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Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium phage-type DT104 among salmonellae causing enteritis in Israel

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SUMMARY

The relative frequency of salmonella strains isolated from hospitalized and non-hospitalized patients in Southern Israel changed during the period, 1994–6. *Salmonella enterica* serotype Typhimurium definitive phage-type 104 (DT104) appeared in Israel in 1994 and became the most prevalent strain in 1996. An outbreak of enteritis due to *Salmonella enterica* serotype Agona occurred in Israel, in October 1994 and lasted for 4 months. The relative frequency of *Salmonella enterica* serotype Enteritidis remained almost constant during these years, with seasonal fluctuations only.

The importance of the increase in the prevalence of Typhimurium DT104 has been the epidemic spread of a multiresistant strain of R-type ACT (A, ampicillin; C, chloramphenicol; T, tetracycline) belonging to this phage-type. Since 1995 the frequency of Typhimurium DT104 isolates that possess, in addition to the above R-type, a chromosomally encoded resistance to the quinolone drug, nalidixic acid, increased tenfold. In 1996, 27% of the Typhimurium DT104 isolates were of R-type ACTN. *S.* Enteritidis exhibited over 95% susceptibility to at least eight of the most commonly used antibiotic drugs, and none of the isolates was resistant to quinolone or fluoroquinoline.

INTRODUCTION

Non-typhoidal salmonellosis outbreaks have become of increasing importance in many European countries [1–3], in Japan [4] and in the USA [5]. *Salmonella enteritica* serotype Enteritidis has become the most commonly reported serotype causing salmonelloses in humans throughout the world [6]. *Salmonella enterica* serotype Typhimurium definitive type 104 (DT104) is now the second most prevalent salmonella strain in human infections reported worldwide [7].

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A large number of reports are published annually documenting the acquisition of antibacterial resistance by previously susceptible bacterial species [8]. A multidrug-resistant Typhimurium DT104 strain of R-type ACSSuT (A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulphonamides; T, tetracycline) was reported in the UK [9]. Since 1992, an increasing rate of isolates has acquired additional resistance to trimethoprim (R-type ACSSuTTm). In 1995 a high rate of DT104 isolates, already multiresistant, carried additional chromosomally encoded resistance to ciprofloxacin [9].

A similar pattern of growing rate of resistance was reported in the USA [10]. The World Health Organ-

ization (WHO) aware of the acquisition and spread of resistance in pathogens has listed key organisms and key antibacterial drugs for surveillance. Among the combinations worthy of epidemiological studies in local laboratories, WHO includes salmonella with one of the following antibacterial drugs; ampicillin, chloramphenicol, trimethoprim, ceftriaxone and ciprofloxacin [8].

Salmonellosis is steadily rising in Israel and in 1996 the incidence rate was one laboratory-confirmed case per 1000 inhabitants per year [11]. Submission of salmonella isolates from peripheral laboratory to the Israeli Ministry of Health *Salmonella* Reference Laboratory for further characterization is mandatory but for different practical and economic reasons related to the activity of the peripheral laboratories only a part of the human isolates of salmonella finally reach the reference laboratory. An evaluation study carried out recently showed that in 1995, 57% of the salmonella strains isolated all over the country were transferred to the *Salmonella* Reference Laboratory [12]. The most prevalent serotypes isolated in Israel from humans, in 1996, were Typhimurium (28%), Enteritidis (22%) and Virchow (16%) [13].

The objective of this study was to examine the epidemiology of the most common salmonella strains that were isolated from hospitalized and non-hospitalized patients in Southern Israel, in 1994–6, and to follow the changes in the patterns of antibiotic susceptibility of the most prevalent strains, during this period, in accordance with the WHO recommendations.

MATERIALS AND METHODS

Bacterial isolates and epidemiological background

A total of 648, 400 and 407 salmonella were isolated in the microbiology laboratory of Soroka Hospital in Beer-Sheva in the years 1994, 1995 and the last 8 months of 1996, respectively. Only one isolate from each patient, as identified by first and second name, ID number, data and location of isolation, was counted.

Soroka laboratory serves a population of about 600000 inhabitants of Southern Israel. The population is very heterogenous and live in big and small urban centres, and in various agricultural communities (Kibbutzim and villages). Soroka Medical Centre serves as a regional hospital and provides laboratory services to community outpatient clinics in the area.

Salmonella spp. from stool samples of hospitalized and non-hospitalized patients was isolated using standard bacteriological procedures. The samples were inoculated on MacConkey agar directly and on Shigella–Salmonella agar, after overnight incubation in Selenite broth. Representatives of all non-lactose fermenting and/or H_2S producing colonies on MacConkey and Shigella–Salmonella agars were tested for salmonella by routine morphological, biochemical and serological testing. After being serogrouped at the microbiological laboratory of the Soroka Medical Centre, the isolates were sent to the Salmonella Reference Laboratories of the Israeli Ministry of Health for further characterization. All isolates that were not contaminated upon arrival at the reference laboratory were serotyped according to Kauffmann's scheme [14] while phage typing of Typhimurium and Enteritidis strains was carried out according to Sechter's scheme [15]. Phage type 2(4+) of Typhimurium according to Sechter's scheme is defined as phage type DT104 according to schemes employed by the German National Centre for Salmonella, Robert Koch Institute, the Laboratory of Enteric Pathogens of the British Central Public Health Laboratory and the Danish Statens Veterinaere Serumlaboratorium. This conclusion has been drawn after the above-mentioned Salmonella Reference Laboratories defined identically, as Typhimurium DT104, representative isolates identified in Israel as Typhimurium 2(4+).

Antimicrobial susceptibility

Salmonella isolates were tested *in vitro* for susceptibility to ampicillin (10 μg/disk) (A), ceftriaxone (30 μg/disk) (Cef), tetracycline (30 μg/disk) (T), gentamicin (10 μg/disk) (Gen), chloramphenicol (30 μg/disk) (C), resprim (sulphamethoxazole + trimethoprim 25 μg/disk) (R), nalidixic acid (30 μg/disk) (N) and ciprofloxacin (5 μg/disk) (Cip) or ofloxacin (5 μg/disk) (O) by the Kirby–Bauer disk diffusion method [16]. The tests were carried out on the Mueller–Hinton medium using Oxoid antimicrobial susceptibility disks (Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 0PW, England).

RESULTS

Epidemiology

Thirty different serotypes were identified among 606 independent salmonella isolates (only one isolate from one source) that were tested out of 648 organisms

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Table 1. *Main salmonella serotypes in Southern Israel,* 1994–6*

	1994		1995		1996†	
Serotypes	*n*	%	*n*	%	*n*	%
S. agona	194	32·0	42	12·6	1	0·3
S. blockley	29	4·8	11	3·3	7	2·2
S. enteritidis	125	20·6	78	23·4	78	22·7
S. hadar	17	2·8	38	11·4	27	7·9
S. infantis	19	3·1	29	8·7	1	0·3
S. typhimurium	23	3·8	48	14·4	140	40·8
S. virchow	147	24·3	34	10·2	58	16·9
Others	52	8·6	53	15·9	31	9·0
Total	606	100·0	333	100·0	343	100·0

* Isolates of salmonella from stool cultures.
† 1 May to 31 December 1996.

isolated in 1994. In 1995, 333 out of 400 independent salmonella isolates were tested and 26 serotypes were identified. In the last 8 months of 1996, 343 out of 407 salmonella isolates were defined and grouped into 24 serotypes.

The three dominant strains in 1994 were *Salmonella enterica* serotype Agona (32·0%), Virchow (24·3%) and Enteritidis (20·6%) (Table 1). An outbreak of salmonellosis due to Agona occurred in Israel toward the end of 1994 and lasted 4 months. The vehicle for the epidemic agent was a ready to eat savoury snack, the same food item associated with the international spread of Agona in 1994–5 [17, 18]. Enteritidis B3 was the dominant phage type, consisting of 69% of this serotype. One half of the Typhimurium isolates belonged to Typhimurium definitive-type 104 (DT104).

The prevalence of Agona declined significantly on February 1995 while the prevalence of Typhimurium in 1995 quadrupled compared to its 1994 rate (Table 1). This increase was attributed entirely to Typhimurium DT104 strain. Typhimurium was the most prevalent serotype (40·8%) among the 24 different serotypes identified during the last 8 months of 1996 (Table 1) with DT 104 being the dominant phage type (90%).

Antimicrobial susceptibility

The antibiotic susceptibility pattern of Enteritidis did not change in the years 1994–6. Over 95% of the isolates were completely susceptible when tested to ampicillin, tetracycline, gentamicin, chloramphenicol, resprim, and nalidixic acid. None of the Enteritidis isolates tested (125, 78, and 78 in 1994, 1995 and 1996, respectively) was resistant to cephalothin, ciprofloxacin or ofloxacin (data not shown).

Table 2. *R-types of* S. typhimurium *DT104 in Southern Israel 1994–6*

		% Isolates of R-type*				
Year	Number of isolates	ACT	ACTN	ACN	Other patterns	Drug-sensitive
1994	11	90·9	0	0	0	9·1
1995	39	82·0	2·6	2·6	12·8	0
1996	114	58·8	27·2	0·9	10·5	2·6

* A, ampicillin; C, chloramphenicol; T, tetracycline; N, nalidixic acid.

Typhimurium DT104 was resistant (90·9%) to ampicillin, chloramphenicol and tetracycline (R-type ACT) since its first appearance in Israel in 1994. In 1995, 2 out of 39 isolates (5·1%) exhibited resistance also to nalidixic acid, and in 1996, 31 out of 114 isolates examined (27·2%) were resistant to 4 out of 8 antibacterial drugs tested (R-type ACTN, Table 2). No resistance to ciprofloxacin was found among the nalidixic resistant strains isolated in Israel when tested by the disk diffusion method.

DISCUSSION

Non-typhoidal salmonellosis became a major cause of enteric infections in many countries. In the recent years there were many reports on rising incidence and severity of salmonellosis contracted mainly through the consumption of raw or undercooked contaminated food of animal origin [1, 20].

A retrospective study of all salmonella isolated from stool samples obtained at the Soroka Medical Centre over the period 1994–6 revealed a constant rise in the prevalence of Typhimurium since 1994 (4, 14 and 41% in 1994, 1995 and the last 8 months of 1996, respectively). A similar trend of increase in the prevalence rate of Typhimurium among all salmonella serotypes has been documented in whole Israel (4, 13 and 28% in 1994, 1995, and 1996, respectively) [13] and also reported from Europe [7] and the USA [5]. This increase in the relative frequency of Typhimurium, in Israel as in the other countries, has been attributed entirely to the emergence of Typhimurium DT104. Interestingly, according to the annual reports of the Salmonella Reference Laboratory of the Israeli

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Ministry of Health, the relative frequency of Typhimurium isolated from animal sources increased from 11% in 1994 to 15% in 1995 and 1996. DT104 represented 14, 40 and 44% of the isolates of Typhimurium from chickens in 1994, 1995 and 1996, respectively [13].

The relative frequency of Enteritidis fluctuated around 20% during this period. Following the end of the 1994 outbreak [17, 18], a sharp drop in the prevalence of Agona was noticed. The distribution of these serotypes in the southern part of Israel was similar to that of the entire country, as recorded by the Salmonella Reference Laboratory of the Israeli Ministry of Health [13].

In Italy, Enteritidis was the most common serotype of salmonella in 1991–4, accounting for 64·8% of the outbreaks [2]. In England and Wales Enteritidis in humans peaked in 1992 and was still the most common salmonella serotype in 1995. Since the beginning of the 1990s, Typhimurium DT104 emerged as the most significant serotype in England and Wales [7].

Typhimurium DT104 appeared in Israel in 1994 as a multi-resistant strain of R-type ACT. Within 2 years, resistance to nalidixic-acid developed and by the second half of 1996, 28·1% of DT104 isolates were already resistant to the quinolone.

A similar epidemic spread of a multidrug resistant Typhimurium DT104 occurred in the UK. Out of 3500 human isolates, 98·8% (90·1% in Israel) were multidrug resistant in 1995. A growing resistance to quinolone drugs has been observed in Typhimurium since 1994. In 1995, 3·9% of the humane isolates, that were already multiresistant, acquired resistance to nalidixic acid as well [21]. In Israel, 5·2% of Typhimurium DT104 exhibited resistance to nalidixic acid in 1995.

Threlfall and colleagues reported an increase in the rate of resistance (1% in 1994 to 6% in 1995) to ciprofloxacin (MIC 0·25 mg/l) [9]. No resistance to ciprofloxacin was found among the nalidixic resistant strains isolated in Israel when tested by the disk diffusion method [16]. Neither was fluoroquinolone resistance detected in the multiresistant Typhimurium DT104 isolates from humans in the USA [10].

Resistant mutants selected with any fluoroquinolone exhibit cross-resistance to the other quinolonic compounds at variable levels [22]. In Israel nalidixic acid resistant mutants, are still susceptible to ciprofloxacin, using a cut-off of 0·5 mg/l.

It is assumed that resistance of Typhimurium to the various antibiotics is acquired at the level of the animal reservoir (cattle, poultry, sheep and pigs) [23]. Identical or similar antibiotic compounds including fluroquinolones are used in veterinary and human medicine. In Israel, ciprofloxacin and ofloxacin are widely used in human medicine. A similar compound, enrofloxacin, has been approved for veterinarian use since 1993 and is currently employed by veterinarians for treatment of a broad range of infections occurring in cattle and poultry.

Fluoroquinolone resistant bacteria that are selected in animals can be transmitted to humans, as was suggested in the case of quinolone resistant campylobacter [24]. This phenomenon is specially worrisome in light of the findings that multistep selection of resistant strains is responsible for the emergence of high level resistance to fluoroquinolones [22].

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REFERENCES

1. Communicable Disease Surveillance Centre, Public Health Laboratory Service. Salmonella in humans. England and Wales: quarterly report. CDR 1994; **4**: 204–8.
2. Scuderi G, Fantasia M, Filetici E, Anastasio MP. Foodborne outbreaks caused by salmonella in Italy, 1991–4. Epidemiol Infect 1996; **116**: 257–65.
3. Liebisch B, Schwarz S. Molecular typing of *Salmonella enterica* subsp. enterica serovar Enteritidis isolates. J Med Microbiol 1996; **44**: 52–9.
4. Suzuki Y, Ishihara M, Matsumoto M, Arakawa S, Saito M, Ishikawa N, Yokochi T. Molecular epidemiology of *Salmonella enteritidis*. An outbreak and sporadic cases studied by means of pulsed-field gel electrophoresis. J Infect 1995; **31**: 211–7.
5. Hedberg CW, David MJ, White KE, MacDonald KC, Osterholm MT. Role of egg consumption in sporadic *Salmonella enteritidis* and *Salmonella typhimurium* in Minnesota. J Infect Dis 1993; **167**: 107–11.
6. Rodrigue DC, Tauxe RV, Rowe B. International increase in *Salmonella enteritidis*: a new pandemic? Epidemiol Infect 1990; **105**: 21–7.
7. World Health Organization. Multi-drug resistant *Salmonella typhimurium*. WHO Fact Sheet 1997; **139**: 1–4.
8. Williams JD. *Quo vadis* with bacterial resistance? Drugs 1995; **49** (suppl 2): 1–9.

9. Threlfall EJ, Frost JA, Ward LR, Rowe B. Increasing spectrum of resistance in multiresistant *Salmonella typhimurium*. Lancet 1996; **347**: 1953–4.
10. Centers for Disease Control and Prevention. Multidrug-resistant *Salmonella* serotype *typhimurium* – United States, 1996. MMWR 1997, 11 April.
11. Israel Ministry of Health. Department of Epidemiology. Weekly Epidemiological Record No. 52/1997.
12. Israel Ministry of Health. Israel Centre for Diseases Control. Evaluation of the laboratory-based surveillance of salmonellosis, shigellosis and campylobacteriosis in Israel. Publication no. 103, December 1996.
13. Israel Ministry of Health. Central Laboratories. Salmonella Reference Laboratory. Annual reports. 1994–1995 and 1996.
14. Kauffman F. The bacteriology of enterobacteriaceae. Baltimore: The Williams & Wilkins Company, 1966: 131.
15. Sechter I, Gerichter ChB. Phage typing of *Salmonella typhimurium* in Israel (1964–66). Annal L'Inst Pasteur **113**: 399–9.
16. Bauer AW, Kirby WWM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966; **45**: 493–6.
17. Shohat T, Green MS, Merom D et al. International epidemiological and microbiological study of outbreak of *Salmonella agona* infection from a ready to eat savoury snack. BMJ 1995; **313**: 1107–9.
18. Threlfall EJ, Hampton MD, Ward LR, Rowe B. Application of pulsed-field gel electrophoresis to an international outbreak of *Salmonella agona*. Emerging Infect Dis 1996; **2**: 130–2.
19. Cowden JM, Lynch D, Joseph CA et al. Case-control study of infections with *Salmonella enteritidis* phase type 4 in England. BMJ 1989; **299**: 771–3.
20. Centers for Diseases Control and Prevention. Outbreaks of *Salmonella* serotype Enteritidis infection association with consumption of raw shell eggs – United States, 1994–1995. MMWR 1996; **45**: 737–42.
21. MacConnell J. Risk of untreatable infection is growing. Lancet 1996; **347**: 1471.
22. Acar JF, Goldstein FW. Trends in bacterial resistance to fluoroquinolones. Clin Infect Dis 1997; **24** (Suppl 1): 567–73.
23. Threlfall EJ, Frost JA, Ward LR, Rowe B. Epidemic in cattle and humans of *Salmonella typhimurium* DT 104 with chromosomally integrated multiple drug resistance. Vet Rec 1995; **134**: 577.
24. Piddock LJV. Quinolone resistance and *Campylobacter* spp. J Antimicrob Chemother 1995; **36**: 891–8.