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ORIGINAL ARTICLE

A 9-year study of shigellosis in Northeast Malaysia: Antimicrobial susceptibility and shifting species dominance

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Abstract
Aims In Malaysia, *Shigella* spp. is the third most common bacterial agent responsible for childhood diarrhoea. This study was conducted to determine the prevalence and antimicrobial susceptibility patterns of *Shigella* spp. isolated from patients admitted to the Hospital Universiti Sains Malaysia from January 2001 to December 2009.
Subjects and methods A hospital-based retrospective study was used. Stool samples from patients were cultured using a standard culture method. *Shigella* spp. isolates were identified by biochemical and serological methods, and the antimicrobial susceptibility pattern was evaluated using the Kirby-Bauer disc-diffusion method.
Results A total of 138 *Shigella* spp. were isolated from a total of 14,830 routine stool specimens, yielding an isolation rate of 0.93% that corresponded to 9.99% of the 1,381 bacterial pathogens isolated. Of these isolates, *S. sonnei* was the predominant species, followed by *S. flexneri* and *S. boydii*. Seasonal variation was noticed, and no significant differences were detected in the demographic data for *S. flexneri* and *S. sonnei*. The susceptibility of all isolated *Shigella* strains was tested against seven antibiotics. Ceftriaxone (99.1%), ciprofloxacin (98.4%), and nalidixic acid (93.8%) were effective against the *Shigella* strains, whereas tetracycline and trimethoprim-sulfamethoxazole exhibited high frequencies of resistance (58.4% and 53.8%, respectively).
Conclusion This study is important for public health education aimed at reducing the morbidity and mortality associated with *Shigella* spp. infection. Our results also will be helpful for paediatricians and microbiologists in the selection of appropriate antibiotics for the management of diarrhoea.

Keywords *Shigella* · Prevalence · Antimicrobial susceptibility · Diarrhoea

Introduction

Shigellosis, an intestinal infection caused by bacteria, is a global human health problem. It is one of the major epidemic diseases in the history of mankind, and it is one of the most common killing diseases of children all over the world. Shigellosis is the most important cause of bloody diarrhoea worldwide, especially in underdeveloped and developing nations with substandard hygiene and poor quality of water supplies; it also affects travelers from industrialised countries (Bennish and Wojtyniak 1991; Niyogi 2005). The four species of *Shigella* differ epidemiologically. *S. dysenteriae* is primarily associated with epidemics of serotype 1 and is associated with the highest fatality rate (5–15%) (Ingersoll et al. 2002). *S. flexneri* predominates in areas of endemic infection and is the most frequently isolated species in developing countries (Faruque et al. 2002; Niyogi and Pazhani 2003; Zafar et al. 2005), and *S. sonnei* has been implicated in source outbreaks in developed countries (Katouli et al. 1989; Hale et al. 1991; Bonfiglio et al. 2002; Ekdaha and Andersson 2005; Hamamoto et al. 2000). S. *boydii* is

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associated with source outbreaks in Central and South America and is rarely isolated in North America (Kalluri et al. 2004). Kotloff et al. (1999) reported the percentage distribution of S. *flexneri*, *S. sonnei*, *S. boydii*, and *S. dysenteriae* to be 60%, 15%, 6%, and 6%, respectively, in developing countries, and 16%, 77%, 2%, and 1%, respectively, in developed countries. Outbreaks generally are associated with contaminated water, food, overcrowded communities, food handlers, and flies (Kapperud et al. 1995; Shears 1996).

Shigella infection occurs by the faecal-oral route. The minimum infective dose is extremely low; as few as 10–100 bacilli can initiate the disease, as it can survive gastric acidity better than other enterobacteria. The bacilli infect the epithelial cells of the villi in the large intestine and multiply inside them, spreading laterally to adjacent cells and penetrating into the lamina propria (Warren et al. 2006). *Shigella* infection can lead to illness ranging from mild, self-limited diarrhoea to severe dysentery with frequent passage of blood and mucus, high fever, abdominal pain, abdominal cramping, malaise, chills, nausea, vomiting, tenesmus, and, in rare cases, bacteraemia. Complications of shigellosis occur most frequently in children, the elderly, and immunocompromised patients. Prompt treatment with effective antimicrobial agents may shorten the duration of clinical symptoms and reduce the spread of infection (Replogle et al. 2000).

A definitive diagnosis of shigellosis can be made by isolating the organism from a stool sample. Shigellosis is one of the few enteric infections for which antimicrobials are prescribed. *Shigella* species have managed to survive the antibiotic era via an ingenious mechanism of resistance that has complicated the selection of empirical agents for its treatment (WHO 2001, 2005; Sivapalasingam et al. 2006; Kansakar et al. 2007; Kuo et al. 2008; Djie-Maletz et al. 2008; Sire et al. 2008). Moreover, antimicrobial resistance among *Shigella* spp. varies from region to region (Bhattacharya et al. 2005; Pazhani et al. 2005; Taneja 2007). Thus, when choosing an appropriate antibiotic to treat shigellosis, it is important to understand the local antimicrobial resistance. Herein, we report the prevalence of various *Shigella* species as well as the local antibiotic susceptibility pattern in Northeast Malaysia.

Materials and methods

Study design and subjects

A retrospective study was conducted by collecting data from the Department of Medical Microbiology and Parasitology, Hospital Universiti Sains Malaysia (HUSM), Malaysia. The institute, an 800-bed tertiary-care teaching hospital, provides low-cost medical care to the regional population of the Northeastern region of Malaysia as well as to the population of the border area of the neighboring country, Thailand. Stool specimens were collected from patients with clinically suspected dysentery who were admitted to HUSM between January 2001 and December 2009. Most of these patients visited HUSM with complaints of abdominal pain, vomiting, diarrhoea, and fever. Faecal specimens were collected from patients of all age groups. Demographic data such as age and sex, and the season during which the illness occurred were collected by questionnaire. These data were analysed using SPSS version 12 (SPSS Inc., USA). The association among *Shigella* spp., age, and sex was analysed using Fisher's exact test. The differences among the groups were considered to be statistically significant at $p<0.05$.

Sample collection

During the study period, faecal samples were collected from patients in a clean, wide-mouth, sterile container. Freshly

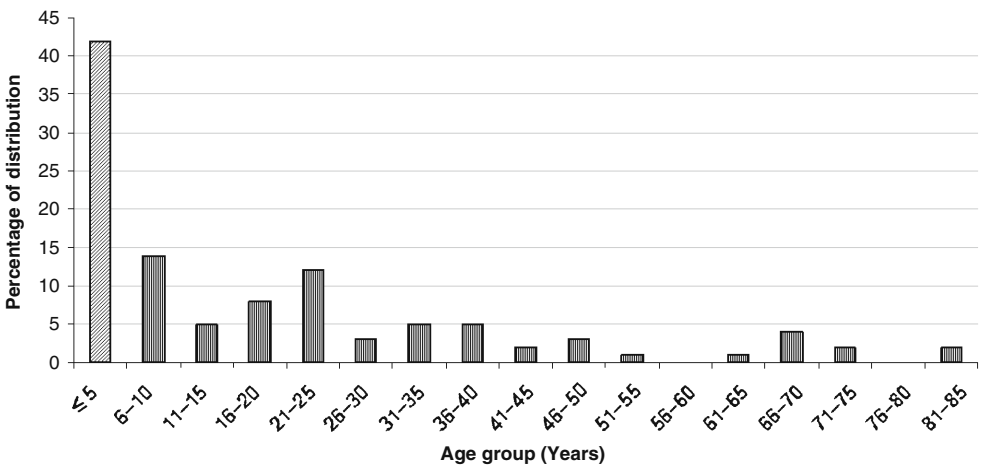

Fig. 1 Distribution of *Shigella* isolates (n=120) by age group from HUSM from 2001–2009

Table 1 Distribution of *Shigella* isolates by age group and gender

		S .flexneri	*S. sonnei*	P-value
Age group (n=120)	Paediatrics	n=33 (57.9%)	n=33 (52.4%)	0.585
	Adult	n=24 (42.1%)	n=30 (47.6%)	
Gender (n=135)	Male	n=28 (41.8%)	n=27 (39.7%)	0.862
	Female	n=39 (58.2%)	n=41 (60.3%)	

collected faecal specimens were transported within 2 h of collection to the clinical laboratory for further processing.

Bacteriological examination

Stool specimens were plated on MacConkey and deoxycholate citrate agar (DCA) using a sterile inoculation loop. At the same time, another subsample was enriched in selenite F broth and incubated overnight at 37°C. The next day, enriched broth was subcultured on MacConkey agar and DCA, and incubated overnight at 37°C. Colonies morphologically resembling *Shigella* spp. were further evaluated with biochemical tests using triple sugar iron (TSI), urea agar slant, methyl red (MR), Simmon's citrate agar slant, and sulphur indole motility (SIM) media. Colonies were serologically confirmed by slide agglutination with appropriate group-specific polyvalent antisera, followed by type-specific monovalent antisera (Denka-Seikan, Tokyo, Japan). Non-serotypable isolates were further identified by API 20E (BioMerieux, France).

Drug susceptibility test

Antibiotic susceptibility of the *Shigella* isolates was tested using the Kirby-Bauer disc-diffusion method on Muller-Hinton agar plates following the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2005). An isolate that is able to grow in the presence of an antibiotic in the medium is considered to be resistant. Resistance or sensitivity is defined according to the size of the zone of inhibition around the discs, and it correlates with the standard minimal inhibitory concentration (MIC), which is the lowest concentration that visibly inhibits bacterial growth. This correlation permits extrapolation from this simple test to the more complicated agar or broth dilution MIC test. A control strain of *Escherichia coli* (ATCC 25922) was included in the test as a quality control. Commercially manufactured discs (Oxoid, Hampshire, England) containing antimicrobial agents were used, and their concentrations in μg/ml were as follows: ampicillin-10 (AMP), ciprofloxacin-5 (CIP), trimethoprim-sulphamethoxazole-25 (SXT), nalidixic acid-30 (NAL), chloramphenicol-30 (CHL), ceftriaxone-30 (CRO), and tetracycline-30 (TCY). For NAL, only data for the years 2001, 2002, and 2006–2009 were included.

Results

Bacteriological examination

During the 9-year study period, a total of 14,830 routine stool samples were collected from patients with clinically suspected dysentery, and laboratory examinations of these samples were carried out to determine the prevalence of *Shigella* isolates. A total of 138 *Shigella*-specific strains were isolated from 1,381 isolates positive for bacterial pathogens, which corresponded to 9.99% of the total bacterial pathogens isolated. *Shigella* strains were isolated from specimens from symptomatic patients of all age groups. *S. sonnei* was the most prevalent isolate (n=69, 50.0%), followed by *S. flexneri* (n=68, 49.3%) and *S.*

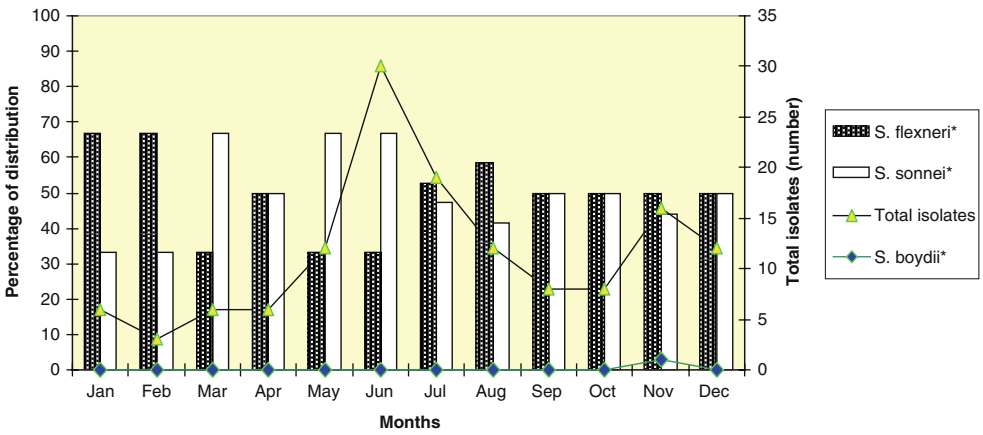

Fig. 2 Distribution of *Shigella* isolates based on months (accumulation for the year 2001 to 2009). *The distribution of each *Shigella* species is expressed in percentage, and the total isolates are expressed in number

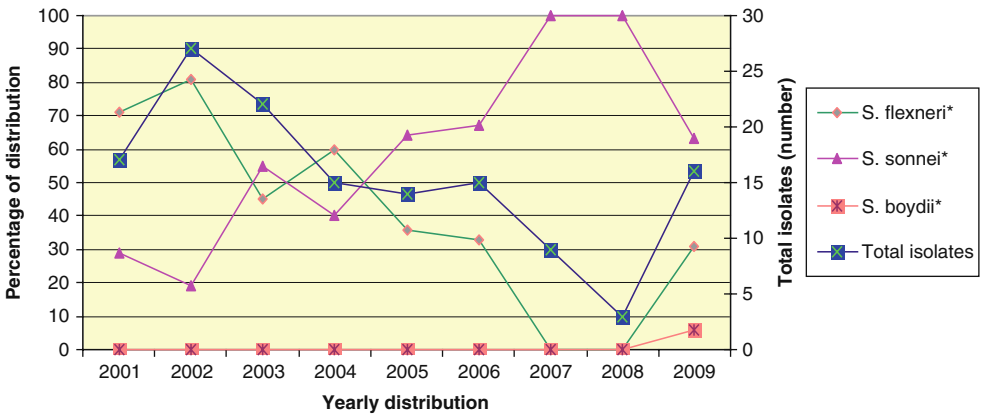

Fig. 3 Yearly distribution of *Shigella* isolates. *The distribution of each *Shigella* species is expressed in percentage, and the total isolates are expressed in number

boydii (n=1, 0.7%). Eighty-six *Shigella* strains (61.8%) were isolated from paediatric patients younger than 15 years of age, and 42.0% of the total positive samples was obtained from children ≤5 years old. Figure 1 shows the distribution of *Shigella* isolates by age group in 5-year age intervals. In this study, demographic data for *S. flexneri* and *S. sonnei* were not significantly different (Table 1).

Seasonality and yearly distribution

In Malaysia, environmental conditions remain almost the same throughout the year, with the exception of the rainy season in November and December. Figure 2 shows that the seasonal distribution of infection with *Shigella* spp. Shigellosis occurred throughout the year, but was more prevalent during the months of May to August, followed by the rainy season.

Figure 3 shows the yearly distribution of the *Shigella* isolates. *S. flexneri* was the most common species isolated in 2001 (70.6%), 2002 (81.5%), and 2004 (60.0%). In 2003, a shift occurred, and *S. sonnei* replaced *S. flexneri* as the most prevalent species. Subsequently, a gradual decreasing trend for *S. flexneri* was observed until 2008. In 2009, *S. sonnei* remained the dominant species, although a few strains of *S. flexneri* were isolated.

Drug susceptibility

Figure 4 shows the results of the tests of antimicrobial susceptibility of *Shigella* isolates against seven antimicrobial agents. The highest sensitivity was detected for CRO (99.1%), followed by CIP (98.4%) and NAL (93.8%). High sensitivity of *Shigella* isolates to CHL (68.0%) and AMP (67.4%) also was detected. The most frequent antibiotic resistance was observed for TCY and SXT, with resistance rates of 58.4% and 53.8%, respectively. Resistance to one or more drugs was observed in 100% of the 138 isolates. The frequency of resistance for TCY, SXT, AMP, and CHL was the highest compared to the other antibiotics.

A comparative analysis of the sensitivity of the *Shigella* isolates against the individual antibiotics was performed. The highest sensitivity of *S. sonnei* occurred with CRO (98.4%), followed by CHL (96.9%), CIP (96.8%), AMP (95.5%), and NAL (95.2%). The resistance percentage for *S. sonnei* was greatest for TCY (52.7%), followed by SXT (52.9%). *S. flexneri* was most sensitive to CIP (100%) and CRO (100%), followed by NAL (96.2%). *S. flexneri* was most resistant to TCY (73.3%), SXT (54.0%), CHL (66.0%), and AMP (60.0%). *S. boydii* showed the highest sensitivity to CIP, CRO, and NAL, and was resistant to the remaining antimicrobial agents tested.

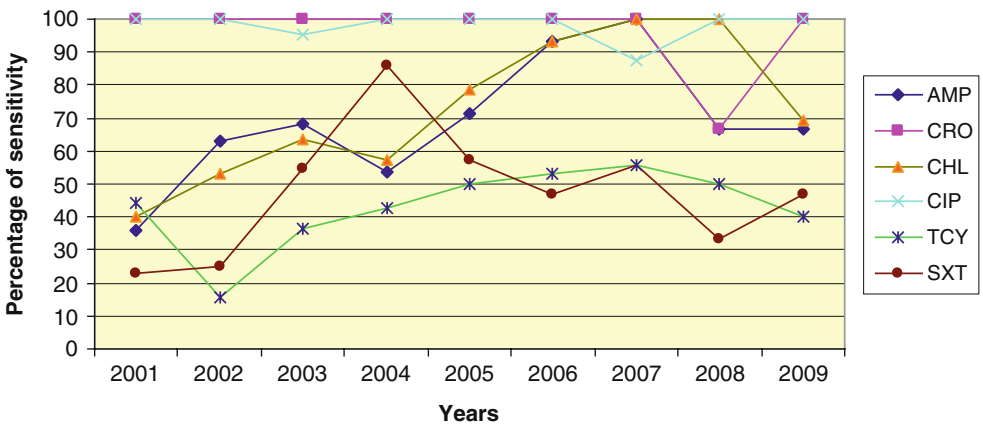

Fig. 4 Antibiotics sensitivity pattern of *Shigella* isolates. AMP (ampicillin); CIP (ciprofloxacin); SXT (trimethoprim-sulfamethoxazole); CHL (chloramphenicol); CRO (ceftriaxone); TCY (tetracycline)

Discussion

Shigellosis is the most communicable of the bacterial diarrhoeas. According to the WHO, an estimated 165 million episodes of shigellosis occur annually, resulting in 1.1 million fatalities (Kotloff et al. 1999). Because shigellosis is highly contagious, awareness of the prevalence of the disease, the identity of the dominant strain, and the antimicrobial susceptibility of the dominant strain is crucial to ensuring proper clinical treatment and patient management, and for epidemiological investigation purposes.

Shigella spp. still accounts for a significant proportion of bacillary dysentery in many tropical and subtropical countries. In the Lee and Puthacheary (2003) study of bacterial enteropathogens in a Malaysian urban hospital, *Shigella* spp. were isolated from 1.4% (386 isolates) of total stool specimens, corresponding to 13% of the 2,986 isolates positive for bacterial pathogens. In our study the isolation rate was 0.93%, corresponding to 9.99% of the 1,381 isolates positive for bacterial pathogens. The possible underestimation of *Shigella* spp. prevalence can result from both delayed transportation and non-use of a transport medium. Over the course of the 9-year study, the isolation rate did not vary for *S. sonnei* (50.0%) and *S. flexneri* (49.3%), and *S. dysenteriae* was not detected at all.

The monthly distribution of *Shigella* spp. did not play an important role in shigellosis prevalence. The infection occurred throughout the year, with the highest isolation in June followed by a second peak in November (i.e., two peaks each year). In 2001, 2002, and 2004, *S. flexneri* was the most common of the four species, whereas in 2003 and from 2005 to 2009, *S. sonnei* was the dominant species. This finding differs from those of studies conducted in other developing countries (Faruque et al. 2002; Niyogi and Pazhani 2003; Zafar et al. 2005), where *S. flexneri* was the most frequently isolated species; however, our results match findings in developed countries (Katouli et al. 1989; Hale et al. 1991; Hamamoto et al. 2000; Bonfiglio et al. 2002; Ekdaha and Andersson 2005).

In our study, *Shigella* spp. were isolated from stool specimens of symptomatic patients of all age groups (from <1 month to >85 years of age) seen in our hospital. Of these, the majority of *Shigella*-specific strains were obtained from paediatric patients younger than 15 years of age, and the highest prevalence was seen in the ≤5 years age group. This finding was in agreement with the results of Lee and Puthacheary's (2002) study of patients with diarrhoea admitted to the University of Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia. Their study showed that diarrhoea due to *Shigella* is a disease that affects all ages, but that it is commonly seen in paediatric patients. Other studies also have reported that the highest prevalence of *Shigella* is seen in the ≤5 years age group (Arya et al. 1977; Mutanda et al. 1979; MoezArdalan et al. 2003).

Shigella spp. were most sensitive to the antibiotics CRO, CIP, and NAL, and the sensitivity did not vary much among species or strains; this finding is similar to the results reported by Fulla et al. (2005). Resistance to individual drugs varied among the three *Shigella* species, and this was especially true for AMP and CHL. All three *Shigella* species also exhibited a very high rate of resistance to TCY and SXT. A similar pattern of resistance has been reported in Pakistan, Turkey, India, and Nepal (Ayser and Guriz 1998; Fulla et al. 2005; Pazhani et al. 2005). We found significant differences in the antimicrobial susceptibility of *S. flexneri* and *S. sonnei* to AMP and CHL. Overall, *S. flexneri* was more frequently resistant to TCY, SXT, AMP, and CHL, alone or in combination, than was *S. sonnei*. A possible explanation is that infections due to *S. sonnei* are clinically less severe than infections due to the other *Shigella* species, making exposure to selective pressure from antibiotics less likely.

These results strongly suggest that TCY and SXT can no longer be used to treat cases of severe diarrhoea and dysentery at HUSM. Although our sample size may not reflect the genuine spectrum of *Shigella* spp. in this part og Malaysia, our results are significant: To our knowledge, this study is the first to describe the prevalence of *Shigella* spp. and their antimicrobial resistance patterns in Northeastern Malaysia.

In conclusion, our study demonstrates that *S. sonnei* is currently the predominant species at HUSM. The antimicrobial resistance pattern suggests widespread resistance of *Shigella* to TCY and SXT. However, recommendations about antimicrobial treatment must be updated regularly based on surveillance results.

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Conflict of interest All of the authors contributed to, read, and approved the final, submitted version of the manuscript, and they do not have any conflict of interest.

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