A Changing Picture of Shigellosis in Ho Chi Minh City, Viet Nam; Shifting Species Dominance, Antimicrobial Susceptibility and Clinical Presentation

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Abstract

Shigelllosis remains considerable public health problem in some developing countries. The nature of the gastrointestinal pathogens causing shigellosis suggests that they are highly adaptable when placed under selective pressure in a human population. This is demonstrated by variation and fluctuations in the serotype and antimicrobial resistance profile of organisms circulating in differing setting in endemic locations. Antimicrobial resistance in *Shigella* spp. is a constant threat, with reports of organisms in Asia being resistant to multiple antimicrobials and new generation therapies. Here we compare microbiological, clinical and epidemiological data from three different studies conducted in Southern Viet Nam over a 14 year period. Our data demonstrates a shift in dominant infecting species (*S. flexneri* to *S. sonnei*) and resistance profile of the organisms circulating in Southern Viet Nam. We find that there was no significant variation in the syndromes associated with either *S. sonnei* or *S. flexneri*, yet the clinical features of the disease are more severe in later observations. Our findings suggest that the clinical presentation of shigellosis in this setting may be now more pronounced and may be related to the antimicrobial resistance profile. These data highlight the socio-economic development of Southern Viet Nam and should guide future vaccine development and deployment strategies.
Background

Shigellosis is an ongoing global public health problem. Due to the fecal-oral transmission route of the organisms, the overwhelming burden of shigellosis is found in resource-poor settings with inadequate sanitation [1, 2]. With an estimated number of episodes exceeding 90 million per annum in Asia alone, shigellosis represents a significant proportion of the total number of bacterial gastrointestinal infections worldwide [3]. Unlike other related bacteria which can cause a specific disease syndrome in specific locations (e.g. Salmonella Typhi [4]) it is disease which “bridges the gap” between industrialized and developing countries. A report from the National Center for Infectious Diseases in the United States of America found the incidence of shigellosis to be 7.6 cases per 100,000 persons in 1993 [5].

The Shigellae are gram negative, non-motile bacilli of the larger bacterial family Enterobacteriaceae. *flexneri* are regarded to be the most abundant globally and are known to predominate in developing countries [3]. *S. sonnei* is the most commonly isolated species in developed countries, representing over 70% of the total isolates in the United States of America and Israel [5, 6]. The disease syndrome associated with these organisms includes fever, headache, malaise, anorexia and occasionally vomiting, followed by excretion of profuse watery diarrhea proceeding bloody and / or mucoid diarrhea [7]. All the members of the genus Shigella are pathogens restricted to infecting humans (and higher primates) and exert their effects on the gastrointestinal mucosa via the production of a multitude of virulence factors, including enterotoxins and effector proteins [8, 9]. The most virulent member of the shigellae is *S. dysenteriae*, this species is
associated with the most severe infection syndrome and unlike other *shigellae* has the
ability to produce and secrete the shiga exotoxin [10, 11].

In a recent publication by von Seidlein *et al.* the authors found a change in
dominant *Shigella* species related to the location in Asia (*S. sonnei* predominated in
Thailand, *S. flexneri* was dominant in other Asian countries) and fluctuations in *S.*
*flexneri* serotypes in the same location over the duration of the study [12]. The authors
concluded that “*Shigella* appears to be more ubiquitous in Asian impoverished
populations than previously thought and antibiotic-resistant strains of different species
and serotypes have emerged” [12]. Such findings have important implications for
treatment and prevention strategies of shigellosis.

On a larger scale, the *Shigellae* are a group of dynamic and fluctuating organisms,
in which the overall bacterial population appears to be adaptable [13], changeable and
have a high recombination rate with a large amount of imported genetic material in the
genome architecture [14]. The *Shigellae* are highly promiscuous regarding their ability to
accept horizontally transferred genetic material. Like *E. coli* the *Shigellae* are successful
recipients of numerous plasmids, which may be transferred from other enteric organisms
in the gastrointestinal tract [15]. This is supported by evolutionary evidence in which the
*Shigellae* are a branch of the *E. coli* family, having developed a pathogenic phenotype by
the acquisition of a virulence plasmid and other gene loci and genomic compensatory
mechanisms [16, 17].

It is known that the circulating species and serotypes may be considered a marker
of the socio-economic climate in an individual setting [18]. It is clear that Viet Nam, in
particular Ho Chi Minh City, has undergone rapid development since the early 1990`s.
To understand the nature of bacterial and clinical nature of shigellosis in Southern Viet Nam we have amassed and compared microbiological and epidemiological data from three individual studies on childhood shigellosis conducted in the same locations spanning a 14 year period from 1995 to 2008.

Methods

Study sites and settings
The primary location was the pediatric gastrointestinal infections ward at the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City in Southern Viet Nam. The HTD is a 500 bed tertiary referral hospital treating patients from the surrounding provinces and from the districts within Ho Chi Minh City. The secondary location was Dong Thap Provincial (DTP) Hospital in Dong Thap province, approximately 120 Km from the HTD in Ho Chi Minh City. Both locations are urban settings, however owing to the referral patterns, both hospitals have a wide catchment area and accept patients from the locality and other rural provinces in the vicinity. The three studies were performed either in both locations or solely at the HTD.

Studies contributing data for analysis
Data from three independent studies were combined and compared. All three studies were overseen and directed by the same physician (HV). All patients enrolled in the three studies were treated as inpatients and there were no fatalities. The initial study (referred to as study A from here onwards) was performed only at the pediatric ward at HTD from January 1995 to August 1996. Study A was a treatment study comparing regimes of nalidixic acid and ofloxacin in Vietnamese children for the treatment of shigellosis. The
enrollment and clinical observations for this randomized controlled trial are as described previously [19]. Briefly, children that were aged >3 months and < 15 years, admitted to HTD with fever and bloody diarrhea (bloody diarrhea defined >3 loose stools with obvious blood) for <5 days were entered into the study provided that their parents or guardian gave fully informed consent. Additional strains for microbiological assessment only (nine in total) were collected for comparison within the same period of the study duration from DTP. Overall 80 strains were isolated from enrolled children over this period; clinical data was available for analysis on 63 patients with culture confirmed shigellosis.

The secondary study (referred to as study B from here onwards) was conducted only at the HTD, between March 2000 and December 2002. Study B was a clinical and microbiological investigation of the etiology of diarrhea in the pediatric population admitted to the HTD in Ho Chi Minh City. Whilst the treatment criteria for this descriptive study were not controlled (> 90% of patients received treatment with fluroquinolones (norfloxacin or ofloxacin)), the remainder of the criteria for admission to the study were comparable. The obvious variation in the enrollment for this study was that children were enrolled on the basis of having any diarrheal syndrome, rather than specifically targeting those with dysentery and suspected shigellosis. In total over 1,500 children were enrolled and monitored in study B, *Shigella spp.* were the most common bacterial isolated over this period (Ha Vinh and Stephen Baker, *unpublished data*). 114 *Shigella* isolates were recovered during this period; clinical data was available for analysis on 113 patients.
The final study (referred to as study C from here onwards) in which data was combined for analysis was conducted at the HTD and at DTP between June 2006 and December 2008. Study C was a randomized controlled trial for the treatment of non-complicated shigellosis with ciprofloxacin and gatifloxacin in Vietnamese children (Ha Vinh and Stephen Baker, *unpublished data*). The inclusion criteria were as study A. 103 isolates were collected during this period and clinical data on all admitted children was available for analysis.

All three studies were approved ethical assessment by the Scientific and Ethical Committee of the Hospital for Tropical Diseases and Oxford University Tropical Ethics Committee (OXTREC).

**Microbiological methods**

From all studies, stool samples were collected from patients and cultured directly on the day of sampling. Initial isolation was as below, however, all bacterial isolates were stored in glycerol at -80°C and re-serotyped and checked for consistency with the original antimicrobial susceptibility profile for the purposes of this work. All specimens were processed and checked in the microbiology laboratory of the HTD.

Samples were cultured overnight in selenite F broth (Oxoid, Basingstoke, UK) and onto MacConkey and XLD agar (Oxoid) at 37°C. Colonies suggestive of *Salmonella* or *Shigella* (non-lactose fermenting) were sub-cultured on to nutrient agar and were identified using a ‘short set’ of sugar fermentation reactions (Kliger iron agar, urea agar, citrate agar, SIM motility-indole media (Oxoid)). After incubation for 18 – 24 h at 37 °C, the test media were read for characteristic *Shigella* reactions and API 20E test strips of biochemical reactions (Biomerieux, Paris, France) were used to confirm the identity of
Shigella spp. Serologic identification was performed by slide agglutination with polyvalent somatic (O) antigen grouping sera, followed by testing with available monovalent antisera for specific serotype identification as per the manufacturers recommendations (Denka Seiken, Japan).

Antimicrobial susceptibility testing of all Shigella isolates against ampicillin (AMP), chloramphenicol (CHL), trimethoprim – sulfamethoxazole (SXT), tetracycline (TET), nalidixic acid (NAL), ofloxacin (O FX; and ceftriaxone (CRO) was performed by disk diffusion following standardized Clinical and Laboratory Standards Institute methods [20]. The minimum inhibitory concentrations (MICs) were additionally calculated for all isolates by E-test, according to manufacturer’s recommendations.

Clinical observations and statistical analysis

Clinical data was recorded on specialized clinical report forms for all three studies by clinical staff involved in the studies. The data collected was related to basic details of the patient, age (months), sex, location of residence and weight (kg). A history from all patients was also recorded, including; duration of illness prior to admission to hospital (days), fever (defined as a prolonged temperature > 37.5 °C), abdominal discomfort, vomiting, watery diarrhea (defined as defined as three or more loose bowel movements during a 24-h period), bloody or mucoid diarrhea (defined as >3 loose stools with obvious blood or mucus), estimated number of episodes of diarrhea before attending hospital, convulsions believed to be related to fever and / or infection and if there was any known pretreatment with antimicrobials. A white blood cell count was performed on all patients and stools were examined by microscopy to identify white and red blood cells (these observations were scored on scale from zero to four, zero without cells, four with
the most. Time in hours (from initial investigation in hospital) to the ceasing of bloody / mucoid and watery diarrhea was recorded. Duration of hospital stay was recorded in days post admission; patients were only discharged when all clinical symptoms had resolved completely.

Data were double entered into Microsoft Excel for storage and manipulation. Mapping data was entered, analyzed and draw in MapInfo software (Pitney Bowes MapInfo Corporation, USA). For intergroup comparisons, Chi-square tests were used for comparison of categorical variables. For the analysis of continuous variables, Wilcoxon rank sum, and Kruskal-Wallis test were used for non-normally distributed data. A p-value of less than 0.05 (two-tailed) was considered significant. Statistical analysis was performed in R (http://www.r-project.org/).

Results

Epidemiological findings

Data was combined from three childhood shigellosis studies performed in two locations in Southern Viet Nam; all children enrolled in all three independent studies were treated as inpatients and required antimicrobial therapy to treat infection, there were no fatalities. Study A was performed from January 1995 to August 1996. Microbiological data was available for analysis from 80 strains isolated in this period, of which 66 of the shigellosis patients were resident in Ho Chi Minh City and nine were resident in Dong Thap province. The remainders of patients were from other local provinces. In study B, 114 Shigella strains were isolated between March 2000 and December 2002. 102 of these strains were isolated from children living in Ho Chi Minh City; the remainders were
patients admitted to the hospital from other local provinces. From study C, (June 2006 to December 2008) 103 *Shigella* strains were isolated, 60 of the patients were resident in Ho Chi Minh City, 36 were resident in Dong Thap province and 7 were resident in other local provinces.

Over the duration of the three studies spanning 14 years, 228 *Shigellae* were isolated from children living within 13 districts that constitute Ho Chi Minh City (Figure 1). Whilst the distribution of the location of the residences of these patients is biased by referral patterns and people attending the local hospital (HTD is one of several hospitals in the City where children may be treated for gastrointestinal infections), the majority of children attending HTD with culture confirmed shigellosis came from the three districts within the locality of the Hospital (districts 5, 6 and 8), which constitutes a total population of over 800,000 people. In total, the majority of the patients resided in district eight (n = 88) within approximately 6 Km of the hospital. There was no significant change in the locality of patients over the three studies, or any relationship between serotype and location of the residence of the patients (even in those residing outside Ho Chi Minh City).

The median age of children with culture confirmed shigellosis from all the combined study data was 24 months, the age range was from 3 months up to 154 months (Figure 2). The number of children requiring hospital treatment as inpatients for shigellosis declined significantly after 36 months of age. These observations are consistent with other epidemiological findings of *Shigella* infections in Viet Nam and other Asian countries, which show that the majority of the burden of shigellosis is in children under the age of 3 years [12]. The combined data from studies A, B and C
demonstrated some seasonality related to the times of peak infection, with the majority of cases (> 60 %) occurring in the wet season (May and September), peaking in August (Figure 3). This pattern suggests a possible influence of seasonal precipitation and temperature on the transmission capabilities of *Shigella* spp. potentially in food or water, in this location.

**Microbiology and antimicrobial susceptibilities**

In total, 297 *Shigella* strains were isolated from studies A, B and C. Of which three were *S. Boydii*, 136 were *S. flexneri*, 149 were *S. sonnei* and nine were untypeable. There was no shigellosis caused by *S. dysenteriae* over the period of the three studies; this was in accordance with the clinical observations made at the time of the investigations (lack of complications associated with the organisms capable of producing the shiga toxin). There was a significant species shift from *S. flexneri* to *S. sonnei* between study A (29 % *S. sonnei*) and study C (78 % *S. sonnei*) with an approximate 1:1 ratio of *S. flexneri* to *S. sonnei* in the intermediate period (figure 4) (*p* <0.0001).

Apart from serotype one only being found in strains from study A there was no evident fluctuations in *S. flexneri* populations between the three studies, which has been shown to occur in other endemic settings where the majority of shigellosis is related to infection with *S. flexneri*. The most commonly isolated *S. flexneri* serotype was serotype 2a representing 43.38 % of all the *S. flexneri* strains isolated (Table 1).

There was a significant change in the profile of the proportions of organisms demonstrating resistance to the seven antimicrobials tested (Figure 5). There were sequential increases in the number of *Shigellae* isolated that were resistant to nalidixic acid, ofloxacin and ceftriaxone. In study C, 23 % of strains were resistant to ceftriaxone
and 68% were resistant to nalidixic acid (Figure 5a). There was an additional overall
increase in the number of antimicrobials that the organisms were resistant to, out of the
seven tested (Figure 5b and Figure 5c). In study A, 62% of all *Shigellae* were resistant to
three or more of the seven antimicrobials tested, this percentage increased to 87% in
study B and decreased slightly to 83% in study C, the overall increase was statistically
significant (p = <0.0001) (Figure 5b). The proportion of organisms that were resistant to
trimethoprim – sulfamethoxazole and tetracycline was unchanged between the three
studies (all greater than 95% resistance in all groups) (Figure 5a). Between study A and
study C, there were significant decreases in the proportions of organisms resistant to
ampicillin, decreasing from 75% to 48% (p = 0.0003), and chloramphenicol, decreasing
from 66% to 30% (p = <0.0001).

There was a discernible change in sensitivity patterns over time related to the
*Shigella* species isolated (Table 2). *S. flexneri* was significantly more likely to be resistant
to ampicillin in study A, study C and when combined over all three studies. *S. flexneri*
was also significant more likely to be resistant to chloramphenicol in study B, study C
and overall (Table 2). The combined data demonstrated that *S. sonnei* was significantly
more likely to be resistant to both trimethoprim – sulfamethoxazole and ceftriaxone,
despite ceftriaxone resistance not becoming evident till study C. The overall pattern of
reversion of sensitivity to ampicillin and chloramphenicol was mainly observed with
respect to *S. sonnei* isolates. The reversion of *S. sonnei* sensitivity to older generation
antimicrobials (such as ampicillin) may account for the overall sensitivity patterns of *S.*
*flexneri* being different to *S. sonnei* (Figure 5c).
An increase in the number of organisms resistant to multiple antimicrobials over time was seen in both *Shigella* species. However, between study A and study C, *S. flexneri* was more likely to be resistant to more antimicrobials than *S. sonnei* (*p* = 0.001). Resistance to multiple antimicrobials increased from two to three out of the seven tested from study A to C for *S. sonnei* and from four to five from the seven antimicrobials tested from study A to C for *S. flexneri* (Figure 5c).

**Clinical features associated with shigellosis**

Data from clinical observations was combined and analyzed from all three studies; this permitted a comparison of some of the clinical features of the patients with confirmed shigellosis in over the period of the three studies. Data were available for analysis from 279 patients; 63 patients from study A, 113 patients from study B and 103 patients from study C (Table 3). These data demonstrated several changes in disease profile over the three studies. There was a statistically significant increase in age, which corresponded with an increase in weight of the children from study A to study C (Table 3). There was a decrease in the number of days of history of the disease symptoms prior to admission to hospital (decrease in median days from 2 to 1). There was a statistically significant increase in the number of children with watery diarrhea (44% to 71% from study A to C), abdominal pain (52% to 76% from study A to C) and febrile convulsions (4% to 20% from study A to C). These clinical features combined suggest a progressively more severe infection syndrome from 1995 to 2008. Additionally, patients in study C, had higher white blood cell counts (median $13.1 \times 10^3$ / mm$^3$ in study C, compared to a median $10 \times 10^3$ / mm$^3$ in study A). Over time, patients had a higher density of white cells in their stool and had longer stays in hospital (an increase from 3 to 5 median days...
from study A to study C). The combined duration of disease (days of history + duration of stay in hospital) was significantly increased from study A to study C.

The increase in the severity of the disease was related a change in antimicrobial resistance profile of the organisms and a change in the dominant *Shigella* species isolated (*S. sonnei* becoming more widespread). Therefore, these data suggest that a more severe disease pattern may be related to infection with *S. sonnei*. To account for any variation in disease syndrome that may be species specific, the data were analyzed to compare the clinical syndromes related to species, i.e. comparing the combined data from *S. flexneri* to *S. sonnei* infections (Table 4). The data presented in Table 4 demonstrate only subtle differences between the syndromes synonymous with the two differing species. *S. flexneri* shows an increase in the number of days of illness prior to admission in hospital, the number of episodes of diarrhea, an increase in the duration of mucoid / bloody diarrhea and the duration of stay in hospital. These data imply that a change in the clinical profile of the infection is not dependent on differing symptoms offered by infection by two different *Shigella* species. This is in spite of the fact that infection with *S. sonnei* is generally considered to be milder than infection with *S. flexneri*.

**Discussion**

Our findings demonstrate that the epidemiology of shigellosis infection is similar in Southern Viet Nam to other locations in Asia; the main burden of infection being in children under three years of age [12, 18, 21, 22]. The median age of patients in this investigation was 24 months, two years less than a previous in Nha Trang, Central Viet Nam; it was additionally observed that a similar species shift may be occurring in this
location with respect to sites in China, Pakistan and Bangladesh [12]. A discrepancy in age in the two settings may be related to the epidemiological study being performed with ongoing community surveillance, rather than those admitted to hospital for treatment. We also found a pattern of infection which correlated with the rainy season. The observation that *Shigella* infections generally coincide with the wet season in a tropical setting has been noted before in an urban setting in North Jakarta, Indonesia [21]. Transmission of *Shigella* has been linked to wastewater and river water in Viet Nam in two independent locations in Viet Nam, Hanoi and Nha Trang respectively [23, 24]. An increase in fecal contamination of the water supply due to increased ground water may account for this pattern. Distance to a water source / river was found to be associated with higher risk in Nha Trang. The majority of patients enrolled in the studies combined here resided in District 8 of Ho Chi Minh City. Although we are unable to draw strong conclusions from the residences of these patients owning to referral and catchment areas of the HTD, district 8 represents the area of the city with the greatest density of canal networks and waterways. Further investigations may demonstrate a causal link between the contamination and location of water sources and local sanitation facilities.

In addition to a serotype shift over time, there was combined effect on antimicrobial resistance; there has been a marked increase in resistance to ceftriaxone and nalidixic acid. We have previously reported the alarming increase in ceftriaxone resistant *Shigellae* in Southern Viet Nam [25]. Whilst nalidixic acid is not used therapeutically, resistance increases the MIC to fluoroquinolones, which are recommended agents for treatment of *Shigella* infections in those that require intervention [26]. Therefore, resistance to nalidixic acid may increase disease duration in those treated with a sub-
optimal dose of fluoroquinolone. Our theory that antimicrobial resistant organisms are
under selective pressure in this population is supported by a sequential decrease in
resistance to older antimicrobial therapies, such as ampicillin and chloramphenicol which
are rarely used in the community to treat gastrointestinal infections. The uncontrolled use
of antimicrobials in this setting may fuel the spread of multiple drug resistant organisms.
However, due to promiscuous nature of the Shigellae it is likely that resistance genes are
transferred regularly to and from other enteric bacteria and maintained by selective
pressure.

Currently there are several potential Shigella vaccines undergoing development,
of which some have already been tested in initial clinical trials [27-30]. The development
and deployment of Shigella vaccines may be hindered by the number of different species
and serotypes circulating in one setting and in differing locations. For example, S.
flexneri serotypes are known to fluctuate over time, as shown in India, Indonesia,
Bangladesh, and Pakistan, [12, 13]. Here, we have demonstrated a longitudinal transition
from S. flexneri to S. sonnei. Vaccination is problematic as primary infection offers only
serotype specific immunity, as demonstrated in healthy volunteers that were infected with
either S. sonnei or S. flexneri [31]. A study concerning a cohort of Chilean children found
infection conferred 76% protective efficacy against re-infection with the same serotype
[32]. Therefore, an option for controlling shigellosis would be the development of a
series of single serotype vaccines which could be implemented in individual locations
with a known serotype profile. Alternatively, the most cost effective method of control
would be the development of a polyvalent vaccine offering protection to a number of
known dominant serotypes capable of tackling the global burden of shigellosis. It is
evident that Viet Nam has undergone rapid economic development, this arguably most
apparent in Ho Chi Minh City. The transition of dominant *Shigella* species supports this
somewhat and may predict a continuing cycle in other areas under going similar
economic development.

**Conclusions**

What we are unable to specifically ascertain from this study is the overall incidence and
greater epidemiological picture of shigellosis in this setting. It is unclear if the incidence
of shigellosis in the pediatric population is actually decreasing due to sanitation
improvements and economic development. On the basis of these data a thorough
epidemiological assessment of burden is warranted to calculate the financial and health
implications of any potential future routine vaccination against shigellosis that may
become available. Notwithstanding the lack of incidence data, here we have shown a
transition in *Shigella* species and antimicrobial resistance dominance overtime, which
appears to predict a more severe clinical disease presentation and prolonged symptoms.

**Competing Interests**

The authors wish to declare there have no competing interests

**Authors Contributions**

NTKN, TVTN, PHD, JIC, NVMH, TTTN, PVM, CTT, PVBB and TSD performed the
microbiological culturing, sensitivity testing and serotyping. MFB, PVTM provided
critical analysis related to this work. HV, CP, LTP, MNL, BLM, VTCA, PVBB, NVVC
and JF conducted the clinical work providing the data for analysis. HV, JF, MFB and SB conceived the study, analyzed and interpreted the data and prepared the manuscript.

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Figure 1 The distribution of household residences of cases of childhood shigellosis 
admitted to the Hospital for Tropical Diseases in Ho Chi Minh City.

Over the period of the three studies we were able to positively identify infecting *Shigella* 
serotypes in the stools of 297 children with symptoms consistent with shigellosis. Of 
these 228 (76.8 %) children lived in the 23 districts that constitute Ho Chi Minh City.

This figure represents the distribution of the homes of patients reporting to the Hospital 
for Tropical diseases with *Shigella* isolated from stool, by district. The percentage of 
cases reporting from each ward is distinguished by gradual shading. The location of the 
Hospital for Tropical Diseases is shown by a yellow star. Large waterways (rivers and 
canals) are shown in dark grey shading.

Figure 2 The combined distribution of sex and age of childhood shigellosis in this study 
Graph depicting the combined age and sex distribution of 297 children with shigellosis. 
The age range was from 3 months to 154 months, with a median of 24 months. There was 
no significant relationship of shigellosis with sex; in total 152 patients were male (51.2 
%) and 145 were female (48.8 %)
The overall seasonal distribution of shigellosis in Southern Viet Nam

There is an association of shigellosis infection with season. Southern Viet Nam has two distinct seasons, wet and dry. The combined data were averaged by calculating the number of months represented to get an overall assessment of the number of cases per month. The seasonal data were provided by hymetdata in Viet Nam, and represent the average rainfall and temperature per month for Ho Chi Minh City.

The distribution of *Shigella* species and serotypes from three childhood shigellosis studies in Southern Viet Nam over fourteen years.

The distribution of *Shigella* species from study A (1995 - 1996) n = 80, study B (2000 - 2001) n = 114 and study C (2007 - 2008) n = 103. The percentages of organisms that are *S. sonnei*, *S. flexneri* are colored red and grey respectively. Other *Shigella* species are colored black. The p Value was calculated using a chi - squared contingency test. *S. sonnei* was more frequently isolated in study C when compared to studies A and B, p <0.0001.

The changing antimicrobial resistance patterns of *Shigella* serotypes.

a) All organisms were tested for susceptibility to seven antimicrobial agents by the disc diffusion and E-tests. The antimicrobials tested were as follows, AMP; Ampicillin, CHL; Chloramphenicol, SXT; Trimethoprim – Sulfamethoxazole, TET; Tetracycline, NAL; Nalidixic Acid, OFX; Ofloxacin and CRO; Ceftriaxone. Graph shows the percentage of resistant (red) and sensitive (grey) organisms isolated from; study A (1995 – 1996), B

b) The proportions of organisms isolated that were resistant to three or more of any combination of the seven antimicrobials tested. Statistical significant changes were calculated using a Kruskal-Wallis test. The collection of organisms (A, B and C) relates to figure 3a.

c) The distribution of the proportion of *S. sonnei* and *S. flexneri* isolates that were resistant to one or more of seven antimicrobials tested. *S. flexneri* strains (red lines) were significantly more likely to be resistant to more antimicrobials that *S. sonnei* (black lines) over both collections compared (p = < 0.0001). *S. sonnei* and *S. flexneri* were significantly more likely to be resistant to more antimicrobials when study C (2007 – 2008) (lines with triangles) was compared to study A (1995 – 1996) (lines with squares).
Table 1 *Shigella flexneri* serotypes circulating in Ho Chi Minh City, 1995 - 2008

<table>
<thead>
<tr>
<th>S. flexneri serotype</th>
<th>Number</th>
<th>Percentage (%)</th>
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<tr>
<td>1a</td>
<td>0</td>
<td>0.00</td>
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<tr>
<td>1b</td>
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</table>

Table 2 Comparison of resistance patterns between three collections of *Shigella* serotypes *flexneri* and *sonnei* isolated in Southern Viet Nam between 1995 and 2008

<table>
<thead>
<tr>
<th>Collection</th>
<th>Serotype (n)</th>
<th>Phenotype b</th>
<th>AMP</th>
<th>CHL</th>
<th>SXT</th>
<th>TET</th>
<th>NAL</th>
<th>OFX</th>
<th>CRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (1995 - 1996)</td>
<td><em>Sonnei</em> (24)</td>
<td>R</td>
<td>11</td>
<td>8</td>
<td>23</td>
<td>23</td>
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<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
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<td>13</td>
<td>16</td>
<td>1</td>
<td>1</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td><em>Flexneri</em> (56)</td>
<td>R</td>
<td>54</td>
<td>10</td>
<td>53</td>
<td>53</td>
<td>9</td>
<td>0</td>
<td>0</td>
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<td>S</td>
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<td>0.1287</td>
<td>0.8102</td>
<td>0.0371</td>
<td>-</td>
<td>-</td>
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<tr>
<td>B (2000 - 2002)</td>
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<td>R</td>
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<td>10</td>
<td>53</td>
<td>52</td>
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<td>0</td>
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<td>2</td>
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<td>-</td>
<td>0.329</td>
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<td>C (2007 - 2008)</td>
<td><em>Sonnei</em> (71)</td>
<td>R</td>
<td>17</td>
<td>5</td>
<td>71</td>
<td>69</td>
<td>51</td>
<td>0</td>
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<td>66</td>
<td>0</td>
<td>2</td>
<td>20</td>
<td>71</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td><em>Flexneri</em> (30)</td>
<td>R</td>
<td>25</td>
<td>28</td>
<td>28</td>
<td>30</td>
<td>19</td>
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<td>1</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<td>Combined</td>
<td><em>Sonnei</em> (148)</td>
<td>R</td>
<td>78</td>
<td>23</td>
<td>147</td>
<td>144</td>
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<td>0</td>
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<td>125</td>
<td>1</td>
<td>4</td>
<td>88</td>
<td>148</td>
<td>135</td>
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<tr>
<td></td>
<td><em>Flexneri</em> (136)</td>
<td>R</td>
<td>125</td>
<td>76</td>
<td>125</td>
<td>132</td>
<td>49</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>11</td>
<td>60</td>
<td>11</td>
<td>4</td>
<td>87</td>
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<td>&lt;0.0001</td>
<td>0.001</td>
<td>0.613</td>
<td>0.365</td>
<td>0.478</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

a p Value calculated using chi-squared contingency test
b Phenotype with respect to Resistance (R) or Sensitive (S)
AMP; Ampicillin, CHL; Chloramphenicol, SXT; Trimethoprim – Sulfamethoxazole, TET; Tetracycline, NAL; Nalidixic Acid, OFX; Ofloxacin, CRO; Ceftriaxone.
### Table 3 Clinical results and observations of *Shigella* infections in Southern Viet Nam

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<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>n = 63</td>
<td>n = 113</td>
<td>n = 103</td>
<td>n = 279</td>
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<tr>
<td>Age (months) b</td>
<td>23 (17 - 48)</td>
<td>21 (14 - 29)</td>
<td>30 (19 - 42)</td>
<td>24 (16 - 36)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>10 (9 - 13)</td>
<td>10 (9 - 12)</td>
<td>11.5 (10 - 15)</td>
<td>10.5 (9 - 13)</td>
<td>0.004</td>
</tr>
<tr>
<td>Male Sex (%)</td>
<td>31 (49)</td>
<td>50 (44)</td>
<td>61 (59)</td>
<td>184 (59%)</td>
<td>0.085</td>
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<td>Patient History</td>
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<td></td>
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<tr>
<td>Days</td>
<td>2 (1 - 7)</td>
<td>2 (1 - 9)</td>
<td>1 (1 - 4)</td>
<td>2 (1 - 9)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Fever (%)</td>
<td>62 (98)</td>
<td>104 (92)</td>
<td>100 (97)</td>
<td>266 (95)</td>
<td>0.09</td>
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<td>Abdominal Pain (%)</td>
<td>33 (52)</td>
<td>41 (36)</td>
<td>79 (76)</td>
<td>153 (54)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vomitting (%)</td>
<td>24 (38)</td>
<td>64 (56)</td>
<td>51 (49)</td>
<td>139 (50)</td>
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<tr>
<td>Watery Diarrhea (%)</td>
<td>28 (44)</td>
<td>67 (59)</td>
<td>74 (71)</td>
<td>169 (60)</td>
<td>0.002</td>
</tr>
<tr>
<td>Bloody / Mucoid Diarrhea (%)</td>
<td>63 (100)</td>
<td>60 (53)</td>
<td>98 (95)</td>
<td>221 (79)</td>
<td>&lt; 0.001</td>
</tr>
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<td>Diarrheal episodes per day</td>
<td>NA</td>
<td>8 (5 - 10)</td>
<td>8 (5 - 10)</td>
<td>8 (5 - 10)</td>
<td>0.595</td>
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<tr>
<td>Convulsions (%)</td>
<td>4 (6)</td>
<td>7 (6)</td>
<td>20 (19.4)</td>
<td>31 (11)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Known pretreatment (%)</td>
<td>3 (5)</td>
<td>8 (7)</td>
<td>4 (4)</td>
<td>14 (5)</td>
<td>0.543</td>
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<td>Clinical Details</td>
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<td>Serotype Sonnei (%)</td>
<td>21 / 63 (33)</td>
<td>55 / 113 (49)</td>
<td>71 / 103 (69)</td>
<td>153 / 279 (55)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>White Cell Count (x 103 / mm3)</td>
<td>10 (8.3 - 15)</td>
<td>10.1 (7.7 - 12.8)</td>
<td>13.1 (10.1 - 17.3)</td>
<td>11.3 (8.7 - 15.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Red Cells in Stool b</td>
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<td>1</td>
<td>1</td>
<td>0.715</td>
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<td>White Cells in Stool b</td>
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<td>3</td>
<td>0.02</td>
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<td>Mucoid Duration (hrs)</td>
<td>31.5 (24 - 53.5)</td>
<td>36 (24 - 54)</td>
<td>28 (18 - 48)</td>
<td>30 (19.5 - 48)</td>
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<td>Diarrhoea duration (hrs)</td>
<td>48.5 (29.25 - 87)</td>
<td>48 (24 - 72)</td>
<td>48 (30 - 72)</td>
<td>48 (26.75 - 72)</td>
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<td>Duration of Illness</td>
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<tr>
<td>Hospital stay (days)</td>
<td>3 (1 - 12)</td>
<td>4 (1 - 15)</td>
<td>5 (2 - 14)</td>
<td>4 (1 - 15)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Disease duration (days) c</td>
<td>4 (2 - 15)</td>
<td>6 (3 - 18)</td>
<td>6 (3 - 15)</td>
<td>6 (2 - 18)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

a p Values calculated using either Chi-square test or Kruskal-Wallis test
b Cells in Stool assessed over a range from not seen to highly prevalent (0 – 4)
cc Disease duration calculated by addition of history of disease and stay in hospital
d Interquartile range values in brackets unless stated
Table 4 Comparing the clinical presentation of *Shigella flexneri* and *Shigella sonnei*

<table>
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<tr>
<th></th>
<th><em>S. flexneri</em></th>
<th><em>S. sonnei</em></th>
<th>p value (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>n = 123</td>
<td>n = 147</td>
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<tr>
<td>Age (months) (^d)</td>
<td>25 (12 - 42)</td>
<td>23 (14 - 36)</td>
<td>0.105</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>11 (8.5 - 14)</td>
<td>10 (9.9 - 13)</td>
<td>0.558</td>
</tr>
<tr>
<td>Male Sex(%)</td>
<td>55 (44.7)</td>
<td>83 (56.5)</td>
<td>0.055</td>
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<tr>
<td><strong>Patient History</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td>2 (2 - 3)</td>
<td>1 (1 - 2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fever (%)</td>
<td>117 (95)</td>
<td>141 (96)</td>
<td>0.761</td>
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<tr>
<td>Abdominal Pain (%)</td>
<td>64 (52)</td>
<td>84 (57.1)</td>
<td>0.48</td>
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<td>Vomitting (%)</td>
<td>60 (48.8)</td>
<td>74 (50.3)</td>
<td>0.78</td>
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<tr>
<td>Watery Diarrhoea (%)</td>
<td>78 (63.4)</td>
<td>86 (58.5)</td>
<td>0.41</td>
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<td>Bloody / Mucoid Diarrhoea (%)</td>
<td>97 (78.9)</td>
<td>117 (80)</td>
<td>0.88</td>
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<td>Diarrhoeal episodes per day</td>
<td>10 (5 - 10)</td>
<td>8 (5 - 10)</td>
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<td>Convulsions (%)</td>
<td>9 (7.3)</td>
<td>21 (14.3)</td>
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<td>Known pretreatment (%)</td>
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<td><strong>Clinical Details</strong></td>
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<tr>
<td>White Cell Count (x 10^3 / mm^3)</td>
<td>10 (8 - 13.6)</td>
<td>12 (10.5 - 15.5)</td>
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<td>Red Cells in Stool (^b)</td>
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<td>1</td>
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<tr>
<td>White Cells in Stool (^b)</td>
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<tr>
<td>Mucoid Duration (hrs)</td>
<td>36 (24 - 53.5)</td>
<td>25 (18 - 48)</td>
<td>0.054</td>
</tr>
<tr>
<td>Diarrhoea duration (hrs)</td>
<td>48 (39 - 72)</td>
<td>48 (27 - 72)</td>
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<td><strong>Duration of Illness</strong></td>
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<td>Hospital stay (days)</td>
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<td>4 (3 - 5)</td>
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<td>Disease duration (days) (^c)</td>
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<td>5 (4 - 7)</td>
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\(^a\) p Values calculated using either Chi-square test or Kruskal-Wallis test
\(^b\) Cells in Stool assessed over a range from not seen to highly prevalent (0 – 4)
\(^c\) Disease duration calculated by addition of history of disease and stay in hospital
\(^d\) Interquartile range values in brackets unless stated
Figure 3
Figure 4


Percentage %

P < 0.0001
Figure 5a

Antimicrobial and Collection

Percentage %

P<0.0001

TET SXT AMP CHL OFX CRO NAL

P=0.0003 P=0.0001
Figure 5b

P<0.0001
Figure 5c

Percentage of Resistant Isolates vs. Number of Antimicrobials to Which Organisms were Resistant

P < 0.0001