within pattern type AHO, three isolates (30, 33, 34) had phenotype (a), and 16 (24–26, 28, 29, 32, 35–44) had phenotype (d). Isolates belonging to minor typing patterns had a distinct antibiotic resistance profile: (a) for AJP (isolate 27) and CHK (isolate 10) (c) for BH_1L (isolate 6), and (d) for AHM, DHK_5, EHK_3, EIN, FHO and GH_2O corresponded to isolates 9, 20, 21, 22, 23 and 31, respectively. No significant correlation was found between typing patterns and the antibiotic susceptibility pattern.

PFGE, RAPD and the antibiotic susceptibility patterns subdivided 44 isolates into 16 clonally related groups. Two of them, GG_1 and GG_9, were predominantly implicated in the course of the study (Figure 4).

Epidemiological investigation

The epidemiological, phenotypical and genotypical characterization of 44 isolates is shown in

Table 1. Distribution of patients over time revealed two clusters of bacteria. The isolates of pattern AHK and phenotype d were associated with a first cluster of ten cases from January to June 1999. Isolates of pattern AHO and phenotype d were associated with a second cluster of 16 cases between July and December 1999. Isolates of both clusters were identified in both inpatients and outpatients and were most frequently found in the pediatric group (11 isolates). The other pattern types were sporadically encountered once, between April and May for pattern types BH_1L, AHM, and CHK (genomic groups 2, 3 and 4, respectively) and between July and August for pattern types DHK_5, EHK_3, EIN, FHO, AJP and GH_2O (genomic groups 5, 6, 7, 8, 10 and 11, respectively) and were finally epidemiologically unrelated (Figure 4).

DISCUSSION

S. Enteritidis, a major food-borne pathogen, has recently emerged as a cause of acute gastroenteritis worldwide [17]. In the present study, the most frequent serovars of *Salmonella* spp., which caused human salmonellosis in Amiens in 1999, were *S.* Enteritidis followed by *S.* Typhimurium. These data are in line with those recorded in a Spanish hospital [18] but differ from our study, in which positive-d-tartrate *S.* Paratyphi B was the third most common serotype [19]. The percentage of *S.* Enteritidis-positive blood cultures in this period was 18%, higher than the percentage of 7% reported previously in Amiens by Canarelli B *et al.* [4] and that of 1.3% in Asturias, by Rodriguez *et al.* [18]. A review of *S.* bacteremia in England and Wales [20] reported that less than 2% of nontyphoid *Salmonella* isolated from humans were from blood culture and emphasized that the greatest number of bloodstream isolates were Enteritidis and Typhimurium.

Most of our *S.* Enteritidis isolates were susceptible to the antibiotics tested. Our findings confirm those of the literature indicating that most isolates were susceptible to a wide range of antimicrobial agents [21]. Nevertheless, 27.2% of the isolates studied were resistant to more than two tested antibiotics. This rate was lower than those of 35% reported elsewhere [18]. The R-patterns AM^R TIC^R $PIP^{I/R}$ $AMC^{I/S}$ observed here would correspond to TEM-type β-lactamase resistance. Ampicillin resistance in *S.* Enteritidis is usually owing to TEM-type β-lactamase encoded by genes on a 34_ 60_, or 100 Mda plasmid [22,23]. The resistance to trimethoprim-sulfamethoxazole and ampicillin, ticarcillin was expressed in three isolates. The literature shows that the resistance to trimethoprim-sulfamethoxazole is encoded by genes on the plasmid or chromosome [23]. It is noteworthy that bacteremia was recorded for all age groups but did not show the bimodal distribution, with a higher incidence at the two extremes, which is usually reported [24,25]. In our investigation bacteremia was only observed in the elderly patients. The relationship between the *S.* Enteritidis genomic group and the age group showed that the isolates belonging to genomic groups ($GG_{1,5,8}$ and $_{10}$) were observed only in the pediatric group, those of $GG_{3,6}$ and $_{11}$ in the young adult group and those of GG_4 and $_7$ in the elderly patients group. The other isolates belonging to the predominant genomic group (GG_1 and GG_9) were shared between the three groups studied.

With respect to focal infections, we found a single pyelonephritis episode caused by *S.* Enteritidis AHK (GG_1) isolate in an 82-year-old female with bacteremia and two episodes classified as intra-abdominal infections as a result of *S.* Enteritidis AHO (GG_9): one case of peritonitis and one case of chronic colitis where *S.* Enteritidis had been isolated from mesenteric lymph nodes and from a biopsy of colon, respectively.

In conclusion, it is evident that there was a small outbreak owing to the successive spread of two epidemic clones having a common ancestor during the study period of 1 year. In addition, interspersed sporadic cases of infection with genetically unrelated strains developed during the same period. It is also clear that most of the gastroenteritis episodes with or without bacteremia which occur in Amiens from time to time are caused by *S.* Enteritidis belonging to predominant genotypes. In the pediatric group, the illness occurred regardless of the presence of predisposing factors and/or underlying disease, unlike the young adults and elderly patients where nontyphoidal salmonellosis more frequently affected patients with some predisposing factor and/or underlying disease.

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