

Research Article

Antigenic Detection of *Salmonella* Infection among Pediatric Patients with Acute Gastroenteritis

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Abstract:

Background: Diarrheal diseases are one of the social problems in developing countries. The pathogens commonly associated with childhood diarrhea are *Salmonella*, *Clostridium difficile*, *Shigella*, *Yersinia* and *Escherichia coli* but the highest attack rate for salmonellosis in infancy.

Objective: The aim of the study was to evaluate the presence of *Salmonella* antigen in acute gastroenteritis in children admitted to a pediatric hospital.

Material and Methods: The study was performed on freshly collected stool samples among 94 acute diarrheal children below two years admitted to AL-Khadymia and AL-Elweya pediatric hospitals from May 2015 to January 2016. A questionnaire was completed for each patient's name, age, gender, clinical data like fever, nausea, vomiting, and abdominal pain. The criteria included hemorrhagic fresh stool sample in addition to containing parasite agent. Fresh stool samples were tested by immunochromatographic assay for antigenic detection of *Salmonella*.

Results: *Salmonella* antigen identified in five stool samples one for male and four for females. All pediatric patients show fever, vomiting and abdominal pain, while the stool consistency distributed to 75.5% watery and 24.5% loosely. Stool samples show 69.1% with blood and 39.9% without blood, 16.9% with pus and 83.1% without pus, 83% with mucous and 17% without mucous. Four cases with giardiasis and 24 cases with entamebiasis and 14 cases with cyst of *E. histolytica* or *G. lamblia* in addition to absence the parasites ova in all stool samples.

Conclusion: *Salmonella* antigen present in five stool samples, all the patients show vomiting, fever, abdominal pain, 65 cases with blood in comparison with, 29 without blood 15 cases with pus in comparison with 79 without pus. 78 cases with mucous in comparison with, 16 without mucous, four cases with goddesses and 24 cases with entamebiasis, 14 cases with cyst of *E. histolytica* or *G. lamblia* in addition to absence the parasites ova in all stool samples.

Keywords: Acute diarrhea, *Salmonella*, Abdominal pain, Immunochromatography.

1. Introduction

World health organization (WHO) defined diarrhea as having three or more loose or liquid stool per day (1). While, Acute diarrhea, defined as increased the defecation till or more times per day and may be accompanied by nausea, vomiting, abdominal pain, malnutrition (2). Acute gastroenteritis diagnosable through clinical manifestation which included diarrhea, vomiting, fever, and dehydration and considered as one of the important diseases of children and infants in areas with lower economic and hygienic level (3). As a rule of diarrhea has an infectious etiology, they are caused by microbial toxin produced in intestinal

mucosa or by contaminated food or by bacterial invasion to mucosal cells of the intestine or by the ingestion of contamination of drinking water and food (4,5,6).

Receptors of intestinal cells and the immunological status of the stomach are predisposing factor of the host which determined the deficiency of immunoglobulin present in the intestine which entering the child's body through maternal milk (7).

In developing countries, most cases of diarrhea are bacterial origin, repeated by viral origin (5-9). Among the bacteriological pathogen *Salmonella* spp. which play an important role in children under five years (10).

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The highest attack rate for salmonellosis is the infancy, with lower incidence of symptomatic infection in patients older than five years (11).

The purpose of the study was to determine *Salmonella* antigens which are the common causative agent of gastroenteritis in children admitted to pediatric hospitals under two years.

2. Materials and Methods

2.1 Study population

During a period of eight months from May 2015 to January 2016, a study was conducted at two pediatric hospitals in Baghdad (AL-Elweya and Al-Khadymia pediatric hospital) on freshly-collected stool samples from a total number of 94 cases of acute diarrhea among children aged less than two years.

A questionnaire was completed for each patient containing the following information: name, age, gender, clinical data (fever, nausea, vomiting, abdominal pain, and diarrhea), macroscopic and microscopic laboratory examinations of stool samples. The inclusion criteria was to include in this study watery stool sample (at macroscopic examination) and a parasite-free stool samples for microscopic examination (using saline and iodine preparations) from the diarrheal cases that were not lasting more than seven days after the onset of illness. The criteria was also, to include reported hemorrhagic fresh stool samples containing parasitic agents (*Giardia lamblia* or *Entamoeba histolytica*) in their stools.

Stool samples were collected in a labeled screw-cap clean container. Stool samples were tested by immunochromatographic assay (purchased from CerTest Biotec, Spain) for antigen detection of *Salmonella* and were done according to the instructions of the manufacturers. Allowing the card-device, test reagents and stool samples to reach room temperature prior to testing. A separate stool collection tube and device were used for each sample and the assay was done right after collection. To detect *Salmonella*, approximately 100mg or 100 microliter of stool sample was put and shaken in collection tube containing the diluents. Four drops or 100µl was dispensed in the circular window of the card. The results (appearance of the colored bands) were read after 10 minutes. This CerTest-*Salmonella* KIT is a qualitative immunochromatographic assay for determination of rotavirus in fecal samples. The membrane in the test band region is precoated with mouse monoclonal antibodies against *Salmonella* antigens. During testing, the sample is allowed to react with the colored conjugates (anti-*Salmonella* mouse monoclonal antibodies-red microspheres) which were pre-dried on the test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the colored particles migrate. In the case of positive result, the specific

antibodies present on the membrane will capture the colored particles and a red colored line becomes visible. The mixture captures the colored particles and a red colored line becomes visible. The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a green-colored band always appear. The presence of this green band serves as 1-verification that sufficient volume is added, 2-that proper flow is obtained and 3-as an internal control for the reagents. Insufficient specimen volume, incorrect procedural or deterioration of the reagents are the most likely reasons for control line failure. Negative results were indicated by only one green band (control line). For positive results, in addition to the green control band, a red band also appeared on the site of result line. A total absence of the control colored band (green) regardless the appearance or not of the result line (red) was evaluated as an invalid result.

3. Results

Diarrheal children according to their gender with acute diarrhea below two years were studied, among them 44 (46.8%) were males and 50 (53.2%) were females. Males to female ratio was 0.87.

Salmonella antigen was revealed in 94 of fecal samples. Among that studied child who has *Salmonella* antigen positive diarrhea 5. One (20%) was males and 4 (80%) were females with male to female ratio 1:4 (Table 1). The results show statistical difference between *Salmonella* positive antigen in both males and female group using chi square test.

Table 1. Diarrheal children according to their gender and Salmonellosis infection.

<i>Salmonella</i> Antigen	Males		Females		Total	%
	No.	%	No.	%		
<i>Salmonella</i> +ve Antigen	1	20.0%	4	80.0%	5	100%
<i>Salmonella</i> -ve Antigen	43	48.3%	46	51.7%	89	100%
Total	44	46.8	50	53.2	94	100%

3.1 Fever

Children with acute diarrhea whom fecal specimens were positive to *Salmonella* antigen or negative develops fever more than those without fever (98.9% versus 1.1%). The result revealed statistically significant differences ($p < 0.01$).

Table 2. Child with acute diarrhea according to fever in their bodies.

Salmonella Antigen	Fever				Total	
	Positive		Negative			
	No.	%	No.	%		
Salmonella +ve Antigen	4	80%	1	20%	5	100%
Salmonella -ve Antigen	88	100%	0	0%	88	100%
Total	92	98.9%	1	1.1%	94	100%

3.2 Abdominal pain

Children with acute diarrhea whom fecal specimens were *Salmonella* positive antigen and *Salmonella* negative antigen develops. Abdominal pain is more than those without abdominal pain (65.6% versus 34.4%). The result revealed significant difference ($p<0.01$).

Table 3. Diarrheal children according to abdominal pain.

Salmonella Antigen	Abdominal pain				Total	
	Positive		Negative			
	No.	%	No.	%		%
Salmonella +ve Antigen	4	80.0%	1	20.0%	5	100%
Salmonella -ve Antigen	57	64.8%	31	35.2%	88	100%
Total	61	65.6%	32	34.4%	93	100%

3.3 Vomiting

All children with acute diarrhea whom fecal specimens were positive to *Salmonella* antigen or negative develops vomiting (100%).

Table 4. Diarrheal children according to vomiting.

Salmonella Antigen	Vomiting		Total	
	No.	%	No.	%
Salmonella +ve Antigen	5	100%	5	100%
Salmonella -ve Antigen	89	100%	89	100%
Total	94	100%	94	100%

3.4 Stool color

Children with acute diarrhea whom fecal specimens were *Salmonella* positive antigen or *Salmonella* antigen negative varies in the stool color 45.6% were brown, 38.9% were green and 15.6% were yellowish. Result shows significant difference ($p<0.01$) among the groups.

Table 5. Diarrheal children according to the color of stool.

Salmonella Antigen	Color			Total
	Brown	Green	Yellow	
Salmonella +ve Antigen	3 60.0%	1 20.0%	1 20.0%	5 100%
Salmonella -ve Antigen	38 44.7%	34 40.0%	13 15.3%	85 100.0%
Total	41 45.6%	35 38.9%	14 15.6%	90 100.0%

3.5 Stool consistency

Children with acute diarrhea whom fecal specimens were *Salmonella* positive antigen and *Salmonella* negative antigen develop watery stool more than loose stool (75.5% versus 24.5%). The results indicated significant difference ($p<0.01$) between groups (Table 6).

3.6 Blood

A Child with acute diarrhea whom fecal specimens were *Salmonella* positive antigen and *Salmonella* negative antigen develop blood in their stool. The

percent of bloody stool was 64.1% versus 30.9% to negative bloody stool. The results indicated significant differences ($p<0.01$) between groups (Table 7).

Table 6. Diarrheal children according to the consistency.

Salmonella Antigen	consistency		Total
	Loose	Watery	
Salmonella +ve Antigen	1 20%	4 80%	5 100%
Salmonella -ve Antigen	22 24.7%	67 75.3%	89 100%
Total	23 24.5%	71 75.5%	94 100%

Table 7. Diarrheal children according to blood in their stool.

Salmonella Antigen	Blood		Total
	Positive	Negative	
Salmonella +ve Antigen	1 20.0%	4 80.0%	5 100.0%
Salmonella -ve Antigen	64 71.9%	25 28.1%	89 100 %
Total	65 69.1%	29 30.9%	94 100 %

3.7 Pus cells

A Child with acute diarrhea whom fecal specimens were *Salmonella* positive antigen and *Salmonella* negative antigen show decrease in cells in their stool ($p<0.01$) in comparison with other groups (16% versus 84%).

Table 8. Diarrheal children according to the presence of pus cells in their stool.

Salmonella Antigen	Pus cell		Total
	Positive	Negative	
Salmonella +ve Antigen	0 0%	5 100%	5 100%
Salmonella -ve Antigen	15 16.9%	74 83.1%	89 100 %
Total	15	79	94

3.8 Mucous

A Child with acute diarrhea whom fecal specimens were *Salmonella* positive antigen and *Salmonella* negative antigen show mucous in their stool ($p<0.01$) in comparison with other groups (83% versus 17%).

Table 9. Diarrheal children according to the presence of mucous in their stool.

Salmonella Antigen	Mucous		Total
	Positive	Negative	
Salmonella +ve Antigen	5 100%	0 0%	5 100%
Salmonella -ve Antigen	73 82.0%	16 16%	89 100 %
Total	78 83.0%	16 17%	94 100%

3.9 Trophozoites

A Child with acute diarrhea whom fecal specimens were *Salmonella* negative antigen or positive show presence of *Giardia lamblia* trophozoites in four cases and 24 cases of *E. histolytica* trophozoites with a percent of 14.3% and 85.7%, respectively (Table 10).

Table 10. Diarrheal children according to the presence of *Giardia lamblia* and *E. histolytica* in their stool.

Salmonella Antigen	Trophozoites		Total
	<i>Giardia lamblia</i>	<i>E. histolytica</i>	
Salmonella +ve Antigen	2 14.3%	12 85.7%	14 100%
Salmonella -ve Antigen	2 14.3%	12 85.7%	14 100%
Total	4 14.20%	24 85.80%	28 100%

3.10 Cysts

A Child with acute diarrhea whom fecal specimens were *Salmonella* negative antigen show presence of protozoan cyst. The percent was 14.9% versus 85.1% data shown in (Table 11) while those whom fecal specimens were *Salmonella* positive antigen not appear the cyst in their stool.

Table 11. Diarrheal children according to the presence of parasites cysts.

Salmonella Antigen	Cyst		Total
	Positive	Negative	
Salmonella +ve Antigen	0 0%	5 100%	5 100%
Salmonella -ve Antigen	14 15.7%	75 84.1%	89 100%
Total	14 14.9%	80 85.1%	94 100%

3.11 Ova

A Child with acute diarrhea whom fecal specimens were *Salmonella* positive antigen and *Salmonella* negative antigen show absence of parasitic ova in their stool.

Table 12. Diarrheal children according to the presence of ova in their stool.

Salmonella Antigen	Ova		Total
	Positive	Negative	
Salmonella +ve Antigen	0 0%	5 100%	5 100%
Salmonella -ve Antigen	0 0%	89 100%	89 100%
Total	0 0%	94 100%	94 100%

4. Discussion

Salmonella spp. was identified in five stool samples of child out of 94 samples. One of them is of male and other four samples are of females (Table 1).

The infections may be due to lack of sanitary facilities and poor living condition is among the major causes of diarrhea (11). The result in line with other studies in India revealed that 58.9% of children suffering from diarrhea caused by *Salmonella* and other enteropathogenic bacteria below two years (12). In a general, *Salmonella* spreads by hospitals, contaminated food, and sewage system. Fever, vomiting occur in all pediatric patients positive or negative to *Salmonella* antigen (Table 2 and 3). The reasons due to endotoxins of bacteria act on the vascular and nervous apparatus resulting in increased permeability and decrease tone of the vessel, then upset thermal regulation and vomiting (12,13,14). All pediatric patients show abdominal pain (Table 4). The reasons due to the ability of bacteria to multiply in the intestine lumen, causing an intestinal inflammation or in some instance associated with irritable bowel syndrome, inflammatory bowel diseases and abdominal cramps (13). Most pediatric patients with diarrhea with positive or negative *Salmonella* antigen show pus, mucous and blood in their stools (Table 5,6,7). In a general, patients with salmonellosis and other enteropathogenic bacteria are often with mucopurulent containing mucous, pus and blood specially when the diarrhea is severed (14). Mostly the pediatric patients show presence of entamebiasis or giardiasis in their stools (Table 10,11). In a general, the former two parasites represented the most common parasites which are transmitted via the ingested unhealthy food (15). Despite the fact, that they exert a big worldwide threat to human population. However, there is no specific vaccination to prevent neither spread nor infection of the disease (16,17). Amoebiasis is riskier infectious disease than others while the cyst of *Entamoeba* can survive for up to a month in soil or for up to 45 min under fingernails. Invasion of the intestinal lining causes amoebic bloody diarrhea or amoebic colitis. If the parasites reach bloodstream, it can spread through the body to another site like liver. When it causes amoebic liver abscess (18,19). Also, giardiasis is transmitted via the fecal oral route by ingestion of cysts (17,18,19) primary route one personal contact and contaminated water and food. The cyst can stay infectious for up to three months in the cold water. The animal plays a role in keeping infections present in environment (20,21).

5. Conclusion

Salmonella antigen present in five stool samples, all the patients show vomiting, fever, abdominal pain, 65 cases with blood in comparison with, 29 without blood 15 cases with pus in comparison with 79 without pus. 78 cases with mucous in comparison with, 16 without mucous, four cases with giardiasis and 24 cases with entamebiasis, and 14 cases with cyst of *E. histolytica* or *G. lamblia* in addition to absence the parasites ova in all stool samples.

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