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Original article

Identification, characterisation and antibiotic susceptibility of shigella species isolated from stool samples in children

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ABSTRACT

Background and Objectives: Shigellosis, an acute diarrhoeal disease, remains a major public health problem in developing countries. The increasing levels of antimicrobial resistance of Shigella isolates have complicated the treatment of shigellosis. The purpose of present study was to determine the incidence of Shigella species and their antimicrobial resistance patterns for the better management of shigellosis in children. **Material and methods:** The study was conducted in a tertiary care hospital in the Department of Microbiology, between June 2009 to May 2010. Stool samples were collected from paediatric age group. Shigella isolation, identification and speciation were done by using simplified phenotypic identification scheme and antibiotic sensitivity pattern of these isolates was studied. **Results :** A total of 128 stool samples were studied, out of which Shigella species accounted for 10.1%, E.coli accounted for 57.8%, Klebsiella species accounted for 20.3% and Pseudomonas species accounted for 15%. Resistance pattern of Shigella species to various drugs were, Co-trimoxazole (76.9%), Doxycycline (69.2%), Amoxicillin (100%), Cefotaxime (69.2%), Ceftriaxone (69.2%), Gentamicin (61.5%), Nalidixic acid (100%) and Ciprofloxacin (69.2%). All the Shigella isolates were sensitive to Amikacin. **Conclusion :** The predominant shigella species isolated in this study was Shigella flexneri. The present study demonstrates that Shigella species showing significant increase in resistance to several commonly used antimicrobial agents. The results suggest reconsideration of the empiric use of these antimicrobial drugs for the treatment of Shigellosis and the findings, stress the need for ongoing drug resistance surveillance.

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1. Introduction

The Genus Shigella is classified in the family Enterobacteriaceae and consists of bacteria that are non-motile, oxidase negative, non-lactose fermenting, gram negative bacilli [1]. Shigella infection is one of the major public health problems worldwide. An estimated 164.7 million shigella episodes have been identified in two-thirds of young children. Shigellosis occurs mainly in developing countries due to poor hygiene and limited access to clean drinking water, whereas in industrialized countries it is mainly because of travel to developing countries and exposure to contaminated foods and/or food handlers [2]. Infection with shigella species cause approximately 6,00,000 deaths world wide annually. Two-thirds of

all cases and most of the deaths occur among children under 10 years of age. Although more prevalent in developing countries, shigellosis is a worldwide problem [3]. The term dysentery was used by Hippocrates to indicate a condition characterised by frequent passage of stool containing blood and mucus accompanied by straining and painful defecation [4]. Humans are the only natural host for shigella and infection occurs by ingestion. Bacillary dysentery is the most communicable of the bacterial diarrhoeas with an infective dose as low as 10-100 bacteria producing disease in healthy adults.[5] Shigellosis is the most communicable of the bacterial diarrhoeas. Humans serve as the natural host and disease is transmitted by the feco-oral route [6]. Shigellosis remains an important public health problem in developing countries with Shigella flexneri in Asian and African Countries and Shigella sonnei in Europe and the United States being of epidemiological importance. Antimicrobial therapy is advocated for Shigellosis to shorten the duration of illness. However, in Asia and Africa, antimicrobial resistance is an emerging problem among Shigella species and treatment options

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are becoming limited globally [7]. Reports of epidemic outbreaks of Shigellosis in India are frequent but studies of sporadic (endemic) cases are uncommon. The magnitude of the problem of Shigella infections in infants and children is thus not adequately defined [14]. So this study is undertaken to know the incidence of Shigellosis in children and its antimicrobial resistance pattern.

2. Materials and methods

The study was conducted in the Department of Microbiology from June-2009 to May 2010. Stool samples were collected in a wide mouth container from children who were clinically diagnosed as suffering from dysentery. Stool samples were immediately sent to the laboratory and isolation and identification of Shigella organisms and antimicrobial susceptibility testing was done.

Microscopic examination of stool samples was made by preparing saline wet mount and iodine wet mount to look for leucocytes, RBC's, ovas and cysts.

Stool samples showing pus cells and RBC's were inoculated on MacConkey agar, XLD agar, SS agar and for enrichment in Selenite F broth. After enrichment in Selenite F broth for 6 hours subculture was done in the MacConkey agar, XLD agar and SS agar and further incubated at 37°C overnight.

All non-lactose fermenters were isolated and subjected for oxidase test. All oxidase negative organisms were subjected to the biochemical tests to identify the isolate. Final speciation was done by doing slide agglutination test using Shigella polyvalent antisera.

Identification of Shigella was done on the basis of Oxidase test, Hanging drop test, Mannitol fermentation test, Nitrate reduction test, TSI test and Methyl red test. Specific antisera (Denken-Seika, Japan. Shigella antisera) were used for serotyping of isolated shigella.

The susceptibility of all the isolated shigella to different antibiotics were determined by Kirby-Bauer's disk diffusion technique as per the CLSI guidelines. The antibiotics used were Amikacin, Amoxycillin, Cotrimoxazole, Doxycycline, Cefotaxime, Ceftriaxone, Gentamicin, Nalidixic acid and Ciprofloxacin.

3. Results

During the 12 month study period a total of 128 stool specimens were examined from paediatric age group, admitted in paediatric ward.

Out of total 128 stool specimens examined, Shigella species accounted for 10.1%, E.coli accounted for 57.8% Klebsiella spp accounted for 20.3% and Pseudomonas spp. accounted for 15%.

Table: 1 List of Species Isolated from stool sample

Species Isolated from stool specimen	No of Isolates
Shigella spp	13
E.coli	74
Klebsiella	26
Pseudomonas	15
Total	128

Of the 13 samples of shigella species, *S. flexneri* (n = 11) was the predominant isolate, followed by *S. boydii* (n = 1) and *S. dysenteriae* (n = 1). Among the shigella flexneri isolates, two isolates were producing gas.

The predominant age group was between 5 years to 10 years and they presented with history of fever, frequent passing of loose stools associated with blood and mucus and pain abdomen.

The overall isolation rate of Shigella spp accounted for 10.1% and the isolation rate between the age group 1 year to 5 years was high compared to other age groups.

Table:2 List of Species Isolated from stool sample

Species Isolated from stool specimen	Age in Years	No of Isolates
<i>S. flexneri</i>	1yr - 5yr	11
<i>S. dysenteriae</i>	2yr	1
<i>S. boydii</i>	10yrs	1
E.coli	<10 month	5
	>10 months - 4yrs	34
	>5yrs - 10yrs	20
Klebsiella spp.	>11yrs - 15yrs	15
	10mon - 4yrs	14
Pseudomonas spp.	>5yrs - 10yrs	12
	10mon - 4yrs	3
	10yrs - 15yrs	12
Total		128

In the present study the resistance pattern of shigella species to the following drugs is Co-trimoxazole (76.9%), Doxycycline (69.2%), Amoxycillin (100%), Cefotaxime (69.2%), Ceftriaxone (69.2%), Gentamicin (61.5%), Nalidixic acid (100%), Ciprofloxacin (69.2%), and this finding correlates with other studies (Table 6). All the shigella isolates were sensitive to Amikacin and resistant to Nalidixic acid.

Table - 3 : Sensitivity Pattern of Shigella Isolates To Different Antibiotics

Species Isolated from stool specimen	Sensitive (S) (%)	Moderately sensitive (MS) %	Resistance (R)%
Amikacin	13 (100)	-	-
Cotrimoxazole	1(7.69)	2(15.38)	10(76.92)
Doxycycline	-	4(30.7)	9(69.2)
Amoxycilin	-	-	12(100)
Cefotaxime	4(40.7)	-	9(69.2)
Ceftriaxone	4(30.7)	-	9(69.2)
Gentamycin	5(38.4)	-	8(61.5)
Nalidixic acid	-	-	13(100)
Ciprofloxacin	4(30.7)	-	9(69.2)

4. Discussion

The present study shows predominant species belong to *Shigella flexneri* group and two isolates in *shigella flexneri* group produces gas. In a study conducted in Vellore, South India (1997 - 2004) faecal samples from patients of gastroenteritis were processed for enteric pathogens. *Shigella* accounted for 132 (5.4%) of the pathogens isolated and *S.flexneri* was the commonest serogroup (76/132 or 57.6%) followed by *S. Sonnei* (41/132 or 31%) [9].

The present study shows more species belonging to *S.flexneri* group and then followed by *S.boydii* and *S. dysenteriae*.

In a study conducted by H Srinivasa et al showed the isolation rate of *Shigella* species was 4.6% . Of these 56 were from children from 0 to 14 years and rest from adults and elderly patients. *S.flexneri* strains were identified in 84 (64.9%) *shigella* positive cultures, while *S.sonnei* accounted for 29 (21.6%), *S. boydii* 11 (8.2%) and *S.dysenteria* in 5 (3.7%).

Over 70% of *shigella*, isolates were resistant to two or more drugs including Ampicillin and Co-trimoxazole. Resistance rate of Ampicillin was 55.2%, Cotrimoxazole 81.3%, Chloramphenicol 43.3%, Nalidixic acid 61.9% and Ciprofloxacin 20.9%. *S. flexneri* showed highest resistance to Chloramphenicol (52.9%) and Ciprofloxacin (28.7%), *S.sonnei* to Nalidixic acid (82.8%) and Co-trimoxazole (96.6%) and *S.dysenteriae* to Ampicillin (71.4%) [10].

The majority of *Shigella* species were isolated from children aged less than five years, which is similar to other studies [11,22] and also to this current study. The clinical course was found to be severe in children superadded with malnutrition. This calls for an urgent measure to reduce the deaths in children due to shigellosis. Therefore public health strategy should ensure clean water supply, good sewage management and a clean environment.

The changing patterns in the distribution of *Shigella* sero groups and serotypes have been reported from time to time over the past decades, a significant number of *Shigella* isolates, resistant to commonly -prescribed antimicrobials have been reported [45].

In early 1990s, many isolates were susceptible to Nalidixic acid, Norfloxacin, Furazolidone and Gentamycin [13]. In the late 1990s, most isolates especially *S.dysenteriae* type 1, showed an increased resistance to antimicrobials [20,21] but most were susceptible to Ciprofloxacin [76,77]. In one of the study done by Radice M, Gonzealez et al in Argentina in 2001, shows resistance of *Shigella sonnei* isolates to the third generation cephalosporins [50].

Although most patients recover spontaneously from shigella infections, antimicrobial therapy reduces the severity and risk of serious complications and death from the disease.

Practical empirical therapy for shigellosis starts with the administration of either Co-trimoxazole or Ampicillin irrespective of the probable serotype causing dysentery. If there is no clinical improvement within 48 hours a second antibiotic that is effective against shigella in the area should be given. Because of the growing proportion of *Shigella* strains resistant to standard low-cost antibiotic Amoxycillin, Nalidixic acid, Co-trimoxazole, Tetracycline, Chloramphenicol, effective treatment becoming increasingly difficult. WHO is developing studies to determine the efficacy and safety of Azithromycin, a new oral Macrolide antibiotic, against shigella [15-19].

In this study all the isolates were sensitive to Amikacin, and four isolates were sensitive to third generation Cephalosporins such as to Cefotaxime and Ceftriaxone. Also four isolates were sensitive to Ciprofloxacin and Gentamycin. And all thirteen isolates were resistant to Nalidixic acid, Amoxycillin and nine isolates were resistant to Ciprofloxacin. This difference in the sensitivity pattern was due to environment factors and different patterns of antimicrobial usage. There are very few reports on shigellosis in infants and children in whom the infection may not manifest as classical dysentery but also presents as an acute gastroenteritis or acute enteritis. So this study is done to find out the incidence of *Shigella* species in children and their antibiotic pattern. In conclusion, the present study shows *Shigella flexneri* as predominant species and demonstrates that *Shigella* species showing significant increase in resistance to several commonly used antimicrobial agents. The rapid increase in Ciprofloxacin and Nalidixic acid resistance is a major cause of concern. The results suggest reconsideration of the empiric use of these antimicrobial agents for the treatment of Shigellosis. A further study is required with additional drugs such as Norfloxacin, Ofloxacin and

Azithromycin. These drugs may help to formulate the empirical therapy of Shigellosis. Antibiotics may not be necessary for mild cases and if required should be guided by local data. The continuous surveillance of multidrug resistant strains is very important to know the changing antibiotic susceptibility pattern as well as, the resistance pattern also changes with the change in the serogroup. Analysis and periodic reporting is important on proper therapy of shigellosis.

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