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# An 11-year study of shigellosis and *Shigella* species in Taiyuan, China: Active surveillance, epidemic characteristics, and molecular serotyping

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ABSTRACT

A hospital-based surveillance of shigellosis was conducted in Taiyuan from 2005 to 2015. A total of 2655 stool cultures were collected from patients with diarrhea, 115 were identified as *S. flexneri* and 107 were *S. sonnei*. The highest infection rates were found among children under 5 years of age (34.2 %), and during the summer (61.0 %). Six serotypes were identified among *S. flexneri*isolates:1a, 2a, 2b, Xv, X and Y. Serotype 2a and Xv were the dominant serotypes in two periods, 2012–2015 and 2005–2008, respectively. High shigellosis rates over the past decade highlight shigellosis is still a major public health problem in Taiyuan.

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## Introduction

Shigellosis, also known as bacillary dysentery or *Shigella* dysentery, is an enteric bacterial infection caused by a group of *Shigella* bacteria [1,2]. Nowadays, shigellosis is still a major health problem in many parts of world, especially in the developing countries [2]. The World Health Organization (WHO) pointed out that the global number of shigellosis was about 165 million per year, of which 163 million were in underdeveloped and developing countries [3]. A survey revealed that shigellosis was estimated to have caused the death of 34,000 children under the age of 5 in 2013, and 40,000 deaths in people over 5 years of age [4].

*Shigella* is a group of gram-negative, facultative intracellular pathogens [5]. The genus is divided into four species or groups: *Shigella dysenteriae* (*S. dysenteriae*, Group A), *Shigella flexneri* (*S. flexneri*, Group B), *Shigella boydii* (*S. boydii*, Group C), and *Shigella sonnei* (*S. sonnei*, Group D). Group A, B, and C can be further divided into multiple serotypes: *S. dysenteriae* (15 serotypes), *S. flexneri* (16 serotypes), and *S. boydii* (20 serotypes) [6–8]. Group A, B, and D are the major disease-causing species: *S. flexneri* is the most common species accounts for 60% of shigellosis cases in the developing world; *S. sonnei* causes 77% cases in the developed world, and most cases are associated with foreign travelers; *S. dysenteriae* is usually the cause of epidemics of dysentery, particularly in confined populations such as refugee camps [9–11].

A definitive diagnosis of *Shigella*-infection can only be made by isolating and identifying the organism from stool samples [12]. In recent years, PCR is widely used in disease diagnosis and bacteria identification. For example, Suvash et al. reported 4 pairs of primers, which located in *invC*, *RFC*, *wbgZ* and *rfpB*, were used to identify 4 species of *Shigella* [13]. In 2011, Sun et al. created a multiplex PCR method for *S. flexneri* serotyping and constantly improve it in the next few years [14].

China is a developing country with a large population, and shigellosis is a significant public health problem in past decades [1,15]. In Beijing, the drug resistance of *Shigella* was studied by Qu et al. and they reported that over 90% of *Shigella* isolates were

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resistant to at least on of three drugs with widened spectrum [15]. Besides, Li et al. gave us a detailed information of genetic characterization of *S. flexneri* isolates in Guizhou Province [16]. However, few reports of shigellosis have been done in Taiyuan, the capital city of Shanxi Providence where the population is over 4.3 million people. By analyzing the infection rate, temporal trend, high-risk populations, and the predominant circulating serotypes, we hope to provide a scientific basis of shigellosis in Taiyuan and understand ways to prevent shigellosis in Shanxi Province.

## Material and methods

### *Bacterial isolates and identification*

A hospital-based active surveillance was conducted from 2005 to 2015. Fecal specimens were collected from patients with diarrhea and clinically suspected shigellosis from seven major hospitals in Taiyuan. All *Shigella* strains were isolated by selective medium, then analyzed by biochemical tests. Confirmation was made by using both API-32E biochemical test strips (bioMérieux, France) and slide agglutination test using *Shigella* antisera (Denka Seiken, Japan) according to the manufacturer's instructions.

### *Preparation of DNA templates*

DNA templates were prepared directly from bacterial colonies by the boiling method. Briefly, a single colony was suspended in 30 μl of distilled water and boiled at 100 °C for 10 min. The sample was immediately cooled on ice for 5 min and centrifuged at 13,000 × *g* at 4 °C for 10 min. The supernatant, containing DNA, was used as the template for PCR amplification.

### *PCR primers for* S. flexneri *serotyping*

Based on Sun's methods, nine genes were amplified using the specific primers and PCR conditions described below for serotyping of *S. flexneri*: *gtrI*, *gtrII*, *oac*, *gtr IV*, *gtrV*, *gtrX*, *wzx*, *gtrIC*, and *opt* [14]. Primers were synthesized by Sangon Biotech (Shanghai, China) and dissolved in TE buffer (10 mM Tris–Cl, 1 mM EDTA [pH 8.0]) to obtain a 50 μM stock solution. The primer sequences, product sizes, and serotype specificity are shown in Table 1.

### *PCR amplification and detection*

Qiagen multiplex PCR kit (Qiagen, Germany) was used in this study. Each reaction mixture includes 1x PCR Master Mix, 0.2 μM concentrations of each primer, and 2 μl of template DNA in a final reaction volume of 25 μl. PCR amplification was performed using a standard multiplex PCR cycling protocol according to the instruction for the kit: 95 °C for 15 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 90 s, and 72 °C for 60 s, with a final extension of 72 °C for 10 min in a PTC-200 cycler (Bio-Rad, USA). A portion (5 μl) of the reaction mixture with loading buffer, followed electrophoresis in 1.5% agarose gel (Takara, Japan), was visualized by Goldview (Transgen, China) staining.

## Results

### *Specimens collection and preliminary identification for* Shigella *strains*

From 2006 to 2015, a total of 2655 samples were collected and tested for the presence of *Shigella* bacteria in Taiyuan CDC. A total of 222 *Shigella* strains were isolated based on immunologic and biochemical profile (laboratory-confirmed) (Table 2). The average infection rate (the proportion of *Shigella*-infection cases in diarrhea patients) was 8.4%. Of these, *S. flexneri* was the most prevalent species (115 isolates, 51.8%), followed by *S. sonnei* (107 isolates, 48.2%). No *S. dysenteriae* and *S. boydii* were isolated. The highest and lowest infection rates of *Shigella* occurred in 2006 and 2015, respectively. The annual infection rate decreased by approximately 12-fold from 28.3% in 2006 to 2.3% in 2015 (Fig. 1A).

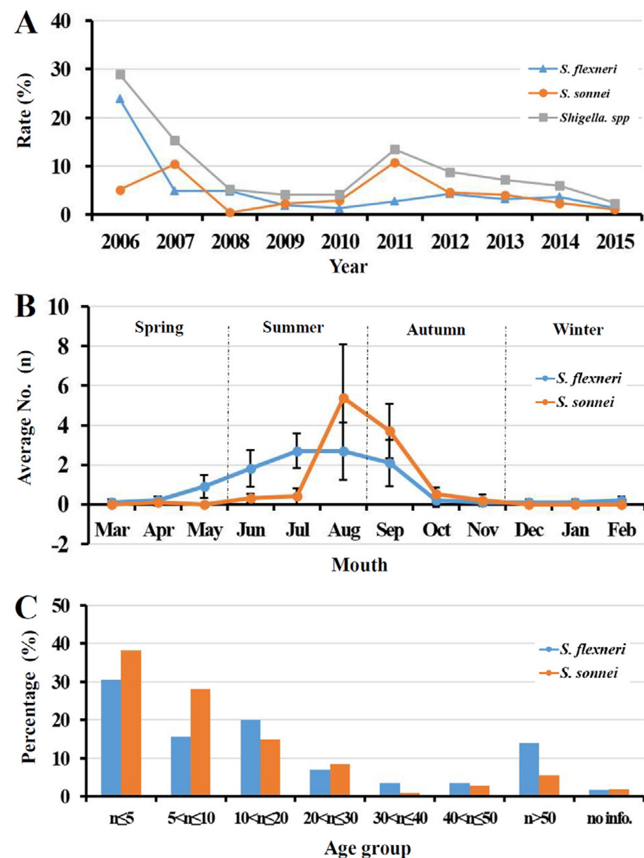


**Fig. 1.** Retrospective analysis of shigellosis in Taiyuan from 2006 to 2015.
A, Infection rate of *Shigella.* Rate (%): the proportion of shigellosis cases in patients with diarrhea.
B, Seasonal distribution of shigellosis. Average No. (n): the average number of shigellosis cases by month of illness onset; Error bars: standard deviation.
C, Demographic features of shigellosis. Percentage (%): the proportion of shigellosis cases by age groups; no info: no information.

### *Seasonal distribution and demographic features*

From 2006–2015, cases of shigellosis presented obvious seasonal characteristic (Fig. 1B). Ninety-three percent of shigellosis cases occurred from May to September, during which 93.2% of cases occurred. Trends of *S. flexneri* infections began to increase slowly from April in general, then rapidly increased until August's peak point, gradually decreased from November to next February (Fig. 1B). But, cases of *S. sonnei*-infection mainly occured in August and September (Fig. 1B).

The proportion of shigellosis varied greatly by age groups, with the highest ratio being 34.2% observed in children <5 years of age, followed by 21.6% in children 5–10 years of age. Besides, the data showed a *S. sonnei*-infection predominance in children <10 years of age, with an *S. sonnei*-to-*S. flexneri* ratio of 1.34:1 (Fig. 1C). But the *S. flexneri*-infection predominance was most obvious in adult >30 years of age, and the proportion of *S. flexneri*-infection was 1.4 times higher than *S. sonnei*-infection of the same age (Fig. 1C). There was no statistical significance was attained ($\chi^2 = 0.9061$, $P = 0.3411$).

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