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Molecular epidemiology of *Salmonella* Typhimurium isolates from human sporadic and outbreak cases

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SUMMARY

The molecular epidemiology of a representative collection of sporadic foreign and domestically acquired *Salmonella* Typhimurium (*S.* Typhimurium) isolates from Norwegian patients in 1996–9 was studied by numerical analysis of pulsed-field gel electrophoresis (PFGE) profiles. Three subclusters (E5, F1 and G1) comprised 47% of the 102 sporadic isolates investigated and 45% of the domestically acquired isolates fell in subclusters E5 and F1. Distinct seasonal and geographic variations were evident for these strains which have been responsible for both local outbreaks (E5) and a national epidemic (F1) where salmonella-infected hedgehogs and birds constituted the suggested primary source of infection. Subcluster G1 was dominated by imported multi-resistant definitive type (DT) 104 isolates. All multi-resistant isolates contained integron-associated gene cassette-structures. This study presents valuable information on the relative significance, geographic distribution and antibiotic resistance features of distinct *S.* Typhimurium clones causing human salmonellosis among Norwegians.

INTRODUCTION

Salmonella has been one of the most important causes of bacterial enteric illness in humans for many years. In Norway, approximately 1500 cases are notified annually [1], but this number clearly underestimates the total number of human cases. In the United States an estimated 1·4 million cases of human salmonellosis occur each year [2]. In Norway as in most industrialized countries, *S.* Enteritidis is the most common serovar, but about 90% of these infections are related to foreign travel. For salmonellosis acquired in Norway, *S.* Typhimurium is the dominant serovar, and the proportion of domestically acquired infections has increased gradually over the past years to reach 45% in 1999 (outbreak-related cases excluded). Strains of this serovar have also been the causal agent in several outbreaks in Norway recent years, so much so that the serovar is now endemic in the country. An increase in isolates of *S.* Typhimurium resistant to several antibiotics, mainly due to the spread of multiresistant definitive phage-type (DT) 104 isolates, has also been reported from several countries, including Norway [3].

The molecular epidemiology of *S.* Typhimurium has not been thoroughly investigated, although the endemic situation has prompted the need to apply discriminative techniques for this purpose [3, 4]. Biochemical profiling, serotyping and phage typing are routinely used in reference laboratories for the identification and characterization of salmonella isolates, but their overall low discriminative power make these methods of limited use as discriminative tools in epidemiological studies. Several methods for molecular fingerprinting of salmonella strains have been reported including plasmid profiling, restriction endonuclease analysis of plasmid or chromosomal DNA,

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ribotyping, IS200 typing, amplified-fragment length polymorphism (AFLP) and pulsed-field gel electrophoresis (PFGE) among others [5–11]. The PFGE technique is acknowledged to be highly discriminative and of epidemiological relevance making it one of the most reliable typing procedures. The application of discriminating, molecular techniques could also be an important tool to unravel epidemic patterns, trace sources of infection and aid the development of reasonable intervention strategies to reduce the presence and spread of *S*. Typhimurium infections in Norway.

In the present study, the molecular epidemiology of representative strains of *S*. Typhimurium from 1996–9 including isolates from humans sporadically infected abroad and in Norway were analysed mainly by macrorestriction profiling by PFGE. Strains representing the eight major outbreaks in 1987–2000 were also included to compare the diversity of *S*. Typhimurium strains involved in outbreaks and sporadic infections. Isolates resistant to selected antibiotics were investigated for the presence of class 1 integron-associated gene cassettes to determine the frequency and diversity of these genetic constructs in antibiotic resistant *S*. Typhimurium. Clustering of the PFGE macrorestriction profiles (MRPs) in a dendrogram enabled quantification of similarities between band profiles and easy analysis of cluster characteristics.

METHODS

Salmonella isolates

A list of all reported human cases of *S*. Typhimurium infection in 1996–9 was obtained from the National Notification System for Infectious Diseases (MSIS) which receives continuous notifications of all culture-confirmed human cases of salmonellosis in Norway. The list was sorted chronologically by date of illness onset. Indigenous cases were defined as patients with no reported history of travelling abroad before the onset of illness while imported cases included patients who developed symptoms abroad or shortly after their return home. For the purpose of this study, we selected every fifth consecutive indigenous case (60 cases in total) and every tenth imported case (42 cases in total). Outbreak related strains were excluded to avoid selection bias. For each selected case, the corresponding bacterial isolate was obtained from the National Salmonella Reference Laboratory at the National Institute of Public Health, which verifies, types and collects all salmonella isolates in Norway. We also included a selection of human isolates representing the eight *S*. Typhimurium outbreaks in Norway in 1987–2000 (family outbreaks excluded). Phage-typing was performed on all multiresistant isolates and other selected isolates according to the methods described by Callow [12] and extended by Anderson and colleagues [13, 14].

Antimicrobial susceptibility

Antimicrobial susceptibility was tested by a tablet diffusion method according to the manufacturer's guidelines (Rosco diagnostics, Taastrup, Denmark). The antimicrobials used were: ampicillin (A), chloramphenicol (C), gentamicin (G), ciprofloxacin (Cip), cefoxitin (Cef), tetracycline (T), and trimethoprim-sulfamethoxazole (Tr/sulfa). Isolates resistant to A, C and T were also tested for resistance to streptomycin (S) and sulphonamides (Su). A 4-group system was applied for the interpretation of the inhibition zones with break points determined as recommended by the Norwegian Working Group on Antibiotics [15]. Isolates in groups 1 and 2 were considered susceptible, isolates in groups 3 and 4 were resistant.

Genotyping

DNA preparation, restriction enzyme digestion with *Xba*I and PFGE was performed as previously described [16]. Lambda DNA (Sigma, St Louis, MO, USA) served as a molecular size standard in all PFGE experiments. After electrophoresis, PFGE gels were stained with ethidium bromide and photographed with GelDoc 2000 using the Quantity One software (Bio–Rad, Hercules, CA, USA).

Isolation of plasmid DNA was performed as described by Birnboim and Doly [17]. After electrophoresis in conventional, horizontal 1% agarose gels, bands were stained with ethidium bromide and plasmid profiles visualized and photographed under UV light.

Strains resistant to one or more antibiotics were investigated for the presence of class 1 integron associated gene cassettes. The primers 5′CS and 3′CS [18] were used to amplify integrated gene cassettes in the regions between the 5′ conserved sequence (CS) and 3′CS. The PCR was performed on a Perkin–Elmer GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) with 100 ng template DNA

under the following conditions: 94 °C for 5 min, 25 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 30 sec; and a final extension at 72 °C for 7 min. The amplified products were subjected to conventional gel electrophoresis in 2% agarose gels at 100 V, stained with ethidium bromide and visualized under UV light.

Computerized numerical analyses of PFGE data

Images obtained by GelDoc 2000 using Quantity One software were saved in TIFF format and transferred to the GelCompar II software (Applied Maths, Kortrijk, Belgium) for computer analyses. Similarity between fingerprints was determined by the Dice coefficient. A band position tolerance of 1·5% was used for analysis of PFGE patterns. Dendrograms were generated by the unweighted pair group method with arithmetic averages (UPGMA). Single band differences were treated as distinct MRPs. Capital letters (A–M) were used to designate the main genetic lineages of *S.* Typhimurium isolates in the dendrogram, while each distinct MRP was assigned by a numerical suffix.

RESULTS

Molecular epidemiology of sporadic *S.* Typhimurium isolates

The 102 epidemiologically unrelated human *S.* Typhimurium isolates gave rise to 46 distinct MRPs. The clusters defined by the MRPs are shown in a dendrogram (Fig. 1). A relatively tight clustering with all isolates being within a window of similarity of approximately 70% was observed. Thirteen main clusters (designated A–M) were formed at the 83% similarity level. The majority of isolates (80%, $n = 82$) were grouped into three clusters: E (20%, $n = 20$), F (39%, $n = 40$) and G (22%, $n = 22$). Within the clusters E, F and G, certain subclusters represented by isolates with identical MRPs dominated. The most prevalent subclusters were E5, F1 and G1 that accounted for 75%, 40% and 77% of the isolates within each of their respective genetic lineages. These three subclusters comprised 47% of the 102 sporadic *S.* Typhimurium isolates investigated. The remaining 10 main clusters (A, B, C, D, H, I, J, K, L and M) each included between 1 and 5 isolates (Fig. 1).

A thorough examination of the dominant subclusters E5, F1 and G1 revealed significant geographical variations. Twelve of the 15 subcluster E5 isolates were acquired in Norway, exclusively isolated from sporadic cases in two counties in Western Norway. Subcluster F1 also contained a majority of domestic isolates and 15 of 16 F1 isolates were acquired in Norway. However, these isolates were geographically widespread in the country being isolated from patients in six separate counties from Eastern, Western, Central and Northern Norway. Nine of the 16 isolates were from three counties in Western and Central Norway. Isolates within subclusters F1 and E5 accounted for 45% of the investigated domestic cases in 1996–9.

The 17 isolates comprising subcluster G1 had a wide global occurrence; 31% ($n = 13$) of the isolates acquired abroad (10 countries) belonged to this characteristic subcluster while less than 7% ($n = 4$) of the domestic isolates belonged to subcluster G1. Thus, a significantly higher proportion of DT104 infections was acquired abroad ($P = 0{\cdot}0022$ Fisher's exact test). Interestingly, a significant increase of isolates of the DT104 clone was observed during the period (1996–9). While this clone constituted only four isolates in the years 1996 and 1997, 13 isolates of this clone were recovered in 1998/9 ($P = 0{\cdot}0372$ Fisher's exact test).

A distinct seasonality was observed for isolates comprising certain genetic subclusters. The 15 domestic subcluster F1 isolates all appeared between January and June with 80% ($n = 12$) of the cases in the period January to April. In comparison, domestic cases caused by isolates of the second most dominant subcluster, E5, appeared between April and November predominating in July/August (10/13; Fig. 2). Subcluster G1 isolates occurred throughout the whole year although 59% (10/17) appeared in July through September.

Interestingly, PFGE analysis of human isolates involved in a nationwide outbreak in 1987 traced to contaminated chocolate bars [19] had identical MRPs to the sporadic subcluster F1 isolates in this study. The 1987 epidemic strain belonged to a group previously termed variant 1 based on plasmid profile and biochemical properties [4]. Fourteen of the 15 subcluster F1 isolates also had a plasmid profile identical to the variant 1 epidemic strain.

Outbreak strains *vs.* sporadic strains

Five different MRPs were encountered among human isolates representing the eight domestic outbreaks in 1987–2000. Seven of the outbreaks each contained isolates with a single MRP, while one outbreak

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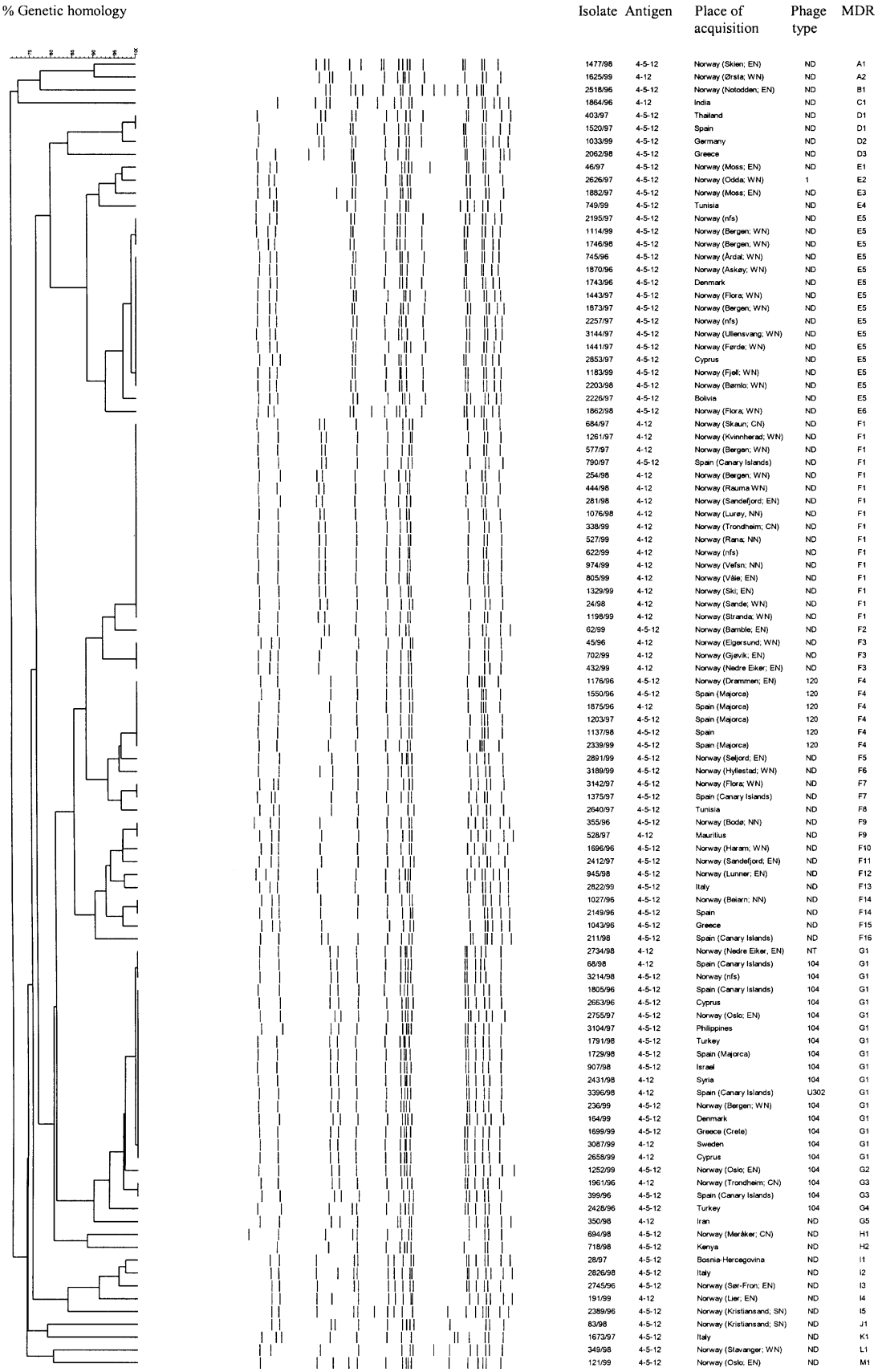

Isolate	Antigen	Place of acquisition	Phage type	MDR
1477/98	4-5-12	Norway (Skien; EN)	ND	A1
1625/99	4-12	Norway (Ørsta; WN)	ND	A2
2518/96	4-5-12	Norway (Notodden; EN)	ND	B1
1864/96	4-12	India	ND	C1
403/97	4-5-12	Thailand	ND	D1
1520/97	4-5-12	Spain	ND	D1
1033/99	4-5-12	Germany	ND	D2
2062/98	4-5-12	Greece	ND	D3
46/97	4-5-12	Norway (Moss; EN)	ND	E1
2626/97	4-5-12	Norway (Odda; WN)	1	E2
1882/97	4-5-12	Norway (Moss; EN)	ND	E3
749/99	4-5-12	Tunisia	ND	E4
2195/97	4-5-12	Norway (nfs)	ND	E5
1114/99	4-5-12	Norway (Bergen; WN)	ND	E5
1746/98	4-5-12	Norway (Bergen; WN)	ND	E5
745/96	4-5-12	Norway (Årdal; WN)	ND	E5
1870/96	4-5-12	Norway (Askøy; WN)	ND	E5
1743/96	4-5-12	Denmark	ND	E5
1443/97	4-5-12	Norway (Flora; WN)	ND	E5
1873/97	4-5-12	Norway (Bergen; WN)	ND	E5
2257/97	4-5-12	Norway (nfs)	ND	E5
3144/97	4-5-12	Norway (Ullensvang; WN)	ND	E5
1441/97	4-5-12	Norway (Førde; WN)	ND	E5
2853/97	4-5-12	Cyprus	ND	E5
1183/99	4-5-12	Norway (Fjell; WN)	ND	E5
2203/98	4-5-12	Norway (Bømlo; WN)	ND	E5
2226/97	4-5-12	Bolivia	ND	E5
1862/98	4-5-12	Norway (Flora; WN)	ND	E6
684/97	4-12	Norway (Skaun; CN)	ND	F1
1261/97	4-12	Norway (Kvinnherad; WN)	ND	F1
577/97	4-12	Norway (Bergen; WN)	ND	F1
790/97	4-5-12	Spain (Canary Islands)	ND	F1
254/98	4-12	Norway (Bergen; WN)	ND	F1
444/98	4-12	Norway (Rauma WN)	ND	F1
281/98	4-12	Norway (Sandefjord; EN)	ND	F1
1076/98	4-12	Norway (Lurøy; NN)	ND	F1
338/99	4-12	Norway (Trondheim; CN)	ND	F1
527/99	4-12	Norway (Rana; NN)	ND	F1
622/99	4-12	Norway (nfs)	ND	F1
974/99	4-12	Norway (Vefsn; NN)	ND	F1
805/99	4-12	Norway (Våle; EN)	ND	F1
1329/99	4-12	Norway (Ski; EN)	ND	F1
24/98	4-12	Norway (Sande; WN)	ND	F1
1198/99	4-12	Norway (Stranda; WN)	ND	F1
62/99	4-5-12	Norway (Bamble; EN)	ND	F2
45/96	4-12	Norway (Eigersund; WN)	ND	F3
702/99	4-12	Norway (Gjøvik; EN)	ND	F3
432/99	4-12	Norway (Nedre Eiker; EN)	ND	F3
1176/96	4-5-12	Norway (Drammen; EN)	120	F4
1550/96	4-5-12	Spain (Majorca)	120	F4
1875/96	4-12	Spain (Majorca)	120	F4
1203/97	4-5-12	Spain (Majorca)	120	F4
1137/98	4-5-12	Spain	120	F4
2339/99	4-5-12	Spain (Majorca)	120	F4
2891/99	4-5-12	Norway (Seljord; EN)	ND	F5
3189/99	4-5-12	Norway (Hyllestad; WN)	ND	F6
3142/97	4-5-12	Norway (Flora; WN)	ND	F7
1375/97	4-5-12	Spain (Canary Islands)	ND	F7
2640/97	4-5-12	Tunisia	ND	F8
355/96	4-5-12	Norway (Bodø; NN)	ND	F9
528/97	4-12	Mauritius	ND	F9
1696/96	4-5-12	Norway (Haram; WN)	ND	F10
2412/97	4-5-12	Norway (Sandefjord; EN)	ND	F11
945/98	4-5-12	Norway (Lunner; EN)	ND	F12
2822/99	4-5-12	Italy	ND	F13
1027/96	4-5-12	Norway (Beiarn; NN)	ND	F14
2149/96	4-5-12	Spain	ND	F14
1043/96	4-5-12	Greece	ND	F15
211/98	4-5-12	Spain (Canary Islands)	ND	F16
2734/98	4-12	Norway (Nedre Eiker, EN)	NT	G1
68/98	4-12	Spain (Canary Islands)	104	G1
3214/98	4-5-12	Norway (nfs)	104	G1
1805/96	4-5-12	Spain (Canary Islands)	104	G1
2663/96	4-5-12	Cyprus	104	G1
2755/97	4-5-12	Norway (Oslo; EN)	104	G1
3104/97	4-5-12	Philippines	104	G1
1791/98	4-5-12	Turkey	104	G1
1729/98	4-5-12	Spain (Majorca)	104	G1
907/98	4-5-12	Israel	104	G1
2431/98	4-12	Syria	104	G1
3396/98	4-12	Spain (Canary Islands)	U302	G1
236/99	4-5-12	Norway (Bergen; WN)	104	G1
164/99	4-5-12	Denmark	104	G1
1699/99	4-5-12	Greece (Crete)	104	G1
3087/99	4-12	Sweden	104	G1
2658/99	4-12	Cyprus	104	G1
1252/99	4-5-12	Norway (Oslo; EN)	104	G2
1961/96	4-12	Norway (Trondheim; CN)	104	G3
399/96	4-5-12	Spain (Canary Islands)	104	G3
2428/96	4-5-12	Turkey	104	G4
350/98	4-12	Iran	ND	G5
694/98	4-5-12	Norway (Meråker; CN)	ND	H1
718/98	4-5-12	Kenya	ND	H2
28/97	4-5-12	Bosnia-Hercegovina	ND	I1
2826/98	4-5-12	Italy	ND	I2
2745/96	4-5-12	Norway (Sør-Fron; EN)	ND	I3
191/99	4-12	Norway (Lier; EN)	ND	I4
2389/96	4-5-12	Norway (Kristiansand; SN)	ND	I5
83/98	4-5-12	Norway (Kristiansand; SN)	ND	J1
1673/97	4-5-12	Italy	ND	K1
349/98	4-5-12	Norway (Stavanger; WN)	ND	L1
121/99	4-5-12	Norway (Oslo; EN)	ND	M1

Fig. 1. Dendrogram based on PFGE (*Xba*I) macrorestriction profiles (MRPs) of the 102 sporadic isolates under study. Antigenic factors, country of acquisition (with available geographic details in parentheses; EN, Eastern Norway; SN, Southern Norway; WN, Western Norway; CN, Central Norway; NN, Northern Norway; nfs, no further specified) and phage type (NT, not phage typeable; ND, not determined) are indicated.

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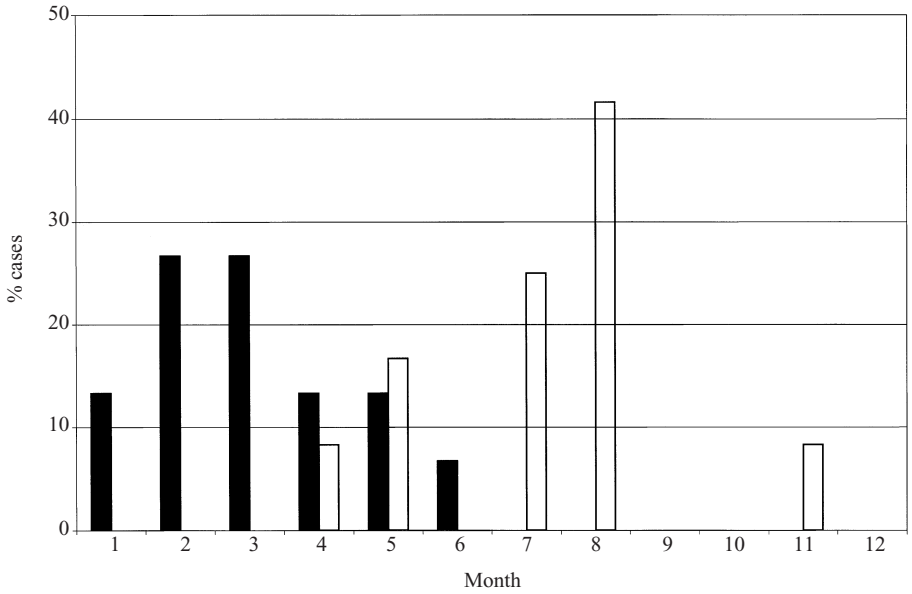

Fig. 2. Seasonal distribution (monthly) of human cases caused by isolates of the two most prevalent MRPs (F1 ($n = 15$) and E5 ($n = 12$)) among patients with no reported history of travel abroad. Values on the y axis are percentages of the total number of cases within each MRP. Black bars represent cases with isolates of MRP F1; white bars represent cases with isolates of MRP E5.

Table 1. *Resistance type, integron-associated gene cassettes and macrorestriction profiles of antibiotic resistant* S. *Typhimurium isolates acquired in Norway and other countries*

Resistance type*	No. of isolates	Place of acquisition		Amplicons (kb)†						Macrorestriction profile (MRP)
		Norway	Foreign	0·2	0·4	1·0	1·2	1·6	2·0	
ACSSuTCef	1	0	1	(+)	−	+	+	−	−	G1
ACSSuTG	2	0	2	(+)	−	+	+	−	−	G1
ACSSuTTr/sulfa	2	0	2	(+)	−	+	+	−	−	G1
ACSSuT	14	6	8	(+)	−	+	+	−	−	G1, G2, G3, G4
	4	1	3	(+)	−	−	−	−	+	F4
ACST	1	0	1	(+)	−	−	−	−	+	F4
ACT	1	0	1	(+)	−	+	+	−	−	G1
AT	1	0	1	(+)	−	−	−	−	+	F1
	1	1	0	(+)	(+)	−	−	−	−	A2
TTr/sulfa	1	0	1	(+)	−	(+)	−	−	−	D3
	1	0	1	(+)	−	−	−	−	−	D1
A	1	0	1	(+)	−	−	−	−	−	H2
T	1	0	1	(+)	−	−	−	−	−	D1
	2	2	0	(+)	(+)	−	−	−	−	I3, A1
Tr/sulfa	2	1	1	(+)	−	−	−	+	−	I4, C1

* Antibiotic abbreviations are shown in Materials and Methods.
† Amplicons of weak intensity are shown in parentheses.

included strains with two similar profiles. Isolates within six of these outbreaks fell in four MRPs that were also observed among sporadic isolates: E2 (2 outbreaks), E5 (1 outbreak), F1 (1 outbreak) and F10 (2 outbreaks). Isolates of one outbreak had a distinct and unique DNA pattern. Both the similar DNA profiles, E2 and E5, were encountered among strains from another outbreak in Western Norway in 1999. The observed band differences in isolates from this outbreak could be due to 'clonal turnover'. However,

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distinct and separate phage types (in parentheses) for outbreak isolates within each of the clusters E2 (DT1) and E5 (variant of DT42), were obtained. The epidemiological data further support that these are distinct clones. In the outbreak from which isolates within the subclusters E2 and E5 were detected, hedgehogs were the probable infecting source. Isolates of the subclusters E2 and E5 were never detected in the same individual, neither humans nor hedgehogs, but were in all cases isolated from different individuals in geographically close but separate regions. Two outbreaks where the infecting agent has been isolates of clusters E2 and E5 probably originated in salmonella infected hedgehog populations [20].

Antibiotic susceptibility and integrons

Thirty-four percent ($n = 35$) of the isolates were resistant to one or more antibiotics leading to 11 resistance phenotypes (Table 1). Seventy-one percent ($n = 25$) of these were multiresistant (3–6 resistances) with 92% ($n = 23$) being resistant to the five-drug pattern ACSSuT, typical for DT104 isolates [21]. Seven of these were acquired in Norway, while five isolates, all of foreign origin, were also resistant to additional antibiotics.

The multidrug-resistant isolates grouped within the subclusters G1–G4 (G1:16 isolates; G2:1 isolate; G3:2 isolates; G4:1 isolate) and F4 (6 isolates; Fig. 1). Eighteen of the multidrug-resistant MRP G1–G4 isolates were DT104, one was phage type (PT) U302 and one isolate was non-typeable. A single DT104 isolate was sensitive to all antibiotics tested, but had an MRP (G3) identical to a multidrug resistant DT104 isolate. Five multidrug resistant isolates (4 of R-type ACSSuT) were of phage type DT120 and grouped in the unique subcluster F4. Four of the sporadic subcluster F4 isolates were from patients with a history of travel to Majorca, Spain, prior to illness onset while the last case was reported not to be associated with foreign travel.

A variety of putative integron-associated gene cassettes were present in the 35 antibiotic resistant isolates (Table 1). All the 20 multidrug-resistant subcluster G1–G4 isolates produced two intense PCR products of about 1·0 and 1·2 kb, characteristic of DT104 and PT U302 isolates. Gene cassettes of different sizes were also identified in antibiotic resistant isolates with other MRPs. Four of the PFGE subcluster F4 isolates had the R-type ACSSuT although PCR identified a gene cassette of about 2·0 kb in all six isolates (Table 1). For two strains both resistant to Tr/sulfa with profiles C1 (foreign) and I4 (domestic), respectively, a PCR product of 1·6 kb was obtained (Table 1). In addition to the intense PCR fragments reported above, all tested isolates contained a weaker PCR product of about 200 bp. Weak signal PCR products of about 400 bp (3 isolates) and 1000 bp (1 isolate) in isolates containing no other class 1 integron-associated gene cassettes were also observed. The possible, integron-associated gene cassette nature of these fragments awaits detailed characterization and the presence of these amplicons is therefore shown in parentheses in Table 1.

DISCUSSION

A representative collection of both domestic and foreign acquired *S*. Typhimurium strains causing human salmonellosis in Norwegians during 1996–9 was subjected to cluster analyses of MRPs obtained by PFGE. Antibiotic resistant strains were analysed for the presence of class 1 integrons to obtain an overview of the dissemination of integrons in antibiotic resistant *S*. Typhimurium isolates. The compiled collection of numerical fingerprint data, antibiotic resistance and surveillance data, including geographical differences and seasonal variations, could be of importance to set priorities and focus efforts for the detection of main reservoirs and sources to limit the spread of *S*. Typhimurium and other bacterial infectious agents.

A problem common to most methods arises in evaluating the significance of small genotypic or phenotypic changes of isolates under comparison. Thus, the clonal nature of many bacteria generating minor interstrain banding pattern differences may make the interpretation and evaluation of fingerprint data subjective. Given the genetic homogeneity of salmonella populations [22], proposed interpretive guidelines [23] were considered too stringent for the analysis and discrimination of serovar Typhimurium strains. A combination of methods including plasmid profile analysis and phage typing were performed to complement the PFGE(*Xba*I) data where appropriate. The stability of PFGE patterns was indicated by the presence of common subclusters throughout the study period, and that outbreak related isolates shared the same PFGE patterns. Numerical analyses of fingerprint data would enable increased accuracy for

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describing epidemiological and clonal relationships of *S.* Typhimurium.

All isolates under study clustered within a window of similarity of approximately 70%. This was similar to a previous PFGE(*Xba*I) fingerprint report on *S.* Typhimurium [11]. Despite the tight clustering of isolates, a large number of domestic *S.* Typhimurium clonal variants were observed. This could indicate the presence of a variety of *S.* Typhimurium reservoirs and sources in Norway, although certain subclusters dominated. The significant dominance of domestic cases among subcluster E5 and F1 isolates along with the distinct geographic distribution of these clones could indicate the presence of specific domestic and /or local reservoirs of these strains. However, these *S.* Typhimurium variants may also be regularly present abroad, but their low incidence in this investigation may be explained by the frequent occurrence of other *S.* Typhimurium clones in foreign countries often visited by Norwegians and where the salmonella incidence is usually higher than in Norway.

For subcluster F1 isolates, a conspicuous seasonality with 81% of the cases reported in the period January to April was observed. This is in accordance with a previous study reporting that 76% of sporadic, domestic cases of *S.* Typhimurium infections from 1966–96 to appear in the period January to April [4]. A strain previously termed variant 1 (based on biotyping and plasmid profiling), caused a nationwide epidemic in 1987 where chocolate bars were identified as the vehicle of transmission [19]. Variant 1 isolates infected elevated the number of cases (10–26 per year) in the years 1988–93, but this was followed by a significant decline in subsequent years. Isolates from the 1987 epidemic and 15 domestic sporadic isolates of this study had the identical PFGE pattern F1. Furthermore, 14 of the 15 subcluster F1 sporadic isolates had the identical plasmid profile characteristic for variant 1 strains. We conclude that variant 1 isolates still remain one of the most important contributors to *S.* Typhimurium infections in Norway. A previous case-control study suggested an epidemiological link between human and avian cases [4]. Recent studies support the view that wild birds may be an important reservoir and responsible for the wide geographic distribution of variant 1 human infecting salmonella (T. Refsum et al., unpublished observations).

Subcluster G1 included 4 isolates of domestic origin and 13 isolates from 10 other countries indicating the presence of a worldwide *S.* Typhimurium clone. All except two isolates belonged to phage type DT104 along with the four isolates with the related DNA profiles G2–G4. The DT104 clone has rapidly spread worldwide [21, 24, 25] and may be regarded as endemic also in Norway although the annual number of domestically acquired isolates has not exceeded 15 [1]. The incidence of this clone causing sporadic infections among humans in Norway is low compared to cases attributed to foreign travel where it dominates among *S.* Typhimurium. The elevated number of human cases in the period July to September may reflect a predictably high foreign travel frequency among Norwegians within this period of the year.

The high incidence of subcluster F1 sporadic isolates was not reflected in outbreaks. The 1987 outbreak was the only one detected where infecting *S.* Typhimurium had the F1 DNA profile. For the other two most frequent MRPs among sporadic isolates, E5 and G1, only isolates from two outbreaks, both with the E5 profile, were encountered. Interestingly, PFGE revealed six sporadic isolates with the distinct MRP F4. Four of these isolates were from patients with reported travel to Majorca before illness onset. The fingerprint data suggest that this clone has been responsible for regular human infections in Majorca during the study period and also indicates the probable presence of a common reservoir or source of this clone. It is tempting to speculate that there exists an epidemiological connection between all six isolates within this subcluster although the sixth isolate apparently was of domestic origin. This study demonstrates that molecular subtyping by PFGE may elucidate possible epidemiological connections between isolates that may have remained overlooked.

The two outbreaks caused by subcluster E5 isolates were detected in Western Norway in 1999 and 2000 with seagulls and hedgehogs, respectively, as the probable sources of infection [26, 27]. Hedgehogs were also the source of infection in a 1996 outbreak caused by isolates with the similar PFGE pattern E2. A recent investigation suggests that hedgehogs are likely to be a primary source of specific *S.* Typhimurium clones of human clinical significance in certain geographical areas [20]. Birds infected with these and other *S.* Typhimurium clones of the same serotype and PFGE profile as isolates from human cases have also been observed and may be a source for widespread dissemination of certain clones (Refsum et al., unpublished observations).

There have been reports that the incidence of multiresistant DT104 isolates with additional resis-

tances has increased in recent years [28, 29]. In this study, five isolates acquired abroad, all DT104, expressed resistance to other antibiotics in addition to the ACSSuT core resistance pattern to antibiotics (Table 1). None of these isolates was acquired in Norway; 18% ($n = 11$) of the *S.* Typhimurium isolates acquired in Norway were resistant to antibiotics compared to 57% ($n = 24$) of isolates acquired abroad. More than 50% of the resistant isolates originating in Norway were multiresistant and of phage type DT104. This supports recent findings showing a generally low-level, although increasing, spectrum of resistance dominated by phage type DT104 strains [3]. Since all multiresistant isolates were grouped into a limited number of distinct DNA patterns, PFGE may be used for the detection and monitoring of multidrug resistant strains. Although various clones of DT104 and related phage types have been reported [28, 30], this study indicates that the majority of DT104 isolates is still part of the epidemically spreading multiresistant clone [24, 29, 31].

The genetic homogeneity of prevalent multidrug resistant *S.* Typhimurium phage types represents a challenge for discriminative molecular fingerprinting. Genetic constructs associated with integrons may possibly be used in combinations with other typing methods to increase the discriminative fingerprinting of antibiotic resistant *S.* Typhimurium isolates and to trace the spread of epidemic clones. Integrons are responsible for a substantial proportion of antimicrobial resistance and several integron structures have been detected in various salmonella serovars [32]. The sizes of the class 1 integron-associated gene cassettes in the DT104 isolates were 1·0 and 1·2 kb. This is consistent with previously characterized integrons encoding resistance to streptomycin-spectinomycin and ampicillin, respectively, predominant among DT104 strains [31–33]. Other gene cassettes of about 1·6 kb (two isolates) and 2·0 kb (six isolates) were present. The 1·6 kb gene cassette in two isolates resistant to Tr/sulfa may contain a *dfrA* gene encoding trimethoprim resistance and previously detected on a 1·6 kb cassette in *S.* Typhimurium [32]. The weak PCR product of approximately 200 bp observed in all tested isolates may be identical to the recently reported 210 bp fragment containing part of the *purG* gene (encoding phosphoribosylformyl-glycinamidine synthetase) [34]. Studies are in progress to investigate the nature of the integron structures in multiresistant salmonella isolates and to determine if the weak intensity PCR fragments (200 bp, 400 bp, 1·0 kb) represent gene cassettes or are the result of nonspecific primer matching.

In the present study, valuable information of the relative significance and geographic distribution of distinct *S.* Typhimurium clones causing human salmonellosis among Norwegians has been obtained. We report that near half of the domestically acquired, *S.* Typhimurium isolates from sporadically infected human cases grouped within only two subclusters (E5 and F1) which demonstrated specific characteristics regarding geographic and seasonal distribution. Among cases with infection acquired abroad, the worldwide endemic DT104 clone was predominant. Antibiotic resistant isolates were more prevalent among strains acquired abroad than isolates from domestic cases. Integrons were present in all multiresistant isolates. This study demonstrates that molecular fingerprinting could be a valuable part in both short- and long-term surveillance of *S.* Typhimurium, and that the application of detailed, reproducible tools for fingerprint analyses is of paramount importance for increased insights into the molecular epidemiological features of *S.* Typhimurium.

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