STILL SMOULDERING: ENTERIC FEVERMandira Chakraborty¹, Indrani Bhattacharyya², Palash Das³, Dipankar Paul⁴, Sangeeta Das Ghosh⁵**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: We have isolated Salmonella Typhi from blood culture of ten cases of Enteric fever within a period of three months at Calcutta School of Tropical Medicine. The individual cases were from different geographical areas. Case 2, Case 5 and case 7 were from Dumdum. Isolation of ten cases within such a short period of three months between March, April & May 2014 prompted us to report them.

KEYWORDS: Enteric Fever, Blood Culture, Fever, Hepatomegaly, Salmonella Typhi.

CASE 1: The patient was a 20 year old young man with a history of diarrhoea for 17days and dysphagia for 21 days. He was admitted with a history of high non-remitting fever for the last 3 days. He was diagnosed to be HIV positive 1 year back. He had a history of disseminated Koch's for which he had received a full course of ATT Drugs. Now he is on ART (Tenofovir, Lamivudine and Efavirenz). On examination he had 102°F fever and hepatosplenomegaly with oral candidiasis. Stool examination for OPC was negative. LFT showed increased SGOT with no other abnormality. Blood culture was advised.

CASE 2: A 32year old lady was admitted with history of gradually increasing fever for 22 days. Over the last 4 days the increase in fever was almost in a classical step ladder pattern. On examination she had 103°F temperature. Her abdomen was diffusely tender but there was no organomegaly. LFT was normal; USG upper abdomen was normal. Blood culture was advised.

CASE 3: A 56 year old male was admitted with history of high spikes of fever with chill and rigor for last 5 days and loose motion for the last 2 days. On examination he was found to have fever and mild anemia. Hepatomegaly was noted. Blood for malaria parasite was negative. LFT was normal, USG showed hepatomegaly. Blood culture was advised.

CASE 4: A 17 year old boy was admitted with history of fever, anorexia and lethargy for 14 days. For the last 3 days he was having high grade fever. On examination he was found to have fever 103 °F with hepatosplenomegaly. LFT was normal; USG showed hepatosplenomegaly. Blood culture was advised.

CASE 5: A 40 year old male was admitted with history of high spikes of fever for 4 days with chill and rigor, vomiting for 3 days and loose motion for 2 days followed by constipation. Patient was not immunocompromised. On examination mild anemia with fever 100 °F was found and there was no organomegaly. LFT was normal; USG was normal; CRP 44.7 IU. Blood for malaria parasite was negative. Blood culture was advised.

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CASE 6: A 14 year old school going boy was admitted with a low grade of fever for 7 days followed by high spikes with chill and rigor for last 3 days along with loose motion for last 2 days. On examination, he was found to have fever. There was no organomegaly but diffuse abdominal tenderness was found. LFT was normal. USG was normal. No malaria parasite was found. Blood culture was advised.

CASE 7: A 13 year old girl presented with high grade intermittent fever for 18 days along with loose motion and vomiting for last 10 days. On examination, she had fever with epigastric tenderness. Liver was 2 cm palpable. LFT was normal. USG was normal. No malaria parasite was found. Blood culture was advised. While taking history, we found that her mother and brother also suffered from Enteric fever 1 month back for which they were treated and cured.

CASE 8: A 42 year old lady was admitted with history of high spikes of fever for 5 days with chill and rigor, vomiting and loose motion for 3 days. Her abdomen was diffusely tender but there was no organomegaly. LFT was normal. USG upper abdomen was normal. Blood culture was advised.

CASE 9: A 71 year old lady was admitted with anorexia and lethargy for 10 days. For the last 3 days she was having high grade fever. On examination she was found to have fever 103 ° F with hepatosplenomegaly. LFT was normal; USG showed hepatosplenomegaly. Blood culture was advised.

CASE 10: A 10 year old boy was admitted with a low grade fever for 18 days followed by high spikes with chill and rigor for last 3 days along with loose motion for last 2 days No malaria parasite was found. Blood culture was advised.

MATERIALS AND METHODS: 5–10 ml of blood was collected by venepuncture using aseptic technique and inoculated directly into blood culture bottles containing 50 ml Thioglycollate broth for culture.

In all the ten cases, blood culture was advised by the physician. Subculture from Thioglycollate broth onto MacConkey agar following 5 days of incubation yielded non-lactose fermenting colonies. Colonies were large, 2-3 mm in diameter, circular, low convex and smooth. Blood agar shows non-hemolytic colonies.

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Gram stain from the colonies showed gram negative rods, about 1-3 X 0.5 μ m in size.

Motility by hanging drop preparation - positive

Biochemical reactions:

Catalase- positive

Oxidase- negative

A. Indole - negative

B. Triple sugar iron media- alkali/acid with small amount of H₂S showing Moustache Sign.

C. Urease – negative

D. Citrate – negative

Fig3: Biochemical Reactions

E. Glucose fermentation- acid without gas

F. Sucrose - not fermented

G. Lactose - not fermented

**Fig4: Biochemical Reaction;
Sugar Fermentation.**

H. Moeller's aminoacid base

I. Lysine Decarboxylation- positive

J. Ornithine Decarboxylation – negative

K. Arginine Decarboxylation - positive

Fig5: Biochemical Reactions; Amino acid Decarboxylation test

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Salmonella Typhi was suspected. Then serotyping was done by slide agglutination test according to Kauffmann-White scheme.

A loop full of Poly O antiserum added to a normal- saline emulsion from a 24 hour culture

Agglutination occurs (visible clumps)

A loop full of O antiserum (Factor 9/Group D) is added to another emulsion

Agglutination occurs

Flagellar antiserum (anti d-serum) added to another emulsion

Agglutination occurs

Confirms Salmonella enterica serotype Typhi. 9, 12[Vi]: d: -

Antibiotic sensitivity test was performed by the Kirby-Bauer technique according to Clinical and Laboratory Standards Institute guidelines. The organisms in all the 10 cases were sensitive to Azithromycin (15µg), Chloramphenicol (10µg), Ceftriaxone (30µg), Cotrimoxazole (25 µg) and Amoxicillin (10µg). Only one case was resistant to Ciprofloxacin (5 µg), others were sensitive.

DISCUSSION: The disease remains a serious public health problem in Southern and Southeast Asia where the incidence can be as high as 100 cases/100,000 populations per year. It is estimated that enteric fever causes 22 million episodes of illness and more than 200,000 deaths globally each year.^{1,2} It is endemic in all parts of India and still constitutes a significant health hazard.³ Enteric Fever is an acute febrile illness mainly caused by Salmonella enterica serotype Typhi, Paratyphi A, Paratyphi B, Paratyphi C and rarely by S. Barielly, S. Dublin, S. Enteritides, S. Eastbourne, S. Oransenburg, S. Panama, S. Saintpaul, S. Sendai, S. Typhimurium.

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In India Enteric Fever is mainly caused by *S.Typhi* and *S.ParatyphiA* and the proportion of Typhoid to Paratyphoid A is about 10:1.⁴ *Salmonellae* though frequently exist in the river, sewage, & other waters but they do not significantly multiply there and under suitable environmental conditions they can survive in soil and water for years. The organism is also isolated from vegetables and fruits and is an important contaminant of animal protein feed supplements.⁵

Risk factors for acquiring infection are contaminated water or ice, food & drinks purchased from street vendors, raw fruits & vegetables grown in fields fertilized with sewage, ill household contacts, lack of hand washing & toilet access and evidence of previous *H. pylori* infection that can cause decrease in gastric acidity chronically.⁶

The most common mode of transmission is by person to person spread in community or hospitals through ingestion of water or food that has been contaminated by faeces or urine of patients and carriers⁵. It can also be transmitted sexually among male partners⁶. ID 50 is 10^3 to 10^6 bacilli. The incubation period is 10-14 days, ranging from 3 to 21 days depending on the host immune status and size of inoculum⁵. Once the bacilli reach the small intestine, it penetrates the mucus layer of gut. It traverses the intestinal layer through phagocytic microfold (M) cells, that reside in the Peyer's patches, by triggering the formation of membrane ruffles in normally non-phagocytic epithelial cells. The ruffles thus formed reach out and enclose adherent bacteria within large vesicles by a process of bacteria mediated endocytosis (BME).⁷

Salmonellae encode a type III secretion system (T3SS) within *Salmonella* pathogenicity island 1 (the SPI-1 T3SS), which is required for bacteria-mediated endocytosis and intestinal epithelial invasion. Two SPI-1 translocated proteins, Sip C and Sip A, promote membrane ruffling and *Salmonella* invasion through direct interactions with the actin cytoskeleton. Following bacteria internalization, a fraction of the *Salmonella*-containing vesicles transcytose to the basolateral membrane, and the apical epithelial brush border reconstitutes.

In addition to invasion of intestinal epithelial cells, *Salmonella* serotypes clinically associated with gastroenteritis induce a secretory response in intestinal epithelium and initiate recruitment and transmigration of neutrophils into the intestinal lumen. Several SPI-1 translocated proteins that contribute to intestinal inflammation and fluid secretion have been identified. Stimulation of Rho GTPase signaling by Sop E and Sop E2 also leads to activation of microtubule-associated protein kinase pathways and movement of the proinflammatory transcription factor nuclear factor- κ B (NF- κ B) to its site of action in the nucleus.

In addition to its role in invasion, the inositol polyphosphatase activity of Sop B leads to accumulation of d-myo-inositol-1, 4, 5, 6-tetrakisphosphate in epithelial cells. The increased concentration of this compound ultimately leads to an increase in cellular basal chloride secretion, with associated fluid flux. Following *Salmonella* invasion, intestinal inflammation may also result from activation of the innate immune system through stimulation of proinflammatory receptors present on phagocytes and the basolateral surface of intestinal epithelia. This includes activation of Toll-like receptor 4 (Tlr4) by lipopolysaccharide and Toll-like receptor 5 (Tlr5) by bacterial flagellin. Intestinal inflammation probably contributes to fluid secretion and diarrhoea through disruption of the epithelial barrier and increased water flux by an exudative mechanism.

It can survive within the macrophage by sensing the environmental signals that trigger alteration in regulatory system of phagocytosed bacteria. Also by Type III secretory system, it can remodel the vacuole containing the organism promoting bacterial survival and replication.⁶

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The bacillus then enters the mesenteric lymph nodes where it multiplies and through thoracic duct, enters the bloodstream to cause Primary bacteremia, followed by seeding of the bacilli in spleen, lymph nodes, liver, bone marrow, lungs, gall bladder & kidney where it again multiplies and at the end of incubation period causes Secondary bacteremia resulting in clinical manifestation of Enteric Fever.⁴

The onset of fever and abdominal pain, probably result from secretion of cytokines by macrophages and epithelial cells in response to bacterial products that are recognized by innate immune receptors when a critical number of organisms have replicated. Over time, the development of hepatosplenomegaly is likely to be related to the recruitment of mononuclear cells and the development of a specific acquired cell-mediated immune response to *S. Typhi* colonization.

The recruitment of additional mononuclear cells and lymphocytes to Peyer's patches during the several weeks after initial colonization/infection can result in marked enlargement and necrosis of the Peyer's patches, which may be mediated by bacterial products that promote cell death as well as the inflammatory response.

The infiltration of mononuclear cells in the small intestine differentiates it from non-typhoidal *Salmonella* where an infiltration of polymorphonuclear leukocytes occurs in both small and large intestine.⁶ Severe gastroenteritis accompanied with septicemia in up to 40 percent in some outbreaks is the most common presentation in developing country. Mortality rate is up to 30 %.⁵

Early symptoms include progressive onset of fever, headache, abdominal discomfort, loss of appetite, constipation followed by diarrhoea, dry cough, malaise and rash along with relative bradycardia.^{2,3}

The case fatality rate is 30% in the absence of treatment and less than 1% with antibiotics.⁸

Prevention is based on access to safe water and hygienic food handling practices.⁹

Vaccination in high-risk areas is a potential control strategy recommended by WHO for the short to intermediate term. For identification of the causative agent, choice of clinical sample depends upon the duration of illness. For less than 1 week, blood culture is recommended. Blood culture is positive in 90% of cases and positive blood culture is diagnostic of Enteric Fever.⁴

Antimicrobial resistance continues to emerge in *S. Typhi* and *S. Paratyphi*, resulting in loss over time of the value of traditional first-line drugs and fluoroquinolones. Decreased ciprofloxacin susceptibility and, more recently, fluoroquinolone resistance have led to greater use of third-generation cephalosporin.¹⁰ Surveillance studies demonstrate considerable geographic variation in the proportion of *S. Typhi* isolates that are MDR in the same region, with sites in India, Pakistan, and Vietnam having higher rates of MDR isolates than sites in China and Indonesia.¹²

CONCLUSION: Enteric fever still remains a major health problem in India. The most common mode of transmission is by person to person spread in community or hospitals through ingestion of contaminated water or food, so prevention is based on access to safe water and hygienic food handling practices. Although Ty21a and Vi polysaccharide vaccines are effective, the development of cheap, safe vaccines with efficacy among infants that can provide protective immunity after a single dose and that could be easily adapted for Expanded Programs of Immunization would facilitate adoption into national programs.

Until about 1960, nearly all salmonellae were sensitive to a wide range of antimicrobial agents but since 1962 resistance, frequently plasmid mediated, has appeared in salmonellae

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worldwide. The multi-drug resistant strains have now become important agents of hospital cross infections.

A particular property of the majority of these multi drug resistant strains is the possession of a plasmid of the F_Ime incompatibility group coding for multiple resistance. Fortunately the strains of our cases were sensitive to most of the first line antibiotics, except one case which was found to be resistance to Ciprofloxacin, but the historical adaptation of *Salmonellae* to patterns of antimicrobial use suggests that vigilance for the emergence of resistant strains is warranted.

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