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Blood invasiveness of *Salmonella enterica* as a function of age and serotype

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SUMMARY

We explored the dual influence of the patient's age and the infecting serotype on the blood invasiveness patterns of non-Typhi *Salmonella enterica* (NTS). Blood invasiveness ratio (BIR) was calculated as the ratio between the number of blood and blood + stool isolates. Analysis of 14951 NTS isolates showed that the BIR increased drastically above the age of 60 years, reaching levels 3·5–7 times higher compared to age group <2 years. Different patterns of age-related invasiveness were observed for the five most common NTS serotypes (Enteritidis, Typhimurium, Virchow, Hadar, Infantis). Among children <2 years, the BIR was highest for serotype Virchow and lowest for serotype Hadar, while in persons $\geqslant 60$ years it was highest for serotypes Enteritidis and lowest for serotype Infantis. The tendency of NTS serotypes to invade the bloodstream was significantly influenced by the patient's age, however the impact of age differed for various NTS serotypes.

INTRODUCTION

Non-Typhi *Salmonella enterica* (NTS) is a major public health problem in industrialized countries [1–4]. The most common manifestation of NTS infection is gastroenteritis, which is usually a self-limiting and benign disease, but invasion beyond the gastrointestinal tract occurs in approximately 5% of patients with salmonellosis [5, 6]. The most common manifestation of invasive NTS is bacteraemia, followed by meningitis, osteomyelitis, endocarditis, arthritis, urinary-tract infection and pneumonia [5, 6].

Bacteraemia occurs in persons of all ages, but it is more common at the extremes of life. However, the implications and outcome of bacteraemia in children compared to adults are very different [7]. NTS bacteraemia in childhood usually occurs in previously healthy young children, it appears in the context of gastroenteritis, and has a favourable outcome. In adults, NTS bacteraemia tends to affect patients with one or more predisposing conditions, especially those with impaired cellular immunity, is not always preceded by gastroenteritis, and carries a high mortality rate, up to 33–40% [7–9].

The ratio of blood isolates to the total number of isolates for a specific *Salmonella* serotype is a marker of the blood invasion potential. For example, a high proportion of blood to total isolates has been described for serotype Typhi (31 blood per 100 total isolates) and Paratyphi A (59 per 100), as well as several NTS serotypes, including Cholerasuis (62–74 per 100) and

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Dublin (25–40 per 100) [10, 11]. Yet, the majority of NTS serotypes have a much lower proportion, usually not exceeding 5 blood per 100 total isolates [10, 11]. In the following study we explored the dual influence of the patient's age and the infecting serotype on the blood invasiveness of NTS.

METHOD

Data source

Salmonella isolates from all human sources in Israel are passively submitted to a single laboratory, the Government Central Laboratories, Public Health Services, Israel Ministry of Health, Jerusalem, where serological identification is performed according to the Kauffmann–White scheme [12]. NTS isolates from human blood and stool sources received at the Government Central Laboratories from 1 January 1995 to 31 December 1999 were the basis for the present study. Information on the source of isolation was required for all analyses, and the patients' age – for age-specific analyses. For every patient, isolates of the same serotype and from the same source were counted only once in the same year. Data on the breakdown of the Israeli population by age group were retrieved from the Israeli Central Bureau of Statistics [13]. We defined 'annual isolation rate' as the number of NTS isolates submitted to the Government Central Laboratories per 100 000, based on the pertinent census of the Israeli Central Bureau of Statistics. 'Blood invasiveness ratio' (BIR) was defined as the number of submitted blood isolates divided by the number of stool + blood isolates of the same serotype, and expressed per 100 blood + stool isolates. The χ^2 test was used to compare BIRs within and between age groups and $P \leqslant 0{\cdot}05$ was considered significant.

RESULTS

Between 1 January 1995 and 31 December 1999, 22 738 human NTS isolates from blood or stool were submitted to the National Laboratory. The average annual isolation rate was 76·2 per 100 000 persons for blood + stool isolates, and 2·0 per 100 000 for blood isolates alone. The serotype was defined for 21 165 isolates (93·1%), and remained unknown for 1573 (6·1%). Ninety-six different serotypes were responsible for salmonellosis-related illnesses during the study years. The most prevalent NTS serotypes were Enteritidis (22·9%), Typhimurium (20·1%), Hadar (12·9%), Virchow (12·2%) and Infantis (6·9%), which accounted for 75·1% of all submitted human stool + blood isolates.

Table 1. *Distribution and BIR of 14 951 non-Typhi* Salmonella enterica *serotypes, Israel 1995–1999*

Serotype	No. blood isolates (%)	No. blood + stool isolates (%)	BIR*
Enteritidis	116 (35·7)	3545 (23·7)	3·27
Typhimurium	63 (19·4)	2994 (20·0)	2·10
Hadar	19 (5·8)	2011 (13·5)	0·94
Virchow	54 (6·6)	1858 (12·4)	2·91
Infantis	21 (6·5)	1100 (7·4)	1·91
Agona	2 (0·6)	494 (3·3)	0·40
Blockley	2 (0·6)	385 (2·6)	0·52
Bredeney	12 (3·7)	369 (2·5)	3·25
9,12:l,v:-	8 (2·5)	236 (1·6)	3·39
Tenessee	1 (0·3)	153 (1·0)	0·65
Newport	3 (0·9)	118 (0·8)	2·54
Montevideo	0	108 (0·7)	0
Haifa	3 (0·9)	103 (0·7)	2·91
Emek	0	95 (0·6)	0
Anatum	0	94 (0·6)	0
Eastbourne	4 (1·2)	91 (0·6)	4·40
Saintpaul	2 (0·6)	83 (0·6)	2·41
Heidelberg	1 (0·3)	80 (0·5)	1·25
Kottbus	1 (0·3)	77 (0·5)	1·30
Senftenberg	0	72 (0·5)	0
Other	13 (4·0)	885 (5·9)	1·47
Total	325 (100)	14 951 (100)	2·17

* Blood invasiveness ratio = blood/100 blood + stool isolates.

The patients' age was known for 14 951 (65·8%) isolates, and this proportion did not differ significantly between the various serotypes, except for Agona (39·2%). The distribution of blood and stool isolates and the invasiveness ratio of the 20 most common submitted NTS serotypes for patients with known age are presented in Table 1. Only five isolates of serotype Dublin (four from stool and one from blood) were submitted during the study years, and none of serotype Cholerasuis. Overall, the invasiveness ratio of the most common Israeli serotypes was below 5, with an average of 2·2.

The average annual incidence of the submitted blood + stool and blood isolates was greatly influenced by age (Fig. 1). The highest incidence of blood and stool isolation was found in the age group <2 years (493·9 per 100 000), and decreased dramatically with age. The incidence of blood isolation had a

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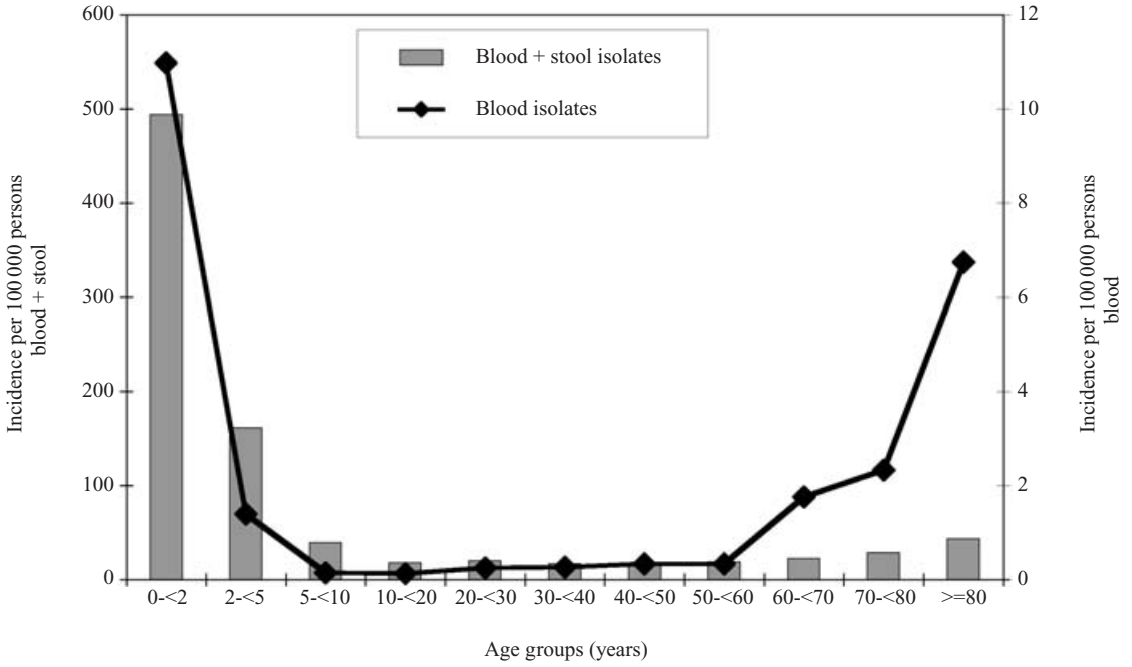

Fig. 1. Age-specific isolation rates of NTS from blood and stool, Israel 1995–1999.

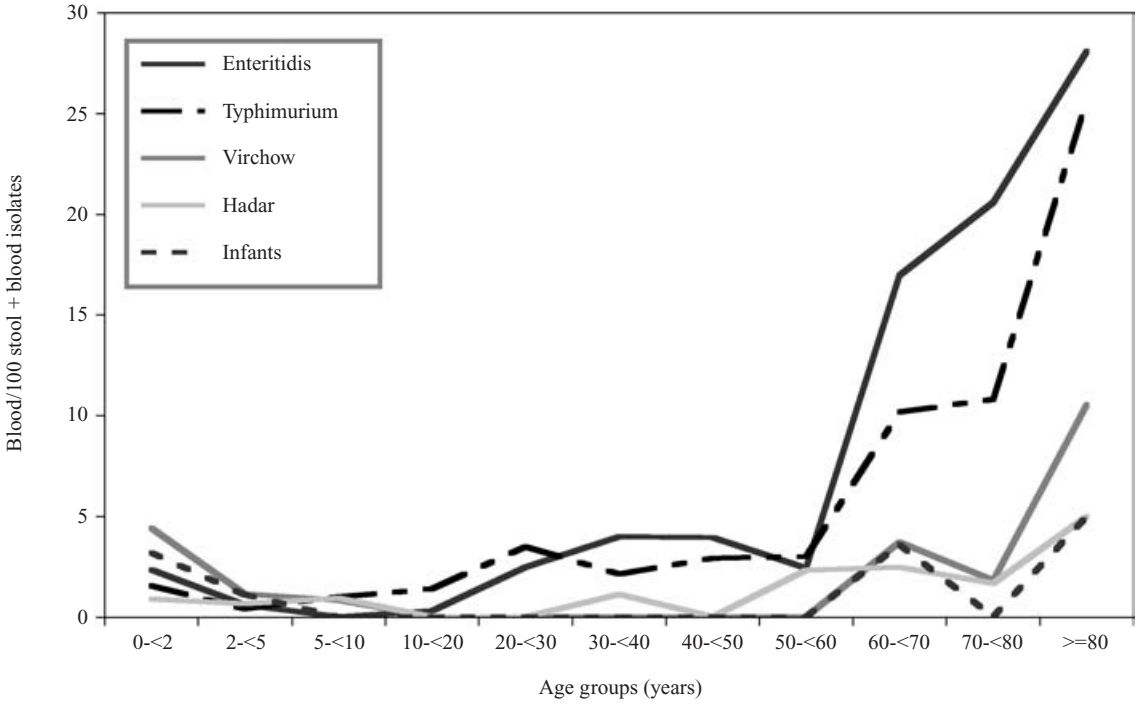

Fig. 2. BIR of NTS isolates by age and serotype.

bimodal pattern: a high incidence was observed in the age group <2 years (11 per 100 000) and again in the age group $\geqslant 80$ years (7 per 100 000). The BIR was also markedly influenced by age (Fig. 2). It increased drastically above the age of 60 years, reaching levels of 3·5–7 times higher (7·7–15·5 per 100 000) compared to age group <2 years (2·2 per 100 000). This trend was statistically significant ($P<0{\cdot}0001$). However, the pattern of change with age was not the same for all serotypes. Among the five most prominent serotypes

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Table 2. *BIR of non-Typhi* Salmonella enterica *isolates as a function of age and serotype*

Serotype	No. blood isolates	No. blood + stool isolates	BIR*	*P* value
Age group <2 years				
Enteritidis	21	895	2·35	
Typhimurium	21	1373	1·53	
Virchow	43	975	4·41	<0·001
Hadar	8	897	0·89	
Infantis	17	533	3·19	
Age group 2 to <60 years				
Enteritidis	30	2347	1·28	
Typhimurium	18	1449	1·24	
Virchow	4	736	0·54	0·095
Hadar	6	933	0·64	
Infantis	2	487	0·41	
Age group ⩾60 years				
Enteritidis	65	303	21·45	
Typhimurium	24	172	13·95	
Virchow	7	147	4·76	<0·001
Hadar	5	181	2·76	
Infantis	2	80	2·50	

* Blood invasiveness ratio = blood/100 blood + stool isolates.

(Fig. 2), Enteritidis and Typhimurium revealed a more pronounced increase with age, while serotypes Hadar and Infantis showed much smaller changes. At the other extreme of life, among children 2 years or younger, all serotypes had a low BIR compared to the elderly.

The BIR of the five prominent serotypes was further compared between three major age groups, age group <2, 2 to <60 and ⩾60 years (Table 2). In the group of children <2 years, the BIR was highest for serotype Virchow (4·4 blood per 100 blood + stool isolates) and lowest for serotype Hadar (0·9 per 100), ($P<0·001$). In the age group 2 to <60 years, the BIR was low for all serotypes, and the differences were not statistically significant. In the oldest group the highest ratio was observed for serotypes Enteritidis (21·5) and Typhimurium (14·0), and the lowest for serotypes Hadar (2·8) and Infantis (2·5). These differences were statistically significant ($P<0·001$).

DISCUSSION

We have shown that the patterns of blood invasiveness by NTS are dynamic and are determined by both the patients' age and the unique properties of the individual serotypes. Various serotypes had different patterns at the two extremes of life. Serotypes Enteritidis and Typhimurium had a low BIR in young children but were more than ten times higher in patients of 60 years or older. Serotypes Hadar and Infantis had a low BIR in childhood, and only a small increase in the elderly. Serotype Virchow had a different pattern: it was the serotype with the highest BIR in early childhood, and showed moderate increase with age.

Blaser and Feldman have also reported different patterns of age-related BIRs for several NTS serotypes submitted to the Centers for Diseases Control and Prevention in 1968–79 [10]. These included serotypes Cholerasuis, Montevideo and Newport, which all showed an increase in BIR as a function of age. These serotypes, however, are responsible for only a small proportion of NTS infection in Israel (Table 1 [14]) as well as the United States, Canada and many European countries [15, 16].

Our results add another angle to our understanding of the different characteristics of NTS bacteraemia in the extremes of life. In early childhood the high incidence of bacteraemia can mostly be explained by the high incidence of gastroenteritis, since at this age faecal isolation is high and the invasiveness ratio of the common NTS serotypes is low. In contrast, for the older age groups, the increase in the incidence of bacteraemia is mostly due to the increase in BIR of the most prevalent serotypes (Figs 1 and 2).

The impact of age on blood invasiveness was also reflected by the relative frequency of NTS blood isolates among the various age groups (Table 2). While serotype Enteritidis was the most frequent blood isolate among most age groups, and particularly in persons of 60 years or older, serotype Virchow predominated in children younger than 2 years. This finding concurs with our previous report from a single institute in Israel [7] and with a recent report from Southern Israel [17]. The predominance of serotype Virchow as a cause of bacteraemia and extraintestinal infection among children was also reported from the United Kingdom [18], and Australia [19]. In addition, invasive disease due to this serotype in children and infants was reported from Spain [20, 21] and Greece [22]. However, serotype Virchow was absent in a series of paediatric NTS bacteraemia from other parts of the world, including the United States [23], Taiwan [8], Thailand [24] or Malaysia [25]. These geographic differences are probably due to the interplay between

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the relative frequencies of NTS serotypes and their respective blood-invasive potential.

The tendency of serotype Virchow to invade the bloodstream and to cause systemic disease in previously healthy children and infants is of concern. Except for bacteraemia, the other most common extraintestinal manifestation with this serotype is meningitis, which tends to occur in very young infants [19–21, 26]. Other complications were rarely described, and these include septic arthritis, sacroiliitis, osteomyelitis, prevertebral abscess, cholecystitis and pericarditis [22, 27–29]. Fatality among children was only rarely reported [27]. Another source for concern is the increasing resistance of serotype Virchow to multiple antibiotic agents [30, 31].

The main limitation of our study is that it was based on passive submission of *Salmonella* isolates to the Government Central Laboratories. It has been estimated that the submission rate is only 57% [32], and this can result in an underestimate of the annual isolation rate. Another potential bias is the possibility of increased submission of blood isolates, but this would be true for all age groups. Moreover, blood to stool isolation ratios in our study were similar to those previously reported [10, 11]. Incomplete information of patients' age is another limitation of our study and may have contributed to lower age-specific isolation rates. The proportion of missing age data was similar for all five major NTS serotypes (average, 32·6%), thus comparisons between the various serotypes should be valid.

We believe that the fan-like pattern of the serotype-specific invasiveness ratio at the extremes of life (Fig. 2), strongly support a true interaction between the host (age) and the parasite (serotype) factors.

The differences in the blood-invasive potential of the individual NTS serotypes can be only partially explained by the presence of virulence plasmids, since they were not found in all serotypes, and could not explain all cases of bacteraemia [33, 34]. Indeed they could not be demonstrated in serotype Virchow [33, 34], which is highly invasive in children. The reasons for the age-related differences in the blood invasiveness and distribution of the NTS serotypes are even less understood. Differences in food consumption as well as susceptibility of the host may play important roles [6]. Future research should focus on the factors responsible for these variations, in order to establish better policies to contain the NTS epidemic and reduce the heavy burden on the paediatric population.

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REFERENCES

1. Tauxe RV. Foodborne illnesses. Strategies for surveillance and prevention. Lancet 1998; **352** (Suppl 4): SIV10.
2. Tauxe RV. *Salmonella enteritidis* and *Salmonella typhimurium*: successful subtypes in the modern world. In: Scheld WM, Craig WA, Hughes JM, eds. Emerging infection 3. Washington, DC: ASM Press, 1999: 37–52.
3. Slutker L, Alterkruse SF, Swerdlow DL. Foodborne diseases. Emerging pathogens and trends. Infect Dis Clin North Am 1998; **12**: 199–216.
4. Fierer J, Krause M, Tauxe R, Guiney D. *Salmonella typhimurium* bacteremia: association with the virulence plasmid. J Infect Dis 1992; **166**: 639–642.
5. Fierer J, Swancutt M. Non-typhoid *Salmonella*: a review. Curr Clin Top Infect Dis 2000; **20**: 134–157.
6. Miller SI, Pegues DA. *Salmonella* species, including *Salmonella typhi*. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett's principles and practice of infectious diseases, 5th ed. Philadelphia: Churchill Livingstone, 2000: 2344–2360.
7. Shimoni Z, Pitlik S, Leibovici L, et al. Non-typhoid *Salmonella* bacteremia: age related differences in clinical presentation, bacteriology and outcome. Clin Infect Dis 1999; **28**: 822–827.
8. Lee S-C, Yang P-H, Shien W-B, Lasserre R. Bacteremia due to non-typhi *Salmonella*: analysis of 64 cases and review. Clin Infect Dis 1994; **19**: 693–696.
9. Ramos JM, Garcia-Corbeira P, Aguado JM, Arjona R, Ales JM, Soriano F. Clinical significance of primary vs. secondary bacteremia due to nontyphoid *Salmonella* in patients without AIDS. Clin Infect Dis 1994; **19**: 777–780.
10. Blaser MJ, Feldman RA. *Salmonella* bacteremia: reports to the Center for Disease Control, 1968–1979. J Infect Dis 1981; **143**: 743–746.
11. Threlfall EJ, Hall ML, Rowe B. *Salmonella* bacteraemia in England and Wales, 1981–1990. J Clin Pathol 1992; **45**: 34–36.
12. Kauffmann F. Classification of bacteria. A realistic scheme with special reference to the classification of *Salmonella* and *Escherichia* species. Copenhagen: Munksgaard, 1975.
13. Statistical Abstracts of Israel. Publications of the Central Bureau of Statistics (no. 51). 2000.
14. Publication of the Central Laboratories NSC, Israeli Ministry of Health, Jerusalem, Israel. *Salmonella* 1997–2000. December 2001.
15. Olsen SJ, Bishop R, Brenner FW, et al. The changing epidemiology of *Salmonella*: trends in serotypes isolated from humans in the United States, 1987–1997. J Infect Dis 2001; **183**: 753–761.

16. Gill N, Reilly B, Smith H. Enter-net quarterly Salmonella report 01/1-4. International surveillance network for the enteric infections – *Salmonella* and VTEC O157. 2001.
17. Yagupsky P, Maimon N, Dagan R. Increasing incidence of nontyphi *Salmonella* bacteremia among children living in southern Israel. Int J Infect Dis 2002; **6**: 94–97.
18. Ispahani P, Slack RC. Enteric fever and other extraintestinal salmonellosis in University Hospital, Nottingham, UK, between 1980 and 1997. Eur J Clin Microbiol Infect Dis 2000; **19**: 679–687.
19. Messer RD, Warnock TH, Heazlewood RJ, Hanna JN. *Salmonella* meningitis in children in far north Queensland. J Paediatr Child Health 1997; **33**: 535–538.
20. Usera MA, Echeita A, Aladuena A, et al. Interregional foodborne salmonellosis outbreak due to powdered infant formula contaminated with lactose-fermenting *Salmonella virchow*. Eur J Epidemiol 1996; **12**: 377–381.
21. Ruiz J, Nunez ML, Sempere MA, Diaz J, Gomez J. Systemic infections in three infants due to lactose-fermenting strains of *Salmonella virchow*. Eur J Clin Microbiol Infect Dis 1995; **14**: 454–456.
22. Bitsori M, Galanakis E, Maraki S, Raissaki M, Velivassakis E, Sbyrakis S. Invasive *Salmonella virchow* infection in childhood. Scand J Infect Dis 2001; **33**: 862–865.
23. Zaidi E, Bachur R, Harper M. Non-typhi *Salmonella* bacteremia in children. Pediatr Infect Dis J 1999; **18**: 1073–1077.
24. Sirinavin S, Chiemchanya S, Vorachit M. Systemic nontyphoidal *Salmonella* infection in normal infants in Thailand. Pediatr Infect Dis J 2001; **20**: 581–587.
25. Lee WS, Puthucheary SD, Boey CC. Non-typhoid *Salmonella* gastroenteritis. J Paediatr Child Health 1998; **34**: 387–390.
26. Ashdown LR, Ryan PJ. Invasive disease due to *Salmonella virchow*: a north Queensland problem. Med J Aust 1990; **153**: 330–335.
27. Neuwirth C, François C, Laurent N, Pechinot A. Myocarditis due to *Salmonella virchow* and sudden infant death. Lancet 1999; **354**: 1004.
28. Genizi J, Miron D, Colodner R, Lumilsky D, Bykov S, Horowitz Y. [*Salmonella virchow* sacroiliitis and bacteremia in a child]. Harefuah 2002; **141**: 685–688.
29. Beiler HA, Kuntz C, Eckstein TM, Daum R. Cholecystolithiasis and infection of the biliary tract with *Salmonella* Virchow – a very rare case in early childhood. Eur J Pediatr Surg 1995; **5**: 369–371.
30. Martin MC, Gonzalez H, Alvarez-Riesgo JA, Mendoza MC. *Salmonella* serotype Virchow causing salmonellosis in a Spanish region. Characterization and survey of clones by DNA fingerprinting, phage typing and antimicrobial resistance. Eur J Epidemiol 2001; **17**: 31–40.
31. Threlfall EJ. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. FEMS Microbiol Rev 2002; **26**: 141–148.
32. Survey of the monitoring system for enteric infections in Israel. Publication of the Israeli Central for Disease Control, Israel Ministry of Health, Petach Tikva, December 1996 (Publication no. 103).
33. Chiu CH, Lin TY, Ou JT. Prevalence of the virulence plasmids of nontyphoid *Salmonella* in the serovars isolated from humans and their association with bacteremia. Microbiol Immunol 1999; **43**: 899–903.
34. Guiney DG, Fang FC, Krause M, Libby S, Buchmeier NA, Fierer J. Biology and clinical significance of virulence plasmids in *Salmonella* serovars. Clin Infect Dis 1995; **21** (Suppl 2): S146–S151.