Isolation And Identification of Salmonella typhi From Patients with Typhoid Fever in Babylon City in Iraq

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Abstract
A total of 263 clinical samples were collected from the blood of patients with typhoid fever who attended Al-Hillah Surgical Teaching Hospital and private laboratories in AL-Hillah / Babylon province, during the period from February to August 2021. Only 50 (19%) of 263 people tested positive for *S.typhi* infection using blood culture, biochemical tests, and the VITEK-2 compact system. According to the findings, clinical samples were divided into 50 (19%) positive blood cultures for *S.typhi* and 213 (81%) negative cultures for *S.typhi*. The Widal test yielded 100% positive results for *S.typhi*, whereas the blood culture yielded only 23% positive results.
Keyword: S.typhi, typhoid fever, Hillah, blood culture, VITEK-2

1. Introduction

Typhoid fever, which causes diarrhea, enteric fever, and septicemia, is a serious public health concern in many low- and middle-income countries. The availability of antibiotic treatment, as well as better water quality and sanitation, are long-term remedies to this problem, and vaccination in high-risk regions is a viable control method [1]. Typhoid fever is a potentially lethal illness of the gastrointestinal tract and circulatory system caused by harmful microorganisms. *Salmonella enterica* serotype *typhi* is a gram-negative, non-capsulated, rod-shaped, facultative anaerobe of the enterobacteriaceae family with flagella, somatic, and outer coat antigens that only lives in humans because it is an infectious illness spread orally through person-to-person contact, contaminated food, or contaminated water [2,3]. These bacteria may infect food products of animal origin like poultry and dairy products [4]. Typhoid fever is not a specific clinical condition because the presenting signs and symptoms are numerous and similar to those of other common febrile diseases, such as malaria and dengue fever, and can only be identified with certainty by isolating the pathogen from clinical specimens in humans. Clinical studies have revealed that this disease stimulates both the digestive mucosal and humeral reactions, both of which play an important role in pathogen control [5].

2. Material and Methods

1- Patients and Clinical Samples

A total of 263 samples were collected from the blood of patients with typhoid fever who attended Al-Hillah Surgical Teaching Hospital and private laboratories in AL-Hillah/Babylon province, during the period from February to August 2021. Ten ml of blood was drawn from patients suffering from typhoid fever symptoms aged 15–65 years. 5 ml of blood was injected into bottles containing 20 ml of BHI broth and incubated for 3 days at 37°C. If positive, each sample was inoculated using the direct method of inoculation on MacConkey, Blood, XLD, and SS agar and inoculated for 24 hours at 37°C. These samples were compared with 50 samples of apparently healthy individuals who appeared to be disease-free.

2-Serology Test

The Widal test was used to identify typhoid antibodies in blood samples. The presence of typhoid antibodies in fresh blood samples was determined using the O and H antigens.

3- Isolation and Identification of *S.typhi*

Blood cultures were evaluated on a regular basis for the following signs of microbial development: turbidity, gas production, color change, and other signs of microbial growth. Before reporting negative results, cultures should be cultured for at least seven days. Blood agar and MacConkey agar were used to subculture probable positive bottles. Pale MacConkey agar colonies were subcultured on Xylose Lysine Deoxycholate agar and Salmonella Shigella agar and inoculated at 37oC for 24–48 hours.

4- VITEK-2 Compact System

The VITEK-2 compact system was used to confirm the manual biochemical test findings, and it was recently employed to identify bacteria [6]. It was provided with the essential identification data base for all standard identification tests, allowing for greater efficiency in microbiological diagnosis by minimizing the time and need for any additional tests that are safe for the system's user. This system was created in compliance with the specifications provided by the manufacturer (BioMérieux-France).

3. Results

1- Serologically Test

doi.org/10.31838/hiv23.01.180 Received: 17.09.22, Revised: 03.10.22, Accepted: 03.12.22

According to the findings, clinical samples were divided into 50 (19%) positive blood cultures for *S.typhi* and 213 (81%) negative cultures for *S.typhi* as shown in figure (1).

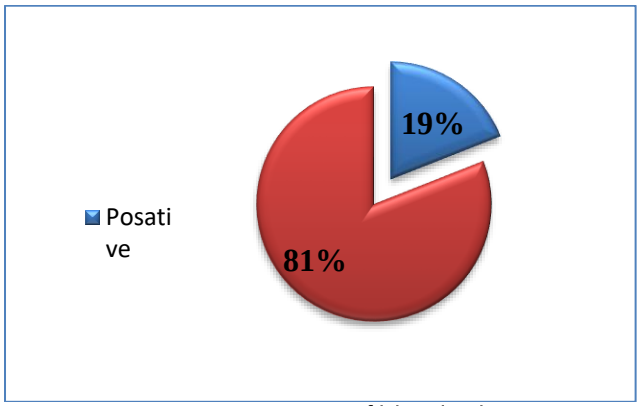


Figure (1): Percentage of blood cultures

The Widal test, which measures antibody responses ≥160 titer to S.typhi H and O antigens, revealed that the blood specimens were positive. The Widal test yielded 100% positive results for S.typhi, whereas the blood culture yielded only 23% positive results.

2- Isolation and Identification of *S.typhi*

Brain Heart Infusion has been used to inoculate the blood samples. As evidence of microbial growth, several changes in the broth have been observed, including turbidity, hemolysis, gas formation, color change, and foul odor. Identification was done by sub culturing of inoculated bottles on MacConkey agar, Blood agar, Salmonella Shigella agar and Xylose Lysine Deoxycholate agar.

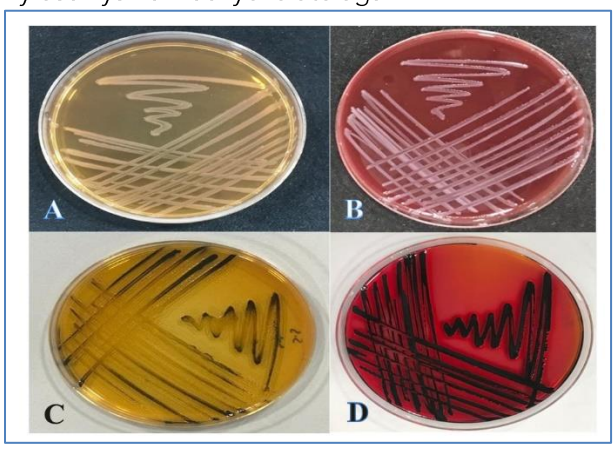


Figure (2): Salmonella typhi in different culture media

A. n Macconkey agar S.typhi appeared as pale colonies (non-lactose fermentation).
B. On blood agar, S.typhi appeared as white colonies (non-hemolytic).
C. On S. S agar, S.typhi formed black colonies with H2S production.
D. On XLD Agar, S.typhi appeared as red colonies with black centers.

3- Biochemical Tests

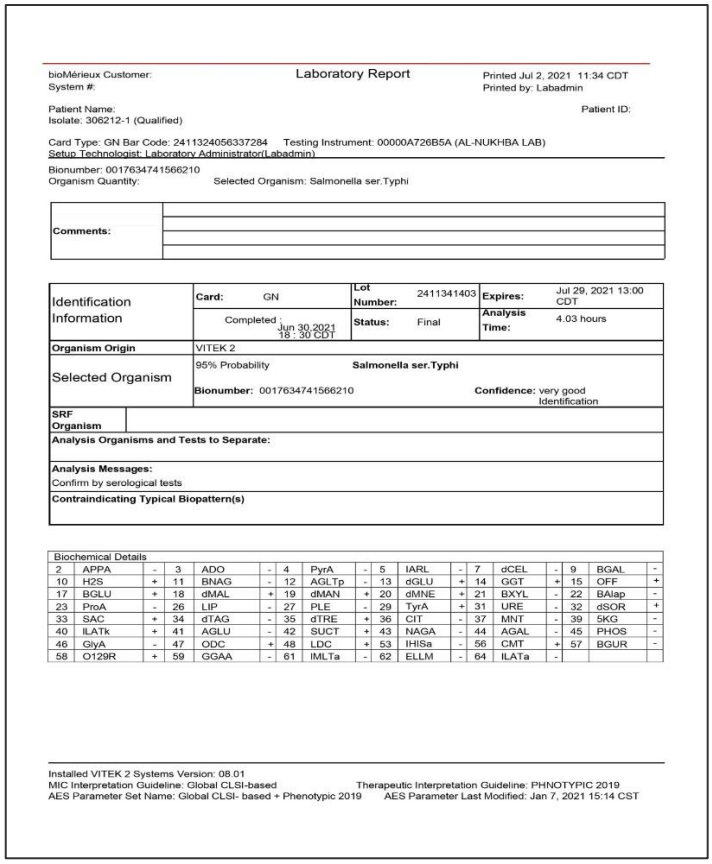
Biochemical tests that are used as complimentary to the identification of *S.typhi* isolates are shown in Table (1). *S.typhi* tested negative for oxidase, indole and Voges-Proskauer test but positive for methyl red, catalase test and citrate test. In triple sugar iron, *S.typhi* isolates produced hydrogen sulfide.

Table (1): Biochemical test for *S.typhi*

Test	Catalase	Indole	Oxidase	Methyl-red	Voges-Proskauer	Citrate	TSI
Result	+	-	-	+	-	+	Alk / acid

3- Identification of *S.typhi* by VITEK-2 Compact System

The automated VITEK-2 compact system with GN-ID cards was used for the final identification, which included 47 biochemical tests and one negative control well. As shown in the appendix, the results show that the blood isolates are *S.typhi*. The isolates were identified using the VITEK-2 compact system auto analyzer system, and the results were analyzed using compact software, which may provide accurate microbial identification. Demonstrates that a phenotypic confirmatory tool with faster results, higher specificities, demonstrated confidence, and less training time than manual microbial identification techniques as showed in figure (3).



bioMérieux Customer: Laboratory Report Printed Jul 2, 2021 11:34 CDT
System #: Printed by: Labadmin
Patient Name: Patient ID:
Isolate: 306212-1 (Qualified)
Card Type: GN Bar Code: 2411324056337284 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)
Bionumber: 0017634741566210
Organism Quantity: Selected Organism: Salmonella ser.Typhi

Comments:

Identification Information	Card: GN	Lot Number: 2411341403	Expires: Jul 29, 2021 13:00 CDT
	Completed: Jun 30 2021 18 : 30 CDT	Status: Final	Analysis Time: 4.03 hours
Organism Origin	VITEK 2		
Selected Organism	95% Probability	Salmonella ser.Typhi	
	Bionumber: 0017634741566210		Confidence: very good Identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages: Confirm by serological tests			
Contraindicating Typical Biopattern(s)			

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-		
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+		
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-		
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+		
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-		
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-		
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-		
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-					

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based Therapeutic Interpretation Guideline: PHNOTYPIC 2019
AES Parameter Set Name: Global CLSI- based + Phenotypic 2019 AES Parameter Last Modified: Jan 7, 2021 15:14 CST

Figure (3): VITEK report

Discussion

The Widal test yielded 100% positive results for S.typhi, whereas the blood culture yielded only 23% positive results. Misleading results when using the Widal test may keep one from making the correct diagnosis due to cross reaction of antigen from other infections with Salmonella antibody. S.typhi has cross-reacting epitopes with other

Enterobacteriaceae, which can lead to false-positive results and is not reliable for typhoid fever diagnosis [7]. The Widal test cannot be relied on to provide a reliable diagnosis [8]. Widal test methods are inexpensive and widely available, making them ideal for poor and developing countries, but they have low sensitivity and specificity [9]. Detect the agglutination of the antigens and antibodies found in the patient's serum needed for confirmation of results with a second test method [10]. S.typhi is most commonly isolated from blood during the first week of illness, but it can also be found during the second and third weeks. Blood culture diagnosis of S.typhi is a more standard and rapid method than stool culture because blood culture sensitivity is higher in the first week of illness. Stool culture is less than 50% sensitive on its own, and urine is even less so [11].

4. Moral Approval

The appropriate ethical permission from the ethics committees of Babylon Health Office, the hospital's ethical committee, as well as patients and their supporters, must be acquired. Furthermore, prior to the collection of samples, all participants engaged in this work are informed, and the agreement necessary for conducting the tests and publishing this work is acquired from each one.

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