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Emerging resistance to newer antimicrobial agents among *Shigella* isolated from Finnish foreign travellers

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SUMMARY

In Finland, most cases of shigellosis are related to travel abroad. Antimicrobial drug resistance of 1814 *Shigella* strains isolated from Finnish patients during 1990–2005 was studied using discs of 12 antimicrobial agents. Since 2000, the E-test has been performed to determine ciprofloxacin minimum inhibitory concentrations of nalidixic acid-resistant isolates. The proportion of multi-resistant strains (resistant to $\geqslant 4$ antimicrobials) was highest among isolates from China and India, but is increasing significantly in other parts of Asia. Resistance to nalidixic acid has become common among the strains from the Far East, and the first isolates also resistant to ciprofloxacin were detected during 2004–2005. All the ciprofloxacin-resistant isolates belonged to the *S. flexneri* 2a serotype. All the nalidixic acid-resistant *S. flexneri* strains had reduced susceptibility to ciprofloxacin, whereas 23% of the nalidixic acid-resistant *S. sonnei* strains were still completely susceptible to ciprofloxacin.

INTRODUCTION

Shigella bacteria are enteropathogens that cause diarrhoea and bacillary dysentery, and humans are their principal reservoir. Endemic and epidemic *Shigella* strains are a common cause of infection and even mortality in the developing countries, whereas in Western countries most of the infections are caused by imported strains [1]. *S. sonnei* (serogroup D, a single serotype), which causes a relatively mild disease, most commonly infects travellers from Western countries [1–5]. *S. flexneri* (serogroup B, consisting of six serotypes with several subtypes) is endemic in most developing countries and causes more mortality than the other *Shigella* spp. *S. dysenteriae* (serogroup A, consisting of 15 serotypes) causes large epidemics in many developing countries. The fourth species, common in some parts of the world, is *S. boydii* (serogroup C, consisting of 20 serotypes). Three predominant strains are globally responsible for the majority of shigellosis cases, namely *S. sonnei*, *S. flexneri* 2a and *S. dysenteriae* type 1 [6].

Although gastroenteritis caused by *Shigella* spp. is normally self-limited, patients are often treated with antimicrobial agents to reduce the duration of the illness and the period of shigella excretion after symptoms subside [7]. However, *Shigella* strains have rapidly developed resistance to the commonly used antimicrobials [8]. Ampicillin and trimethoprim–sulfamethoxazole previously used to treat shigellosis are of limited use today. Nalidixic acid was commonly used in the early 1990s but has since lost its effectiveness in many regions. Fluoroquinolones, especially ciprofloxacin, are the drug of choice in Western countries today. However, *Shigella* strains seem to become rapidly resistant to this group of

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antimicrobials as well. Here we report on the increasing antimicrobial resistance among *Shigella* spp. isolated from patients in Finland between 1990 and 2005.

METHODS

Shigella strains

All of about 30 Finnish routine clinical microbiology laboratories have sent, first voluntarily and since 1995 mandatorily, all their suspected *Shigella* isolates to the National Reference Centre for *Shigella* at the Enteric Bacteria Laboratory of the National Public Health Institute (KTL). The potential history of travel preceding the diarrhoea of a patient was supplied on a special form that always accompanied the strain.

This study was based on 1814 isolates that were obtained during 1990–2005. Standard identification methods were used. Serotypes were determined by using commercially available antisera (Wellcome Diagnostics, Dartford, UK, and since 1995 Denka Seiken, Tokyo, Japan) according to the manufacturer's instructions. If the results of the biochemical identification or of the serotyping were doubtful, the strain was referred to the Laboratory of Enteric Pathogens of the Health Protection Agency (Colindale, London, UK) for verification.

The origin of an isolate was defined in our study as a continent where the infected person had travelled prior to falling ill. The whole of Russia or Former Soviet Union was considered as part of Europe. The American continents were considered as one group since only 10 of the 158 American isolates were from North America. In the course of the study Asia was divided into two: the Far East including China, India and the neighbouring countries (Pakistan, Bangladesh, Nepal, Sri Lanka) and the rest of Asia: other Asian countries including Turkey.

Susceptibility testing

During 1990–1998, antimicrobial susceptibility of *Shigella* isolates to the following 12 antimicrobials was determined: ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline, trimethoprim, ciprofloxacin, nalidixic acid, mecillinam, imipenem, neomycin and ceftriaxone. During 1999–2005, gentamicin and cefotaxime were used instead of neomycin and ceftriaxone. The susceptibility tests were performed by agar diffusion technique, first, on Iso-Sensitest medium using the zone size criteria recommended by the disc manufacturer (A/S Rosco, Taastrup, Denmark) and established by the Swedish Reference Group for Antibiotics [9]. CLSI guidelines [10] and discs from Oxoid (Oxoid, Basingstoke, Hampshire, UK) were first used in 1999 and Mueller–Hinton agar in 2005. In this study, the strains showing intermediate susceptibility were categorized as resistant. Since 2000, minimal inhibitory concentration (MIC) for ciprofloxacin has been determined by E-test (AB Biodisk, Solna, Sweden) to the isolates resistant to nalidixic acid, following the recommended MIC breakpoints, susceptible $\leqslant 1$ mg/l and resistant $\geqslant 4$ mg/l [10]. MIC of 0·125–1·0 mg/l was considered as reduced susceptibility to ciprofloxacin [2].

Statistical methods

χ^2 test (Epi-Info, v. 3.3.2, CDC, Atlanta, GA, USA) was used to evaluate the significance of the increased number of antimicrobial-resistant strains. When an expected cell value was <5, Fisher's exact test was used. Statistical significance was indicated at $P<0{\cdot}05$. The proportion of multi-resistant *Shigella* strains in different areas of the world was modelled using binomial distributions $\mathrm{Bin}(N(t), p(t))$, where $N(t)$ is the total amount of strains in the year t. The probability (proportion of multi-resistant strains) $p(t)$ was modelled using log-link as $\log(p(t)) = a + b*(t-1990)$, where parameters a and b must be estimated. The goodness-of-fit tests were used to assess the fit of the linear time trend in log-scale. The relative annual change in the proportion that was multi-resistant was $\exp(b)-1$.

RESULTS

In Finland, around 100 cases of shigellosis have been reported annually during the last decade. Of the 1869 isolates originally available for the study, the following were excluded: 41 isolates from an outbreak caused by contaminated foodstuff in 2001, nine isolates from an outbreak in a shelter for alcoholics in 1990 and five isolates from a hospital outbreak in 1991. All these outbreaks were caused by *S. sonnei*. Thus, 1814 strains (one isolate per patient) were analysed. Of these, 68% (1238 strains) belonged to *S. sonnei*, 22% (407 strains) to *S. flexneri*, 7% (123 strains) to *S. boydii* and 3% (46 strains) to *S. dysenteriae*.

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Table 1. *Number of* Shigella *strains associated with travelling to different geographic areas during 1990–2005*

	Total from abroad	Far East	Rest of Asia	Africa	Americas	Europe	No known travel
S. sonnei	1075 (87)	140 (11)	343 (28)	303 (24)	119 (10)	170 (14)	163 (13)
S. flexneri	359 (88)	62 (15)	66 (16)	149 (37)	32 (8)	50 (12)	48 (12)
S. boydii	113 (92)	17 (14)	28 (23)	49 (40)	9 (7)	10 (8)	10 (8)
S. dysenteriae	45 (98)	13 (28)	4 (9)	19 (41)	7 (15)	2 (4)	1 (2)
All *Shigella* strains	1592 (88)	232 (13)	441 (24)	520 (29)	167 (9)	232 (13)	222 (12)

The proportion of strains by species originating from various geographical areas is shown in parentheses (%).

Of the 1814 *Shigella* strains analysed, 1592 (88%) were known to be associated with travel abroad: 37% originated from Asia (13% from the Far East and 24% from the other Asian countries), 29% from Africa (most commonly Egypt), 13% from Europe (especially Russia and Estonia), 9% from Latin America and 0·5% from the United States (Table 1). Further, 10% of the patients had no trips outside Finland 4 weeks before the symptoms started and for 2% no suspected location of infection was indicated. While *S. sonnei* was the most common *Shigella* isolate detected and seen relatively equally in travellers returning from all locations, all other *Shigella* spp. were most common in travellers returning from Africa (Table 1). There were no major annual changes in the proportions of different species at each geographic area during 1990–2005 (data not shown).

During the study period, the proportion of multi-resistant shigellas rose globally (Tables 2 and 3). A binomial model from the data indicated that the overall proportion of resistant strains increased significantly ($P<0{\cdot}005$), which was mainly due to the increasing proportion of resistant strains imported from Asia (often from Turkey or Thailand, $P<0{\cdot}001$) (Table 2, Fig. 1). The strains originating from the Far East were usually already multi-resistant at the beginning of the study period, yet the proportion continued to increase ($P<0{\cdot}001$) (Table 2, Fig. 1).

The proportion of resistant strains to all the antimicrobial agents studied increased during the study period. Statistically significant changes in the sensitivity of the strains can be seen with several antimicrobials between the first two and last two years of follow-up (Table 3). However, the frequencies of resistance differed between the species (Table 3). For example, 19% of the *S. sonnei* strains but 12% of the *S. flexneri* strains were nalidixic acid-resistant. In addition, the proportion of multi-resistant (resistant to $\geqslant 4$ antimicrobials) *S. sonnei* strains nearly doubled

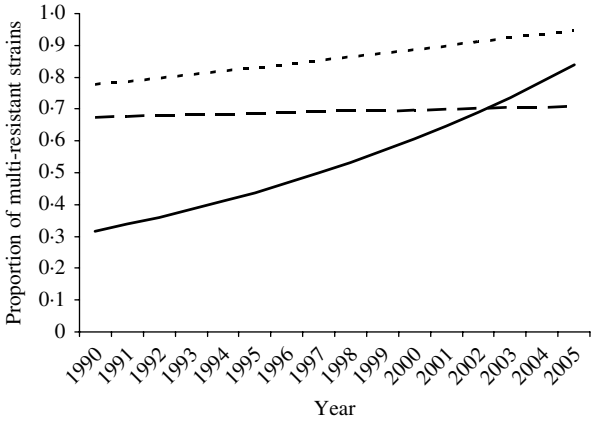

Fig. 1. Predicted proportion of multiresistant strains in the Far East (- - -), the rest of Asia (—) and Africa (– – –).

in 15 years, whereas for the other species the change was not statistically significant. The number of *S. boydii* and *S. dysenteriae* isolates was too small to draw any firm conclusions.

Isolates from all the countries were commonly resistant to several 'traditional' antimicrobials (ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline and trimethoprim). However, most of the strains from the Far East have now developed resistance to one or more of the following antimicrobials: mecillinam, nalidixic acid, ciprofloxacin, gentamicin and cefotaxime. During the years 1990–1997, nalidixic acid resistance of the isolates from travellers returning from the Far East remained low (between 0% and 6%) but in 1998 resistance started to increase so that in 2003 six strains (67%), in 2004 14 strains (61%) and in 2005 20 strains (87%) originating from the Far East were resistant to nalidixic acid (data not shown). In general, only one strain resistant to nalidixic acid per year has been obtained from the other countries.

Resistance to nalidixic acid indicates possible lowered resistance to fluoroquinolones. During

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Table 2. *Number of the isolates from subjects with known history of travel* ($n=1592$) *to certain geographical areas outside Finland and the annual proportion of multi-resistant* (*resistant to* $\geqslant 4$ *antimicrobials*) *strains* (*%*) *in 1990–2005*

Year	Total from abroad	Far East	Rest of Asia	Africa	Americas	Europe	No known travel
1990	262 (44)	22 (77)	131 (24)	59 (69)	18 (56)	32 (50)	45 (58)
1991	145 (62)	16 (75)	40 (43)	57 (68)	13 (69)	19 (68)	23 (65)
1992	121 (55)	17 (71)	32 (28)	30 (67)	13 (54)	29 (62)	15 (87)
1993	100 (54)	12 (75)	45 (40)	22 (59)	7 (43)	14 (79)	8 (25)
1994	86 (62)	14 (79)	31 (45)	18 (83)	10 (30)	13 (77)	14 (64)
1995	59 (68)	14 (79)	18 (61)	12 (75)	7 (43)	8 (75)	7 (86)
1996	75 (65)	13 (92)	23 (70)	20 (90)	2 (0)	17 (18)	16 (63)
1997	73 (59)	18 (100)	13 (38)	28 (54)	11 (18)	3 (100)	16 (56)
1998	62 (50)	10 (90)	22 (27)	6 (33)	14 (43)	10 (80)	9 (33)
1999	63 (67)	3 (100)	10 (80)	29 (48)	6 (83)	15 (80)	5 (100)
2000	55 (75)	12 (75)	6 (67)	20 (85)	9 (44)	13 (92)	13 (100)
2001	166 (77)	18 (100)	29 (90)	71 (75)	17 (94)	31 (45)	11 (55)
2002	66 (68)	8 (100)	21 (71)	23 (57)	6 (50)	8 (75)	12 (75)
2003	58 (66)	9 (78)	6 (67)	28 (54)	9 (89)	6 (67)	4 (100)
2004	95 (82)	23 (96)	8 (63)	41 (76)	14 (86)	9 (89)	11 (100)
2005	106 (78)	23 (91)	7 (57)	58 (78)	11 (64)	7 (86)	15 (73)
All years	1592 (63)	232 (86)	441 (44)	520 (69)	167 (59)	232 (64)	222 (68)

Table 3. *Comparison of antimicrobial resistance* (*%*) *of 702* Shigella *strains isolated during two periods* (*in 1990–1991 and 2004–2005*). *Multi-resistant strains were resistant to* $\geqslant 4$ *antimicrobial agents and pan-susceptible strains were susceptible to all tested agents*

	All *Shigella* strains			*S. sonnei*			*S. flexneri*			*S. boydii*, *S. dysenteriae*		
	1990–91 ($n=474$)	2004–05 ($n=228$)		1990–91 ($n=322$)	2004–05 ($n=149$)		1990–91 ($n=103$)	2004–05 ($n=59$)		1990–91 ($n=49$)	2004–05 ($n=20$)	
Streptomycin	76	91	***	77	95	***	79	86	n.s.	61	80	n.s.
Sulfonamide	74	80	n.s.	78	92	***	68	56	n.s.	63	65	n.s.
Tetracycline	54	85	***	49	86	***	75	88	*	41	70	*
Ampicillin	27	33	n.s.	14	17	n.s.	71	73	n.s.	22	30	n.s.
Chloramphenicol	18	21	n.s.	4	5	n.s.	64	64	n.s.	12	20	n.s.
Trimethoprim	48	84	***	52	95	***	47	69	**	27	45	n.s.
Mecillinam	0	4	***	0	3	**	0	8	**	2	0	n.s.
Nalidixic acid	0	16	***	0	19	***	0	12	***	0	5	n.s.
Ciprofloxacin	0	1	*	0	0	—	0	5	*	0	0	—
Neomycin	0	n.d.	—	0	n.d.	—	0	n.d.	—	0	n.d.	—
Gentamicin	n.d.	2	—	n.d.	2	—	n.d.	3	—	n.d.	0	—
Ceftriaxone/Cefotaxime	0	0	—	0	0	—	0	0	—	0	0	—
Imipenem	0	0	—	0	0	—	0	0	—	0	0	—
Multiresistant	51	82	***	46	89	***	74	78	n.s.	33	45	n.s.
Pansusceptible	19	5	***	19	3	***	14	7	n.s.	29	15	n.s.

Significance of the change in the proportion of resistant strains during the indicated times, measured by χ^2 test: *** $P<0{\cdot}001$, ** $P<0{\cdot}01$, * $P<0{\cdot}05$; n.s., not significant; —, not tested; n.d., not determined.

2000–2005, a total of 65 nalidixic acid-resistant strains were found and 86% of them originated from Far East countries. In 2000–2001 susceptibility to ciprofloxacin had decreased (MIC $\geqslant 0{\cdot}125$ mg/l) in 44%, in 2002–2003 in 62% and in 2004–2005 in 83% of the nalidixic acid-resistant strains. The first isolate highly resistant to ciprofloxacin (MIC 32 mg/l) was encountered in 2004 and the second (MIC 6 mg/l) in

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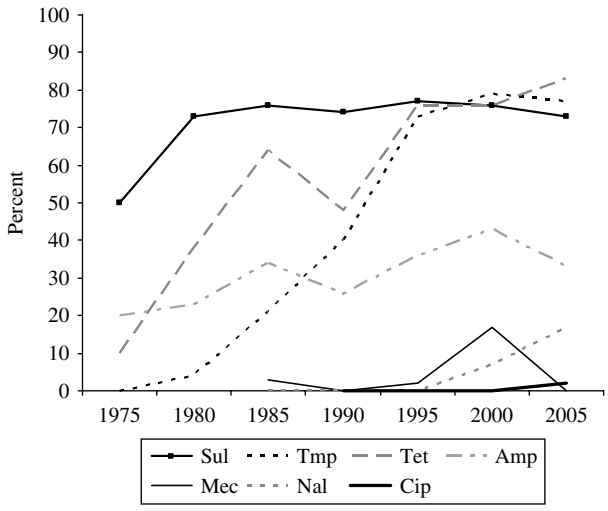

Fig. 2. Development of resistance to some clinically important antimicrobial agents in Finnish *Shigella* isolates during 1975–1988 [11] and 1990–2005 (this study). Sul, sulfonamide; Tmp, trimethoprim; Tet, tetracycline; Amp, ampicillin; Mec, mecillinam; Nal, nalidixic acid; Cip, ciprofloxacin.

2005. The ciprofloxacin resistance of these two plus one other strain (MIC 3 mg/l) was also detected by the disc method. All these isolates belonged to the *S. flexneri* 2a serotype. During 2004–2005 the rest of the *S. flexneri* strains ($n=9$) also showed reduced susceptibility to ciprofloxacin, whereas 23% of the *S. sonnei* strains ($n=26$) were still completely susceptible to ciprofloxacin (MIC $<0{\cdot}125$ mg/ml).

We have tested resistance to gentamicin since 1999, and during 2004–2005 five gentamicin-resistant strains were detected. Two strains belonged to *S. flexneri* (from China) and three to *S. sonnei* (two from China and one from Egypt). In addition, in 2002 we received a *S. sonnei* strain originating from Egypt that was resistant to cefotaxime and to five other antimicrobials (ampicillin, streptomycin, sulfonamide, tetracycline and trimethoprim). Several mecillinam-resistant *Shigella* strains have been seen since 1990. Thus, resistance to representatives of nearly all groups of antimicrobial agents was detected in *Shigella*. Of the 12 antimicrobials tested, only resistance to imipenem was not detected during the study period.

DISCUSSION

In this study we used the so-called 'epidemiological susceptibility panel' of 12 different antimicrobial discs for *Shigella* antimicrobial susceptibility testing. A similar panel is also frequently used in other countries belonging to the Enter-net international surveillance network for human gastrointestinal infections. The antimicrobial panel showed that the proportion of multi-resistant *Shigella* strains is growing and, moreover, resistance to several new antimicrobials is emerging. Development of resistance to some clinically important antimicrobials in *Shigella* isolates from Finns has been followed since 1975. The current data, together with this previous study [11], showed that, for example, the development of resistance to trimethoprim increased from 0% to nearly 80% in 20 years (Fig. 2). So far, the resistance to nalidixic acid has also risen from 0% to nearly 20% in 10 years. However, the resistance to nalidixic acid is much more common in the Far East than in the other areas of the world. Similarly, ciprofloxacin resistance is now increasing in the Far East, especially among *S. flexneri*. As a consequence, Finnish travellers who have been to those areas can now be infected with a ciprofloxacin-resistant strain.

Ciprofloxacin is currently the recommended antimicrobial for the treatment of shigellosis worldwide [7, 12], and also in Finland. Although several studies have established the tolerability and efficacy of fluoroquinolones in children during short-term treatments [13, 14], the use of cephalosporins has been recommended for children instead of ciprofloxacin [2]. Thus far we have detected only one strain resistant to a third-generation cephalosporin. However, it is of concern when considering the alternatives for treatment with ciprofloxacin. It can be expected that *Shigella* strains will rapidly develop resistance to cephalosporins as well, since the emergence of extended-spectrum of β-lactamases have been reported in recent years, for example, from Korea in *S. sonnei* isolates [15], from Argentina in *S. flexneri* isolates [16] and from Bangladesh in *S. sonnei*, *S. flexneri* and *S. boydii* [17].

The Finnish *Shigella* isolates commonly originate from the countries popular among Finnish tourists, especially Egypt, Turkey, India, Tunisia and Estonia. The antimicrobial resistance patterns detected among these isolates reflect the resistance situation and usage of antimicrobial agents in the countries the infections were obtained from. Each year we also receive *Shigella* isolates from Finns who have not travelled abroad before contracting shigellosis. The antibiotic resistance patterns of these strains are similar to those

of foreign origin; these people may have received their infection as a secondary infection or from imported foodstuffs. The total number of detected cases of shigellosis has decreased during the study years; the exact reasons for this are unknown. However, increased hygiene standards at holiday resorts and increased awareness of personal hygiene have possibly led to this positive development.

South and East Asia seem to be the areas where the new resistances emerge. In some areas of China, virtually all *Shigella* strains are resistant to nalidixic acid [18, 19]. Moreover, a high prevalence of resistance to ciprofloxacin has been reported in China, in one study 6% [19] and in another as much as 20% [20]. Resistance to gentamicin was also reported to be rather common (13%), since oral gentamicin is widely used to treat bacillary dysentery in China, even if its therapeutic efficacy is controversial [20]. In China and in parts of India, *S. flexneri* 2a is the dominant *Shigella* type [20, 21]. In Calcutta, in 2004, 92% of the *S. flexneri* strains were resistant to nalidixic acid and 41% to ciprofloxacin, in contrast to the *S. sonnei* strains, which were all resistant to nalidixic acid but none to ciprofloxacin [21]. Similarly, in Bangladesh, during 1999–2003, more than 60% of *S. sonnei* isolates were resistant to nalidixic acid but none to ciprofloxacin or other newer antimicrobials [22]. However, ciprofloxacin-resistant *S. sonnei* strains have recently been reported from Japan [23]. The difference in the resistance patterns of *S. sonnei* and *S. flexneri* was also reflected in the strains Finnish travellers were infected with.

We found that isolates originating from the Americas, Europe or Africa were often multi-resistant but rarely exhibited resistance to nalidixic acid or reduced susceptibility to ciprofloxacin. This agrees with previous reports on the situation in these areas [24–28]. However, in the Middle East and parts of Africa the incidence of resistance seems to be increasing [29, 30]. Studies conducted in other Western countries, where the majority of shigellosis cases are related to travel abroad, have also reported on a growing prevalence of multi-resistant strains and, especially, an increase in nalidixic acid resistance [2–4]. In England and Wales, of the 912 *Shigella* strains isolated in 2002, 13% of *S. sonnei* and 10% of the other strains were resistant to nalidixic acid. All these strains were reported to have reduced susceptibility to ciprofloxacin (MIC 0·125–1·0 mg/l), although no clinically significant resistance (MIC $\geqslant$2 mg/l) was detected [2].

Since virtually all the *Shigella* isolates from the Finnish travellers were available to our study, we were able to analyse where the Finns became infected with shigellosis and what the resistance patterns of these isolates were. Naturally, our study does not give a full picture of the situation in the various destination countries. This could only be provided by an improved surveillance system in each of these countries. However, in our study, certain differences in the antimicrobial resistance were clearly seen. First, the possibility of ciprofloxacin resistance has to be taken into consideration when treating patients from Asia, especially from China or India. Second, *S. flexneri* strains are more probably ciprofloxacin resistant than the strains of the other species. This knowledge offers a basis for empiric treatment recommendations. However, our study also showed that the antimicrobial resistance situation is changing, thus, in the future, constant surveillance will be needed in Finland as well as in other countries.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Ekdahl K, Andersson Y.** The epidemiology of travel-associated shigellosis – regional risks, seasonality and serogroups. *Journal of Infection* 2005; **51**: 222–229.
2. **Cheasty T, Day M, Threlfall EJ.** Increasing incidence of resistance to nalidixic acid in shigellas from humans in England and Wales: implications for therapy. *Clinical Microbiology and Infection* 2004; **10**: 1033–1035.
3. **Sivapalasingam S, *et al.*** High prevalence of antimicrobial resistance among *Shigella* isolates in the United States tested by the National Antimicrobial Resistance Monitoring System from 1999 to 2002. *Antimicrobial Agents and Chemotherapy* 2006; **50**: 49–54.
4. **Hirose K, *et al.*** Antimicrobial susceptibility of *Shigella sonnei* isolates in Japan and molecular analysis of *S. sonnei* isolates with reduced susceptibility to fluoroquinolones. *Antimicrobial Agents and Chemotherapy* 2005; **49**: 1203–1205.
5. **Janda JM, Abbott SL.** *The Enterobacteria*, 2nd edn. Washington, DC: ASM Press, 2006, pp. 411.

6. **Sur D, *et al.*** Shigellosis: challenges and management issues. *Indian Journal of Medical Research* 2004; **120**: 454–462.
7. **Heymann DL.** *Control of Communicable Diseases Manual*, 18th edn. Washington, DC: APHA, 2004, pp. 700.
8. **Niyogi SK.** Shigellosis. *Journal of Microbiology* 2005; **43**: 133–143.
9. **Swedish Reference Group for Antibiotics.** *Antimicrobial Susceptibility Testing of Bacteria – A Reference and a Methodology Manual*. Stockholm, Sweden: The Swedish Medical Society and Statens Bakteriologiska Laboratorium, 1990.
10. **CLSI.** Performance standards for antimicrobial susceptibility testing; M100-S16. Clinical and Laboratory Standards Institute, 2006.
11. **Heikkilä E, *et al.*** Increase in trimethoprim resistance among *Shigella* species, 1975–1988: analysis of resistance mechanisms. *Journal of Infectious Diseases* 1990; **161**: 1242–1248.
12. **WHO.** Shigellosis: disease burden, epidemiology and case management. *Weekly Epidemiological Record* 2005; **80**: 93–100.
13. **Phavichitr N, Catto-Smith AG.** Acute gastroenteritis in children: what role for antibacterials? *Pediatric Drugs* 2003; **5**: 279–290.
14. **Leibovitz E.** The use of fluoroquinolones in children. *Current Opinion in Pediatrics* 2006; **18**: 64–70.
15. **Kim S, *et al.*** Occurrence of extended-spectrum β-lactamases in membranes of the genus *Shigella* in the Republic of Korea. *Journal of Clinical Microbiology* 2004; **42**: 5264–5269.
16. **Andres P, *et al.*** Extended-spectrum β-lactamases in *Shigella flexneri* from Argentina: first report of TOHO-1 outside Japan. *International Journal of Antimicrobial Agents* 2005; **25**: 501–507.
17. **Rahman M, *et al.*** Extended-spectrum β-lactamase-mediated third generation cephalosporin resistance in *Shigella* isolates in Bangladesh. *Journal of Antimicrobial Chemotherapy* 2004; **54**: 846–847.
18. **Wang XY, *et al.*** Occurrence of shigellosis in the young and elderly in rural China: results of a 12-month population-based surveillance study. *American Journal of Tropical Medicine and Hygiene* 2005; **73**: 416–422.
19. **von Seidlein L, *et al.*** A multicentre study of *Shigella* diarrhoea in six Asian countries: disease burden, clinical manifestations, and microbiology. *PLOS Medicine* 2006; **3**: e353.
20. **Wang X, *et al.*** Trend and disease burden of bacillary dysentery in China (1991–2000). *Bulletin of the World Health Organization* 2006; **84**: 561–568.
21. **Pazhani GP, *et al.*** Species diversity and antimicrobial resistance of *Shigella* spp. isolated between 2001 and 2004 from hospitalized children with diarrhoea in Kolkata (Calcutta), India. *Epidemiology and Infection* 2005; **133**: 1089–1095.
22. **Talukder KA, *et al.*** Antibiotic resistance and genetic diversity of *Shigella sonnei* isolated from patients with diarrhoea between 1999 and 2003 in Bangladesh. *Journal of Medical Microbiology* 2006; **55**: 1257–1263.
23. **Ahmed AM, *et al.*** Genetic characterization of multidrug resistance in *Shigella* spp. from Japan. *Journal of Medical Microbiology* 2006; **55**: 1685–1691.
24. **Streit JM, *et al.*** Prevalence and antimicrobial susceptibility patterns among gastroenteritis-causing pathogens recovered in Europe and Latin America and *Salmonella* isolates recovered from bloodstream infections in North America and Latin America: report from the SENTRY Antimicrobial Surveillance Program, 2003. *International Journal of Antimicrobial Agents* 2006; **27**: 367–375.
25. **Brooks JT, *et al.*** Surveillance for bacterial diarrhea and antimicrobial resistance in rural Western Kenya, 1997–2003. *Clinical Infectious Diseases* 2006; **43**: 393–401.
26. **Naik DG.** Prevalence and antimicrobial susceptibility patterns of Shigella species in Asmara, Eritrea, Northeast Africa. *Journal of Microbiology, Immunology and Infection* 2006; **39**: 392–395.
27. **Putnam SD, *et al.*** Antimicrobial susceptibility trends among *Escherichia coli* and *Shigella* spp. isolated from rural Egyptian paediatric populations with diarrhoea between 1995 and 2000. *Clinical Microbiology and Infection* 2004; **10**: 804–810.
28. **Ahmed SF, *et al.*** Epidemiology and genetic characterization of *Shigella flexneri* strains isolated from three paediatric populations in Egypt, 2000–2004. *Epidemiology and Infection* 2006; **134**: 1237–1248.
29. **Ashkenazi S, *et al.*** Growing antimicrobial resistance of *Shigella* isolates. *Journal of Antimicrobial Chemotherapy* 2003; **51**: 427–429.
30. **Eja ME, *et al.*** Incidence of enteric bacterial pathogens in water found at the bottom of commercial freezers in Calabar, southeastern Nigeria. *Southeast Asian Journal of Tropical Medicine and Public Health* 2006; **37**: 394–399.