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Frequency of Isolation of Shigella Serogroups/Serotypes and their Antimicrobial Susceptibility Pattern in Children from slum areas in Karachi

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Abstract

Objectives: To assess the frequency of serogroups and serotypes, as well as the antimicrobial susceptibility pattern of Shigella species isolated from known cases of diarrhoea and dysentery from Karachi, Pakistan.

Methods: A cross-sectional study was conducted between January 2002 and March 2003 at Aga Khan University on stool samples received from children with diarrhoea and dysentery from four low socio-economic areas (Sultanabad, Rehri Goth, Hijrat and Sherpao colony) of Karachi. Stool samples yielding growth of Shigella species, were further identified for serotypes by slide agglutination. Antibiotics susceptibility was performed by Kirby Bauer disk diffusion method.

Results: Out of 4688 stool samples received, 193 (4.1%) were positive for Shigella species. Shigella flexneri was the predominant serogroup (58%) followed by Shigella sonnei (16%), Shigella boydii (15%) and Shigella dysenteriae being the least common (11%). A number of serotypes were isolated in each serogroup, 8 serotypes in S. flexneri, 8 serotypes in S. dysenteriae, 9 serotypes in S. boydii, and Phase 1 & 2 were found in S. sonnei. 17% isolates remained non-serotypeable. All isolates were susceptible to Ofloxacin and Ceftriaxone, high rate of resistance was observed in Cotrimoxazole (87.75%) and Ampicillin (55.5%). Emerging resistance against Nalidixic acid (39%) was observed.

Conclusion: Shigella still accounts for a significant proportion of bacillary dysentery in many tropical and sub-tropical countries. Serotype identification can help in devising strategies such as development of effective vaccine for controlling this problem. Increasing antibiotic resistance against commonly prescribed drugs signify that treatment options have become difficult in cases of severe dysentery (JPMA 55:184;2005).

Introduction

Shigella species are a major cause of diarrhoea and dysentery worldwide and are also responsible for significant morbidity and mortality in the developing world.^{1,2} WHO bulletin concluded that, 99% of the estimated 165 million cases of Shigella diarrhoea annually occurs in developing countries. Majority (69%) of episodes are seen in children under five years of age.³ This is attributable to personal hygiene and sanitary conditions which promote spread of organisms like Shigella and other enteric pathogens.

The genus is divided into four serogroups with 47 serotypes: A (S dysenteriae, 12 serotypes); B (S flexneri, 15 serotypes), C (S boydii 18 serotypes); and D (S sonnei, two antigenic types, phase1 and phase2).^{4,5} This serotyping scheme uses the polysaccharide O antigen found in the outer part of the cell wall.

Serological analysis of Shigella has long been used to characterize isolates for epidemiological and diagnostic purposes.^{4,5} In addition, knowledge of prevalent serotype may also assist in devising strategies such as development of vaccine to prevent infection especially when the immunity to disease is only serotype specific.⁶⁻⁸ The prevalence of a particular species of Shigella differs in various geograph-

ical areas. The WHO bulletin 1999 reported S. flexneri, the main serogroup found in developing countries (median 60% of isolates), followed by S. sonnei (median 15%), S. dysenteriae and S. boydii with equal frequency (median 6%).³ However data from Spain, Israel and the United States consistently demonstrate that S. sonnei was the most common serogroup found in industrialized countries (median 77%), followed by S. flexneri (median 16%), S. boydii (median 2%) and S. dysenteriae (median 1%).³

Frequency of antibiotic resistance among Shigella species is growing and has been reported in various studies globally.⁹⁻¹¹ Local data of antimicrobial susceptibility of frequently used drugs like ampicillin, tetracyclines, chloramphenicol, co-trimoxazole and nalidixic acid have been published earlier¹²⁻¹⁴ but reports of antimicrobial susceptibility to nalidixic acid, fluoroquinolones and 3rd generation cephalosporins seems to be lacking from Pakistan.

Studies published in Pakistan have discussed prevalent Shigella groups but common serotypes from different geographic regions have not been reported.^{15,16} Therefore, we conducted a study and the frequency of serogroups and various prevalent serotypes of Shigella isolated from Karachi, Pakistan with antimicrobial susceptibilities are reported.

Subjects and Methods

A cross-sectional study was conducted at the clinical microbiology laboratory of a tertiary care hospital. Stool samples were collected from children with diarrhoea from four low socio-economic areas (Sultanabad, Rehri Goth, Hijrat and Sherpao colony) of Karachi during January 2002 to March 2003.

All submitted stool samples received in transport media (Phosphate buffered saline) were inoculated on MacConkey, Xylose-Lysine Deoxycholate (XLD) agar and for enrichment in Selenite-F broth and then incubated at 37°C for 24 hours in aerobic environment. After overnight incubation, Selenite-F broth was subcultured on Salmonella-Shigella agar (oxid).¹⁷ Colonies morphologically suggestive of Shigella species were identified by conventional biochemical reactions (urea, citrate, triple sugar iron, indole, motility) and slide agglutination tests using antisera from Denka Seiken Co.Ltd, Japan. Non-serotypable isolates were further checked by API 20 E (Bio Murex, France).

Antibiotics susceptibility was performed by Kirby Bauer disk diffusion method, against Ampicillin (10ug), Chloramphenicol (30ug), Ceftriaxone (30ug), Cotrimoxazole (25ug), Nalidixic acid (30ug) and Ofloxacin (5ug).¹⁸ Statistical analysis was done using SPSS version 10.0 for Windows.

Results

During the study period, 4688 stool samples were received and 193 samples were found to be positive for Shigella species giving the isolation rate of 4.1%. Shigella flexneri was the most predominant serogroup (58%) followed by Shigella sonnei (16%), Shigella boydii (15%) and

1 (13%) and 2 (13%). Amongst S. dysenteriae 8 serotypes were identified, and the most prevalent subtype was S. dysenteriae 7 (42%). Nine serotypes were found in S. boydii and most frequent isolate was S. boydii 2 (20%). Eighty percent S. sonnei belonged to phase 1 (Table 2).

Thirty three isolates (17%) suggestive of Shigella by biochemical reactions (including API 20E) and antisera could not be further subtyped and finally remained non-serotypable (Table 2).

Further analysis of data demonstrated that none of the Shigella was found resistant to ofloxacin and ceftriaxone. Highest percentage of antimicrobial resistance was noted against co-trimoxazole (mean 87.75% with the range of 72-100%) and ampicillin (mean 55.5% with range of 8-88%). Resistance against nalidixic acid and chloramphenicol were comparatively lower with the mean of 39% and 11.25% respectively (Figure).

On further analysis S. flexneri was found most

Figure. Antimicrobial resistance (%) of various group of Shigella.

Table 1. Isolation of Serogroups from various study areas (%).

Serogroups	Hijrat Colony	Rehri Goth	Sultanabad	Sherpao Colony	Total	P value
Shigella flexneri	33 (68)	20 (46)	32 (61)	28 (54)	112 (58)	0.001
Shigella sonnei	7 (14)	5 (11)	7 (13)	13 (25)	31 (16)	0.26
Shigella boydii	6 (14)	10 (23)	8 (15)	5 (9)	29 (15)	0.27
Shigella dysenteriae	2 (4)	8 (18)	5 (9)	6 (11)	21 (11)	0.30

Shigella dysenteriae being the least common isolate (11%). Isolation rate of Shigella flexneri from Hijrat colony (68%) was significantly more compared to other areas (P value 0.001), isolation of all other serogroups from all areas did not show any significant difference (Table 1).

Amongst S. flexneri, 8 serotypes with multiple sub-serotypes were found, the commonest being S. flexneri type

resistant while S. sonnei appeared to be most susceptible to tested drugs. Amongst S. sonnei 87-100% isolates were found susceptible to most of the antimicrobials except co-trimoxazole in which case almost all strains demonstrated resistance (Figure).

Discussion

Shigella still accounts for a significant proportion of

Table 2. Serogroups and serotypes of Shigella.

S. flexneri (n=112)	No.	S. boydii (n=29)	No.	S. dysenteriae (n=21)	No.	S. sonnei (n=31)	No.
S.flexneri 1	15						
S.flexneri 1a	2	S. boydii 1	2	S. dysenteriae 1	1	S. sonnei 1	25
S.flexneri 1b	5	S. boydii 2	6	S. dysenteriae 2	2	S. sonnei 2	3
S.flexneri 2	9	S.boydii 2c	1	S. dysenteriae 3	1	S. sonnei non-sero-type-able	3
S.flexneri 2a	7	S. boydii 3	1	S. dysenteriae 4	3	Total	31
S.flexneri 2b	6	S. boydii 4	1	S. dysenteriae 7	9		
S.flexneri 3	5	S. boydii 7	1	S.dysenteriae 8	1		
S.flexneri 3a	6	S. boydii 8	3	S.dysenteriae 12	1		
S.flexneri 3b	1	S. boydii 9	1	S.dysenteriae 14	1		
S.flexneri 3c	2	S.boydii 10	2	S.dysenteriae non-			
S.flexneri 4	9	S.boydii 14	3	sero-type- able	2		
S.flexneri 4a	3	S. boydii Non-		Total	21		
S.flexneri 4b	1	sero-type-able	8				
		Total	29				
S.flexneri 6	14						
S.flexneri.7	5						
S.flexneri 8	1						
S.flexneri 10	1						
S.flexneri non- sero-typeable	20						
Total	112						

bacillary dysentery in the developing world. During our study period, a total of 193 Shigella species were isolated from 4688 stool samples giving the isolation rate of 4.1%, which is similar to other studies published in India (3.2%).^{19,20}

Analysis of our data showed that *S. flexneri* was the most prevalent serotype (58%) followed by *S. sonnei* (16%). Though it is in contrast to the finding of developed world but is similar to that in other countries where diarrhoeal diseases are endemic too.^{21,22}

Amongst *S. flexneri*, multiple serotypes were isolated from clinical samples and the highest prevalence was found to be of serotypes 1 and 2 followed by 3, 4 and 6. However predominance of serotypes 3, 4 and 6 of *S. flexneri* had not been reported earlier in Pakistan. Recently a change in trend has also been reported from Bangladesh and other parts of the world.^{22,23} These findings support the importance of continuous monitoring of Shigella serogroups and serotypes.

Out of 112 *S. flexneri*, 20 (18%) of the isolates remained non-serotypable by antisera (Denka Seiken)

though biochemically had excellent identification profile by API20 E. While, it is known that isolation of rare serotypes of Shigella particularly of flexneri is not uncommon. Recently Talukdar²⁴ from Bangladesh has reported the emergence of 1c strain. Unfortunately, the antiserum of 1c is not commercially available. Considering the emergence of 1c from countries like Bangladesh, there are possibilities that these non-type-able isolates could be of 1c subtype. For the final identification of these strains, specific antisera and molecular methods for genotyping would be required.²⁴

Non sero-typeable Shigella was also found among other groups. *S. boydii* (8 out of 29), *S. dysenteriae* (2 out of 21) and *S. sonnei* (3 out of 21). Unfortunately, by the routine laboratory methods these isolates remained nontypable. All of the tested strains were isolated from cases with dysentery, which reinforces need to have more comprehensive and cost effective ways to identify these isolates in clinical laboratories of developing world.

Isolation rate of Shigella flexneri from Hijrat colony was significantly more as compared to other areas (P value 0.001). Isolation rate of other serogroups from different areas did not show any significant difference. Higher rate of

isolation of one species from one locality of same geographic region is not unusual and this phenomenon has already been reported earlier from Bangladesh.¹⁴

Review of available data shows a global rise in antimicrobial resistance. Various studies from different parts of the world reported increase in resistance among different species of *Shigella* against commonly used drugs like ampicillin, tetracycline, chloramphenicol and co-trimoxazole.¹³⁻¹⁵ This study also reveals higher rate of resistance particularly against co-trimoxazole and ampicillin as compared to the previous local report (3.5% and 7%).^{12,16}

Therefore, these results strongly suggest that ampicillin and co-trimoxazole cannot be used as first line drugs in cases of severe diarrhoea and dysentery in our setting. And this inference is in accordance with the findings from other countries where these drugs have already proven ineffective against *S. flexneri* and *S. sonnei*.⁷⁻⁹

Resistance to nalidixic acid was found among different groups of *Shigella* with variable frequency. Resistance to nalidixic acid has been reported previously from Far east⁹, Santiago²⁵ and also from neighbouring countries like India.^{19,20} In this region, nalidixic acid is an inexpensive and frequently used drug for community acquired diarrhoea. It is sad, as acquiring resistance against nalidixic acid in a poor country like Pakistan will make the treatment options for a family physician more difficult and expensive.

Fortunately, no resistance was found against third generation cephalosporins and fluoroquinolones, which is consistent with the international data. It also implies that at least a few drugs can be empirically used in seriously ill patients.

Our results indicate that detection of a variety of serotypes and nontypable strains highlights the need of continuous monitoring of trends of prevalent strains. We are hoping that in future this information will facilitate the successful development of specific vaccine against commonly occurring strains.

In addition, presence of a large number of nontypable *Shigella* isolates indicate that the commercially available typing antisera are not sufficient to recognize all prevalent serotypes and a need for development of more sensitive methods for *Shigella* typing may be required.

There is a need to establish regional or national surveillance program to document the resistance rates and trends. Moreover, these rates should be available for all family physicians and paediatricians to ensure appropriate use of antimicrobials. In addition, government agencies should monitor and restrict the use of drugs by making policies, providing guidelines and also by training the health care workers in selection of an agent.

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