

## ORIGINAL ARTICLE

# New Method for Diagnosis of Salmonella Gastroenteritis in Pediatric Patients

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## ABSTRACT

### Key words:

Non- typhoidal salmonella - Gastroenteritis - MSRVR agar - Salmonella single tube

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**Background:** Non- typhoidal salmonella infection is a major cause for acute bacterial gastroenteritis in children. There is no ideal method for diagnosis of salmonella infection and the development of highly selective media continues to be of particular importance for diagnostic and treatment purposes. **Objective:** Was to assess the performance of two media, the first one was the MSRVR agar as a screening medium for isolation of salmonella from stool samples of pediatric patients with gastroenteritis and the second medium was the new salmonella single tube to identify *Salmonella* spp. **Methodology:** Stool samples were subjected to stool analysis, isolation of *Salmonella* species on selective culture media (XLD, SS & MSRVR). Different biochemical reactions were used to identify *Salmonella* spp; the first was the conventional biochemical reactions and the second was the newly suggested salmonella single tube. Conventional PCR was done to detect the genus salmonella and the serovars enteritidis and typhimurium. **Results:** Comparing the results of the three selective culture media, the validity values observed with MSRVR medium were superior to those obtained by the other two media. *Salmonella* single tube yielded 100% sensitivity, specificity, positive predictive and negative predictive values with reliability equivalent to that of the conventional 5 tubes method. *Salmonella* typhimurium was prevalent in 75% of positive patients while *Salmonella* enteritidis was detected in 25%. **Conclusion:** When MSRVR agar was evaluated in comparison to other selective media, it had the highest values in terms of performance. Direct plating of MSRVR agar produced the same performance of indirect plating therefore, direct plating could shorten the detection time of *Salmonella* spp. Using salmonella single tube ensures simple and rapid processing in one step and is very suitable for mass screening purposes.

## INTRODUCTION

Gastrointestinal infections are widespread in the community and have numerous economic consequences, and remain one of the leading causes of childhood morbidity and mortality in most developing countries <sup>1</sup>. According to the United Nations Children's Emergency Fund (UNICEF) and World Health Organization (WHO), there are nearly 2 billion cases of diarrheal disease worldwide every year and 1.9 million children younger than 5 years of age die from diarrhea each year, mostly in developing countries <sup>2</sup>.

*Salmonella* enteric serovar typhi (*S. typhi*) and the different pathovars of *S. paratyphi* are commonly referred to as typhoidal *Salmonella*. These agents are the primary cause of typhoid fever and are restricted to human hosts. *Salmonella* serovars outside this group are typically referred to as non typhoidal salmonella (NTS) serovars and are considered to have the potential to interact with human and nonhuman hosts causing

salmonella-induced food poisoning called salmonellosis<sup>3</sup>.

Most cases of human salmonellosis are foodborne, but infections may also be acquired through direct or indirect animal contact. People at any age are susceptible to the diarrhea, intestinal cramping, and intestinal epithelial erosion associated with salmonellosis. The disease is often self-limiting, but can cause prolonged complications. The young children, the elderly and immune-compromised individuals are most at higher risk for complications <sup>4</sup>.

Faster detection and identification are the cornerstones for the fight against human pathogens <sup>5</sup>. There is no ideal method for diagnosis of salmonella infection and the development of highly selective media continues to be of particular importance for diagnostic and treatment purposes. Plating media depend upon the detection of characteristic byproducts or behavior of the pathogens for their isolation. For example, agar media containing chromogenic substrates for salmonella-specific enzymes have been developed; however,

chromogenic media are less sensitive, but more specific when compared to conventional media <sup>6</sup>.

Regarding behavior of the pathogens, semisolid medium for isolating salmonella, based on its motile behavior had been developed. Modified Semisolid Rappaport-Vassiliadis (MSRV) agar is one of them, it is a modification of Rappaport-Vassiliadis enrichment broth for detecting motile *Salmonella species* in food products and feces. The efficacy of the medium is based on the ability of *Salmonella species* to migrate through the medium ahead of other competing motile organism, thus producing opaque halos of growth surrounding the inoculum <sup>7,8</sup>.

When H<sub>2</sub>S producing colonies is detected, serological typing and more detailed biochemical testing are performed. Edwards and Fife <sup>9</sup> introduced their excellent medium: Lysine iron agar (LIA). It was the first medium that tests the two most important biochemical characteristics of the genus salmonella i.e. lysine decarboxylation, and H<sub>2</sub>S production, as well as glucose fermentation. They suggested their LIA medium- that is lactose free- as an alternative to TSI to solve the problem of missing identification of the pathogenic arizona cultures because of failure to precipitate H<sub>2</sub>S due to excessive acidity resulting from lactose fermentation that occurs in TSI tube.

Microbiologists can conclude that; mild acidity resulting from fermentation of a low concentration of glucose (e.g. 1.0 gm/L) allows all H<sub>2</sub>S +ve species to produce black precipitate in both of TSI and LIA. In contrast; excessive acidity resulting from fermentation of high concentration of lactose or any other sugar (e.g. 10.0 gm/L) prevents precipitation of the black ferrous sulfide although H<sub>2</sub>S is produced. This conclusion was the basis to suggest an acidic medium; that changes by glucose fermentation to exaggerated acidity to prevent any growing H<sub>2</sub>S +ve colony (except salmonella) from precipitating black ferrous sulfide. Salmonella should be an exception because it decarboxylates lysine, gradually changes pH to mild acidity- then to neutral- allowing precipitation of ferrous sulfide that changes color from yellow to black. This new medium {Salmonella single tube; Acidic Lysine Tryptophan Iron Motility Agar (ALTIMA tube) } aims to introduce a single tube to discriminate salmonella from the numerous other species of the family Enterobacteriaceae that produce H<sub>2</sub>S (black) colonies on selective and differential culture plates <sup>10</sup>.

The aim of this study: was to assess the performance of two media, the first was the Modified Semisolid Rappaport Vassiliadis agar (MSRV) as a screening medium for isolation of salmonella from stool samples of pediatric patients with gastroenteritis and the second was the new salmonella single tube to identify *Salmonella spp.*

## METHODOLOGY

### Study design

This cross sectional study was carried out during the period from December 2017 to December 2018 in Clinical & Chemical Pathology and Pediatrics Departments of Banha University Hospital. One hundred infant and child with symptoms & signs of gastroenteritis with strong evidence for the presence of bacterial dysentery were included in the study. They were 65 male and 35 female with their age ranged from 1 month to 16 year with the mean age of  $10 \pm 1.4$ . Enrolling procedures comprised full history taking and clinical examination. The study design was approved by the local Ethics Committee of Faculty of Medicine, Banha University.

### Laboratory methods:

Stool samples were collected, transported immediately to the microbiology laboratory and subjected to:

**Stool analysis:** was done according to the Standard Operating Procedures (SOPs) of WHO (2006a) <sup>11</sup> which included macroscopic and microscopic examination.

### Isolation of *Salmonella species*

#### Selective culture media:

All stool samples were inoculated in selenite broth, then plated onto selective media {Xylose-Lysine-Desoxycholate (XLD) agar and Salmonella-Shigella (SS) agar} (Oxoid). All inoculated plates were incubated aerobically at 37 °C for 24 hours and lactose non fermenter H<sub>2</sub>S producing colonies were selected for biochemical reactions <sup>12</sup>.

#### Modified Semi-solid Rappaport-Vassiliadis Agar (MSRV) (CM1112, Oxoid):

It is used for the selective enrichment of *Salmonella species* from feces and environmental samples. The efficiency of the medium is based on the ability of salmonellae to migrate through the selective medium ahead of competing Gram-negative, motile organisms, thus producing opaque halos of growth. Novobiocin supplement (SR0181, Oxoid), low pH, high magnesium chloride concentration and malachite green inhibit the growth of Gram-positive flora <sup>13</sup>.

Samples streaked into MSRV agar plates both directly and indirectly after inoculation of stool into BPW (ISO- CM1049). Motile salmonella colonies are characterized by white/grey, turbid zones radiating from the inoculum, which are surrounded by a white halo with a sharply defined border as shown in figure 2.

#### Identification of the isolates:

**Conventional biochemical reactions:** (five tubes): triple sugar iron agar, lysine Iron agar, motility indole ornithine, citrate utilization and urease tests (Oxoid) <sup>12</sup>.

### **Salmonella single tube; Acidic Lysine Tryptophan Iron Motility Agar (ALTIMA)**

ALTIMA tube incorporates the following tests: 1- Glucose fermentation, 2- Lysine decarboxylation (LDC), 3- Tryptophan deamination (TDA), 4- Indole production, 5- H<sub>2</sub>S production and 6 - Motility test<sup>10</sup>.

**ACIDIC:** The medium has acidic pH (5.5 ± 0.2), the un-inoculated tube is yellow and the pH indicator is phenol red. When any species of the family Enterobacteriaceae is grown in this medium; the original acidity is exaggerated and the yellow color persists.

**LYSINE:** Decarboxylation yields an alkaline amine (cadaverine) that gradually opposes the acidity. LDC +ve bacteria e.g. *E.coli*, and *Klebsiella pneumoniae* raise pH towards mild acidity, then to neutral and later to alkalinity and the color turns into purple.

Except for *Salmonella paratyphi* A; all species of the genus salmonella are LDC +ve. Decarboxylation of lysine raises pH to mild acidity suitable for ferrous sulfide to precipitate. As early as 8-10 hours; a black precipitate starts to be visualized preceding any purple color change of the pH indicators. Within 24 hours the color of the whole medium changes into (coal) black that masks the underlying purple.

**TRYPTOPHAN:** L- tryptophan is incorporated in the new medium to add two useful tests: indole production and detection of the enzyme tryptophan deaminase activity.

**IRON:** the medium contains sufficient amounts of sodium thiosulphate and ferric ammonium citrate to ensure production of hydrogen sulfide.

**MOTILITY:** The new medium is a semisolid agar to allow observing motility in absence of black precipitate.

A single, well isolated colony (non-lactose, H<sub>2</sub>S positive; from selective medium) was picked by a straight needle loop and stabbed into Salmonella single tube, incubated at 37°C for 24 hours. Interpretation: the tube was inspected

If the medium remains yellow = LDC -ve e.g. *Citrobacter* and *Proteus species* as shown in figure 3.

The medium changes into black = LDC +ve then Kovac's reagent was added:

- A cherry red surface layer = Indole positive reaction = Edwardsiella (or rarely Morganella).

- A yellow surface layer = Indole negative reaction = *Salmonella species* as shown in figure 4.

### **Conventional PCR to detect the genus salmonella and the serovars enteritidis and typhimurium**

**DNA extraction:** Using Gene Jet Genomic DNA Purification kit (Thermo-Scientific, E.U). The extraction procedures were done according to manufacturer's instructions.

**Primers:** were designed for amplification of DNA to detect the genus salmonella and the serovars enteritidis and typhimurium using conventional PCR. The pair of primers used to detect Salmonella at genus level was the

invasion protein A gene (InvA), widely distributed in *Salmonella species*<sup>14</sup>. The pair of primers specific for *Salmonella enteritidis* was the Insertion Element (IE)-1 DNA sequence which is found only in this serovar<sup>15</sup>. The pair of primers used to detect *S. typhimurium* was designed using the sequence involved in flagellin synthesis gene (the Flic-C)<sup>16</sup>.

Target	Primer	Sequence 5' to 3', as synthesized
Inv-A	Inv-A-F	CGGTGGTTTTAAGCG TACTCTT
	Inv-A-R	CGAATATGCTCCACAAGGTTA
IE-1	IE-1-F	AGTGCCATA CTTTAAATGAC
	IE-1-R	ACTATGTTCGATACGGTGGG
Flic-C	Flic-C-F	CCCGCTTACAGGTGGACTAC
	Flic-C-R	AGCGGGTTTTTCGGTGGTTGT

**Amplification of DNA:** Amplification was done using 2x HotStart Taq PCR master mix supplied by **TIANGEN Biotech (Beijing) CO., Ltd.** Amplification was done in Veriti 96 well thermalcycler (Applied Biosystem) according to manufacturer's instructions.

#### **Interpretation:**

Appearance of a band at 796 bp indicated presence of Inv-A gene that document the presence of bacteria of the genus salmonella.

Appearance of a band at 432 bp indicated presence of Flic-C gene that document the presence of *Salmonella typhimurium*.

Appearance of a band at 316 bp indicated the presence of IE-1 gene that document the presence of *Salmonella enteritidis*.

#### **Statistical analysis**

The collected data were tabulated and analyzed using SPSS version 16 software (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages. Z test for independent proportions (ZProp.) was used to analyze categorical variables. Cohen kappa test was used to assess degree of agreement between the studied techniques. ROC curve was constructed to assess the performance of evaluated methods for isolation and identification of salmonella. The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant).

## **RESULTS**

The positive rate for salmonella detection by XLD agar and SS agar was 18% (18/100 sample) and 19% (19/100 sample) respectively, however higher rate (20%) was obtained with the MSR/V medium. The diagnostic performance of the three selective culture media was done taking PCR as the gold standard method and revealed that the values observed with MSR/V medium were superior to those obtained by the other two selective culture media as shown in table 1.

Two samples tested negative by XLD agar but positive by PCR, XLD agar failed to detect two (10%) out of 20 confirmed salmonella positive stool samples (two false negative results). Three samples tested negative by SS agar but positive by PCR (three false negative results) and two samples tested positive by SS agar while they were negative by PCR method (2 false positive results).

The agreement between MSR/V agar and XLD agar for isolation of *Salmonella species* was 98 % (kappa = 0.935) as shown in table 2 and the agreement between the MSR/V agar and SS agar was 95% (kappa = 0.841) as shown in table 3, while the agreement between XLD agar and SS agar was 97% (kappa = 0.901) as shown in table 4 and all are of high significant statistical value (<0.001 HS).

Different biochemical reactions were used to discriminate suspected salmonella from other species of the family Enterobacteriaceae that are non-lactose fermenters and H<sub>2</sub>S producing colonies on selective culture plates; the first was the conventional biochemical reactions which could identify 20 isolates as being *Salmonella species* (20/100 sample, 20%). The second was the new salmonella single tube which yielded 100% sensitivity, specificity, positive predictive and negative predictive values with reliability equivalent to that of the conventional 5 tubes method as shown in figure 1.

Using conventional PCR to confirm the genus salmonella and to detect the serovars enteritidis and typhimurium, the two serovars were detected; *Salmonella typhimurium* was prevalent in 75% (15/20) of positive patients while *Salmonella enteritidis* was found in 25% (5/20) as shown in figure 5.

A large majority of patients (75%) (15/20) were in school children older than 5 years of age and particularly from 12 to 16 year (50%) (10/20) and this result was of significant statistical value. Males were more affected (65%) than females (35%) with male to female ratio of (1.86: 1) but this was of no statistical significance.

About 70% (14/20) of Salmonella positive cases showed fecal WBC count more than 60 / HPF and 35% of Salmonella positive cases showed fecal WBC count more than 100/ HPF and this result was of high significant statistical value (P<0.001). In addition, CRP was tested in all patients and found positive in 80% of them.

The majority of patients with salmonella gastroenteritis suffered in addition to diarrhea from abdominal pain (100 %), fever (80%), mucus in stool (80%) and decreased activity (60%).

**Table 1: Diagnostic performance test for the three selective culture media using PCR as the gold standard method**

	XLD	SS	MSRV
<b>Sensitivity</b>	90%	85%	100%
<b>Specificity</b>	100%	97.5%	100%
<b>Positive predictive value</b>	100%	89.5%	100%
<b>Negative predictive value</b>	97.5%	96.3%	100%
<b>Accuracy</b>	98%	95%	100%
<b>AUC</b>	0.95	0.912	1.0
<b>95%CI</b>	0.87-1.0	0.82-1.0	1-1
<b>P</b>	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)
<b>TP(Sensitivity)</b>	90%	85%	
<b>TN(Specificity)</b>	100%	97.5%	

**Table 2: Results of MSR/V medium versus XLD agar**

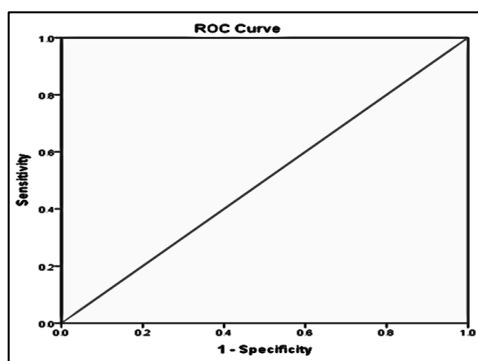
		MSRV		TOTAL
		Positive	Negative	
<b>XLD</b>	Positive	18, 90%	0, 0%	18, 18%
	Negative	2, 10%	80, 100%	82, 82%
	Total	20 100.0%	80 100.0%	100 100.0%
FET (P)		<0.001 (HS)		
Agreement (%)		98%		
Kappa, its P value		0.935, <0.001 (HS)		

**Table 3: Results of MSR/V agar versus SS agar**

		MSRV		TOTAL
		Positive	Negative	
<b>SS</b>	Positive	17, 85%	2, 2.5%	19, 19%
	Negative	3, 15%	78, 97.5%	81.81%
	Total	20, 100%	80, 100%	100,100%
FET (P)		<0.001 (HS)		
Agreement (%)		95%		
Kappa, its P value		0.841 (<0.001HS)		

**Table 4: Results of SS agar medium versus XLD agar**

		XLD		TOTAL
		Positive No., %	Negative No., %	
<b>SS</b>	Positive	17, 94.4%	2, 2.4%	19, 19%
	Negative	1, 5.6%	80, 97.6%	81, 81%
	Total	18, 100%	82, 100%	100,100%
FET (P)		<0.001 (HS)		
Agreement (%)		97%		
Kappa, its P value		0.901 , <0.001 (HS)		



**Fig. 1:** Performance of salmonella single tube in comparison to the reference method.



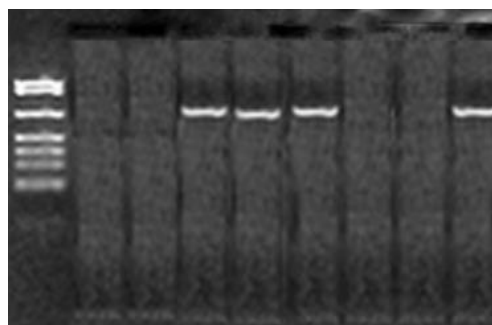
**Fig. 2:** A plate of MSRV agar showing motile *Salmonella spp* which appears as a grey-white, turbid zone extending from the inoculated area.



**Fig. 3:** The salmonella single tube appears yellow after incubation (LDC -ve) e.g. *Citrobacter* and *Proteus* species.



**Fig. 4:** The salmonella single tube changes into black after incubation (LDC +ve) and on using Kovac's reagent: indole negative reaction = *Salmonella species*



**Fig. 5:** *Salmonella typhimurium* (Flic-C gene), specific band at 432 bp

## DISCUSSION

The detection of enteric pathogens has relied upon culture techniques to isolate bacteria. A variety of selective, non-selective and differential media, in addition to enrichment broths, are traditionally used by clinical microbiology laboratories for the screening of stool. When a pathogen is detected, serological typing and more detailed biochemical testing are performed and data from these tests facilitate epidemiological analyses and can help prevent epidemics and management of severely ill and immuno-compromised patients<sup>17</sup>.

In spite of the high grade of reliability and performance of immune based and molecular based methods for diagnosis of salmonella, yet, they are of high cost, tedious work, in need for sophisticated instruments and well trained laboratory professionals, which cannot be available in developing countries or in mass screening purposes, so many trials should be done to get rapid, cheap and easily manipulated techniques for effective detection of salmonella<sup>5</sup>.

In our study the positive rate for salmonella isolation by XLD agar and SS agar were 18% (18/100 sample) and 19% (19/100 sample) respectively, however, MSRV agar could detect higher rate (20%) than SS agar and XLD agar.

Comparing the results of the three selective culture media, we found, the sensitivity, specificity, positive predictive, negative predictive values and the accuracy observed by MSRV agar (100% for each) were superior to those obtained by the other two selective culture media. Ruiz et al.<sup>18</sup> found that sensitivities and specificities of MSRV, and SS agar were 95.1%, and 98.1%, and 69.1%, and 99.3%, respectively. MSRV was the most sensitive (100%) medium tested and was very specific for isolation of non-typhoidal salmonellae from stool specimens comparable to Hektoen Enteric agar, Rambach agar, Xylose-lysine-Tergitol 4 agar, Novobiocin-brilliant green-glycerol-lactose agar as observed by Dusch and Altwegg<sup>13</sup>. Similarly, Srijan et al<sup>19</sup> suggested that the use of MSRV was more efficacious over MacConkey, Hektoen Enteric and XLD

agars for isolation of non-typhoid *Salmonella species* from human stools.

From a public health perspective, faster detection times are essential to prevent the spread of infectious diseases or the identification of a continuing source of infection<sup>20</sup>. In the present study, results indicated that screening of samples for the presence of salmonellae on XLD and SS media is labor-intensive because of the high number of colonies (e.g., *Proteus spp.* and *Citrobacter spp.*) that resemble *Salmonella species*. This facts lead to an additional costs for subsequent identification<sup>13</sup>, Direct plating of MSR/V agar produced the same performance of indirect plating hence, direct plating could shorten the detection time of *Salmonella spp.* Additionally, other studies suggested that enrichment broth be used only during outbreaks, for screening carriers or in clinically warranted situations<sup>21</sup>.

The prevalence of salmonella in our study was 20%, this percentage was closely related to the isolation rate of Aguirre et al<sup>20</sup> who reported 19.3% (n=83/430). However, lower rates (1.8%) & (11.3%) were detected by Maddocks et al<sup>22</sup> and Ruiz Gomez et al<sup>23</sup> respectively & higher rate (29.3%) was reported by Stuart and Pivnick<sup>24</sup>. This higher rate in our study may be explained by the use of samples under more selective conditions than other studies which used samples from consecutive patients with gastroenteritis without any previous selection.

Different biochemical reactions were used to discriminate *Salmonella species* from other species of the family Enterobacteriaceae; the first was the conventional biochemical reactions which could identify 20 isolates as being *Salmonella species* (20%). The second one was the newly suggested salmonella single tube, which showed 100% agreement with the results of the conventional reactions. After assessment of both strategies by the molecular method, it was found that the sensitivity, specificity, positive predictive and negative predictive values of the salmonella single tube were 100% with reliability equivalent to that of the traditional 5 tubes method. The high sensitivity is a desired characteristic for a screening test, whereas high specificity is desired requirement for a confirmatory test.

Salmonella single tube; an acidic, semisolid agar is sufficient alone for biochemical identification of the majority of salmonella strains. The new medium was administered as single tube for stab inoculation which ensures simple and rapid processing in one step, not needing much technical skills. Followed by overnight incubation and simple way of interpretation (salmonella gives black color along the whole length of the tube, with negative indole reaction upon addition of the Kovac's reagent) making it very suitable for mass screening purposes.

We think, using salmonella single tube will save time, afford easy manipulation, and make no need for high skills. Salmonella single tube is also of low cost, and can be easily made and processed in small size microbiology laboratories, especially in the set of screening with relatively high number of suspicious isolates. Procop et al<sup>25</sup> reported that The Single Tube Study (STS) system seems promising for screening of lactose-negative stool isolates that may represent salmonella or shigella.

In the present study, conventional PCR was used to detect the serovars enteritidis and typhimurium, the two serovars were detected; *Salmonella typhimurium* was prevalent in 75% (15/20) of positive patients while *Salmonella enteritidis* was found in 25% (5/20). Zheng et al<sup>26</sup> reported that *S. typhimurium*, known by its clinical importance and is a highly prevalent serotype in outbreaks both in the US and in Asian countries. Lu Ren et al<sup>27</sup> also found *Salmonella typhimurium* was the predominant serotype, accounting for 82.4% of isolates. On the other hand, Ran et al<sup>28</sup> found that serotype enteritidis (31%) and serotype typhimurium (26%) were the most common and Li et al<sup>29</sup> reported that enteritidis (38.9%) and typhimurium (29.7%) were the most common serotypes. This inconsistency may be due to the regional variation, the changing epidemiological trend and the specific patient population.

A large majority of patients in our study (75%) (15/20) were in school children older than 5 years of age and particularly from 12 to 16 year (50%) (10/20). In harmony with our results, Scharff et al<sup>30</sup> found higher incidence of salmonella infection in late childhood and adolescence and stated that, contamination of water supply and consumption of contaminated food are possible causes. In contrast, Lu Ren et al<sup>27</sup> observed that about half (52.1%) of the patients with salmonella gastroenteritis presented at <12 months of age.

In the present study males were more affected (65%) than females (35%) with males to females' ratio of (1.86: 1). This result was similar to that of Lu Ren et al<sup>27</sup> who found males predominance in patients with salmonella gastroenteritis. On the other hand, Khanum; et al<sup>31</sup> reported slightly higher incidence of salmonellosis among females compared to males (54.5% and 45.5 % respectively).

In this study, salmonella positive cases showed higher fecal WBC count than salmonella negative cases and this finding was of high significant statistical value ( $P < 0.001$ ). In addition, CRP was tested in all patients and found positive in 80% of them. In a study done by Lu Ren et al<sup>27</sup> laboratory investigations revealed that the majority (89.4%) of patients had elevated CRP and microscopic stool examination showed increased WBC and RBC in 62.7% and 33.1% of patients, respectively.

## CONCLUSION

When MSR/V agar evaluated in comparison to other selective media, it was found to have the highest values in terms of performance. Direct plating of MSR/V agar produced the same performance of indirect plating therefore, direct plating could shorten the detection time of *Salmonella spp.* Regarding the single tube study, salmonella single tube yielded 100% sensitivity, specificity, positive predictive and negative predictive values with reliability equivalent to that of the conventional 5 tubes method. Using salmonella single tube ensures simple and rapid processing in one step and is very suitable for mass screening purposes.

**Conflicts of interest:** The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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