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Original Article

Comparison of the antibiotic resistance patterns among Shigella species isolated from pediatric hospital between 1995-1999 and 2009-2013 in North-West of Iran

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Abstract

Introduction: This study was conducted to determine the frequency and pattern of antimicrobial susceptibility of Shigella spp. isolated from pediatric hospital in two different time periods between March 1995 to March 1999 and March 2009 to March 2013 in North-West of Iran.

Methods: The stool specimens were collected and examined for shigellosis by biochemical tests, and antibiogram was conducted according to Clinical and Laboratory Standards Institute protocol. One hundred and thirty-nine Shigella spp. isolated from year 1995 to 1999 and 38 Shigella spp. isolates collected from year 2009 to 2013 and examined for serotyping and antibiotic resistance pattern.

Results: According to serotyping results Shigella flexneri isolated in 98.6% of isolates in the first time period, followed by Shigella boydii and Shigella sonnei (0.7%) but in the second time period just 47.3% were S. flexneri and 39.5% were S. sonnei, 7.9% were S. boydii and 5.3% of isolates were Shigella dysenteriae. Results indicated significantly increase in resistance to ceftizoxime, chloramphenicol, and amikacin (P = 0.004, 0.010, and 0.004 respectively), also, in Shigella isolates isolated in the second time period showed an increase in multidrug resistant (MDR) isolate and frequency of MDR isolates increased to 95.0% in the second time period.

Conclusion: We are facing with the increase in resistance to antibiotics in Shigella spp. especially MDR isolates. These results showed changing pattern of resistance in Shigella isolates and needs for planning and design antibiotics stewardships for controlling Shigellosis, especially in pediatric hospitals.

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Introduction

Shigella species are Gram-negative, non-sporulating, rod-shaped bacteria that belong to the family Enterobacteriaceae[1] and are important pathogens that are responsible for 5-10% of diarrheal diseases and dysentery occurring all over the world.[2] Shigellosis is still an important public health problem, especially in developing countries.[3-5] It is also leading to annual deaths of 3-5 million children younger than 5 years of age in these countries,[4-6] where there is substandard hygiene and unsafe water supplies[3,7] and use of antimicrobial agents is often indiscriminate.[6] The progressive increase in antibiotic resistance among enteric pathogens in developing countries is a research priority of the diarrheal disease control program of the World Health Organization.[6]

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Since its first report in studies conducted in the 1950s, multidrug resistant (MDR) transmitted by plasmids among Shigella species has been reported from many countries.[3,8,9]

Moreover, an increase in resistance against many different drugs has been observed in the last two decades.[3] Until 2007, fluoroquinolones, β-lactams, and a combination of trimethoprim and sulfamethoxazole, cotrimoxazole (SXT) represented the drugs of choice for treatment of shigellosis.[2] However, the therapy employing these drugs is becoming compromised by the emergence of strains resistant to these commonly used antibiotics and becoming MDR. This rapid emergence of MDR strains is largely due to empirical treatment with antibiotics, and the antimicrobials may not be completely effective.[6] Thus, the knowledge of antimicrobial resistance pattern is important for the better management of shigellosis.[6,10]

The purpose of this study was to determine the changes of antimicrobial resistance patterns of Shigella species isolated from Tabriz Pediatric Hospital, Iran, as the central pediatric hospital in North-West of Iran from March 1995 to March 1999 and March 2009 to March 2013 for the better management of shigellosis.

Methods

This retrospective study was conducted in the Bacteriology Laboratory, Tabriz Pediatric Hospital (from a pediatric ward), in two time periods from March 1995 to March 1999 and March 2009 to March 2013. In brief, stool specimens were collected from inpatients and outpatients (one single isolate per individual) and after isolation in blood agar were inoculated on plates of MacConkey agar (BBL® 211391) and Salmonella-Shigella agar (BBL® 211596). The plates were incubated at 37° C for 24 h and suspected colonies were further examined by conventional biochemical tests.[11,12] The isolates were tested for susceptibility to antimicrobials using Kirby-Bauer disc diffusion methodology according to the criteria recommended by the Clinical and Laboratory Standards Institute protocol of antibiogram,[13] using the following antimicrobial agents (all antibiotic discs provided from Mast Group, UK): tetracycline (TET), ampicillin (AMC), gentamicin (GEN), amikacin (AMK), SXT, chloramphenicol (CHL), ceftizoxime (ZOX), and cephalexin (LEX) in the first time period (March 1995 to March 1999) and AMC, GEN, AMK, TET, SXT, ZOX, LEX, CHL, ciprofloxacin (CIP), nalidixic acid (NAL), cefixime (CFM), cephalotin (CEF), ceftazidime (CAZ), and cefotaxime (CTX) in the second time period (March 2009 to March 2013).

Escherichia coli ATCC 25922 was used as a control for antibiotic susceptibility determination. MDR was defined as resistance in an isolate to more than two unrelated drugs.[13]

Serogroups of Shigella was identified by serotyping with antiserums provided by Bahar Afshan Company (Bahar Afshan Co., Tehran, Iran). Serotyping was done by latex agglutination for detecting serogroups included serogroup A: Shigella dysenteriae, serogroup B: Shigella flexneri, serogroup C: Shigella boydii, serogroup D: Shigella sonnei. All procedure was done according to manufactures instruction.

All data were analyzed with SPSS for Windows (version 13, SPSS Inc., Chicago, IL, USA). The significance of differences between resistance patterns of isolates was determined using the chi-square test (or Fisher exact test). The significance level was defined as $P \leq 0.05$.

Results

Stool specimens were received and processed in the first-time period (number of isolates = 139) and the second time (number of isolates = 38).

During the first time, 61.2% Shigella species were isolated from male patients and 38.8% were from females and in the second-time, the isolation rate of Shigella spp. From male and female patients were 63.2% and 36.8%, respectively.

Shigella spp. isolates belonging to S. flexneri were the more common (98.6%), followed by S. boydii and S. sonnei (0.7%) in the first time and in the second time 47.3% were serotyped as S. flexneri and 39.5% as S. sonnei and 7.9% as S. boydii and 5.3% were S. dysenteriae. In susceptibility patterns of Shigella isolates, from 139 isolates of Shigella

isolated in the first time showed a high proportion of resistant isolates to TET (95.0%), AMC (95.0%), SXT (88.5%), CEF (64.7%), and CHL (56.1%), and a low proportion of resistant isolates to GEN (5.0%), AMK (2.2%), and ZOX (5.8%).

Of the 38 isolates of Shigella isolated during the second time period, 97.4% were resistant to AMC, 96.2% to TET, 94.7% to SXT, 63.2% to CFM, 63.2% to LEX, 50.0% to CTX, 47.4% to CEF, 47.4% to CAZ, 36.8% to NAL, 34.2% to CHL, 29.0% to ZOX, 23.7% to AMK, 13.1% to GEN and 5.3% to CIP. These results indicated significantly increase in resistance to ZOX, CHL, and AMK (P = 0.004, 0.010, and 0.004, respectively) (Table 1). In the second time, more than 50.0% of isolates were resistant to more than seven types of antibiotics (Figure 1). Overall, Shigella species isolated in the second time reveals the increased resistance to the most of antimicrobials compared with the first time period.

Discussion

Shigellosis still accounts for a significant proportion of morbidity and mortality, especially in developing countries.[10,14] Four species of the genus Shigella, S. dysenteriae, S. flexneri, S. boydii, and S. sonnei cause a wide spectrum of illnesses ranging from watery diarrhea to fulminant dysentery.[2,5,15] The frequency of occurrence of Shigella species differs by country and in different populations within a country.[2,16] In this retrospective study was demonstrated a high level of antimicrobial resistance in Shigella species isolated from stool samples during two time periods between 1995-1999 and 2009-2013 in Tabriz Pediatric Hospital as central pediatric hospital in North-West of Iran. In the present study, S. flexneri was the predominant isolate with a mean prevalence of 98.6%, followed by S. boydii (0.7%) and S. sonnei (0.7%) in isolates isolated from 1995 to 1999 but in the second time period our results represented S. flexneri as 47.3% of responsible in all Shigella infections in children and S. sonnei with 39.5% was the next most common isolate, followed by S. boydii (7.9%) and S. dysenteriae (5.3%) respectively. S. flexneri is dominant to isolate in developing countries such as Bangladesh, Pakistan, Indonesia, India, Africa, and Iran[17-19] while S. sonnei is the major Shigella isolate in developed countries.[17] Results of the present study shows change in the pattern of species

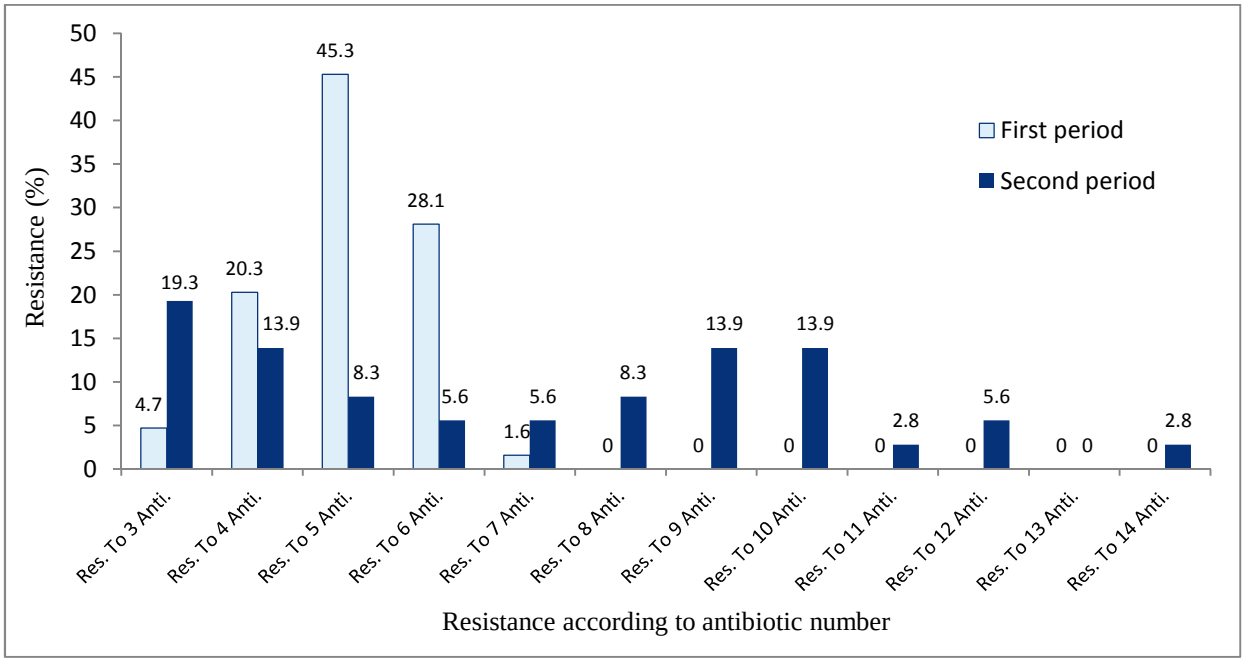

Figure 1. Frequency of multidrug resistant Shigella spp. in two time periods that the first time period was from 1995 to 1999 and the second time period was from 2009 to 2013

Res: Resistance; Anti: Antibiotics

isolated in Iran, and we had grown in isolation of S. sonnei from patients with shigellosis in this region.

In this study, low rate of resistance to GEN, AMK, and ZOX was observed in investigated isolates during both time periods and also in the second time our isolates were sensitive to CIP that these findings were in agreement with reports of other studies indicating Shigella isolates are sensitive to these antimicrobials.[3,10,20-22]

In the present study also resistance was observed for TET, AMC, and SXT during both time periods that high prevalence of resistance to SXT was proved in different studies,[21] for example, a high frequency of resistance was seen in Shigella spp. to SXT in Belgium and Ethiopia.[2,3,23] This high resistance to SXT can be due to longtime use of this antibiotic in our wards and community in diarrhea infections.

According to the results, Shigella isolates isolated in the second time period showed a significant increase resistance to the most of antibiotic in comparison with those isolated isolates in first time period. These indicate lower possible choices for treatment of shigellosis infections that can be due to empirical prescriptions of antibiotics for such infections that can cause a disaster in enteric infections in the near future. The frequency of MDR isolates increased from approximately 92.0% in the first time period to 95.0% in the second time. This increase was clearly associated with the emergence of the CIP, NAL, CTX, CEF, CAZ, CFM, AMK, and LEX MDR profile (Figure 1).

Our data are relatively in agreement with those of other studies showing the same trends of increases in the incidence caused by similar MDR profiles.[2,24]

This increasing incidence of MDR has led to tremendous interest in the genetics and mechanisms of resistance evolved by bacteria to counteract the effects of antimicrobial agents. Although Shigella isolates isolated in the second time indicated the increase resistance to the most of antimicrobials, but there have been significant statistical changes in ZOX, AMK, and AMK (P = 0.004, P = 0.010, and P = 0.004, respectively) (Table 1).

Table 1. Comparison of the antimicrobial resistance of isolates between the time periods 1995-1999 (n = 139) and 2009-2013 (n = 38)

Antimicrobial agent	Resistance rate (%)		P
	2009-2013	1995-1999	
Tetracycline	97.3	95.0	0.530
Ampicillin	97.4	95.0	0.530
Ceftizoxime	29.0	5.8	0.004
Cephalexin	63.2	64.7	0.850
Cotrimoxazole	94.7	88.5	0.170
Chloramphenicol	34.2	56.1	0.010
Gentamicin	13.1	5.0	0.170
Amikacin	23.7	2.2	0.004

Conclusion

These results showed that we were facing with the increase in resistance to antibiotics in Shigella spp. isolates like other Enterobacteriaceae members and changing pattern of resistance in Shigella isolates. Therefore, we need for planning and design antibiotics stewardships for controlling Shigellosis, especially in pediatric hospitals.

Conflict of Interests

Authors have no conflict of interest.

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