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Emerging antibiotic resistance in *Salmonella* Typhimurium in Norway

T. M. LEEGAARD*, D. A. CAUGANT, L. O. FRØHOLM, E. A. HØIBY AND J. LASSEN

Department of Bacteriology, National Institute of Public Health, Oslo, Norway

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SUMMARY

The antimicrobial resistance of 809 *Salmonella* Typhimurium isolates collected from humans in Norway between 1975 and 1998 was studied. The material was subdivided into domestic and foreign isolates according to whether the patient had recently travelled abroad or not. In imported isolates the largest increase in resistance was in 1996 when 35% of the isolates were multi-resistant. The first multi-resistant isolate acquired in Norway appeared in 1994, but already in 1998 23% of the isolates domestically acquired were multi-resistant, and a majority were *S.* Typhimurium DT104. We found no ciprofloxacin resistance in domestically acquired isolates. Amplified fragment length polymorphism analysis was performed on selected multi-resistant isolates. The method discriminated well between different multi-resistant isolates, but not between DT104 isolates. Resistant and multi-resistant *S.* Typhimurium were until 1998 essentially recovered from patients who had travelled abroad, but multi-resistant isolates, mainly DT104, are now also being transmitted within the country.

INTRODUCTION

The incidence of human salmonellosis in Norway (population 4·5 million) is still low, with about 1500 cases registered yearly, but the epidemiological situation seems to run parallel to the situation observed elsewhere in Europe with an increase in the incidence of salmonellosis over the past 20 years [1]. The increase in the incidence of salmonellosis in Norway is largely due to an increase in the number of *S.* Enteritidis infections, of which about 90% are acquired abroad. *S.* Typhimurium is the second most prevalent serovar among salmonella recovered from humans in Norway. The number of *S.* Typhimurium infections has been stable over the last 20 years with about 200 infections registered yearly. One exception was 1987 when an increased number of cases were observed as a result of a nation-wide outbreak linked to contaminated chocolate [2]. In 1998 *S.* Typhimurium constituted 12% of all salmonella isolates [3]. While in 1982 only 5% of the *S.* Typhimurium isolates were domestically acquired, the proportion has increased gradually over the past years to reach 35% in 1998. In fact, *S.* Typhimurium is the only serovar of salmonella that is endemic in Norway.

An increase in resistance of salmonellas to antimicrobial agents has been reported from several countries during the last 10 years [4–9]. The main reason for this increase has been the emergence of salmonellosis caused by *S.* Typhimurium definitive phage-type (DT) 104. This phage-type typically is resistant to five antibiotics, ampicillin (A), chloramphenicol (C), streptomycin (S), sulphonamides (Su)

* Author for correspondence: Department of Bacteriology, National Institute of Public Health, P.O. Box 4404 Torshov, N-0403 Oslo, Norway.

and tetracycline (T), often referred to as ACSSuT-resistance. In addition, resistance to trimethoprim and ciprofloxacin has recently been observed [10]. *S.* Typhimurium DT104 has now been reported from an increasing number of countries, amongst others Great Britain, Germany, France, Denmark, Finland, the Czech Republic, Israel and the USA [5, 7, 9, 11–15]. In Great Britain *S.* Typhimurium DT104 now is the second most common cause of human salmonellosis after *S.* Enteritidis PT4 [10], and in the United States the prevalence of *S.* Typhimurium isolates with the five-drug pattern of resistance was 34% in 1996 [15].

The purpose of this study was to determine whether a change in antimicrobial susceptibility of *S.* Typhimurium isolated from humans in Norway had occurred in recent years. As most cases of *S.* Typhimurium infections in Norway have been acquired abroad, we compared the antimicrobial resistance of isolates from patients who had acquired their infection outside Norway with isolates acquired in Norway. In addition, we wanted to determine whether domestic transmission of *S.* Typhimurium DT104 occurred in Norway.

Most isolates of *S.* Typhimurium are genetically very similar [16], especially those of *S.* Typhimurium DT104 which represents a single clone that spreads epidemically [9]. Various typing methods have been applied in order to distinguish resistant variants of *S.* Typhimurium DT104 and study their spread. Most success has been achieved with pulsed-field gel electrophoresis (PFGE), which differentiated five patterns among *S.* Typhimurium DT104 [17]. In this study we analysed selected isolates with amplified fragment-length polymorphism (AFLP) to see whether this method could reveal the origins of domestically acquired *S.* Typhimurium DT104 isolates and differentiate them from other phage-types.

METHODS

Bacterial isolates

In Norway, all clinical bacterial isolates of salmonella recovered from humans by the medical microbiological laboratories have been submitted to and stored at the National Salmonella Reference Laboratory (NSRL) at the National Institute of Public Health since the middle of the 1960s. We studied a total of 809 human isolates of *S.* Typhimurium submitted to the NSRL between 1975 and 1998. From the isolates received in the years 1975, 1980 and 1985, and yearly from 1990 to 1995, we selected a total of 174 *S.* Typhimurium isolates. From each of these 9 years, all available isolates up to 10, from patients who had not travelled outside Norway and 10 isolates from patients who had recently travelled abroad were selected randomly. All isolates from the years 1996 ($n = 211$), 1997 ($n = 221$) and 1998 ($n = 203$) were studied regardless of the patients' travel history. For all patients information regarding travel abroad was collected from the National Notification System for Communicable Diseases. Patients with a history of foreign travel who developed symptoms in the incubation period after their return home were defined as imported cases [18]. Since 1995 more complete information, e.g. on the patients' travel history prior to the onset of the illness, has been requested from physicians treating patients with positive stool samples. If no information regarding travel is submitted, a questionnaire is sent to the physician retrospectively. In 1998, travel information was available for 93% ($n = 189$) of the isolates.

All isolates were identified as belonging to serovar Typhimurium according to standard procedures [19]. Phage-typing was performed on selected resistant isolates from 1998, according to the methods described by Callow [20] and extended by Anderson and colleagues [21, 22]. *S.* Typhimurium DT104 was characterized by sensitivity to typing phages 12, 13 and 18, using the 30 typing phages of the Anderson basic set. Typing phage 35 did not react in our laboratory. Therefore seven isolates were submitted to the German Salmonella Reference Laboratory, Robert Koch-Institut, for verification. All were designated *S.* Typhimurium DT104. Subsequently, all *S.* Typhimurium isolates with ACSSuT-resistance reacting with phages 12, 13 and 18 were designated as *S.* Typhimurium DT104.

Antibiotic susceptibility

Antibiotic susceptibility was tested by a tablet diffusion method according to the manufacturer's guidelines (A/S Rosco, Taastrup, Denmark). The antimicrobials used were ampicillin, chloramphenicol, cefoxitin, ciprofloxacin, gentamicin, streptomycin, sulphadiazine, tetracycline and trimethoprim-sulphamethoxazole. The results were classified according to the four-group system recommended by the Norwegian Working Group on Antibiotics [23]. Isolates in groups 1 and 2 were considered susceptible. Isolates

Table 1. *Percentage of 809* S. *Typhimurium isolates from humans in Norway resistant to the antibiotics tested**

Year	No. isolates	A	C	Cf	Ci	G	S	Su	T	Sxt
1975–90	76	2·6	5·3	0	0	0	3·9	3·9	7·9	1·3
1991–5	98	13·0	12·0	0	0	1·0	7·0	19·4	30·6	9·0
1996	211	35·5	31·8	0·5	0·9	0·5	34·1	41·2	43·6	5·7
1997	221	27·6	24·0	0	1·4	1·4	23·1	36·2	37·1	15·4
1998	203	35·0	26·6	0	0	2·5	30·0	34·0	50·7	8·9

* Antibiotic abbreviations: A, ampicillin; C, chloramphenicol; Cf, cefoxitin; Ci, ciprofloxacin; G, gentamicin; S, streptomycin; Su, sulphonamides; T, tetracycline; Sxt, trimethoprim-sulphamethoxazole.

that were resistant to four or more antibiotics were considered multi-resistant. We checked the susceptibility to ciprofloxacin with Etest (AD Biodisk, Solna, Sweden) in all isolates from 1998 that were resistant to ampicillin and chloramphenicol ($n = 54$), and in four isolates where resistance to ciprofloxacin had been found by tablet diffusion.

AFLP

Selected multi-resistant isolates were analysed with an AFLP method, as previously described [24]. The restriction enzymes were *Eco*RI and *Mse*I (New England Biolabs, Beverly, MA, USA), and capillary electrophoresis was run on an ABI-310 Genetic Analyzer (Perkin–Elmer Inc., Norwalk, Conn., USA). In total 52 isolates were analysed. The isolates were mainly *S.* Typhimurium DT104 isolates ($n = 32$) from patients with and without a history of recent foreign travel. In addition, other multi-resistant isolates ($n = 19$) and one strain that was resistant to three antibiotics only were run as comparison. Most ($n = 11$) of the other multi-resistant isolates had the same ACSSuT resistance pattern as the DT104 isolates. Two fully susceptible isolates were used as control strains.

RESULTS

Antibiotic susceptibility

The percentages of isolates resistant to the tested antibiotics are shown in Table 1. The highest proportion of resistance was seen for tetracycline (over 50% of the isolates in 1998). Relatively similar proportions of resistance were found for ampicillin, chloramphenicol, streptomycin and sulphonamides (between 27 and 35% of the isolates in 1998). A marked increase in resistance to these five drugs can be seen from the earliest to the most recent years. In Figure 1, the percentage of resistant isolates with respect to place of acquisition of infection is shown for the different time periods. For most antibiotics, major differences in the percentage of resistance were found between isolates acquired in Norway and isolates acquired abroad. Only 4 isolates were resistant to ciprofloxacin; 1 from 1996 and 3 from 1997, all from patients with a history of travel to an Asian country, China (2 isolates), Thailand and the Philippines. No ciprofloxacin resistance was found in domestically acquired isolates: all MICs were $\leqslant 0{\cdot}032$ μg/ml except for one isolate that had a MIC of $0{\cdot}25$ μg/ml.

The percentages of *S.* Typhimurium isolates fully susceptible to all antibiotics (S), or resistant to 1, 2, 3 or $\geqslant 4$ antibiotics from 1975 to 1998 are shown in Figure 2. The proportion of fully sensitive isolates decreased from 87% before 1990 to only 47% in 1998. Correspondingly, only one multi-resistant isolate was found before 1990, but since 1990 the number of resistant isolates has increased gradually, and in 1998 30% of the isolates were multi-resistant.

The first multi-resistant isolate from a patient who had not travelled outside Norway appeared in 1994 and the percentage of multi-resistant *S.* Typhimurium isolates recovered from patients who had not travelled outside Norway increased from 14% (10/71) and 12% (9/77) in 1996 and 1997 respectively, to 23% (16/71) in 1998. In comparison, 36% (42/118) of the isolates acquired abroad in 1998 were multi-resistant.

Phage-types of multi-resistant isolates

Table 2 shows the phage-types of multi-resistant *S.* Typhimurium isolates in 1998, according to the place of acquisition of the strain. In 1998 a total of 71 isolates were acquired in Norway and 118 while travelling abroad. For 14 isolates travel information was not available. Thirty-one of 60 (52%) of the

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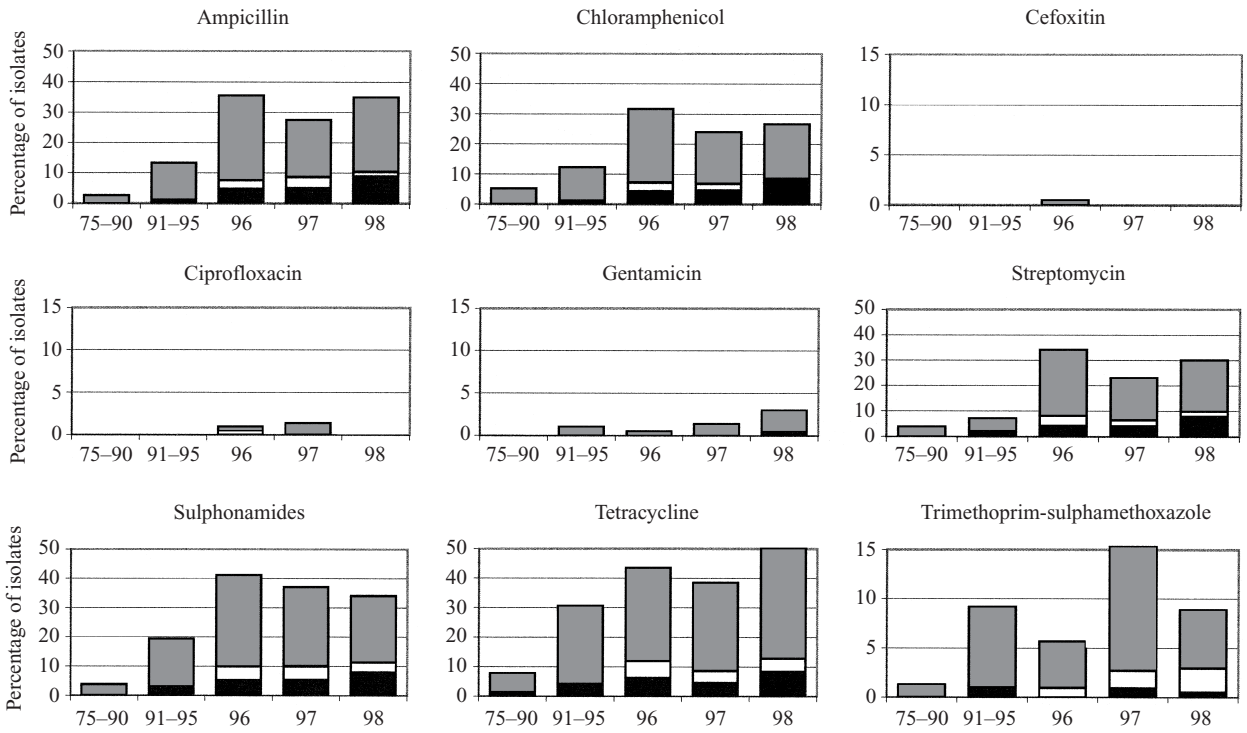

Fig. 1. Percentage of resistant *S*. Typhimurium isolates in Norway, 1975–98, with respect to place of acquisition of infection. ■, indicates acquisition in Norway; (▩), indicates acquisition abroad; □, indicates that it is unknown where the infection has been acquired.

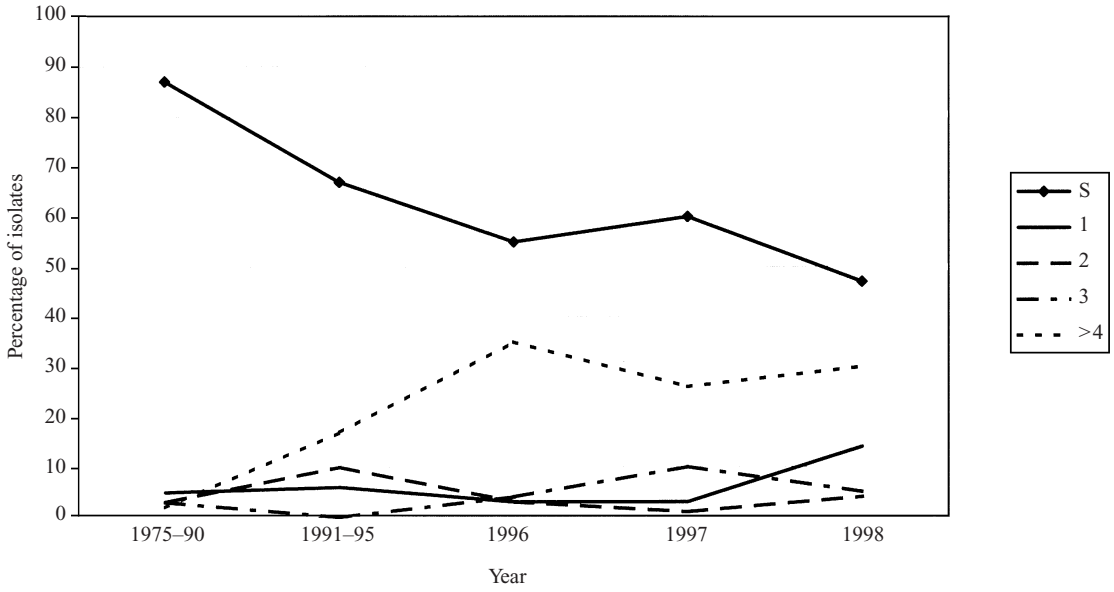

Fig. 2. The percentage of *S*. Typhimurium isolates fully susceptible to all antibiotics (S), and resistant to 1, 2, 3 or ⩾ 4 antibiotics from 1975 to 1998.

multi-resistant isolates were *S*. Typhimurium DT104. In patients who had acquired their infection in Norway, *S*. Typhimurium DT104 were responsible for 12 out of 71 (17%) of all *S*. Typhimurium infections, and 12 out of 16 (75%) of the multi-resistant isolates. In patients who had acquired their infection outside. Norway the corresponding figures were 18 out of 118 (15%) and 18 out of 42 (43%). All isolates identified as *S*. Typhimurium DT104 had the characteristic ACSSuT resistance pattern.

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Table 2. *Phage-types of 60 multi-resistant* S. *Typhimurium isolates from humans in 1998*

	No. isolates	Phage-type		
		DT104	Other	UT	ND*
Acquired in Norway	16	12	0	4	0
Acquired abroad	42	18	6	9	9
No travel information	2	1	0	0	1

* ND, not done; UT, untypable: no reaction with any of the 30 phages or the combination of phages did not correspond with any of the standard phage types; Other: 5 isolates – DT120, 1 isolate – DT12.

Table 3. *AFLP patterns of antibiotic resistant* S. *Typhimurium isolates grouped according to phage-type and antibiogram. In total 52 isolates from humans were analysed of which 30 were acquired in Norway and 22 abroad*

AFLP group	Antibiogram*	Phage-type†	No. of isolates acquired in Norway/abroad
I	ACSSuT	DT104	22/10
	ACSSuT	No reaction	2/0
	ACSSuT	DT120	1/2
	ACSSuT	DT12	0/1
	ACSu	DT120	1/0
II	ACSSuTSxt	No reaction	0/3
	ACSSuT	No reaction	1/0
	ACSSuT	DT120	0/1
III	ACCiGSSuTSxt	DT12	0/1
	ACSSuTSxt	No reaction	0/1
IV	ACSSuT	DT120	1/0
V	ACSSuT	DT120	1/0
VI	ACSSuT	Non-typable	0/1
VII	ACSSuTSxt	No reaction	0/1
VIII	ACGSSuT	DT120	0/1
IX	ACGSuTSxt	No reaction	1/0
X	—	ND	2/0 (controls)

* For abbreviations of antibiotics, see Table 1.
† DT, definitive type; No reaction, no reaction with any of the 30 phages; Non-typable, the combination of phages did not correspond with any of the standard phage types; ND, not done.

AFLP

Results of the AFLP analysis of selected multi-resistant isolates are shown in Table 3. Based on visual analysis we grouped the patterns and used Roman numerals, ranging from I to X, to differentiate these groups. Figure 3 illustrates the different patterns of the three most prevalent AFLP groups (I, II and III). Thirty-eight isolates with ACSSuT resistance had the same AFLP pattern (I), which was shared with one ACSu isolate. All 32 isolates assigned to *S.* Typhimurium DT104 had the same AFLP pattern (I), whereas the 11 isolates with ACSSuT resistance that were not DT104, had many different AFLP patterns (I, II, IV–VI). Different AFLP patterns were found for all isolates with resistance to more than five antibiotics (II–III, VII–IX), as well as for the fully susceptible control strains (X).

DISCUSSION

By selecting 10 isolates from patients with a history of foreign travel and 10 isolates from patients without a history of foreign travel, a substantial proportion of

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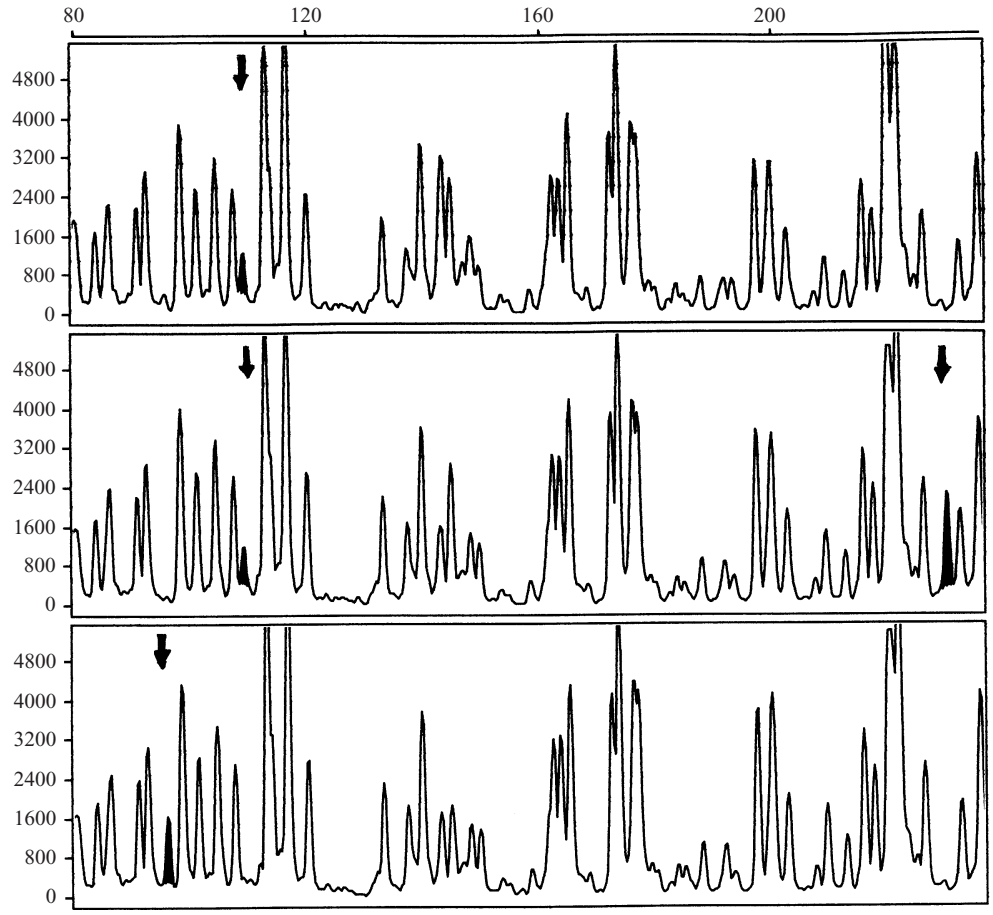

Fig. 3. A graphical presentation of the three most prevalent AFLP groups (I, II and III). The differences between the groups has been indicated by marking the characteristic fragments.

the isolates from the years between 1975 and 1995 has been investigated. This should at least be true for the domestically acquired isolates collected at the NSRL: about 200 human *S.* Typhimurium isolates were registered yearly during the investigated period, except in 1987 when a nationwide outbreak occurred [2]. A maximum of 20% of these isolates was acquired in Norway.

Before 1990 there were few resistant isolates compared to the later years. We therefore put together the isolates collected up to and including 1990 into one group and the isolates from 1991 through 1995 in another group to make the change in resistance easily visible. Also in other European countries increased antimicrobial resistance emerged in the same period. Possibly, the increase started a little earlier in Southern Europe. In Spain ampicillin resistance in *S.* Typhimurium from humans rose from 10 to 15% in 1986–8 to above 50% in 1989–91 [4], and in Germany the rate of fully susceptible isolates decreased from 73% in 1987 to 48% in 1996 [5]. In Norway the increase in antimicrobial resistance was first noted in isolates acquired abroad in 1991–2, but the largest increase in resistance among imported isolates was seen in 1996 when 35% of the isolates were multi-resistant. The increase in antimicrobial resistance in isolates acquired in Norway started shortly afterwards. The first domestically acquired multi-resistant isolate appeared in 1994, but already in 1998 23% of the isolates from patients without a history of travel abroad were multi-resistant.

In addition to human isolates, all salmonella isolates of non-human origin are referred to the NSRL. As only very few *S.* Typhimurium isolates of food or animal origin are referred to the NSRL per year, and antibiotic resistance in these isolates is very unusual, salmonella isolates from the food-chain were not included in this study. In 1998, there were 19 non-human *S.* Typhimurium isolates. Eight of the isolates were from the food-chain of which two were multi-resistant (ACSSuT- and ACSSuTSxt-resistance). The isolate with ACSSuT-resistance was from minced meat from Norway, and the isolate with ACSSuTSxt-resistance was from imported duck breast. Neither of

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the two were *S.* Typhimurium DT104. Previous investigations have documented that the prevalence of salmonella in the Norwegian food chain is very low [1].

The emergence of the multi-resistant *S.* Typhimurium DT104 in the last decade is of international concern [15, 25]. A multi-resistant pathogen from the food chain that spreads rapidly throughout the entire industrialized world is worrisome. The more food-handling is industrialized the more important it is to understand and stop the spread of pathogens like *S.* Typhimurium DT104. Our study shows that *S.* Typhimurium DT104 is transmitted within Norway too. Until recently it was assumed that this was not the case. As regular phage typing of multi-resistant isolates has only been done since 1998, it is difficult to tell when the actual increase in *S.* Typhimurium DT104 isolates in Norway began. It is also impossible from our examinations to tell when domestic transmission of *S.* Typhimurium DT104 first took place. But, due to the fact that no multi-resistance in domestically transmitted isolates was found before 1994, we assume that the multi-resistant clone of *S.* Typhimurium DT104 was introduced to Norway in 1994 or later.

In England and Wales, the incidence of ciprofloxacin resistance in *S.* Typhimurium DT104 increased from 0·6% in 1994 to 13% in 1997 [11]. In Norway it seems that ciprofloxacin resistance has not yet been established. As shown in a recent study, adding nalidixic acid to the test panel can discover early fluoroquinolone resistance [26]. In the NSRL a nalidixic acid tablet is nowadays included to the panel of antimicrobial drugs when resistance to chloramphenicol is found as has recently been recommended by an international expert group [27].

Various genetic typing techniques have been used to type *S.* Typhimurium isolates, especially DT104. None of these seems to have been able to give additional information as to how the epidemic strain DT104 spread, even though a certain heterogeneity has been found, mainly with analyses of plasmid profile and PFGE [6, 17, 28]. It has been suggested that the same multi-resistant clone has spread throughout both the human and the animal ecosystem [9]. However, recent findings based on multiple techniques showed that about 5% of *S.* Typhimurium DT104 isolates are genetically diverse, but that the majority is still part of the epidemically spreading multi-resistant clone [29]. Even though PFGE has been able to differentiate up to five different variants of *S.* Typhimurium DT104, this has not revealed any new information on the spread of this multi-resistant variant of *S.* Typhimurium [17]. We wanted to see if the AFLP technique could reveal how these isolates had spread and established themselves in Norway, but were unable to subdivide the *S.* Typhimurium DT104 isolates with this technique. Anyway, these findings support the theory that *S.* Typhimurium DT104 is a single clone that spreads epidemically. AFLP does discriminate well between the different phage-types, can be automated and hence has large potential in an outbreak situation. Possibly other combinations of restriction enzymes could yield better results, and give us a tool to differentiate between outbreak isolates and other isolates and help us understand the spread of DT104. Until then PFGE seems to give the best, although not very satisfactory results.

In conclusion, there was an increase in the number of domestically acquired multi-resistant human *S.* Typhimurium isolates in Norway in 1998. In imported isolates the increase in resistance was most noticeable in 1996 when 35% of the isolates were multi-resistant. Multi-resistant isolates, essentially *S.* Typhimurium DT104, are now being transmitted within Norway, but still the majority of the resistant and multi-resistant isolates are imported.

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