Novel strain of *Shigella dysenteriae* serotype 7 from India

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Abstract

We describe a strain of *Shigella dysenteriae* serotype 7 which had novel biochemical and genetic characters. Unlike other *S. dysenteriae,* it produced gas, fermented mannitol, was a late-lactose fermenter and harboured the *set 1A* and *set 1B* genes. The significance of such atypical strains is that they are difficult to identify. If such strains are missed, they could prove to be a serious public health problem because the infectious dose is very low and they may harbour integrons contributing to drug resistance.

Keywords: Catalase, gas, late-lactose, SheT1, *Shigella dysenteriae*
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The differentiation of *Shigella* into its four species is based on their different reactions in simple biochemical tests and the serologic activity of the O polysaccharide of their lipopolysaccharide. Currently there are 15 serotypes in *S. dysenteriae,* six serotypes with 14 subserotypes in *S. flexneri,* 19 serotypes in *S. boydii* and a single serotype of *S. sonnei* [1,2]. They harbour many genetic elements on their invasion plasmid as well as on their chromosomes [1]. Isolation of atypical serotypes of *S. flexneri* have been reported from Korea, Russia, Bangladesh and China [3–6], and a new serovar of *S. dysenteriae* has been reported from Bangladesh [7]. Here we report a strain of *S. dysenteriae* serotype 7 which had novel biochemical and virulence characters.

The strain on primary isolation from a diarrhoeic stool specimen on MacConkey agar showed colonies 2 to 3 mm in size which were flat topped with irregular margins and remained pale up to 16 to 18 hours after incubation. These colonies turned pink after 24 to 48 hours, indicating late-lactose fermenting behavior. On deoxycholate citrate agar, initially pale non-lactose-fermenting colonies were observed, which also turned pink after 24 to 48 hours. On xylose lysine deoxycholate agar, red colonies with a low convex surface were noted. Gram stain revealed the presence of short Gram-negative bacilli which were found to be nonmotile. The organism produced catalase, was oxidase negative, produced indole, did not utilize citrate and did not hydrolyze urea. Kligler iron agar showed a K^+/A^- reaction indicative of gas production; lysine and ornithine were not decarboxylated; arginine was not hydrolyzed; mannitol was fermented; and ortho-nitrophenyl β-d-galactopyranoside (ONPG) test was positive. The strain fermented lactose after an incubation of more than 24 hours, which explained the late-lactose fermenting behaviour. All these biochemical tests were performed using conventional media and chemicals (Himedia, Mumbai, India). The strain agglutinated with *S. dysenteriae* polyvalent A antisera (which covers serotypes 1 to 7; Denka-Seiken, Tokyo, Japan). Using the monovalent specific antisera, the strain agglutinated with type 7 monovalent antiserum (Denka-Seiken). Disc diffusion testing performed using the Kirby-Bauer method as per the Clinical Laboratory Standards Institute guidelines [8] against ampicillin 10 μg, ceftriaxone 30 μg, ciprofloxacin 5 μg and cotrimoxazole 25 μg showed that the strain was sensitive to all the antibiotics tested. 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* and *Shigella* enterotoxin 2 or *sen* (SheT2) gene; and *InvE* (signature gene of enteroinvasive *Escherichia coli*) [9–12] (Table 1). The strain harboured the *ipaH, sen, set 1A, set 1B* and *ial* genes. No other pathogen was isolated from the stool specimen.

Multiple novel biochemical and virulence characters were identified in the present strain. It produced gas and fermented mannitol, characters which have not been reported in the species *dysenteriae* (*Shigella* serogroup A). It also fermented lactose after 24 hours of aerobic incubation with a positive ONPG test (late-lactose fermenter), characters reported only in *S. sonnei* and rarely in serotype 1 of *S. dysenteriae* [1]. Because the strain had biochemical characters similar to those of enteroinvasive *Escherichia coli* (EIEC), the *InvE* gene (a signature gene of EIEC) was targeted by PCR. However, the absence of

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TABLE 1. Primers used in PCR for identification of virulence genes

Virulence gene	Primer	Oligonucleotide sequence (5′–3′)	Size of amplified product (bp)	Reference
set 1A	SheT1A-F	TCACGCTACCATCAAAGA	309	[10]
	SheT1A-R	TATCCCCCTTTGGTGGTA		
set 1B	SheT1B-F	GTGAACCTGCTGCCGATATC	147	[10]
	SheT1B-R	ATTAGTGGATAAAAATGACG		
ipaH	Shig-F	TGGAAAAACTCAGTGCCTCT	423	[9]
	Shig-R	CCAGTCCGTAAATTCATTCT		
stx_1	stx_1-F	ACCCTGTAACGAAGTTTGCG	140	[11]
	stx_1-R	ATCTCATGCGACTACTTGAC		
sen	SheT2-F	ATGTGCCTGCTATTATTTAT	799	[9]
	SheT2-R	CATAATAATAAGCGGTCAGC		
ial	*ial*-F	CTGGATGGTATGGTGAGG	320	[9]
	ial-R	GGAGGCCAACAATTATTTCC		
InvE	*InvE*-F	CGATAGATGGCGAGAAATTATATCCCG	766	[12]
	InvE-R	CGATCAAGAATCCCTAACAGAAGAATCAC		

the *InvE* gene negated the possibility that this could be an EIEC strain. The presence of *ial, ipaH* and *sen* (SheT2) further confirmed that this belonged to the genus *Shigella*. There is no record of *set 1* (SheT1) being reported from *S. dysenteriae* in the literature. It has previously been reported only from *S. flexneri* (commonly serotype 2a) [7,9,10]. Sequence BLAST analysis of the target genes *set 1A, set 1B* and *ipaH* matched with that of GenBank accession number CP004057, and the *ial* gene matched with that of GenBank accession number NC016833. We used the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST) for database searching. The GenBank accession numbers for the submitted sequences of the current isolates are as follows: for the *set 1A* gene KR822808, for the *ipaH* gene KR269602 and for the *ial* gene KT013267. The strain did not harbour the *stx* gene.

Out of all the *Shigella* species isolated by our centre, *S. flexneri* comprised 90.54%, *S. sonnei* 5.40%, *S. dysenteriae* 2.70% and *S. boydii* 1.35%. Antimicrobial resistance is frequently encountered in these isolates. As demonstrated in previous studies from this laboratory, the majority (79%) of the isolates of *Shigella* were resistant to ampicillin and cotrimoxazole, while 50% were resistant to ciprofloxacin. (The minimum inhibitory concentration of ciprofloxacin was found to be 16 μg/mL.) It was found that 3% of the isolates of *S. flexneri* were resistant to ceftriaxone (minimum inhibitory concentration of >256 μg/mL) and were confirmed to be extended-spectrum β-lactamase producers that harboured the *CTX-M-15* gene [13,14]. Unlike many of the earlier isolates from our region, the present strain of *S. dysenteriae* was sensitive to all the antibiotics tested.

In general, there are a number of factors which contribute to the emergence of new strains. Of these, human migration associated with trade and industrialization has been well studied [6]. Genetic recombination can arise as a result of selective pressure on the bacterium from environmental factors or can be phage mediated, resulting in aberrant strains. For example, in *S. flexneri,* the O polysaccharides of the different serotypes and subserotypes are actually polymers of the same basic tetrasaccharide unit. As a result of the phage-mediated insertion of α-glycosyl or O-acetyl residues at specific positions in this basic tetrasaccharide unit, different group and type-specific variants arise [15].

The significance of atypical strains are that the identification of such strains is difficult. Missing such variant strains could result in a serious public health problem [6,16]. Redefining the classification of *Shigella* by developing a comprehensive classification scheme using a combination of biochemical, serologic and molecular characters must be undertaken because more new variants are being discovered across the globe [7].

Conflict of interest

None declared.

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