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ORIGINAL RESEARCH

Isolation and identification of *Shigella spp.* from human fecal samples collected from Pantnagar, India

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Abstract

Aim: To isolate and identify *Shigella species* from faecal samples based on cultural and biochemical tests.

Material and Methods: For the isolation of *Shigella spp.*, faecal samples from cattle, poultry and humans were collected from various locations of Pantnagar. Fecal specimens were processed according to standard protocols.

Results: Out of 511 fecal samples (311 human, 100 cattle and 100 poultry faecal samples) analyzed, 8 isolates of *Shigella species* were confirmed on the basis of Gram stain, morphology, cultural identification and biochemical characters. All the 8 *Shigella* isolates were obtained from human stool samples giving a prevalence rate of 2.57%.

Conclusion: Under the conditions of the current study, *Shigella* species were prevalent in human population although in small numbers, whereas it was not isolated from cattle and poultry samples.

Keywords: cultural and biochemical characters, diarrhoea, faecal samples, *Shigella*

Introduction

Shigellosis, an acute diarrhoeal disease, is caused by Gram-negative bacterium, Shigella, belonging to the family Enterobacteriaceae, with four species viz. Shigella dysenteriae (serogroup A), Shigella flexneri (serogroup B), Shigella sonnei (serogroup C) and Shigella boydii (serogroup D). Shigella sonnei has become the most dominant serotype causing shigellosis in asian countries in recent years [1]. Shigella dysenteriae, implicated in epidemics, leads to death. [2]. Environmental risk factors of shigellosis include water supply, sanitation, and household environment including fly aggregation [3]. Shigellosis also leads to the development of some complications like lethal toxic encephalopathy or Ekiri syndrome [4] and haemolytic uraemic syndrome (HUS) [5].

Shigella has become a public health concern because of the development of multiple antimicrobial resistant strains, emphasizing the importance of continuous monitoring of the pathogen. Resistance and reduced susceptibility to β-lactam antibiotics is mainly caused by the production of CTX-M-type ESBLs [6]. Vaccination seems to be the only sensible prophylactic approach for controlling shigellosis. Unfortunately, there is still no safe and efficacious vaccine available. Outer membrane vesicles are among the promising candidates to be used for vaccination against shigellosis [7]. First-generation virG-based live attenuated Shigella strains have been successfully tested in phase I and II clinical trials and are a leading approach for Shigella vaccine development [8].

The prominent pathogenic feature of Shigella is its ability to invade a variety of host intestinal cells, including the enterocytes, macrophages and dendritic cells, which lead to severe inflammatory responses in intestinal tissue [9]. Intracellular Shigella movement is facilitated by directing host cell actin polymerization exclusively at one pole of the bacteria by a process known as actin-based motility. The force generated by the polymerizing actin is sufficient to propel Shigella through the cytoplasm and into neighboring cells [10]. All species of Shigella cause acute bloody diarrhea by invading and causing patchy destruction of the colonic epithelium. This leads to the formation of micro-ulcers and inflammatory exudates, and causes inflammatory cells (polymorphonuclear leucocytes, PMNs) and blood to appear in the stool. The diarrhoeal stool contains 10^6- 10^8shigellae per gram. Once excreted, the organism is very sensitive to environmental conditions and dies rapidly, especially when dried or exposed to direct sunlight.

Materials and Methods

Sample collection: For the isolation of Shigella organism, faecal samples were collected from various locations of Pantnagar. The sterlized sample collection containers were distributed to the residents of Pantnagar in the evening and the stool samples were obtained next morning. The sample collection process lasted for more than three months and in total 311 human stool samples, 100 cattle and 100 poultry faecal

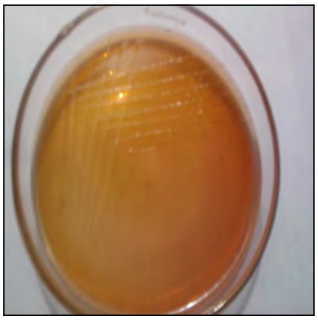

Fig-1. Plate showing colourless colonies of *Shigella* on MLA

Fig-2. Plate showing colourless colonies of *Shigella* on XLD

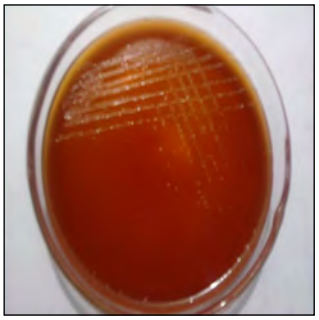

Fig-3. Plate showing colourless colonies of Shigella on SSA

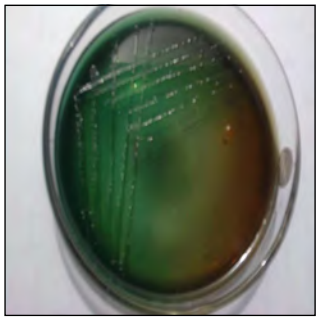

Fig-4. Plate showing green coloured colonies of *Shigella* on HEA

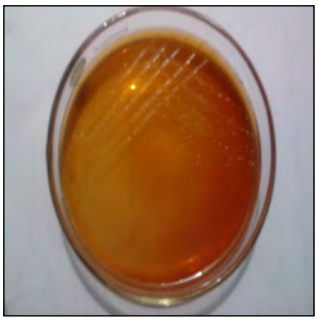

Fig-5. Plate showing colourless colonies of *Shigella* on DCA

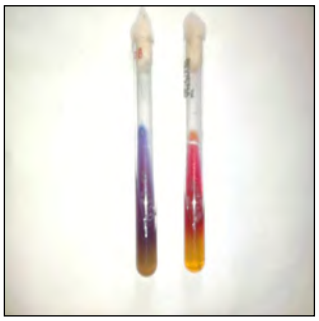

Fig-6. Culture tubes showing K/A reaction on LIA and TSI slants

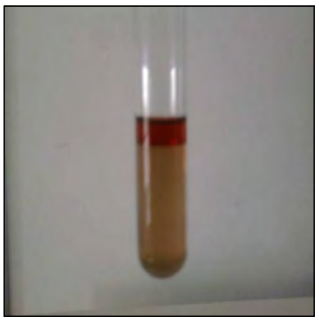

Fig-7. Development of red coloured ring in Indole Test

Fig-8. Development of red colour in MR Test

samples were collected. After collection, the samples were brought to Laboratory maintaining cold chain and processed on the same day for isolation of the *Shigella* organisms.

Media, chemicals and reagents: In order to isolate *Shigella species;* various bacteriological media, chemicals and reagents used in the present study were obtained from Hi-media, Difco and Sisco Reference Labaratory. All media used in the present study were prepared according to the instructions provided by the manufacturing firms and checked for sterility.

Cultural isolation of the organisms: Fecal specimens were processed according to published protocols. [11]. One loopful of faecal sample was streaked on Mac Conkey Lactose Agar (MLA) and Xylose Lysine Deoxycholate Agar (XLD) and incubated at 37°C for 24 h. The MLA plates showing the presence of convex and colourless colonies were considered for further identification. Similarly, the XLD plates showing the presence of translucent or red coloured colonies were considered for further identification. The colonies showing the desired morphology and colour were again re-streaked on the above media to obtain pure culture. Suspected colonies were re-streaked on other selective media i.e. Hektoen Enteric Agar (HEA), Salmonella-Shigella Agar (SSA) and Deoxycholate Citrate Agar (DCA).

Biochemical identification of isolates: Colonies showing characteristic appearance on selective media were sub-cultured on Kligler iron agar (KIA) and Triple sugar iron agar (TSI). To obtain true reactions in KIA and TSI, a pure culture was used to inoculate the respective media. Using straight platinum wire, one colony was picked up and inoculated into each of the test media. The KIA and TSI slants were inoculated by stabbing the butt and streaking the surface of the slant and incubating at 37°C for 24 h. Other biochemical tests, viz; MR, VP, Indole, Citrate, Urease and Motility were also conducted by inoculating putative isolates.

Results and Discussion

In the present investigation, as many as 511 samples comprising of human stool (311), cattle (100) and poultry (100) faecal samples collected from Pantnagar were examined for the presence of *Shigella*. The isolation of *Shigella* species was attempted from the collected samples by direct plating on differential (MLA and XLD) and selective (HEA, DCA and SSA) media without involving any enrichment medium. After inoculation of samples on the differential media (MLA and XLD) appearance of convex and colourless colonies (Fig.1-2) were suspected to be *Shigella* species and were selected for further streaking on selective media (HEA, DCA and SSA). The colonies exhibiting colourless appearance on DCA and SSA and green colour on HEA media. (Table-1; Fig.3-5) were selected for further identification and confirmation.

In the present study, no enrichment was used for the isolation of *Shigellae* considering the work of Mehlman I.J. *et.al*. [12] who reported that the use of enrichment for the isolation of shigellae were neither very specific nor sensitive. BAM (Bacteriological Analytical Manual) method also recorded that an

Table-1. Characteristic colony appearance on various culture media

Sr. No.	Media used	Colony appearance
1.	MLA	Convex, colourless colonies
2.	XLD	Colourless colonies
3.	HEA	Green colonies
4.	SSA	Colourless colonies
5.	DCA	Colourless colonies

Table-2. Characteristic colony appearance on various culture media

Test Media	Result
Urease test	Negative (No change in colour)
Mannitol motility test	Negative (No turbidity and no colour change)
Indole Test	Positive (Red ring Development)
Methyl Red Test	Positive (Red colour Development)
Voges-Proskauer Test	Negative (No pink colour Development)
Citrate Utilization Test	Negative (No growth and change in colour)

effective selective enrichment procedure for all *Shigellae* is still not available [13].

The suspected isolates of *Shigella* were primarily obtained by using MLA and XLD media, while SSA, DCA and HEA media were used for further confirmation. These findings were in agreement with the observations of [14] who recommended the parallel use of at least two plating media differing in selectivity. They suggested that XLD agar was the best culture medium for isolation of shigellae. XLD agar together with MacConkey agar was used for the detection of *Shigella* from stools in the present study. The use of MLA, XLD and SSA for the isolation of *Shigella* species was carried out elsewhere also [15].

The colonies suspected to be of *Shigella* species were subjected to biochemical tests which consisted of TSI, LIA, urease, methyl red, indole, Simmons Citrate, Voges-Proskauer and mannitol motility tests. The suspected cultures which were inoculated on TSI and LIA slants were observed for reaction after incubation for 24h at 37^0C. Alkaline slant and acidic butt without production of gas or H_2S on TSI and LIA slants was observed as shown in Fig 6.

Urease agar slant was inoculated with the suspected cultures and incubated at 37^OC for a duration upto 5 days for the appearance of colour change. No change in colour clearly indicated that the suspected organisms were urease negative. Similarly, neither clouding of the medium nor colour change was observed in the medium inoculated for mannitol motility test, indicating that the organism was non-motile and also mannitol was not fermentated.

All the isolates showed positive reactions in indole test as pink coloured ring was observed (Fig-7). Similarly, the appearance of red colour in MR test indicated a positive reaction (Fig-8). However, VP test was found to be negative with no pink colored colonies being observed. No growth as well as colour change was noticed when citrate utilization test was done indicating that the test organism were not able to utilize sodium citrate. The results depicting biochemical characterization of the isolates are shown in Table-2.

Conclusion

As many as 511 (311 human stool, 100 cattle and 100 poultry faecal) samples were collected from Pantnagar and nearby areas and examined for the presence of *Shigella* species. Out of 311 human stool samples analyzed, eight samples (2.57%) yielded *Shigella* isolates. All these 8 isolates belonged to human samples and none of the cattle (100) and poultry (100) faecal samples showed the presence of pathogen. The results obtained in the present study indicated that the presence of *Shigella flexneri* infection was limited to human beings only, as none of the cattle and poultry sample yielded *Shigella* organisms. Low prevalence of virulent *S flexneri* infection in human samples points towards the good hygienic practices being followed.

Authors' contribution

The present research work was carried out by AG during his post graduation under the guidance of SPS. The other authors also contributed equally. All authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interest.

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