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Case Report

A Case Report of Dysentery Caused by an Unusual Shigella Isolate from South India

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Abstract

Background: Shigellosis is one of the leading causes of mortality and morbidity among children less than five years (under-5 children). Available literature report Atypical serotypes of *Shigella flexneri* from various parts of the world. At present, there are 17 serotypes in *S. dysenteriae*, 6 serotypes and 14 subserotypes in *S. flexneri*, 20 serotypes in *S. boydii* and a single serotype of *S.sonnei*, 2 phases and 5 biotypes (a, b, e, f and g). Here, we report a strain of mannitol non-fermenting *S.flexneri* serotype 4 in a one-year-old girl child presented with high-grade fever and loose stools.

Clinical Description: A one-year-old girl child with a known history of absence seizures presented with high-grade fever and loose stools for two days, about 8-9 episodes per day, foul-smelling, mucoid in nature, stained with fresh blood, and abdominal pain. The child also had 2-3 episodes of vomiting, non-bilious, non-blood-stained and contained food particles. Stool sample was plated onto MacConkey agar (MAC) and Xylose Lysine Deoxycholate Agar (XLD agar) and incubated aerobically at 37°C for 16-18 hours.Non lactose fermenting and red colonies were seen in MAC and XLD respectively which was identified subsequently as *Shigella flexneri* by serogrouping, but was a mannitol non fermentor. The isolate was found to harbour multiple virulence genes by PCR.

Conclusion: The child was continued on oral cefixime for 10 days but has not improved following which she was started on Inj. Ceftriaxone 100 mg/kg/day for 5 days and the child improved and discharged subsequently.

Keywords: Atypical Shigella; Type IV Shigella Flexneri; Non-Mannitol Fermentor; Shigellosis; Virulence Genes PCR

Case Report

Shigellosis is one of the significant causes of mortality and morbidity among children less than five years of age, predominantly in developing countries due to overcrowding and poor sanitation. *Shigella,* a non-motile Gram-negative bacillus belonging to the family Enterobacteriaceae, has four species, namely, *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei* (designated as serogroups A, B, C and D, respectively). The distribution of *Shigella* species varies globally. *S. flexneri* is most common among all the species isolated across developing countries, whereas *S. sonnei* is the prominent species in developed countries [1]. At present, there are 17 serotypes in *S. dysenteriae*, 6 serotypes and 14 subserotypes in *S. flexneri*, 20 serotypes in *S. boydii* and a single serotype of *S.sonnei*, 2 phases and 5 biotypes (a, b, e, f and g) [2]. Reports of Atypical serotypes of *S.flexneri* are available from various parts of the world like Korea, Russia, Bangladesh and China [3-7]. Similarly, novel serovars of *S.dysentriae* have been reported from Bangladesh and Puducherry [8,9]. These were proven to harbour multiple virulence factors on their invasion plasmids and chromosomes. Here, again we report a strain of *S.flexneri* serotype 4, which was a mannitol non-fermentor and harboured multiple virulence genes.

Clinical Description

A one-year-old girl child with a known history of absence seizures and global developmental delay presented to a tertiary care hospital with high-grade fever and loose stools for two days, about 8-9 episodes per day, foul-smelling, mucoid in nature, stained with fresh blood, and abdominal pain. The child also had 2-3 episodes of vomiting, non-bilious, non-blood-stained and contained food particles. No history of similar illnesses in the past and no history of similar illnesses among the family members. On examination, the child was afebrile, and pulse rate was 140 beats/min, respiratory rate was 38 breaths/ min, blood pressure was 80/42 mmHg, SpO2 was 97%, and capillary refilling time was 3 seconds. Cardiovascular, respiratory and per abdomen examination were normal. The child was started on oral cefixime 10 mg/kg/day after collecting a stool sample which was sent for bacteriological culture.

In the macroscopic examination, the stool was semi-formed, mucoid and blood-tinged. Microscopic examination revealed plenty of pus cells and red blood cells. Subsequently, the stool sample was plated onto MacConkey agar (MAC) and Xylose Lysine Deoxycholate Agar (XLD agar) as well as inoculated into the Selenite F enrichment broth. The plates and the broth were incubated aerobically at 37°C for 16-18 hours, following which the plates were read. Pure growth of 2-3 mm in size, circular, low convex pale non-lactose fermenting colonies with smooth surface and entire margins were seen on MAC. On XLD, red colonies were noted. A similar type of growth was seen in the Selenite F subculture as well. Gram stain from the colony revealed short gram-negative bacilli. The organism produced indole, did not utilize citrate or hydrolyze urea and in Kligler iron agar produced alkaline slant with acid butt without gas or H_2S. The same reaction was seen in lysine iron agar. The Orthonitrophenyl β-d-galactopyranoside (ONPG) test was negative. Mannitol was not fermented and non-motile in the Mannitol motility medium (MMM). The colonies were subjected to Matrix-Assisted Laser Desorption/Ionization-Time Of Flight mass spectrometry (MALDI-TOF MS -VITEK® MS, version 3.0, BioMérieux, Marcy-l'Étoile, France) and was identified as *Escherichia coli* as it is a known fact that MALDI-TOF misidentifies *Shigella* species as *E.coli*. The identification was reconfirmed by another automated identification system i.e VITEK® 2 ID card (version 8.01, BioMérieux, Marcy-l'Étoile, France) and the isolate was identified as *Shigella* group with 97% probability, which also confirmed the non-fermentation of mannitol. The strain had agglutinated with *Shigella flexneri* polyvalent B antisera (Difco, Becton Dickson, United States). Further, the strain agglutinated with type 4 monovalent antiserum (Denka-Seiken, Tokyo, Japan). Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method against Ampicillin 10 µg, Ciprofloxacin 5 µg, Cotrimoxazole 23.75/1.25 µg, Ceftriaxone 30 µg and Cefixime 30 µg. Epsilometer test (E test) was performed for determining minimal inhibitory concentration (MIC) of azithromycin and interpreted as per Clinical Laboratory Standards Institute breakpoints [10]. The isolate was susceptible to ciprofloxacin, ceftriaxone, cefixime, azithromycin and resistant to ampicillin and cotrimoxazole.

Conventional Polymerase Chain Reaction (PCR) was performed to detect the virulence genes; namely, *Shigella* enterotoxin 1 (SheT1) comprising the two subtypes *set 1A* (SheT1A) and *set 1B* (SheT1B); *stx, ipaH, ial, invE* and *Shigella* enterotoxin 2 or sen (*SheT2*) gene. The details of the primers used for the identification of virulence genes are listed in table 1 [11,12]. The isolate was found to harbour all the virulence genes. as depicted in figure 1 The products of PCR amplification were subjected to sequencing using an ABI 3730 XL sequencer (Applied Biosystem, Foster, CA,

USA) at Eurofins genomics private limited, India. The sequences generated were assembled and compared using the NCBI's Basic Local Alignment Search Tool (BLAST). Translation of DNA sequences into protein sequences was done using EXPASY translate. The translated protein sequences were assembled and compared using the protein Basic Local Alignment Search Tool (pBLAST) of the NCBI. Aligned sequences were searched in NCBI-BLAST (megablast) for the similarity of significant matches in the database. The sequences of *ipaH*, *sen*, *set1*, *ial* genes showed 100% similarity with *Shigella flexneri* strain FDAARGOS 714 plasmid (accession number - CP055125.1) whereas the sequence of the gtr4 gene had 100% homology with *Shigella* phage *SfIV* complete genome (accession number -KC814930.1).

Management and Outcome

The child was continued on oral cefixime 10 mg/kg/day for 10 days but has not improved. Blood in stool persisted, following which she was admitted and started on Inj.Ceftriaxone 100 mg/kg/day for 5 days. The child improved and repeat stool culture did not yield any further isolation of *Shigella flexneri.* The child was discharged subsequently. The *S. flexneri* are predominantly mannitol fermentors, except a few serotypes like 4 and 6, but their isolation

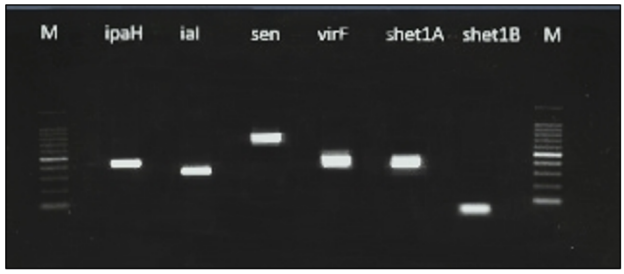

Figure 1: Gel electrophoresis image showing bands for multiple virulence genes.

Lane 1 and 8: 100 basepair DNA ladder

Lane 2: *ipaH* (423 bp)

Lane 3: *ial* (320 bp)

Lane 4: *sen* (799 bp)

Lane 5: *virF* (450 bp)

Lane 6: *shet 1A* (309)

Lane 7: *shet 1B* (147 bp).

Virulence genes	Primers	Oligonucleotide Sequence (5'-3')	Size of amplified product (bp)	Location	Reference
ipaH	Shig-F Shig-R	F: TGGAAAAACTCAGTGCCTCT R: CCAGTCCGTAAATTCATTCT	423	Plasmid and chromosome	11
ial	ial-F ial-R	F: CTGGATGGTATGGTGAGG R: GGAGGCCAACAATTATTTCC	320	Plasmid	
sen	SheT2-F SheT2-R	F: ATGTGCCTGCTATTATTTAT R: CATAATAATAAGCGGTCGC	799	Plasmid	
virF	virF- F virF- R	F: CAATGACGGTTAGCTCAGGCA R: AAAGACGCCATCTCTTCTCGAT	450	Plasmid	
set 1A	SheT1A -F SheT1A -R	F: TCACGCTACCATCAAAGA R: TATCCCCCTTTGGTGGTA	309	SHI-1 pathogen island	
set 1B	SheT1B -F SheT1B -R	F: GTGAACCTGCTGCCGATATC R: ATTTGTGGATAAAAATGACG	147	SHI-1 pathogen island	
gtr4	Gtr4 - F Gtr4 - R	F: ATGTTCCTCCTTCTTCCTTT R: TCCTGATGCTACCTTATCCA	905	chromosome	12

Table 1: Primers used in PCR for identification of virulence genes.

is relatively rare from clinical samples compared to the other serotypes [5].

Discussion

In the present case, despite the isolate being susceptible to cefixime *in vitro*, there was a therapeutic failure when given orally. The possible reason would be that gastrointestinal absorption of antibiotics will be affected during severe mucosal inflammation/ involvement [13]. The clinical importance of these atypical strains is that they can be easily missed during identification, thereby posing a severe public health hazard. Hence, there is a need to keep an open eye throughout the identification process and not rely on automated identification systems alone due to their limitations. Conventional systems of studying biochemical reactions could be more awarding with such clinical isolates.

Lessons Learnt

- To take into cognizance, unusual biochemical characters among suspected *Shigella* isolates. Such isolates should be characterized using molecular tool available for determining the virulence factors.
- Atypical *Shigella* need to be reported/ documented.
- Clinically, Atypical *Shigella* may or may not have unusual manifestations.

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None.

Conflict of Interest

The authors do not have any conflicts of interest to declare.

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