

Identification and Characterization of *Salmonella typhi* Infection from Warangal and Adjoining Districts Based on Conventional and Rapid Methods

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(Received: March 03, 2012; Accepted: April 15, 2012)

ABSTRACT

Salmonella infections are highly transmitting due to uptake of contaminated food, water, and poor hygienic conditions. The severity of salmonella diseases (typhoid) increasing day to day life. The *Salmonella typhi* is major creature playing crucial role in distribution of typhoid fever. The virulent antigens of *S typhi* are responsible for generation of typhoid. The present assessment consists of series of biochemical and immunological examinations of concerned organism to know the rate of infections in individuals including male and female. The observations made by captivating age, sex, and habit of individuals in to considerations. The preliminary work is carried out by compiling the blood and urine samples from patients, subjected to Widal test for confirmation and urine sample is used for culturing of salmonella and blood samples used for immunological tests. On culturing of 50 urine samples, 40 isolates were showing salmonella species and remaining 10 isolates were recognized as *E. coli* species. Identification and differentiation was done based on staining, motility and biochemical tests. Immunological tests were mainly processed to know the antigen reactivity.

Keywords: *Salmonella typhi*, Typhoid, Antigens, Biochemical and immunological examinations. Widal test, Blood and Urine samples.

INTRODUCTION

Salmonella is a genus of Gram-negative rod-shaped bacteria of the family Enterobacteriaceae. They cause a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia. Gastroenteritis associated with food-borne outbreaks is probably the most common clinical manifestation of the infection¹⁻⁵. The genus salmonella includes a large of pathogens of human beings as well as other mammals which are antigenetically related to one other. The salmonella typhi is a gram negative bacilli which are nonacid fast, non-capsulated and nonsporulating⁶. Cell size rises from 2-4 μm x 0.6 μm . The principal habitat of the salmonellae is the intestinal tract of humans and animals. Salmonella serovars can be found predominantly in one

particular host can be ubiquitous, or can have an unknown habitat⁷. Salmonella infections in humans vary with the serovars, the infectious dose, the nature of contaminated food, and the host status. Certain serovars are highly pathogenic for humans⁸. Infants, immunosuppressed patients, and those affected with blood disease are more susceptible to salmonella infection than healthy adults⁹. In the pathogenesis of typhoid the bacteria enter the human digestive tract, penetrate the intestinal mucosa, and are stopped in the mesenteric lymph nodes (10). The bacterial cell multiplication occurs, and part of the bacterial population lyses, from the mesenteric lymph nodes, viable bacteria and LPS (endotoxin) released in to blood stream resulting in septicemia release of endotoxin is responsible for cardiovascular collapse and typhoid due to the action on the

ventriculus neurovegetative centers¹¹. The incidence of food borne salmonella infection remains relatively high developed countries because of commercially prepared food or ingredients for food. Any contamination of commercially prepared food will result in a large scale infection. In under developed countries, food borne salmonella intoxications are less spectacular number of individuals infected¹². While individuals may contact salmonella food poisoning from contaminated foods, the diseases proves most threatening infants, the elderly, and individuals with weakened with immune systems¹³. Salmonella is also involved in contamination of all kinds of meat where proper canning is not performed; it causes salmonellosis, when contaminated meat is consumed by the individuals¹⁴.

MATERIAL AND METHODS

The methodology comprises of collection of urine and blood samples, which were widal positive. In Most of the patients Salmonella H antigens mainly presented. The collected urine samples were subjected for culture and biochemical tests. Urine samples were inoculated onto the nutrient media and incubated at 37 C for 12to 24hrs observed for growth on the medium based on the growth and morphological appearance confirmatory test were performed

Biochemical test like IMVIC tests were performed to know the presence of salmonella in collected samples. Other specific media was used to conform and antimicrobial activity was performed to see the drug resistance patterns in collected isolates. Indole test was performed by inoculating the experimental organism in to tryptophan broth by adding kovacs reagent, resulting the formation of cherry red coloured compound, it indicates the liberation of Indole (10) (Fisher Scientific Ltd, Mumbai, 07/2009). Methyl red and voges proskauer tests are performed simultaneously on the same medium by inoculating salmonella strain in to the MR- VP broth and incubated at 35°C for 48 hrs, at the end of incubation period, 1-2 drops of Methyl red indicator (Sd fine Chem ltd, 07/2008), is added to the broth, allowed to the completion of reaction 15-30 minutes by exposing to the oxygen (10). Citrate test is processed by preparing the Simmons

citrate agar medium (FinarchemLtd, 10/2007), inoculating the bacterial culture in to agar slants, incubated for 48 hours at 37°C (10) (Fig IV).

H₂S production test is performed. Preparing SIM agar deep tubes and organism is inoculated and incubated at 25°C for 48 hours, after incubation period, observation of tubes taken place for colour change along the stab. Some of the lactose fermentation tests, glucose fermentation tests also performed to 50 collected samples. This methodology is includes only urine samples, little amount of urine sample is inoculated in peptone water and used for biochemical analysis. After these tests

Sample placed on MacConkey (Fisher Scientific Ltd, Mumbai, 07/2009) EMB Agar (Merck specialities Ltd, Mumbai 07/ 2009) Medias to study the colony characters. The biochemical tests were performed to confirm the salmonella typhi infections. Samples were diluted and/or homogenized in TSB medium, and isolates obtained by Salmonella selective enrichment in Rappaport–Vassiliadis (RV)¹¹ medium after 24 h incubation at 43°C. Isolates were grown on Xylose–Lysine–Deoxycholate (XLD) medium, for isolation of enteric pathogens¹². Media included a H₂S indicator, and incubation was extended to 48h to increase visibility of H₂S production. Presumptive Salmonella isolates were grown in Selenite–Cystine¹³ broth at 37°C for 24 h. Salmonella– Shigella (SS) agar plates were inoculated from positive orange tubes and incubated at 37°C for 24–48 h. Colorless nonlactose- fermenter colonies were used to inoculate Kligler’s iron agar tubes¹⁴ and incubated at 37°C for 24 h. Alkaline/ acid, H₂S, with/without gas isolates were tested on Christensen’s urea agar¹⁵ for the detection of urease activity after 24 h incubation at 37°C. Differentiation between Salmonella subspecies was made using biochemical tests. The serotyping of Salmonella strains followed the Kauffmann–White scheme ([16]. Dispens- O-Disc, susceptibility test system (Difco Laboratories) was used to observe antibiotic resistance. All strains were preserved in Luria–Bertani broth amended with 15% glycerol and stored at –70°C. Further immunological tests performed mainly to know the antigen reactivity. For this slide agglutination and latex agglutination tests were processed, blood serum sample which were

isolated from the typhoid patients were used for these purpose.

Immunological tests

Immunological tests essentially performed by agglutination tests, by using latex agglutination tests (Medsorce Biochemicals Ltd, Delhi, 09/2008) H antigen reactivity determined. All the 50 blood specimens collected from patients and processed in our microbiology laboratory. 70 days period were included in the bacteriological study. These were derived from patients with various symptoms such as enteric fever, septicemia, meningitis, pyrexia and other conditions. 5 ml of Blood sample from each patient was inoculated in to 80 ml of Brain heart infusion broth and kept in open environment; 5 ml of blood inoculated in another brain heart infusion broth and maintained anaerobic condition. After 1 day, culture broth was gram stained by making a loop full of smear and subcultures were made on blood and Macconkey agar. Any growths of subcultures were made on days 2 and 5 other days when broth become turbid¹⁷. Latex agglutination was carried out on these broths showed the presence of salmonella antigens. For the antigen detection by latex agglutination includes various amounts of Salmonella LPS (0- 0.8ug/ml), 0.1 M Glycine (Merck specialities Ltd, Mumbai 09/ 2009) - 0.9% NaCl (Sd fineChem Ltd,07/2008), buffer containing 0.1% Tween 20(Merck specialities Ltd, Mumbai 07/ 2009) and 1% serum- albumin, normal urine obtained from healthy adults, human sera

obtained from patients suffering from typhal infections¹⁸. 1% of latex particles was sensitized in 0.1 M glycine- 0.9 sodium chloride buffer (pH 8.2) , with an equal volume of Salmonella H monoclonal Ig. The antibodies were obtained from mouse ascities fluid that was partially purified by precipitation with cold 50% saturated Ammonium sulphate and subsequently by cryoprecipitation (19). The latex antibody mixture was incubated at room temperature for 2hour on a mixture, after which 1% bovine serum albumin was added, and incubation continued for another hour. The latex particles were then washed in 0.1 M Glycine- 0.9% NaCl buffer and finally suspended in a 1% bovine serum albumin and 0.2% sodium azide (Merck specialities Ltd, Mumbai 07/ 2009), kept incubation for 1-3 hours with shaking continuous latex at room temperature or without shaking at 37°C at the end of the incubation at 4°C for 2 hrs, the tubes were examined for agglutination²⁰. This was indicated by a presence of agglutinated material at the bottom of tube. The latex particle concentration was 0.01% µg of LPS. Widal test is also performed to know the dilutions. (Figure V)

RESULTS AND DISCUSSION

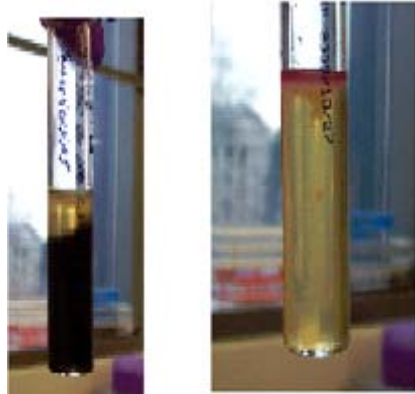
Samples were collected from the patients who had attended to different clinics of Warangal and its adjoining districts hospitals how had complains with fever and chills from past three days there blood and urine samples were collected for

Table 1: Biochemical tests for patients urine sample

S. No	Culture	Liquid culture	Indole test	Methyl Red Test	Vi Test	Citrate Test	H2S production Test
1	Clearly in White	Turbidity low	-	+	-	-	+
2	Light yellow	well	-	+	-	-	+
3	white	well	-	+	-	-	+
4	Thick yellow	well	-	+	-	-	+
5	white	low	-	+	-	-	+
6	White	high	-	+	-	-	+
7	Light yellow	high	-	+	-	-	+
8	yellow	high	-	+	-	-	+
9	Thickyellow	high	-	+	-	-	+
10	Light yellow	high	-	+	-	-	+

Bio chemical Tests for *Salmonella typhi*

Indole test for salmonella typhi



Negative

Positive

Fig. 1: Indole test



Fig. 2: Voges prauskar test

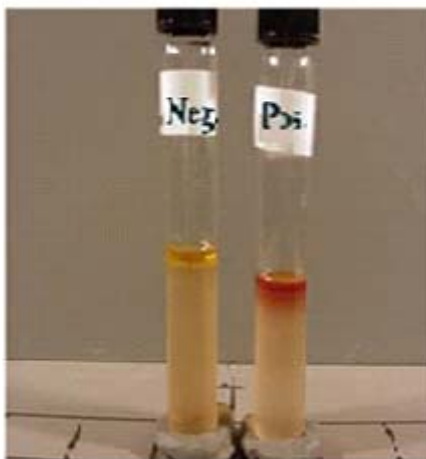


Fig. 3: Methyl red test



Negative

Positive

Fig. 4: Citrate test



Fig. 5: Latex agglutination tube

diagnosis of typhoid. All the blood samples were subjected for widal test all the 50 blood samples were shown positive for widal test there titer were varied from 60 to 120 ratio. All the 50 patients shown positive for widal, urine sample were collected and subjected for culture on Nutrient medium and incubated the plates for 24hrs at 36c in an incubator based on the growth and morphology on culture plates this colonines were transferred to Nutrient broth and performed different microbial tests like staining, motility, biochemical, selected media for bacterial identification. By Gramstaining all the isolated obtained during growth were grains negative and motile bacteria. Out of 50 bacterial

isolates 40 isolates were showed Indole negative, methyl red, voges prauskar and H₂S positive. Based on these biochemical examinations it observed that all the 40 isolates were representing the Salmonella species. All the 40 isolate were subjected on the selected media XLD, MacConkey and blood agar media, growth on the above mentioned Medias were representing the salmonella morphology like salmonella. Remaining 10 samples showed the Positive results for Indole and citrate tests, it indicates the presence of *E. coli* in typhoid patients.

Biochemical tests (Table I). Remaining 30 samples showing similar results for biochemical tests, only they differ in colony appearance and liquid culture turbidity. In latex agglutination test, out 50 samples, 9 blood sample were analyzed and showing more agglutination at LPS 400 ng/ml. Widal test dilutions for antigen H, antigen O found 1:80 and 1:160.

CONCLUSION

These biochemical and immunological

examinations were mainly conducted to know the people suffering with typhoid infection in Warangal and Karimnagar region, where located in Andhra Pradesh, India. The further work is conducted to isolation of antigens from salmonella typhi and their purification by different molecular techniques.

ACKNOWLEDGEMENTS

The implementation and success of this project could not have been possible without the patient guidance of J.MALATI, Department of Microbiology, Shivani Pharmacy College, Warangal. We are indebted to Sri Dr.Singaracharya, Head of Microbiology Department, Kakatiya University, Warangal for the full-fledged cooperation. We express our profound gratitude to Principal of Shivani Pharmacy College, for providing excellent lab facility. It's our privilege to keep on record our deep sense of gratitude and warm regards to all the faculty members for their encouragement. We are also very thankful to district hospitals of Karimnagar and Warangal for providing urine and blood cultures of patients.

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