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Isolation and identification of *Salmonella* serovars from clinical samples

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Abstract
This study was carried out to determine the prevalence, distribution, and identification of *Salmonella* serovars in diarrheagenic in different hospitals of Mahakaushal region. A total of 40 clinical samples were collected from typhoid patient of different hospitals like N. S. C. Bose Govt. Medical Hospital, Govt. Hospital Narsinghpur, Govt. Hospital Katni, Govt. Hospital Sagar, and Govt. H. G. Hospital located in Mahakaushal region. Out of these, only 16 samples showed the presence of *Salmonella* after screening with selective enrichment technique. In order to detect *Salmonella* strains, positive differential and selective biochemical tests such as H_2S production, gas production from glucose, citrate consumption and carbohydrate fermentation (including manitol, dolsitol, sorbitol, arabinose and glucose) and negative biochemical tests such as urease test, indole production and carbohydrate fermentation (including sucrose and lactose) were used. A total of nine Serovars of *Salmonella*e *S. typhi, S. cholerasius, S. houtnae, S. bongori, S. salmae, S. indica, S. typhimurium, S. arizonae, S. diarizonae* were identified. It was observed that *Salmonella* isolated from stool had developed simultaneous resistance to commonly used antibiotics. This has led to enormous morbidity in Mahakaushal region.

Keywords: Mahakaushal, serovars, *Salmonella,* typhoid

Introduction

The procedures used to identify *Salmonella* are laborious, time-consuming and require a number of biochemical and serological tests to confirm presumptive isolates. On the other hand, tests using molecular tools have been useful in reducing the steps and the time needed for the detection, identification and characterization of specific pathogens. They were demonstrates the utility of the PCR-RFLP technique for determining the serotypes of *Salmonella* (Miguel *et al*., 2008) [4]. DNA preparation protocol was rapid, sensitive, required minimal handling and reduced interference from produce associated inhibitors of real-time PCR. A rapid and simple DNA extraction procedure to detect *Salmonella* species and Listeria monocytogenes from fresh produce using real-time PCR (Dwarkin *et al.,* 2008) [1]. Samples of food cultures are collected on membrane filters and assayed by molecular hybridization to labeled probes. The probes consist of DNA sequences which are unique to the genus *Salmonella* and are widely distributed in the genus. Isolation and identification of *Salmonella typhi* in faeces involves the use of several types of culture medium as well as biochemical and serological testing (Ewing and Martin, 1974)[7]. A simplified method of detecting faecal *S. typhi* would be of value, especially when the presence of other intestinal pathogens is unlikely. Two such circumstances are the search for asymptomatic *S. typhi* carriers associated with sporadic cases of typhoid fever and the examination of stools from known typhoid carriers. Recently, bacteria in mixed culture have been identified by means of immuno precipitates that form about their colonies during growth on agar in which specific or cross reacting antiserum has been incorporated. This technique, termed the antiserum-agar technique for the detection of *S. typhi* in faeces (Nolon *et al*., 1980) [6].

Material and Methods

Sample Collection

A total of 40 clinical samples were collected from typhoid patient of different hospitals like N. S. C. Bose Govt. Medical Hospital, Govt. Hospital Narsinghpur, Govt. Hospital Katni, Govt. Hospital Sagar, and Govt. H. G. Hospital located in Mahakaushal region.

25 grams of stool samples were taken in 225 ml of buffered peptone water. The urine was collected in sterile vials whereas blood samples in EDTA containing tubes from typhoid positive patients by visiting different hospitals in Mahakaushal region.

Isolation of *Salmonella* species

The standard procedures (ISO-6579; 2002) followed for the isolation of *Salmonella* from clinical samples are described as under-

- Pre-enrichment in non-selective medium (buffered peptone water).
- Selective enrichment in Tetrathionate broth and Rappaport Vassiliadis soy peptone (RVS) broth.
- Sub-cultivation on Xylose Lysine Desoxycholate (XLD) agar.

1. Non selective pre-enrichment

Buffered peptone water is used as pre-enrichment medium.

Weigh out 25 gm stool sample, and put it into a sterile flask and add 225 ml of buffered peptone water to obtain 1 part sample + 9 part buffer. Mix it well and incubate at 37ºC for 18-24 hr.

2. Selective enrichment

For selective enrichment two media were used.

1. Tetrathionate broth and 2. Rappaport Vassiliadis soy (RVS) peptone broth

Transfer 1.0 ml of Pre-enrichment in 10 ml of Tetrathionate broth and incubate it at 37ºC±0.5ºC for 18-24 hr. Tetrathionate broth is a selective enrichment of *Salmonella.* It is inhibitory to *S. typhi, S. pullorum* and *S. gallinarum.*

Transfer 0.1 ml of Pre-enrichment culture medium in 10 ml of Rappaport Vassiliadis broth and incubate at 41.5ºC±0.5ºC for 18-24 hr.

3. Sub-cultivation on Xylose Lysine Desoxycholate (XLD) agar plates

Sodium desoxycholate is the selective agent and phenol red is the pH indicator. The indicative principle is based on lactose, sucrose and xylose fermentation, H_2S production and lysine decarboxylation. If H_2S is produced from sodium thiosulphate, black FeS (ferrosulfide) will develop. *Salmonella* ferments xylose, but not lactose and sucrose, decarboxy late lysine and produces H_2S. *Salmonella* suspect colonies grow as red colonies with a black center.

Take 10µl of inoculated and incubated selective enrichment (Rappaport Vassiliadis soy peptone broth and Tetrathionate broth) and spread it onto the XLD agar plate, incubate it at 37ºC for 18-24 hr.

Identification of *Salmonella* species

After isolation of clonies from XLD medium, in order to detect *Salmonella* strains, positive differential and selective biochemical tests such as H_2S production, gas production from glucose, citrate consumption and carbohydrate fermentation (including manitol, dolsitol, sorbitol, arabinose and glucose) and negative biochemical tests such as urease test, indole production and carbohydrate fermentation (including sucrose and lactose) were used. For this aim, bacteria were cultured in the nutrient broth medium at 37ºC for 18-24 hr and hence, their positive or negative states were distinguished according to phenotypic changes.

For the identification of *Salmonella* biochemical test were performed by using KB 011 Hi media kit.

Biochemical test

For biochemical test Hi *Salmonella* kit (KB O11 Hi Media, 2009-10) were used. These kits contain 12 different biochemical test media wells named as-Methyl red, Voges Prosuker, Urease, H_2S production, Citrate, Lysine, ONPG, Lactose, Arabinose, Maltose, Sorbitol and Dulcitol.

- For biochemical test firstly bacterial suspensions is prepared by transferring loop full of pure culture on Nutrient broth medium and incubate it for 18-24hrs at 37^0 C.
- In all wells, 50 µl bacterial suspensions is added and incubate the kit for 24hr at 37 ºC.
- After incubation in first well methyl red indicator and in second well beret reagent 1 and 2 are added.
- After 10-15 minutes the colour change in all 12 wells is noted as shown in figure I (a, b &

Results

Isolation of *Salmonella*

A total 40 clinical samples of blood, urine and stool were collected from 40 patients admitted during March, to April, 2014 in different hospitals of Mahakaushal region (Table 1). Out of these, only 16 samples showed the presence of *Salmonella*e after screening with selective enrichment technique.

Identification of *Salmonella* serovar

The biochemical tests for these 16 isolates were performed by using Hi media KB 011 kit. The test results are presented in Table 2 & 3. A total of 9 serovars of *Salmonella*e *S. typhi, S. cholerasius, S. houtnae, S. bongori, S. salmae, S. indica, S. typhimurium, S. arizonae, S. diarizonae* were identified.

A basic set of six biochemical tests (Indol, urease, hydrogen sulphide production, acetoin, ornithine decarboxylase and β-galactosidase) were selected from an original set of fourteen tests and found to give more than 97% accurate reactions within 18 h of incubation for the identification of bacteria belonging to Enterobacteriaceae. This set permits identification on the genus and species level for *Escherichia, Shigella, Salmonella, Edwardsiella, Citrobacter* and *Proteus*. For the *Klebsiella-Enterobacter-Serratia* group, where a species diagonsis was considered desirable, eight additional tests (Esculin, Raffinose, Sorbitol, Malonate, Citrate, Lysine decarboxylase, Deoxyribonuclease and Motility) permitting identification within 18 to 72 h were added. The use of this two-step test set for the identification of almost 6000 strains isolated from clinical specimens during an eight month period is reported (Lindberg *et al*., 2005) [3]. In 1993 Munoz *et al*., developed DNA-DNA hybridization test identify *Salmonella* in food. This test requires an initial pre-enrichment of food samples in nutrient broth but does not require selective enrichment.

Table: 1: Number of clinical samples collected from different hospitals

S. No.	Month	March			April			Total Sample
	Place of collection	Blood	Urine	Stool	Blood	Urine	Stool	
1.	N. S. C. Bose Govt. Medical Hospital	2	1	2	1	1	3	10
2.	Govt. Hospital Narsinghpur	2	0	1	2	1	1	7
3.	Govt. Hospital Katni	1	1	2	2	1	1	8
4.	Govt. Hospital Sagar	2	1	1	1	0	2	7
5.	Govt. H. G. Hospital	1	0	3	1	1	2	8

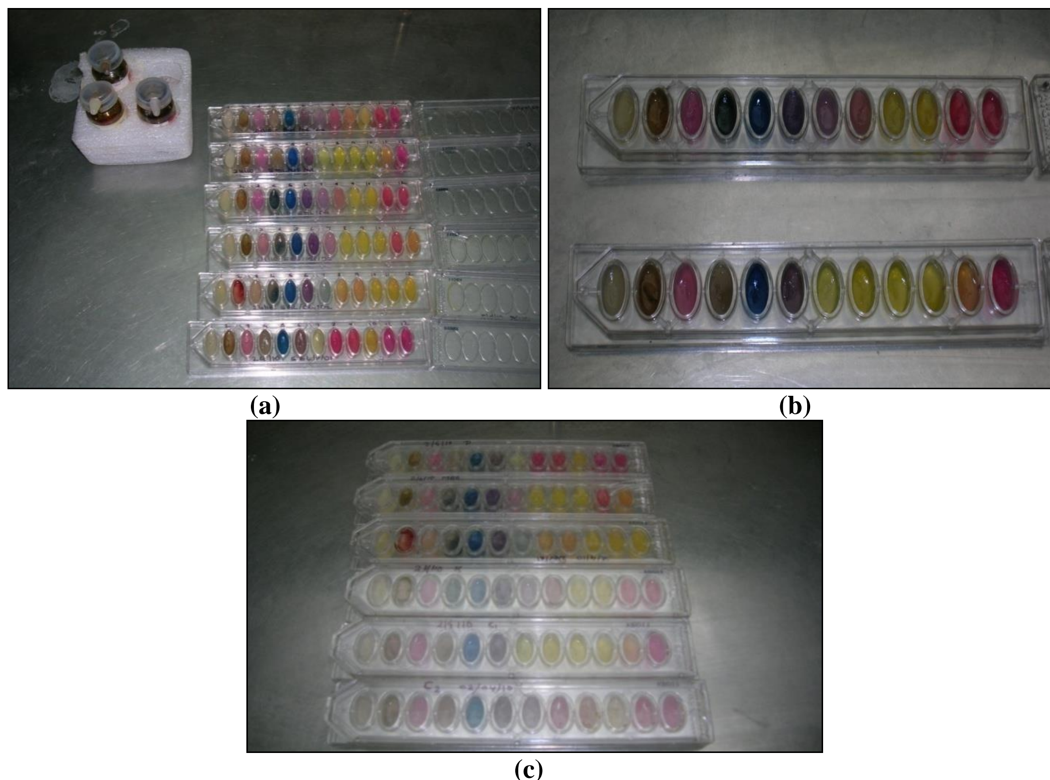
Table 2: Biochemical tests of *Salmonella* serovars

Name of the test	EBL S1	EBL S2	EBL S3	EBL S4	EBL S5	EBL S6	EBL S7	EBL S8
MR	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-
H_2S Production	+	+	+	+	+	+	+	+
Citrate	-	+	+	-	-	+	+	-
Lysine	+	+	+	+	+	+	+	+
ONPG	-	-	-	-	-	+	V	-
Lactose	-	-	-	-	-	-	-	-
Arabinose	-	+	+	-	-	+	+	-
Maltose	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Dulcitol	-	+	-	-	-	-	+	-
Name of Serovars	*S. typhi*	*S. cholerasius*	*S. houtenae*	*S. typhi*	*S. typhi*	*S. bongori*	*S. salmae*	*S. typhus*

Table: 3: Biochemical tests of *Salmonella* serovars

Name of the test	EBL S9	EBL S10	EBL S11	EBL S12	EBL S13	EBL S14	EBL S15	EBL S16
MR	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-
H_2S Production	+	+	+	+	+	+	+	+
Citrate	-	V	+	+	-	+	+	+
Lysine	+	+	+	+	+	+	+	+
ONPG	-	V	-	+	-	+	+	+
Lactose	-	V	-	V	-	-	V	V
Arabinose	-	+	+	+	-	+	+	+
Maltose	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Dulcitol	-	V	V	-	-	-	-	-
Name of Serovars	*S. typhi*	*S. indica*	*S. typhimurium*	*S. arizonae*	*S. typhi*	*S. bongori*	*S. arizonae*	*S. diarizonae*

+ = positive, - = negative, V = variable
Samples = EBL S1 – EBL S16



(a) **(b)** **(c)**

Fig 1: (a, b & c) Photographs showing biochemical test of different isolates by Hi Media KB 011 Hi *Salmonella* kit.

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