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Antimicrobial resistance of *Shigella flexneri* serotypes in Israel during a period of three years: 2000–2002

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SUMMARY

This is a surveillance study of the antimicrobial resistance of the *S. flexneri* group in the context of its serotype diversity. It includes 1422 isolates, which were sent to the National Shigella Reference Centre (NSRC) by hospitals and outpatient clinics in Israel during a 3-year period (2000–2002). The strains were identified and classified according to the prevalence and antigenic structure of their serotypes. All samples were checked for resistance to ampicillin (AMP), trimethoprim–sulphamethoxazole (TMP–SMX), ceftriaxone (CRO), tetracycline (TE), nalidixic acid (NAL), and chloramphenicol (C) by the disk diffusion method of Bauer et al. There were significant differences in their resistance to the individual antimicrobials with resistance to AMP, TE and C being lower among the strains of serotype 6 than among those of serotypes 2a and 1b. The resistant phenotypes were also serotype-specific. The similarities both in individual and in phenotype resistance between the rare and the prevalent serotypes (but not serotype 6) may be attributed to their antigenic relatedness. The serospecificity of the antimicrobial resistance was not affected by external factors such as seasonality and source (hospital or outpatient laboratory) of the isolates, and the age and sex of the patients. The serotype-specific approach can assist in properly assessing the problem of the antimicrobial resistance of the *Shigella flexneri* group and may prove useful for the empirical therapy of shigellosis. The observed interdependency between resistance and the antigenic specificity and relatedness of the *S. flexneri* serotypes requires additional investigation.

INTRODUCTION

In endemic regions the serological diversity of the *Shigella flexneri* group increases the burden of infections through variations in the serotypes of the circulating strains [1–3]. Studies of such changes as well as of cross reactions between 14 of the 15 serotypes in the group (with the exception of serotype 6) have been conducted mostly with the aim of developing an effective vaccine, while investigations of antimicrobial resistance usually deal with the *S. flexneri* group [4–7].

In Israel shigellosis is endemic, which is related to the character of the region and expressed in its year-round occurrence [8–10]. However, in contrast to other endemic areas the prevalent serogroup is *S. sonnei*, while *S. flexneri* is second with 8–10% of the cases [10, 11]. Over the years a number of mostly hospital-based studies have dealt with various aspects of shigellosis in the country, including antimicrobial resistance of the *S. flexneri* group [12–14]. The aim of this investigation is to analyse resistance in relation to the serological diversity of *S. flexneri*, using data from

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the routine surveillance carried out by the National Shigella Reference Centre (NSRC).

MATERIALS AND METHODS

Bacterial strains

The strains were sent by hospital and outpatient clinical laboratories in Israel. This is a routine procedure, mandated by existing public health regulations, according to which the NSRC must confirm all *Shigella* isolates and identify those of groups A, B and C to the level of serotypes and subserotypes [10, 15].

Biochemical identification

The sending laboratories performed the initial identification using standard methods such as growing the bacteria on selective media (MacConkey sorbitol agar, *Salmonella–Shigella* agar and selenite broth), and inoculating the suspicious colonies into triple sugar iron agar slants. The smaller establishments carried out the biochemical identification with the EnteroPlus biphasic reagent (Novamed Ltd, Jerusalem, Israel), and the bigger facilities with the API and VITEK identification systems (bioMérieux, Marcy l'Etoile, France). Using standard methods, the NSRC verified the results of the sending laboratories in 98% of the cases [16].

Serological identification

At the sending laboratories the strains were checked by the slide agglutination method with *S. flexneri* polyvalent B antisera (Denka Seiken, Tokyo, Japan; Murex-Remel, Lenexa, KS, USA and Bio-Rad, Hercules, CA, USA) [17, 18]. Using the same method the NSRC carried out the final identification with a set of *S. flexneri* type and group antisera (Denka Seiken), capable of identifying 13 serotypes [18]. Additional group antisera (SIFIN, Berlin, Germany) were used for strain, which were untypable with the Denka Seiken products.

Antimicrobial susceptibility tests

The disk diffusion method of Bauer et al. [19] was used with Muller–Hinton agar (Difco, BD Biosciences, Sparks, MD, USA) and commercially prepared disks (Oxoid, Basingstoke, UK), containing ampicillin (AMP, 10 μg), trimethoprim–sulphamethoxazole (TMP–SMX, 25 μg), ceftriaxone (CRO, 30 μg), tetracycline (TE, 30 μg), nalidixic acid (NAL, 30 μg) and chloramphenicol (C, 30 μg). The zones of inhibition were interpreted according to the guidelines of the National Committee on Clinical Laboratory Standards (NCCLS) [20]. An *Escherichia coli* strain, ATCC 25922, was used as control in each run.

Statistical analysis

Individual and phenotype resistance of the isolates in the groups with the prevalent and rare serotypes and correlations with the seasonality and hospital/outpatient distributions of the strains as well as the sex and age of the patients were examined by the χ^2 test and Fischer's exact test. Two-tailed tests were applied.

RESULTS

The study included all 1422 isolates of *S. flexneri* received at the NSRC during the reviewed period (Table 1). The prevalent serotypes were 2a (38·7%), 6 (31·8%) and 1b (19·7%), which was concurrent with data from a previous investigation [21]. The rarely isolated serotypes were divided by common group antigens according to the formula used by Denka Seiken [22]. The type (i.e. I–VI) and group (i.e. 3,4, 6 and 7,8) antigens defining the serotypes and subserotypes of the *S. flexneri* group are shown in Table 2. Ewing's formulae were used as reference in the overall evaluation of the *S. flexneri* antigens [16, 17]. The number of the isolates with prevalent and rare serotypes, their individual resistance (%) and most common resistant patterns (%) are shown in Table 1.

Prevalent serotypes

The resistance of the isolates was significantly serotype specific – among the serotype 6 strains there was higher resistance to TMP–SMX than to AMP, TE and C ($P < 0{\cdot}001$). In contrast, both the 2a and the 1b isolates had higher resistance to AMP, TE and C, than to TMP–SMX ($P < 0{\cdot}001$, Table 1). The resistance phenotypes also differed according to serotype, with the multidrug-resistance pattern of AMP/TMP–SMX/TE/C being the most common for serotype 2a (55·0%), phenotype AMP/TE/C for serotype 1b (47·0%) and phenotype TMP/SMX alone for serotype 6 (52·1%, Table 1).

We checked the durability of the serotype-specific effect on resistance by investigating the influence of external factors linked to the host and the host's surroundings.

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Table 1. *Resistance (%) to individual antimicrobials and distribution of the predominant resistance patterns (phenotypes)*

	Prevalent serotypes			Rare serotypes			
	2a ($n=551$) Group antigen 3,4	6 ($n=453$) Group antigen —	1b ($n=280$) Group antigen 4,6	1a, 4a, 5a, Y ($n=53$) Group antigen 3,4	3b, 4b ($n=27$) Group antigen 6	2b, 3a, X ($n=58$) Group antigen 7,8	*S. flexneri* group NSRC study 1990–1995 [15] ($n=970$)
Antimicrobials							
AMP	96·4	38·4	96·1	79·2	100·0	55·2	63·5
TMP–SMX	71·0	88·5	36·4	41·5	44·4	31·0	51·3
TE	80·2	32·0	93·2	58·5	93·0	53·4	54·2
NAL	0·5	—	1·8	—	—	7·0	0·7
C	93·3	32·0	82·5	49·0	93·0	50·0	48·9
Predominant resistance patterns (phenotypes) (%)							
TMP–SMX alone	2·3	52·1	0·3	3·7	—	—	11·3
AMP/TMP–SMX	0·7	6·2	—	17·0	3·7	1·7	36·3
AMP/TE	0·5	0·4	12·5	1·9	—	—	48·8
AMP/C	5·0	—	0·3	5·7	3·7	—	48·6
TMP–SMX/TE	0·4	0·7	0·3	—	—	1·7	25·9
TMP–SMX/C	—	0·4	—	—	—	—	24·8
AMP/TMP–SMX/TE	0·5	0·7	0·3	9·4	3·7	1·7	23·6
AMP/TMP–SMX/TE/C	55·0	23·4	30·3	11·3	37·0	17·2	18·8
AMP/TE/C	23·2	3·3	47·0	32·1	51·8	25·9	—
AMP/TMP–SMX/C	11·1	3·1	3·6	—	—	1·7	—
Full sensitivity	1·0	6·4	2·5	13·2	—	43·1	17·7

AMP, ampicillin; TMP–SMX, trimethoprim–sulphamethoxazole; TE, tetracycline; NAL, nalidixic acid; C, chloramphenicol.

Seasonality

In Israel the climatic conditions define two seasons: warm (May to October) and cool (November to April) [21]. The NSRC received more isolates in the warm season (343 of serotype 2a, 182 of serotype 1b and 296 of serotype 6). During this period resistance to AMP was higher among the 2a strains (99·0%, $P<0·001$), and lower in the serotype 6 group (31·4%, $P<0·001$). During the cool period resistance to TE was lower in the 2a group (75·5%, $P<0·02$) and higher in the serotype 6 group (46·5%, $P<0·001$). The resistance to TMP–SMX of the 2a and 6 strains was not significantly affected by seasonal changes, but in the 1b group it was low in the cool season (22·4%, $P<0·001$).

The majority of the prevalent resistance phenotypes were identified during the warm season: 277 among 2a isolates, 138 among 1b isolates, and 232 among serotype 6 isolates. Significant seasonal variations were found in the groups of serotypes 1b and 6: during the warm season the multidrug-resistance pattern AMP/TMP–SMX/TE/C was more common among the 1b strains (47·5%, $P<0·001$), and during the cool period it was more common among the isolates of serotype 6 (24·1%, $P<0·01$).

Table 2. *Antigenic structure of S. flexneri*

	Antigenic structure	
Serotypes	Denka Seiken [22]	W. H. Ewing [17]
1a	I: 4	I: 1,2,4,5,9…
1b	I: 4,6	I: 1,2,4,5,6,9…
2a	II: 3,4	II: 1,3,4…
2b	II: 7,8	II: 1,7,8,9…
3a	III: (3,4), 6,7,8	III: 1,6,7,8,9…
3b	III: (3,4), 6	III: 1,3,4,6,7,8,9…
3c	—	III: 1,3,4,6…
4a	IV: 3,4	IV: 1,3,4…
4b	IV: 6	IV: 1,6…
5a	V: 3,4	
5b	V: 7,8	V: 1,5,7,9…
5 non a, non b	—	
6	VI: (4)	VI: 1,2,4…
Variant X	—: 7,8	—
Variant Y	—: 3,4	—

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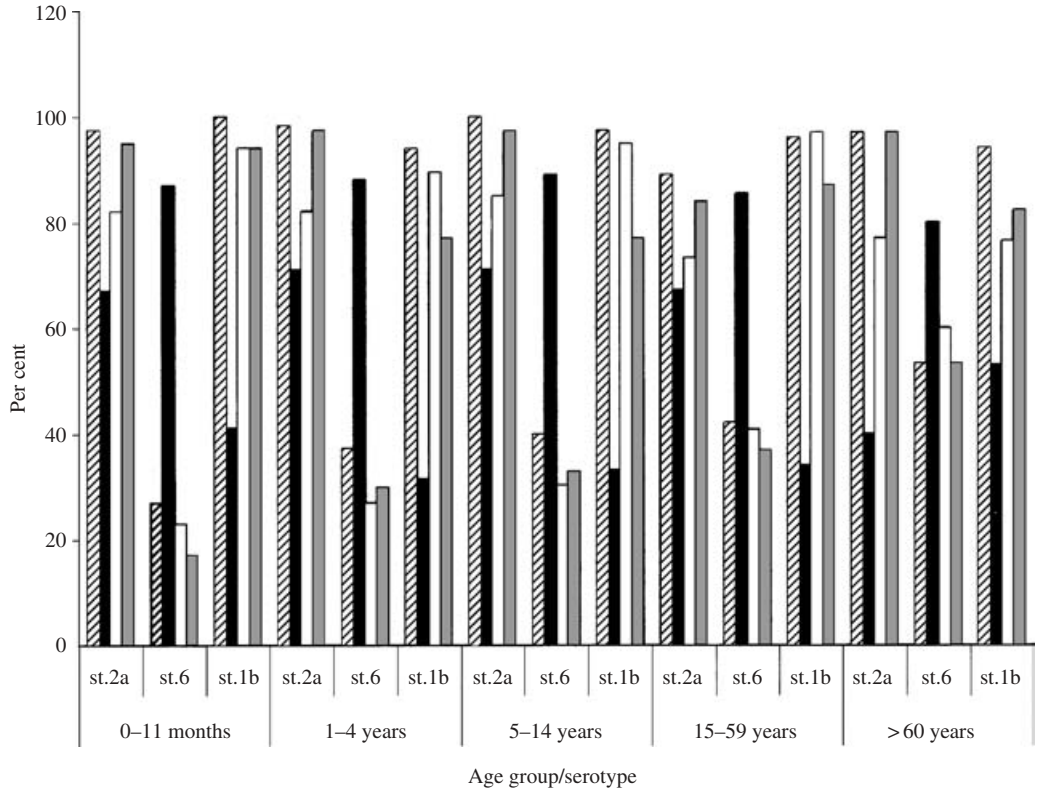

Fig. Antimicrobial resistance of the prevalent *S. flexneri* serotypes and age (%). ▨, Ampicillin; ■, trimethoprim–sulphamethoxazole; □, tetracycline; ▩, chloramphenicol.

Hospital/outpatient distribution

The outpatient clinics sent the majority of the isolates, i.e. 309 with serotype 2a, 170 with serotype 1b and 296 with serotype 6. The hospital isolates of serotype 2a had higher resistance to TMP–SMX (75·2%, $P<0·05$), and also more of the multidrug-resistance pattern AMP/TMP–SMX/TE/C (60·6%, $P<0·025$). Among the outpatient strains in the serotype 6 group there was higher resistance to AMP (42·0%, $P<0·05$).

Age and sex

The serospecific individual resistance of the isolates (as shown in Table 1) was repeated in the age groups (Fig.). Ageing and sex did not have a significant effect on both individual and phenotype resistance.

Rare serotypes

Because of small numbers, the isolates with rare subserotypes were divided by common group antigens (Table 1). The strains in each of the three groups had significantly different resistance to AMP, TMP–SMX, TE and C (Table 1, $P<0·001$ for groups 3,4 and 6, and $P<0·025$ for group 7,8). The distribution of the resistance phenotypes was not significant. All isolates were susceptible to CRO and resistance to NAL, although slightly higher than in the NSRC study of 1990–1995, was still very low (Table 1).

DISCUSSION

As distinct from other investigations of the antimicrobial resistance of *S. flexneri*, this study takes into account the antigenic structure of the bacterium. It is known that the O antigens of all *S. flexneri* serotypes (except serotype 6) consist of a repeating (common) tetrasaccharide, which is identical to subserotype Y [23–25]. The other serotypes are the result of substitutions of the repeating unit with D-glucose or O-acetyls (or both), providing the basis for their 'type' (i.e. I–V) and 'group' (i.e. 3,4, 6, and 7,8) antigens [4, 5]. The type antigens are shared between members of the same serotype (e.g. serotypes 1a and 1b), and the group antigens are found in members of different serotypes (e.g. group antigen 6 is present in serotypes 1b, 3a, 3b and 4b) [26, 27]. Somehow, this

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structural relatedness may bear upon the similarities in individual and phenotype resistance of strains of the rare and prevalent serotypes, except for serotype 6, which is a separate case in spite of many common features (including DNA-relatedness). There are major structural differences both in its basal structure and in the O-specific side-chain, which have not been found in any other *S. flexneri* serotype [26–28]. As observed in this study, both the individual and the phenotype resistance of serotype 6 strains seem to reflect these differences. Moreover, the biotype diversity of serotype 6 (bioserotypes Boyd 88, Manchester and Newcastle) may have an effect on resistance, which has to be investigated [22]. Even in the absence of more tangible evidence at this stage, the suggested connections between resistance to individual antimicrobials such as AMP, TMP–SMX and C, or the reliability of TMP–SMX, i.e. resistance as indication of multidrug resistance, may require a re-evaluation [29, 30].

According to the empirical evidence in this study it may be inferred that there is interdependence between the antigenic diversity of the *S. flexneri* group and its antimicrobial resistance. Additional and more detailed investigations of the antigenic structure and the role of integrons, plasmids or other resistance genes are needed in order to prove its existence.

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