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Low Antimicrobial Resistance of Shigella Isolates In a Tertiary Care Hospital in Ahmedabad, Gujarat- A 3 Year Study

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ABSTRACT

Background and objectives: Shigella play an important role as a causative organism of acute gastroenteritis, which is a global health problem with significant morbidity and mortality especially in developing countries and also is the most communicable of the bacterial diarrheas. The purpose of present study was to determine the incidence of Shigella species and their antimicrobial resistance patterns in the patients presenting with acute gastroenteritis to our tertiary care centre in Ahmedabad.

Materials and methods: The study included all patients with acute gastroenteritis who visited a tertiary care hospital in Ahmedabad during a 3-year period (Jan' 2013- Dec'15). The isolates was confirmed as *Shigella* by biochemical reaction and slide agglutination test using specific antisera. Antibiotic sensitivity was determined by Kirby-Bauer disc diffusion method as per CLSI guidelines.

Discussion and 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 like ampicillin, cotrimoxazole and chloramphenicol but still very less resistance for fluoroquinolones and 3rd generation cephalosporins.

Key words: Shigella, gastroenteritis, resistance.

INTRODUCTION

Shigellosis is the most communicable of the bacterial diarrheas. Humans serve as the natural host, and disease is transmitted by the fecal-oral route, with as few as 200 viable organisms being able to cause disease.[1] Epidemiology reports show that about 140 million people suffer from shigellosis with estimated 600,000 deaths per year worldwide.[2] 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.[3] The emergence of multiple drug resistance to cost-effective antimicrobials against *Shigella* is a matter of concern in developing countries.[4] Besides the temporal changes in the antibiogram of *Shigella* species, it is well-known that antimicrobial

Susceptibility patterns in *Shigella* may differ between geographical areas. Such differences are

never stable and may change rapidly, especially in places where antimicrobials are used excessively, particularly in developing countries.[5] Thus, it is important to identify species as well as antibiotic susceptibility pattern of Shigella, to ensure proper treatment for the patient and to reduce further emergence of resistance.

As no data from our region is available, the present study was carried out to know the prevalence of Shigella from the stool samples of the patients of acute gastroenteritis and their antibiotic susceptibility pattern so as to enable the clinicians to choose appropriate antibiotics.

MATERIALS AND METHODS

During Jan 2013 to 15th Dec 2015, 3062 stool samples were received from the patients presenting with acute gastroenteritis to the tertiary care hospital in Ahmedabad. The samples were processed within two hours of receiving and were analysed according to standard method[6]. They were inoculated on differential and selective media like Mac Conkey agar, Xylose Lysine Deoxycholate (XLD) agar and Thiosulphate Citrate Bile-salt Sucrose (TCBS) agar. They were also subjected to routine microscopy and examined for pus cells, RBCs, trophozoites, ova and cysts. The culture plates were incubated for 37°c for 24 hrs. Suspected colonies on inoculated plates (i.e Lactose non-fermenting colonies on Mac-conkey Agar and pink colonies on XLD) were confirmed for Shigella by biochemical tests. Biochemical tests included Triple sugar iron, Citrate utilization, Urease, Phenylalanine pyruvic acid (PPA), indole test, 1% sugar fermentation, amino acid decarboxylation, Motility Indole Ornithine (MIO) medium and Lysine iron agar. Presumptive Shigella isolates were serotyped by slide agglutination tests by polyvalent and monovalent anti-sera from Denka Seiken.

Antimicrobial susceptibility testing and resistance patterns of the *Shigella* isolates to various antimicrobials were determined by Kirby Bauer disc-diffusion technique[7]. Antimicrobial discs used included Ampicillin (10 mcg), Ciprofloxacin (5 mcg), Levofloxacin (5 mcg), Ofloxacin (5 mcg), Cotrimoxazole (25 mcg) Chloramphenicol (30 mcg) &Cefotaxime(30 mcg) as per CLSI guidelines 2013[8].

RESULTS

Total 3062 stool samples were received during January'13 to 15th December'15 of the patients with suspected acute gastroenteritis. 519 stool showed positive results in culture of which Shigella sp was isolated in 99 (19.07%) stool samples. Of these 52 were male (52.5%) and 47 (47.4%) were female.

Table 1: Various Shigella species isolated

Shigella pecies	Isolates (%)
Sh. Dysentriiae	5 (5.05)
Sh. Flexneri	74 (74.7)
Sh. Sonnei	11 (11.1)
Sh. Boydii	4 (4.04)
Shigella sp.	5 (5.05)

Table: 2 Age distribution of isolate

Age group	Isolates (%)
<1 yrs	11(11.11%)
1-5 yrs	22(22.22%)
5-10 yrs	4(4.04%)
10-15 rs	6(6.06%)
15-40 yrs	32(32.3%)
>40 yrs	24(24.2%)

Table 3: Absolute sensitive strains of Shigella

Species	Absolute sensitive (%)	Resistant
Shigella flexneri	13 (17.56%)	61 (86.4%)
Shigella sonnie	2 (18.18%)	9 (81.8%)
Shigella dysentriae	2 (40%)	3(60%)
Shigella boydii	1(25%)	3 (75%)
Shigella sp	1 (20%)	4 (80%)

Table 4: Antimicrobial susceptibility pattern in different species

Antimicrobial drug	Sh. Flexneri (n=74)		Sh. Sonnie (n=11)		Sh dysentriae (n=5)		Sh. Boydii (n=4)		Shigella sp (n=5)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ampicillin	38 (51.3)	36 (48.6)	9 (81.8)	2 (18.1)	3 (60)	2 (40)	3 (75)	1 (25)	2 (40)	3 (60)
Ciprofloxacin	66 (89.1)	8 (10.8)	11 (100)	0 (0)	4 (80)	1 (20)	4 (100)	0 (0)	4 (80)	1 (20)
Ofloxacin	68 (91.8)	6 (8.1)	11 (100)	0 (0)	3 (60)	2 (40)	4 (100)	0 (0)	4 (80)	1 (20)
Levofloxacin	67 (90.5)	7 (9.4)	11 (100)	0 (0)	5 (100)	0 (0)	4 (100)	0 (0)	4 (80)	1 (20)
Cotrimoxazole	22 (29.7)	52 (70.2)	3 (27.2)	8 (72.7)	4 (80)	1 (20)	2 (50)	2 (50)	2 (40)	3 (60)
Chloramphenicol	45 (60.8)	29 (39.1)	11 (100)	0 (0)	5 (100)	0 (0)	4 (100)	0 (0)	4 (80)	1 (20)
Cefotaxime	68 (91.8)	6 (8.1)	10 (90.9)	1 (9.09)	3 (60)	2 (40)	4 (100)	0 (0)	4 (80)	1 (20)

Shigella flexneri was the most common isolate (n=74) followed by Shigella sonnei (n=11), Shigella dysentriae (n=5) and Shigella boydii (n=4). Five isolates were not agglutinable by any of the antisera, hence reported as Shigella sp. (Table 1). Shigella was isolated in various age groups of which it was most commonly found in 15-40 yrs age group (n=32) as shown in Table 2 and Table 3.

The antimicrobial susceptibility pattern in various species isolated was as shown in Table 4 and 5.

Table 5: Antimicrobial susceptibility in all isolates

Antimicrobial drug	Shigella sp (N=99)	
	Sensitive	Resistant
Ampicillin	55 (55.5%)	44 (44.4%)
Ciprofloxacin	89 (89.9%)	10 (10.1%)
Ofloxacin	90 (90.9%)	9 (9.1%)
Levofloxacin	91 (91.9%)	8 (8.1%)
Cotrimoxazole	33 (33.3%)	66 (66.1%)
Chloramphenicol	69 (69.6%)	30 (30.3%)
Cefotaxime	89 (89.9%)	10 (10.1%)

DISCUSSION

In the present study, Shigella flexneri was the most common species isolated followed by Shigella sonnie. This finding was similar to the study conducted in Vellore, South India (1997-2004), in which the faecal samples from patients of gastroenteritis were processed for enteric pathogens. Shigella accounted for 5.4% of pathogens isolated, Shigella flexneri(57.6%) being the commonest followed by Shigella sonnei (31%).[9] Also another study done in Karnataka in 2012 showed similar findings with Shigella flexneri being the most common isolate.[10]

The majority of Shigella species were isolated from patients belonging to 15-40 years age group (32.3%) . Also a study by Reema et al in Assam(2013) showed similar findings with Shigella commonly isolated in patients with age group of 11-40 yrs(64.78%).[11]

Looking at the antimicrobial susceptibility pattern, though cotrimoxazole resistance was highest which is similar to other studies across India, Resistance to other antibiotics was relatively low as compared to other studies.[10,11,12,13] About 19.19% of Shigella sp. showed absolute sensitivity as shown in Table:2. Shigella flexneri showed maximum resistance compared to other species. Overall Resistance to Ampicillin (44.4%) is much lesser than resistance represented by various other studies.[10,11,12,13] Similarly resistance to quinolones (10.1%,9.1% and 8.1%) and 3rd Generation cephalosporins (10%) is very low in comparison to other studies.[10,12,13] However study from Wilson et al has shown even lesser resistance which might be due to a relatively early period of study i.e 2006 (Table:5) .[14] Resistance is increased during last few years due to injudicious and indiscriminate use of antibiotics.

As shown in Table 5, other studies also showed maximum resistance to Ampicillin and cotrimoxazole, similar to our study. Ciprofloxacin and cephalosporin resistance in our study was less while chloramphenicol resistance was almost similar to other studies. Overall resistance isolates were less in our study compared to other studies. It could be explained by the fact that this tertiary care hospital cures lay population of west Ahmedabad, that harbors a large proportion of low socioeconomic status. This hospital being only the tertiary care hospital receives them in naive condition. Patients visit hospital at the onset of illness without treated with antibiotics elsewhere and hence low level of resistance is seen.

Antimicrobial therapy is the cornerstone of treatment of Shigellosis and also it helps to prevent the more serious complications and infections[15]. The guiding principle for the choice of antimicrobials in developing countries includes cost, availability of the drug and pattern of resistance in the community. Practical empirical therapy for shigellosis starts with the administration of Cotrimoxazole or Ampicillin irrespective of the probable serotype causing dysentery. Because of the growing proportion of Shigella strains resistant to standard low-cost antibiotic Ampicillin, Cotrimoxazole, tetracycline and Chloramphenicol, effective treatment is becoming increasingly difficult [16,17,18 ,19,20] According to our study findings and as other studies mentioned above, there is an increase in resistance to various commonly used anti-microbials like cotrimoxazole, ampicillin and chloramphenicol. So, identification of species and resistance pattern is utmost important to avoid any wrong treatment and ensure quick recovery of the patient.

Also some degree of regulation of antimicrobial use is necessary as most antimicrobials are available over the counter and can be bought without any prescription. There is a need to educate both general public and health practitioners that most cases of diarrhoea do not require antimicrobials[12]. As currently, in case of resistant to first line antibiotics there is an available option like fluoroquinolones and 3rd generation cephalosporins. But a marked increase in resistance to fluoroquinolones and 3rd generation cephalosporins due to ESBL production, would leave limited treatment options for those with life-threatening bacterial infections.

Table: 5 Comparison of antimicrobial resistance with other studies:

Antibiotics	Present study	Urvashi et al (2011)[12]	Bhattacharya et al (2012)[13]	Wilson et al (2006)[14]	Patil & Lava et al. (2012)[10]	Nath Ret al. (2013)[11]	Khan et al (2003)[17]
Ampicillin	44.4%	88.6%	100%	53%	100%	93.6%	51%
Ciprofloxacin	10.1%	67.9	82%	2.4%	-	11.3%	0
Ofloxacin	9.1%	-	80%	-	-	6.5%	-
Levofloxacin	8.1%	-	-	-	69.2%	-	-
Cotrimoxazole	66.6%	84.9%	80%	80.7%	76.92%	92%	74%
Chloramphenicol	30.3%	54%	44%	39.7%	-	12.9%	-
Cefotaxime	10.1%	17.9%	12%	0%	69.2%	4.9%	-

CONCLUSION

It is concluded that Shigella flexneri is a predominant isolate in patients with acute gastroenteritis in Ahmedabad. Though we had less resistance isolates compared to other studies as discussed above, any pitfall in continuous monitoring of susceptibility pattern of Shigella isolates and injudicious use of antimicrobials may definitely increase the prevalence of resistance isolates. Thus, prescribing antimicrobials without susceptibility testing should be avoided. .

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