

ORIGINAL ARTICLE

Patterns of Antimicrobial Resistance and Molecular Detection of ipaH Shigella Strains in Diarrhoeal Children in Sylhet

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Abstract:

Background: Despite the efforts of SDG and MDGs, diarrhoea remains a leading cause of morbidity and mortality among children under five.

Aim: The present study aimed to observe the antimicrobial susceptibility patterns and molecular detection of ipaH producing *Shigella* species from under 5 children with diarrhoea.

Methods: This cross-sectional observational study was carried out from July 1, 2021, to June 30, 2022, in the Department of Microbiology in collaboration with the Department of Paediatrics at Sylhet MAG Osmani Medical College Hospital, Sylhet. The target population comprised all children with diarrhoea admitted to the Inpatient Department of Paediatrics. 98 sample were selected based on inclusion and exclusion criteria and data were collected by a pre-designed questionnaire. Stool samples were collected promptly upon admission or prior to the commencement of any antibiotic therapy. The specimens underwent culture, Gram staining, and biochemical tests to identify *Shigella* species, followed by antimicrobial susceptibility testing and molecular analysis (PCR) for the ipaH gene.

Result: The mean age of 2.84 ± 2.45 years with 56.1% male children. *Shigella* species were isolated from 13 cases (13.3%). All *Shigella* spp isolates produced ipaH gene. *Shigella* was found maximum in bloody diarrhoea (40.0%) ($p=0.003$). Out of 13 isolates all (100.0%) were sensitive to cefixime, ciprofloxacin and cefepime; 92.3% were sensitive to cefuroxime, ceftriaxone and mecillinam; 84.6% to azithromycin and amoxicillin-clavulanic acid; 38.5% to nalidixic acid; 30.8% to tetracycline and ampicillin and 23.1% to trimethoprim-sulfamethoxazole.

Conclusion: Diverse antibiotic susceptibility pattern of *Shigella* spp., was observed in the study sample.

Keywords: antimicrobial resistant, bloody diarrhea, ipaH *Shigella*

Introduction

Diarrhoea remains a leading cause of morbidity and mortality among children. Globally, diarrhoea accounts for nearly 1.7 billion cases annually, resulting in over 525,000 deaths in children under five.¹ In Bangladesh, diarrhoea prevalence among children is 6.9%,² with bacterial pathogens, particularly *Shigella* species, being significant contributors. Annually, an estimated 80-165

million shigellosis cases occur globally, resulting in 600,000 deaths.³ In Bangladesh, *Shigella* is endemic, causing up to 300,000 cases yearly and contributing to 20% of diarrhoea-related deaths among children.⁴

Shigella species invade intestinal cells through virulence factors such as invasion plasmid antigen H (ipaH). ipaH is present in multiple copies on both the plasmid and chromosome of *Shigella* species.⁵ Major manifestations of shigellosis is diarrhoea, which may be bloody, mucoid or watery, and is associated with fever and abdominal pain.

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The emergence of multidrug-resistant (MDR) *Shigella* strains has complicated treatment, with reported resistance against ciprofloxacin and azithromycin.⁶ At present, the first line recommended agents for treating shigellosis are ciprofloxacin or azithromycin, however a study conducted in Bangladesh revealed that rise in resistant strains of *Shigella* towards ciprofloxacin from 0% in 2004 to 44% in 2010.⁷

Molecular detection methods, particularly polymerase chain reaction (PCR), provide high sensitivity and specificity for identifying ipaH-producing *Shigella*. Such techniques can enhance diagnostic accuracy and enable timely administration of appropriate antibiotics, especially in MDR cases. This study aimed to determine the antimicrobial susceptibility patterns and detect ipaH-producing *Shigella* in stool samples from children with diarrhoea in Sylhet, Bangladesh.

Methodology

This cross-sectional observational study was conducted for 1 year, in the Department of Microbiology in collaboration with the Department of Paediatrics at Sylhet MAG Osmani Medical College Hospital, Sylhet; after obtaining ethical permission from Ethical Committee of Sylhet MAG Osmani Medical College Hospital. The selected population included all diarrhoeal children admitted to the Inpatient Department of Paediatrics, excluding those attending the outpatient department. Children aged below six months or who had received antibiotics within the previous week were excluded from the study.

A non-probability convenient sampling technique was employed to recruit participants. Patients fulfilling the eligibility criteria were identified from the paediatric inpatient department. After obtaining informed written consent from the guardians, stool samples were collected immediately after admission or before initiating any antibiotic therapy. Samples were collected in sterile, disposable containers without preservatives and transported promptly to the Department of Microbiology. In the laboratory, the specimens underwent culture, Gram staining, and biochemical tests to identify *Shigella* species, followed by antimicrobial susceptibility testing and molecular analysis for the ipaH gene.

Molecular detection of the ipaH gene was performed using polymerase chain reaction (PCR) techniques. Initially, plasmid DNA was extracted from the isolates using the Monarch plasmid DNA purification kit (New England Biolabs, USA) according to the manufacturer's guidelines. The extracted DNA served as a template for

thermal cycling to amplify the target ipaH gene using a PCR mixture comprising a master mix, forward and reverse primers specific to the gene, and the template DNA. Visualization of the amplified gene product was carried out using a Digi gel documentation system. For the PCR assay, a total reaction volume of 50 µl was prepared in sterile micro-centrifuge tubes. Agarose gel electrophoresis was used to analyze the PCR products.

All collected data were processed and analyzed with the help of SPSS (Statistical Package for Social Science) version 26. Continuous variables were summarized using means and standard deviation in case on normally distributed data, or, when data is not be normally distributed. And categorical variables were summarized using frequency distributions. Difference between groups were analyzed using chi-square test for categorical variables. A p-value of less than 0.05 was considered to constitute a statistically significant difference.

Result

Table-1 described that about 52.0% of the participants were aged between 1 to 5 years, 32.6% were between 6 months and 1 year, and 15.3% were above 5 years, with a mean age of 2.84 ± 2.45 years. Male children accounted for 56.1%, while female children comprised 43.9%, resulting in a male-to-female ratio of 1.28:1. Most of the participants (63.3%) belonged to the lower socioeconomic class, followed by 35.7% from the middle class and only 1.0% from the upper class, indicating a higher prevalence of cases among children from economically disadvantaged backgrounds.

Table-1: Distribution of the participants according to sociodemographic characteristics (n=98)

Variables	Frequency	Percentage (%)
Age		
6 months-<1 year	32	32.6
1-5 years	51	52.0
>5 years	15	15.3
mean $\pm$ SD	2.84 ± 2.45	
Sex		
Female	43	43.9
Male	55	56.1
Socioeconomic status		
Lower class	62	63.3
Middle class	35	35.7
Upper class	1	1.0

Out of 98 stool samples, 13 (13.3%) were positive for both *Shigella* spp. and the *ipaH* gene, while the remaining 85 (86.7%) samples tested negative (figure-1).

Shigella spp. and ipaH gene detection

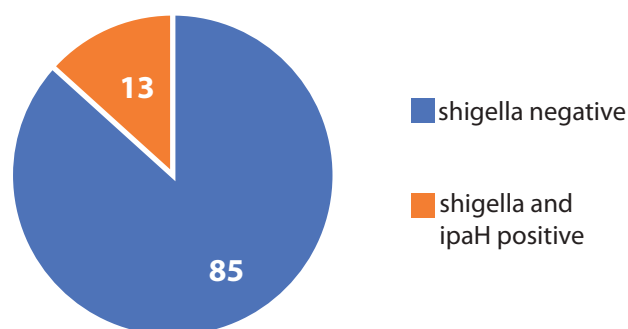


Figure-1: Distribution of the participants according to *Shigella* spp. and *ipaH* gene detection (n=98)

Shigella was most frequently detected in cases of bloody diarrhoea, with 40.0% of samples testing positive, compared to 13.3% in mucoid diarrhoea and 5.7% in watery diarrhoea. The difference in detection rates among diarrhoeal types was statistically significant ($p=0.003$) (table-2).

Table-2: Association of isolated *Shigella* (*ipaH* positive) with types of diarrhea

Diarrhoeal type	Shigella		p-value
	Positive and ipaH positive	Negative	
Mucoid diarrhoea (n=30)	4 (13.3%)	26 (86.7%)	ap=0.003s
Watery diarrhoea (n=53)	3 (5.7%)	50 (94.3%)	
Bloody diarrhoea (n=15)	6 (40.0%)	9 (60.0%)	
Total	13	85	

a= chi square test

s= statistical significance

Figure-2 described antibiotic susceptibility pattern of *Shigella* species showed high resistance to tetracycline, nalidixic acid, ampicillin, and trimethoprim-sulfamethoxazole, with resistance rates of 69.2%, 61.5%, 69.2%, and 76.9%, respectively. In contrast, high sensitivity was observed for ciprofloxacin, cefixime, ceftriaxone, cefepime, and mecillinam, with sensitivity rates exceeding 92.3%. Moderate resistance was noted for azithromycin and amoxicillin-clavulanic acid.

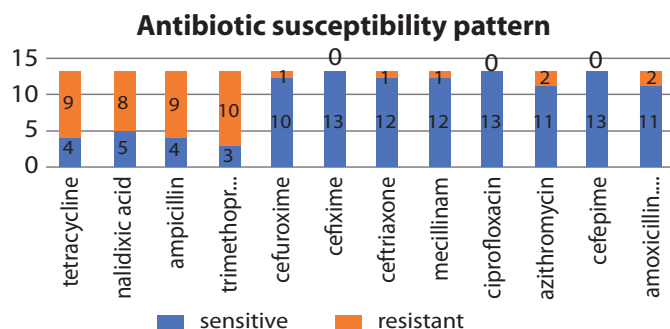


Figure-2: Antimicrobial susceptibility pattern of *Shigella* spp. isolated from diarrhoeal children (n=13)

Discussion:

The age of participants in this study had a mean age of 2.84 ± 2.45 years. Comparable findings were reported by Assefa and Girma, Gebreegziabher et al., Jomezadeh et al. and Kahsay et al.⁸⁻¹⁰ Male children accounted for 56.1% of the participants in this study, with a male-to-female ratio of 1.28:1. This aligns with findings by a couple of articles^{9,11,12} who reported male predominance also.

Regarding the type of diarrhoea, watery diarrhoea was most common (54.1%), followed by mucoid and bloody diarrhea. Similar trends were reported where watery diarrhoea accounted for 62.7%, mucoid 29.2%, bloody 4.6%, and mixed 3.5%, highlighting potential geographic variations in strain distribution.⁹ In the present study, 13 (13.3%) were positive for *Shigella* spp., consistent with findings by Kahsay et al. (13.3%),¹⁰ Sheikh et al. (13.2%),¹³ Debas et al. (14.9%),¹⁴ Mekonnen et al. (14.6%),¹⁵ and Huruy et al. (16.9%).¹⁶ Lower detection rates were reported by a couple of studies (6.9% and 7.1%)^{9,17} while higher rates were observed in studies by Mache et al. (20.1%)¹⁸ and Jafari-Sales and Shariat (26.17%).¹²

All 13 isolates of *Shigella* spp. in this study were positive for the *ipaH* gene, a finding corroborated by studies in Iran¹³ and Bangladesh.⁴ A multicenter study in six Asian countries reported *ipaH* gene detection in 90% of 427 *Shigella* culture-positive specimens.¹⁹

Cefixime, an orally administered third-generation cephalosporin, is considered effective for outpatient treatment. High sensitivity rates of *Shigella* spp. to cefixime were reported, even 100% in some studies²⁰ including in Bangladesh,²¹ while Jafari-Sales and Shariat observed a lower sensitivity rate of 72.6%.¹² Fluoroquinolones, particularly ciprofloxacin, are preferred for empirical treatment of acute diarrhea in adults due to their broad efficacy and favorable safety profile. This study and others, reported 100% sensitivity of *Shigella* spp. to ciprofloxacin.⁹ However, Jafari-Sales and Shariat observed a reduced sensitivity rate of 75%,¹²

Cefuroxime demonstrated 92.3% sensitivity in this study, though comparable studies are limited. Similarly, ceftriaxone, another third-generation cephalosporin, showed 92.3% sensitivity in this study, aligning with findings of 98–100% sensitivity by a couple of studies.^{9,21} Sensitivity to cefepime was universal (100%) in this study. Mecillinam showed 92.3% sensitivity, consistent with the 97% sensitivity reported by Rahman et al. (2007).²¹ Sensitivity to azithromycin and amoxicillin-clavulanic acid was observed at 84.6% in this study, with Rahman et al. reporting slightly higher rates for both drugs (84% and 100%, respectively).²¹

Resistance to tetracycline was high (69.2%), corroborating similar findings by Jafari-Sales and Shariat (67.9%)¹² and Gebreegziabher et al. (77.8%).⁹ Ampicillin resistance was also substantial (69.2%), with higher resistance rates reported by Abebe et al. (76.5%)²² and Gebreegziabher et al. (88.9%).⁹ Similarly, trimethoprim-sulfamethoxazole resistance was 76.8%, aligning with findings by Abebe et al. (64.7%)²² and Jafari-Sales and Shariat (92.8%).¹² Nalidixic acid resistance was notable at 61.5%, comparable to other original articles.²²

Conclusion:

Shigellosis was significantly associated with bloody diarrhoea, predominantly affecting children under five years from lower socioeconomic backgrounds. PCR demonstrated high sensitivity in detecting *Shigella* spp., which exhibited resistance to trimethoprim-sulfamethoxazole, ampicillin, tetracycline, and nalidixic acid but remained highly sensitive to ciprofloxacin, cefixime, cefepime, and ceftriaxone.

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