Shift in serotype distribution of Shigella species in China, 2003–2013

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Abstract

We identified 2912 Shigella isolates from diarrhoeal patients in China during 2003–2013. The most common species was Shigella flexneri (55.3%), followed by Shigella sonnei (44.1%); however, S. sonnei is becoming increasingly prevalent. Among the S. flexneri isolates, serotypes 2a and X variant (-7,8, E1037) were the two most prevalent serotypes, and serologically atypical isolates were also commonly identified. Overall, S. sonnei, S. flexneri 2a and S. flexneri X variant (-7,8, E1037) accounted for 76.1% of all Shigella isolates, and their prevalence increased from 54.0% during 2003–2004 to 84.1% during 2011–2013. A change was observed in the serotype distribution of Shigella in China during this period, and we propose an ideal strategy to inform the development of a broadly effective Shigella vaccine candidate.

Keywords: Atypical serotype, serotype distribution, shift, Shigella, vaccine development

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The Shigella genus is composed of four species, i.e. Shigella flexneri, Shigella dysenteriae, Shigella boydii, and Shigella sonnei, which are the causative agents of shigellosis. Shigella infections require a low inoculum (as few as ten organisms), and this facilitates person-to-person transmission [1]. Emerging multidrug-resistant Shigella isolates with resistance to fluoroquinolones and extended-spectrum cephalosporins have exacerbated the public health impact of shigellosis, leading to increased morbidity, mortality, and treatment costs [2,3]. It is estimated that, annually, ~164.7 million cases of shigellosis occur worldwide, with 125 million cases and 14,000 deaths in Asia [2,4]. In China, shigellosis is still endemic, and is one of the top three notifiable infectious diseases, with nearly half a million cases reported each year [5,6]. Thus, shigellosis remains an important public health threat globally, especially in China.

Because of clinical severity, disease burden, the emergence of antimicrobial resistance and the global failure to develop new, effective antimicrobial drugs, greater emphasis should be placed on prevention, especially vaccine development. Complicating vaccine development is the fact that there are at least 50 recognized Shigella serotypes, as well as several provisional subserotypes, and the serotype distribution of Shigella species differs by geographical region, and fluctuates over time [7]. Therefore, it is vital to better understand the serotype distribution of Shigella species at a national level to inform rational vaccine development. As China further strengthens its role as an economic powerhouse, it is a good choice for investigating the serotype distribution of Shigella isolates to inform vaccine development. However, in recent years, limited nationwide data in different spatiotemporal contexts available have made the epidemiological situation unclear, although scattered data have been reported [3,8]. Therefore, we investigated the serotype distribution of Shigella species at a national level in the period 2003–2013.

In China, from 2003 to 2013, a total of 2912 Shigella isolates were identified from diarrhoeal patients attending sentinel hospitals participating in a national pathogen-monitoring system, representing different economic levels across China. The Shigella isolates were identified by the use of API 20E biochemical strips (bioMérieux, Marcy l’Etoile, France). Sero-typing was performed by slide agglutination with a commercial antiserum kit (Denka Seiken, Tokyo, Japan); untypeable isolates were characterized with a panel of monoclonal antibodies against S. flexneri (MASF; Reagensia AB, Stockholm, Sweden). The most common species identified was S. flexneri (n = 1610, 55.3%), followed by S. sonnei (n = 1284, 44.1%); these two species accounted for 99.4% of all recovered Shigella isolates. S. boydii (n = 13, 0.4%) and S. dysenteriae (n = 5, 0.2%) were rarely encountered (Table 1). Among the 1610 S. flexneri isolates...
isolates, serotypes 2a (n = 500, 31.1%) and X variant (-7,8, E1037; n = 433, 26.9%) were the two most prevalent serotypes, followed by serotypes 1a (n = 144, 8.9%), X (-7,8; n = 108, 6.7%), and 2b (n = 95, 5.9%) (Table 1). S. flexneri X variant (-7,8, E1037), a newly identified serotype, now seems to be widely prevalent in China [6,9].

Obvious regional variation was observed among the Shigella species and serotypes. S. sonnei appeared to be more prevalent in central and south-eastern China, whereas S. flexneri was more prevalent in southern, north-western, and south-western regions of China (Table 1). Among the S. flexneri serotypes, the most common serotypes in south-eastern, north-western and central regions of China were all serotype 2a, whereas in northern and north-eastern regions, the most common serotype was S. flexneri X variant (-7,8, E1037); the most common serotypes in southern and south-western regions were 1a and 1b, respectively (Table 1). Moreover, we also observed a clearly changing trend in the prevalence of Shigella isolates over time (Table 2). Alarming, S. sonnei isolates were recovered with a greater frequency over time, increasing from 17.4% during 2003–2004 to 58.2% during 2011–2013; at the same time, the frequency of recovery of S. flexneri decreased. Among the S. flexneri isolates, the prevalence of S. flexneri 2a and S. flexneri X variant (-7,8, E1037) seemedly fluctuated over time, but during 2007–2013 the overall proportion of these two serotypes remained at ~60% of all S. flexneri isolates (Fig. S1). S. sonnei, along with S. flexneri 2a and S. flexneri X variant (-7,8, E1037), increased in frequency from 54.0% during 2003–2004 to 84.1% during 2011–2013 (Table 2).

Furthermore, it is interesting that we identified a total of 172 serologically atypical isolates, accounting for 5.9% of all Shigella isolates (Table 1). These isolates were confirmed by biochemical tests and serotyping as Shigella species. Among the atypical isolates, 46 (1.6%) were identified as S. flexneri serotype

### Supplementary Table 1: Species and serotype distribution of the Shigella isolates in China during 2003–2013

<table>
<thead>
<tr>
<th>Species/serotype</th>
<th>No. (%)/isolates</th>
<th>Southeast (n = 2012)</th>
<th>Northwest (n = 204)</th>
<th>North (n = 440)</th>
<th>South (n = 864)</th>
<th>Central (n = 127)</th>
<th>Northeast (n = 213)</th>
<th>Southwest (n = 440)</th>
<th>Total (n = 2912)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. flexneri</td>
<td></td>
<td>511 (42.2)</td>
<td>536 (68.7)</td>
<td>255 (58.0)</td>
<td>169 (82.8)</td>
<td>52 (40.9)</td>
<td>46 (53.5)</td>
<td>41 (63.1)</td>
<td>1610 (53.3)</td>
</tr>
<tr>
<td>1a</td>
<td></td>
<td>48 (4.0)</td>
<td>25 (3.2)</td>
<td>8 (1.8)</td>
<td>43 (21.1)</td>
<td>8 (6.3)</td>
<td>10 (11.6)</td>
<td>2 (3.2)</td>
<td>144 (4.9)</td>
</tr>
<tr>
<td>1b</td>
<td></td>
<td>1 (0.1)</td>
<td>37 (4.7)</td>
<td>0</td>
<td>2 (1.0)</td>
<td>1 (0.8)</td>
<td>0</td>
<td>25 (39.7)</td>
<td>66 (2.3)</td>
</tr>
<tr>
<td>1c</td>
<td></td>
<td>15 (1.2)</td>
<td>2 (0.3)</td>
<td>0</td>
<td>0</td>
<td>3 (3.5)</td>
<td>0</td>
<td>2 (0.7)</td>
<td>20 (0.7)</td>
</tr>
<tr>
<td>2a</td>
<td></td>
<td>221 (18.2)</td>
<td>191 (24.5)</td>
<td>32 (7.3)</td>
<td>29 (14.2)</td>
<td>15 (11.8)</td>
<td>5 (5.8)</td>
<td>7 (11.1)</td>
<td>500 (17.2)</td>
</tr>
<tr>
<td>2b</td>
<td></td>
<td>32 (2.6)</td>
<td>37 (4.7)</td>
<td>8 (1.8)</td>
<td>3 (1.5)</td>
<td>9 (7.1)</td>
<td>6 (7.0)</td>
<td>0</td>
<td>95 (3.2)</td>
</tr>
<tr>
<td>Serotype 2 variant</td>
<td></td>
<td>34 (2.8)</td>
<td>7 (0.9)</td>
<td>1 (0.2)</td>
<td>4 (2.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>46 (1.6)</td>
</tr>
</tbody>
</table>

### Supplementary Table 2: The prevalence of Shigella sonnei and Shigella flexneri serogroups in China over time

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>S. sonnei</td>
<td></td>
<td>41 (17.4)</td>
<td>112 (26.6)</td>
<td>208 (42.3)</td>
<td>312 (43.6)</td>
<td>611 (58.2)</td>
<td>1284 (44.1)</td>
</tr>
<tr>
<td>S. flexneri</td>
<td></td>
<td>194 (82.6)</td>
<td>309 (73.4)</td>
<td>281 (57.1)</td>
<td>390 (54.5)</td>
<td>436 (41.6)</td>
<td>1610 (55.3)</td>
</tr>
<tr>
<td>S. sonnei + S. flexneri</td>
<td></td>
<td>235 (100.0)</td>
<td>421 (100.0)</td>
<td>489 (99.4)</td>
<td>702 (98.2)</td>
<td>1047 (99.8)</td>
<td>2089 (99.4)</td>
</tr>
<tr>
<td>2a and X variant</td>
<td></td>
<td>127 (54.0)</td>
<td>273 (64.8)</td>
<td>389 (79.1)</td>
<td>546 (76.4)</td>
<td>882 (84.1)</td>
<td>2217 (76.1)</td>
</tr>
</tbody>
</table>

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2 variant (II:3,4,7,8) [9], 27 (0.9%) were 4s (\textasciitilde E1037) [5,9], and 20 (0.7%) were 1c [10]; 79 isolates (2.7%) remained untypeable.

During the 1991–2000 period in China, Wang et al. determined that the most prevalent species of Shigella was \textit{S. flexneri} (86%), followed by \textit{S. sonnei} (12%); the predominant serotype among \textit{S. flexneri} was 2a (80%) [11]. However, our study showed a significant shift in the species and serotype distribution of \textit{Shigella} in China during the 2003–2013 period. We found that \textit{S. sonnei} accounted for 44.1% of all \textit{Shigella} isolates, and increased in frequency over time (ranging from 17.4% to 58.2%), while the prevalence of \textit{S. flexneri} declined (ranging from 82.6% to 41.6%). Among the \textit{S. flexneri} isolates, serotypes 2a and X variant (\textasciitilde 7,8, E1037) were the two most common serotypes, although their frequencies fluctuated with time. Consistent with previous studies, \textit{S. sonnei} was more prevalent in economically developed regions, whereas \textit{S. flexneri} remained prevalent in the less developed regions [3,7]. Notably, we also observed the emergence and prevalence of several atypical variants of \textit{Shigella} in China, accounting for 5.9% of all \textit{Shigella} isolates. These alarming trends may be largely attributed to the recent unprecedented changes in China, resulting from rapid but unbalanced socio-economic development. Accordingly, these trends, coupled with the emergence of multidrug resistance, have resulted in a changing disease burden of shigellosis in China. With the globalization of world trade and human travel, this new situation in China will also have an impact on other countries. Existing interventions, such as improved sanitation and hygiene, cannot on their own control the spread of \textit{Shigella}, although they can reduce the shigellosis burden [7,11]. In this context, innovative strategies, particularly vaccine development, are likely to have a significant impact.

Previous studies demonstrated that protection against \textit{Shigella} infections is O-antigen dependent, and some serotype-based vaccines are under development [12,13]. These have been confirmed not only providing significant protection against epidemiologically important species but also providing cross-protection among the \textit{S. flexneri} serotypes [13]. This finding suggests that choosing the most common serotypes for inclusion in a multivalent vaccine could provide coverage for most \textit{Shigella} species and serotypes. Livio et al. evaluated the potential for a quadrivalent vaccine including O-antigens from \textit{S. flexneri} serotypes 2a, 3a and \textit{S. flexneri} serotype 6, as well as \textit{S. sonnei}, which can provide direct coverage against \textasciitilde 64% of the \textit{Shigella} isolates from the Global Enteric Multicenter Study, and cross-protection against up to 88% of all \textit{Shigella} isolates. In this study, we observed a distribution of species and serotypes of \textit{Shigella} in China that was different from that of the Global Enteric Multicenter Study. \textit{S. sonnei}, \textit{S. flexneri} 2a and \textit{S. flexneri} X variant (\textasciitilde 7,8, E1037) were the most prevalent serotypes in China, and the overall prevalence of these serotypes accounted for 76.1% of all \textit{Shigella} isolates. More importantly, the proportion of these three serotypes increased over time, and during the 2011–2013 period it increased to 84.1% of all recovered \textit{Shigella} isolates (Table 2). \textit{S. dysenteriae}, \textit{S. boydii}, \textit{S. flexneri} 6 and the untypical \textit{S. flexneri} isolates were rarely encountered, accounting for only 3.8% of all \textit{Shigella} isolates. Excluding these serotypes from a vaccine should therefore be acceptable for public health interventions. Therefore, we propose that an effective \textit{Shigella} vaccine should include, as a minimum, a combination of \textit{S. sonnei}, \textit{S. flexneri} 2a and \textit{S. flexneri} X variant (\textasciitilde 7,8, E1037); these should provide direct coverage against 76.1% of all \textit{Shigella} isolates in China. Additionally, cross-protection by a shared type antigen (type II antigen; \textit{S. flexneri} 2b and \textit{S. flexneri} 2 variant) and by shared group antigens (group antigens 3, 4, 7, 8, and E1037; \textit{S. flexneri} 1a, 1b, 1c, 3a, 4a, 4s, 5b, X, and Y) can increase the overall coverage to up to 96.1% of all \textit{Shigella} isolates.

In conclusion, we have demonstrated a change in species and serotype distribution of \textit{Shigella} in China, and provided data for the development of a broadly effective \textit{Shigella} vaccine candidate.

**Transparency declaration**

The authors declare that they have no conflicting interests in relation to this work.

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**Appendix A. Supplementary data**

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2014.10.019.
References