

Prevalence of *Shigella* species and Antimicrobial Resistance Patterns of Isolated Strains from Infected Pediatrics in Tehran

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ABSTRACT

Background: *Shigella* is an infectious food-borne pathogen that can cause a severe diarrhea illness called shigellosis. The increasing levels of antimicrobial resistance of *Shigella* isolates have complicated the treatment of shigellosis. The antimicrobial resistance patterns of *Shigella* species vary according to geographic region and in the same place over time, leading to a therapeutic problem.

Objectives: This study attempts to determine the prevalence of shigellosis and the antimicrobial susceptibility pattern of isolated strains.

Materials and Methods: This cross-sectional study, with a 12-month duration, between August 2010 and August 2011 was carried out on 9131 infants with acute diarrheal disease. Stool samples were inoculated on selective and differential media. The *Shigella* isolates were identified and confirmed by biochemical laboratory tests. Subsequently, serotyping was performed with group specific antisera. Drug sensitivity test was carried out according to CLSI (2010) recommendations by using the standard disc diffusion method.

Results: *Shigella* spp. were isolated from 90 (1%) of the 9131 stool samples collected from children with acute diarrhea. Among *Shigella* isolates, the most common subgroup was *S. sonnei* 63 (70%). Our results showed that imipenem, ciprofloxacin, ceftazidime and ceftizoxime are most effective antimicrobial agents against *Shigella* sp. The most frequent resistance observed, was towards co-trimoxazole (92.2%), ampicillin (65.6%) and tetracycline (65.6%)

Conclusion: Since antibiotic resistant profile of *Shigella* varies geographically and also over time within a single country, regular, continuous surveillance is necessary. The results of accurate surveillance should be used to guide policies for antibiotic prescription.

Keywords: Children, Shigellosis, *Shigella* SPP., Antimicrobial Susceptibility

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► Article type: Research Article; Received: 12 Feb 2013; Revised: 06 Apr 2013; Accepted: 24 Apr 2013; Epub: 21 May 2013; Ppub: 05 Aug 2013

► Implication for health policy/practice/research/medical education:

This research was carried out on infants with acute diarrheal disease for determination of shigellosis frequency and antimicrobial resistance pattern of isolates. Our results showed that imipenem, ciprofloxacin, ceftazidime and ceftizoxime are most effective antimicrobial agents against *Shigella* sp. Regular, continuous surveillance is necessary in shigellosis. The results from accurate surveillance should be used to guide policies for antibiotic prescription.

► Please cite this paper as:

Mardaneh J, Abbas Poor S, Afrugh P. Prevalence of *Shigella* species and Antimicrobial Resistance Patterns of Isolated Strains from Infected Pediatrics in Tehran. *Int J Enteropathog.* 2013; 01(01): 28-31. DOI: 10.17795/ijep10705

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1. Background

Shigella is an infectious food-borne pathogen that can cause a severe diarrhea illness called shigellosis (1-3). It is responsible for approximately 165 million cases of diarrhea annually, of which 163 million are in developing countries and 1.5 million are in industrialized ones (4, 5). In developing countries, 69% of all cases are children under the age of 5 years (6, 7). Annually, 1.1 million deaths occur worldwide (3, 5, 6). Factors influencing the emergence or decrease of epidemic shigellosis are not obvious, and *Shigella* spp. are generally believed to have only a human or primate host. Primarily, shigellosis is transmitted from person to person through the fecal-oral route, but it may also spread indirectly by fecal contamination of water or food (8, 9). Many individuals who are infected with *Shigella* expand fever, painful bloody or mucous diarrhea, and stomach cramps (3, 10). *Shigella flexneri* like *Kingellakingae*, that cause septic arthritis in children, can develop post-infectious arthritis in about 2% of infected individuals which can lead to chronic arthritis (11, 12).

Initially, *Shigella* invades (by virulence plasmid) the large intestine epithelium (8, 13). Invasive strains arrive to epithelial cells which follows intracellular proliferation, intra- and intercellular spreading, and finally cause host cell death (14, 15). Generally, intestinal infection caused by *Shigella* spp. is self-limited; however, antimicrobial therapy reduces the period and intensity of disease symptoms, decreases excretion of bacteria and prevents potentially lethal complications (4). The disease is often worse in children and medical treatment is sometimes necessary in severe cases. Over the past decades, *Shigella* species have become progressively resistant to most widely-used antimicrobials (16, 17). The increasing levels of antimicrobial resistance of *Shigella* isolates have complicated the treatment of shigellosis. The antimicrobial resistance patterns of *Shigella* species differ according to geographic area and in the same place over time, leading to therapeutic problems (2, 18-20). Periodic regional monitoring of disease with serotype breakdown and regular periodic antibiotic-susceptibility testing patterns of isolates to guide local empirical therapy are important factors for the adequate control of shigellosis. Therefore, this study attempts to determine the prevalence of Shigellosis and antimicrobial susceptibility pattern of isolated strains.

2. Objectives

This study attempts to determine the prevalence of shigellosis and the antimicrobial susceptibility pattern of isolated strains.

3. Materials and Methods

3.1. Study population

This cross-sectional study, during a 12-month period, between August 2010 and August 2011 studied 9131 infants with acute diarrheal disease admitted to Bahrami chil-

dren's hospital affiliated to Tehran University of Medical Sciences, Tehran, Iran. Before sample collection, the entire study population was registered with number codes.

3.2. Sampling

Stool samples were collected in stool vials, on the day of hospital admission prior to the administration of antimicrobials. Fecal specimens were inoculated into Cary-Blair transport medium and stored at 4°C and processed within 24 h. The samples were sent to the microbiology laboratory for diagnostic processing.

3.3. Microbiological studies

Isolation and identification: For isolation of *Shigella*, stool samples were inoculated on selective and differential media including *Salmonella-Shigella* (SS) agar, xylose lysine desoxycholate (XLD) Agar and Hektoen Enteric Agar (HEA) (Merck, Co. Germany). Cultured plates were incubated at 35°C for 18-48 h. The *Shigella* isolates were identified and confirmed by biochemical laboratory tests. Subsequently, serotyping was performed with group specific antisera (Bahar Afshan, Iran) by using slide agglutination test.

Antimicrobial susceptibility testing: Drug sensitivity test was performed according to CLSI (2012) (23) recommendations by using the standard disc diffusion method. Antimicrobial agents used in this study, were ampicillin (AMP), 10 µg; ceftazidime (CAZ) 30 µg; cefotaxime, (CTX) 30 µg; ceftriaxone, (CRO) 30 µg; cefixime, (CFM) 5 µg; ceftizoxime, (CT) 30 µg; imipenem (IMI) 10 µg; tetracycline (T), 30 µg; ciprofloxacin (CIP), 5 µg; trimethoprim-sulfamethoxazole (SXT), 25 µg; nalidixic acid (NA) 30 µg.

4. Results

Shigella sp. were isolated from 90 (1%) of the 9131 stool samples collected from children with acute diarrhea. Among *Shigella* isolates, the most common subgroup was *S. sonnei* (70%), followed by *S. flexneri* (28.9%), *S. boydii* (1.1%) and *S. dysenteriae* (0%) respectively (Table 1).

Table 1. Prevalence of *Shigella* Subgroups Isolated From Children with Acute Diarrhea

<i>Shigella</i> sp.	No. (%)
<i>S. sonnei</i>	63 (70)
<i>S. flexneri</i>	26 (28.9)
<i>S. boydii</i>	01.00 (11.0)
<i>S. dysenteriae</i>	0.00
Total	90 (100)

Overall, 5662 (62%) patients were male and 3469 (38%) female. *Shigella* sp. was isolated frequently from children under 5 years of age, who accounted for 57.7% of all isolates. The results showed that imipenem, ciprofloxacin, ceftazidime and ceftizoxime are most effective antimicrobial agents against *Shigella* sp. The most frequent re-

sistance observed was to co-trimoxazole (92.2%), ampicillin (65.6%) and tetracycline (65.6%) (Table 2).

Table 2. Antimicrobial Susceptibility Profile of *Shigella* Subgroups Isolated From Children with Acute Diarrhea

Antibiotics	Sensitive, (%)	Resistant, (%)
Ampicillin	31 (34.4)	59 (65.6)
Ceftriaxone	69 (76.7)	21 (23.3)
Cefixime	67 (74.4)	23 (24.4)
Co-trimoxazole	07.0 (7.8)	82 (92.2)
Nalidixic Acid	59 (65.6)	31 (34.4)
Cefotaxime	70 (77.8)	20 (22.2)
Ceftazidime	83 (92.2)	07.0 (7.8)
Ceftizoxime	83 (92.2)	07.0 (7.8)
Imipenem	89 (98.9)	01.0 (1.1)
Tetracycline	31 (34.4)	59 (65.6)
Ciprofloxacin	88 (97.8)	20 (22.2)

5. Discussion

Shigella is a major cause of bacillary dysentery throughout the world and is responsible for 5-10% of diarrheal illness in many regions. It has been estimated that annually 91 million people worldwide become in contact with *shigella* and approximately 580,000 cases of shigellosis are reported among travelers from industrialized countries (4, 21). *Shigella* is highly adapted to humen, with humans and primates in captivity being the only known natural hosts. The minimal infectious dose is less than 200 bacterial cells, facilitating transmission of disease in areas where there are overcrowding, low hygiene and poor sanitation (22, 23).

The geographical repartition and the pathogenicity of the four *Shigella* spp. are different (24). *S. flexneri* is the most commonly isolated species in the developing world and the most frequent cause of morbidity and mortality. In general, illness caused by *S. sonnei* is less severe. The frequencies of *S. flexneri*, *S. sonnei*, *S. boydii*, and *S. dysenteriae* are 16, 77, 2, and 1% in developed countries and 60, 15, 6, and 6% in developing countries, respectively. In developing countries, the most common serotype of *S. flexneri* is 2a, followed by 1b, 3a, 4a, and 6.29, (25).

In our study, *S. sonnei* (70%) was the most common, followed by *S. flexneri* (28.9%), *S. boydii* (1.1%) and *S. dysenteriae* (0%), respectively. This information is important because *S. sonnei* and *S. flexneri* are the most common species and they should be taken into account for therapy. Our isolates were most resistant to co-trimoxazole, tetracycline and ampicillin (>65%) which is compatible with other studies. Although resistance to β -lactams may arise in *Shigella* as a result of mutations in genes encoding penicillin-binding proteins (PBPs) that reduce the affinity of PBPs for the antibiotics, the predominant and most clinically

significant mechanism of resistance to beta-lactam antibiotics in gram-negative bacteria is the production of β -lactamases (26).

In tetracycline resistant *Shigellaspp.*, genes carried on transposons and/or plasmids encode transmembrane proteins that efflux the antibiotic by an energy dependent manner (27). Co-trimoxazole is a common drug used as an empirical therapy for treatment of shigellosis. The extensive use of Co-trimoxazole has contributed to the emergence of resistant *Shigella* strains. Resistance to Co-trimoxazole can be chromosomally mediated, but the predominant mechanism in *Shigella*, is attainment of an additional, plasmid encoded, variant DHFR enzyme (especially DHFR I) (27). Widespread use of antimicrobial drugs and horizontal gene transfer has led to *Shigella* species resistance to generally used antibiotics. Resistance patterns are influenced by geographic area, year that isolates were obtained, classes of antimicrobial agents, and pressure exerted by antimicrobial use.

Our results showed the antimicrobials that remain effective against *Shigella* are imipenem, ciprofloxacin, ceftazidime and ceftizoxime. The antimicrobial resistance profile differs between geographical locations; this may be due to the occurrence and spread of antimicrobial-resistant clones. There is no complete relevance between *in vitro* antibiotic susceptibility results and clinical efficacy. Several antimicrobial agents that were effective *in vitro* have been ineffective clinically. Using an ineffective antibiotic, that is one to which the organism is resistant or that is clinically ineffective, may imprint a risk. Further to any potential systemic side-effects of the antibiotic, it may affect the gastrointestinal normal microflora. There is document reporting that the normal microflora compete with the infecting *Shigella*; (25) thus, ineffective antimicrobials may actually deteriorate shigellosis by selectively inducing *Shigella* proliferation.

In many developing countries antibiotics are readily accessible without a medical prescription. Self-prescribing of antimicrobial agents are common, primarily for non-specific illnesses and for complaints such as headache, fever, diarrhea, minor respiratory and gastrointestinal infections and abdominal pain. Moreover, antibiotics may have a low quality, due to decay, counterfeiting, or lack of bioequivalence in the case of generic drugs. Thus, use of such drugs results in suboptimal serum levels, which induces selection for antimicrobial resistance. An increasingly ambulant population supplies opportunities for the rapid spread of multidrug resistant microorganisms in areas where unlimited antibiotic use is common, especially in regions where many people may asymptotically anchor *Shigella*. Crowding and unsuitable sewage waste induces the spread of resistant *Shigellaspp.* and this supplies opportunities for genetic exchange among bacteria, facilitating the dissemination of antibiotic resistance determinants. Since the antibiotic resistant profile of *Shigella* differs geographically as well as over time

within a single country, regular (annually or monthly) continuous surveillance is necessary. The results of accurate surveillance should be used to reconsider guideline policies for antibiotic prescription.

Acknowledgements

This research received financial and technical support from the Bahrami Hospital, Tehran, Iran.

Authors' Contribution

All authors listed have contributed sufficiently to the project.

Financial Disclosure

There is no conflict of interest.

Funding Support

Bahrami Hospital, Tehran, Iran.

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