Antimicrobial susceptibility of *Shigella* isolates in eight Asian countries, 2001-2004

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Background and Purpose: Shigellosis is a major health problem in developing countries, causing 91 million episodes and 414,000 deaths in Asia annually. Because of increasing trends towards drug resistance, this study was undertaken to monitor local resistance patterns of *Shigella* isolates from 8 Asian countries.

Methods: Ninety eight *Shigella* isolates collected from 8 centers in 8 Asian countries from July 2001 to July 2004 were analyzed in terms of serogroup distribution and antimicrobial susceptibility.

Results: The most common serogroup of *Shigella* isolates was *Shigella flexneri* (49/98, 50%), followed by *Shigella sonnei* (44/98, 45%). The highest resistance rate was found for trimethoprim-sulfamethoxazole (81%), followed by tetracycline (74%) and ampicillin (53%). Overall, 76 *Shigella* isolates (78%) were multidrug-resistant strains; *S. flexneri* had a higher multidrug resistance rate than *S. sonnei* (74% vs 23%). Increasing ciprofloxacin and ceftriaxone resistance was observed; approximately 10% and 5% of isolates were resistant to ciprofloxacin and ceftriaxone, respectively. Five ceftriaxone-non-susceptible strains (from Taiwan [3], Hong Kong [1] and The Philippines [1]) and 10 ciprofloxacin-non-susceptible strains (from Hong Kong [2], The Philippines [1], Korea [2], Vietnam [4] and Sri Lanka [1]) were isolated.

Conclusions: High rates of multidrug resistance and steady increases in ceftriaxone and ciprofloxacin resistance of *Shigella* are serious pubic health concerns in Asian countries. Continuous monitoring of resistance patterns among *Shigella* isolates is necessary.

Key words: Asia; Microbial sensitivity tests; *Shigella*

Introduction

Shigellosis is a major health problem in developing countries and is associated with high morbidity and mortality. The World Health Organization (WHO) has estimated that 3.5 million children die from diarrheal disease each year, 600,000 of them from shigellosis alone. Shigellosis is caused by *Shigella* spp., including *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*. Each *Shigella* serogroup contains many serotypes. The prevalence of the *Shigella* serogroup varies over time and in different geographical areas. *S. sonnei* is the most prevalent (>80%) in the developed countries. In developing countries, *S. flexneri* is the most common. *Shigella* infections can lead to illness ranging from mild, self-limited diarrhea to severe dysentery with frequent passage of blood and mucus, high fever, cramps, tenesmus, and in rare cases, bacteremia. The severity of disease is determined in part by the infecting species; infections due

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to *S. dysenteriae* usually progress to dysentery, which may also occur in infections caused by *S. flexneri*, whereas *S. boydii* and *S. sonnei* generally cause a self-limited, watery diarrhea. Complications of shigellosis are seen most frequently in children, the elderly, and the immunocompromised [1].

Antibiotic treatment is usually indicated for individuals with moderate or severe symptoms of shigellosis [2], because it can reduce the duration and severity of symptoms, excretion of organisms [3], and prevent potentially lethal complications [1]. However, increasing antimicrobial resistance of the *Shigella* spp. has been reported worldwide [4-8]. Because of the lack of epidemiologic data regarding antimicrobial resistance of *Shigella* isolates in Asian regions, this study was undertaken to analyze serogroup distribution and antimicrobial susceptibility of *Shigella* isolates from 8 Asian countries collected during the period 2001-2004.

Methods

Bacterial isolates
From July 2001 to July 2004, a total of 98 *Shigella* isolates from clinical specimens of patients with shigellosis were collected by the Asian Network for Surveillance of Resistant Pathogens (ANSORP). There were 8 centers in 8 Asian countries participating in the ANSORP study — Samsung Medical Center, Seoul, Korea; Chang Gung Children's Hospital, Tao-yuan, Taiwan; National University of Singapore and Singapore General Hospital, Singapore; Chulalong-korn University, Bangkok, Thailand; University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam; Research Institute for Tropical Medicine, Manila, The Philippines; Princess Margaret Hospital, Hong Kong; and University of Colombo, Colombo, Sri Lanka.

Serotyping
Shigella isolates were biochemically identified by standard methods and grouped serologically by a slide agglutination test with antisera (Becton Dickinson and Company, Franklin Lakes, NJ, USA).

Antimicrobial susceptibility test
Antimicrobial susceptibility testing was performed with the standard disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) [9]. A total of 6 antimicrobials, ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, cef-triaxone, ciprofloxacin, and tetracycline, were tested.

Minimal inhibitory concentration (MIC) was determined by broth microdilution test. NCCLS criteria of zone diameter and MIC values were used to classify the isolates as susceptible, intermediate or resistant. The isolates in the "intermediate" category were regarded as "resistant" for analysis in this study.

Results

A total of 98 *Shigella* isolates collected during the period from July 2001 to July 2004 were analyzed. Of these, 6, 36, 11, 7, 12, 9, 16, and 1 were obtained from Korea, Taiwan, Singapore, Vietnam, The Philippines, Hong Kong, Sri Lanka, and Thailand, respectively. As shown in Fig. 1, *S. flexneri* accounted for 50% of the isolates, representing the most common serogroup, followed by *S. sonnei* (45%). *S. sonnei* was found more frequently than *S. flexneri* in Taiwan and Sri Lanka, accounting for 56% (20/36) and 68% (11/16) of the isolates, respectively. One *S. dysenteriae* isolate resistant to ampicillin was found in Singapore. Two *S. boydii* isolates, which were also from Singapore, were sensitive to all 6 antibiotics tested. We further analyzed the distribution of serotypes in each *Shigella* serogroup. It was found that the most common serotype of *S. flexneri* was 2a (60%) and that of *S. sonnei* was phase II (70%).

The susceptibility of the *Shigella* isolates to 6 antibiotics tested is shown in Table 1. Seventy six of 98 *Shigella* isolates (78%) were multidrug-resistant strains (resistant to at least two antibiotics). Forty seven (48%) were resistant to both ampicillin and trimethoprim-sulfamethoxazole. A total of 11 isolates (11%) were susceptible to all antibiotics tested. The highest resistance rate was found with trimethoprim-sulfamethoxazole (81%), followed by tetracycline (74%) and ampicillin (53%). The five ceftriaxone-non-susceptible strains were isolated from Taiwan (3), Hong Kong (1), and The Philippines (1). Ten
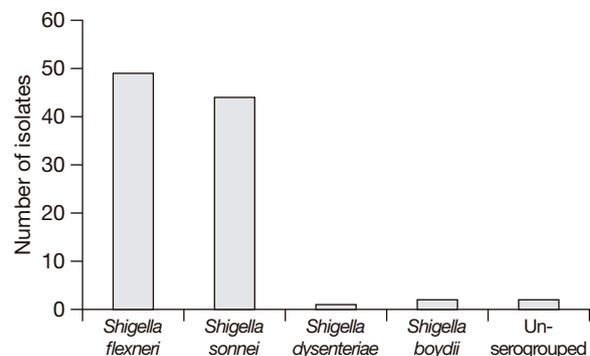

Fig. 1. Distribution of *Shigella* serogroups in the 8 Asian countries.

Table 1. Antimicrobial susceptibilities of *Shigella* isolates (n = 98)

Antimicrobial agent	Resistant isolates No. (%)	MIC (µg/mL)		
		Range	MIC_{50}	MIC_{90}
Ampicillin	52 (53)	0.5->128	64	>128
Chloramphenicol	37 (38)	1->28	4	>128
Trimethoprim-sulfamethoxazole	78 (81)	<0.12/2.37->4/76	>4/76	>4/76
Tetracycline	76 (74)	0.25->64	>64	>64
Ceftriaxone	5 (5)[a]	<0.12->128	<0.12	<0.12
Ciprofloxacin	10 (10)[b]	<0.06->64	<0.06	2

Abbreviations: MIC = minimal inhibitory concentration; MIC_{50} = MIC inhibiting 50% of isolates; MIC_{90} = MIC inhibiting 90% of isolates
[a]Two *Shigella* isolates were intermediately resistant.
[b]Four *Shigella* isolates were intermediately resistant.

ciprofloxacin-non-susceptible strains were isolated from Hong Kong (2), The Philippines (1), Korea (2), Vietnam (4), and Sri Lanka (1).

Because *S. flexneri* and *S. sonnei* accounted for most *Shigella* isolates, their antimicrobial susceptibility patterns were compared with each other. Overall, 73% of *S. flexneri* and 30% of *S. sonnei* were resistant to ampicillin. Sixty seven percent of *S. flexneri* and 7% of *S. sonnei* were resistant to chloramphenicol. Sixty seven percent of *S. flexneri* and 27% of *S. sonnei* were resistant to both ampicillin and trimethoprim-sulfamethoxazole. In addition, values for MIC inhibiting 90% of isolates of *S. flexneri* for chloramphenicol and ciprofloxacin were higher than those of *S. sonnei* (data not shown).

As shown in Table 2, 74% of *S. flexneri* were resistant to at least three of the antibiotics tested. The most common antimicrobial resistance pattern was ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline. Twenty three percent of *S. sonnei* were resistant to at least three antibiotics. The most common antimicrobial resistance pattern was trimethoprim-sulfamethoxazole, tetracycline. Multidrug resistance to at least three drugs was more common among *S. flexneri* than *S. sonnei* (74% vs 23%).

Discussion

S. sonnei is the predominant cause of shigellosis in developed countries and more common in children than in adults [10]. But in developing countries with low socioeconomic conditions, *S. flexneri* is the predominant species. In Taiwan, approximately 250 to 500 cases of shigellosis were identified annually from 1995 to 2000, with an average annual incidence of 1-3 cases per 100,000 persons [11,12]. The majority (51.0%) occurred in children younger than 9 years

Table 2. Antimicrobial resistance patterns of *Shigella flexneri* and *Shigella sonnei*

Resistance pattern	*S. flexneri* (n = 49) No. (%)	*S. sonnei* (n = 44) No. (%)
Amp, Chl, Sxt, Tet, Ctr, Cip	2 (4)	0 (0)
Amp, Chl, Sxt, Tet, Cip	4 (8)	0 (0)
Amp, Chl, Sxt, Tet	24 (50)	0 (0)
Amp, Sxt, Tet, Ctr	0 (0)	3 (7)
Amp, Chl, Tet	3 (6)	0 (0)
Amp, Chl, Sxt	0 (0)	2 (5)
Amp, Sxt, Tet	3 (6)	5 (11)
Amp, Sxt	0 (0)	1 (2)
Amp, Tet	0 (0)	2 (5)
Sxt, Tet	7 (14)	16 (36)
Tet, Ctr	0 (0)	2 (5)
Tet	1 (2)	1 (2)
Sxt	3 (6)	5 (11)
No resistance	2 (4)	7 (16)

Abbreviations: Amp = ampicillin; Chl = chloramphenicol; Sxt = trimethoprim-sulfamethoxazole; Tet = tetracycline; Ctr = ceftriaxone; Cip = ciprofloxacin

of age, and were caused by *S. flexneri* and *S. sonnei*, which accounted for 73.3% and 26.5% of the total strains isolated in Taiwan, respectively [11]. However, in this study, we observed the trends of increased prevalence of *S. sonnei* (56%) and decreased prevalence of *S. flexneri* (44%), although the isolate number from Taiwan was small.

Increasing antimicrobial resistance has complicated the selection of empiric antibiotics for the treatment of shigellosis. In 2000, data from Oregon, United States revealed a high rate of resistance to ampicillin (59%), trimethoprim-sulfamethoxazole (63%), and of multidrug resistance (13%) among *Shigella* isolates [6]. A higher multidrug-resistant rate (78%) among *Shigella* isolates was found in our study. Although only limited numbers of strains were examined, this result showed a clear trend that multidrug-resistant *Shigella* in Asian countries has been increasing, and this may be due to a worsening situation with regard to antibiotic overuse in both humans and animals. However, there were some limitations in this study. Some countries such as Thailand provided only a small number of isolates. This collection bias may cause overestimation of drug resistance of *Shigella* isolates in these countries.

There have been several previous reports about the evolution of ciprofloxacin and ceftriaxone resistance among *Shigella* strains. Ciprofloxacin resistance was documented in approximately 0.5% of *Shigella* isolates in Israel from 1998 to 2000 (4.0% of *S. flexneri* and 0.2% of *S. sonnei*) [13], 0.06% in the United States from 1999 to 2002 (0.3% of *S. flexneri*) [14] and 0.2% in Asia from 2000 to 2004 (1.6% of *S. flexneri* and 0.2% of *S. sonnei*) [15]. We found no previous report of ceftriaxone resistance in Asia, but 2% of *S. sonnei* in the United States from 1995 to 1998 were resistant to ceftriaxone.

Higher rates of resistance to ciprofloxacin (10%) [12% of *S. flexneri* and 5% of *S. sonnei*] and ceftriaxone (5%) [4% of *S. flexneri* and 7% of *S. sonnei*] were demonstrated in this study. In Taiwan, 27% of *S. sonnei* were resistant to ceftriaxone and no strain showed ciprofloxacin resistance from 1999 to 2002 [11]. This study also found no ciprofloxacin resistance among *Shigella* isolates from Taiwan.

According to the WHO revised guidelines for the control of shigellosis (2005), ciprofloxacin is now the drug of choice for all patients with bloody diarrhea, irrespective of their age [16]. Although use of ciprofloxacin in pediatric patients is limited by concerns about the potential risk of damage to growing cartilage [17,18], fluoroquinolones are generally safe for the treatment of shigellosis in children [19,20]. Ceftriaxone and azithromycin are considered the second-line drugs for the treatment of multidrug-resistant strains of Shigella in all age groups [16]. Because high rates of resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole and tetracycline among *Shigella* isolates exist worldwide, these drugs are no longer recommended as empiric therapy for shigellosis [16].

In conclusion, empiric antimicrobial therapy for shigellosis requires knowledge of the antimicrobial resistance pattern of *Shigella* strains circulating locally. Physicians should be aware of the high multidrug resistance rate of *Shigella* spp., especially the increasing resistance to ceftriaxone and fluoroquinolones. *S. flexneri* is still the most prevalent species in most Asian countries and its higher antimicrobial resistance rate compared with *S. sonnei* is a cause for concern. Continuous monitoring of resistance patterns at national and international levels is necessary to control the spread of resistance in *Shigella*.

Acknowledgment

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