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Analysis of Outer Membrane Protein of *Salmonellatyphi* Isolates from Drinking Water

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***Abstract*— Drinking water is most essential for life. The *Salmonella* species from different drinking water samples at different areas of Chennai are collected. These are found major in the roadside hotels drinking water. The current study were undertaken to isolate the *Salmonella* species, protein profiles of isolate, isolation of outer membrane protein and to compare the outer membrane protein among the isolates. The band formation of compared protein was found to be in between 43 to 66 KD.**

***Index Terms*— drinking water, Salmonella, outer membrane, protein.**

I. INTRODUCTION

Salmonella are typical members of the *Enterobacteriaceace* consist of *bacilli* that parasites the intestine of a large number of vertebrate species and infect human beings, leading to enteric fever gastroenteritis, septicemia with or without fecal suppuration and the carrier state. It is a gram-negative rod about 1-3 to 0.05-0.8 M in size and motile with peritrichous flagella. Somatic 'O' Antigen 'Vi' surface Antigen 'H' Antigen is present in flagella. It is a heat labile protein. When mixed with specific antisera it agglutinates rapidly producing loose fluffy clumps. This is used for serological identification. 'O' Antigen is the capsular phospholipids protein polysaccharide complex present in cell wall. This antigen is common for all the species of *Salmonella* genus. 'Vi' surface Antigen was isolated strains of Typhoid *bacilli* form Vi antigen as a covering layer outside their cell wall. This antigen is an acidic polysaccharide. When fully developed it renders the bacteria agglutinable by Vi antibody and inagglutinably by O antibody. **Incubation Period**- incubation period 7 to 10 days, Primary bacteraemia, Secondary bacteraemia. **Onset-** The interval between ingestion of the organism and the onset of illness varies with the size of the infecting dose. It can be as short as 3 days or as long as 50 days, but is usually about two weeks. The onset is usually insidious. **Clinical Syndrome** – Although *Salmonella* can cause a wide spectrum of clinical illness there are four major syndromes, each with its own diagnostic and therapeutic problems, which are best considered separately as shown in figure 1. They are Enteric fever, Gastero-enteritis, Bacteraemiawith or without Metastatic infection, and the asymptomatic carriers. Rapid detection and identification of the etiological agent, *Salmonella typhi* is essential in diagnosis and for treatment to reduce morbidity and mortality. The definitive diagnosis of the disease requires the isolation of *Salmonella typhi* from the blood, feces, urine or other body fluids. Blood culture is generally recognized as the best procedure for definitive diagnosis of early typhoid fever. Positively is generally obtained in about 45-50 percent patients even in well- equipped laboratories.

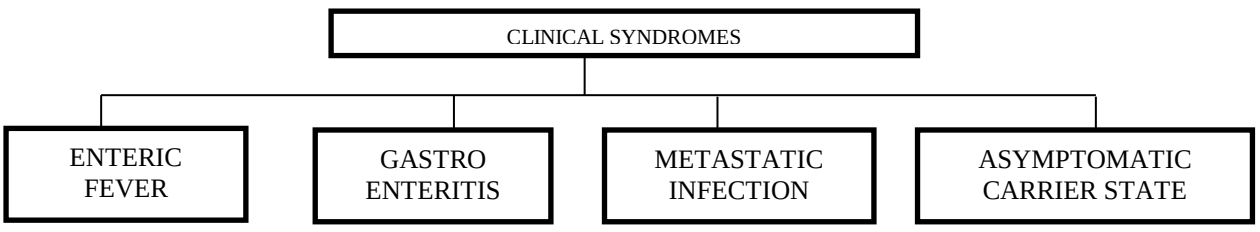

Fig.1 Clinical Syndrome

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II. IMPLEMENTATION

The implementation is explained in step by process as follows:

Step 1: Collection of Sample

The Hotel drinking water of 25 samples collected around Chennai. The water samples are collected in a sterile container and transported to the laboratory for the further process.

Step 2: Isolation and Identification of *Salmonella species* for water samples

A. Streaking on various selective media

Selective media facilitate the isolation of particular species from mixed inoculums. The Enriched samples from the selenite-F-Broth were streaked on to the selective media.

B. *Salmonella-Shigella* Agar(SSA)

Salmonella-Shigella agar is one of the selective media for the isolation of enteric pathogens like *Salmonella* and *Shigella* from water sample. Medium contains high concentration of bile salts, sodium citrate and brilliant green inhibits gram positive and any gram negative coliforms organism. Lactose is the sole carbon source and neutral red is the pH indicator. Sodium trio sulphate is sulfur source.H_2S production is shown by the formation of black precipitate with ferric citrate.

C. Deoxycholate Citrate Agar (DCA)

It is selective media for *Salmonella* and *Shigella species*. It produce colorless colony with black centered for *SalmonellaSpecies* and without black centered colonies for *Shigella species*.

Step 3: Testing

I. Preliminary Test

Preliminary test was performed on the next day by inoculating the culture from the selective media to the peptone water. Inoculated peptone water was incubated at 37^0C for

6-9hrs.

A. Gram's staining

This technique was introduced in 1884 by Christian Gram, a Danish scientist.

The organisms on culture were stained using gram's staining method. This technique is used to distinguish between grams positive and grams negative organism.

- A Thin smear was made on the grease free slide
- Slide was flooded with crystal violet and allowed to stand for one minute and washed.
- Then flooded wit gram's iodine for one minute and washed.
- Then decolorized with 95% of ethanol and wash immediately.
- Counter stained Safranine was allowed to stand for one minute.
- Slider was dried and observed under microscope.

B. Motility

The hanging drop technique was followed to observe motility of the bacteria with which they are classified as motile and non-motile organism.

- A drop of culture was placed on the cover slip.
- Then invert the cavity slide over the cover slip.
- Life the slide careful and gently.
- Edge of the drop was focused and observed for motility.

C. Oxidase Test

Oxidase is an enzyme possessed by some bacteria, which forms the part of the electron transport system. It oxidizes the reagent tetra methyl Para phenyl diamine dihydrochloride to colored product indophenol.

- Oxideas disc was placed on a clean glass slide which is placed in the petriplate.
- With the help of a clean applicator stick test, positive and negative controls were placed from the 24 hrs culture plate.
- Color change was observed.

D. Catalase Test

Some organisms possess the enzyme catalase that splits hydrogen peroxide into oxygen and water. When small amount of organism is introduced into H_2O_2 rapid elaboration of oxygen bubbles.

- A clean glass slide was placed in the petri dish.
- Saline suspension of test, positive and negative controls were made.
- Immediately drop of 3% hydrogen peroxide was added using the dropper.
- To all three suspension each.

II. Biochemical Reactions

A. Indole Test

Some bacteria contain tryptohanase, which act upon the tryptophan present in the peptone water and convert into indole and indole acetic acid and this indole reacts with the aldehydes in kovac's reagents to form a cherry red colored ring.

Peptone broth was prepared and distributed 2 ml in sterile tubes.

- Autoclaved at 121^0C for 15min.
- Then the culture was inoculated and incubated at 37^0C for overnight.
- Few drops of Kovac's reagent was added which forms a red colored.
- Ring at the top.

B. Methyl Red Test

Organism belonging to the family *Enterobacteriaceae* ferment Glucose to produce mixed acid such as acetic lactic, succinic and formic acids. As a result of which the indicators like Methyl Red detect the final product.

- MR-VP broth was prepared 2ml was dispensed in sterile tubes.
- The tube was sterilized, and the culture was inoculated.

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- The tubes were incubated overnight at 370C.
- Few drops of Methyl Red reagent were added, Color Change was observed.

C. Voges Proskauer Test

Some of *Enterobacteriaceae* produce 2,3 –butylene glycol and acetoin by the termination of glucose of other carbohydrates. These products are more neutral in nature and not much drop in the pH is noticed. The end products are detected by the addition of VP Reagents.

- MR-VP both was prepared dispensed in tubes was sterilized.
- The culture was inoculated and incubated overnight at 37^0C
- Then 0.2ml of 5% LPH-napthol and 0.2ml of 40% potassium hydroxide were added.
- The color change was noticed.

D. Citrate Utilization Test

Certain organisms utilize citrate as a sole source of carbon producing acetate and other alkaline carbonates which in turn changes the color of indicator eye bromothymol blue from its original green color to blue.

- Citrate agar was prepared, dispensed in tubes and then sterilized.
- Later slants were prepared by allowing it to solidify in a slanting position.
- The organism was inoculated and incubated overnight at 37^0C.
- Color change was observed.

E. Triple Sugar Iron Agar

Some bacteria liberate sulphur from sulphur containing amino acids or other sulphur containing compounds. The sulphur is used as final hydrogen acceptor leading to formation of hydrogen sulphide, which can be detected in the TSI medium.

- TSI medium was prepared, dispensed in tubes and then sterilized.
- Later slants were prepared with 1 inch butt.
- Single colony was picked up and stabed down the center of the agar butt.
- And then streaked on the surface of the slant.
- Inoculated tubes were incubated overnight at 37^0C.

F. Sugar Fermentation Test

Organism utilize various sugars for there growth and carry out sugar fermentation. Sugar fermentation can be visualized by the color change in the indicator bromocresol purple because of acid production. Gas production is visualized by bubble formation inside the Durham's tube.

- 90ml of sterile peptone water was prepared.
- 1gm of sugar was weight and dissolved in 10ml of distilled water.
- This 0.1ml of sugar solutions was added to 90ml of prepared peptone water.
- The 0.1ml bromocresol purple indicator dye was added.
- This sugar solution was dispensed in tubes.
- Durham's tubes were placed and then sterilized.
- Later cultures were inoculated and incubated overnight at 37^0C

Both acid and gas production and noticed.

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III. RESULT

In the present study, about 25 drinking water samples were process for examination of *Salmonella typhi* isolate of those six samples yield positive result for *Salmonella typhi*. The isolate were identified by biochemical test and characteristic growth on selective media(table 1,2 and 3, Plate-Ia,b,Plate-II c,d, Plate-III e,f,g).

Protein profile of S*almonella typhi* isolate

Salmonella typhi isolate that isolated from drinking water was further studied on molecular level to find out heterogeneity among the isolate based on the protein profile about 24 percentage (table –III) of drinking water samples collected in Chennai were found to be positive for the presence of *Salmonella typhi* isolate the outer membrane protein of *Salmonella typhi* was studied by SDS phage and compared with standard molecular marker. The protein patterns in SDS phage showed variations on among the isolates and all protein bands were detected at the range of 43 to 66KD (plate-IV,h).

TABLE-I

Cultural Characteristics of the *Salmonella* isolate in Various Medias

Media	Colony formation
Salmonella Shigella agar(SS)	Black centered colonies
Deoxycholate citrate agar (DCA)	Colorless, smooth, shiny, Translucent colonies

TABLE-2

Prevalence of *Salmonella typhi* in Water Samples of

Total number of sample used	Total number of positive for *Salmonella typhi*	Prevalence of *Salmonella typhi* in percentage
25	6	24%

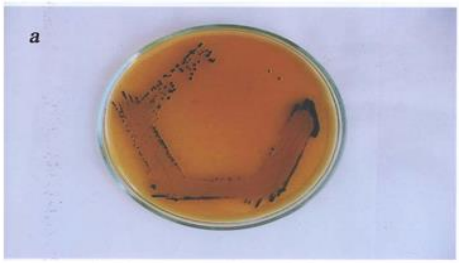
TABLE 3

Shows Bio chemical characters of *Salmonella typhi*

Isolate	Preliminary Test				Biochemical Test									
					Sugar fermentation				IMVIC					
S.typhi	G	M	C	O	G	L	MAL	S	I	MR	VP	C	TSI	U
	-ve rod	+	+	-	+	-	+	-	-	+	-	-	AK/AH_2S	-

G-Gram Staining, M- Motility, C-Catalase, O-Oxidase, G-Glucose, L-Lactose, MAL- Maltose,, MAN-Mannitol, S-Sucrose, I-Indole, C-Citrate Utilization, TSI- Triple Sugar iron, U-Urease.

Plate - I



Growth of *Salmonella typhi* on SSagar

Growth of *Salmonella typhi* on DCA

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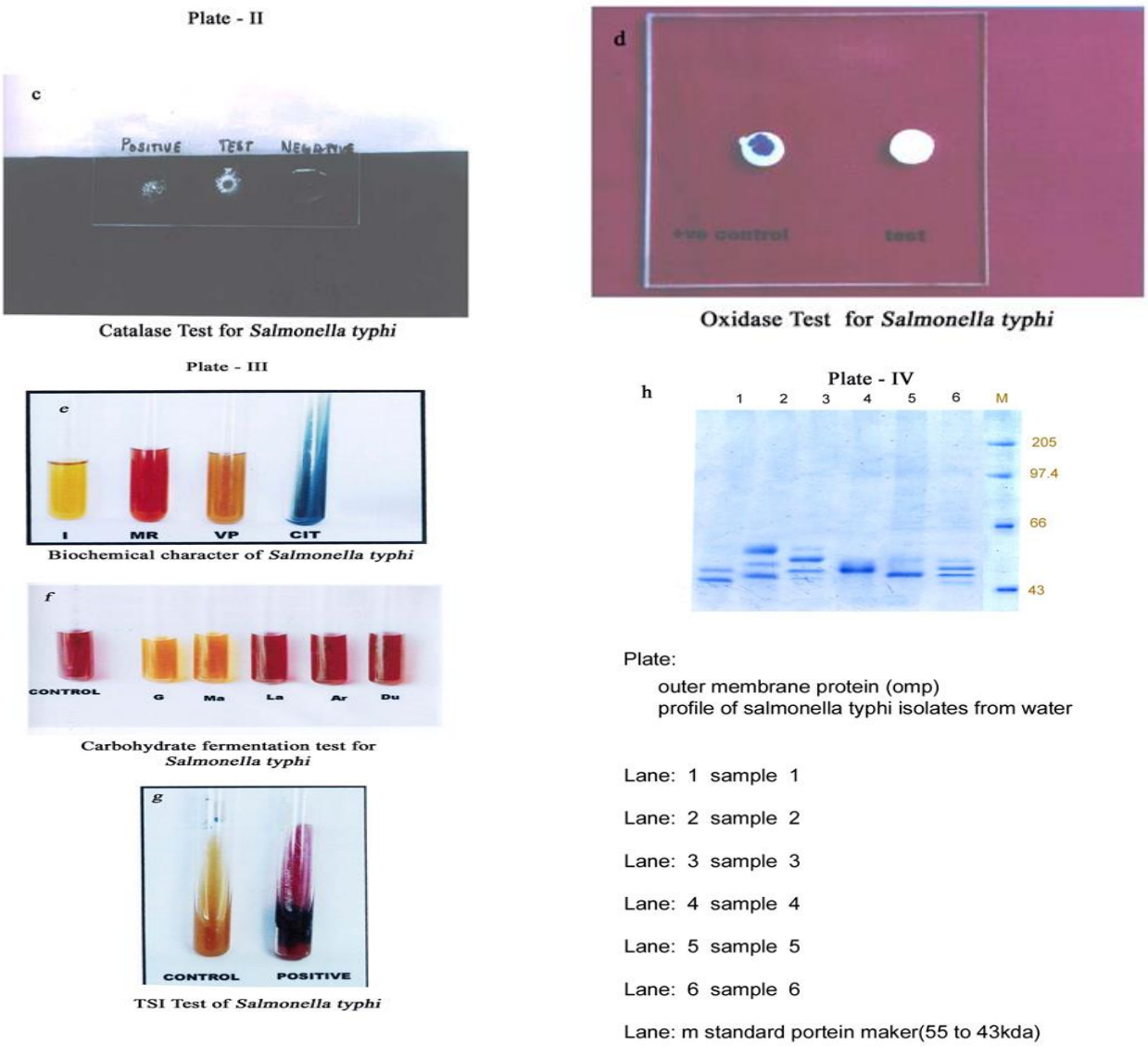

Fig.2 Plates with growth and test analysis.

IV. CONCLUSION

Drinking water sample were collected from different areas of Chennai. A total of 25 samples were collected and in it six samples were showing the presence of *Salmonella typhi.* These samples, which were collected, were plated in the nutrient agar plates. Then the colonies formed in the plates were again streaked in the selective media. The incidence of *Salmonella typhi* was found mostly in the roadside hotels drinking water. The plates showed colony formation in the selective media were isolated their biochemical characteristics were studied. The biochemical tests showed the presence of *Salmonella typhi* in seven plates. The isolates were then inoculated in brain heart infusion agar and then outer membrane protein isolated from isolate. *Salmonella* isolate can cause serious hazards to public health. To create awareness and improve hygiene status the present study was carried out. For further molecular study of outer membrane protein of *Salmonella typhi* isolates. SDS phage was done and the band formation was observed. The band formation was compared with the standard marker and the band of outer membrane

protein was found to be in between 43 to 66 KD. This confirms that the outer membrane protein of *Salmonella typhi* has only one type of protein in them. All the six samples showed their bands in the similar pattern.

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