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Determination of Antibody Titration between Clinical and Community-Based Patients for O, H, AH and BH Antigens in *Salmonella* Samples

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ABSTRACT

The aim of the study is to determine the baseline antibody titer of *Salmonella* bacteria in different positive samples with a view to establishing the significant titer for Widal agglutination test in Kashmir. The Widal test was performed on each serum. The slide agglutination test was first done and then positive samples were further subjected to tube agglutination for quantitative titration. The most commonly utilized diagnostic test for enteric fever is a Widal test, which detects agglutinating antibodies against the O, H, AH and BH antigens of *S. typhi*. The results of the Widal test showed that out of 413 samples 219 were positive for Antigen O, 165 were positive for Antigen H, 17 were positive for Antigen AH and 12 were positive for Antigen BH. The highest percentage cases were with Antigen-O whereas the lowest percentage was found in Antigen-BH. The difference between clinical and community-based patients have been studied.

Keywords: Aetiology, Antigen, Enteric fever, Morbidity, *Salmonella typhi*, Widal test.

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INTRODUCTION

The *S. typhi* genomics work published in 2015 has shown the spread of the H58 lineage into Africa from Asia, predicting that antimicrobial resistance is a problem that is likely to increase over time[1]. The work presented in this supplement should raise the awareness of typhoid fever in rural locations in Africa and follow-up studies should be conducted in other rural settings that were not sampled through TSAP. However, further studies on typhoid fever are confounded by the available diagnostic testing methods[2]. The ongoing investments by the Bill and Melinda Gates Foundation and other donors will hopefully yield better diagnostic tests that can be used at the point of care without the need for cumbersome and insensitive blood culture methods[3]. The TSAP study results show a great need for these vaccines and hopefully provide further impetus to move the agenda to make these vaccines available to the populations in the greatest need. The possible future vaccination strategies in Africa include pre-emptive, responsive (in the case of an outbreak) and food handler immunization[4].

Typhoid fever is still a deadly disease in developing countries, particularly in India. Although, the pediatric population is most affected by this disease, yet the disease is an important cause of morbidity and mortality in adult populations also. There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to *Salmonella*[5]. In brief, *Salmonella* is facultative anaerobe, gram-negative flagellated rod-shaped bacterium which is about 2-3 x 0.4-0.6 µm in size [6,7]. It is among the most commonly isolated foodborne pathogens associated with fresh fruits and vegetables.

The disease typhoid fever is an orally transmitted infectious disease caused by the bacteria *S. typhi*. It is usually caused by consuming contaminated water and food. As *S. typhi* bacteria can survive in water for days, contamination of surface water such as sewage, freshwater and groundwater act as a major etiological agent of typhoid. Defecation in open places is another notable cause of typhoid transmission. Unpacked food, cut fruits kept uncovered for some time are an important cause of contamination in most developing countries. The papaya has a neutral pH and its cut surface can support the growth of various microorganisms. Inhabiting in a congested locality or household is significantly related with typhoid fever[8]. Again, the habit of washing vegetables and compulsory use of sanitary latrine for defecation have been found to prevent typhoid[9].

Typhoid fever continues to be a global health problem, with an estimated 12 to 33 million cases occurring worldwide each year. Incidence of typhoid fever has been estimated at approximately 17 million cases with 6,00,000 associated deaths occurring annually[10]. Typhoid fever was so called because it resembled typhus. The first detailed description of typhoid was given by William Jenner in 1850. The term enteric fever was introduced in 1869. The *S. typhi* was discovered by Eberth in 1880 and was first cultured by Gafiy 1884[11]. The prevalence of typhoid fever is increasing tremendously in Kashmir region because of poor sanitation, contaminated food, and water, lack of medical awareness and unhygienic education. The aim of this study is to find out the prevalence of typhoid fever in the Kashmir valley. The samples were carried out from different categories of patients, Including males, females, and children with the age group between 5 to 80 years and were examined in a laboratory using various tests.

MATERIALS AND METHODS

Collection, processing of samples

The samples obtained from males, females, and children with the age group between 5 to 80 years were collected for epidemiological investigations in Kashmir province state of Jammu and Kashmir- India. The total sample size of 676 was collected from various hospitals, clinical laboratories, and communities.

Identification

The standard Gram Staining, Slide test, and Tube test were performed for identification of *Salmonella* as per standard methodology.

Widal test

The Widal tube agglutination test was done on all sera collected from different laboratory diagnosis methods using commercially available antigens (Tulip diagnostics, India). 0.4ml of two-fold serially diluted patient's sera (1:20 to 1:1280) in 0.9% normal saline were tested by adding an equal volume of antigen. A negative control was included in each batch of the test. The tubes were incubated at 37ºC for 24 h and then examined for agglutination[12]and higher for anti TH antibodies were taken as a cut of value to indicate a recent infection of typhoid fever.

RESULT AND DISCUSSION

Widal Baseline titre

The laboratory diagnosis of typhoid fever depends upon either the isolates of *S. typhi* or the detection of titers of agglutinating serum antibodies against the anti - O, anti - H, anti-AH and anti BH of *S. typhi*. The titer value of *S. typhi* - O for the two groups, includes patients group table 1 and community control group table 2 shows the serum titer values. The titer value of *S. typhi* H for the two groups includes patient group table 3 and community control group table 4 shows the serum titer values. The titer value of *S. typhi* AH for the two groups includes patient group table 5 and community control group table 6 shows the serum titer values. The titer value of *S. typhi* BH for the two groups includes patient group table 7 and community control group table 8 shows the serum titer values.

Table 1. Serum titre value among patients against O

S. No.	Age Group (in years)	Serum titre (n = 400)						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
1	<1 to 10 (33)	6	9	7	10	1	--	--
2	11 to 20 (40)	7	8	12	11	2	--	--
3	21 to 30 (52)	2	3	31	13	3	--	--
4	31 to 40 (146)	3	5	94	30	9	5	--
5	41 to 50 (115)	1	3	82	20	6	3	--
6	51 to 60 (10)	2	1	5	1	1	--	--
7	61 to 70 (03)	1	2	--	--	--	--	--
8	71 to 80 (01)	--	--	1	--	--	--	--
9	> 80 (-)	--	--	--	--	--	---	--
Total (n = 400)		22(5.5)	31(7.75)	232(58)	85(21.25)	22(5.5)	8(2)	

Table 1 shows that the age group of <1 to 10 the highest number of samples i.e., 10 out of 33 gives a titer value of 1:80. And in the age group of 11 to 20 the highest number of samples i.e., 12 out of 40 gives a titer value of 1:40, in the age group of 21 to 30 the highest number of samples i.e., 31 out of 52 gives a titre value of 1:40, in the age group of 31 to 40 the highest number of samples i.e., 94 out of 146 gives a titer value of 1:40, in the age group of 41 to 50 the highest number of samples i.e., 82 out of 115 gives a titer value of 1:40, in the age group of 51 to 60 the highest number of samples i.e., 5 out of 10 gives a titer value of 1:40, in the age group of 61 to 70 the highest number of samples i.e., 2 out of 3 gives a titer value of 1:20 and in the age group of 71 to 80 the highest number of samples i.e., 1 out of 1 gives a titer value of 1:40 . The highest number of cases falls on the serum titer of 1:40 (232 cases), followed by 1:80 (85), 1:20 (31), 1:10 (22), 1:160 (22) and 1:320 (8).

Table 2. Serum titer value among Community controls against O

S. No.	Age Group (in years)	Serum titre (n = 400)						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
1	<1 to 10 (20)	2	5	11	2	--	--	--
2	11 to 20 (27)	4	8	6	9	--	--	--
3	21 to 30 (45)	3	4	15	19	2	2	--
4	31 to 40 (135)	6	12	46	62	8	1	--
5	41 to 50 (110)	1	9	43	51	4	2	--
6	51 to 60 (59)	5	6	38	8	2	--	--
7	61 to 70 (02)	1	1	--	--	--	--	--
8	71 to 80 (01)	1	--	--	--	--	--	--
9	> 80 (01)	--	--	--	1	--	---	--
Total(n = 400) %		23(5.75)	45(11.25)	159(39.75)	152(38)	16(4)	5(1.25)	---

Table 2 shows that the age group of <1 to 10 the highest number of samples i.e., 11 out of 20 gives a titer value of 1:40. And in the age group of 11 to 20 the highest number of samples i.e., 9 out of 27 gives a titer value of 1:80, in the age group of 21 to 30 the highest number of samples i.e., 19 out of 45 gives a titre value of 1:80, in the age group of 31 to 40 the highest number of samples i.e., 62 out of 135 gives a titer value of 1:80, in the age group of 41 to 50 the highest number of samples i.e., 51 out of 110 gives a titer value of 1:80, in the age group of 51 to 60 the highest number of samples i.e., 38 out of 59 gives a titer value of 1:40, in the age group of 61 to 70 the highest number of samples i.e., 1 out of 2 gives a titre value of 1:10 and 1:20 and in the age group of 71 to 80 the highest number of samples i.e., 1 out of 1 gives a titer value of 1:10 and in the age group of > 80 the highest no. of samples is 1 out of 1 gives a titer value of 1:80. The highest number of cases falls on the serum titer of 1:40 (159 cases), followed by 1:80 (152), 1:20 (45), 1:10 (23), 1:160 (16) and 1:320 (5).

Table 3. Serum titre value among patients against H

S. No.	Age Group (in years)	Serum titre (n = 400)						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
1	<1 to 10 (33)	4	7	13	9	--	--	--
2	11 to 20 (40)	9	11	8	11	1	--	--
3	21 to 30 (52)	1	4	28	18	1	--	--
4	31 to 40 (146)	5	12	45	78	5	1	--
5	41 to 50 (115)	6	8	19	74	7	1	--
6	51 to 60 (10)	1	3	2	3	1	--	--
7	61 to 70 (03)	--	1	1	1	--	--	--
8	71 to 80 (01)	--	--	--	1	--	--	--
9	> 80 (-)	--	--	--	--	--	---	--
Total(n = 400)		26(6.5)	46(11.15)	116(29)	195(48.75)	15(3.75)	2(0.5)	

Table 3 shows that the age group of <1 to 10 the highest number of samples i.e., 13 out of 33 gives a titer value of 1:40. And in the age group of 11 to 20 the highest number of samples i.e., 11 out of 40 gives a titre value of 1:20 and 1:80 in the age group of 21 to 30 the highest number of samples i.e., 28 out of 52 gives a titer value of 1:40, in the age group of 31 to 40 the highest number of samples i.e., 78 out of 146 gives a titer value of 1:80, in the age group of 41 to 50 the highest number of samples i.e., 74 out of 115 gives a titer value of 1:80, in the age group of 51 to 60 the highest number of samples i.e., 3 out of 10 gives a titre value of 1:20 and 1 :80, in the age group of 61 to 70 the highest number of samples i.e., 1 out of 3 gives a titre value of 1:20,1:40 and 1 :80 in the age group of 71 to 80 the highest number of samples i.e., 1 out of 1 gives a titer value of 1:80 . The highest number of cases falls on the serum titre of 1:80 (195 cases), followed by 1:40 (116), 1:20 (46), 1:10 (26) ,1:160 (15) and 1:320(2).

Table 4. Serum titer value among Community controls against H

S. No.	Age Group (in years)	Serum titre (n = 400)						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
1	<1 to 10 (20)	--	2	8	10	--	--	--
2	11 to 20 (27)	3	11	7	5	1	--	--
3	21 to 30 (45)	5	3	27	9	1	--	--
4	31 to 40 (135)	10	22	35	60	7	1	--
5	41 to 50 (110)	6	18	32	47	6	1	--
6	51 to 60 (59)	7	14	21	16	1	--	--
7	61 to 70 (02)	1	--	1	--	--	--	--
8	71 to 80 (01)	--	--	--	1	--	--	--
9	> 80 (01)	--	--	1	--	--	---	--
Total(n = 400) %		32(8)	70(17.5)	132(33)	148(37)	16(4)	2(0.5)	---

Table 4 shows that the age group of <1 to 10 the highest number of samples i.e., 10 out of 20 gives a titer value of 1:80. And in the age group of 11 to 20 the highest number of samples i.e., 11 out of 27 gives a titer value of 1:20, in the age group of 21 to 30 the highest number of samples i.e., 27 out of 45 gives a titre value of 1:40, in the age group of 31 to 40 the highest number of samples i.e., 60 out of 135 gives a titer value of 1:80, in the age group of 41 to 50 the highest number of samples i.e., 47 out of 110 gives a titer value of 1:80, in the age group of 51 to 60 the highest number of samples i.e., 21 out of 59 gives a titer value of 1:40, in the age group of 61 to 70 the highest number of samples i.e., 1 out of 2 gives a titre value of 1:10 and 1:40 and in the age group of 71 to 80 the highest number of samples i.e., 1 out of 1 gives a titer value of 1:80 and in the age group of >80 the highest no. of samples i,e 1 0ut of 1 gives a titer value of 1:40. The highest number of cases falls on the serum titer of 1:80 (148 cases), followed by 1:40 (132), 1:20 (70), 1:10 (32), 1:160 (16) and 1:320 (2).

Table 5. Serum titre value among patients against AH

S. No.	Age Group (in years)	Serum titre (n = 400)						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
1	<1 to 10 (33)	5	6	12	10	--	--	--
2	11 to 20 (40)	9	13	11	7	--	--	--
3	21 to 30 (52)	4	8	18	20	1	1	--
4	31 to 40 (146)	8	9	40	81	7	1	--
5	41 to 50 (115)	6	7	29	65	6	2	--
6	51 to 60 (10)	1	2	4	2	1	--	--
7	61 to 70 (03)	1	1	1	--	--	--	--
8	71 to 80 (01)	--	--	--	1	--	--	--
9	> 80 (-)	--	--	--	--	--	---	--
Total (n = 400)		34(8.5)	46(11.5)	115(28.75)	186(46.5)	15(3.75)	4(1)	

Table 5 shows that the age group of <1 to 10 the highest number of samples i.e., 12 out of 33 gives a titer value of 1:40. And in the age group of 11 to 20 the highest number of samples i.e., 13 out of 40 gives a titer value of 1:20, in the age group of 21 to 30 the highest number of samples i.e., 20 out of 52 gives a titre value of 1:80, in the age group of 31 to 40 the highest number of samples i.e., 81 out of 146 gives a titer value of 1:80, in the age group of 41 to 50 the highest number of samples i.e., 65 out of 115 gives a titer value of 1:80, in the age group of 51 to 60 the highest number of samples i.e., 4 out of 10 gives a titer value of 1:40, in the age group of 61 to 70 the highest number of samples i.e., 1 out of 3 gives a titre value of 1:10,1:20 and 1:40 and in the age group of 71 to 80 the highest number of samples i.e., 1 out of 1 gives a titer value of 1:80. The highest number of cases falls on the serum titre of 1:80 (186 cases), followed by 1:40 (115), 1:20 (46), 1:10 (34), 1:10 (27) , 1:160 (15) and 1 :320(4).

Table 6. Serum titer value among Community controls against AH

S. No.	Age Group (in years)	Serum titre (n = 400)						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
1	<1 to 10 (20)	3	7	8	2	--	--	--
2	11 to 20 (27)	5	8	10	3	1	--	--
3	21 to 30 (45)	7	9	12	16	1	--	--
4	31 to 40 (135)	6	20	34	69	4	2	--
5	41 to 50 (110)	4	15	26	58	6	1	--
6	51 to 60 (59)	2	10	19	25	3	--	--
7	61 to 70 (02)	--	--	1	1	--	--	--
8	71 to 80 (01)	--	--	--	1	--	--	--
9	> 80 (01)	--	--	1	--	--	---	--
Total(n = 400) %		27(6.75)	69(17.25)	111(27.75)	175(43.75)	15(3.75)	3(0.75)	---

Table 6 shows that the age group of <1 to 10 the highest number of samples i.e., 8 out of 20 gives a titer value of 1:40. And in the age group of 11 to 20 the highest number of samples i.e., 10 out of 27 gives a titer value of 1:40, in the age group of 21 to 30 the highest number of samples i.e., 16 out of 45 gives a titre value of 1:80, in the age group of 31 to 40 the highest number of samples i.e., 69 out of 135 gives a titer value of 1:80, in the age group of 41 to 50 the highest number of samples i.e., 58 out of 110 gives a titer value of 1:80, in the age group of 51 to 60 the highest number of samples i.e., 25 out of 59 gives a titer value of 1:80, in the age group of 61 to 70 the highest number of samples i.e., 1 out of 2 gives a titre value of 1:40 and 1: 80, in the age group of 71 to 80 the highest number of samples i.e., 1 out of 1 gives a titer value of 1:80 and in the age group >80 the highest no. of samples is 1 out of 1 gives a titre value of 1:40. The highest number of cases falls on the serum titer of 1:80 (175 cases), followed by 1:40 (111), 1:20 (69), 1:10 (27), 1:160 (15) and 1:320 (3).

Table 7. Serum titre value among patients against BH

S. No.	Age Group (in years)	Serum titre (n = 400)						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
1	<1 to 10 (33)	10	18	4	1	--	--	--
2	11 to 20 (40)	14	16	6	4	--	--	--
3	21 to 30 (52)	8	15	16	12	1	--	--
4	31 to 40 (146)	20	56	35	32	3	--	--
5	41 to 50 (115)	16	44	29	22	2	2	--
6	51 to 60 (10)	4	2	2	1	1	--	--
7	61 to 70 (03)	--	1	1	1	--	--	--
8	71 to 80 (01)	--	--	--	1	--	--	--
9	> 80 (-)	--	--	--	--	--	---	--
Total(n = 400)		72(18)	152(38)	93(23.25)	74(18.5)	7(1.75)	2(0.5)	

Table 7 shows that the age group of <1 to 10 the highest number of samples i.e., 18 out of 33 gives a titer value of 1:20. And in the age group of 11 to 20 the highest number of samples i.e., 16 out of 40 gives a titer value of 1:20, in the age group of 21 to 30 the highest number of samples i.e., 16 out of 52 gives a titre value of 1:40, in the age group of 31 to 40 the highest number of samples i.e., 56 out of 146 gives a titer value of 1:20, in the age group of 41 to 50 the highest number of samples i.e., 44 out of 115 gives a titer value of 1:20, in the age group of 51 to 60 the highest number of samples i.e., 4 out of 10 gives a titer value of 1:10, in the age group of 61 to 70 the highest number of samples i.e., 1 out of 3 gives a titre value of 1:20, 1:40 and 1:80 and in the age group of 71 to 80 the highest number of samples i.e., 1 out of 1 gives a titre value of 1:80. The highest number of cases falls on the serum titer of 1:20 (152 cases), followed by 1:40 (93), 1:80 (74), 1:10 (72), 1:160 (7) and 1:320 (2).

Table 8. Serum titer value among Community controls against BH

S. No.	Age Group (in years)	Serum titre (n = 400)						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
1	<1 to 10 (20)	8	6	4	2	--	--	--
2	11 to 20 (27)	11	9	3	4	--	--	--
3	21 to 30 (45)	16	12	8	8	1	--	--
4	31 to 40 (135)	14	20	42	54	4	1	--
5	41 to 50 (110)	10	24	33	37	5	1	--
6	51 to 60 (59)	7	19	14	18	1	--	--
7	61 to 70 (02)	--	--	1	1	--	--	--
8	71 to 80 (01)	--	--	--	1	--	--	--
9	> 80 (01)	--	--	1	--	--	---	--
Total (n = 400) %		66(16.5)	90(22.5)	106(26.5)	125(31.25)	11(2.7)	2(0.5)	---

Table 8 shows that the age group of <1 to 10 the highest number of samples i.e., 8 out of 20 gives a titer value of 1:10. And in the age group of 11 to 20 the highest number of samples i.e., 11 out of 27 gives a titer value of 1:10, in the age group of 21 to 30 the highest number of samples i.e., 16 out of 45 gives a titre value of 1:10, in the age group of 31 to 40 the highest number of samples i.e., 54 out of 135 gives a titer value of 1:80, in the age group of 41 to 50 the highest number of samples i.e., 37 out of 110 gives a titer value of 1:80, in the age group of 51 to 60 the highest number of samples i.e., 19 out of 59 gives a titer value of 1:20, in the age group of 61 to 70 the highest number of samples i.e., 1 out of 2 gives a titre value of 1:40 and 1:80, in the age group of 71 to 80 the highest number of samples i.e., 1 out of 1 gives a titer value of 1:80 and in the age group >80 the highest no. of samples i,e 1 out of 1 gives a titre value of 1:40. The highest number of cases falls on the serum titer of 1:80 (125 cases), followed by 1:40 (106), 1:20 (90), 1:10 (66), 1:160 (11) and 1:320 (2).

For the diagnosis of typhoid fever Widal test is the second most widely used after blood culture [13]. A study showed a single Widal test titer against 'O' antigen is 1:160[14]. Another study shows the widal baseline titer for anti-O is more or equal to 1:160 and for anti-H is more or equal to 1:320 and also it showed baseline titre for *S. typhi* is 1:160 for 'O' and 1:160 for 'H'[13]. A recent study showed the cut off value for anti-O titer is 1:80 [15]. Besides, our previous studies[16,17] concluded that out of 192 positive cases (IgM positive for typhidot), 189 were found positive for IgM and 3 were negative by ELISA method. Similarly, out of 40 positive cases (IgG positive for typhidot), 38 were found positive for IgM and 2 were negative by ELISA method. Thus the study found that the prevalence of typhoid was found higher in females than in males and children, and the other study concluded that in 263 normal individuals, C-reactive protein was below 5 mg/dl. In 168 typhoid positive samples the C-reactive protein was found to be higher than 6 mg/dl whereas in 245 typhoid positive samples the C-reactive protein was found to be less than 6 mg/dl.

CONCLUSION

The Widal baseline titer in this locality, in order to determine a threshold above which the antibody titer is considered as significant. The maximum percentage of antigens were detected of somatic antigen which was greater than flagellar antigen. There is a wider scope of community-based patients also in Kashmir province for the reason that of unawareness.

Conflict of interest

The authors declare no conflict of interest

REFERENCES

1. Wong VK, Baker S, Pickard DJ, et al. Phylogeographical analysis of the dominant multidrug-resistant H58 clade of *Salmonella typhi* identifies inter- and intracontinental transmission events. Nat Genet 2015; 47:632–9.
2. Keddy KH, Sooka A, Letsoalo ME, et al. Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-Saharan African sites. Bull World Health Organ 2011; 89:640–7.
3. Andrews JR, Ryan ET. Diagnostics for invasive *Salmonella* infections: current challenges and future directions. Vaccine 2015; 33:C8–15.
4. Date KA, Bentsi-Enchill A, Marks F, Fox K. Typhoid fever vaccination strategies. Vaccine 2015; 33:C55–61.
5. Bhunia AK., Foodborne microbial pathogens: Mechanisms and pathogenesis. United States of America: Springer Science + Business Media, LLC. 2008.
6. Yousef AE., Carlstrom C., *Salmonella*. In Yousef, A. E. and Carstrom, C. (Eds.). Food microbiology: A laboratory manual. 2003; 167-205.
7. Montville TJ., Matthews KR., Food microbiology: an introduction. 2nd ed. Washington, USA: ASM Press.Mweu E, English M. 2008.
8. Hosoglu S., Celen M., Geyik MF., Risk factors for typhoid fever among adult patients in Diyarbakir, Turkey. Epidemiol Infect. 2006; 134:612-6.
9. Ram PK., Naheed A., Brooks WA et al. Risk factors for typhoid fever in a slum in Dhaka, Bangladesh. Epidemiol Infect. 2007; 135:458-65.
10. Gerald L., Mandel., John E., Bennett., Rapheal D., Principles and Practice of Infectious Diseases, 5th edition, Volume-2, Churchill Living Stone. 2000; (210):2344-2363.
11. Wilson JC., A treatise on the continued fevers, Wood, New York. 1881.
12. Corkill G., Rapley R., The Manipulation of Nucleic Acids: Basic Tools & Techniques in Molecular Bio Methods Handbook Second Edition. Ed: Walker J M, Rapley R (NJ: Humana Press). 2008.
13. Drancourt M., Bollet C., Carlioz A., Martelin R., Gayral JP., Raoult D., 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J Clin Microbiol. 2000; 38:3623–3630.
14. Mignard S., Flandrois JP., 16S rRNA sequencing in routine bacterial identification: a 30-month experiment. J Microbiol Methods. 2006; 67:574–581.
15. Fox GE., Wisotzkey 1D., Jurtshuk, P., (1992). How close is close: 16s rRNA sequence identity may not be sufficient to guarantee species identity. Int J Syst Bacteriol. 1992; 42, 166-170.
16. Nisar AW., Veeramanikandan V., Jaianand K., Balaji P., Evaluation of clinical profile and response of various antibiotics in Typhoid fever. International Journal of Medical Science and Diagnosis Research. 2018; 2(4): 166-171.
17. Veeramanikandan V., Nisar AW., Jaianand K., Balaji P., Prevalence and different laboratory diagnosis methods for the determination of typhoid fever in Kashmir region of Jammu and Kashmir, India. Journal of Medical science and Clinical Research. 2018; 06(08): 861-866.