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Frequency of isolation of various subtypes and antimicrobial resistance of Shigella from urban slums of Karachi, Pakistan

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Summary
Objectives: Shigellosis remains a major public health problem in developing countries. Antimicrobial resistance has complicated the empirical treatment. Knowledge of serotypes is crucial in vaccine development, as cross-protection between various serotypes is limited. Therefore we conducted a prospective study to determine the frequency of isolation of Shigella serotypes and antimicrobial resistance.
Methods: Stool samples from 8155 individuals, collected through a surveillance study conducted in four slums of Karachi from January 2002 to March 2004, were cultured. *Results:* Shigella was isolated in 394 (4.8%) of 8155 patients presenting with diarrhea. Two hundred and forty-two (62%) isolates were *Shigella flexneri*, 72 (18%) were *Shigella sonnei*, 43 (11%) were *Shigella boydii*, and 37 (9%) were *Shigella dysenteriae*. Thirteen *S. flexneri* serotypes were identified, of which the most frequent were 2a (38), 6 (37), and 1b (25), followed by 2b (23). Only 22 (5.6%) Shigella isolates were found to be pan-susceptible. Large proportions of isolates were resistant to co-trimoxazole (89% *S. flexneri*, 81% *S. dysenteriae*, 80% *S. sonnei*, and 56% *S. boydii*) and ampicillin (87% *S. flexneri*, 68% *S dysenteriae*, 35% *S. boydii*, and 4% *S. sonnei*).
Conclusions: Concurrent circulation of multiple strains with high resistance is worrying and mandates surveillance at the national level to facilitate the control of shigellosis.
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Introduction

Shigellosis, an acute invasive enteric infection caused by Shigella, is recognized as a major public health problem.[1–3] Ninety-nine percent of shigellosis episodes occur in developing countries. The majority of cases and of deaths occur among children less than five years of age.[1]

Serological analysis of Shigella has long been used to characterize isolates for epidemiological and diagnostic purposes.[4,5] The genus is divided into four serogroups with 47 serotypes: A (*S. dysenteriae*, 12 serotypes), B (*S. flexneri*, 15 serotypes), C (*S. boydii*, 18 serotypes), and D (*S. sonnei*, one antigenic type, phase 1 and phase 2). For the development of vaccine-based control strategies it is essential to understand the prevalent serotypes, since immunity against shigellosis is serotype-specific.[6–10] The prevalence of a particular species of Shigella differs in various geographical areas. In 1999 Kotloff and coworkers reported *S. flexneri* as the main serogroup found in developing countries (60%), followed by *S. sonnei* (15%), and *S. dysenteriae* and *S. boydii* with equal frequencies (6%).[6] However, data consistently demonstrate that *S. sonnei* is the most common serogroup found in industrialized countries (77%), followed by *S. flexneri* (16%), *S. boydii* (2%), and *S. dysenteriae* (1%).[6]

A definitive diagnosis of shigellosis can be made by isolating the organism from stool. It is one of the few enteric infections for which antimicrobials are prescribed. Antimicrobial resistance has complicated the selection of empirical agents for its treatment.[1,2,11–15] Also, antimicrobial resistance amongst Shigella species varies from region to region.[16–18] Previously effective drugs such as tetracycline, ampicillin, and co-trimoxazole have become largely ineffective, and the recent emergence of resistance against nalidixic acid has further narrowed the choice of effective agents.[14–16] For the choice of an appropriate antibiotic it is important to understand the local antimicrobial resistance patterns. An understanding of serogroups and serotypes is crucial in vaccine development, as cross-protection between various Shigella serotypes is very limited. In preparation for a vaccine trial we conducted surveillance in four urban slums of Karachi. We report herein the frequency of distribution of various serogroups and serotypes of Shigella, as well as local susceptibility patterns.

Materials and methods

Four slum communities (Rehri Goth, Sherpao Colony, Sultanabad, and Hijrat Colony) located in Karachi, Sindh Province, Pakistan were included in this surveillance study. Rehri Goth and Sherpao Colony are in the southeast of Karachi. Hijrat Colony and Sultanabad, two contiguous urban slums, are near the business center and port of Karachi. In 2002 the combined catchment area for this surveillance study had a population of 59 584, of which 8381 (14%) were children under the age of five years. In each of the four communities a treatment center was established for the purpose of surveillance. A passive surveillance was conducted at these treatment centers by enrolling all patients visiting the study clinics.

Households of patients enrolled at surveillance clinics were visited weekly, and the head or a representative was asked about diarrhea cases in members of the household in the preceding 7 days. If a diarrhea case was reported, the patient was asked to come to the study clinic. Consenting patients of all age groups with diarrhea or dysentery presenting to the study clinics were eligible to participate in the study. For every patient presenting with diarrhea, a case report form (CRF) describing demographics and medical history was completed, and two rectal/stool swabs or stool specimens were obtained. All consenting patients with a history of dysentery or diarrhea for three days or more were eligible for study enrollment. Stool samples or rectal swabs from patients presenting between January 2002 and March 2004 were included in the analysis. All samples were processed in the Clinical Microbiology Laboratory of the Aga Khan University Hospital.

Two specimens, either rectal swabs or stool, were obtained from each patient with diarrhea. Specimens were immediately placed in buffered glycerol saline (BGS) and transported to the clinical laboratory in a cool box. On arrival, specimens were plated on MacConkey and Salmonella–Shigella agar. For enrichment, samples were also inoculated into selenite-F broth and were incubated at 37 °C for 24 h in an aerobic environment. After overnight incubation, selenite-F broth was further subcultured on Salmonella–Shigella agar (Oxoid). Biochemical reactions of suggested lactose non-fermenters and oxidase-negative colonies were evaluated in triple sugar iron, urea, lysine, indole, and motility medium. Colonies were serologically confirmed by slide agglutination with appropriate group-specific polyvalent antisera, followed by type-specific monovalent antisera (Denka-Seiken, Tokyo, Japan). Non-serotypable isolates were further checked by API20E (BioMerieux, France). Antibiotic susceptibility testing was performed by Kirby–Bauer disk diffusion method, against ampicillin (10 μg), chloramphenicol (30 μg), ceftriaxone (30 μg), co-amoxyclav (30 μg), co-trimoxazole (25 μg), nalidixic acid (30 μg), and ofloxacin (5 μg).[19] *Escherichia coli* ATCC strain 25922 was used as the control.

Data management and analysis

All results were double-entered into custom-made data entry programs (FoxPro, Microsoft, Redmond, WA, USA). The data management programs included error as well as consistency and range check programs. Statistical analysis was done using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered significant.

Ethics

Verbal consent was obtained from each participant (parent or guardian for children) following an explanation of the purpose of the study. The study received approval from the Aga Khan University Ethics Committee and from the Secretariat Committee for Research Involving Human Subjects, World Health Organization, Geneva, Switzerland.

Results

During the study period, a total of 8155 stool samples were received and 394 specimens were found to be positive for *Shigella* species, giving an average isolation rate of 4.8%. Two

hundred and forty-two (62%) isolates were *S. flexneri*, 72 (18%) were *S. sonnei*, 43 (11%) were *S. boydii*, and 37 (9%) were *S. dysenteriae*.

The percentage of *S. flexneri* was 67% in Rehri Goth, 65% in Sultanabad, 58% in Sherpao, and 54% in Hijrat Colony. Thirteen *S. flexneri* serotypes were identified, of which the most frequent were 2a (38), 6 (37), and 1b (25), followed by 2b (23). *S. sonnei* phase 1 (56/72) was the most prevalent serosubtype. Eleven serotypes were found in *S. boydii* and the most frequent isolates were *S. boydii* 1, 2, and 8. Eight serotypes were identified in *S. dysenteriae* and the most prevalent subtype was 7 (9/37); Figure 1.

Overall 78 (20%) isolates suggestive of Shigella (groups A–D) by biochemical reactions, conventional as well as API20E, and serology could not be further subtyped and remained untypable (*S. flexneri* 64/242 (26%), *S. boydii* 5/43 (12%), *S. dysenteriae* 6/37 (16%), and *S. sonnei* 3/72 (04%); Figure 1.

Among all isolates, only 22 (5.6%) were found to be pansusceptible. Large proportions of isolates were resistant to co-trimoxazole (81% *S. flexneri*, 81% *S. dysenteriae*, 75% *S. sonnei*, and 56% *S. boydii*) and ampicillin (87% *S. flexneri*, 68% *S. dysenteriae*, 35% *S. boydii*, and 4% *S. sonnei*); Figure 2. Forty-two percent of isolates were resistant to both drugs (co-trimoxazole and ampicillin). The frequency of isolation of multidrug-resistant isolates in individual groups was 76% in *S. flexneri*, 62% in *S. dysenteriae*, 26% in *S. boydii*, and 4% in *S. sonnei*.

Resistance to chloramphenicol was common in *S. flexneri* (50%), *S. dysenteriae* (41%), and *S. boydii* (28%), but very low in *S. sonnei* (3%). Low resistance was found to co-amoxyclav (25% *S. flexneri*, 12% *S. dysenteriae*, 10% *S. boydii*, and 5% *S. sonnei*). Nalidixic acid resistance was variable, with *S. sonnei* (13%) having the highest rate of resistance followed by *S. dysenteriae*, *S. flexneri* (3%), and *S. boydii* (2%); Figure 2. Very low resistance was found to ofloxacin (0% *S. dysenteriae*, $\leq$3% *S. flexneri*, 2% *S. boydii*, and 7% *S. sonnei*). None of the isolates were ceftriaxone-resistant.

When tested for average resistance to all antibiotics (ampicillin, co-trimoxazole, nalidixic acid, chloramphenicol, co-amoxyclav, nalidixic acid, and ofloxacin), *S. flexneri* was the most resistant and *S. sonnei* was the most susceptible group to all tested drugs – 41% (out of 242 isolates) versus 18% (out of 72 isolates) with a p value of $\leq$0.0001. *S. dysenteriae* was the second most resistant species and *S. boydii* was the next, with rates of 34% and 22%, respectively.

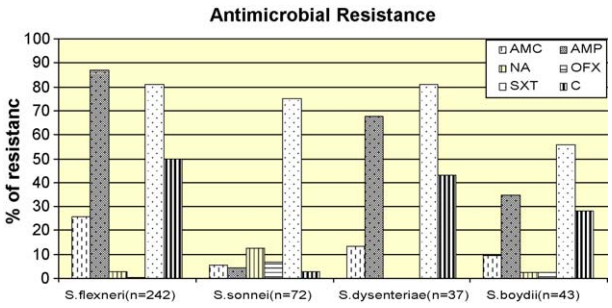

Figure 2 Antimicrobial resistance in Shigella isolates in Karachi, Pakistan (AMC, co-amoxyclav; NA, nalidixic acid; SXT, co-trimoxazole; AMP, ampicillin; OFX, ofloxacin; C, chloramphenicol).

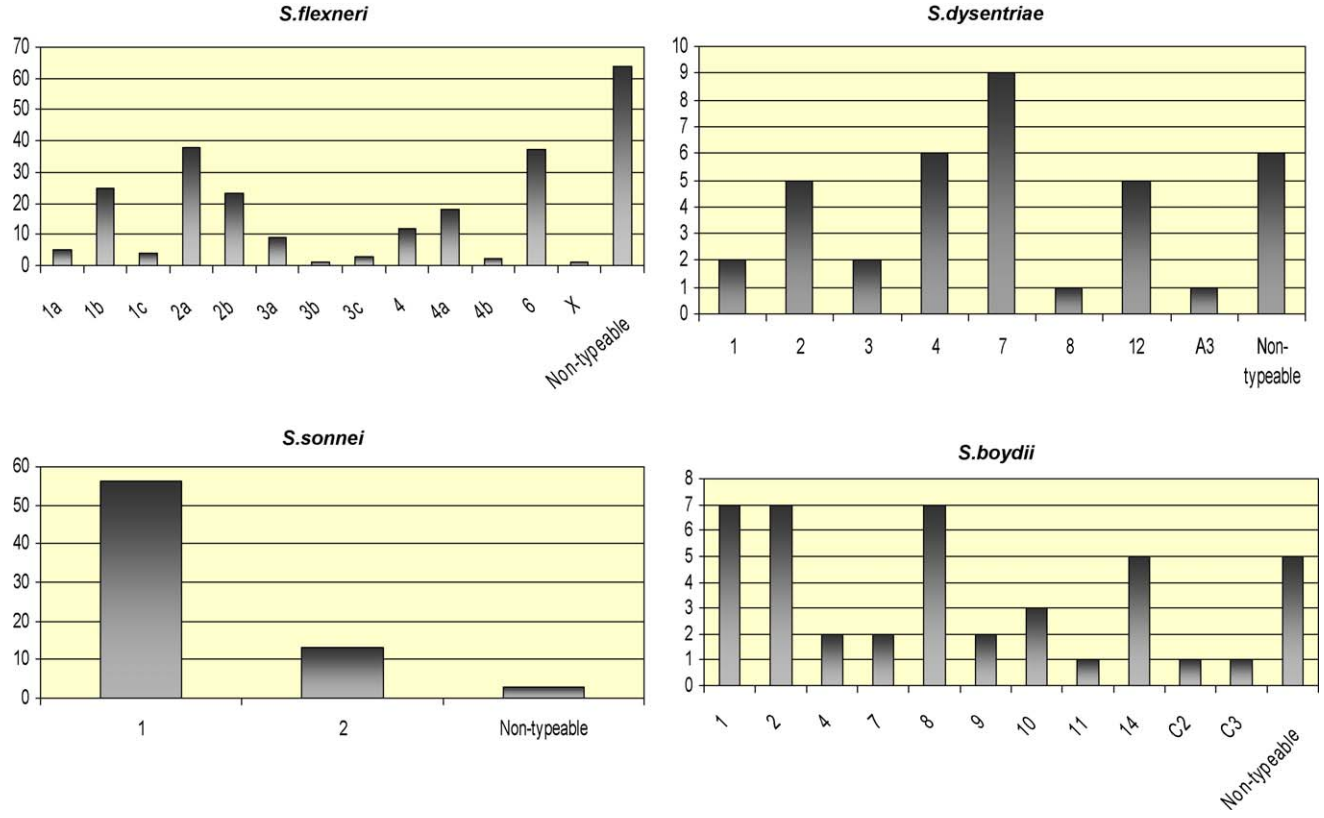

Figure 1 Frequency of isolation of various serotypes of *Shigella* species in Karachi, Pakistan.

Discussion

This is the first study reporting the prevalence of Shigella serotypes in Karachi, Pakistan. We report an isolation rate of 4.8%, which is similar to other studies published in India (7.7%) and Nepal (4%).[16,17] *S. flexneri* was most frequently isolated, which is consistent with previous reports on Shigella prevalence in resource-limited countries.[1,6]

The distribution of Shigella serogroups *flexneri*, *boydii*, and *dysenteriae* was highly heterogeneous. Furthermore, a substantial number of Shigella isolates were untypable. Emergence of new subtypes is common in *Shigella* species.[20–22] Recently Woodward et al. in Canada and Grimont et al. in France reported new serotypes of *Shigella boydii*.[23,24] A change in the trend and emergence of new serotype 1c has also been reported from Bangladesh.[25]

These findings highlight the importance of continuous monitoring of emerging isolates. However identification of these isolates requires specific antisera and molecular typing, which remain beyond the capability of the majority of clinical diagnostic laboratories in developing countries.[25] There may well be a need to establish a reference molecular laboratory at a national level.

Various studies have reported an increase in antimicrobial resistance amongst different species of Shigella against commonly used drugs, including ampicillin, tetracycline, chloramphenicol, and co-trimoxazole.[11–16,26–28] This study also reveals a very high rate of resistance, particularly against co-trimoxazole and ampicillin. Resistance to individual drugs is very high in *S. flexneri* in comparison to earlier reports (7% to co-trimoxazole and 10% to ampicillin) from Pakistan published in 1998 and 2003.[29,30] These results strongly suggest that ampicillin and co-trimoxazole can no longer be used empirically in cases of severe diarrhea and dysentery in Karachi. It is very likely that high antimicrobial resistance is the direct outcome of irrational use and ready over-the-counter availability of antibiotics including ampicillin and co-trimoxazole in the community.

The relative antimicrobial resistance of various *Shigella* species may vary geographically. We found statistically significant differences in the antimicrobial susceptibility of *S. flexneri* and *S. sonnei* strains. This is in contrast to reports published from Israel and Bangladesh, but consistent with observations in several other countries.[31–36]

Resistance to nalidixic acid has previously been reported from Iran, Hong Kong, Vietnam, China, Chile, and also from neighboring India.[29,34–38] We found variable resistance in different Shigella serogroups. No nalidixic acid resistance was observed in Bangladesh in 1985, increasing to 100% resistance in 1997.[14] In Pakistan, nalidixic acid is an inexpensive and frequently used drug for community-acquired diarrhea. Resistance to nalidixic acid is distressing, as acquiring resistance against nalidixic acid in a resource-limited country will make the treatment options for family physicians and pediatricians more difficult and costly.

Fluoroquinolone resistance has been reported from the neighboring countries of India and Iran.[16,28] The emergence of fluoroquinolone resistance, though very low at present, is alarming, as resistant enteric organisms disseminate quickly in compromised hygienic and living conditions. Fortunately, no resistance was found against third-generation cephalosporin, which is consistent with the international data. In 2003, a report published from the northern part of Pakistan stated that 10% of Shigella isolates were sensitive to all antimicrobials.[30] We found the scenario has further worsened, as only 5.6% of our isolates remained pan-susceptible.

In conclusion, the present study demonstrates that *S. flexneri* is currently the predominant species in Karachi, Pakistan. Antimicrobial resistance patterns suggest widespread resistance of Shigella to multiple clinically relevant antimicrobials. Recommendations on antimicrobial treatment must be regularly updated based on surveillance results.

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Conflict of interest: No conflict of interest to declare.

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