The Pharma Innovation Journal 2017; 6(1): 93-97

www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2017; 6(1): 93-97

www.thepharmajournal.com
Received: 17-11-2016
Accepted: 18-12-2016

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Study of antibiotic sensitivity pattern of *Salmonella typhi* and *Salmonella paratyphi* isolated from blood samples in Dhaka city

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Abstract
The purpose of this study was to find out the prevalence and antibiotic sensitivity pattern of *Salmonella typhi* and *Salmonella paratyphi* isolated from blood samples of typhoid suspected patients of both sexes in Dhaka city, Samples were inoculated on MacConkey agar media and Blood agar media for primary identification of *Salmonella* species. Depending on colony formation, pigmentation, elevation and margins, colonies were presumably identified. Then the presumed isolates were further tested for more confirmation. All isolates were examined through biochemical tests and susceptibility test was performed by using different antimicrobial agents. Out of total 350 samples 123(35.14%) were positive for *Salmonella typhi* and *Salmonella paratyphi*, among the positive samples 112 (91%) were *Salmonella typhi* and 11 (9%) were *Salmonella paratyphi*. The growth positive rate in the samples of the two genders for *Salmonella typhi*- Male: 64% and Female: 36%, and for *Salmonella paratyphi* male- 63.6% and female- 36.4%. The samples of *Salmonella typhi* were most sensitive to Ceftriaxone, Ciprofloxacin and Gentamycin followed by, Cefixime, Cotrimoxazole, Chloramphenicol and Ampicillin and was least sensitive to Nalidixic acid followed by Azithromycin. *Salmonella paratyphi* was found to be most sensitive to Cefixime, Ceftriaxone, Ciprofloxacin and Gentamycin followed by Chloramphenicol, Cotrimoxazole and Ampicillin and was least sensitive to both Nalidixic acid and Azithromycin. This study showed that in the city of Dhaka males are more likely to be affected by typhoid fever than females. This study also showed the percentages of patients being affected by *Salmonella typhi* are more than *Salmonella paratyphi*.

Keywords: Blood samples, biochemical tests, antibiotic-susceptibility

1. Introduction
Typhoid fever is very common in the developing countries of the world like Bangladesh. It is caused by *Salmonella typhi*, a Gram-negative bacterium. A very similar but often less severe disease paratyphoid is caused by *Salmonella paratyphi* [1, 2]. *Salmonella paratyphi* is the most common cause of paratyphoid fever. Infective dose of paratyphoid bacilli is relatively larger than typhoid bacilli and therefore water borne spread of paratyphoid fever is rare [3, 4]. Most of the incidents are due to ingestion of contaminated food stuffs. Modes of infections and development of carrier states are almost the same as typhoid fever [5].

Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract and can spread the infection directly to other people by contaminating food or water. Travelers visiting developing countries are at greatest risk for getting typhoid fever [6].

On a global scale, at least 16–20 million cases of typhoid fever occur annually, resulting in approximately 600,000 deaths. The disease may occur in all ages, with the highest incidence found particularly in children [7].

Since all the signs and symptoms of typhoid fever are nonspecific, a definitive diagnosis of the disease depending on clinical presentation alone is very difficult [8]. Therefore, laboratory-based investigations are essential for supporting the diagnosis of typhoid fever. The "gold standard" for diagnosis of typhoid fever is the isolation of *Salmonella typhi* from appropriate samples including blood, bone marrow aspirates, stool and urine [9, 10].

The purpose of this study was to find out the prevalence and antibiotic sensitivity pattern of *Salmonella typhi* and *Salmonella paratyphi* isolated from collected blood samples of both males and females in Dhaka city.

2. Materials and Methods

2.1 Sample Collection

All samples were collected from Local blood donation centers of Dhaka. Samples of patients those had clinical symptoms of microbial infection were collected. Samples of both sexes were collected. Blood samples were collected according to WHO guidelines. The samples were ordered by microbiology department of Prime Asia University for research purpose only. To use the samples for research all the guidelines of the university were followed by the authors.

2.2 Study period

8th September 2015- 20th January, 2016

2.3 Study Site

Prime Asia University, Dhaka

2.4 Samples quantity and type of specimen

A total of 350 clinical isolates were tested from patients. The specimen type that included in this study was blood.

2.5 Type of patients

Both male & female

Male= 175, Female= 175

2.6 Methods and working procedure

2.6.1 Sterilization:

All the media were sterilized (for 15 minutes) by using autoclave. Glass materials sterilized at 180 degree Celsius for 1 hour in a hot air oven prior to use. All solutions were sterilized under the same condition.

2.6.2 Bactec technique

After collection the bottles were put in the Bactec machine where it was incubated at 37 °C and agitated continuously. In case of unloading positive bottle; when there is any growth, both the machine and the computer will indicate the growth by alarm message on the computer screen.

2.6.3 Microbiological culture media

Two culture media were used in this study. These are MacConkey agar and Blood agar.

Table 1: Colony characteristics of Salmonella species on different agar media

Name of pathogen	Colony characteristics on MacConkey agar media	Colony characteristics on Blood Agar media
Salmonella	Circular, low convex, smooth, translucent, colorless due to absence of lactose fermentation.	Red colonies, some with black centers. The agar itself will turn red due to the presence of Salmonella type colonies.

2.7 Microscopic study

2.7.1 Gram staining (Morphology): Small drop of distilled water was placed on a slide. Then one loop full isolated colony was taken and smeared over the surface of slide. The smear was allowed to dry thoroughly. The smear was fixed quickly through the Bunsen flame three times. After cooling the smear was stained. Between each staining reagent the smear was washed under gently running tap water. Staining and reagent were applied as per following sequence:

1. Ammonium oxalate (Crystal violet) (60 sec)
2. Gram's iodine (60 sec)
3. 95% Ethanol (30 sec)
4. Safranin (45 sec)

Then it was air dried and observed under a compound microscope

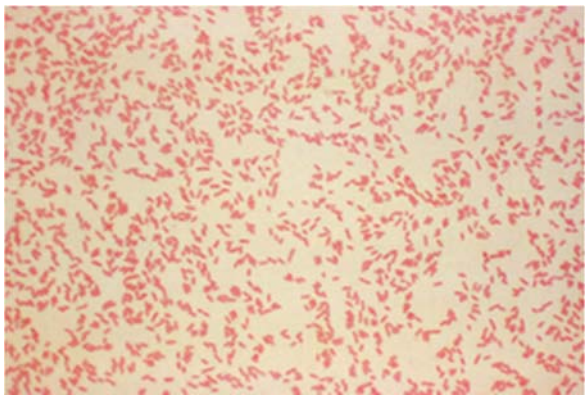

Fig 1: Gram reaction of isolates

2.8 Isolation and Identification of Microorganisms

Identification of bacterial isolate was carried out by different bio-chemical test such as Triple sugar Iron (TSI) test, Motility Indole Urease (MIU) test, Citrate utilization test, Oxidase test and Catalase test.

2.8.1 Catalase

To find out if the particular bacterial isolate is able to produce catalase enzyme, small inoculum of bacterial isolate is mixed into hydrogen peroxide solution (3%) and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. It was done by picking a pure colony by a sterile loop and immersing it in 2 drops of 3% H_2O_2 solution in a glass slide. Production of bubbles indicated the positive results [11].

2.8.2 Oxidase

The test was done to detect the presence of cytochrome oxidase in the organism. A single colony was picked up with a sterile toothpick and rubbed on to whatman filter paper that is soaked with 2-3 drops of N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride Positive result was recognized by a dark purple color within 5-10 seconds [12].

2.8.3 Motility Indole Urea (MIU)

One isolated colony was touched with a sterile wire and stabbed into semisolid agar medium very carefully down the tube, without touching the bottom. The tube was incubated at 37 °C for 18 to 24 hours. Non-motile bacteria generally give growths that are confined to the stab-line, have sharply defined margins and leave the surrounding medium clearly transparent. Motile Bacteria typically give diffuse, hazy growths that spread throughout the medium rendering it slightly opaque [13].

2.8.4 Triple Sugar Iron (TSI) test

The test was done to determine the ability of an organism to attack a specific carbohydrate incorporated in a basal growth medium, with or without the production of gas, along with the determination of possible hydrogen sulfide (H_2S) production. This test is used, in conjunction with others, for the identification of enteric pathogens. TSI agar was prepared by Lactose, Sucrose and Glucose in the concentration of 10:10:1

(i.e. 10 part Lactose (1%), 10 part Sucrose (1%) and 1 part Glucose (0.1%). One isolated colony was touched with a sterile wire and inoculated by stab-and-streak inoculation into agar very carefully. The tube was incubated at 37 °C for 18 to 24 hours [14].

2.8.5 Citrate Utilization test
Simmons citrate agar tests the ability of organisms to utilize citrate as a carbon source. Simmons citrate agar contains sodium citrate as the sole source of carbon, ammonium di-hydrogen phosphate as the sole source of nitrogen and the pH indicator bromothymol blue. If the medium turns blue, the organism is citrate positive. If there is no color change, the organism is citrate negative [15].

2.9 Determination of antibiotic Susceptibility of Salmonella isolates
Susceptibility of *Salmonella* isolates to different antimicrobial agents was measured in vitro by the Kirby-Bauer method which allowed rapid determination of the efficacy of drug by measuring the zone of inhibition that result from diffusion of the antimicrobial agent into the medium surrounding the disc. Commercially available antimicrobial discs were used for the test [16].

2.10 Antimicrobial agents used
Ampicillin-10 mcg, Azithromycin- 15 mcg, Cefixime- 5 mcg, Ceftriaxone-30 mcg, Ciprofloxacin-5 mcg, Chloramphenicol-30 mcg, Gentamycin-30 mcg, Nalidixic acid-30 mcg, Cotrimoxazole-25 mcg

2.11 Inoculation of the Mueller-Hinton agar plate with test organism
The isolated colony from the various media was inoculated on the media by spreading technique.

2.12 Application of discs to inoculated agar plates
a. The antimicrobial discs were dispensed into the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. The discs must be distributed evenly so that they are not closer than 24 mm to each other. Not more than 5 discs were used in a 100 mm plate because some of the drugs diffuse almost instantaneously. A disc should not be relocated once it has come into contact with the agar surface.
b. The plates were inverted and placed in an incubator set to 37 degree Celsius for overnight.

3. Results and Discussion
3.1 Results

Table 2: Results of biochemical tests for *Salmonella typhi* and *Salmonella paratyphi*

Etiological agents	Catalase	Oxidase	TSI	MIU	Citrate utilization
Salmonella typhi	+ve	-ve	Alkaline slant & acid with H_2S & no gas produced.	M: +ve I: -ve U: -ve	-ve
Salmonella paratyphi	+ve	-ve	Alkaline slant & acid without H_2S & gas produced.	M: +ve I: -ve U: -ve	-ve

Table 3: Percentage of Positive Result of *Salmonella* spp.

No of total specimens	No of *salmonella* spp. negative specimens	No of *salmonella spp.* positive specimens	Percentage of *salmonella spp.* positive specimens
350	227	123	35.14%

Table 4: Percentages of Etiological Agents

Etiological Agents	No. of isolates	Percentage %
Salmonella typhi	112	91 %
Salmonella paratyphi	11	9 %

Table 5: Percentages of *Salmonella typhi* positive patients according to sex

No of *Salmonella typhi* positive patient	No of male patient	% of male patient	No of female patient	% of female patient
112	72	64 %	40	36 %

Table 6: Percentages of *Salmonella paratyphi* positive patients according to sex

No of *Salmonella paratyphi* positive patient	No of male patient	% of male patient	No of female patient	% of female patient
11	7	63.6 %	4	36.4 %

Antibiotic susceptibility patterns of isolated Salmonella
After 24 hour of incubation, inoculated Muller – Hinton agar plates were observed according to Kirby–Bauer method to determine the antibiotic susceptibility.

Table 7: Antibiotic Sensitivity and Resistance Pattern for *Salmonella typhi* and *Salmonella paratyphi*

Antibiotics (conc.)	*Salmonella typhi*						*Salmonella paratyphi*				
	Sample	S	%	R	%		Sample	S	%	R	%
Cefixime (5 mcg)	112	110	98.2	2	1.9		11	11	100	0	0
Ceftriaxone (30 mcg)	112	112	100	0	0		11	11	100	0	0
Ciprofloxacin (5 mcg)	112	112	100	0	0		11	11	100	0	0

Gentamycin (30 mcg)	112	112	100	0	0		11	11	100	0	0
Ampicillin (10 mcg)	112	78	73.2	34	26.9		11	10	89.9	1	10.1
Chloramphenicol (30 mcg)	112	92	82.1	20	15.4		11	10	89.9	1	10.1
Cotrimoxazole (25 mcg)	112	95	85	17	13.5		11	9	81.8	2	18.2
Azithromycin (15 mcg)	112	11	10	101	90.3		11	2	18.2	9	81.8
Nalidixic acid (30 mcg)	112	6	5.3	106	96.1		11	1	9.1	10	90.1

S= Sensitive, R= Resistant,

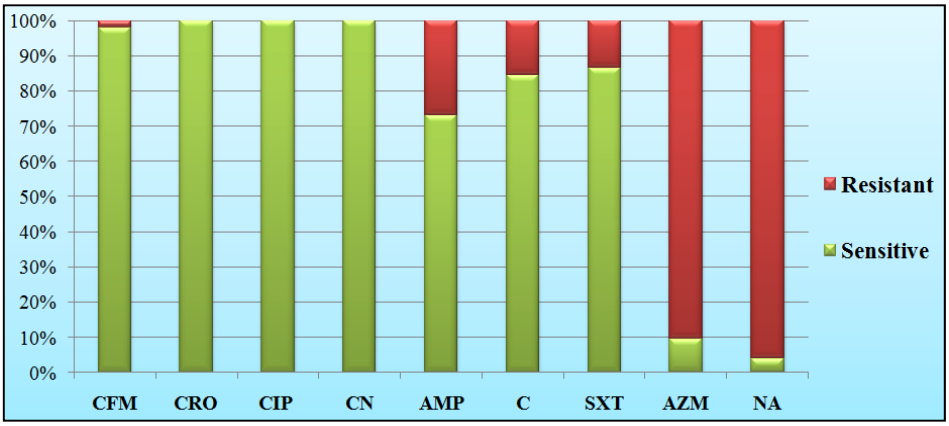

Fig 2: Antibiotic susceptibility pattern of *Salmonella typhi*

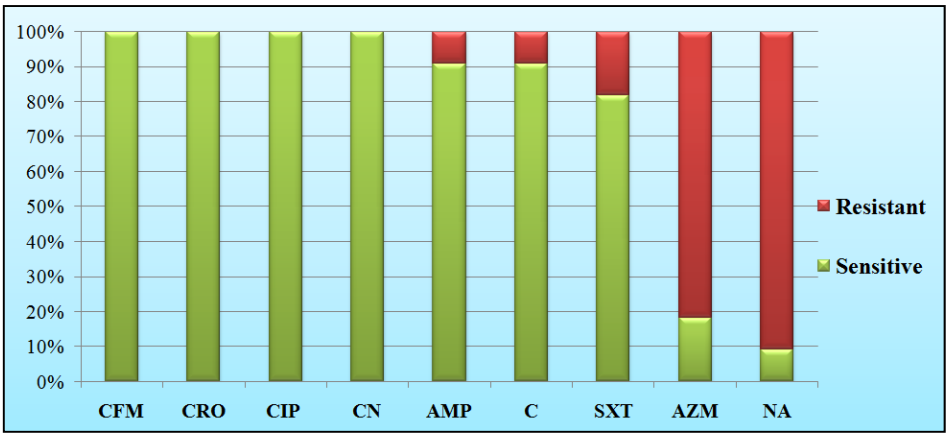

Fig 3: Antibiotic susceptibility pattern of *Salmonella paratyphi* **Green=** Sensitivity, **Red=** Resistance

3.2 Discussion

Blood samples of 350 typhoid suspect patients of both sexes were studied. Among them 123 specimens were analyzed to observe the antibiotic susceptibility because they had significant growth of *Salmonella* species.

The samples were inoculated on MacConkey agar media and Blood agar media for primary identification of *Salmonella* species. Depending on colony formation, pigmentation, elevation and margins, colonies were presumably identified. Then the presumed isolates were further tested for more confirmation. All isolates were examined through biochemical tests- TSI (Triple Sugar Iron) test, MIU (Motility Indole Urea) test, Citrate utilization test, Catalase test and oxidase test.

Salmonella typhi is believed to cause typhoid fever in most of the cases. This study also showed the same result because 112 patients of 123 patients were infected by *Salmonella typhi* and rest of the 11 patients was infected by *Salmonella paratyphi*. So the percentages of patients affected by *Salmonella typhi* and *Salmonella paratyphi* were respectively 91% and 9%.

This study has also shown that males were affected slightly more than females. Usually males remain outside of home more than females and as a result males are exposed to the contaminated food and water more than females. Both *Salmonella typhi & Salmonella paratyphi* almost similarly infected both males and females.

The samples of *Salmonella typhi* were most sensitive to Ceftriaxone, Ciprofloxacin and Gentamycin followed by, Cefixime, Cotrimoxazole, Chloramphenicol and Ampicillin and was least sensitive to Nalidixic acid followed by Azithromycin. *Salmonella paratyphi* was found to be most sensitive to Cefixime, Ceftriaxone, Ciprofloxacin and Gentamycin followed by Chloramphenicol, Cotrimoxazole and Ampicillin and was least sensitive to both Nalidixic acid and Azithromycin.

4. Conclusion

This study showed that in the city of Dhaka males are more likely to be affected by typhoid fever than females. This study also showed the percentages of patients affected by *Salmonella typhi* are more than *Salmonella paratyphi*. The samples of *Salmonella typhi* and *Salmonella paratyphi* were both sensitive to most of the antibiotics. However, to achieve more accurate information further researches should be carried out about this topic.

5. Conflict of Interest

The authors declare that there is no conflict of interest about this article with any institution.

6. Acknowledgement

Authors are grateful to the Local blood donation centers of Dhaka for providing the blood samples and Prime Asia University for providing the equipment and laboratory space for conducting this research.

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