International Journal of Molecular and Clinical Microbiology 7(1) (2017) 803-808

International Journal of
Molecular and Clinical Microbiology

Identification of *Shigella* species and their antibiotic resistance patterns among dysenteric patients in Baradaran Rezaei Hospital of Damghan, Northeast of Iran

Mansoor Khaledi[1], Reza heidari-soureshjani[2], Hanieh Asaadi[3], Abdollah Ardebili[4], Sajjad Yazdansetad[4*]

1. MS.c. in Microbiology, Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran.
2. BSc Student, Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran.
3. Department of Microbiology and Parasitology, Faculty of Medicine Bushehr, Iran.
4. Department of Microbiology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

ARTICLE INFO

Article history:
Received 17 October 2016
Accepted 28 May 2017
Available online 1 June 2017

Keywords:
Shigella,
Antibiotic resistance,
Dysentery,
Damghan

ABSTRACT

Shigella is a major cause of dysentery across the world. Appropriate antibiotic treatment of shigellosis depends on resistance patterns. The present study was conducted to identify *Shigella* species and their antibiotic resistance patterns among dysenteric patients in Rezaei Hospital of Damghan. Isolation of *Shigella* species was conducted by specific culture medium and biochemical tests. The *Shigella* species were determined by specific antiserum with agglutination on slide. Then, susceptibility to different antibiotics, i. e. nalidixic acid, ciprofloxacin, ampicillin, tetracycline, co-trimoxazole and ceftriaxone, was tested. The antibiotic susceptibility tests were carried out using the Kirby-Bauer standard method on Mueller-Hinton agar. In this study, 29 *Shigella* species were found in 91 stool samples of the patients. Determination of *Shigella* spp. by specific antiserum showed *S. flexneri* (group B) in 13 cases, *S. dysenteriae* (group A) in 10 cases, and *S. sonnei* (group D) in 6 cases, while no case of *S. boydii* (group C) was found. The antibiotic resistance tests indicated that resistance to co-trimoxazole, tetracycline, ampicillin, nalidixic acid, ciprofloxacin and ceftriaxone was 75.8%, 65.5%, 55.1%, 6.8%, 3.4% and 0% respectively. According to lower resistance to ciprofloxacin and ceftriaxone, it seems that the fluoroquinolone antibiotic, as the first choice, and the third-generation cephalosporin, as the second choice, were suitable for treatment of shigellosis, but regarding the multidrug-resistance likelihood and antibiotic resistance patterns variation in *Shigella* strains, it is recommended to perform the organism susceptibility test to the antibiotic before treatment.

1. Introduction

Shigella species are non-motile, narrow, and gram-negative microorganisms from *Enterobacteriaceae* family, with four species, *Shigella sonnei*, *Shigella flexneri*, *Shigella dysenteriae*, and *Shigella boydii*, identified by somatic on surface antigens and fermentation type of carbohydrates. It is remarkable that a small number of these microorganisms can cause shigellosis (Connie and George, 2000; Nelson, 2000).

*Corresponding author: Sajjad Yazdansetad
Tel: +98 9144263864
E-mail address: sajjad.yazdansetad@gmail.com

Shigellosis is an acute inflammatory and infectious colitis, often presenting with dysentery and caused by one of the four species of the genus *Shigella*, with a higher prevalence in children. In temperate and warm regions especially rainy season, the increased prevalence of this infection is a factor that plays a role in its spread. Observance of personal hygiene is the most important way to control the infection (Deniss et al., 2005; Gerald et al., 2000; Richard et al., 2004; Hartman et al., 2003). Over 140 million shigellosis cases are reported each year; this infection has led to nearly 6 million deaths in children under 5 years, especially in developing countries, while its associated mortality can be minimized by appropriate diagnosis and early treatment (Kottlof et al., 1999; Bradbury et al., 1984; Craun et al., 2005; Mandell and Bennett, 1990).

The emergence of strains resistant to the antibiotics conventionally used to treat shigellosis is one of the most important problems for treatment of this disease (Mandell and Bennett, 1990). Prescription of antibiotics must be done after detection of antibiotic susceptibility of these strains because different strains of this bacterium exhibit different patterns of drug resistance (Heidari-Soureshjani et al., 2016). Use of ineffective antibiotics can lead to the spread of infection due to incomplete treatment (Kuroki and Hart, 2001). The drug resistance in different regions depends on several factors, including use of antibiotics, how they are administered, and prevalence of infectious diseases (Mandell and Bennett, 1990). The first antibiotic resistance was reported in Japan in 1995. Studies have shown that 85.4-89.4% of the strains of this bacterium have acquired resistance to certain antibiotics (Matsushita et al., 1999).

A study in Hong Kong reported resistance to antibiotics ampicillin and trimethoprim-sulfamethoxazole 59%, and studies on *S. dysenteriae* in Saudi Arabia have reported resistance to nalidixic acid 94% (Chu et al., 1998; El Bushra and Bin, 1999). The research findings in Iran have been consistent; according to a study conducted on *Shigella* specimens taken from patients referring to Mofid Children's Hospital in Tehran, resistance to cotrimoxazole was reported 98.5%, to nalidixic acid 10%, and to ampicillin 84.6% (Qadamli, 1999). In a study on different species in Zanjan, the highest sensitivity (88.8%) was found for ciprofloxacin, and the highest resistance (100%) for ampicillin (Jamshidi and Matbooei, 2008). In several studies, resistance to antibiotics that have been mostly prescribed in the past such as ampicillin, cotrimoxazole, and nalidixic acid has increased, and in most countries ampicillin has been set aside as a daily treatment for Shigellosis (Mache et al., 1997; Ghaemi et al., 2007).

In the light of the drug resistance of various *Shigella* species in several areas and the importance of the issue for the type of prescribed antibiotics by physicians and the World Health Organization's recommendation of annual determination of drug resistance, this study was conducted to identify the species of *Shigella* and their antibiotic resistance patterns in patients with dysentery in a hospital in Damghan, central Iran.

2. Materials and Methods

2.1. Sampling

This study is a cross-sectional study, conducted during one year, from 1391 to 1392. The specimens of patients with diarrhea who were admitted to the hospital were taken by rectal swabs and transferred to the Cary Blair medium. Then, the samples were immediately transferred to the laboratory and stored at 4°C.

Inclusion criteria: dysentery specimens obtained from patients suffering from dysentery or acute diarrhea associated with fever, abdominal cramps, nausea and vomiting in health care centers (Fatahi et al., 2015).

Exclusion criteria: Consumption of antibiotics before sampling, specimens without a label and questionnaire, transferring the specimens not in compliance with the cold chain, sampling after the initial 24 h of the onset of symptoms, and receiving the sample 72 h after collection (Fatahi et al., 2015).

2.2. Microscopy Test

First, the specimens were examined microscopically, and then placed in *Shigella* selective and differential media, McConky, xylose lysine deoxycholate (XLD), and deoxycholate citrate (DCA). Selenite F was used to culture and isolate pure colonies.

After isolation of the colonies with *Shigella's* properties, standard biochemical tests were used

to confirm the genus of *Shigella* (Bopp et al., 2003). Then, the *Shigella* species and heads were determined by species-specific antiserum test (MAST) by slide agglutination.

2.3. Antimicrobial resistance

Antibiotic susceptibility test was carried out using Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Merck co.), according to Clinical and Laboratory Standards Institute (CLSI) guidelines using antibiotic discs (Padtan Teb co.), i. e. nalidixic acid (NAL, 30 µg), ciprofloxacin (CIP, 30 µg), ampicillin (AMP, 10 µg), tetracycline (TET, 30 µg), co-trimoxazole (CoT, 20 µg), and ceftriaxone (CRO, 30 µg). Quality control strains *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included in each test. The minimum inhibitory concentrations (MICs) of the antibiotics were determined.

3. Results

3.1. Species distribution

Of the 91 stool specimens, 29 (31.8%) contained *Shigella* isolates. Among these isolates, 13 (44.8%) were *S. flexneri* (group B), 10 (34.4%) were *S. dysenteriae* (group A), and 6 (20.6%) were *S. sonnei* (group D), while no case of *S. boydii* (group C) was found.

3.2. Antimicrobial resistance

Our results showed that 22 of the 29 (75.8%) *Shigella* isolates were resistant to co-trimoxazole, 19 isolates (65.6%) to tetracycline, 16 isolates (55.1%) to ampicillin, two isolates (6.8%) to nalidixic acid, and one isolate to ciprofloxacin, while all the isolates were sensitive to ceftriaxone. In the 22 resistant Shigella isolates to co-trimoxazole, *S. flexneri* was found in 11 cases; *S. dysenteriae* in nine cases, and *S. sonnei* in 2 cases. In the 19 resistant isolates to tetracycline, *S. flexneri* was found in six cases; *S. dysenteriae* in 10 cases, and *S. sonnei* in three cases. In the 16 resistant isolates to ampicillin, *S. flexneri* was found in six cases; *S. dysen-teriae* in nine cases, and *S. sonnei* in one case. In two resistant isolates to nalidixic acid, *S. flexneri* was found in one case and *S. dysenteriae* found in 1 case. Of 1 resistant *Shigella* isolate to ciprofloxacin, *S. dysenteriae* was found in one case only. The antibiotic resistance percentages of all *Shigella* isolates (groups A, B, C and D) are showed in diagram 1. The distribution of antibiotic resistance patterns of *S. flexneri*, *S. dysenteriae*, *S. sonnei* and of all *Shigella* isolates is shown in Tables 1,2,3 and 4 respectively.

Table 1. Antibiotic resistance patterns of *S. flexneri* isolates (group B) in *S. flexeneri* (group B) (total isolates 13)

Antibiotic	No.(%) of AB resistant
Nalidixic acid	1(7.6%)
Ciprofloxacin	0(0%)
Ampicillin	6(46.1%)
Tetracycline	6(46.1%)
Co-trimoxazole	11(84.6%)
Ceftriaxone	0(0%)

Table 2. Antibiotic resistance patterns of *S. dysenteriae isolates* (group A) (total isolates 10)

Antibiotic	No.(%) of AB resistant
Nalidixic acid	1(10%)
Ciprofloxacin	1(10%)
Ampicillin	9(90.0%)
Tetracycline	10(100.0%)
Co-trimoxazole	9(90.0%)
Ceftriaxone	0(0%)

Table 3. Antibiotic resistance patterns of *S. sonnei* isolates (group A) (total isolates 6)

Antibiotic	No.(%) of AB resistant
Nalidixic acid	0 (0%)
Ciprofloxacin	0 (0%)
Ampicillin	1 (16.6%)
Tetracycline	3 (50.0%)
Co-trimoxazole	2 (33.3%)
Ceftriaxone	0 (0%)

Table 4. Antibiotic resistance patterns of all *Shigella* isolates (groups A, B, C and D) (total 29)

Antibiotic	No.(%) of AB resistant
Nalidixic acid	2(6.8%)
Ciprofloxacin	1(3.4%)
Ampicillin	16(55.1%)
Tetracycline	19(65.5%)
Co-trimoxazole	22(75.8%)
Ceftriaxone	0(0%)

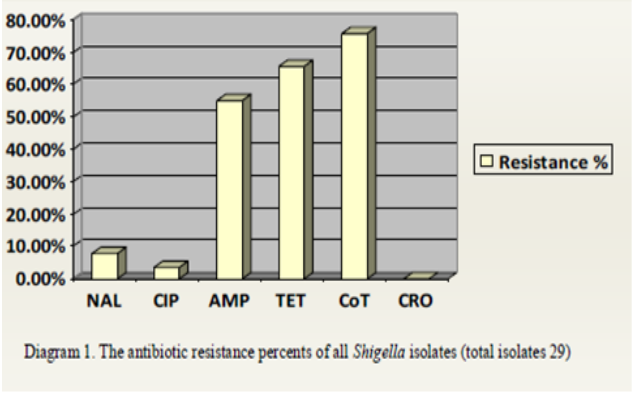

Diagram 1. The antibiotic resistance percents of all *Shigella* isolates (total isolates 29)

4. Discussion

Increased emergence of resistance to antimicrobial agents among *Shigella* spp. is considered a major threat for the control of shigellosis. Indiscriminate use of drugs and horizontal gene transfer have resulted in *Shigella* species' resistance to commonly used antibiotics. First, both sulphonamides and tetracycline were effective on shigellosis, but as strains quickly acquired resistance to these drugs, ampicillin and trimethoprim–sulfamethoxazole were used as well. The strains of all *Shigella* species worldwide have become resistant to all these inexpensive antimicrobials, and quinolones such as norfloxacin or ciprofloxacin are one of the few remaining effective drugs (Bradbury et al., 1984; Keusch and Bennish, 1989). In addition, changing patterns of antimicrobial susceptibilities of *Shigella* isolates have caused serious problems related to selection of an appropriate drug for the treatment of shigellosis. In a hospital in Tehran, 7.6% of the stool specimens of patients with enterocolitis were positive, but in our study the corresponding figure was obtained 31.8%. In a study conducted in Zahedan, *S. flexneri* was seen in 69.4% of the patients, *S. dysenteriae* in 21.8%, *S. boydii* in 7.5%, and *S. sonnei* in 1.3%. The corresponding figures in our study were obtained 44.8%, 34.4%, 0%, and 20.6%, respectively (WHO, 2005). Moreover, in the study of Zahedan, according to antibiotic resistance test, the resistance rate of co-trimoxazole, ampicillin, nalidixic acid, ciprofloxacin, and ceftriaxone was obtained 57.1%, 99.3%, 1.3%, 0%, and 0%, respectively. The corresponding figures in our study were obtained 75.8%, 55.1%, 6.8%, 3.4%, and 0%, respectively (Eghbal et al., 2009). In a study conducted in China on 77,600 specimens, 1,635 (2.1%) were positive for *Shigella*, *S. flexneri* was found in (569, 34.7%) of the pations, *S. boydii* in (3, 0.2%), and *S. Sonnei* in (1,066, 65.1%). According to antibiotic resistance test, the resistance rate of streptomycin, trimethoprim, ampicillin, and nalidixic acid was obtained (98.7%), (98.0%), (92.1%), and (91.7%), respectively (Zhang et al., 2014). In a study coducted in Nepal, 14.1% of stool specimens of patients with *Shigell*osis were positive. *Shigella flexneri*, *Shigella dysenteriae*, *Shigella boydii* and *Shigella sonnei* were accounted respectively for 43.07%, 27.69%, 21.53% and 7.69% of the total number of *Shigella* isolates. According to antibiotic resistance test, the resistance rates of ampicillin (84.62%), nalidixic acid (95.38%), ciprofloxacin (46.15%), co-trimoxazole (81.54%) were found (Khan et al., 2014).

In this study, 29 *Shigella* spp. were isolated from dysenteric patients and resistance patterns to several therapeutic antibiotics such as nalidixic acid, ciprofloxacin, ampicillin, tetracycline, co-trimoxazole and ceftriaxone were investigated. Much evidence of recent studies in our country and worldwide have shown that *S. flexneri*, *S. sonnei*, and *S. dysenteriae* species were dominant. The results correspond to our results. Also, our study showed that resistance to ampicillin, co-trimoxazole and tetracycline antibiotics were greater than that to quinolones, fluoroquinolones, and cephalosporins. In the present study, we found greater resistance to co-trimoxazole (75.8%) and tetracycline (65.5%) and lower resistance to nalidixic acid (quinolone) (6.7%) and ciprofloxacin (fluoroquinolone) (3.4%), and no resistance to ceftriaxone (cephalosporin). The results have been consistent with our findings. Due to the emergence of resistance to antibioticts in *Shigella*, as a great problem, an inclusive strategy for resistance control involving regulation of drug availability, ensurance of antimicrobial drug quality, adequate surveillance, and discouragement of antimicrobial abuse should be promoted.

Conclusions

According to lower less resistance to the ciprofloxacin and ceftriaxone, it seems that the

fluoroquinolone, as the first choice, and the third-generation cephalosporin, as the second choice, were suitable for treatment of shigellosis, but regarding the multidrug-resistance likelihood and antibiotic resistance patterns variation in *Shigella* strains, it is recommended to perform the organism susceptibility test to the antibiotic before treatment.

Refereces

Bopp, C.A., Brenner, F.W., Fields, P.I., et al., (2003). Escherichia, shigella, and salmonella. Manual clin microbial. 8: 654-71.

Bradbury, W.C., Pearson, A.D., Marko, M.A., et al., (1984). Investigation of a Campylobacter jejuni outbreak by serotyping and chromosomal restriction endonuclease analysis. J clin microbial. 19(3): 342-346.

Chu, Y.W., Houang, E.T., Lyon, D.J., et al., (1998). Antibiotic resistance in *S.flexneri* and Sh.Sonnei in Hong Kong in 1986 to 1995. Antimicrob Agents Ch. 42(2):440-3.

Connie, R.M., George, M., (2000). textbook of Diagnostic Microbiology, 2th Ed, Saunders Company. Philadelphia: USA; 73: 484,496,963.

Craun, G.F., Calderon, R.L., & Craun, M.F. (2005). Outbreaks associated with recreational water in the United States. J. Environ. Health Res. 15(4): 243-262.

Deniss, K., Eugene, B., Anthony, S.F., et al. (2005). Harrison's Principles of Internal Medicine,16th ed, McGraw-Hill Companies. New York: USA, 902-6.

Eghbal, G.M., Borji, A., Naghavi, A., et al., (2009). Antimicrobial resistance of shigella species isolated from diarrehal patiens in zahedan. Zahedan J Res Med Sci, 3 (1): 65-72.

El Bushra, H.E., & Bin, S.A., (1999). Intrafamilial person-to-person spread of bacillary dysentery due to Shigella dysenteriae in southwestern Saudi Arabia. East Afr Med J. 76(5): 255-259.

Fatahi, A., Ajami, A., Bozorgzad, M., et al. (2015). A Comparison between Culture and Multiplex PCR for Detection and Identification of Shigella Species in Patients with Shigellosis from Isfahan Province in 2014-2015. J Med Microbial Infect Dis. 3(1): 23-28.

Gerald, L.M., John, E.B., Raphael, D.M., (2000). Douglas, and Bennett's Principles and Practice of Infectious diseases, 5th ed, Churchill Livingstone Company. Philadelphia: USA, 2363-8.

Hartman, A.B., Essiet, I.I., Isenbarger, D.W., & Lindler, L.E., (2003). Epidemiology of tetracycline resistance determinants in Shigella spp. And enteroinvasive Escherichia coli: characterization and dissemination of tet (A)-1. J clin microbial. 41(3): 1023-1032.

Heidari-Soureshjani, R., Obeidavi, Z., Reisi-Vanani, V., Ebrahimi Dehkordi, S., Fattahian, N., & Gholipour, A. (2016). Evaluation of antibacterial effect of sesame oil, olive oil and their synergism on Staphylococcus aureus in vitro. Advanced Herbal Medicine, 2(3): 13-19.

Jamshidi, A., Matbooei, A., (2008). [Shigella spp frequency,serotyping and antibiotic resistance pattern in acute diarrheic patients in Zanjan shahid beheshti hospital, during 2003-2007] Persian. ZUMS.; 62 (16):77-84.

Keusch, G.T., & Bennish, M.L., (1989). Shigellosis: recent progress, persisting problems and research issues. Pediatr Infect Dis J. 8: 713–9.

Khan, S., Singh, P., Ansari, M., & Asthana, A. (2014). Isolation of Shigella species and their resistance patterns to a panel of fifteen antibiotics in mid and far western region of Nepal. Asian Pac J Trop Dis. 4(1): 30-34.

Kottlof, K.L., Winickoff, J.P., Ivanoff, B., et al., (1999). Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. Bull World Health Organ. 77: 651 666.

Kuroki, S., & Hart, C.A., (2001). Global aspects of antimicrobial-resistant enteric bacteria. Curr Opin Infect Dis. 14(5): 579-586.

Mache, A., Mengistu, Y., and Cowley, S., (1997). Shigella serogroups identified from adult diarrhoeal out-patients in Addis Ababa, Ethiopia: antibiotic resistance and plasmid profile analysis. East Afr Med J. 74: 179-182.

Mandell, G.L., Bennett, J.E., (1990). Principles and practice of infectious diseases.3th ed. New York: Churchill Livingstone. p: 380.

Matsushita, S., Konishi, N., Yanagawa, Y., et al. (1999). Strains of Shigella sonnei recently isolated in Tokyo. Kansenshogaku zasshi. J Japan Assoc Infect Dis. 73(5): 414-420.

Ghaemi, E.O., Aslani, M.M., Moradi, A.V., Dadgar, T., Livani, S., Mansourian, A.R., & Ahmadi, A. R. (2007). Epidemiology of Shigella-associated diarrhea in Gorgan, north of Iran. Saudi Journal of Gastroenterology, 13(3), 129.

Qadamli, P., (1999). [prevalenc of clinical symptoms and antimicrobial sensitivity of shigella in children.] Persian. JQUMS. 10:62-67.

Richard, E.B., Robert, M.K., Hal, B.J., (2004). Nelson Textbook of Pediatrics, 7th Ed, Saunders Company. Philadelphia: USA. 912-20.

World Health Organization. (2005). Guidelines for the control of shigellosis, including epidemics due to Shigella dysenteriae type 1. Geneva: WHO; Available at: http://www.who.int/ child _adolescent_health/documents/9241592330/en /(accessed December 2008).

Zhang, J., Jin, H., Hu, J., et al. (2014). Antimicrobial resistance of Shigella spp. from humans in Shanghai, China, 2004–2011. Diagn microbial infect dis. 78(3): 282-286.