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## Serogroup distribution of *Shigella* in Ile-Ife, southwest Nigeria

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### ABSTRACT

**Background:** Shigellosis is endemic throughout the world and *Shigella* spp. is among the most common pathogens responsible for bacterial diarrhoeal diseases. Death attributed to shigellosis is common in developing countries, where affected populations are immunologically compromised due to poor nutrition and background infections.

**Aim:** To investigate the serogroup distribution of *Shigella* spp. recovered from clinically diagnosed cases of gastroenteritis and acute diarrhoea among children (0–5 years) in Ile-Ife, southwest Nigeria between September 2003 and September 2006.

**Methods:** The isolates were identified and characterized biochemically and serologically.

**Results:** Out of 102 *Shigella* isolates identified, 45 (44%) were *S. flexneri*, 26 (25%) were *S. dysenteriae*, 19 (19%) were *S. boydii*, 6 (6%) were *S. sonnei* and 6 (6%) were untypable strains.

**Conclusions:** We conclude that *Shigella* serogroups can be considered an important aetiological agent of acute diarrhoea and mortality among children in Ile-Ife, southwest Nigeria.

**KEYWORDS:** *Shigella* spp. serogroup distribution, Ile-Ife

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### Introduction

*Shigella* is named after Kiyoshi Shiga, who was the first scientist to isolate *Shigella dysenteriae* type 1 in the year 1896 during a large epidemic of dysentery in Japan.<sup>1</sup> Shigellosis is a collective description for infectious diseases caused by members of the bacterial genus *Shigella*, whose transmission occurs mostly via the faecal–oral route. Most *Shigella* outbreaks are associated with over-crowding, poor personal hygiene and abysmal conditions in daycare centres, nursing homes, custodial institutions, cruise ships, aboriginal reservations and refugee camps<sup>2</sup> with contaminated food or water serving as the vehicle for infection. The infectious dose of *Shigella* spp. is low, with 10–100 bacteria/mL sufficient to cause the disease.<sup>3</sup> In developed countries, shigellosis transmission has also been linked to oro-genital and oro-anal sexual contact between men<sup>4</sup> and more recently with underlying human immunodeficiency virus (HIV) infection.<sup>5</sup>

Shigellosis is endemic throughout the world and it is among the most common pathogens associated with bacterial diarrhoeal diseases.<sup>6</sup> It is particularly common in developing countries where affected populations are immunologically compromised due to poor nutrition and background infections, leading to high morbidity attributed to shigellosis.<sup>1,4,7</sup>

Although epidemic *Shigella* dysentery is the most serious manifestation of *Shigella* infection in developing countries, the majority of *Shigella* infections are due to endemic shigellosis. Epidemiological reports have shown that shigellosis is responsible for approximately 165 million cases annually, of which 163 million (98.8%) are in developing countries and 1.5 million in the industrialized countries.<sup>7</sup> It has also been estimated that between 600,000 and 1.1 million (mean 850,000) people die annually from *Shigella* infection<sup>7–8</sup> and nearly 580,000 cases of shigellosis are reported among travellers from industrialized nations travelling to developing countries.<sup>9</sup>

The genus *Shigella* comprises four subgroups that historically have been considered species, and these four *Shigella* spp. are recognized as pathogenic to humans. Subgroup A is referred to as *S. dysenteriae*, subgroup B as *S. flexneri*, subgroup C as *S. boydii* and subgroup D as *S. sonnei*.<sup>10</sup> Both *S. sonnei* and *S. boydii* are usually associated with mild illness of short duration in which the stool may be watery or bloody.<sup>11</sup> *S. flexneri* is generally more severe, lasts longer, causes dysentery more frequently and is the principal cause of endemic shigellosis in many developing countries.<sup>12</sup> Of all serotypes of *Shigellae*, particularly type 1 *S. dysenteriae*,

attracts special attention for its endemic and epidemic-causing potential as well as its high attack rate, high case-fatality rate, and various complications.<sup>13</sup>

Significant changes in the global epidemiology of *Shigellae* spp. have been observed over the past decades of the 20th century. In industrialized regions, *S. dysenteriae* was first replaced by *S. flexneri*, and then *S. sonnei*;<sup>14</sup> and has been predominantly involved in common source sporadic outbreaks<sup>12</sup> accounting for a prevalence rate of 77%.<sup>15</sup> *S. flexneri* remains the leading cause of shigellosis in most of the developing world<sup>16</sup> and has been reported in approximately 10% of all diarrhoeal episodes among children younger than 5 years of age.<sup>12</sup> According to Peirano et al.,<sup>6</sup> the frequency of *S. flexneri*, *S. sonnei*, *S. boydii* and *S. dysenteriae* was 60%, 15%, 6% and 6%, respectively (30% of *S. dysenteriae* cases were type 1) in developing countries; and 16%, 77%, 2% and 1%, respectively in the developed nations. Evidence has shown that in developing nations, the predominant serotype of *S. flexneri* is 2a, followed by 1b, 3a, 4a and 6.<sup>17</sup>

The current study was undertaken to investigate the serogroup distribution of *Shigella* spp. isolated from clinically diagnosed cases of gastroenteritis and acute diarrhoea in Ile-Ife—a semi-urban area in southwest Nigeria.

**Methods**

Patient enrolment and sample collection was carried out from September 2003 to September 2006. A total of 300 consecutive stool specimens were collected from children with acute diarrhoea admitted to the Children’s Emergency Ward at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, southwest Nigeria.

*Bacteriological procedures*

Rectal swabs or fresh stool samples were collected into Stuart transport medium (Oxoid, England) and processed within 2 hours of collection following the procedure described by Chompook et al.<sup>18</sup> and Iwalokun et al.<sup>19</sup> The samples were simultaneously inoculated directly on MacConkey agar (Oxoid, England) and Salmonella–Shigella agar, and were incubated at 37 °C overnight. Overnight cultures were tested for enteropathogens by employing standard techniques as outlined by Bopp et al.<sup>20</sup>

*Biochemical characterization*

Colonies morphologically resembling *Shigellae* were further subcultured and identified by biochemical reactions on API 20E.

*Serogrouping*

Serogroups were determined by using commercially available antisera kits (Sanofi Diagnostics Pasteur, France and Pro-Lab Diagnostics, England). Strains were subcultured on Salmonella–Shigella agar (Oxoid, England) and serotyping was performed after ~18 hours of incubation at 37 °C, by using the slide agglutination tests as described by the manufacturers.

**Results**

Of the 300 diarrhoeal stool samples screened, a total of 102 (34%) *Shigella* strains were identified. Clinical symptoms and other characteristics of the 102 patients with *Shigella* spp.

**Table 1: Clinical symptoms, other characteristics and age distribution of 102 patients with *Shigella* spp. isolated from their stool specimens**

Clinical and other characteristics	Patients					Total (n=102)
	0–12** (n=47)	13–24** (n=18)	25–36** (n=14)	37–48** (n=13)	49–60** (n=10)	
<b>Diarrhoea</b>						
• Frequency*	6.9±0.9	4.9±0.7	5.0±2.4	6.4±3.2	6.0±2.6	5.8±2.0
• Watery stool	28 (59.5)	11 (61.1)	8 (57.1)	9 (69.2)	6 (60.0)	62 (60.8)
• Mucus in stool	7 (14.9)	5 (27.8)	3 (21.4)	5 (38.5)	3 (30.1)	20 (19.6)
• Blood in stool	1 (2.1)	7 (38.9)	3 (21.4)	3 (23.1)	3 (30.1)	14 (13.7)
Vomiting	40 (85.1)	13 (72.2)	11 (78.6)	6 (46.2)	6 (60.0)	76 (74.5)
Anorexia	1 (2.1)	3 (16.7)	2 (14.3)	7 (53.8)	2 (20.0)	14 (13.7)
Lethargy	20 (42.6)	9 (50.0)	3 (21.4)	8 (61.5)	6 (60.0)	46 (45.1)
Abdominal pain	0	3 (16.7)	1 (7.1)	11 (84.6)	5 (50.0)	20 (19.6)
Temperature >38.5 °C	5 (10.6)	3 (16.7)	0	6 (46.2)	3 (30.0)	17 (16.7)
<b>Therapy received</b>						
• Intravenous fluid	100	100	100	100	100	102 (100)
• Oral rehydration solution	25 (53.2)	5 (27.8)	9 (64.3)	1 (7.7)	3 (30.0)	43 (42.2)
• Antibiotics	100	100	100	100	100	102 (100)
Death	4 (8.5)	2 (11.1)	2 (14.3)	1(7.7)	1 (7.7)	10 (9.8)

\* Mean±SD number of loose or watery stools per 24 hours

Figures in parentheses indicate percentages

\*\* Age in months

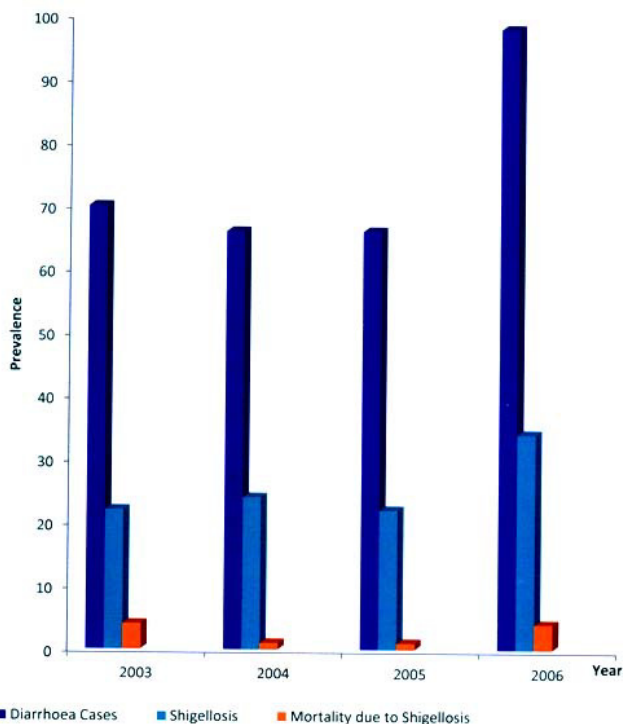


Figure 1: Prevalence of diarrhoea and incidence of shigellosis with attendant mortality due to shigellosis from children presented at the OAUTHC, Ile-Ife, southwest Nigeria between September 2003 and September 2006.

isolated from their stool specimen are summarized in **Table 1**. Fever, blood in stool, and abdominal pain were significantly higher ( $p < 0.002$ ,  $p < 0.0001$  and  $p < 0.05$ , respectively), and mucus in stool was significantly lower ( $p < 0.04$ ) in patients whose specimen yielded *Shigella* spp. as compared to the rest of the patients. Other characteristics and clinical symptoms did not show a significant difference between the above-mentioned groups. Among the patients with *Shigella*, fever, blood in stool, anorexia and abdominal pain were significantly higher among those who were aged  $\geq 37$  months ( $p < 0.001$ ), while vomiting was more prevalent among those aged  $< 37$  months ( $p < 0.001$ ). The physicians prescribed intravenous fluid and antibiotics to all the patients.

**Figure 1** shows the prevalence of diarrhoea and the incidence of shigellosis with attendant mortality due to

**Table 2: Age and sex distribution of subjects with *Shigella* serogroups isolates**

Age (months)	Isolates		Sex		Serogroups				Untypable
	No.	%	Men	Women	A	B	C	D	
0–6	7	6.86	4	3	2	2	1	1	1
07–12	40	39.22	22	18	10	20	7	2	1
13–18	10	9.80	6	4	2	4	2	1	1
19–24	8	7.84	4	4	5	3	0	0	0
25–30	7	6.86	4	3	2	5	0	0	0
31–36	7	6.86	2	5	1	3	2	0	1
37–42	7	6.86	4	3	1	2	2	1	1
43–48	6	5.88	4	2	1	3	2	0	0
49–54	3	2.94	1	2	1	1	1	0	0
55–60	7	6.86	5	2	1	2	2	1	1
Total	102	100	56	46	26	45	19	6	6

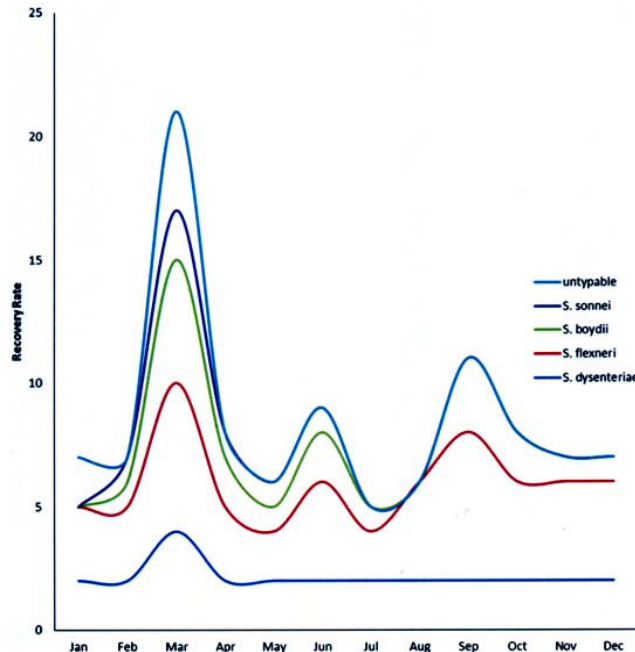


Figure 2: Monthly distribution of *Shigella* strains isolated from stools of children with diarrhoea between September 2003 and September 2006.

shigellosis, among the children enrolled in this study. Of the 102 children infected with *Shigella* spp., 10 (9.8%) were lost to their illness. Age-distributed mortality rate revealed that 2 (14.3%), 2 (11.1%) and 4 (8.5%) deaths occurred in children of 25–36 months, 13–24 months and 0–12 month(s) of age, respectively (table not shown). Fatality rate obtained was highest in patients with *S. dysenteriae* (70%) with a mortality rate of 30% associated with *S. flexneri*. None of the children infected with either *S. boydii* or *S. sonnei* died.

The frequency distribution of serogroups A, B, C and D is shown in **Table 2**. The serogroups prevalence of the 102 *Shigella* isolates was as follows: *S. flexneri* strains were the most frequently encountered [ $n = 45$  (44.12%)], while *S. dysenteriae*, *S. boydii* and *S. sonnei* accounted for 26 (25.49%), 19 (18.63%) and 6 (5.88%) isolates, respectively. Six (5.88%) of the isolates were untypable.

**Figure 2** shows the month-wise distribution to highlight the number of *Shigella* serogroups isolated per season. The

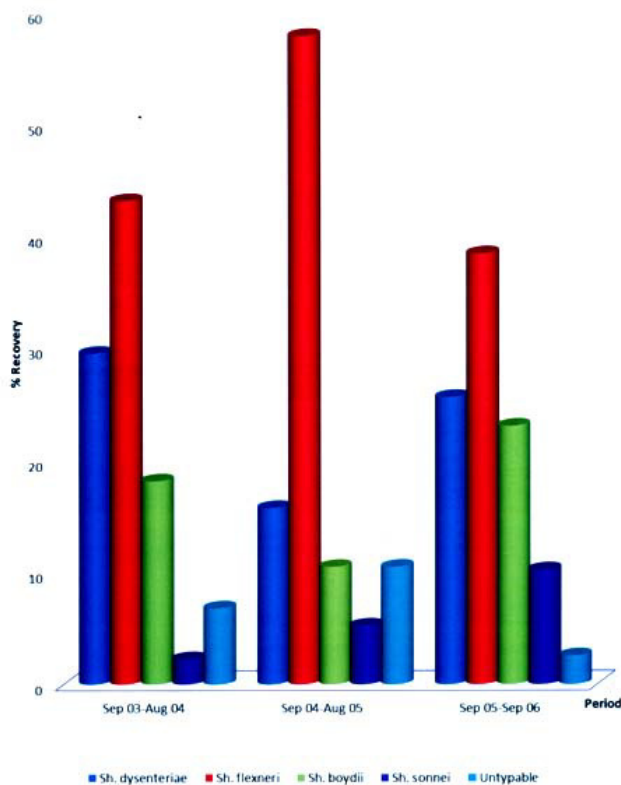


Figure 3: Trends in the relative frequencies of the various *Shigella* serogroups recovered from Ile-Ife, southwest Nigeria between September 2003 and September 2006.

highest incidence of *Shigella* infections was found in March (20.59%), while the lowest numbers were observed in July (4.90%). **Figure 3**, on the other hand, illustrates the annual trend of *Shigella* serogroup isolation.

## Discussion

In the present study, we investigated the serogroup distribution of 102 isolates of *Shigella* isolated in Ile-Ife, a semi-urban settlement, in southwest Nigeria, from . Shigellosis, like in many other developing countries, is among the main health problems in Ile-Ife and accounts for about 34% of the disease incidence in the study area.

The speciation showed that serogroup B—*S. flexneri* is the most common species [45 (44.1%)] among the total cases of shigellosis observed during the study period. Serogroup A—*S. dysenteriae* and serogroup C—*S. boydii* were identified in 26 (25.5%) and 19 (18.6%) cases, respectively; while 6 (5.9%) cases were caused by serogroup D—*S. sonnei*. Six (5.9%) of the isolates were serologically untypeable with the available commercial antisera. This pattern of shigellosis indicates that *S. flexneri* is the predominant and most active serogroup in Ile-Ife which is consistent with the patterns of isolates reported from other parts of Nigeria by Enabulele et al.,<sup>5</sup> Iwalokun et al.,<sup>19</sup> Egah et al.,<sup>21</sup> and Ogunlesi et al.<sup>22</sup> It is pertinent to point out that the order of serogroups distribution observed in this study had also been reported earlier in several studies done elsewhere, especially in other parts of Africa as reported by Shapiro et al. from Rwanda,<sup>23</sup> Kenya;<sup>24</sup> Yismaw et al. from

Ethiopia<sup>25</sup> and Opintam and Newman from Ghana.<sup>26</sup> The observed results are also in keeping with the prevalence data reported from other developing countries,<sup>18,27,28</sup> thus emphasizing the continued predominance of *S. flexneri* in developing nations. Our study findings indicate that *S. flexneri* is the predominant *Shigella* spp. recovered from patients with acute diarrhoea in developing countries with an isolation rate of 50%–70% of all *Shigella* isolates recovered.<sup>6,29</sup>

This result is, however, in contrast with studies from developed countries where *S. sonnei* is the predominant species isolated in *Shigella*-associated diarrhoea, and *S. flexneri* is the second most prevalent *Shigella* isolate.<sup>30</sup> For instance, *S. sonnei* has been documented to account for 64% of all *Shigella* spp. isolated in the USA,<sup>31</sup> and 80% in European countries.<sup>32</sup> This is in line with the reports that *S. sonnei* is dominant in more economically successful countries and the epidemiological decrease in the proportion of *S. flexneri* and *S. dysenteriae* strains in the industrialized countries has been attributed to the improvement in hygiene and living conditions in these countries.<sup>6</sup> In addition, Naik<sup>33</sup> has attributed to improved sanitary conditions the progressive substitution of the most pathogenic *Shigella* spp. (e.g. *S. flexneri*) by less virulent ones (e.g. *S. sonnei*). These observations imply that the circulation of the more virulent *Shigella* spp. tends to depreciate with improvements in hygiene and living conditions as in developed countries, to be replaced by the less virulent *Shigella* strains and a significantly lower prevalence of *Shigella*-associated diarrhoea.<sup>34</sup>

Unlike other places across the world, where surveillance of *Shigella* species has revealed a distinct shift in the circulating species associated with socioeconomic development over time,<sup>35</sup> *S. flexneri* continues to remain the predominant species in Nigeria, indicating no significant improvement in socioeconomic living conditions of our populace over the past two decades.<sup>19</sup> Although we have not included a detailed description and comparison of the sanitary condition of our subjects' living conditions, it would suffice to indicate that sanitary conditions are inevitably abysmal in such communities lacking a secure water supply and proper waste disposal system. It is also worth noting that the manually dug shallow wells, which are the major source of drinking water in these communities, are highly vulnerable to contamination with faecal pathogens, including *Shigella* spp. Acute shortage of clean potable water forces such communities to be parsimonious with their water supply, thus neglecting the basic tenets of hygiene including hand-washing, which can interrupt transmission of pathogens such as *Shigella*.<sup>22</sup> Most of the subjects in such communities rely on ponds, streams and other surface sources of water for bathing, washing clothes and maintaining livestock thus contaminating these water sources with infectious human and animal wastes. *Shigella* infection is contracted due to poor personal hygiene, consuming contaminated food and water and person-to-person contact. Similar to other enteric pathogens, *Shigella* can also be transferred by flies,<sup>36</sup> fingers and fomites.<sup>6</sup>

The results of this study are also in agreement with the results of studies from various parts of Nigeria, which have shown that *S. dysenteriae* is the second most prevalent species

in Lagos and Ilesa, southwest Nigeria,<sup>19,22</sup> in Calabar, south Nigeria<sup>5</sup> and in Enugu, southeast Nigeria;<sup>37</sup> but these are in contrast to the study from north-central Nigeria, which detected *S. boydii* as the second most common species in Jos.<sup>21</sup> This pattern of geographical species diversity among *Shigella* isolates within a country has been reported in earlier studies carried out in India<sup>38</sup> and Brazil.<sup>6,39</sup>

This study has shown that *S. boydii* serogroup C is also an important aetiological agent in Ile-Ife, southwest Nigeria and its isolation rate [19 (18.6%)] is similar to [11 (17.7%)] earlier reports by Iwalokun et al.<sup>19</sup> from Lagos. These observations also support the report that *S. boydii* accounts for several cases of human disease in developing countries.<sup>40</sup> Recent reports from Canada indicate that there has been a steady increase in *S. boydii* isolation rate starting in 1999 and this species is being most frequently recovered from individuals travelling to developing countries such as Cuba, Ethiopia, India, Guatemala and Mexico.<sup>7</sup>

The present study showed a very low occurrence of *S. sonnei* infections [6 (5.9%)]. This is in line with the report that *S. sonnei* occurs more commonly in developed countries, often emerging at epidemic levels or as sporadic outbreaks.<sup>21</sup> *S. sonnei* is responsible for about 77% of *Shigella* infections in industrialized countries.<sup>27</sup>

## Conclusions

Based on our findings in this study, we conclude that *Shigella* serogroups can be considered an important aetiological agent of acute diarrhoea and mortality among children in Ile-Ife, southwest Nigeria. Given the gravity of the situation, it is imperative to prevent this infection through promotion of health education among food-handlers as well as close attention should be given to the improvement of good hygiene practices among the general populace.

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