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RESEARCH ARTICLE OPEN ACCESS

Speciation and Antibiotic Susceptibility Pattern of *Shigella* Isolates in a Rural Tertiary Care Hospital, Kolar

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Abstract

Shigellosis is the most important cause of bacillary dysentery. It's a major public health problem. It is endemic in many developing countries resulting in a significant amount of mortality and morbidity. The incidence is 90 million with 6,00,000 deaths world over. In India, there is an emergence of resistance to *Shigella* resulting in limitation of treatment. To study the prevalence of *Shigella* species and their antibiogram in stool samples obtained from patients treated for dysentery at Rural Tertiary Care Hospital. A retrospective analysis of the *Shigella* isolates obtained from 400 stool samples from patients with dysentery , treated at Rural Tertiary Care Hospital from 2015 -2019 was conducted. The isolates were identified by conventional methods and antibiotic susceptibility testing was performed by Kirbybauers disk diffusion method and analysed. The percentage of isolation of *Shigella* species at our centre was 5% with *Shigella flexneri* being the predominant species isolated. A progressive pattern of resistance was observed. 100% strains of *Shigella flexneri* and *Shigella sonnei* were resistant to cotrimoxazole and nalidixic acid. 75% of *Shigella flexneri* were resistant to fluoroquinolones and 25% resistant to third generation cephalosporins. 100% isolates were sensitive to azithromycin. In view of rampant resistance observed among *Shigella* species, it is a need to test these isolates for antibiotic susceptibility and treat the patients based on the antibiogram.

Keywords: Shigellosis, dysentery, antibiotic resistance

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INTRODUCTION

Shigellosis is a gastrointestinal infection caused by genus *Shigella*.[1] One of the most common causes of morbidity and mortality among children in developing countries is acute gastroenteritis due to *Shigella* species. *Shigella* is a gram-negative bacilli that belongs to the family *Enterobacteriaceae* and is generically very similar to *Escherichia coli*.[2] Genus *Shigella* is divided into *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei.*[3] The mode of transmission is faeco-oral and the infective dose is as low as 10-to 100 bacilli. The pathogen colonizes and disrupts the intestinal epithelium resulting in fever, bloody diarrhoea and tenesmus.[4] One of the most common causes of morbidity and mortality among children in developing countries is acute gastroenteritis due to *Shigella* species. It predominantly affects children below 5 years, elderly and immunocompromised. It accounts for 1.1 million deaths in developing countries, of which 60% is constituted by children below 5 years of age.[5] The mortality and morbidity due to Shigellosis is preventable by oral rehydration and appropriate antimicrobial agents. WHO reports of Multidrug-resistant (MDR) *Shigella* in India, and other developing countries has become a therapeutic challenge in the treatment of Shigellosis.[1,6] So, this study is undertaken to know about the prevalence of *Shigella* species and their antibiogram in stool samples obtained from patients treated for dysentery at Rural Tertiary Care Hospital.

This study helps us understand the changing trend of antibiotic resistance among *Shigella* isolates in a rural background where antibiotic usage is comparatively low.

Objectives

To study the prevalence of *Shigella* species and their antibiogram in stool samples obtained from patients treated for dysentery.

MATERIALS AND METHODS

A retrospective analysis of the *Shigella* isolates from stool culture of patients with dysentery, treated at RL Jalappa Hospital from May 2015 – June 2019 was conducted. Ethical clearance was obtained from the institutional ethics committee.

Inclusion criteria

Shigella isolates from patients who presented with dysentery were included

Exclusion criteria

Incomplete patient data.

20 isolates of *Shigella* from patients who presented with dysentery were included in the study and were identified by conventional culture methods. The stool specimens were inoculated on Blood agar, Mac Conkey agar, Xylose lysine deoxycholate (XLD) agar directly. They were also inoculated into enrichment media, Selinite F broth and subcultured to MacConkey and XLD agar. Suspected colonies of *Shigella* was identified by standard biochemical tests and confirmed by specific antiserum (Denka -Seiken, Japan). Antibiotic susceptibility testing was done by Kirby Bauer disk diffusion technique as per CLSI (Clinical and Laboratory standard institute) guidelines using the following antibiotics, ampicillin (Amp)10µg, cotrimoxazole (Cot) 25µg, nalidixic acid (Na) 30µg, ciprofloxacin (Cip) 5µg, and ceftriaxone (Ctr) 30µg (Hi Media Laboratories, India). An additional drug azithromycin (Az) 15µg was tested as an investigational drug to find the usefulness of the drug as second line drug in case of pan drug- resistance. These isolates were further confirmed by NICED (National Institute of Cholera and Enteric Disease) Kolkotta. Further, their antibiotic susceptibility pattern was analysed as per CLSI (Clinical and Laboratory standard institute) guidelines. The data was retrieved from the Medical records department.

Statistical Analysis

A descriptive analysis of the data was done.

RESULTS

A total number of 400 stool samples were cultured between May 2015-June 2019. 20 (5%) isolates of *Shigella* species were obtained from patients with dysentery, treated at Rural Tertiary Care Hospital. Out of the 20 isolates, the predominant serotype was *Shigella flexneri*, 16 (80%) followed by *Shigella sonnei,* 4 (20%). A gradual increase in the number of isolates from 2015 to 2019 was observed as shown in (Table 1). The majority of the isolates 18 (80%)

Table 1. Distribution of *shigella* isolates during 5 years period

N=20		*Shigella flexneri* (6) 80%		*Shigella sonnei* (4) 20%
2015	2	*Shigella flexneri* 4A	(1)	
		Shigella flexneri 2A	(1)	NIL
2016	2	*Shigella flexneri* 1B	(1)	*Shigella sonnei* Phase 2(3)
		Shigella flexneri 2A	(1)	
2017	3	*Shigella flexneri* 2A	(2)	NIL
		Shigella flexneri 3A	(1)	
2018	2	*Shigella flexneri*	(1)	NIL
		Shigella flexneri	(1)	
2019	7	Shigella flexneri 2A	(3)	*Shigella sonnei* Phase 2(1)
		Shigella flexneri	(4)	

Note: A and B denotes the serotypes of *Shigella.*

Table 2. Resistance pattern in *shigella* isolates (N=20)

Antibiotic	*Shigella flexneri* (16)	*Shigella sonnei* (4)	Total (20)
AMPICILLIN	13(81.25%)	NIL	13
COTRIMOXAZOLE	16(100%)	4(100%)	20
NALIDIXIC ACID	16(100%)	4(100%)	20
CIPROFLOXACIN	12(75%)	NIL	12
CEFTRIAXONE	4(25%)	NIL	4
AZITHROMYCIN	16(100%)	NIL	16

Note: The figure shown in the bracket is resistant.

out of 20 isolates were from the adult population. All the 20 isolates showed 100% resistance to cotrimoxazole and nalidixic acid as shown in (Table 2). 4 (25%) isolates of *Shigella flexneri* showed resistance to ceftriaxone, 2 of the 4 were ESBL producers. All the 20 (100%) isolates were sensitive to azithromycin.

DISCUSSION

Global estimation of shigellosis is 164.7 million annually. [7] One of the major public health problems in developing countries is shigellosis. It is transmitted through contaminated food and water and direct contact with infected individuals. In the present study, the rate of isolation was 5% which is higher than the rate quoted by Taneja et al.[8] The overall percentage of isolation of *Shigella* from different parts of our country ranges from 3 to 6%. It has been shown by many studies that shigellosis is prevalent among children less than 5 years.[9] Contrary to the above observation made, the majority of the isolates 18 (80%) out of 20 were from the adult population in the present study.

The most common serotype isolated in our study is *Shigella flexneri* followed by *Shigella sonnei.* Madhavan et al. has reported *Shigella sonnei* as the predominant serotype in Kerala.[8] *Shigella sonnei*[9] was found to be the predominant species in Thailand, Vietnam and Srilanka attributed to improved sanitary conditions. From various studies across the world, it is found that there is a shift from *Shigella flexneri* to *Shigella sonnei* in countries with improved sanitation.[9]

The trend of antibiotic resistance was progressive in our study. An alarming increase in the resistance pattern of *Shigella flexneri* was observed. 13 (81%) of the *Shigella flexneri* strains were MDR strains resistant to more than three classes of antibiotics. 12 (75%) of the strains were resistant to ciprofloxacin. Fluoroquinolones were recommended by the WHO as drug of choice in 1990 but the emergence of resistance

to fluoroquinolone has limited its usage and has mandated the use of ceftriaxone. The emergence of fluoroquinolone resistance is probably due to the over use of these drugs for diarrhoea and urinary tract infections. 5.9% of fluoroquinolone resistance was observed in 2004 which increased to 48.5% in 2007.[9] Gururaj et al. proved that there was a uniform mutation in Gyr A among the fluoroquine resistant strains.[10] Presently some of the strains have developed resistance to third-generation cephalosporins. Madhavan et al.[11] showed that 17% of *Shigella* isolates in his study were resistant to third-generation cephalosporin as compared to 25% in our study. Mandal J et al. showed 3% of cephalosporin resistance which was lesser in comparison to our study. ESBL production was observed in 2 (12.5%) isolates of *Shigella flexneri* as compared to 3% in the study conducted by Mandal J et al.[12] The greatest danger faced with MDR strains are the limited therapeutic options available to the treating physician.[13] In a rural setup, the cost of procuring higher antibiotics results in delayed recovery and prolonged hospital stay, on the isolation of an MDR strain.

As CLSI recommends azithromycin to be tested as an investigational drug, azithromycin sensitivity was performed on all the isolates obtained in the year 2019 and 100% sensitivity was observed. Although there is a smaller sample size this work has gained success to fulfil the purpose of this work to achieve better treatment

Limitation of the Study

The sample size is the limitation of the study. Extrapolation of the results could have been better with a larger sample size.

CONCLUSION

Shigella flexneri has become increasingly resistant to antimicrobials. WHO in 2005 recommended ciprofloxacin as the first line treatment for shigellosis in children and ceftriaxone as a second line drug for children. Azithromycin as a second line drug for adults. Shigellosis should be treated with antibiotics as it shortens the duration of clinical symptoms and decreases fecal excretion of the organism and minimises transmission. Provision for improved sanitation and clean drinking water is needed to prevent shigellosis. There is a impending need for updation of the guidelines for treating shigellosis in the developing countries

This work would ameliorate the treatment for dysentery, it exhibits a significance in the treatment among rural areas where in many cases are not diagnosed and treated effectively. Instead of the limitation due to the smaller sample size this work is very fruitful for the study of the prevalence of *Shigella* species and antibiogram which is very important for the proper treatment of patients with dysentery.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

PS designed the study and implemented the concepts. PS and BR did the literature search, Data acquisition, Data analysis and wrote the manuscript. BPM, AN and PS reviewed and edited the manuscript.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Sri Devaraj URS Medical College, Tamaka, Karnataka, India.

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