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DETECTION OF TYPHOID FEVER BY IMMUNO CHROMATOGRAPHIC TEST (ICT) AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *Salmonella typhi* IN CHITTAGONG METROPOLITON CITY

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ABSTRACT

Typhoid fever caused by the *Salmonella typhi* is a disease of global distribution and one of the major health problems in many developing countries. In this study, clinically suspected thirty five patients were enrolled from different diagnostic centers of Chittagong city. We evaluated the reliability of Immuno Chromatographic Test (ICT) compared to different diagnostic methods like blood/serum culture and serological/Widal test for early detection of the disease. A total of 18 (51.43%) and 20 (57.14%) samples were found positive in culture method and Widal test, respectively, whereas 31 (88.57%) were found positive in ICT. Moreover, 13 culture negative and 11 Widal test negative samples were found positive in ICT. As the ICT showed satisfactory results compared to the other methods used in this survey, it can be considered as a reliable method for the early detection of typhoid infection. The study revealed several antibiotic resistant patterns by standard disc diffusion method, 12 (66.66%) out of 18 culture positives were found multi-drug resistant.

Key words: *Salmonella typhi*, ICT, Typhoid.

INTRODUCTION

The etiological agent of typhoid is *Salmonella enteric* serovar*typhi* belongs to the *Enterobacteriaceae* family. Typhoid fever is clinically characterized by high fever and a myriad of other non-specific features, including abdominal pain and constipation, headache, myalgia and arthralgia, cough, lymphadenopathy and rash. Depending on the clinical setting and quality of available medical care, some 5-10% of typhoid patients may develop serious complications, the most frequent being intestinal hemorrhage or peritonitis due to intestinal perforation. The infection is most common in young children and elderly people with peak

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incidence in summer and fall (rainy season) (Jerrold 2010). In Bangladesh, the incidence of typhoid fever among children annually was found 210/100,000 children of >5 years of age and 1870/100,000 of <5 years of age (Brooks 2005).

Since the clinical picture of typhoid fever may be confused with many other febrile infections such asmalaria, dengue fever, leptospirosis, melioidosis and the rickettsioses, a definitive diagnosis of the disease depending on the clinical presentation alone is very difficult. Therefore, laboratory-based investigations are essential for supporting the diagnosis of typhoid fever. In the perspective of Bangladesh, it is presumed that the diagnosis of typhoid fever is usually based on clinical presentation**s** as well as Widal test, both of which are associated with numerous limitations (Jenkins 2009).

Although culture of blood or serum remains to be the gold standard in the diagnosis of typhoid fever, thet facility for culture is not widely available and also time consuming (Chau 2007). The Widal test is used to demonstrate rising titres of antibodies to flagellar (H), Vi and somatic (O) antigens in typhoid and paratyphoid fever (Saha 1996). An increased O antibody level signifies acute infection, whilst an increased Vi antibody level may indicate the serotype of the infecting organism. Widal test has limited use, because Vi and O antibody levels may rise non-specifically due to cross reactions with other *enterobacteriaceae*. Thus, there is always a need for the development of a simple, rapid, reliable and sensitive diagnostic method for the early diagnosis of typhoid fever, especially in the endemic areas. The rapid and early immune diagnosis of typhoid fever can be done by the detection of anti-salmonella antibodies by immune chromatographic test or ICT (Jesudason *et al*. 2002) which does not require any specialized laboratory or highly skilled personnel and can be done in field areas also. The test simultaneously detects and differentiates the IgG and IgM antibodies to *S. typhi* specific-antigen in whole blood (Ismail 1991). The detection of IgM reveals acute typhoid fever in the early phase of infection and the IgG detection reveals late phase as well as the carriage of infection, while the detection of both IgG and IgM suggests acute typhoid in the middle phase of the infection (Saha 1999). Its usefulness has been shown to detect the anti-salmonella antibodies as early as 4 days of fever onset (Collee 1996). Thus, detection of anti-salmonella antibodies (IgM and IgG) by immune chromatographic test may be an appropriate adjunct for the clinical diagnosis of typhoid fever. In this study, the performance of the

ICT method in the diagnosis of typhoid fever was compared with those of the conventional culture based method and Widal test.

MATERIALS AND METHODS

Patients and specimens

Thirty five (35) clinically suspected cases of typhoid fever patients' serum sample swere collected from Chevron Clinical Laboratory (Pte) Ltd., Popular Diagnostic Center Ltd., CSCR (Pvt.) Ltd. and Chattogram Metropolitan Hospital Limited of Chattagram city. The patients were illiterate, living in unhygienic surroundings, drinking raw water, not habitual of washing hand properly and presented clinical features which included fever, chills, rigor, altered bowel habit, raised spot on the trunk, headache and myalgia.Serum samples of ten healthy people were also collected from different areas of Chittagong city as control specimen.

Enrichment Culture

Tetrathionate Broth is selected as a selective enrichment broth for isolation of *Salmonella typhi* and other *Salmonellae* from clinical specimens. The medium selectively inhibits coliforms and permits unrestricted growth of enteric pathogens. The medium was prepared by following manufacturer's instruction and dispensed in sterile tubes. One ml of diluted serum sample was taken and transferred into the tubes containing Tetrathionate broth medium. Then the tubes were placed into a shaking incubator at 37℃ temperature for 24 hours.

Plating of enrichment culture

One loopful of the selective enrichment culture was streaked from each sample onto LB agar. Following incubation, a single colony was transferred into a nutrient agar plate.

Identification of Salmonella typhi by bacteriological method

Salmonella typhi was identified by culturing the bacterial colonies in MacConkey agar media, Blood Agar base medium, XLD (Xylose LysineDeoxycholate) medium. Only those isolates which gave positive result in all selective media were selected for morphological and biochemical tests. A known *S. typhi* was also cultured as positive control to compare the test samples by bacteriological method.

Morphological identification

The bacterial isolate was Gram-stained and observed under a light microscope. (Gram1884).

Biochemical tests

The selected isolates were characterized biochemically by following the standard method of Catalase (Holt *et al*. 1994; Collins *et al.* 1987), Indole (Mac Faddin *et al*. 1980), Methyl-Red (MacFaddin *et al*. 1980), Voges-Proskauer (Voges and Proskauar, 1989, Lennette 1985), Citrate utilization (MacFaddin *et al.*1980). Mannitol motility (MacFaddin *et al.,* 1980) and TSI slant tests (MacFaddin *et al.* 1980, Hajna 1945) and compared with the positive control.

Bacterial (Slide) agglutination test (Widal test)

A small drop of O antisera (approximately 20 µl) was added on a glass slide. Culture was transferred from slant to the drop of antiserum and mixed well with a sterile loop. Then the slides were observed for 5-10 seconds. The reaction was read with the naked eye by holding the slide in front of a light source against a black background (indirect illumination).

Immuno Chromatographic Test (ICT) method

Immuno chromatographic test (Typhoid IgG/IgM combo rapid test ®USA) is a rapid and qualitative test assay for the detection of *S. typhi* antibody in whole blood/serum specimen (Jesudason *et al*. 2002) The test cassette consists of a colored conjugate pad containing recombinant *S. typhi* H antigen and O antigen conjugated with colloid gold (Typhoid conjugates) and rabbit IgG gold conjugates. Firstly, the test device was placed on a clean, flat surface. Then, one drop of diluted serum sample was put into the sample well of the test unit. The test unit was kept under inoculation for 15 minutes. Results were recorded after 15 minutes.

Antibiotic sensitivity test by disc diffusion method

Mueller-Hinton agar was prepared by following manufacturer's instruction. The antibiotic discs used were Amikacin (30 µg/disc), Amoxicillin (30 µg/disc), Azithromycin (15 µg/disc), Cefixime (5 µg/disc), Ceftriaxone (30 µg/disc), Cefuroxime (30 µg/disc) and Nalidixic acid (30 µg/disc).

Sixteen hours old bacterial culture in LB broth medium was taken by a sterilized cotton bud and spread into the medium so that the surface of the media was fully covered by bacterial culture. Then the commercially available antimicrobial disks

containing different antibiotics were placed onto the medium of the plate using a sterilized forcep. The plate with lid was sealed with parafilm and incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

Complaints and population study of typhoid fever

The present study was conducted on a total 35 individuals of different age and sex, of clinically suspected typhoid fever. Among the 35 suspected cases of typhoid fever, almost all of the cases complained with fever (30 cases), followed by headache (20 cases), vomiting (15 cases) and abdominal discomfort (8 cases) for 3-10 days (Fig -1).

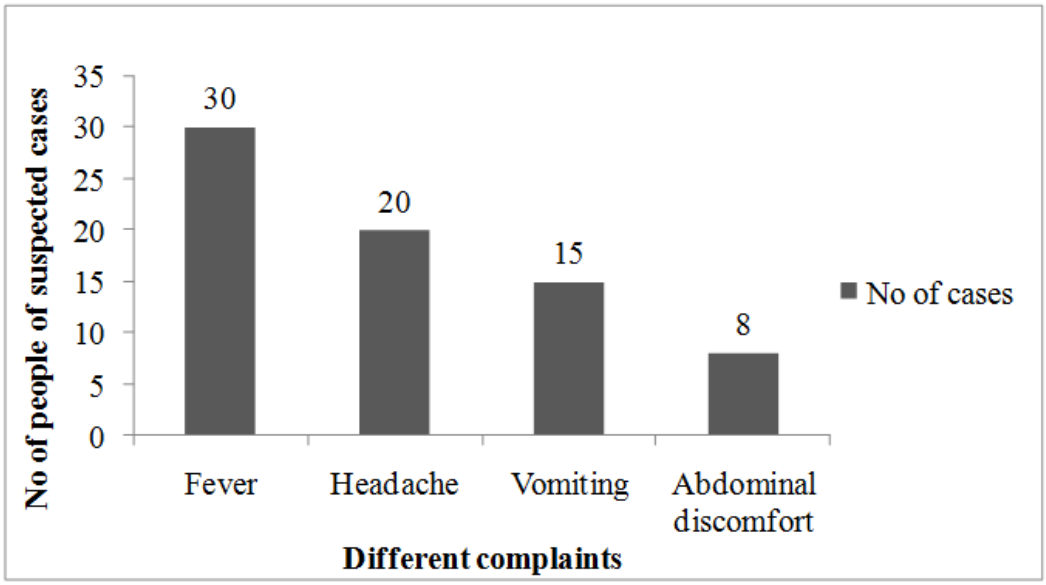

FIGURE 1: DIFFERENT SYMTOMS S OF THE SUSPECTED PATIENTS OF TYPHOID FEVER

Age distribution of 35 suspected cases showed that the majority of the respondents of the cases (15; 42.85%) belonged to the age group of 1 to 5, **(**5; 14.28%), 6 to 15, (10; 28.57%), 16 -20 years (7; 20%) and the age group of >30 years (Fig-2). This findings correlate with the observation made by Saha *et al*. (2003) who found that children between 2-3 years of age are most susceptible age group (35.6%). The child aged group < 5 years were more prone to infection,

which may be due to a lack of immunity transferred through mother's milk or the non consumption of potable drinking water especially in the rural areas.

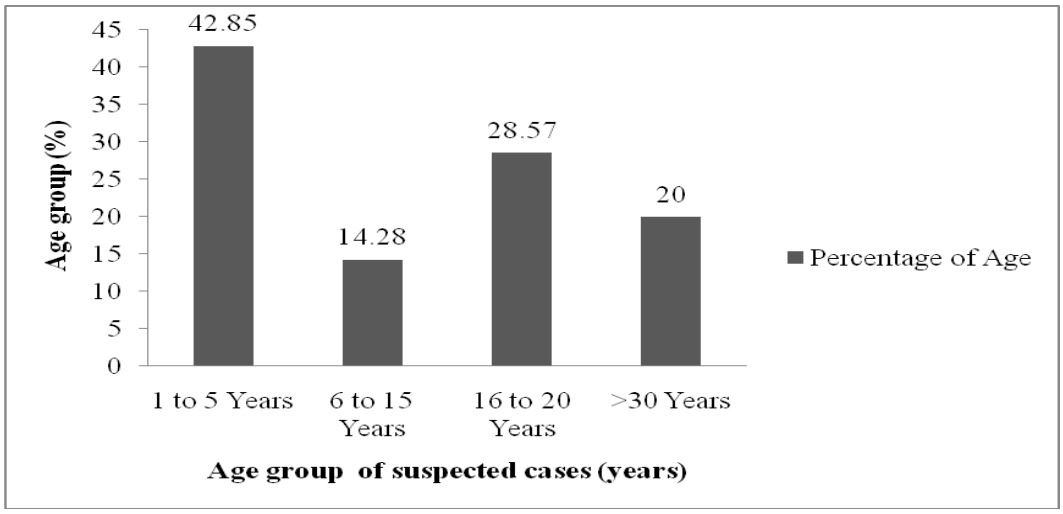

FIGURE 2: AGE GROUP DISTRIBUTION OF STUDY POPULATION

In the present study, among 35 samples, male were found more susceptible for typhoid fever than female and most prominently, the ages between 1 to 5 years are more affected by typhoid fever. Distributing the sex of study population, it is showed that the infection rate with the male were 19 (54.28%) was higher than that of the female 16 (45.71%) among the cases studied. Another study done by Hornick *et al.* (1991) which also showed similar result that the infection rate was slightly higher in male.

The socio-demographic characteristics of suspected cases of typhoid fever in the present study showed that the majority of the respondents were pre-school children and were illiterate, belonged to lower class, used unhygienic sanitation condition and drinking from tube well. Similar findings also were reported by Sur *et al.* (2007) that showing the illiteracy rates were highest in the cases of typhoid fever. It is presumed that typhoid fever is a major health problem in all those parts of the world where safe drinking water and sanitation is inadequate. By maintaining hygienic lifestyles and proper sanitation this disease may be controlled to some extent.

Bacteriological and biochemical analyses

Serum sample in Tetrathionate broth medium after 24 hours of incubation at 37°C was observed for the opaque turbidity and compared with that of control medium without any bacterial inoculation. The organism produced turbidity.Growth in 25 out of 35 samples was observed in treatment tubes. Ten control samples of healthy subjects exhibited negative results in Tetrathionate broth.The organism was identified as *Salmonella typhi* by culturing it on several media. In culture method, among 35 suspected samples, 18 samples (51.43%) were showed growth in all media. Other samples were found to show variable growth. After culturing the bacterial samples in selective media, 18 out of 35 (51.42%) showed positive results. These 18 samples were selected for Gram staining and biochemical tests. Eighteen selected samples were observed and found to be Gram-negative and rod shaped bacteria.A series of biochemical tests were carried out for the biochemical characterization of selected bacterium.In biochemical test, 18 (51.23%) selected samples out of suspected 35 samples showed positive result for *Salmonella* spp. Theresults of bacteriological identification methods are shown inTable 1.

TABLE 1. RESULTS OF MORPHOLOGICAL AND BIOCHEMICAL TESTS OF BACTERIAL ISOLATES

Isolates	Colony Character			Morphology		Bio-chemical test							Comment
	MA	**BA**	**XLD**	**G**	**Shape**	**CT**	**IT**	**MT**	**VPT**	**CUT**	**MMT**	**TSI**	
Sample-1	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-2	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-3	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-4	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-5	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-6	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-8	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-10	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-12	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-14	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-15	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-18	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*

Sample-20	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-24	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-25	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-26	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-30	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*
Sample-33	Pale	White	R with B center	G^-	Rod	+	-	+	-	-	+	+	*Salmonella typhi*

Note:

MA = MacConkey Agar
BA = Blood Agar
XLD = Xylose Deoxycholate Agar
G = Gram staining
CT = Catalase Test
IT = Indole Test
R = Red, B = Black
MT = Methyl- red Test
VPT = Voges-Prokauer Test
CUT = Citrate Utilization Test
MMT = Mannitol Motility Test
TSI = Triple Sugar Iron Test
+ (Positive), - (Negative)
S = Sample

Comparison of the agglutination (Widal test) and Immuno Chromatographic Test (ICT)

Typhoid fever, caused by *Salmonella typhi*, is an important cause of morbidity and mortality among the people in many developing countries. The disease may occur in all ages, with the highest incidence found particularly in children. In addition, infection is most common in young children. A rapid and reliable method for the detection of *S. typhi* is essential for early diagnosis. We used three different types of diagnostic methods for the detection of *S. typhi* from typhoid patients.

By **s**lide agglutination test, among the 35 enrichment positive samples from the suspected cases, 20 (57.14%) were positive for O antigen that indicates *S. typhi*. Among 35 samples, 15 (42.86%) from the suspected cases were negative. The result of Widal test is shown in Figure 3. Widal test has limited use because Vi and O antibody levels may rise non- specifically due to cross reactions with other *enterobacteriaceae*. Jenkins *et al*. (2009) presumed that the diagnosis of typhoid fever is usually based on clinical presentation as well as Widal test, both of which are associated with numerous limitations. Kawano *et al*. (2007) also stated that due to standardization the Widal test has some limitations. By ICT, among 35

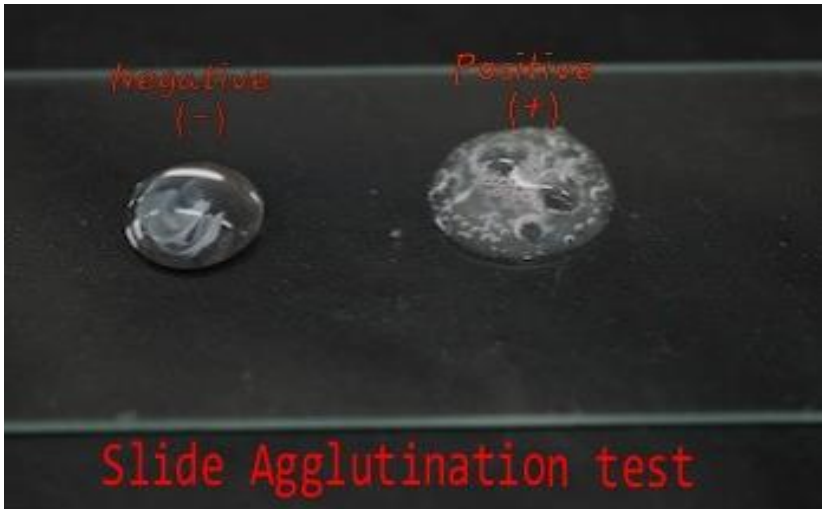

FIGURE 3: BACTERIAL AGGLUTINATION TEST (LEFT ONE CONTROL)

serum samples from the suspected cases, 31 (88.57%) were positive for IgM of *S. typhi*and 11 (31.43%) were positive for IgG (Table 2).Of the ICT positive cases, it was found that 31 (100%) cases had IgM antibody, 7 (20%) cases had both IgM and IgG antibody, whereas control samples were found to be negative for antibody by ICT method. In a study from India by Nakhla*et al*. (2011) showed similar result in which the sensitivity and specificity of ICT (IgM) was 80% and 71.4%, respectively. Abdoel *et al*. (2007) showed that the sensitivity of ICT improved with the duration of illness from 30.8% for samples collected during the first 4-5 days of illness to 45.5% for samples collected between days 7 and 9, and to 84.6% for the samples collected more than 9 days after the onset of illness.

Fig - 4 is presenting the comparative findings of three detection methods and in our study. Immuno Chromatographic Test (ICT) showed maximum positive results (31; 88.57%) followed by bacterial culture method (51.43%) and Widal test (20; 57.14%). These findings suggest that, the ICT method is more efficient and sensitive than the other two methods used for *S. typhi* detection.

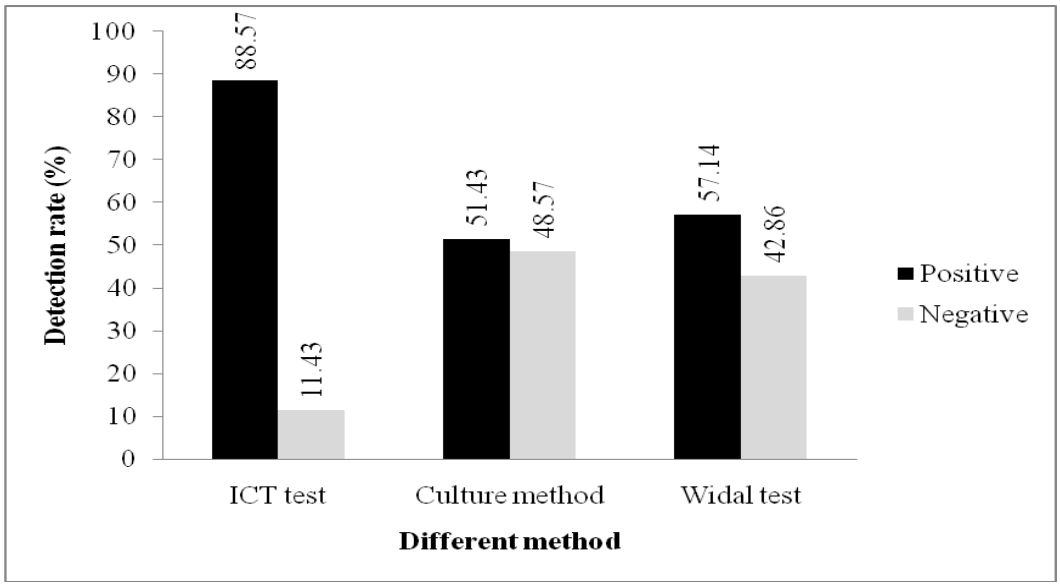

FIGURE 4: COMPARISON OF DIFFERENT DIAGNOSIS METHODS OF *S. TYPHI*

Jesudason *et al*. (2002) stated that ICT (Immuno Chromatographic Test) has been studied in many countries and found to be of significantly higher sensitivity and specificity. An evaluation of ICT (Typhidot) in India was found to be 100% sensitive and 80% specific compared to a blood culture as gold standard. In this study, out of 17 cultures negative typhoid cases 13 (76.47%) were positive by ICT method**. S**imilar results were also reported by Anggraini *et al*. (2004) where they showed that ICT (IgM) was positive for 66% among culture negative samples that were clinically diagnosed to be typhoid fever. On the other hand, 18 (90%) out of 20 widal positive patients were found positive for ICT. Only 2 Widal positive patients were found negative for ICT. The negative ICT in these cases were perhaps due to the antibodies which did not yet reach detectable levels in the first week of fever. In our study, out of 35, 4 cases were negative for ICT (IgM). The negative ICT in this case may be due to the inadequate antibodies production. The detailed resultsof ICT of *S. typhi*shown inTable 2.

TABLE 2: RESULTS OF ICT FOR IDENTIFICATION OF IgM ANTIBODY OF *S. TYPHI*

Sample	IgM (Positive)	IgG (Positive)	Both IgM&IgG (Positive)
Sample-1	Positive	-	-
Sample-2	Positive	-	-
Sample-3	Positive	-	-
Sample-4	Positive	Positive	Positive
Sample-5	Positive	-	-
Sample-6	Positive	-	-
Sample-7	Positive	-	-
Sample-8	Positive	Positive	Positive
Sample-9	Positive	-	-
Sample-10	Positive	-	-
Sample-11	Positive	-	-
Sample-12	Positive	Positive	Positive
Sample-13	Positive	-	-
Sample-14	Positive	-	-
Sample-15	Positive	-	-
Sample-16	Positive	-	-
Sample-17	Positive	-	-
Sample-18	Positive	-	-
Sample-19	Positive	Positive	Positive
Sample-20	Positive	-	-
Sample-21	Positive	-	-
Sample-22	Positive	Positive	Positive
Sample-23	Positive	-	-
Sample-24	Positive	-	-
Sample-25	Positive	-	-
Sample-26	Positive	Positive	Positive

Sample-27	Positive	-	-
Sample-28	Positive	-	-
Sample-29	-	Positive	-
Sample-30	Positive	-	-
Sample-31	-	Positive	-
Sample-32	-	Positive	-
Sample-33	Positive	-	-
Sample-34	-	Positive	-
Sample-35	Positive	Positive	Positive

Note:

IgM - Immunoglobin M (antibody)

IgG – Immunoglobin G (antibody

Though Widal test is performed widely, it has some limitation**s** due to poor standardization and difficulty in interpretation on a single sample. Considering the practical situation of laboratory diagnosis, detection of Salmonella antibodies by ICT has been found to be quite reliable, easy to perform and a good adjunct to clinical suspect in early days of fever. The blood or serum culture though less sensitive and technically demanding is the gold standard method for diagnosis of typhoid fever but ICT appears to be highly sensitive, specific and superior to culture and widal method.

Drug sensitivity of Salmonella typhi

In this study, most of the isolates (88.88%) of *S. typhi* were found resistant to amoxicillin because of beta lactamase production (Fig-5). A similar study was reported by Mastsumoto*et al*. (1999) in which isolates were found resistant to amoxicillin due to beta lactamase. About 94.44% of the isolates showed resistance to cefixime**.** 83.33% of the isolates showed susceptibility to amikacin similar to the report about *in vitro* activity of gentamicin and amikacin against *Salmonella typhi* by Mandal*etal*. (2009). In this study, 77.77% of the isolates showed susceptibility against azithromycin. Same has been reported that more than 99% of the isolates of *Salmonella typhi* were susceptible to azithromycin in another study by *Parry et al*. (2015). In this study, 55.55% of the isolates of

S. typhi were found resistant to ceftriaxone. As consequences of extensive use of

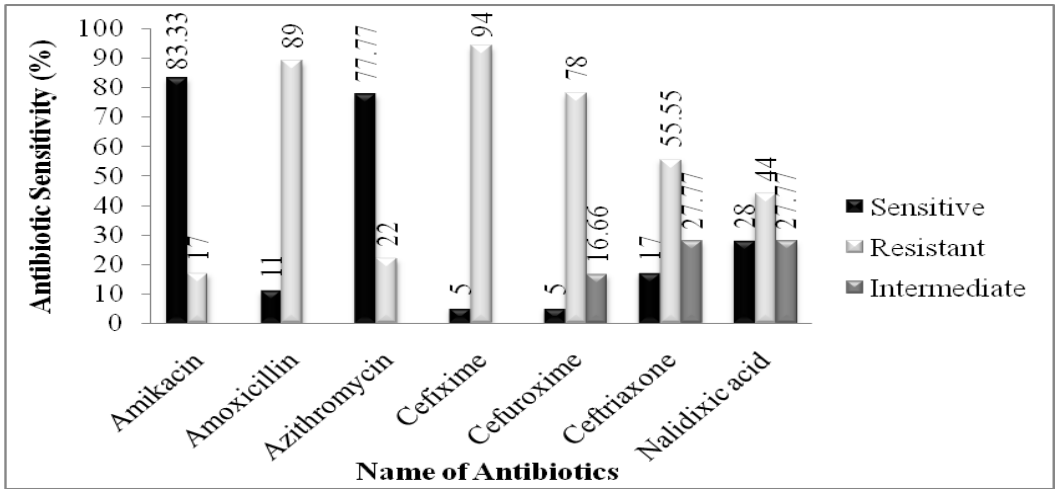

FIGURE 5: ANTIBIOTIC SENSITIVITY PATTERN (%) OF *SALMONELLA TYPHI* ON MH AGAR

ceftriaxone and other third generation cephalosporin, resistance is being reported with increasing frequency all over the world. Another study also reported (Saha 1999) about the high incidence of ceftriaxone resistant strain of *S. typhi* in Bangladesh. Most of the isolates (80%) also showed resistance to cefuroxime. A comparative study reported about the prevelance and antibiotic susceptibility pattern of *Salmonella typhi* among patients attending a military hospital in Minaa showed that most of the isolates were resistant to cefuroxime (Adabara 2012). In our study, 27.77% of the isolates were susceptible to nalidixic acid. Nalidixic acid susceptibility test to screen ciprofloxacin resistance in *Salmonella typhi* showed similar result in a study where about 34 out of 95 isolates showedsusceptibily to nalidixic acid (Kapil 2002). In the present study, 12 out of 18 culture positive samples showed multidrug resistance (Table-3). Similar findings were reported by Rao*et al*. (1993) who showed 80 out of 102 samples were multidrug resistance.

The summary of antibiotic sensitivity test of *Salmonella typhi* against several commercial antibiotic discs is shown in Table-3. Among 18 culture positive samples, most of the samples were found susceptible to Amikacin and Azithromycin but resistant to Cefixime, Cefurixime, Ciftriaxoneand Nalidixic acid. Only twelve samples were found multi-drug resistant (MDR).

TABLE 3: ANTIBIOTIC SENSITIVITY TEST OF *SALMONELLA TYPHI* AGAINST SOME COMMERCIAL ANTIBIOTIC DISCS

Sample	Amikacin	Amoxicillin	Azithromycin	Cefixime	Cefuroxime	Ceftriaxone	Nalidixic Acid	MDR
S-1	S	R	S	S	R	S	S	_
S-2	S	R	S	R	R	R	I	MDR
S-3	S	R	S	R	R	I	R	MDR
S-4	S	R	S	R	R	R	R	MDR
S-6	S	R	S	R	I	R	I	_
S-7	R	R	R	S	R	R	R	MDR
S-8	S	R	S	R	I	R	S	_
S-10	S	R	S	R	R	I	R	MDR
S-12	S	R	S	R	R	R	R	MDR
S-14	S	R	R	R	R	R	I	MDR
S-15	S	R	S	R	S	R	R	MDR
S-18	R	R	R	S	R	R	R	MDR
S-20	S	S	S	R	R	R	S	_
S-24	S	R	S	R	R	R	R	MDR
S-25	S	R	S	S	R	R	I	_
S-26	R	R	R	S	I	R	R	MDR
S-30	S	R	S	R	R	R	I	MDR
S-33	S	S	S	R	R	I	R	_

Note:
S = Sample
S = Sensitive
I = Intermediate
R = Resistant
MDR = Multi Drug Resistant

Our findings represent that socio-demographical conditions contributes to the transmission of typhoid fever and shows directions for the early diagnosis and management of this disease. It is concluded that detection rate of antibody by ICT method is quite satisfactory, so this test can be applicable for field level use. Efforts should be made to establish antibody (IgM) detection from whole blood by ICT method at field level, especially in the endemic areas of Bangladesh. ICT

can be a suitable method for rapid diagnosis of typhoid fever. However, there are some drawbacks in ICT method due to inadequate antibodies production which could not be detected by ICT. Sensitivity and specificity of ICT in suspected typhoid cases were found much higher than other methods.

DECLARATION

The authors hereby declare that the research findings presented here has been conducted by them in the Department of Genetic Engineering and Biotechnology, University of Chittagong. The contents of the paper were not published before or submitted for publication in any other journal.

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