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RESEARCH ARTICLE

RISK FACTORS OF TYPHOID FEVER AMONGST PATIENTS IN THE ALLAHABAD REGION, INDIA

[1,*]Ausama Abed Alkadhum Alajeely, [2]Rubina Lawrence, [3]Ebenezer Jeyakumar and [4]Navodita G. Maurice

[1] Department of General Science, Babylon University, Iraq
[2,3]Department of Microbiology and Fermentation Technology, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Naini, Allahabad-211007
[4]Department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Naini, Allahabad-211007

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ABSTRACT

252 blood samples collected from patients of different localities of Allahabad region were found to be positive for typhoid fever when tested by Widal test. The causative agent*Salmonella* species were cultured from the blood samples and then were identified by using standard procedures. The isolates were identified as *S. typhi*, *S. paratyphi*A, *S. typhimurium* and *S. bongori*. Age, socio-economic status and seasonal variations were identified as significant risk factors associated with incidence of *Salmonella* infection. Blood samples were collected from both males and females belonging to the age groups from <1 to 50 years where the infection rate of typhoid fever was found to be higher among children. Socio-economic strata showed difference in incidence of *Salmonella* species with the low category showed highest number of isolates. Peak period of typhoid fever was found in June while a lower peak was noted in the month of November.

INTRODUCTION

Typhoid fever is a common bacterial disease occurring worldwide. It is a serious systemic disease, spreading *via* faecal-oral route (Crump *et al*., 2004). According to a survey conducted by WHO, the annual global burden of typhoid is of 22 million new cases, reported every year among which 5% are fatal (Ivanoff, 2003). The disease is characterized by the onset of prolonged high fever, severe headache, malaise and abdominal pain (Dutta *et al*., 2000). The illness often causes diarrhea, especially in younger children, whereas constipation is common in older children and adults (WHO, 2003). PCR amplification of the bacterial DNA from blood of the commonly available diagnostic tests, Widal test and other serological diagnostic methods are limited because of the low specificity of the test. There are reports of a large number of false-positive cases especially in areas where typhoid fever is endemic and in patients exposed to typhoid fever earlier (Clemens *et al*., 1999). The risk factors of enteric fever include age, educational status of parents, consumption of street vendor's food, water intake, history of contact with patient of enteric fever in family and availability of Dispensary Facility (Ayaz *et al*., 2007). The highest attack rate occurs in children aged 8-13 years. Older people appear to be relatively immune, presumably because of frequently reinforced acquired immunity through numerous sub-clinical exposures to *S. typhi* (Parry *et al*., 2005).

The occurrence of infections was common in summer months (rainy season) (Prajapati *et al*., 2008). In the present study we determined the prevalence of the different species of *Salmonella* and also accessed various risk factors associated with typhoid infection.

MATERIALS AND METHODS

A total of 252 samples of peripheral blood positive for Widal test (Rapid slide test) were collected from patients attending OPD at local hospitals in Allahabad, India, during March 2011 to February 2012. Age, sex, malaise, headache, diarrhea, chills, anorexia, vomiting, abdominal pain, rash, etc. of each patient was recorded in a predesigned performa in sterile vials containing 10% sodium citrate.

Corresponding author: Ausama Abed Alkadhum Alajeely
General Science Department, Babylon University, Iraq

Bacterial Isolation

An aliquot of 1 ml was inoculated in Brain Heart Infusion and incubated at 37˚C for 24 hours. Positive broths were sub cultured on S-S agar and MacConkey agar and kept overnight at 37˚ C. The colonies showing typical characters were isolated for morphological and biochemical identification (Ewing *et al*., 1986). The second portion of the sample was used for serological identification by the Widal test.

Widal Test

Three milliliters of the blood samples were centrifuged at a (6000 rpm) for 5 min. in order to separate the serum from the blood cells. The test was carried out using Beacon Diagnostics febrile antigen kit (India). The rapid slide screening test was first carried out, followed by the tube agglutination test according to the manufacturer's specifications. All the positive results were subjected to the tube agglutination test (Olopoenia *et al*., 2000).

Biochemical Identification

The bacterial colonies on inoculated plates were identified on the basis of cultural, morphological and biochemical characteristics. Colony's characteristics like colony size, type of margin, elevation, texture and color of the colonies on S- S Agar and MacConkey Agar were recorded. The isolates were then processed for IMViC test, urease test, catalase Production, motility test, gelatin liquefaction, triple sugar, carbohydrate fermentation and decarboxylase test (Brenner *et al*., 2005).

Molecular Identification

The isolates identified on the basis of morphological and biochemical characteristics were further confirmed by molecular screening (Massi *et al*., 2005).

Isolation of Genomic DNA

Genomic DNA from the cultured bacteria in 200 µl of Tris-EDTA (TE) buffer for PCR was extracted according to the instructions given in GeneiUltrapure™ Bacterial Genomic DNA Purification kit (HiMedia Labs, Mumbai). The purity of the extracted DNA was checked by measurement of A260 and by agarose gel electrophoresis.

The PCR Mix Composition

The bacterial DNA extract and control was amplified using 0.5 µM primers 16S rDNA fragment. The PCR mixture (50 µl) contained bacterial DNA, PCR buffer [10 mMTris/HCl (pH 8.3), 50 mM KCl, 3 mM MgCl, and 0.01% gelatin], 200 µM of each dNTP, and 1.0 U AmpliTaq Gold enzyme. The mixtures were amplified using *Taq* DNA polymerase. for 35 cycles at 94ºC for 1 minute, 55ºC for 1 minute, and 72ºC for 2 minutes, with a final extension at 72ºC for 10 minutes in an automated thermal cycler. An aliquot of 10 µl of each amplified product was electrophorese in 2% (wt/vol) agarose gel, with a DNA Molecular Weight Marker (StepUp™500bp DNA ladder) in parallel (Massi *et al*., 2005).

DNA Sequencing

The PCR product was sequenced using forward, reverse and internal primer. Sequence data was aligned and analyzed with the filter option for *Salmonella* in the nucleotide BLASTN programme **(**Altschul *et al*., 1990). The PCR products were loaded on 1.0% agarose gel along with StepUp™500bp DNA ladder.

RESULTS

A total of 252 patients were selected for the study and on the basis of the information collected in predesigned performa129 were male and remaining 123 were female patients. Majority of the patients showed characteristic symptoms of typhoid fever (Table 1).

Table 1. Epidemiological features in patients clinically diagnosed for typhoid

S. No.	Parameters	*Salmonella* infected patients	
		Male n=129	Female n=123
A. Previous History			
1.	Vaccination	2 (1.55)	0 (0.00)
2.	Onset of symptoms	127 (98.44)	119 (96.74)
3.	Previous enteric fever	17 (13.18)	10 (8.13)
4.	Previously known Typhoid carrier	12 (9.30)	9 (7.31)
5.	Antibiotic use	42 (32.56)	54 (43.90)
B. Symptoms			
1.	Fever	117 (90.69)	118 (95.93)
2.	Malaise	78 (60.46)	71 (57.72)
3.	Headache	59 (45.73)	61 (49.59)
4.	Diarrhoea	59 (45.73)	51 (41.46)
5.	Chills	71 (55.03)	77 (62.60)
6.	Anorexia	66 (51.16)	62 (50.40)
7.	Vomiting	16 (12.40)	30 (24.39)
8.	Abdominal Pain	57 (44.18)	56 (45.52)
9.	Rash	4 (3.10)	1 (0.81)

*Figures in parenthesis indicate percentage

Of the 252 blood samples, 167 (66. 26%) were tested positive by Widal test and 51 of 167 (30.53%) showed positive cultures for *Salmonella*. On the basis of the morphological and biochemical characteristics of the colonies obtained on S-S agar and MacConkey agar, four *Salmonella* species were identified *viz*. *S. typhi, S. paratyphi* A*, S. typhimurium* and *S. bongori*. The prevalence of the individual species corresponded to 52.94% for *S. typhi*, 27.45% for *S. paratyphi* A, 15.68% for *S. typhimurium* and 3.92% for *S. bongori*. One isolate each from the *Salmonella* spp. identified on the basis of cultural, morphological and biochemical characteristics was further identified according to the molecular weight of the base pairs. The amplified PCR products carried out using universal bacterial 16S rRNA primers and visualized by UV illumination showed the expected bands of about 1500 bp (Fig. 1). The results demonstrated correct genus identification of examined *Salmonella* isolates.Based on nucleotides homology and phylogenetic analysis, the isolates samples were detected to *Salmonella enteric* 27(52.94%), *Salmonella sp.* 14(27.45%), *Salmonella typhimurium* 8(15.68%) and *Salmonella bongori* 2(3.92%). Distribution of patients on the basis of their age revealed maximum number of subjects to fall in the age group of 11-15 years. Out of the total number of positive samples, maximum incidence was recorded in the age group of 41-45

Table 2. Incidence of *Salmonella* spp. with respect to age

Age (Years)	Total number of blood samples N=252	Total number of positive samples N=167	Total number of positive culture N=51	*Salmonella* spp.			
				Salmonella enterica	*Salmonella species*	*Salmonella Typhimurium*	*Salmonella bongori*
<1	10	7	4 (7.8)	1 (3.7)	1 (7.1)	2 (25.0)	0 (0.0)
1 – 5	23	15	7 (13.8)	2 (7.4)	3 (21.5)	2 (25.0)	0 (0.0)
6 – 10	27	17	8 (15.7)	4 (14.8)	3 (21.5)	0 (0.0)	1 (50.0)
11 – 15	32	19	8 (15.7)	7 (26.0)	0 (0.00)	1 (12.5)	0 (0.0)
16 – 20	21	17	6 (11.8)	4 (14.8)	1 (7.1)	1 (12.5)	0 (0.0)
21 – 25	24	13	2 (3.9)	0 (0.0)	0 (0.00)	1 (12.5)	1 (50.0)
26 – 30	22	16	4 (7.8)	2 (7.4)	2 (14.3)	0 (0.0)	0 (0.0)
31 – 35	20	8	1 (2.0)	1 (3.7)	0 (0.00)	0 (0.0)	0 (0.0)
36 – 40	26	19	5 (9.8)	3 (11.1)	2 (14.3)	0 (0.0)	0 (0.0)
41 – 45	25	21	4 (7.8)	3 (11.1)	1 (7.1)	0 (0.0)	0 (0.0)
46 – 50	22	15	2 (3.9)	0 (0.00)	1 (7.1)	1 (12.5)	0 (0.0)

*Figures in parenthesis indicate percentage

Table 3. Incidence of *Salmonella* spp. with respect to socio-economic status

Socio-economic status	Total number of blood samples N=252	Total number of positive samples n=167	Total number of positive culture N=51	*Salmonella* spp.			
				Salmonella enterica	*Salmonella* sp.	*Salmonella Typhimurium*	*Salmonella bongori*
Low	118	77 (46.11)	30 (58.8)	16 (59.3)	8 (57.1)	5 (62.5)	1 (50.0)
Middle	89	51 (30.54)	13 (25.5)	6 (22.2)	4 (28.6)	2 (25.0)	1 (50.0)
High	45	39 (23.35)	8 (15.7)	5 (18.5)	2 (14.3)	1 (12.5)	0 (0.0)

*Figures in parenthesis indicate percentage

years and least in patients of < 1 years of age (P<0.01). In patients tested positive for *Salmonella* by culture method belonged to the age groups of 6-10 and 11-15 years with significantly higher incidence of the disease (P<0.01) (Table 2). The distribution pattern of *Salmonella* spp. in the study group with respect to gender showed a higher incidence in male patients as compared to female patients in the samples found positive by Widal test and culture methods but the difference was found to be statistically non-significant (P>0.05) (Fig. 2).

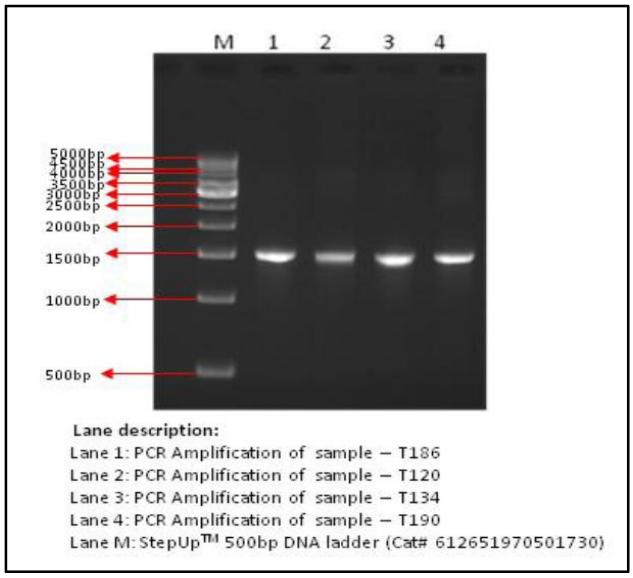

Figure 1. Gel electrophoresis of PCR amplification of *Salmonella* species

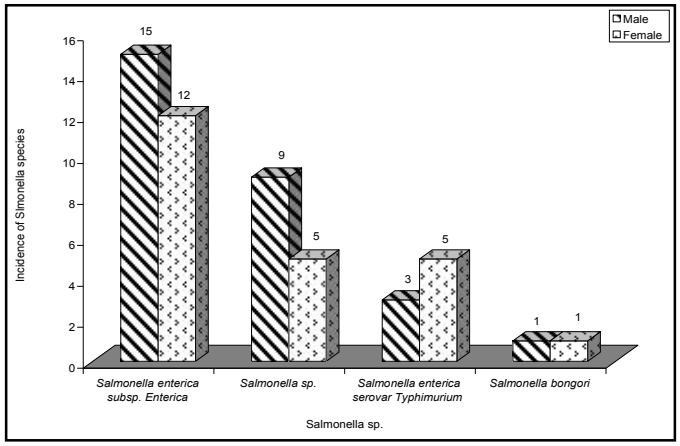

Figure 2. Incidence of *Salmonella* spp. isolates with respect to sex of patients

Majority of the patients selected for the study belonged to the lower socio-economic strata (Table 3). Significant difference was observed in the incidence of *Salmonella* spp. being highest in the lower socio economic group and least in the higher socio-economic population (P<0.01).The monthly distribution of number of samples positive for *Salmonella* spp. on the basis of Widal test collected during March 2011 to Feb 2012 showed maximum number of samples (16.7%) collected during the month of June were positive followed by 11.3% and 10.7% samples collected during the months of May and July as positive. Lower incidence was recorded during winters from November to February (4.2-5.9%) (Fig.3). The data were found to be significant at 5% and 1% levels of significance.

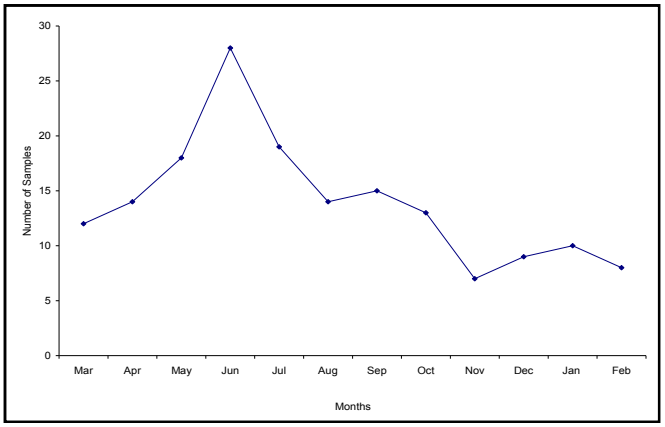

Figure 3. Distribution of positive samples collected during the months March 2011 to February 2012

DISCUSSION

In our study 252 subjects were selected for the analysis of onset of symptoms of typhoid fever and we found that majority of patients showed symptoms of fever, malaise, chills, anorexia and headache and our results were found to be in quick agreement with the study of Ackers *et al*. (2000) who also reported that fever was the most frequent symptom followed by malaise in their study conducted in United States from June 1996-May 1997. Diagnosis of typhoid fever was done by isolating the causative bacteria from the patients most often from blood, but also from urine, stool, or bone marrow. Infected persons can develop sustained fever of up to 104°F (40° C), weakness, stomach pain, and headache. However, a rash (rose spots) may also accompany the infection (Huang *et al*., 2009). In our study 66.26% samples were tested positive by Widal test while 20.23% were found to be culture positive for *Salmonella* species. Manga *et al*. (2006) found 84.3% and 25.9% samples as positive by Widal test and cultural methods however, only 3.2% samples were found culture positive for *Salmonella* in the study of Peletiri and Ibecheozor (2012).

The first evaluation conducted by Song *et al*. (1993) by using PCR as a diagnostic tool for typhoid fever. A molecular method for the detection of *Salmonella* serovars in specimens appears to be an attractive addition to current methods. However, PCR is not commonly reported for the routine identification of *Salmonella* (Ali *et al*., 2009). In our study PCR amplification was used for the detection of *Salmonella* sp. and 27 (52.94%) for *Salmonella enterica*, 14 (27.45%) for *Salmonella sp.*, 8(15.68%) for *Salmonella typhimurium* and 2 (3.92%) for *Salmonella bongori positive results* were obtained while, Kumar *et al*. (2008) found 140 (80%) patients infected by *Salmonella enteric* subspecies *enterica* serovar *Typhi* and 16(9%) by *Salmonella* serovar Paratyphi A and remaining 11% were infected by other *S. enterica* serogroups*, typhimurium*, Paratyphi C and other group E *Salmonella* in their study conducted in north India .Majority of the subjects falling in the age groups of 6-10 and 11-15 years were tested positive for *Salmonella* spp. in our study and our observations are comparable with the findings of Ekdahl *et al*. (2005) who found that the highest individual risk for paratyphoid fever lies in the age group of 7-18 years, followed 19-45 years.

Bhutta *et al*. (1996) and Walsh *et al*. (2000) found the peak incidence of *Salmonella* sp. among children of 5-15 years of age. However, Breiman *et al*. (2012) reported maximum incidence among children of 2-4 and 5-9 years age group. Ochiai *et al*., (2008) reported in their multicentric trial that the mean age of typhoid was significantly lower in south Asian sites as compared to the South East and North East Asian sites and suggested that is an inverse correlation between typhoid incidence and mean age of patients.

According to De Las Casas *et al*. (1999) teenagers and young adults have a more adventurous lifestyle and are therefore, they are at higher risk for food borne diseases as contaminated food from restaurants and street vendors is the main transmission route for paratyphoid fever. A higher probability of *Salmonella* infection was found among males as compared to females in our study and similar findings have been found in the studies of Ayaz *et al*. 2006 and Prajapati *et al*. 2008. This could be because of greater exposure to infection due to the occupation and food habits of men as explained by Khan *et al*. (1999). Ekdahl *et al*. (2005) however, reported that gender is not a risk factor for enteric fever and so is not in agreement with our study. A higher incidence of *Salmonella* infection was recorded in patients belonging to lower socio-economic strata and our finding is in agreement with the findings of Vollaard *et al*. (2004) who found that typhoid fever is more frequent among the people of a lower income category. Kothari *et al*. (2008) stated that typhoid fever is a disease of developing countries associated with poor public health and low socio-economic indices. Abera *et al*. (2009) reported that majority of food handlers are responsible for transmission of *Salmonella* infection with low educational levels.

Our study is in accordance with the study of Ekdahl *et al*. (2005) who also reported that both typhoid and paratyphoid fevers have a seasonal pattern with a distinct risk peaking in June. The seasonal pattern was marked with disease peakin summer and the dry season in the highly endemic areas. In a study conducted by Vollaard *et al*. (2004) and Lin *et al*. (2000) in Indonesia and Vietnam a higher local risk of typhoid and paratyphoid fevers was seen during the dry season however, Sinha *et al*. (1999) reported peak incidence of typhoid fever during the monsoon season *i.e*. from July to November. Prajapati *et al*. (2008) suggested contamination of drinking water and environment with faecal matter is the possible reason for increased incidence of the disease during the rainy season. A study conducted by Mohanty *et al*. (2006) in Delhi, reported maximum incidence between April to June and stated that the streets in the city become flooded with rainwater contaminated with overflow of sewage due to non-existent or insufficient sewerage system.

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REFERENCES

Abera, B., Biadegelgen, F. and Bezabih, B. 2010.Prevalence of *Salmonella typhi* and intestinal parasites among food handlers in Bahir Dar Town, Northwest Ethiopia. *Ethiop. J. Health. Dev.*; 24: 46-50.

Ackers, M.L., Puhr, N.D., Tauxe, R.V. and Mintz, E.D.2000. Laboratory based surveillance of *Salmonella* serotype Typhi infections in the United States: antimicrobial resistance on the rise. JAMA., 283:2668-2673.

Ali, A., Haque, A., Sarwar, Y., Mohsin, M., and Bashir, S.2009.Multiplex PCR for differential diagnosis of emerging typhoidal pathogens directly from blood samples. *Epidemiol Infect.*, 137(1):102-107.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. 1990. Basic local alignment search tool.*J. Mol. Biol.*, 215: 403-10.

Ayaz, A., Pervaiz, M.K., Azad, M.D. and Pervaiz, G. 2006. Risk factors of enteric fever in children less than 15 years of age. *J. Statist*., 13:84-100.

Bhutta, Z.A., Punjwani, N. and Lindblad, B.S. 1996. Concomitant bacteremia as a risk factor in diarrheal disease mortality; a case control study of hospitalized children. *Acta Paediatr.*, 85: 809-813.

Breiman, R.B., Cosmas, L., Njuguna, H., Audi, A., Olack, B. and Ochieng J.B. 2012.Population based incidence of typhoid fever in an urban informal settlement and a rural area in Kenya: Implication for typhoid vaccine see in Africa. Plos ONE., 7: 1-10.

Brenner, D.J. and Farmer, I.J.J. 2005.Family Enterobacteriaceae. In Bergey's Manual of Systematic Bacteriology, 2nd Edn,, The Gamma proteobacteria(edited by Garrity GM, Brenner DJ, Krieg NR and Staley JT). Springer, New York. pp.587-607.

Clemens, J., Hoffman, S., Ivanoff, B., Klugman, K., Levine, M.M. and Neira, M. 1999.Typhoid fever vaccines. Vaccine., 17: 2476¬2478.

Crump, J.A., Luby, S.P. and Mintz, E.D. 2004. The global burden of typhoid fever. Bull. *W.H.O.*, 82: 346-353.

De Las Casas, C., Adachi, J. and Dupont, H. 1999. Review article: traveller's diarrhoea. Aliment. Pharmacol. Ther., 13:1373-1378.

Dutta, U., Garg, P.K., Kumar, R. and Tandon, R.K. 2000.Typhoid carriers among patients with gallstones are at increased risk for carcinoma of the gallbladder. *Am. J. Gastroenterol.*, 95: 784-787.

Ekdahl, K., De Jong, B. and Andersson, Y. 2005. Risk of travel associated typhoid and paratyphoid fevers in various regions. *J. Travel. Med.*, 12: 197-204.

Ewing, W.H. 1986. The genus of *Salmonella*. Edwards and Ewing identification of the Enterobacteriaceae. differentiation of enterobacteriaceae by biochemical reactions. 4th Ed., Elsevier Science Publishing Co., New York.pp.181-245.

Huang, Z., Sutton, S.E., Wallenfang, A.J., Orchard, R.C., Wu, X. and Feng, Y.2009.Structural insights into host GTPase isoform selection by a family of bacterial GEF mimics. Nat. Struct. Mol. Biol., 16: 853-860.

Ivanoff, B.C. 2003. The diagnosis, prevention, and treatment of typhoid fever. (WHO) Geneva, Switzerland. pp. 1-30.

Khan, M., Coovadia, Y.M., Connolly, C. and Sturm, A.W. 1999.Influence of sex on clinical features, laboratory findings and complications of typhoid fever. *Am. J. Trop. Med. Hyg.*, 61:41-46.

Kothari, A., Pruthi, A. and Chugh, T.D. 2008. The burden of enteric fever. *J. Infect. Dev. Ctries.*, 2: 253-259.

Kumar, S., Rizvi, M. and Berry, N. 2008.Rising prevalence of enteric fever due to multidrug resistant *Salmonella*: an epidemiological study. *J. Med. Microbiol*., 57: 1247-1250.

Lin, F.Y., Vo, A.H., Phan, V.B., Nguyen, T.T., Bryla, D. and Tran, C.T. 2000. The epidemiology of typhoid fever in the Dong Thap Province, Mekong Delta region of Vietnam. *Am. J. Trop. Med.Hyg.*, 62: 644-648.

Manga, S.B., Ameh, I.G., Bashir, S., Muazu, A.G., Danjuma, B. and Ibrahim, M.L. 2008. Antibiotics susceptibility of *Salmonella* isolates from Widal test positive patients at the Federal Medical Center, Gusau. *Jos J Med.*, 5: 123-129.

Massi, M.N., Shirakawa, T., Gotoh, A., Hatta, M. and Kawabata, M.2005.Identification and sequencing of *Salmonella enteric* serotype Typhi isolates obtained from patients with perforation and non-perforation Typhoid fever. Southeast. Asia. *J. Trop. Med. Public. Health,* 36:118-122.

Mohanty, S., Renuka, K., Sood, S., Das, B.K. and Kapil, A. 2006. Antibiogram pattern and seasonality of *Salmonella* in a North Indian tertiary care hospital. Epidemiol Infect., 134: 961-966.

Ochiai, R.L., Acosta, C.J. and Danovaro-Holliday, M.C. 2008.A study of typhoid fever in five Asian countries: disease burden and implications for control. *Bull World Health Organ.*, 86: 260-68.

Olopoenia, L.A. and King. A.L. 2000. Widal agglutination test 100 years later: still plagued by controversy. *Postgrad. Med. J.*, 76: 80–84.

Parry, C.M., Tran, T.H., Gordon, D., Nicholas, J.W. and Jeremy, J.F. 2005. Medical progress: Typhoid fever. *New Eng. J. Med*., 347: 32-59.

Peletiri, I.C. and Ibecheozor, N.K.O. 2012.The a etiology of enteric fever in Abuja, North Central, Nigeria. *Afr. J. Clin. Exp. Microb*., 13: 150-155.

Prajapati, B., Rai, G.K., Rai, S.K., Upreti, H.C., Thapa, M. and Singh, G. 2008.Prevalence of *Salmonella typhi* and *paratyphi* infection in children; a hospital based study. Nepal. *Med. Coll. J*., 10: 238- 241.

Sinha, A., Sazawal, S. and Kumar, R. 1999. Typhoid fever in children aged less than 5 years. Lancet., 354: 734-37.

Song, J.H., Cho, H., Park, M.Y., Na, D.S., Moon, H.B. and Pai, C.H. 1993. Detection of *Salmonella typhi* in the blood of patients with typhoid fever by polymerase chain reaction. *J. Clin. Microbiol*., 31:1439-1443.

Vollaard, A.M., Ali, S. and Van Asten, H. A. 2004. Risk factors for typhoid and paratyphoid fever in Jakarta, Indonesia, JAMA.,291: 2607-2615.

Walsh, A.L., Phiri, A.J. and Graham, S.M. 2000. Bacteremia in febrile Malawian children: clinical and microbiologic features. *Pediatr. Infect. Dis. J*., 19: 312-318.

World Health Organization (WHO). 2003. The diagnosis, treatment and prevention of typhoid fever. Communicable Disease Surveillance and Response Vaccine and Biological. pp. 7-18.

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