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Serotype distribution and characteristics of antimicrobial resistance in *Shigella* isolated from Henan province, China, 2001–2008

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SUMMARY

The serotype distribution and susceptibility to 14 antimicrobial agents of 526 isolates of *Shigella* spp. from four hospitals in Sun county, Henan province, China during 2001–2008, were analysed to identify associations of serotypes with resistance trends. *S. flexneri* was the most frequent species (92·4%), the remainder was *S. sonnei*. The prevalent serotype of *S. flexneri* was 2a (26·7%). Almost all (>99%) isolates were resistant to tetracycline, nalidixic acid and pipemidic acid; >80% were resistant to chloramphenicol, amoxicillin and co-trimoxazole but less than 5% were resistant to polymyxin B, furazolidone, cefotaxime and gentamicin. *S. flexneri* showed statistically significant higher resistance than *S. sonnei* to amoxicillin, ampicillin, chloramphenicol and ciprofloxacin but resistance to co-trimoxazole was more common in *S. sonnei* than in *S. flexneri*. These results emphasize that monitoring of emerging resistance in *Shigella* isolates is essential for timely and appropriate recommendations for antimicrobial therapy.

Key words: China, epidemiology, resistance, serotypes, *Shigella*.

INTRODUCTION

Acute dysentery caused by *Shigella* spp. continues to be a frequent cause of enteritis in developing countries [1–3]. In China, shigellosis is the sixth most common cause of death from infectious disease, accounting for up to 1·7 million episodes of bacillary dysentery annually, with about 200 000 patients admitted to hospitals [4, 5]. Four species are recognized, namely *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. Except for *S. sonnei*, each species contains multiple serotypes based on the structure of the O antigen [6]. Until recently at least 47 serotypes of all *Shigella* have been identified, of which 15 belong to *S. flexneri* [7]. In China, *S. flexneri* serotype 2a was the most prevalent but other serotypes such as 1a, 3a and variant X are also of major importance [8–11]. Serological typing of *S. flexneri* and antimicrobial resistance monitoring have long been used for the epidemiological characterization of strains. Prompt appropriate antimicrobial treatment may shorten the duration of clinical symptoms and carriage and reduce the spread of infection [12]. However, in recent years *Shigella* isolates have become progressively more resistant to most of the first-line drugs used [13, 14] with the outcome that tetracycline, sulfonamides, ampicillin and co-trimoxazole are no longer recommended for empirical treatment [15]. The emergence of such resistance poses a major difficulty in the choice of an appropriate antimicrobial for treatment due to shifts in the prevalence of the different serotypes and changes in the resistance patterns [16]. Knowledge of

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the distribution of *Shigella* serotypes in clinical isolates is therefore of epidemiological importance. The major objective of this study was to analyse the patterns and trends in antimicrobial resistance in *Shigella* isolated from a rural area of China, and to describe the distribution of *Shigella* serotypes associated with resistant phenotypes.

METHODS

Shigella isolates

Shigella isolates ($n=526$) were recovered from 2485 faecal samples from patients with diarrhoea in four rural hospitals of Henan province, China during 2001–2008 (see Supplementary Table S1 for resistance patterns). Fresh faecal specimens were collected by swabbing mucous or blood from stool of study participants of all ages. Samples were inoculated into Cary–Blair medium and all isolates were identified according to standard microbiological and biochemical methods [17], and typed by slide agglutination test with *Shigella* polyvalent grouping (Mast Group Ltd, UK) and monovalent antisera (Denka Seiken Co. Ltd, Japan).

Antimicrobial susceptibility testing

Susceptibility to antimicrobial agents was determined by the disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [18]. The antibiotics used were cefamezin (CFZ, 30 μg), cefotaxime (CTX, 30 μg), amoxicillin (AMC, 25 μg), ampicillin (AMP, 10 μg), gentamicin (GM, 10 μg), chloramphenicol (C, 30 μg), tetracycline (TE, 30 μg), co-trimoxazole (SMZ, 25 μg), polymyxin B (PB, 300 μg), nalidixic acid (NAL, 30 μg), pipemidic acid (PI, 30 μg), ciprofloxacin (CIP, 5 μg), tobramycin (TOB, 10 μg), and furazolidone (F, 10 μg). *Escherichia coli* ATCC 25922 was used as the control strain for susceptibility tests. Designation of isolates as susceptible, intermediately resistant, or resistant was based on CLSI guidelines, but those showing intermediate zones of growth inhibition were interpreted as resistant.

Statistical analysis

The database software programme SPSS v. 13.0 (SPSS Inc., USA) was used for statistical analysis and all data entry was performed in duplicate. For the comparison of mean rank differences in resistance to each drug for each year with serotypes, the study (2001–2008) was divided into eight groups and serotypes into 16 groups. For non-parametric tests, the Kruskal–Wallis one-way analysis of variance was employed to compare the mean rank differences in resistance to each drug for each year and for different serotypes. A P value of $<0{\cdot}05$ was considered statistically significant.

RESULTS

Serotypes distribution

Two *Shigella* spp. were identified in the 526 isolates; *S. flexneri* was by far the most frequent species, accounting for 92·4% with *S. sonnei* accounting for the remainder. Each of the 15 serotypes of *S. flexneri* were identified with serotypes 2a (130/486, 26·7%) and 4c (121/486, 24·9%) being the most common (Table 1). The incidence of *S. sonnei* fluctuated over the study period from no isolates recovered in three of the eight years to 13 isolates in 2006. The prevalent serotypes of *S. flexneri* also varied from 2001 to 2008 with 3a being the most frequent in 2001 (42·1%) but 2a was consistently the most common type from 2006 to 2008. Four serotypes (1c, 5, 5a, 6a) were represented by one or two isolates only (Table 1).

Epidemiological characteristics

Shigella-positive patients ranged from age 2 months to 83 years (mean 18·6 years), and 54·6% were male. Infants and children from ages 0 to 10 years accounted for 55·2% of *S. sonnei* and 34·9% of *S. flexneri* cases; 20·6% of *S. flexneri* and 13·8% of *S. sonnei* isolates were from subjects aged >50 years. All isolates were recovered between May and October in the study years.

Resistant trends and profiles

Table 2 shows that >99% of all isolates were resistant to tetracycline, nalidixic acid and pipemidic acid, and >80% were resistant to chloramphenicol, amoxicillin, ampicillin and co-trimoxazole. Most isolates were susceptible to cefotaxime and gentamicin (resistance <5%), and polymyxin B and furazolidone (resistance $<0{\cdot}5$%). Resistance to cefamezin and cefotaxime emerged in 2003, and fluctuated greatly (2·1–30·4%) in the following years. There was a

Table 1. *The serotype distribution of* Shigella *isolates during 2001–2008*

Serotype	2001 ($n=19$)	2002 ($n=49$)	2003 ($n=144$)	2004 ($n=60$)	2005 ($n=80$)	2006 ($n=55$)	2007 ($n=56$)	2008 ($n=63$)	Total ($n=526$)
S. flexneri	19 (100)	49 (100)	137 (95·1)	51 (85)	80 (100)	42 (76·4)	50 (89·3)	58 (92·1)	486 (92·4)
F1a	1 (5·3)	5 (10·2)	8 (5·6)	4 (6·7)	12 (15)	8 (14·5)	10 (17·9)	11 (17·5)	59 (12·1)
F1b	0	1 (2)	6 (4·2)	0	0	0	1 (1·8)	0	8 (1·6)
F1c	0	0	0	0	0	1 (1·8)	0	0	1 (0·2)
F2a	5 (26·3)	15 (30·6)	43 (29·9)	6 (10)	14 (17·5)	11 (20)	17 (30·3)	19 (30·2)	130 (26·7)
F2b	0	1 (2)	1 (0·7)	5 (8·3)	4 (5)	7 (12·7)	6 (10·7)	6 (9·5)	30 (6·2)
F2ab	0	0	1 (0·7)	0	4 (5)	2 (3·6)	0	2 (3·6)	9 (1·9)
F3a	8 (42·1)	7 (14·3)	0	0	0	0	0	0	15 (3·1)
F4	0	0	0	0	1 (1·3)	2 (3·6)	0	6 (5·5)	9 (1·9)
F4c	0	0	51 (35·4)	11 (18·3)	36 (45)	4 (7·3)	10 (17·9)	9 (14·3)	121 (24·9)
F5	0	2 (4·1)	0	0	0	0	0	0	2 (0·4)
F5a	0	1 (2)	0	0	0	0	0	0	1 (0·2)
F5b	4 (21·1)	14 (28·6)	1 (0·7)	0	1 (1·3)	0	0	0	20 (4·1)
F6a	1 (5·3)	0	0	0	0	1 (1·8)	0	0	2 (0·4)
Fx	0	3 (6·1)	16 (11·1)	20 (33·3)	7 (8·8)	5 (9·1)	5 (8·9)	4 (6·3)	60 (12·3)
Fy	0	0	10 (6·9)	5 (8·3)	1 (1·3)	1 (1·8)	1 (1·8)	1 (1·6)	19 (3·9)
S. *sonnei*	0	0	7 (4·9)	9 (15)	0	13 (23·6)	6 (10·7)	5 (7·9)	40 (7·6)

Numbers within parentheses indicate percentage in the current year.

Table 2. *Resistant trends of* Shigella *strains to different antibiotics*

Drugs	2001 ($n=19$)	2002 ($n=49$)	2003 ($n=144$)	2004 ($n=60$)	2005 ($n=80$)	2006 ($n=55$)	2007 ($n=56$)	2008 ($n=63$)	Total ($n=526$)	*P*
CFZ	0	0	3 (2·1)	0	4 (5)	3 (5·5)	17 (30·4)	10 (15·9)	37 (7·0)	0·000*
CTX	0	0	3 (2·1)	0	2 (2·5)	3 (5·5)	5 (8·9)	5 (7·9)	18 (3·4)	0·024*
AMC	18 (94·7)	45 (91·8)	133 (92·4)	51 (85)	76 (95)	41 (74·5)	48 (85·7)	57 (90·5)	469 (89·2)	0·017†
AMP	18 (94·7)	46 (93·9)	133 (92·4)	51 (85)	80 (100)	41 (74·5)	51 (91·1)	62 (98·4)	482 (91·6)	0·000†
GM	0	2 (4·1)	3 (2·1)	0	8 (10)	1 (1·8)	6 (10·7)	4 (6·3)	24 (4·6)	n.s.
TOB	1 (5·3)	2 (4·1)	3 (2·1)	4 (6·7)	12 (15)	2 (3·6)	3 (5·4)	11 (17·5)	38 (7·2)	n.s.
C	19 (100)	48 (98)	134 (93·1)	51 (85)	80 (100)	55 (100)	48 (85·7)	57 (90·5)	492 (93·5)	0·000†
TE	19 (100)	48 (98)	144 (100)	59 (98·3)	79 (98·8)	55 (100)	54 (96·4)	63 (100)	521 (99·0)	n.s.
SMZ	19 (100)	40 (81·6)	130 (90·3)	47 (78·3)	65 (81·3)	45 (81·8)	34 (60·7)	42 (66·7)	422 (80·2)	0·000†
PB	0	0	1 (0·7)	0	0	0	0	0	1 (0·2)	n.s.
NAL	18 (95)	49 (100)	144 (100)	60 (100)	80 (100)	55 (100)	56 (100)	63 (100)	525 (99·8)	n.s.
PI	18 (95)	49 (100)	144 (100)	60 (100)	80 (100)	55 (100)	56 (100)	63 (100)	525 (99·8)	n.s.
CIP	0	2 (4·1)	16 (11·1)	19 (31·7)	28 (35)	16 (29·1)	22 (39·3)	36 (57·1)	139 (26·4)	0·000*
F	0	0	0	0	1 (1·3)	0	0	0	1 (0·2)	n.s.

AMP, Ampicillin; AMC, amoxicillin; C, chloramphenicol; CFZ, cefamezin; CIP, ciprofloxacin; CTX, cefotaxime; F, furazolidone; GM, gentamicin; NAL, nalidixic acid; PB, polymyxin B; PI, pipemidic acid; SMZ, co-trimoxazole; TE, tetracycline; TOB, tobramycin.

n, Total number of strains; n.s., not significant.

Numbers within parentheses indicate percentage.

* Statistically significant increase mean rank in resistance to the antibiotic in question during the years of comparison.

† Significantly decreasing.

significant overall decrease in resistance for amoxicillin, ampicillin, chloramphenicol and co-trimoxazole and conversely there was a significant increase in resistance to cefamezin, cefotaxime and ciprofloxacin. All isolates were resistant to two or more agents and 50 different susceptibility patterns were recorded ranging in frequency from single isolates to three patterns together accounting for 68·7% of isolates. Common resistance patterns included a combination of ampicillin, amoxicillin, chloramphenicol, tetracycline, co-trimoxazole, nalidixic acid and pipemidic acid.

The percentages of *S. flexneri* and *S. sonnei* resistant to antibiotics are given in Table 3. The proportion of isolates with resistance to specific drugs varied by species. *S. flexneri* showed higher resistance than *S. sonnei* to amoxicillin (96·1% *vs.* 2·5%, $P<0·05$), ampicillin (97·1% *vs.* 25%, $P<0·05$), chloramphenicol (97·5% *vs.* 20·0%, $P<0·05$), and ciprofloxacin (21·8% *vs.* 5·0%, $P<0·05$), but resistance to co-trimoxazole, cefamezin, and gentamicin was more common in *S. sonnei* than *S. flexneri* isolates. Overall *S. flexneri* serotype 1b strains compared to other serotypes showed the least resistance to ampicillin, amoxicillin, chloramphenicol, nalidixic acid and pipemidic acid; one isolate only of variant Y showed resistance to polymyxin B and furazolidone.

DISCUSSION

The distribution of *Shigella* spp. appears to vary with geographical region. For example, a national survey in the USA reported that 80% of more than 1500 isolates were *S. sonnei* with 18% *S. flexneri*; *S. boydii* and *S. dysenteriae* together accounted for <2% of isolates [19]. However, in developing countries such as in sub-Saharan Africa [20], *S. flexneri* often predominates and surveys report frequencies of 73% in Peru [21] and >60% in Kolkata, India [22]. These differences are most likely associated with socio-economic factors, geography, and population density. The predominance of *S. flexneri* 2a has also been reported elsewhere [23, 24]. In the present study, this serotype along with 4c and variant X ranked in the top three most common S. flexneri serotypes during the period 2001–2008 and serotype 2a has remained dominant since 2006. Five serotypes of *S. flexneri* were detected at the start of the survey in 2001 and eight in 2008. The fluctuation of serotypes over the survey may explain the high incidence of dysentery in the study area but social and public health factors cannot be discounted. Nevertheless, these changes in serotype prevalence underscore the importance of surveillance monitoring as part of public health strategies for reducing the incidence of shigellosis and, moreover, provide important data relevant to vaccine development.

The development of resistance to antimicrobial drugs by *Shigella* necessitates a continuous surveillance programme. It is clear from the study that treatment of infections caused by *S. flexneri* in this province of China was compromised by widespread resistance to ampicillin, amoxicillin, chloramphenicol and tetracycline, the drugs most widely used for treatment of shigellosis. Resistance to these drugs remained high and relatively steady over the survey period, with only minor fluctuations. It may be possible to reverse this trend of resistance as was achieved in Israel where an almost complete ban on the use of chloramphenicol for the treatment of shigellosis brought about a steady decrease in the number of *Shigella* strains resistant to this drug, reaching almost zero with a concomitant increase in susceptibility [25]. Our study indicated that resistance of *Shigella* isolates to cefamezin, cefotaxime and ciprofloxacin is increasing significantly, as in the USA [26], and multidrug resistance is common. Recently, third-generation cephalosporin resistance in *Shigella* isolates has rapidly emerged in India and in parts of China [27, 28] and this is most likely due to overuse of these agents in healthcare and other sectors such as agriculture owing to a lack of legislative guidelines limiting their use [11]. On the other hand, our data suggest that cephalosporins still have a place along with polymyxin B for the treatment of shigellosis in this region of China, but the finding of ceftriaxone-resistant strains of *S. flexneri* early in the survey as well as reports from Shenyang in 2000 and in France in 1995 [29, 30] suggest that cephalosporins should be used with caution.

The data show that the resistant phenotypes were also serotype-specific. Overall, *S. flexneri* was more frequently resistant to ampicillin, amoxicillin, chloramphenicol and ciprofloxacin, alone or in combination, than *S. sonnei*. A possible explanation is that *S. sonnei* infections are clinically less severe than *S. flexneri*, and most cases would not receive antimicrobial therapy thus negating the selective pressure from antibiotic therapy as shown by several other studies [10, 31–33]. Interestingly, resistance to co-trimoxazole was more common in *S. sonnei* than *S. flexneri* isolates but the reason for this is unknown.

Table 3. *The resistant phenotypes of* Shigella *serotypes*

	Serotype																	
Drugs	F1a ($n=59$)	F1b ($n=8$)	F1c ($n=1$)	F2a ($n=130$)	F2b ($n=30$)	F2ab ($n=9$)	F3a ($n=15$)	F4 ($n=9$)	F4c ($n=121$)	F5 ($n=2$)	F5a ($n=1$)	F5b ($n=20$)	F6 ($n=2$)	Fx ($n=60$)	Fy ($n=19$)	*Flexneri* ($n=486$)	*Sonnei* ($n=40$)	*P*
CFZ	4 (6·8)	1 (12·5)	0	9 (6·9)	4 (13·3)	1 (11·1)	1 (6·7)	0	5 (3·8)	0	0	1 (5)	0	2 (3·3)	2 (10·5)	30 (6·2)	7 (17·5)	0·007
CTX	2 (3·4)	0	0	5 (3·8)	3 (10)	1 (11·1)	0	0	2 (1·5)	0	0	0	0	1 (1·6)	1 (5·3)	15 (3·1)	2 (5)	n.s.
AMC	57 (96·6)	4 (50)	1 (100)	127 (97·7)	30 (100)	8 (88·8)	14 (93·3)	9 (100)	118 (97·7)	2 (100)	1 (100)	17 (85)	2 (100)	60 (100)	18 (94·7)	468 (96·1)	1 (2·5)	0·000
AMP	57 (96·6)	4 (50)	1 (100)	127 (97·7)	30 (100)	9 (100)	14 (93·3)	9 (100)	120 (99·2)	2 (100)	1 (100)	20 (100)	2 (100)	58 (96·7)	18 (94·7)	472 (97·1)	10 (25)	0·000
GM	2 (3·4)	0	0	4 (3·1)	0	0	0	0	4 (3)	0	0	0	0	2 (3·3)	0	12 (2·4)	12 (30)	0·000
C	57 (96·6)	7 (87·5)	1 (100)	125 (96·2)	30 (100)	9 (100)	15 (100)	9 (100)	118 (97·7)	2 (100)	1 (100)	20 (100)	2 (100)	60 (100)	18 (94·7)	474 (97·5)	8 (20)	0·000
TE	59 (100)	8 (100)	1 (100)	128 (98·5)	30 (100)	9 (100)	15 (100)	9 (100)	121 (100)	2 (100)	1 (100)	20 (100)	2 (100)	58 (96·7)	19 (100)	482 (99·2)	39 (97·5)	n.s.
SMZ	41 (69·5)	4 (50)	0	100 (77)	21 (70·0)	7 (77·8)	13 (86·7)	5 (55·5)	108 (89·3)	1 (50)	1 (100)	15 (75)	2 (100)	42 (70)	14 (73·7)	374 (77·0)	39 (97·5)	0·002
PB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (5·3)	1 (0·2)	0	n.s.
NAL	57 (96·6)	7 (87·5)	1 (100)	126 (97)	30 (100)	9 (100)	15 (100)	9 (100)	118 (97·7)	2 (100)	1 (100)	20 (100)	2 (100)	60 (100)	18 (94·7)	475 (97·7)	40 (100)	n.s.
PI	57 (96·6)	7 (87·5)	1 (100)	126 (97)	30 (100)	9 (100)	15 (100)	9 (100)	118 (97·7)	2 (100)	1 (100)	20 (100)	2 (100)	60 (100)	18 (94·7)	475 (97·7)	40 (100)	n.s.
CIP	21 (35·6)	1 (12·5)	0	64 (49·2)	8 (26·7)	1 (11·1)	0	2 (22·2)	22 (18·3)	0	0	0	0	13 (21·7)	3 (15·8)	135 (27·8)	2 (5)	0·002
F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (5·3)	1 (0·2)	0	n.s.
TOB	5 (8·5)	1 (12·5)	0	9 (6·9)	4 (13·3)	1 (11·1)	1 (6·7)	0	9 (7·6)	0	0	1 (5)	0	4 (6·7)	1 (5·3)	36 (7·4)	2 (5)	n.s.

AMP, Ampicillin; AMC, amoxicillin; C, chloramphenicol; CFZ, cefamezin; CIP, ciprofloxacin; CTX, cefotaxime; F, furazolidone; GM, gentamicin; NAL, nalidixic acid; PB, polymyxin B; PI, pipemidic acid; SMZ, co-trimoxazole; TE, tetracycline; TOB, tobramycin.

n, Total number of strains; n.s., not significant.

Numbers within parentheses indicate percentage.

However, a major limitation of comparing the susceptibilities of the two species is the great discrepancy between the number of isolates of each species; 12 times more isolates of *S. flexneri* than *S. sonnei* were examined. Severe gastroenteritis, some of which is due to *Shigella*, is often treated empirically with ciprofloxacin especially in the community. As a consequence the finding of more than one-quarter of *S. flexneri* isolates to be resistant to ciprofloxacin compromises its empirical use for treatment of shigellosis [13, 22]. Therefore, treatment for severe shigellosis, especially in children and the immunosuppressed, must be guided by continued surveillance data of emerging resistance in *Shigella* isolates and these data should inform timely and appropriate recommendations for antimicrobial therapy.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268812002543.

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DECLARATION OF INTEREST

None.

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