

Antibiotic resistance pattern in *Shigella* species isolated from children with acute diarrhea in Tabriz city, Iran

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ABSTRACT

Aim: *Shigella* is one of the leading causes of acute diarrhea in children worldwide. Antibiotic resistance in this bacterium has increased due to the indiscriminate use of common antibiotics. Therefore, the aim of this study was to investigate the prevalence and pattern of antibiotic resistance in *Shigella* species isolated from children with acute diarrhea in Tabriz hospitals.

Material and Method: In this descriptive cross-sectional study, 321 samples suspected of *Shigella* were collected from children's diarrhea stool samples and identified using standard microbiological and biochemical tests. Antibiotic resistance was also determined using disk diffusion by the Kirby-Bauer method.

Results: A total of 84 samples were positive for *Shigella*. Among them, *S. flexneri* species with 83.3% had the highest frequency and *S. boydii* with 13.1%, *S. sonnei* with 2.4% and *S. dysenteriae* with 1.2% had the lowest frequency. The highest resistance was related to the antibiotics cotrimoxazole (92.85%), tetracycline and ampicillin (67.86%) and the lowest resistance was related to imipenem antibiotics (2.38%) and ceftizoxime (9.52%).

Conclusion: The present study showed that *Shigella flexneri* is the predominant species isolated from children with Shigellosis in hospitals in Tabriz city. Our results also indicate an increase in resistance to common antibiotics. Therefore, it is recommended that antimicrobial susceptibility testing be performed in the study area prior to antibiotic administration.

Keywords: Prevalence, *Shigella*, diarrhea, drug resistance

INTRODUCTION

Diarrhea is one of the most serious intestinal infections in children, which is a major health problem in developing countries and one of the leading causes of illness and mortality (1,2). *Shigella* belongs to the family *Enterobacteriaceae*, gram negative coccobacilli, nonmotile, spore-forming rods, and facultative anaerobes. This bacterium causes shigellosis or bloody diarrhea in humans. *Shigella* species are among the most common Enteropathogenic bacteria that have the most diarrheal infections in developing countries. About 200 million *Shigella* infections and 3 to 5 million deaths occur annually in developing countries, most of which involve children under 5 years of age (3). Shigellosis is recognized as a global problem by the World Health Organization (4). *Shigella* contains four species of *S. flexneri*, *S. dysenteriae*, *S. boydii*, and *S. sonnei*, which can cause shigellosis by the presence of blood in the stool (5). Epidemiological data has shown that *S. sonnei* is the predominant species of *Shigella* in Europe, and the United States. But it is noteworthy that *S. flexneri* is more common in Asian and African countries,

especially Iran (6). In terms of pathogenicity, the bacteria involved in diarrhea are divided into two groups: invasive bacteria and toxin-producing bacteria. Bacterial pathogens are involved in adhesion, invasion and toxin production. *Shigella* penetrates the mucosal epithelium, begins to grow and penetrates into adjacent cells, eventually causing cell damage. These bacteria multiply in the gut or on the surface of small intestinal epithelial cells to produce toxins that cause water and electrolytes to be secreted by gut cells (7). The bacterium can be transmitted directly from person to person, and indirectly through the consumption of contaminated food and water (8).

The most important symptoms of infection with this bacterium include: anorexia, fever, intestinal inflammation, bloody-purulent stools, abdominal pain, and feeling of incomplete emptying of the intestine with anal pain (9). One of the most important problems for the treatment of Shigellosis-positive people today is the development of antibiotic resistance by the plasmids and integrons.

Therefore, susceptibility to resistant strains rarely occurs (7). *Shigella* antibiotic resistance is a growing problem in Asia, Africa and South America (10). Because shigellosis is highly contagious, information about the prevalence of the disease and the antimicrobial susceptibility of the strains is crucial to ensure appropriate clinical treatment, and patient management. Therefore, the aim of this study was to determine the pattern of antibiotic resistance in *Shigella* spp isolated from children with diarrhea in Tabriz city.

MATERIAL AND METHOD

In this descriptive cross-sectional study, 321 samples suspected of *Shigella* were collected from stool (diarrhea) samples during one year from November 2016 to November 2017 by random sampling method in special and disposable sterile containers. Stool samples from sick children admitted to different wards of the hospital with positive culture according to *Shigella*, are collected weekly, and then by obtaining the consent of each patient and filling out their personal and clinical profile form and observing the statute of the Commitment Committee Ethical regarding the secrecy of the name and details of each of the test subjects, we transferred the samples to the specialized microbiology laboratory of the Islamic Azad University, Ahar Branch, and proceeded to isolate and identify the bacteria causing the infection. Samples were examined for macroscopic characteristics (consistency, mucus, and blood) and microscopy (white, and red blood cells). Isolates on Salmonella-*Shigella* agar (SS), McConkey agar, xylose lysine deoxycholate agar (XLD) and selenite-F (SF) (manufactured by Merck Germany) for 1 to 4 days at 35 to 37°C was placed in the incubator. Suspected colonies then grown by biochemical and differential tests including oxidase, catalase, SIM, MR/VP, citrate consumption, TSI, urease, phenylalanine deaminase, decarboxylation of the amino acids ornithine and mannitol, the consumption of lysine decarboxylase, XLD and hydrogen sulfide production (all media were provided by Merck, Germany) were identified.

Antibiotic resistance pattern of all *Shigella* isolates by Kirby Baur standard method, and through the instructions of Clinical and Laboratory Standards Institute (CLSI) (11) and preparation of 0.5 McFarland concentration of bacteria and culture on Mueller hinton agar medium against cefotaxime, ceftazidime, ceftriaxone, ampicillin, ciprofloxacin, ceftizoxime, imipenem, nalidixic acid, tetracycline, cotrimoxazole and cefixime (Mast, UK) were performed at the plate level according to the standard, antibiogram. Results were reported according to CLSI guidelines. In order to statistically analyze the data, the twentieth version of SPSS software and Chi-square and Fisher tests were used. $P < 0.05$ values were considered statistically significant.

RESULTS

Out of 321 stool (diarrhea) samples, 84 (26.17%) samples were positive for *Shigella*. Of these, 46 (54.76%) samples belonged to males and 38 (45.24%) samples belonged to females. The mean age of patients varied from 6.07 ± 2.12 to at least one year to a maximum of 12 years. *S. flexneri* species with 83.3% had the highest frequency and *S. boydii* with 13.1%, *S. sonnei* with 2.4% and *S. dysenteriae* with 1.2% had the lowest frequency. According to Fisher test, there was no statistically significant difference between *Shigella* species in sex and age ($p > 0.05$). Antibiogram results show that the highest resistance is related to the antibiotics cotrimoxazole (92.85%), tetracycline and ampicillin (67.86%) (Figure 1). The results of Fisher's exact test showed there was no significant relationship between *Shigella* and antibiotic resistance ($p > 0.05$).

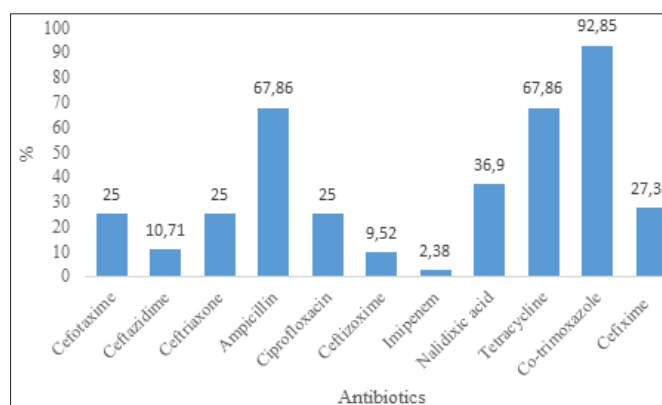


Figure 1. Percentage of antibiotic resistance in *Shigella* bacteria

DISCUSSION

Shigella is the causative agent of Shigellosis, a bacterial diarrhea that develops in adults and is very dangerous in infants and children, and can lead to death if left untreated. The disease is endemic worldwide, affecting 163 million people worldwide each year (12,13). The findings of the present study shows that out of 321 stool (diarrhea) samples, 84 (26.17%) samples are positive for *Shigella*. Of these species, *S. flexneri* with 83.3% had the highest frequency, *S. boydii* with 13.1%, *S. sonnei* with 2.4% and *S. dysenteriae* with 1.2% had the lowest frequency. In developing countries, the prevalence of *S. flexneri* is 60%, *S. sonnei* 15%, *S. boydii* 6% and *S. dysenteriae* 6%. In developed countries, this amount is 16%, 77%, 2, and 1, respectively (4).

Yavari et al. (14), Khorshidi et al. (15), Abbasi et al. (16), Hosseini Nave et al. (17), Kahsay et al. (18), and Tajaddini et al. (19), by examining *Shigella* on patients with diarrhea, the incidence of Shigellosis was reported to be 7.8%, 7.6%, 8.2%, 9%, 13.3% and 14.1%. Which are less than the findings of the present study. In the present study, the most common *Shigella* species are related to *S. flexneri* with 83.3%. Which is in line with the findings of the study of Moosavian et al. (20), in Ahvaz and Hosseini Nave et al. (17), in Kerman.

In 2014, Abbaspour and colleagues isolated 90 *Shigella* cases from a total of 9,131 stool samples in Tehran. Of these, 70% belonged to the *S. sonnei*, 28.9% belonged to the *S. flexneri* and 1.1% belonged to the *S. boydii* (21). In 2020, Karimi-Yazdi et al. (22), reported a prevalence of *S. sonnei* of 78.7%, *S. flexneri* of 19.9%, and *S. boydii* of 1.4%. Abbasi et al. (16), examined multidrug-resistant *Shigella* infection in children with diarrhea, out of 19 positive *Shigella* specimens, 21% belonged to flexneri species and 78.9% belonged to sonnei species. Differences in the frequency of *Shigella* and its genera may be related to age, geography, climate, as well as many other environmental conditions. Facilities such as a public water supply, and sewerage systems, close relationship with the level of health and personal hygiene can be another reason for these differences. *S. sonnei* is generally found in industrialized countries, while *S. flexneri* is highly prevalent in developing countries (23). Analysis of antibiotic resistance data in *Shigella* shows that the highest resistance is related to cotrimoxazole (92.85%), tetracycline and ampicillin (67.86%), and the lowest resistance is related to imipenem 2.38% and ceftizoxime 9.52%. During a study in Tehran, the *Shigella* resistance to common antibiotics was reported as follows: cotrimoxazole 92.2%, tetracycline 65.6%, ampicillin 65.6%, nalidixic acid 34.4%, cefixime 24.4%, Ceftriaxone 23.3%, cefotaxime 22.2%, ciprofloxacin 22.2%, ceftazidime 7.8%, ceftizoxime 7.8%, and imipenem 1.1%. Which is consistent with the findings of the present study (21).

Dolatshahi and Amini from a total of 300 stool samples tested, obtained 60 (20%) *S. sonnei* strains. All isolates (100%) were resistant to streptomycin and nalidixic acid. Also, the results of antimicrobial susceptibility test showed that percentages of resistance to tetracycline, chloramphenicol and ampicillin were 86.7%, 91.8% and 50%, respectively (24). Previous reports in Iran has reported resistance to cotrimoxazole from 92.2% to 94%. Which is close to the findings of the present study (25,26).

According to reports by Ashkenazi et al. (27), in 2003 and Akçali et al. (28), in 2008, ampicillin resistance ranged from 12-20% to 87%. Which is less and more of the findings of the present study, respectively. In a study by Zamanlou et al. (29), and Aggarwal et al. (30), in 2018 and 2016, ciprofloxacin antibiotic resistance reported 4.2% and 56.2%, respectively. Which is less and more from the present study, respectively. According to the findings of Zamanlou et al. (29), and Ranjbar et al. (31), in Iran, the resistance to nalidixic acid antibiotic has been reported as 17.4% and 31%, respectively. Which are less than the findings of the present study.

CONCLUSION

Due to the increasing in prevalence of *Shigella* species, and the increasing resistance of intestinal pathogenic strains to widely use and inexpensive antibiotics, it seems that effective antibiotic treatment is becoming more difficult. Our results therefore raise concerns about the spread of *Shigella* among children with diarrhea in the study area. Therefore, to prevent the spread of these resistant isolates, monitoring of the antimicrobial resistance of *Shigella* species should be considered on an ongoing basis and common antibiotics should be used properly.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study has been approved by the ethical committee of Ahar University of Sciences (permission granted: 12.06.2020, decision no: 2020-02).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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