Shigella Species and Serotypes among Clinical Isolates in Thailand from 2001 to 2005

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Objective: *To study the species and the serotypes of the clinical isolates of Shigella obtained from patients in Thailand*
Material and Method: *The World Health Organization National Salmonella and Shigella Center, Thailand, had confirmed the species and performed serotype identification of 1,913 clinical isolates of Shigella collected from the laboratory network of Department of Medical Sciences and the collaborated hospitals across Thailand from 2001 to 2005.*
Results: *Between the year 2001 and 2005, 728, 481, 160, 247, 297 clinical isolates were tested, respectively. There were 5 isolates of S. dysenteriae (group A), 416 isolates of S. flexneri (group B), 4 isolates of S. boydii (group C) and 1,488 isolates of S. sonnei (group D). A total of 21 Shigella serotypes were identified and there were 3 serotypes in group A, 11 serotypes in group B, 4 serotypes in group C, and 3 serotypes in group D. Throughout these five years, the five common serotypes were S. sonnei Phases I and II, 28.6% (548 isolates); S. sonnei Phase I, 24.6% (470 isolates); S. sonnei Phase II, 24.6% (470 isolates); S. flexneri Type 2a, 10.9% (208 isolates), and S. flexneri Type 3a, 6.3% (121 isolates), respectively.*
Conclusion: *At the national scale in Thailand from 2001 to 2005, S. sonnei was the most frequent Shigella spp. isolated from patients in Thailand. In addition, S. dysenteriae and S. boydii were extremely uncommon. These findings are important in future vaccine development.*

Keywords: *Serotyping, Shigella, Shigella sonnei, Thailand*

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Shigella spp Gram-negative bacillus bacteria in the family Enterobacteriaceae along with *Salmonella* spp and *Escherichia coli*. *Shigella* spp was first reported in 1898 by Kiyoshi Shiga during an epidemic of dysentery in Japan[1,2]. The organism is the causative agent of bacillary dysentery or shigellosis. Clinical manifestations of classic bacillary dysentery include fever, vomiting, abdominal pain, tenesmus (painful straining to pass stool). The stool usually contains blood, mucus, and inflammatory cells which result from invasion of the pathogen into the intestinal mucosa[3]. Transmission takes place by fecal-oral route, generally via person to person by direct contact but sometimes may occur through contaminated vectors such as food, water, flies and fomites.

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Shigella spp are currently classified into four groups as follows: *Shigella dysenteriae* (group A)-the original organism reported by Shiga, *S. flexneri* (group B), *S. boydii* (group C), and *S. sonnei* (group D). All groups cause disease in humans, although with some differences in clinical spectrum. Severe dysentery is most likely due to *S. dysenteria*e, especially those belonging to type 1, but occurs less commonly with *S. flexneri*, and is least likely with *S. sonnei*. *Shigella* bacteremia, an extraintestinal complication occurred in patients in developing countries, was related to the high prevalence of *S. dysenteria*e type 1 and *S. flexneri* infections and the poor nutrition of the host[4].

Shigellosis is still an important cause of gastroenteritis, even in developed countries, resulting in an estimated of 450,000 cases in the United States each year, and more than 6,000 cases require hospitalization[5]. *Shigella* spp appears to be more ubiquitous among impoverished populations in developing countries such as Bangladesh, China, Pakistan, Indonesia, Vietnam, and Thailand. These bacteria cause disease with an annual incidence of 13.2 per 1,000 in children under 5 year and 2.1 per 1,000 in all age groups[6].

The four groups of *Shigella* spp are further classified into numerous serotypes. Serotype identification of *Shigella* spp. is readily performed using agglutination by specific antisera. Today, *S. dysenteriae* has 15 serotypes (*S. dysenteriae* Type 1-15)[7], *S. flexneri* has 13 serotypes (S. *flexneri* Type 1a, 1b, 2a, 2b, 3a, 3b, 3c, 4a, 4b, 5, 6, x and y)[8], *S. boydii* has 20 serotypes (*S. boydii* Type 1-20)[9], and *S. sonnei* has 2 phases (*S. sonnei* Phase I and *S. sonnei* Phase II). Although cases of shigellosis are declining compared to the past, the present serotypes are continuously replaced by new different serotypes in several geographic areas[7,9]. Therefore, *Shigella* serotypes circulating in Thailand are reported here.

Material and Method

Between 2001 and 2005, clinical *Shigella* isolates obtained from the patients, mostly stool cultures, at the laboratory network of Department of Medical Sciences and the collaborating public, private and university hospitals in Thailand were continuously submitted to the World Health Organization (WHO) National *Salmonella* and *Shigella* Center. All isolates were confirmed for the species using standard culture techniques and biochemical tests according to guidelines of the United States' Centers for Disease Control and Prevention[10]. Serotype identification was performed using specific antisera with slide agglutination according to the method of Edwards and Ewing (1972)[8]. The numbers of common *Shigella* serotypes are compared year by year to determine the possible trends of serotype shifting in Thailand.

Results

The authors had performed species confirmation and serotype identification of all 1,913 clinical isolates from 2001 to 2005. For each calendar year, there were 728, 481, 160, 247, and 297 clinical isolates, respectively, submitted to the center. Table 1 shows the frequency of all four groups of *Shigella* species. *S. sonnei* was the most common species isolated, consisting of approximately 80% of all *Shigella* spp each year, while *S. dysentery* and *S. boydii* were very uncommon (Table 1). The serotypes of *S. dysenteriae* isolates from 2001 to 2003 belonged to serotype 2; those in 2005 belonged to serotype 1; while the four isolates of *S. boydii* were serotypes 2, 4, 10, and 12. The serotype distribution of *S. sonnei* and *S. flexneri* are shown in Tables 2 and 3, respectively.

The five common serotypes identified were as follows: *S. sonnei* Phases I and II (28.6% each), *S. sonnei* Phase I (24.6%), *S. sonnei* Phase II (24.6%), *S. flexneri* Type 2a (10.9%), and *S. flexneri* Type 3a (6.3%).

Table 1. All *Shigella* species isolated from 2001 to 2005

Strains	Year					Total, No. (%)
	2001, No. (%)	2002, No. (%)	2003, No. (%)	2004, No. (%)	2005, No. (%)	
S. dysenteriae	1 (0.1)	1 (0.2)	1 (0.6)	-	2 (0.7)	5 (0.3)
S. flexneri	152 (20.9)	79 (16.4)	56 (35.0)	66 (26.7)	63 (21.2)	416 (21.7)
S. boydii	1 (0.1)	1 (0.2)	2 (1.3)	-	-	4 (0.2)
S. sonnei	574 (78.8)	400 (83.2)	101 (63.1)	181 (73.3)	232 (78.1)	1,488 (77.8)
Total	728 (100)	481 (100)	160 (100)	247 (100)	297 (100)	1,913 (100)

Table 2. Serotypes of *Shigella flexneri* isolated from 2001 to 2005

Strains	Year					Total, No. (%)
	2001, No. (%)	2002, No. (%)	2003, No. (%)	2004, No. (%)	2005, No. (%)	
Type 1a	-	7 (8.9)	5 (8.9)	-	2 (3.2)	14 (3.4)
Type 1b	12 (7.9)	9 (11.4)	3 (5.4)	1 (1.5)	-	25 (6.0)
Type 2a	73 (48.0)	48 (60.8)	28 (50.0)	40 (60.6)	19 (30.2)	208 (50.0)
Type 2b	1 (0.7)	3 (3.8)	-	4 (6.1)	3 (4.8)	11 (2.6)
Type 3a	43 (28.3)	10 (12.7)	20 (35.7)	18 (27.3)	30 (47.6)	121 (29.1)
Type 3b	17 (11.2)	-	-	-	-	17 (4.1)
Type 4a	1 (0.7)	-	-	-	-	-
Type 5	1 (0.7)	2 (2.5)	-	-	-	3 (0.7)
Type 6	-	-	-	1 (1.5)	7 (11.1)	8 (1.9)
Type x	-	-	-	-	1 (1.6)	1 (0.2)
Type y	4 (2.6)	-	-	2 (3.0)	1 (1.6)	7 (1.7)
Total	152 (100)	79 (100)	56 (100)	66 (100)	63 (100)	416 (100)

Table 3. Serotypes of *Shigella sonnei* isolated from 2001 to 2005

Strains	Year					Total, No. (%)
	2001, No. (%)	2002, No. (%)	2003, No. (%)	2004, No. (%)	2005, No. (%)	
Phase I	233 (40.6)	115 (28.8)	27 (26.7)	55 (30.4)	40 (17.2)	470 (31.6)
Phase II	59 (10.3)	127 (31.8)	62 (61.4)	82 (45.3)	140 (60.3)	470 (31.6)
Phase I&II	282 (49.1)	158 (39.5)	12 (11.9)	44 (24.3)	52 (22.4)	548 (36.8)
Total	574 (100)	400 (100)	101 (100)	181 (100)	232 (100)	1,488 (100)

Fig. 1-5 demonstrate the distribution of the five common serotypes, compared to the rest of other serotypes isolated in each year.

Discussion

The number of *Shigella* isolates submitted to the WHO National *Salmonella* and *Shigella* Center had drastically decreased during the study period. There was 59.2 percent decrease from 2001 to 2005 (728 isolates in 2001 and 297 isolates in 2005). This trend may represent the decrease in prevalence of *shigellosis* in Thailand. *S. sonnei* was the most common serogroup in each year of the study. In addition, when patterns of *Shigella* serotypes during the study period were analyzed, *S. sonnei* Phase II has increased from 8.1% to 47.1%. In the meantime, *S. sonnei* Phase I, which was commonly isolated in 2001, has been decreasingly isolated from 32.0% to 13.5%. However, *S. sonnei* Phase I could change to be Phase II when the isolates are subcultured from the organisms in preservative media. Therefore, the increase of *S. sonnei* Phase II should be interpreted with caution. Moreover, *S. sonnei* Phases I and II were also common in Thailand because both of these 2 phases could be changed every time when subculture.

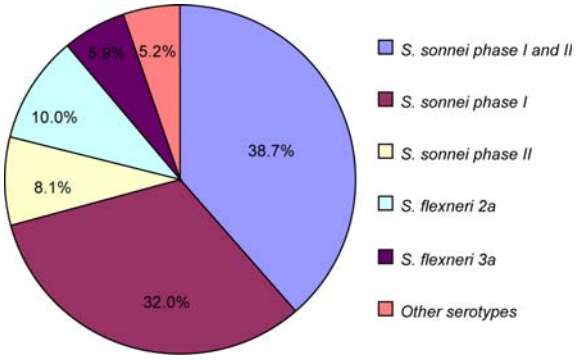

Fig. 1 *Shigella* serotypes isolated in 2001

S. flexneri was the second most common species in the authors data, and *S. flexneri* Type 2a

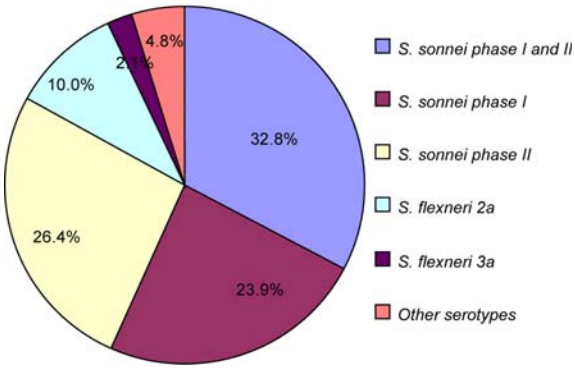

Fig. 2 *Shigella* serotypes isolated in 2002

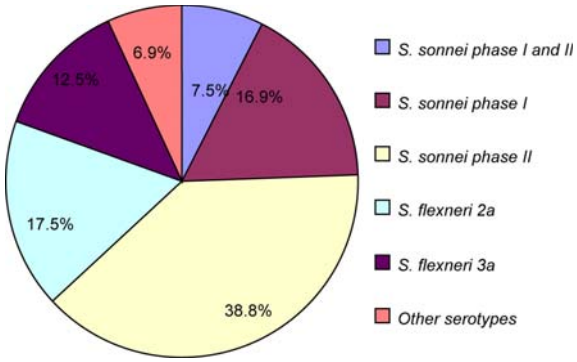

Fig. 3 *Shigella* serotypes isolated in 2003

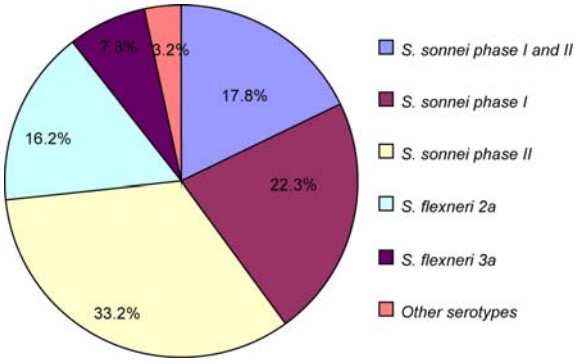

Fig. 4 *Shigella* serotypes isolated in 2004

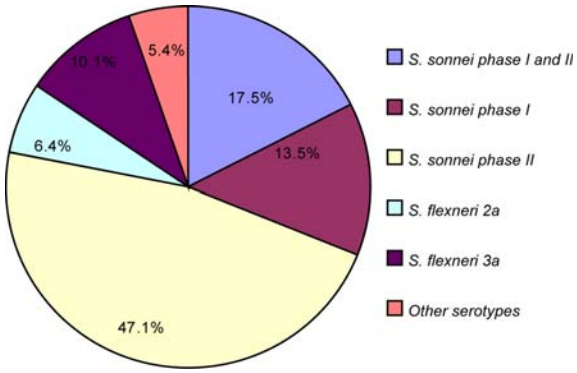

Fig. 5 *Shigella* serotypes isolated in 2005

(50%) and *S. flexneri* Type 3a (29.1%) were the frequent serotypes. The more predominant of *S. sonnei* over *S. flexneri* was consistent with the findings of the population-based surveillance study between 2000 and 2003 in Kaengkhoi District, Saraburi Province, Central Thailand[11]. However, the common serotypes of *S. flexneri* in the Kaengkhoi study were 1b (23%), 2a (36%), and 3b (28%), which differ from the present findings. These differences may be explained by the different sample sizes (416 isolates in the authors' study and 22 isolates in Kaengkhoi study), or it may reflect the differences in the distribution of *Shigella* serotypes in various parts of the country. In a recent multicenter study of shigella diarrhea in six Asian countries including Bangladesh, China, Pakistan, Indonesia, Vietnam, and Thailand[6], *S. flexneri* Type 2a (29%) and *S. flexneri* Type 3a (14%) are the two major serotypes among 1,976 *S. flexneri* isolates. In our data, only 5 clinical isolates of *S. dysenteriae* and 4 clinical isolates of *S. boydii* were isolated during these study periods and the Kaengkhoi study reported no clinical isolates of *S. dysenteriae* or *S. boydii*.

The above findings are in contrast with the data over the past two decades in Thailand, which showed that *S. flexneri* was more frequently isolated than *S. sonnei*[12,13], and *S. dysenteriae* still prevailed. In the developed countries, several previous studies have shown that *S. sonnei* is more dominant than other *Shigella* spp[14]. In a survey that was performed in six developing countries in Asia, *S. flexneri* was the most commonly isolated *Shigella* spp in five countries other than Thailand[6]. It is not clear as to what could be an explanation for the difference in the prevalence of *Shigella* spp in Thailand and other Asian countries in that the patterns in Thailand are similar to those in developed countries. The extent of the contribution of Thai economic transition to be more industrialized or frequent traveling of people among Thailand and other industrialized countries are not known. Moreover, since *S. sonnei* and *S. flexneri* tend to be more resistant to

antimicrobial agents, therefore, selective advantage from extensive use of antimicrobial agents might also be involved in this epidemiological pattern as well.

The study in Kaengkhoi District also reported that more than 90% of *S. sonnei* and *S. flexneri* isolates were resistant to tetracycline and cotrimoxazole (trimethoprim-sulfamethoxazole), and *S. flexneri* was more resistant to ampicillin and chloramphenicol. *S. flexneri* isolates were also reported to be resistant to ciprofloxacin in China (6%), Pakistan (3%), and Vietnam (2%)[6]. Unfortunately, our data on antibiotic susceptibility are not available to be reported at the time being. *Shigellosis* is still a considerable public health burden in developing countries and antimicrobial-resistant strains have continuously emerged. Therefore, the preventable vaccine might be a tool to control *shigellosis*. The data of distribution of *Shigella* spp. and serotypes are very important in vaccine development. Given our findings and the available data, polyvalent or cross-protective *Shigella* vaccine is needed to prevent shigellosis in developing countries.

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ชิเกลล่าสปีซีย์ และซีโรทัยป์จากผู้ป่วยในประเทศไทยระหว่างปี พ.ศ. 2544-2548

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วัตถุประสงค์: *ศึกษาซีโรทัยป์ของเชื้อชิกเกลล่าที่ก่อโรคในผู้ป่วยในประเทศไทย*

วัสดุและวิธีการ: *ศูนย์ทดสอบยืนยันเชื้อซัลโมเนลล่าและชิกเกลล่าได้ตรวจสอบและยืนยันซีโรทัยป์ของเชื้อชิกเกลล่าจำนวน 1,913 ตัวอย่างของผู้ป่วยที่รับจากศูนย์วิทยาศาสตร์การแพทย์ และโรงพยาบาลในเครือข่ายทั่วประเทศระหว่างปี พ.ศ. 2544-2548*

ผลการศึกษา: *เชื้อชิกเกลล่าที่ตรวจในแต่ละปีระหว่างปี พ.ศ. 2544 - 2548 มีจำนวน 728, 481, 160, 247 และ 297 ตัวอย่าง ตามลำดับ ผลการตรวจยืนยันเชื้อสามารถจำแนกเชื้อเป็น Shigella dysenteriae (Group A) จำนวน 5 ตัวอย่าง, Shigella flexneri (Group B) จำนวน 416 ตัวอย่าง, Shigella boydii (Group C) จำนวน 4 ตัวอย่าง และ Shigella sonnei (Group D) จำนวน 1,488 ตัวอย่าง เมื่อวิเคราะห์เป็นซีโรทัยป์ตลอดปีที่ศึกษาพบจำนวน 21 ซีโรทัยป์ที่แตกต่างกัน สำหรับซีโรทัยป์ที่พบบ่อย 5 ลำดับแรก คือ Shigella sonnei Phase I&II ร้อยละ 28.6 (548 ตัวอย่าง), Shigella sonnei Phase I ร้อยละ 24.6 (470 ตัวอย่าง), Shigella sonnei Phase II ร้อยละ 24.6 (470 ตัวอย่าง), Shigella flexneri Type 2a ร้อยละ 10.9 (208 ตัวอย่าง) และ Shigella flexneri Type 3a ร้อยละ 6.3 (121 ตัวอย่าง) ตามลำดับ*

สรุป: *ข้อมูลระดับประเทศของประเทศไทยระหว่างปี พ.ศ. 2544 - 2548 พบเชื้อ Shigella sonnei ก่อโรคในผู้ป่วยมากกว่าเชื้อ Shigella flexneri ในขณะที่พบเชื้อ Shigella dysenteriae และ Shigella boydii ได้น้อยมาก ซึ่งเป็นข้อมูลสำคัญในการพัฒนาวัคซีนในอนาคต*