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Variability of *Shigella flexneri* serotypes in Israel during a period of two years: 2000 and 2001

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SUMMARY

During a period of 2 years (2000 and 2001) 996 *Shigella flexneri* strains from sporadic cases in Israel were sent to the National Shigella Reference Centre (NSRC) by hospital and outpatient clinics. The most common serotypes were 2a, 6 and 1b, accounting for 88·4% of all isolates. They were investigated according to the monthly distribution of the strains, and the age and sex of the patients. The severity of the disease was assessed by a hospital/outpatient distribution (H/Od) of the isolates, based on the location of the sending laboratory. The most affected age groups were 0–11 months and 1–4 years, and the prevalent serotype was 2a, while serotype 6 was dominant in the 5–14 years age group. More cases were registered during the hot season, and there were some serotype-related variations. Overall, 62·1% of the samples were from male patients. Serotype 1b was dominant in the male/female ratio, although it was third in general prevalence. According to the H/Od serotype 2a was more common in hospitalized males and serotype 6 in outpatients, both male and female. These variations, as well as changes in serotype prevalence in the past, underscore the importance of serotype monitoring as part of the public health strategies for reducing the burden of *Shigella flexneri* infections.

INTRODUCTION

Shigella flexneri has been described as the first or the second most common cause of shigellosis depending on geography and socioeconomic factors [1]. It may be as significant for the overall burden of shigellosis as *S. sonnei*, considering the clinical picture of the disease which can be severe with complications and even death, as well as the fact that the real number of cases is usually higher than reported [1–3]. *S. flexneri* is also the prevalent endemic group [1, 4] and vaccine development has been a priority for a number of countries [5–7]. However, the protective immunity, whether natural or induced, is directed primarily against the *Shigella* O antigen and depends upon a diverse antigenic structure, resulting in 15 serotypes [8, 9]. There is also a potential risk of repeated infections with different serotypes [1].

In Israel *S. flexneri* is identified in 8–10% of all cases of shigellosis. Although well behind *S. sonnei*, which with over 80% is the main cause, the endemicity of *S. flexneri* infection is still an important factor in the overall shigellosis morbidity in the country [4, 10–12].

The aim of this study was to investigate in detail the serotype variations of *S. flexneri* strains in an attempt to develop a more focused approach in planning public health strategies as well as addressing the growing problem of antimicrobial resistance.

MATERIALS AND METHODS

Bacterial strains

The *Shigella* strains were isolated by hospitals and outpatient laboratories in Israel during a period of

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2 years – 2000 and 2001. They were identified as belonging to the *S. flexneri* subgroup and sent to the National Shigella Reference Centre (NSRC) in compliance with existing public health regulations [11, 13]. All samples were from sporadic cases. No outbreak of *S. flexneri* infection was reported during the period under review, which coincides with the resumption of testing for antimicrobial resistance of all *S. flexneri* isolates sent to the NSRC.

Biochemical identification

At the sending laboratories, both hospital and outpatient, the faecal samples and/or rectal swabs were processed using standard methods such as growth on selective media (MacConkey–sorbitol agar, *Salmonella–Shigella* agar and selenite broth) followed by inoculation of suspicious colonies into triple sugar iron agar slants. Biochemical identification in the smaller establishments was performed with the Entero-Plus bi-phasic reagent (Novamed, Jerusalem, Israel) and in the bigger facilities – with the Gram-negative identification cards of the API (bioMérieux SA, Marcy l'Etoile, France) and VITEK (bioMérieux) identification systems.

At the NSRC the biochemical identification was verified using standard methods [8, 14]. The results of the sending laboratories were confirmed in 98 % of the cases.

Serological identification

At the sending laboratories the strains were checked by the slide agglutination method [15] with *S. flexneri* polyvalent B antisera (Denka Seiken, Tokyo, Japan; Murex-Remel, Lenexa, KS, USA; or Bio-Rad, Hercules, CA, USA). At the NSRC a complete set of *S. flexneri* type and group antisera (Denka Seiken) was used, allowing for the identification of 13 serotypes [16].

Statistical analysis

Sex and age distributions of the isolates in the *S. flexneri* subgroup and the prevalent serotypes, as well as hospital/outpatient correlations with sex, age and serotype were examined by the χ^2 test. Two-tailed tests were applied. Trends in serotype prevalence and related hospital/outpatient sources were investigated by Pearson's correlation coefficient. Demographic information about the size of the age groups and the male/female distribution as regards the general population was obtained from the Israeli Central Bureau of Statistics [17].

Table. S. flexneri *serotypes during the period 2000–2001*

Serotype	No. of isolates	%
2a	377	37·8
6	299	30
1b	205	20·6
2b	33	3·3
4a	26	2·6
3b	26	2·6
3a	17	1·7
1a	5	0·5
Y	5	0·5
5a	1	0·1
4b	1	0·1
X	1	0·1
Total	996	100

RESULTS

The study includes 996 samples belonging to the *S. flexneri* subgroup, which were confirmed and identified serologically. The information about the patients was received on standard formats, which were checked for completeness and accuracy. Overall the most common serotype was 2a with 37·8 %, followed by serotypes 6 and 1b. The list of all isolated serotypes is presented in the Table.

Seasonality

With no significant difference between the two years, the monthly distributions for the *S. flexneri* group and prevalent serotypes were calculated on the basis of average figures for the whole period (Fig. 1). The higher isolation rates for the *S. flexneri* group were registered between May and October with a peak in August. Serotypes 6 and 1b followed the same trend although there were significant elevations in September ($r = 0{\cdot}75$) and May ($r = 0{\cdot}70$). Serotype 2a peaked in July with a high isolation rate in October and even in December ($r = 0{\cdot}78$).

Age

The affected age groups were selected according to a strata-based division of the world population, which includes different levels of economic development [1]. Precise information about age was obtained for

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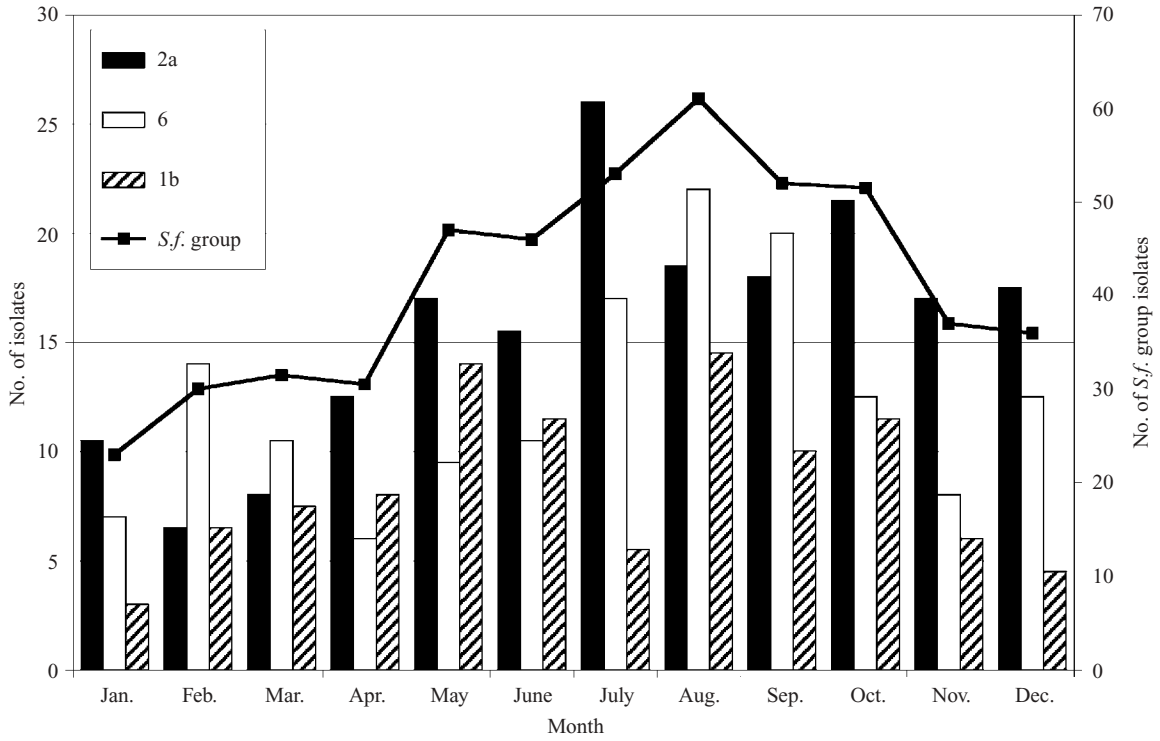

Fig. 1. Monthly distribution of the *S. flexneri* (*S.f.*) serotypes (averages 2000–2001).

95% of the samples. According to the incidence rate, serotype 2a was the most common strain, except for serotype 6 in the 5–14 years age group ($P<0{\cdot}001$). The serotype distribution in the age groups repeated and amplified the incidence patterns, especially the replacement of serotype 6 by serotype 1b in the older age groups.

Sex

In general, 62·1% of the samples in the *S. flexneri* subgroup were from male patients, with similar proportions in the serotype divisions ($P<0{\cdot}001$). Most of the isolates of both males and females came from the 0–11 months and 1–4 years age groups where the prevalent serotype was 2a. The sex distribution in relation to age and serotype showed a pattern similar to the incidence distribution, but here the emergence of serotype 1b, which was second in prevalence in the elderly age group was linked to males (Fig. 2, $P<0{\cdot}001$). This was further emphasized by the male/female ratio in all age groups and especially in the >60 years age group ($P<0{\cdot}001$).

Hospital/Outpatient distribution (H/Od)

This study is based entirely on routine testing at the NSRC and information about the cohorts can only be obtained from the standard formats accompanying the incoming isolates. Since duration of hospitalization, symptoms or other clinical indications are not included, one way to extrapolate the laboratory data on the severity of the disease is to divide the samples according to the location of the sending laboratory. Overall, outpatient laboratories sent 62·4% of the isolates. The H/Od for the *S. flexneri* subgroup was repeated in the serotype divisions with a higher hospitalization rate in the age groups with a greater general susceptibility of the cohorts (Fig. 3, $P<0{\cdot}01$).

The introduction of sex and serotype resulted in a significant H/Od only for the samples from males ($P<0{\cdot}05$), while an additional correlation with age had no effect.

DISCUSSION

Most of the *S. flexneri* isolates in Israel belong to three serotypes. During the period under review 88·4% of all samples were identified as serotypes 2a, 6 and 1b (Table). In order of prevalence serotype 6 was the most common strain in 2000 and serotype 2a in 2001.

Seasonality

Presented as monthly distributions of the samples, seasonality reflects the climatic conditions in the

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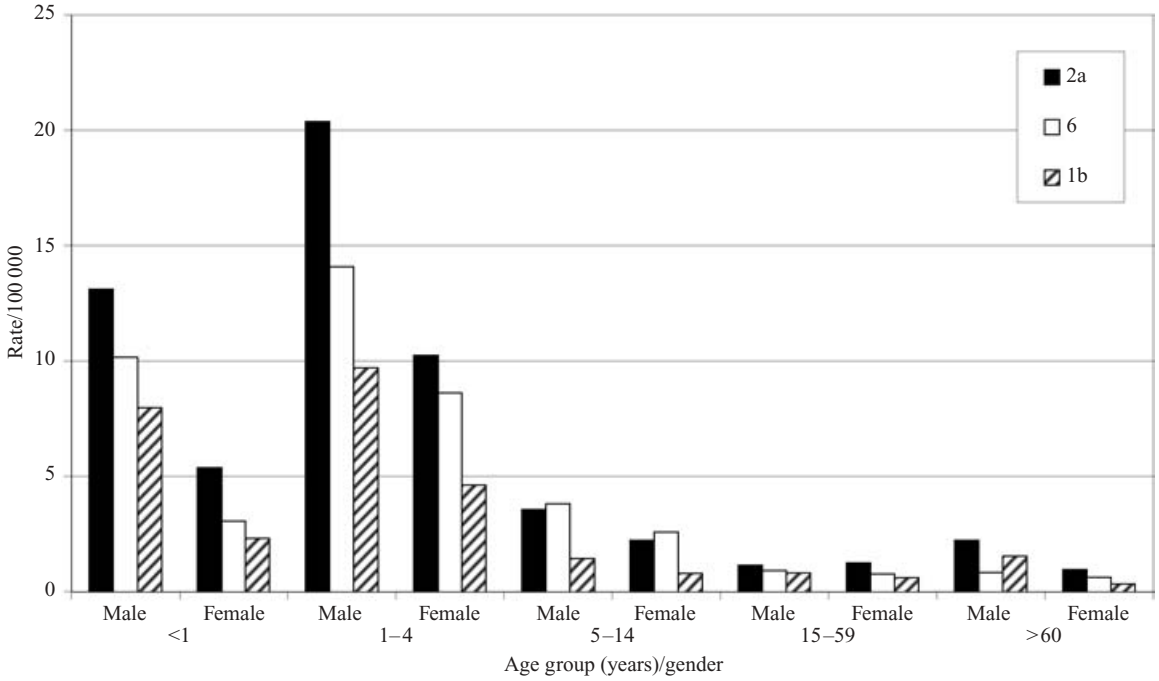

Fig. 2. Age and gender distribution of the prevalent serotypes.

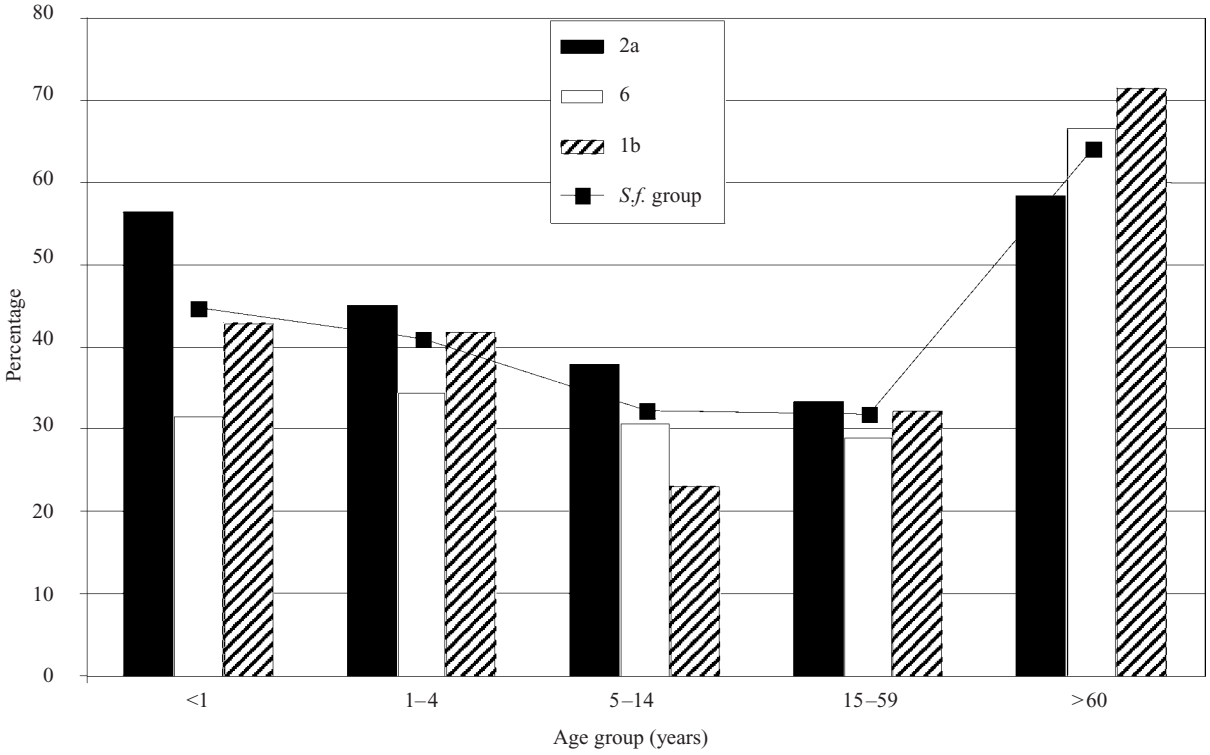

Fig. 3. Hospitalization ratio of the *S. flexneri* (*S.f.*) serotypes.

country, which generally form two seasons – one hot and dry beginning in May and the other a cooler, rainy period starting in November. There have been attempts to define 'winter' and 'summer' or even focus on specific months [11, 18, 19]. However, the monthly trends of serotypes 6 and 1b with significant increases in August, September and May, and of serotype 2a, which peaks in July with elevations in

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October and even December, indicate that, as in other endemic areas, *S. flexneri* shigellosis is an all-year-round disease [6]. On the whole, higher temperatures and humidity coincide with higher morbidity but focusing on a definite time period can be misleading. Overall, serotype 2a was dominant during 8 months and serotype 6 during 4 months. For both serotypes the period of prevalence is evenly divided between the two seasons (Fig. 1).

In previous studies of age-related incidence rates in Israel the highest incidence was registered in the 1–4 years age group although many of the reported cases were not defined by age [4, 11]. This finding is supported by the present study with the addition of the 0–11 months age group (Fig. 2). Thus the age groups, which include 10% of the population, account for almost half of all cases (47%). Reports from other endemic regions also indicate that most cases of shigellosis occur in children between 6 months and 3 years, which links incidence to the time of weaning [6].

In this study the most common serotype is 2a, especially in the >60 years age group. Serotype 6 is prevalent in the kindergarten/early school age group, and is gradually replaced by serotype 1b in the older age groups. The case fatality is 0 in all age groups, which can be attributed to favourable social and economic factors.

In general the sex distribution indicates that more samples come from males. Adding age reveals a pattern similar to the incidence distribution as well as a certain link between serotype 1b and males over 60 years (Fig. 2). On its own the connection may not be convincing as the numbers are low but it is supported by the male/female ratio; serotype 1b is ahead of serotypes 2a and 6 in all age groups and, significantly, in the >60 years group, although it is third in overall prevalence.

The reliability of H/Od as a relative indication of the severity of the disease depends on the level and availability of medical care as well as the veracity of reporting [1]. In Israel, where such conditions exist, the course of the disease, as defined by the H/Od, is generally mild. In the present study the higher hospitalization rates are registered in the very young and the elderly age groups, which may be connected to higher overall susceptibility (Fig. 3). The order of serotype prevalence in the hospitalization ratio indicates that serotype 1b is second in the very young age group, but first in the elderly age group. This means that the second largest group of patients to seek hospitalization because of severe shigellosis may be those challenged by serotype 1b. Another clue to the pathogenicity of the prevalent serotypes is the combination of H/Od with sex, which points to males as being more seriously affected by serotypes 2a and 1b, while serotype 6 appears to cause a milder form of the disease.

The high incidence of *S. flexneri* infections in Israel is attributed to the endemic nature of shigellosis in the region. However, the pattern of the prevalent *S. flexneri* serotypes is not stable. Thus according to the records of the NSRC for a 9-year period (1993–2001) the dominant serotype is 6 while serotype 2a is the most common strain on only two occasions – in 1998 and 2001. This should be considered in planning vaccine prophylaxis, since according to reports of trials with a combination vaccine composed of serotypes 2a and 3a, it did not confer significant protection against a challenge with serotype 6, which lacks the defining acetyl and/or glucosyl groups to the basic Y chain [7, 9].

The pattern of serotype 1b indicates another variation – it emerged as the third most common strain in 1996, replacing serotype 3 (3a and 3b combined), which has since dropped to insignificant numbers (Table). Similar findings have been reported from other endemic regions [19, 20]. A connection between emerging serotypes with sex and/or age may be linked to evolving demographic trends such as ageing or male/female related population shifts. Therefore a strategy aimed at reducing the number of cases, including both vaccine development and public health measures, can only be effective if based on continuous monitoring and investigation of the circulating *S. flexneri* serotypes.

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REFERENCES

1. Kotloff K, Winickoff JP, Ivanoff B, et al. *Shigella* infections: implications for vaccine development and implementation of control strategies. Bull WHO 1999; **7**: 651–666.
2. Ferrecio C, Prado V, Ojeda A, et al. Epidemiologic patterns of acute diarrhea and endemic *Shigella* infections in children in a poor periurban setting in Santiago, Chile. Am J Epidemiol 1991; **134**: 614–627.

3. Noriega FR, Liao FM, Mineval DR, Ren S, Formal SB, Levine MM. Strategy for cross-protection among *Shigella flexneri* serotypes. Infect Immun 1999; **67**: 782–788.
4. Green M, Block C, Cohen D, Slater P. Four decades of shigellosis in Israel: epidemiology of a growing public health problem. Rev Infect Dis 1991; **13**: 248–253.
5. Carlin NIA, Lindberg AA, Bock K, Bundle DR. The *Shigella flexneri* O-antigenic polysaccharide chain. Nature of the natural repeating unit. Eur J Biochem 1984; **139**: 189–194.
6. World Health Organization. Programme for the control of diarrhoeal diseases. In: The Management of bloody diarrhoea in young children. Document WHO/Cdd/94.49. World Health Organization, Geneva, Switzerland, 1994.
7. Van De Verg LL, Bendiuk NO, Kotloff K, et al. Cross-reactivity of *Shigella flexneri* serotype 2a O antigen antibodies following immunization or infection. Vaccine 1996; **14**: 1062–1068.
8. Ewing WH. The genus *Shigella*. In: Edwards and Ewing's identification of Enterobacteriaceae, 4th edn. New York: Elsevier Publishing Co., 1986: 135–172.
9. Simmon DA, Romanovska E. Structure and biology of *Shigella flexneri* O antigens. J Med Microbiol 1987; **23**: 289–302.
10. Dan M, Michaeli D, Treitsmen J. The epidemiology of shigellosis in Israel. Ann Trop Med Parasitol 1988; **82**: 159–162.
11. Ostroi P, Anis E, Green M. Shigellosis in Israel – update 1995. Public Health Rev 1996; **24**: 213–225.
12. Smollan G, Block C. Development of antimicrobial drug resistance among *Shigellae* isolated at an Israeli hospital from 1977 through 1990. Public Health Rev 1992; **18**: 319–327.
13. Mates A, Eyny D, Philo S. Antimicrobial resistance trends in *Shigella* serogroups isolated in Israel, 1990–1995. Eur J Clin Microbiol Infect Dis 2000; **19**: 108–111.
14. Keusch GT, Bennish ML. Shigellosis. In: Evans AS, Brachman ML, eds. Bacterial infections of humans, 2nd edn. New York: Plenum Medical Book Company, 1991: 593–620.
15. Ewing WH, Lindberg AA. Serology of *Shigella*. In: Bergan T, ed. Methods in microbiology, Vol. 14. London: Academic Press, 1984: 113–142.
16. Lefebvre J, Gosselin F, Ismail J, Lorange M, Lior H, Woodward D. Evaluation of commercial antisera for *Shigella* serogrouping. J Clin Microbiol 1995; **33**: 1997–2001.
17. Statistical Abstracts of Israel. Publications of the Central Bureau of Statistics (no. 51); Jerusalem, 2000.
18. Ashkenazi S, May-Zahav M, Dinari G, Gabbay U, Zilberberg R, Samra Z. Recent trends in the epidemiology of Shigella species in Israel. Clin Infect Dis 1995; **7**: 897–899.
19. Talucder KA, Dutta DK, Safa A, et al. Altering trends in the dominance of *Shigella flexneri* serotypes and emergence of serologically atypical *S. flexneri* strains in Dhaka, Bangladesh. J Clin Microbiol 2001; **39**: 3757–3759.
20. Dytta SK, Rajendran K, Roy S, et al. Shifting serotypes, plasmid profiles and antimicrobial resistance patterns of Shigellae strains isolated from Kolkata, India during 1995–2000. Epidemiol Infect 2002; **129**: 235–243.