Case Report

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Microbiology Section

Persistent Diarrhoea due to *Salmonella* Serotype Agona in a Child with Inflammatory Bowel Disease: A Case Report

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ABSTRACT

This case report describes the presentation, diagnosis and review of *Salmonella* serotype Agona in a patient with Inflammatory Bowel Disease (IBD). *Salmonella* serotype Agona is a non typhoidal *Salmonella*, reported globally as a common cause of food-borne outbreaks; however, in India, it is not commonly reported either in outbreak studies or sporadic case reports. A six-year-old male child, known case of IBD, presented with complaints of loose stools along with blood for the past two months. Parents gave a history of intermittent fever, loose stools, abdominal pain, and vomiting for the last one year. Stool sample received in Microbiology Laboratory for routine microscopy, culture and sensitivity. On microscopy, motile Gram-negative bacilli and pus cells were seen; stool was positive for occult blood and negative for parasites. *Salmonella* group B was grown on culture, which was later confirmed by Multi Locus Sequence Typing (MLST) as *Salmonella* serotype Agona. Patient responded to azithromycin and ceftriaxone. It is difficult to distinguish between chronic infective diarrhoea and IBD, as clinically, both can appear the same. Emphasis must be given to the detection of enteropathogens from the stool sample of IBD patients, which will direct the correct management of the patient. *Salmonella* Agona has outbreak potential; hence, vigilant efforts are required for its identification and confirmation.

Keywords: Child, Foodborne disease, *Salmonella* infections

CASE REPORT

A six-year-old male child presented at a tertiary care hospital with complaints of blood in stool for two months. Parents gave a history of intermittent fever and loose stools, decreased oral intake, abdominal pain and vomiting for the last six months. The child had undergone a colonic biopsy one year ago for frequent episodes of loose stools and was diagnosed as a case of chronic IBD and was on treatment. There was past history of treated pulmonary tuberculosis two years ago. There was no history of similar complaints in the family.

On general examination child was pale and malnourished. Vitals and systemic examination were within normal limits. The child was symptomatically treated with ORS and probiotics. A stool sample was sent to the microbiology laboratory for routine microscopy, culture and sensitivity. On routine microscopy, Gram-negative bacilli and pus cells were seen, occult blood was present, and there was no parasitic element.

The stool sample was inoculated on 5% Sheep blood agar, Macconkey Agar (MAC), Xylose Lysine Deoxycholate (XLD) agar and in Selenite F broth. After four hours, subculture from Selenite F broth was done on MAC and XLD agar. All the inoculated plates were incubated at 37°C for 18-20 hours. The next day MAC agar showed growth of two types of colonies. Lactose Fermenting (LF) flat colonies and a Few Non-Lactose Fermenting (NLF), low convex, colonies [Table/Fig-1]. XLD agar showed red colonies with black centre [Table/Fig-2]. Subculture of few NLF colonies done on MAC showed pure growth of NLF colonies [Table/Fig-3]. The NLF colony was oxidase negative, catalase positive [Table/Fig-4] and on microscopy (hanging drop method), motile gram-negative bacilli.

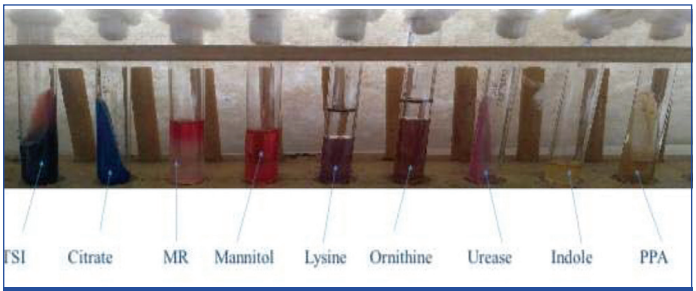
The biochemical reactions of NLF colonies were as follows: TSI: K/A with abundant H_2S; Citrate, Methyl red, Mannitol, Lysine and Ornithine were positive; Indole, Urease and PPA were negative [Table/Fig-5].

[Table/Fig-1]: MAC agar showing Lactose-Fermenting (LF) and Non Lactose Fermenting (NLF) colonies.

[Table/Fig-2]: XLD agar showing red colonies with black centre.

[Table/Fig-3]: MAC agar showing pure Non Lactose Fermenting (NLF) colonies.

[Table/Fig-4]: Catalase test (Positive).



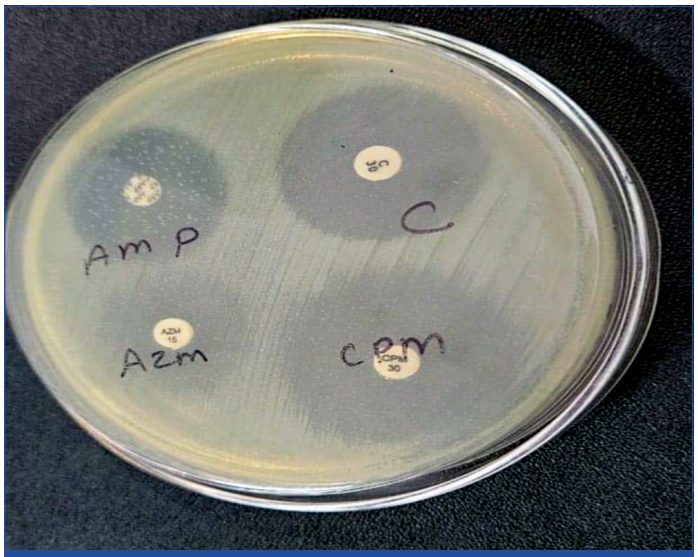
[Table/Fig-5]: Biochemical test.
Left to Right- TSI: K/A with abundant H2S; Citrate, MR, Mannitol, Lysine, and Ornithine were positive; Urease, Indole, and PPA were negative

On the basis of biochemical reactions, the isolate was presumptively identified as Group B *Salmonella* spp. and confirmed with slide agglutination by using *Salmonella* polyvalent O (A-G) and Factor 4 'O' antisera. Slide agglutination was positive with *Salmonella* Polyvalent O (A-G) and Factor 4 'O' antisera; however isolate could not be agglutinated with *Salmonella* H Phase 1 antisera "b" and "i" antisera for confirmation of *S.* paratyphi B or *S.* typhimurium, respectively, which are the most common Group B *Salmonella* serotypes. Antimicrobial sensitivity testing was done as per CLSI guidelines [1] and the isolate was susceptible to ampicillin, ceftriaxone, cefixime, azithromycin, chloramphenicol and cotrimoxazole; however isolate was resistant to ciprofloxacin. Cefixime was checked for Enterobacteriaceae as a part of routine, but not reported [Table/Fig-6].

Antimicrobial	MIC (µg/mL)	Interpretation
Ceftriaxone	≤0.25	S
Cotrimoxazole	≤20	S
Ciprofloxacin	≥4	R

[Table/Fig-6]: Antimicrobial susceptibility profile of *Salmonella* serotype Agona.

Ceftriaxone, cotrimoxazole, and ciprofloxacin were tested using the VITEK 2 Compact system (MIC) and ampicillin, cefixime, azithromycin, and chloramphenicol were tested using the Kirby-Bauer disk diffusion method [Table/Fig-7].



[Table/Fig-7]: Kirby-Bauer disk diffusion method on Mueller–Hinton agar.
AMP: Ampicillin, AZM: Azithromycin, CPM: Chloramphenicol, C: Cefixime

After receiving of Stool culture sensitivity report, the child was put on azithromycin (20 mg/kg/day) for five days and ceftriaxone (50 mg/kg/day) for five days. The child responded to the treatment and was discharged. At follow-up after one month showed sustained clinical improvement.

In the microbiology laboratory, the Group B *Salmonella* isolate could not be confirmed by slide agglutination test with 'H' Phase antisera, as either *S.* paratyphi B or *S.* typhimurium, so the isolate was sent to CMC Vellore for molecular confirmation. At CMC Vellore isolate was subjected to Multilocus Sequence Typing (MLST) and identified as "*Salmonella*. *enterica* serotype Agona." Patient's histopathology report confirmed the diagnosis as ulcerative colitis.

DISCUSSION

Salmonella serotype Agona is one of the rarely reported non typhoidal *Salmonella* from India [2-5]. Transmission to humans occurs via the consumption of raw or inadequately cooked foods of animal origin, or by food items and water contaminated with infected faeces from a reservoir host [6,7]. Though *Salmonella* serotype Agona infection commonly presents as self-limiting gastroenteritis, complications like focal infections, meningitis and persistent diarrhoea have been reported in immunocompromised patients [8,9]. *Salmonella* serotype Agona has caused a number of human disease outbreaks in the European Union, as well as the United States, involving a range of foodstuffs, including ready-to-eat savoury snacks, cereal, air-dried raw beef, infant milk formula, and fennel-aniseed-caraway infusion [10-13].

In the present case, nobody in the family had similar complaints of persistent diarrhoea in the vicinity of the patient's residence. So, this

case is likely to be a sporadic case, clinically manifested because of impaired immunity, attributable to ulcerative colitis and past pulmonary tuberculosis. Clinically, it is very difficult to distinguish between diarrhoea due to infection and ulcerative colitis. Both these conditions can co-exist and one can act as predisposing factor for the development of the other. A study by Tripathi MK et al., has shown a significant correlation between ulcerative colitis and intestinal *Salmonella* sps infection by doing Polymerase Chain Reaction (PCR) tests on intestinal biopsy of chronic IBD patients. They observed *Salmonella* sps in 80% samples. However, their study did not specifically detect *Salmonella* Agona [5].

Various studies have shown that there is an increased risk of IBD after infection by enteropathogens such as *Salmonella*, *Campylobacter*, *Shigella*, *Yersinia enterocolitica,* etc., [14-16]. These infections cause permanent changes in the intestinal microbiota, disruption of the epithelial barrier and alterations of the intestinal immune response. Also, in IBD, there is an increased risk of chronic intestinal infections due to impaired mucosal immunity, which can promote the vicious cycle of infection-impaired mucosal immunity- persistent infection. So, non detection of gastro-pathogens from IBD cases could be attributed to either failure in Laboratory detection of pathogen or appropriate sample collection (biopsy samples are more sensitive than stool). The conventional methods are limited due to the divergence of gastrointestinal flora and hundreds of serotypes of enteropathogens like *Salmonella* spps.

Salmonella enterica serotype Agona is second most commonly reported non typhoidal serotype in the United States and European countries [7,10,11,12]; however, in India, *Salmonella* Agona seems to be under-reported [2-5]. This could be due to difficulty in identification by conventional methods, unavailability of antisera. Conventionally, *Salmonella* serotype Agona would have been reported as Group B *Salmonella* Sps, which includes *Salmonella* paratyphi B and *Salmonella* typhimurium, the most commonly reported serotypes in Group B *Salmonella*. It is impossible to confirm the serotype in the absence of specific antisera and molecular methods. A comparative analysis of the present case with previously reported cases of *Salmonella* Agona summarised in [Table/Fig-8] [3,5,8,11].

Author	Country	Patient profile	Symptoms	Treatment	Outcome
Present case	India	Six-year-old boy with IBD	Persistent diarrhoea, abdominal pain, and blood in stools	Azithromycin + Ceftriaxone	Clinical improvement at follow-up
Tripathi MK et al., [5]	India	IBD patients (biopsy samples)	Diarrhoea, IBD flare	Not specified	*Salmonella* spp. detected in 80% samples
Cooke FJ et al., [8]	UK	Neonate	Meningitis	Antibiotics	Survived
Jourdan-da Silva N et al., [11]	France	Outbreak cases (infants)	Gastroenteritis	Supportive + antibiotics	Large outbreak, recovery in most
Jacob JJ et al., [3]	India	19-year surveillance study	Enteric non typhoidal *Salmonella* infections	Various	Reported resistance trends

[Table/Fig-8]: Comparative analysis of the present case with previously reported cases of *Salmonella* Agona [3,5,8,11].

In addition to this, in India, the occupational, geographic and climatic conditions can favour the transmission of *Salmonella* Agona. Occupations like agriculture, cattle raising, poultry, dairy, etc., poor sanitary conditions and use of seafood at many places across India, are predisposing factors for the transmission of infection [2]. Though all these conditions in India are favourable for transmission of *Salmonella* Agona and it has outbreak potential, it is not commonly reported across India. It may be due to its difficulty in differentiation from Group B *Salmonella* spp by phenotypic methods and the unavailability of molecular typing methods [4]. Indian food processing and cooking methods may have a controlling effect on the possible outbreaks of *Salmonella* Agona [2].

Emphasis needs to be given on the identification of *Salmonella* serotype Agona from cases of gastroenteritis, especially in cases of IBD. This serotype has an outbreak potential. Also, in recent years, there have the reports of multidrug-resistant *S. enterica* serotype Agona, posing a major hazard to human and animal health [17,18]. Hence, it is essential to understand the pathogenicity and antimicrobial resistance of this serotype.

CONCLUSION(S)

In case of IBD, vigilant efforts should be made to recover and identify the possible aetiological agent, like *Salmonella, Campylobacter, Shigella, Yersinia enterocolitica,* etc. Timely recovery and identification of the pathogen and reporting antimicrobial sensitivity patterns will facilitate patient management and a better outcome for the patient. Emphasis needs to be given to the identification of *Salmonella* serotype Agona by molecular methods.

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