Original Article

Surveillance of antibiotic susceptibility patterns among Shigella species in stools of diarrheal children

1. Mahboob Bari 2. Samina Kousar 3. S.A. Jafri 4. S. Junaid Ali

1. Prof. of Biochemistry 2. Asstt. Prof. of Biochemistry, University College of Medicine, The University of Lahore
3 & 4. Prof. of Molecular Biology and Biotechnology, The University of Lahore.

ABSTRACT

Objective: The objective of the present study was to findout the frequency of Shigella spp. in diarrheal patients in Pakistan and the susceptibility of isolated Shigella to different antibiotics: ampicillin, nalidixic acid, meropenem, tetracycline, amikacin, azactam, ciprofloxacin and chloremphenicol.
Design of Study: Experimental Study.
Place and Duration of Study: This Study was conducted at Pediatric Department, Mayo Hospital, Lahore and study was approved by Pakistan Pediatric Association.
Materials and Methods: Stool samples were collected from 50 diarrheal children less than five years of age who were admitted in the Paediatric Department Mayo Hospital Lahore, Pakistan. The samples were cultivated on standard media for Shigella, and Enterobacteriaceae. Susceptibility of Shigella isolates was tested by disk method.
Results: The frequency of isolation was 80% for Shigella species and 20% in others. Shigella. dysenteriae (65%) was the most frequently isolated species, followed by S. flexneri (23%), S. boydii, (10%) and S. sonnei (5%). All Shigella isolates were 100% sensitive to amikacin, azactam and ciprofloxacin, while multiple drug resistance patterns were observed by all four isolates to other antibiotics.
Conclusions: Shigella resistance is increasing against most commonly used antibiotics. Now it is the time not only to explore new drugs but also to invoke awareness about the hazards of unhygienic conditions and self medication.
Key words; Antibiotics, susceptibility, Shigella species, Diarrhea Children, Serotyping

INTRODUCTION

In developing countries, diarrhea is considered to be the major causes of childhood morbidity and mortality. About one billion episodes and 3.3 million deaths occur each year among children under five years of age. This ratio is highest in summer[1]. Overall, these children experienced on an average 2.6 episode of diarrhea per child per year. About 80% of children died of diarrhea in the first two years of their life. Epidemics are usually transmitted occur due to contaminated food and water in crowding area with poor sanitary conditions[2]. Flies are considered to play a major role in the spread of Shigella because of the low infective dose needed to cause diarrhea[3].

Almost all over the world 5 to 10% of dysentery and diarrheal diseases are caused by Shigella pathogens[4]. Diarrhea caused by bacteria Shigella is called Shigellosis and it is the causative factor of diarrhea among young children in developing nations[5]. Shigellosis is an acute gastroenteritis caused by Shigella dysenteriae, Shigella flexneri, Shigella boydii and Shigella soneii [2].

Shigellosis symptoms ranges from abdominal pain, cramps, fever, vomiting to bloody diarrhea and mucus contaminated stool. Some strains produce enterotoxin and shiga toxin. Infections are associated with mucosal ulceration, rectal bleeding and drastic dehydration. Fatality may be as high as 10 to 15 % with some strains[6]. The epidemiology and antibiotic susceptibility of Shigella species changes with the passage of time.

Despite the disease being self-limiting, antibiotic treatment is recommended because it reduces the duration of illness and the transmission rate of the disease by shortening the period of excretion of the pathogen[7]. Appropriate antimicrobial therapy of Shigellosis shorten the duration of fever and it apparently also reduces the risk of developing complications. Significantly reducing the spread of infections but constant use of antibiotics can increase the antibiotic resistance of Shigella sp which can cause major problem. Emergence of multiple drug resistance to cost effective antibiotics against Shigella is a matter of concern for the health authorities in developing countries[8].

The rapid emergence of multidrug-resistant (MDR) strains is largely due to their ability to acquire and disseminate exogenous genes associated with mobile genetic elements such as plasmids, transposons, integrons, and genomic islands[9]. Antibiotic resistance in enteric pathogens is of great concern in developing countries due to indiscriminate use of antibiotics. As Shigella are predominate isolates and show resistance to ampicllin but susceptibility to chloramphenicol with the exception of S. flexneri (susceptible to gentamicin)[10]. Some Shigella strains show resistance to

vibramycin, tetracycline and sensitivity against nalidixic acid, norfloxacin, chloramphenicol, amikacin and aztreonam[11]. Some of the Shigella strains are susceptible to ciprofloxacin and ceftrixone[12].

In Pakistan, self medication and purchase of drugs without prescription is common practice. Thus there is a greater possibility of development of resistant strains due to overuse of antibiotics[13]. The main objectives of this study were to isolate Shigella species from stool samples of diarrheal children of under five year of age and to find out the resistance and susceptibility of Shigell species against different antibiotics.

MATERIALS AND METHODS

Total fifty stool samples were collected in sterile wide mouth containers and rectal swabs from less than five year age group diarrheal children from Pediatrics Department of Mayo Hospital, Lahore, Pakistan. Selenite F broth and specific O (somatic) antigen were used for growth and cauterization of bacterial strains. Stool samples were streaked on SS agar (Salmonella-Shigella) and inoculated in Selenite F broth for sub-culturing and incubated for 24 to 72 hours respectively. Pathogens were identified as they formed colorless colonies on SS agar [14](Ellen et al.).

Characterization of strains

The isolates were characterized by performing conventional bacteriological and biochemical methods[15].

1. Gram's staining
2. Triple sugar iron agar test
3. Citrate utilization test
4. Indole production test
5. Manitol test
6. Urease test

Serotyping

Shigella are composed of four species or serogroup that are referred by A, B, C and D. Serogroup A refers to S. dysenteriae, B refers to S. flexneri, C corresponds to S. boydii and D to S. soneii. Shigelal species were confirmed with polyvalen (serogroup A-D) antisera. Shigella isolates tested by slide agglutination with polyvalent A-D Shigella antisera. Isolates that agglutinate with antisera were reported as presumptive Shigella species[16].

Antibiogram pattern

Antibiogram pattern of isolated strains were determined on Mueller- Hinton agar. Each culture to be tested for antibiotic susceptibility was streaked onto non inhibitory agar medium. For comparison, the anti microbial susceptibilities of isolates from the standard culture were determined by standard disk method. E. coli American Type culture collection (ATCC). Zones of inhibition were determined with the help of list break points of antibiotics[17].

RESULTS

In present study, total fifty stool samples were collected from diarrheal children of less than five year age. Out of 50, 32 were boys (64%) and 18 were girls (36%). Stool samples were collected as *Shigella* is largely excreted through stool. *Shigella* was isolated from a number of commercially prepared plating media used with members of Enterobacteriaceae. *Shigella* species were isolated in 40 samples (80%) and 10 samples contained other members of Enterobacteriaceae (20%) such as *Salmonella* and *E.coli.* All pathogenic forms of *Shigella* were confirmed upto species level by serotyping. All *Shigella* isolates were tested by slide agglutination with polyvalent A-D *Shigella* antisera. Out of forty, 26 (65%) were *S. dysenteriae*, 8 (23%) were *S. flexneri*, 4 (10%) were *S. boydii* and 2(5%) were *S. soneii*. Boys and girls have different rate as presented in Table No.1.

Table No.1: Prevalence and frequency of different bacteria isolated from diarrheal patients

Bacterial species	Number	Boys	Girls
S. dysenteriae	26	17	9
S. flexneri	8	6	2
S. boydii	4	1	3
S. soneii	2	2	0
Others	10	6	4
Total	50	32	18

Identification of selected bacterium

The isolated bacterium was subjected to Gram's staining to screen Gram negative, non capsulated shape and colorless growth. Biochemical tests for identification of selected strain were performed. Results obtained from these tests are given in table No. 2.

Antibiogram pattern

Antibiogram pattern of isolated strains were determined on Mueller- Hinton agar. Commercially available anti-microbial disks were used and incubated at 37 ºC for 24 hours on agar. Zones of inhibition were measured in each case. Most of *Shigella* isolates in the study were found resistant to meropenem and tetracycline and sensitive to amikacin, azactam and ciprofloxacin. Two antibiotics (Chlorempheniсol and Nalidixic acid) showed resistance to *S. dysenteriae* and sensitivity to others strains (Table No.3)

Table No. 2: Biochemical characterization of bacterial strains isolated from diarrheal patients of less than five year of age

Bacteria	Shape	Gram's staining	Manitol	TSI	Citrate test	Ind Test	Urease test
Shigella	Rod	-ve	-	Slant Alkaline Butt Acidic	-ve	-ve	-ve
S. dysenteriae	Rod	-ve	No change	Slant Alkaline Butt Acidic	-ve	-ve	-ve
S. flexneri	Rod	-ve	Acid production	Slant Alkaline Butt Acidic	-ve	-ve	-ve
S. boydii	Rod	-ve	Acid production	Slant Alkaline Butt Acidic	-ve	-ve	-ve
S. sonnei	Rod	-ve	Acid production	Slant Alkaline Butt Acidic	-ve	-ve	-ve

Table NO.3: Susceptibility of antibiotics against different *Shigella* species

Antibiotic used	*S. dysenteriae*	*S. flexneri*	*S. boydii*	*S. sonnei*
Amikacin	S (8%)	S (2%)	S (1%)	S (0.5%)
Azactam	S (10%)	S (3%)	S (2%)	S (0.5%)
Ampicillin	S (5%)	S (1%)	R (0.5%)	R (0.5%)
Chloramphenicol	R (5%)	S (2%)	S (2%)	S (1%)
Ciprofloxacin	S (17%)	S (6%)	S (2%)	S (0.5%)
Meropenem	R (3%)	R (2%)	R (0.5%)	R (0.5%)
Nalidixic acid	R (15%)	S (5%)	S (1%)	S (1%)
Tetracycline	R (2%)	R (2%)	R (1%)	R (0.5%)

DISCUSSIONS

The aim of the present study was to isolate Shigella sp. from fecal samples of diarrhea children less than five years of age. It has been reported that worldwide acute diarrhea disease is the second cause of death among all infectious diseases in children younger than 5 years of age [18]. Total 50 stool samples were collected and subjected to medias for bacterial growth. All isolated species were subjected to Gram's staining and biochemical tests for identification of species. Out of 50 samples 40 contained Shigella sp. and were Gram's negative, rod shaped, with colorless growth and non capsulated. Remaining 10 were belonging to Enterobacteriaceae such as Salmonella and E.coli. It was found that S. dysenteriae was more frequently prevailing strain (65%) than other three after serotyping and boys were more victims of Shigella as compare to girls as given in Table 1. Our findings are combatable with previous studies by Kausar et al.[13] who studied frequency of Shigella species and found to be more frequent (19.1%) and S. dysenteriae was more common than other three strains. Biochemical tests showed different results presented in Table 2. These results are in accordance with findings of Uzma et al.[11] they observed Shigella isolates and found indole test to be negative, oxidase negative, citrate negative, urease negative while TSI as positive.

Antimicrobial therapy is recommended for shigellosis as it can shorten the severity and duration of illness, reduce shedding of the organism, and prevent secondary complication and death. However antimicrobial resistance occurred among Shigella species, since1940s, when sulfonamide resistance among Shigella organism was first detected in Japan 19. In our study, all four isolates showed multiple drug resistance patterns (Table 3). Drug susceptibility of S. dysenteriae showed that their resistance to nalidixic acid, cholramphenicol, tetracycline and meropenem but showed their sensitivity against amikacin, azactam, ciprofloxacin and ampicillin. These results are in conformity with findings of Atif et al.[20] they studied S. dysenteriae S and E. coli resistance against commonly used antimicrobial agents i.e. amoxicillin, chloamphenicol, tetracycline, cotrimoxazole, nalidixic acid, sulfonamide and neomycin and sensitive to amikacin, azactam and ciprofloxacin. S. flexneri isolates were resistant to meropenem and tetracycline while these were found sensitive to nalidixic acid, cholramphenicol, amikacin, azactam, ciprofloxacin and ampicillin. The previous findings quoted by Uzma et al.[11] also showed the same trend, hence substantiates our results.

Antibiotics susceptibility of S. boydii showed resistance to ampicillin, tetracycline and meropenem but give susceptibility towards nalidixic acid, cholramphenicol, amikacin, azactam and ciprofloxacin. These are in conformity with the findings of Urio et al.[10] they also found S. boydii resistant to ampicillin, sulphtriad and cepalothin. Ciprofloxacin and chlorempheniol were the most effective antimicrobial agents against S. boydii.

In our study it was also seen that S. sonii were resistant to ampicillin, tetracycline and meropenm while

susceptible to amikacin, azactam, nalidixic acid, ciprofloxacin and chloremphenicol. These results are in line with Isenbarger et al.[21]studies as they also found S. sonii strains resistant to ampicillin and meropenem and susceptible to nalidixic acid, ciprofloxacin and chloremphenicol.

CONCLUSIONS

It can be concluded from the present study, in heavily populated areas of Pakistan, ecosystem contains high background level of faecal population associated with the transmission of enteric pathogens through water, food, human and animals. In presence of these factors gastroenteritis remains one of the major health problems. *Shigella* resistance is increasing against most commonly using antibiotics. Exploring of new drugs and to inculcate hygienic awareness among the mass is need of the day. Self medication and purchases of drugs needs to be under legal cover.

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Address for Corresponding Author:
Professor Mehboob Bari,
Assistant Professor, Department of Biochemistry,
University College of Medicine,
University of Lahore, 1-KM Defense Road, OFF
Raiwaind Road, Lahore, Pakistan.
Tel: (Office): 042-35321457 Ext.116,
Email: samphdbio@gmail.com
saminakausar@.uol.edu.pk