Original Article

Investigation of clinical characteristics and antibiotic sensitivity pattern of *shigella* and *Escherichia coli* isolated from children with diarrhea hospitalized in Ahvaz

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ABSTRACT

Diarrheal diseases remain the leading cause of death among children under 5 years old. Shigella and *Escherichia coli* are the most common bacterial causes of gastroenteritis in children, particularly in developing countries. Antimicrobial resistance in bacterial pathogens is a worldwide challenge. This study was conducted to investigate the clinical characteristics and antibiotic sensitivity patterns of Shigella and *Escherichia coli* isolated from hospitalized children with acute infectious diarrhea. In this study, 420 patients aged 2 months to 14 years with acute infectious diarrhea hospitalized in Ahvaz Teaching Hospitals during 2021-2022, were included. After history taking and physical examination, stool samples of the patients were cultured and antibiogram tests were performed using the agar disk diffusion method based on the CLSI guidelines. The analysis of stool samples revealed that 7.1% of patients were culture positive, 2.1% for *E. coli* and 5% for Shigella. *Shigella sonnei* was the predominant species followed by *Shigella flexneri*. The highest sensitivity was observed to Ciprofloxacin (90.5% among Shigella isolates and 77.8% among *E. coli* isolates). The rate of resistance in Shigella isolates to Ciprofloxacin, Ceftriaxone and Azithromycin were 9.5%, 62%, 77.1% respectively and in *E. coli* isolates were 22.2%, 55.6%, 88.9% respectively. Considering the increasing of antibiotic resistance, Ceftriaxone and Azithromycin are no longer good choices for empiric treatment of Shigellosis and *E. coli* infections in our region. The rate of resistance to Ciprofloxacin also shows an increase but it is still lower than the reported rate in other regions.

Keywords: Acute diarrhea, clinical characteristics, antibiotic sensitivity pattern Shigella, *E. coli*

Introduction

Diarrhea is a leading cause of mortality among infants and children worldwide. Each year, developing countries experience 2 to 4 billion cases of infectious diarrhea, resulting in 3 to 5 million deaths in children [1]. Understanding the underlying cause of diarrhea is not only crucial for epidemiological monitoring and control but also essential for accurate treatment [2]. Bacterial pathogens are Among the most important infectious agents responsible for acute diarrhea in developing countries.[3] Shigella and *Escherichia coli* are the most common bacterial causes of gastroenteritis in children in Iran and other developing countries [3- 6].

Based on stool characteristics, diarrhea can be classified into two types: watery diarrhea originating from the small intestine and dysentery originating from the large intestine. Viral agents and certain bacterial agents such as *Vibrio cholera* cause watery diarrhea. Dysentery is a type of mucoid diarrhea that is occasionally accompanied by visible blood in the stool and often accompanied by tenesmus and fever. Unlike watery diarrhea, dysentery shows an increased number of leukocytes in the stool exam, usually exceeding 5/HPF and often exceeding 10/HPF. Shigella and Enteroinvasive *Escherichia coli* are common culprits of dysentery, and they may lead to additional symptoms like high fever, encephalopathy, and convulsions [3, 7-9]. Globally, Shigella is responsible for more than one million deaths each year

in developing countries. Shigellosis symptoms can range from mild and self-limiting diarrhea to severe dysentery, accompanied by convulsions, high fever, and encephalopathy. The presence of dysentery and these signs, increases the likelihood of detecting Shigella in stool samples [9, 10].

Based on biochemical and serological characteristics *Shigella* is classified in four serogroups: *Shigella flexneri, Shigella dysenteriae, Shigella boydii* and *Shigella sonnei*.. The prevalence of the species is different in various geographic areas. *S. flexneri* is more prevalent serogroup in developing countries, and the most cases of Shigellosis in developed countries are caused by *S. sonnei*.[2,10-14]

Increasing pattern of antibiotic resistance poses a significant challenge in treating bacterial diarrhea, particularly in cases of shigellosis. Knowing about this pattern is essential for selecting appropriate treatment options. [15, 16]

This study was designed to determine the clinical characteristics and antibiotic sensitivity patterns of Shigella and Escherichia coli isolated from children with acute infectious diarrhea hospitalized in Ahvaz teaching hospitals. Early diagnosis and initiation of suitable treatment are crucial in preventing deaths, complications and the spread of resistant strains.

Materials and Methods

The study population comprised patients aged 2 months to 14 years, who were hospitalized with acute infectious diarrhea at Abuzar and Golestan Teaching Hospitals. Acute diarrhea was defined as the passage of 3 or more loose stools within 24 hours, lasting for less than two weeks [17]. Patients with ≥ 5 white blood cells in their stool exam upon hospitalization were included in the study [18]. Clinical symptoms and signs of patients were recorded and the patient's stool samples were sent to the laboratory in a sterile container for culture. To isolate and differentiate Shigella species, a loop of feces was transferred to Xylose-Lysine Deoxycholate (XLD) Agar and Eosin Methylene Blue (EMB) agar. The cultures were then incubated for 24 hours at 37°C. Additionally, a loop of the sample was transferred to the selenite F medium and incubated for 4 hours, followed by transfer to the SS medium for a 24-hour incubation period at 37°C. The bacterial serogroup was determined using slide agglutination [5, 8, 19].

For the isolation of *Escherichia coli*, the stool sample was first cultured on McConkey agar medium and then incubated for 24 hours at 37°C. Identification was based on standard biochemical tests. All positive *Escherichia coli* species were further identified as pathogenic *Escherichia coli* using a specific antiserum and the agglutination method on a dark background slide, following the protocol provided by the manufacturer [10,19, 20]. The antibiogram test was conducted using the disk diffusion method based on the Laboratory Standards Institute (CLSI) guidelines. Antibiotic disks including Ciprofloxacin, Ceftriaxone, Azithromycin, Gentamicin, Nalidixic Acid, Ampicillin, and Cotrimoxazole were placed on the Mueller Hinton Agar. After 24 hours of incubation, the zone diameters were measured, and the sensitivity and resistance of the bacteria were determined according to the disc manufacturer's instructions [10, 14, 21].

Results and Discussion

During a period of 2 years, 420 hospitalized patients diagnosed with dysentery were investigated. The average age was 4 years and 3 months, with a standard deviation of 2.66. The youngest age was 2 months and the oldest age was 14 years. Out of all the examined patients, 208 (49.5%) were male and 212 (50.5%) were female. **Table 1** shows the frequency distribution of clinical symptoms and signs in studied population.

Stool cultures of 30 patients (7.1%) were positive, of the total positive culture results, 9 samples were related to *E.coli* (2.1%) and 21 samples were related to Shigella (5%). **Table 2** shows the frequency distribution of positive stool culture results considering different Shigella serotypes among study population. Antibiotic sensitivity analysis of isolated *E.coli* and Shigella are shown in **Table 3**. Based on the **Table 3**, the highest antibiotic sensitivity in both types of bacteria was to Ciprofloxacin. Only 9.5% of Shigella isolates were resistant to Ciprofloxacin, 38% showed resistance to Ceftriaxone, 71.4% were resistant to Azithromycin. All of Shigella isolates showed resistance to Cefixime, Cotrimoxazole, Gentamicin, and Ampicillin. All of *E. coli* isolates was resistant to Gentamicin and Ampicillin, 55.6% of them showed resistance to ceftriaxone, 88.9% were resistant to Azithromycin and 22.2% to Ciprofloxacin.

Table 1. Frequency distribution of clinical symptoms and signs among studied population

	Frequency in studied population (n=420)	Frequency in Shigella positive patients (n=21)	P-value	Frequency in *E.coli* positive patients (n=9)	P-value
Fever	410 (97.6%)	21 (100%)	0.46	9 (100%)	0.63
Poor general condition	19 (4.5%)	5 (23.8%)	**0.0001**	2 (11.1%)	**0.01**
Vomiting	284 (67.6%)	18 (85.7%)	0.06	5 (55.5%)	0.51
Tenesus	246 (58.6%)	14 (66.6%)	0.44	6 (66.7%)	0.61
Bloody stool	207 (49.3%)	11 (52.4%)	0.77	7 (77.8%)	0.084
Seizures	22 (5.2%)	3 (14.3%)	0.92	0 (0%)	0.47
Dehydration			**0.03**		0.12
Mild	122 (29%)	6 (28.5%)		0 (0%)	

Moderate	290 (69%)	13 (62%)		9 (100%)	
Severe	8 (1.9%)	2 (9.5%)		0 (0%)	
Encephalopathy	3 (0.7%)	2 (9.5%)	**0.0001**	0 (0%)	0.79

Table 2. Distribution of Positive Stool Culture Results among studied population

Stool culture result	Frequency (%)	Frequency (No)
E. coli	1.2	9
Shigella sonnei	6.2	11
Shigella flexeneri	1.7	7
Shigella dysentery	5	2
Shigella boydii	2	1

Table 3: Frequency and Percentage of Antibiotic Sensitivity for Shigella and *E. coli*.

Antibiotic	**Sensitive *E.coli***		**Sensitive Shigella**	
	%	**No**	**%**	**No**
Ciprofloxacin	**77.8**	**7**	**90.5**	**19**
Ceftriaxone	**44.4**	**4**	**38**	**8**
Azithromycin	**11.1**	**1**	**28.6**	**6**
Nalidixic acid	**22.2**	**2**	**19**	**4**
Cefixime	**33.3**	**3**	**0**	**0**
Co-trimoxazole	**22.2**	**2**	**0**	**0**
Gentamicin	**0**	**0**	**0**	**0**
Ampicillin	**0**	**0**	**0**	**0**

The current study investigated 420 children with acute infectious diarrhea. Stool cultures of 30 patients (7.1%) were positive. Of the total positive culture results, 21 samples were related to Shigella (5%) and 9 samples were related to *E.coli* (2.1%)

In this study, Shigella species were the most prevalent isolates causing acute infectious diarrhea similar to many other previous studies [2, 4, 6, 22]. In some studies, *E.coli* was more frequent than Shigella [3, 23]. The prevalence of Shigella in the study of Jomezadeh *et al*. in Abadan was 5.1% which is similar to our results [20].

We also observed *S. sonnei* as the dominant serogroup of Shigella. (35.5% of total Shigella isolates) followed by *S.flexneri*. (22.6%) The distribution of these serogroups varies geographically, whereby

S. flexneri and *S. sonnei* are predominant in developing and developed countries, respectively [14]. Socioeconomic conditions and general hygiene affect the frequency of Shigella serogroups [13].

Many studies have reported *S. flexneri* as predominant serogroup of Shigella in Iran [1,2,3,4, 20], although recent studies have shown an increasing number of infections caused by *S. sonnei* and predominance of this serogroup [13,15, 24, 25,26]. Evidences suggest that in comparison to S. *flexneri, S. sonnei* has a greater ability to develop resistance to broad-spectrum antimicrobials [11]. A Systematic Review by Salleh *et al.* in 2022 according to current global epidemiology studies demonstrate that *S. sonnei* is increasingly overtaking *S. flexneri* to become the predominant species in Asia [27].

In our study fever was the most common clinical symptoms and signs in studied population (97.6%). All patients with positive cultures had fever. Poor general condition were significantly more prevalent in culture positive patients especially in patients with shigellosis. Severe dehydration and Encephalopathy were significantly more prevalent in patients with shigellosis. The prevalence of bloody diarrhea and seizure in these patients were 52.4% and 14% respectively which were close to the results of nikfar *et al*. (53.5% and 10.4%) and Sangeetha *et al*. (58% and 9%).[1, 28].

In present study, all of the isolated Shigella and *E.coli* were resistant to ampicillin and Gentamicin. The prevalence of resistance in isolated *E.coli* to ciprofloxacin, ceftriaxone and azithromycin were 22.2%, 55.6%, 88.9% respectively. A Systematic Review of the antibiotic resistance patterns for E. coli isolated from Asian diarrheal patients revealed that the pooled prevalence of resistant to amoxicillin, Azithromycine, Ceftriaxone and Ciprofloxacin were 80.9%, 38.9%, 31.7% and 25.7% respectively[29]. In our study rate of resistance to Ciprofloxacin is close to this but resistant to Azithromycine is approximately twice the reported rate by this review.

A study by Zhou in 2018 revealed 77.8% resistance of diarrheagenic *Escherichia coli* to Ampicillin, 57.4% to Cefotaxim, 64.8 to co-trimoxazol and 50% to ciprofloxacin.[30]

In our study the prevalence of resistance in Shigella isolates to Ciprofloxacin, Ceftriaxone and Azithromycin were 9.5%, 62%, 77.1% respectively. A Systematic Review by Salleh *et al*. showed the pooled prevalence of Shigella antibiotic resistance was

highest for Ciprofloxacin (29.8%) and Azithromycin (29.2%), followed by Ceftriaxone (23.8%) respectively [27].

study of Nikfar *et al*. in Ahvaz in 2017 showed The prevalence of resistance in Shigella isolates to Ciprofloxacin, Ceftriaxone and Azithromycin were 1.5%, 51%, 7%.[1] Comparing this with our study shows that resistance to Azithromycin has dramatically increased to the extent that Azithromycin is no longer a good choice for emperic therapy of shigellosis. It seems that this increase in resistance is caused by the excessive use of this drug in society, especially during the outbreak of Covid 19.

The rate of resistance to ciprofloxacin also shows an increase but it is still lower than the reported rate in other regions in recent studies [12, 14, 26, 27].

Study of Abbasi *et al*. in Tehran in 2019 showed that the rates of resistance to Ciprofloxacin and Ceftriaxone were 10.5% and 63.1% respectively that it is very close to the results of our study [13].

In conclusion, our study revealed that due to the increasing rate of Antimicrobial resistance, Ampicillin, Gentamicin, co-trimoxazole, Nalidixic acid, Ceftriaxone and Azithromycin are no longer good choices for empiric treatment of Shigellosis and diarrheagenic *E. coli* infections in our region.

The increasing pattern of multidrug-resistance, highlights the need for planning to control this increasing trend and avoiding excessive and inappropriate use of antimicrobials. Understanding the pattern of antibiotic resistance in each area is crucial for the appropriate prescription of antibiotics, aiming to reduce disease complications, treatment failure and it prevents the spread of resistant strains.

Conclusion

Considering the increasing of antibiotic resistance, Ceftriaxone and Azithromycin are no longer good choices for empiric treatment of Shigellosis and *E. coli* infections in our region. The rate of resistance to Ciprofloxacin also shows an increase but it is still lower than the reported rate in other regions

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Conflict of interest: None

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Ethics statement: The study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Approval Number: IR.AJUMS.HGOLESTAN.REC.1400.025). Parents of all participants signed a written informed consent prior to their enrollment in the study.

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