

The Prevalence of salmonella germs in some districts of Salah al-Din Governorate

Najla'a Fathi Muhsin Al-douri, Yassien Hussain Owied Al-juboory

College of Education for women, Tikrit University, Iraq

Yassien dr 57@tu.edu.iq

Abstract

The results of our current study showed that the numbers and percentages of Salmonella bacteria isolates from urine and blood samples of inpatients and reviewers in Salah Al-Din General in Iraq Hospital in Tikrit and Al-Dur Model Sector Hospital in Al-Dur in Salah Al-Din Governorate amounted to 19 isolates, at a rate of 4.66% of the total samples. The *S.typhimurium* serotype was the most prevalent, with 11 isolates, with a percentage of 5.39% of urine samples isolated, and 2.70% of the total percentage of isolation, followed by *S.typhi* serotype, with 4 isolates, with a percentage of 1.96% of urine samples, and 0.98% of the total percentage of isolation. Then came the *S.paratyphiB* serotype, with a number of 3 isolates, with an isolation rate of 1.47% from urine samples, and from a total isolation rate of 0.74%. Finally, the *S.diarizonae* serotype came with one isolate, with an isolation rate from urine samples of 0.49% and 0.25% of the total isolation rate. These infections were distributed among the villagers and city residents, as the number of isolates in the villages was 13, among the positive isolates, with a rate of 68.42%.

And 6 isolates from the positive isolates in cities, with a rate of 31.58%. The infections were also distributed among infected people who keep animals and infected people who do not keep animals, as the number of positive infections reached 14, with a rate of 73.68% for animal breeders, while the number of positive infections among infected people who did not keep animals was 5, with a rate of 26.32%.

Keywords: Salmonella prevalence, serotypes, housing site, animal husbandry

Introduction

The genus *Salmonella* is one of the largest species of the Enterobacteriaceae family, which causes a wide range of acute and chronic diseases in humans and animals alike.

(Gram *et al.*, 2021)

The types of the genus *Salmonella* are gram-negative, non-spore-forming sticks that grow in the presence or absence of oxygen, i.e. they are facultatively anaerobic (Fong, 2021).

The genus *Salmonella* contains two main species, *Salmonella enterica* and *Salmonella bongori*, with more than 2756 serotypes, 2000 of which can affect humans (Shen *et al.*, 2020).

Salmonellosis is a zoonotic disease, as these bacteria inhabit the intestinal tract of vertebrates (Lauteri *et al.*, 2022).

Sometimes infection with this disease occurs through water or by eating contaminated foods of animal origin, including livestock, pork, chicken and other poultry (Mkangara and Mpenda, 2022), on the other hand, we find that about 50% of the sources of salmonella spread are vegetables and fruits (Dallal *et al.*, 2020).

The disease affects people of all age groups and causes gastroenteritis, symptoms appear After 12-72 hours in the form of abdominal pain, fever, diarrhea, nausea, and vomiting may develop and reach the blood (Bacteremia), which sometimes causes meningitis, which can be fatal (Qian *et al.*, 2020), and may also cause reactive arthritis (ReA) or Reiter's syndrome, which is a reactive inflammation that occurs after infection of the gastrointestinal tract or genitourinary system (Ehuwa *et al.*, 2020).

MATERIALS AND METHODS

The current study included the collection of 408 samples of blood and urine of inpatients and patients at Salah Al-Din General Hospital and Al-Dour Model Health Sector Hospital in Al-Dour, with 204 blood samples and 204 urine samples from the beginning of August 2022 until January 2023. As follows: Take 2 ml of blood using (Tourniquet) and medical syringes, then put the blood in EDTA tubes, then put the blood in the middle of Brain

Heart infusion broth, and incubate the tubes at a temperature of 37 °C for a period of 24 hours.

As for urine samples, they were collected by 3 ml of urine in a tube that was later placed in a centrifuge and a swab was taken from that precipitate by means of a swab and placed in tetrathionate broth and 5 drops of iodine solution were added to it and incubated at a temperature of 37 °C for a period of 24 hours, after which it was transferred by means of a metal carrier (Loop) and planted on the agar culture medium. S.S and XRD medium and incubated at 37°C for 24 hours.

After that, these bacteria were diagnosed on the basis of the morphological characteristics of the culture, which included each of (the size of the colonies, their color, and their edge) (Forbes et al., 2007).

Then it was diagnosed on the basis of biochemical tests such as the indole test according to what was stated in (Leber, 2016), methyl red according to what was stated in (Forbes et al., 2007), the voges-Proskauer test, according to what was stated in (Leber, 2016), and the citrate test, according to what was stated in (Mahon et al., 2011). And the catalase test, according to what was stated in (de la Maza et al., 2020). The urease test according to (Tille, 2017). The growth test on a Triple Sugar iron medium according to (Alfred, 2005). The gelatin analysis test and the sugars fermentation test according to (CruickShank et al., 1975). The motility test according to (Mohan et al., 2011).

After that, it was diagnosed with a more accurate level at the level of the type, through the use of the Vitek device

Results and discussion

Isolation

The results of bacterial isolation showed that the percentage of total isolation of Salmonella bacteria from inpatients and visitors to some hospitals in Salah Al-Din Governorate amounted to 19 isolates, or 4.66% of the total samples.

As the positive urine samples reached 19 isolates, at a rate of 9.31%, while the blood samples were 0 positive samples, at a rate of 0%. As shown in Table (1) and Figure (1), the results of our current study agreed with the

results of (Al-Douri, 2021) study, as the percentage of salmonella infection reached 4.66%, which is identical to the result of (Al-Douri, 2021) study, which amounted to 4.66% of the total number of samples examined.

Table 1: Percentages of isolation of Salmonella bacteria from samples of inpatients and patients

sample type	The number of isolated samples	The number of positive samples	percentage%
Urine	204	19	%9.31
Blood	204	0	%0
Total	408	19	%4.66
** Chi-Square = 38.00 P-Value = 0.0009			

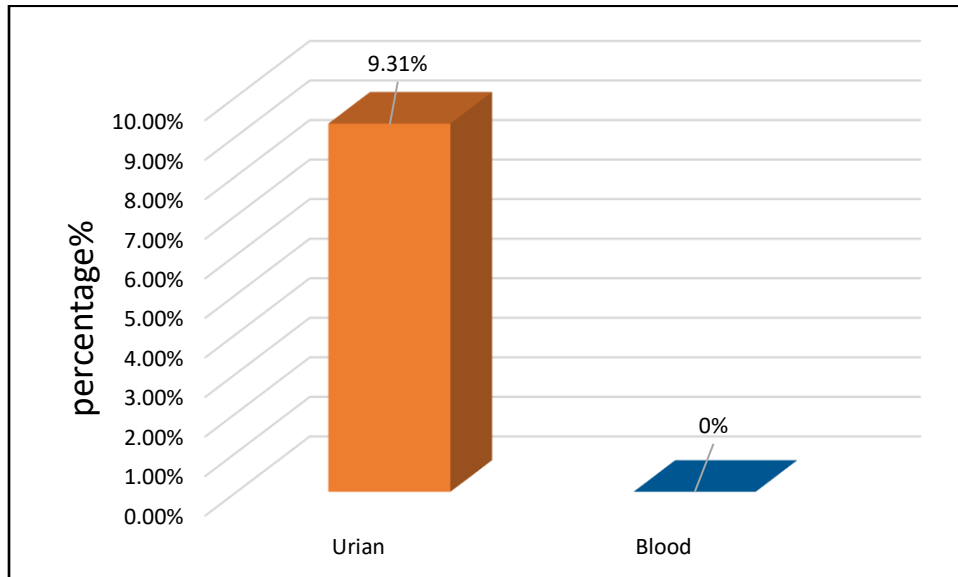


Figure 1: Percentages of isolation of Salmonella bacteria from samples of inpatients and patients

Diagnosis

The bacterial colonies of Salmonella were diagnosed through phenotypic and morphological examinations of the colony growing on the culture

media. On S.Sagar medium, it appeared as transparent, circular colonies with a black center, while on XLD medium, the colonies appeared in a large, red, circular shape with a black center, which agreed with the results of (Andoh *et al.* 2017).

It was also diagnosed on the basis of a set of biochemical tests for the family of Enterobacteriaceae, which included IMViC tests, which include four biochemical tests (Indole, Methyl Red, Voges Proskauer, Simmon citrate) as well as a set of other tests such as oxidase, catalase, gelatin analysis, motility, Urea hydrolysis, hydrogen sulfide gas production, fermentation of a group of sugars (glucose, sucrose, lactose) , The results of the biochemical tests of the Salmonella isolates showed a positive result for each of the catalase, methyl red and citrate tests, except for *S.typhi* (it gave a negative result for citrate), motility test, H₂S gas production, and glucose fermentation. *S.diarizonae* (gave a positive result for the hydrolysis of urea) and the fermentation of sucrose and lactose sugars, and these results were consistent with many studies, including (Al-Obaidi, (2022), and as shown in Table No. (2). Also, isolates were diagnosed in this study using the Vitek2 system and the GP card. /ID of diagnosis, and the results confirmed that the isolates belong to the genus Salmonella. As this technique showed speed and accuracy in diagnosing isolates with a probability ratio of (94%-99%), and the results agreed with many previous studies that used the Vitek2 system in diagnosis, including what was stated in a study (Salman, 2022).

Table 2: Biochemical tests for Salmonella isolates

	<i>S. typhi</i>	<i>S. paratyphi B</i>	<i>S. typhimurium</i>	<i>S. diarizonae</i>
oxidase	-	-	-	-
catalase	+	+	+	+
indole	-	-	-	-
Methyl red	+	+	+	+
Voges Proskauer	-	-	-	-
Citrate	-	+	+	+

Gelatin analysis		-	-	-	-
Motility test		+	+	+	+
urea hydrolysis		-	-	-	+
H2S production		+weak	+	+	+
The fermentation of sugars	glucose	+	+	+	+
	sucrose	-	-	-	-
	lactose	-	-	-	-

Salmonella serotypes isolated

The serotypes of salmonella bacteria have been determined as shown in Table (3), as it was found that the serotype *S. typhimurium* is the most common type, and the number of isolates was 11, with a rate of 2.70%, followed by the serotype *S. typhi*, and the number of isolates was 4, with a rate of 0.98%, followed by the serotype *S. paratyphi B* was 3 isolates with a rate of 0.74%, followed by the *S. diarizonae* serotype with one isolate at a rate of 0.25%, as shown in Figure (2). The results of our study came in agreement with the results of the study (Al-Obaidi, 2022), as the percentage of *S. typhimurium* isolates was 5 out of the total isolate, which is the most common pattern. The results of our current study also agreed with the results of the study conducted by (Ezat *et al.*, 2016) as 300 samples were collected from children between the ages of (one day and 13 years). Twenty-four isolates belonging to the genus Salmonella were diagnosed, among which 14 isolates of the *S. Typhimurium* serotype were identified, with a percentage of 4.66%.

S. typhimurium is one of the most prevalent types "globally and the most affecting" on public health, as the total number of cases of infection with it increases every year because of its ability to Prevalence from animals to humans, or through the food chain, or when associated with food, especially in people who are discouraged. for the immune system or the elderly (Sharif and Tayeb, 2021 and Sadiq and Othman, 2022).

Also, *S. typhimurium* is the most Prevalence type due to its resistance to many antibiotics and possesses resistance and virulence plasmids. In addition, this type of germ has the ability to survive at different temperatures and changing environmental conditions. (Yang *et al.*, 2020)

It also has the ability to invade a group of hosts, including rodents and humans, as it targets different types of cells and is inside the cells capable of active division at different rates or remains dormant to continue. (Luke *et al.*, 2021)

Al-Ahbabi (2019) recorded that *S. typhimurium* is the most common type in animals, with an isolation rate of 32.7% of domestic and wild pigeons.

Likewise, in a study conducted by (Jasim, 2018), the percentage of *S.typhimurium* isolation was 68.75% of the total number of salmonella bacteria isolated from goats in Salah Al-Din Governorate.

Table 3: Number of serotypes of Salmonella bacteria and their percentages

NO	Salmonella serotypes	Number	The percentage of urine excreted	Total percentage
1	<i>S. typhimurium</i>	11	%5.39	%2.70
2	<i>S. typhi</i>	4	%1.96	%0.98
3	<i>S. paratyphi B</i>	3	%1.47	%0.74
4	<i>S. diarizonae</i>	1	%0.49	%0.25
Total		19	%9.31	%4.66
**				
Chi-Square = 15.930 P-Value = 0.001				

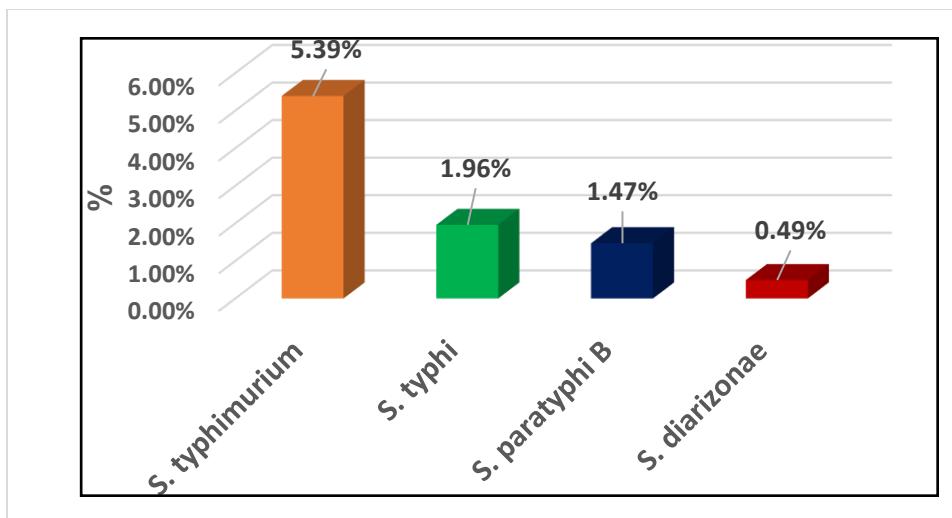


Figure (2) Numbers of serotypes of Salmonella bacteria and their percentages

Distribution of salmonella infections according to the location of residence

The results showed an increase in infections among patients and patients who live in villages with salmonella germs, as the number of positive isolates reached 13, at a rate of 68.42%, while the number of positive isolates among patients and patients who live in cities was 31.58%, as shown in Table (4) and Figure (3).

The results of our current study agreed with the results of the study (Al-Naimi, 2014), in which the percentage of infected people who lived in cities was 31.70%, and those who lived in villages were 68.29%. Our results in this study did not agree with what was stated in the results of the study (Al-Shaik bzainy *et al.*, 2021) as the percentage of infected people who live in cities reached 73.5%, which is high compared to the infected people who live in villages, which amounted to 26.5%. The reason may be due to water pollution and the possibility of a lot of dirt, lack of sanitation, animal husbandry, or appropriate temperature that makes the spread of salmonella germs in a more environment. From another environment (Al Ahbabi, 2019).

Table (4) Distribution of infections with salmonella germs according to the location of residence

Housing location	positive isolates	percentage%
The cities	6	%31.58
villages	13	%68.42
Total	19	%100

*
Chi-Square = 5.158 P-Value = 0.023

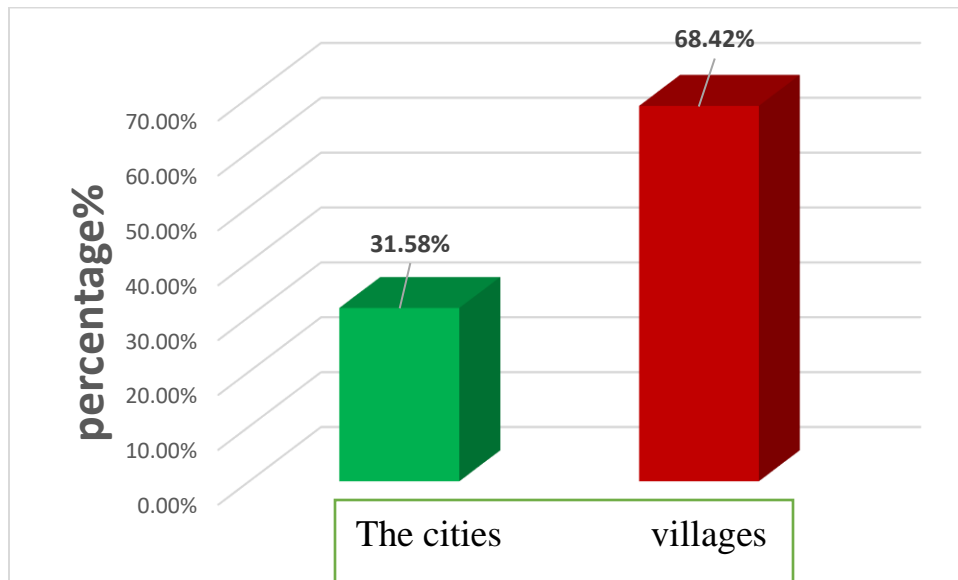


Figure 3: Distribution of Salmonella infections by residence location

Distribution of salmonella infections according to the relationship of those infected with animal husbandry

The results showed an increase in the numbers and percentages of infection with salmonella bacteria among infected people who keep animals, as their number reached 14, with a isolation rate of 73.68%, compared to the numbers and percentage of infected people who did not keep animals, which amounted to 5 isolates, with a rate of 26.32%, as shown in Table (5) and Figure (4).

The results of our current study came in agreement with the results of the study (Al-Naimi), 2014, as the results of her study showed a high infection rate with these germs in patients who keep animals, as the percentage reached 60.97%, while the percentage of infection in patients who did not keep animals reached 39.03%.

The results of our study did not agree with (Schutze *et al.*, 1998) as he reported that there was no difference in injuries between animal breeders and non-animal breeders. According to our belief, raising animals indoors plays a "major" role in achieving infection with salmonella germs, because it is a common disease between humans and animals, especially in recent times, many families have raised animals indoors in cities, but villages are almost devoid of animals.

Table (5) Distribution of infections with salmonella bacteria according to the relationship of those infected with animal husbandry

Relationship to animal husbandry	positive isolates	The percentage of infection
breeder	14	%73.68
Non-breeder	5	%26.32
Total	19	100%
** Chi-Square = 8.526 P-Value = 0.004		

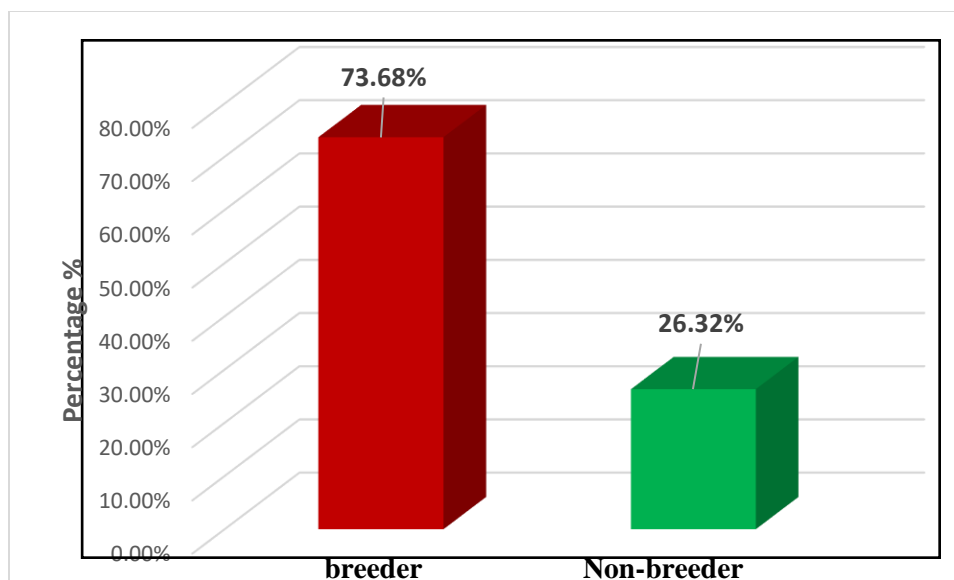


Figure (4) Distribution of infections with Salmonella bacteria according to the relationship of those infected with animal husbandry statistical analysis

The results were analyzed statistically by applying the MINITAB17 statistical program using the Chi-Square test to compare the groups included in the research, and the (ANOVA) F-test to determine the significant differences between the treatments by applying Duncan's multiple test with a probability level of 0.05 (Al-Rawi, 2000).

References :

Gram A.M. ; Wright ,J.A.; Pickering,R.J.; Lam,N.L.; Booty , L. M. ; webster, S. J. and Bryant , C. E. (2021) . Salmonella Flagellin Activates NAIP/NLRC4 and canonical NLRP3 in Flammosomes in Human Macrophages J. Immunol, 2021, 206:631-640

Fong ,W.y.(2021). Exploring the Fitness landscape of gastroenteritis-associated and blood stream in faction- associated *Salmonella enteridis* . Thesis submitted in accordance with the requirements of the University of Liverpool for degree of Doctor of Philosophy .

Shen,Y.;Xu,L.;Li,y.(2020). Biosensors for rapid detection of *Salmonella* in food : A Review. Compr Rev food sci food saf;1-49 .

Lauteri, C.; Maggio, F. ; Serio ,A.; Festino, A.R.; Paparella , A. and Vergara, A.(2022). Overcoming multidrug resistance in *Salmonella Spp.* Isolates obtained from the swine food chain by using essential oils: An *in vitro* study . front Microbiol,12:808286.

Mkangara , M. ; Mpenda, F. N.(2022). Antimicrobial and Cytotoxicity Activities of Medicinal plants against *Salmonella gallinarum* Isolated from chickens. Veterinary Medicine International. Id2294120.

Dallal , M.M.S.; Abdi, M.; Khalilian , M.; Rajabi, Z.; Bakhtiari,R.; Yazdi, M.K.S.; Yaslinifard,S.; Langroudi,S.M.A.(2020) . Isolation Identification and Antibiotic Susceptibility Testing of *Salmonella* Isolated from food borne outbreaks in Enteric patho;8(3):80-83.

Qian, H.; Cheng, S.; Liu,G.; Tan,Z.; Dong, C.; Bao,J.;Hong , J. ; Jin,D.; Bao,C; Gu,B.(2020) . Discovery of seven novel mutations of gyr B , parC and parE in *Salmonella typhi* and paratyphi strains from Jiangsu province of china. Sci. Rep, 10 , 7359.

Ehuwa , O. ; Jaiswal A. K. ; Jaiswal , A. (2021) . Salmonella, Food Safety and Food Handling Practices. Foods , 10, 907 .

Forbes, B.A.; Sahm, D.F. and Weissfeld, A.S. (2007). Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby (Elsevier). USA, P: 171-178.

Leber, A. L. (2016). Clinical Microbiology Procedures Handbook, 4th ed, vol 2 . Washington DC: ASM press

Mahon ,R.C.;Lehman ,C.D.; and Manuselis ,G.(2011). Textbook of Diagnosis Microbiology .4th.Saunders EL Sevier .USA.

De la Maza , L.M. ; Pezzlo , M. T. ; Bittencourt , C. E. ; Peterson , E. M . (2020) . Color Atlas of Medical Bacteriology , Third Edition . ASM press, Washington , DC.

Tille, P.M. (2017). Bailey And Scott's Diagnostic Microbiology. 41th ed. Elsevier, Inc. China. pp 1115 .

Alfred, E.B. (2005). Benson's Microbiological applications in laboratory manual in general microbiology. 9th ed. McGraw-Hill companies. New York.

Cruickshank, R.; Duguid, J. P.; Marmion, B. P. and Swain, R. H. A. (1975). Medical Microbiology.Vol.2. The Practice of Medical Microbiology 12th ed Churchill. Livingstone (publ.),London.

Al-Douri, Noha Sabry. (2021). Bacterial and parasitic infections of patients within Salah al-Din Governorate and their impact on thyroid hormones and measurement of some biochemical parameters. Master Thesis, College of Education for Women, University of Tikrit.

Andoh, L.A.; Ahmed , S. ; Olsen, J.E. ; Obiri-Danso, K.; Newman, M. J.; Opintan , J. A. ; Barco, L.; Dalsgaard, A. (2017) . Prevalence and Characterization of *Salmonella* among humans Ghana. Tropical Medicine and Health, 45:3 .

Al-Obeidi, Harith Rabah Ahmed (2022). Isolation and identification of non-typhoidal salmonella bacteria from humans and study of their immunological effect in rabbits. Master Thesis, College of Education for Women, Iraqi University, Iraq.

Salman, R.S.(2022). Antimicrobial and Antibiofilm , Activity of some probiotics prebiotics and Biosynthesized Silver Nanoparticles against Multidrug Resistant *Salmonella typhi* . College medicine , AL-Iraqia University . Iraq.

Ezat, H.; Mezal.; Zaman, K.; Hanan. and Manal ,B.; Saleh. (2016). Identification, Antimicrobial Resistance Of Salmonella Enterica Isolated From Diarrheal Children In Thi-Qar Province During 2015. Collage of Nursing - Thi-Qar Univ. Iraq. ejpmr, 3(6), 01-6 .

Sharif, Y.H.; Tayeb, B.A.(2021). Estimation of limit of detection of *Salmonella typhimurium* in artificially contaminated chicken meat by cultured-based and polymerase chain reaction techniques . Iraqi Journal of Veterinary Sciences, Vol. 35, No. 4, 2021 (621-625).

Sadiq, M.S.;Othman, R.M.(2022). Phylogenetic tree constructed of *Salmonella enterica* subspecies *enterica* isolated from animals and humans in Basrah and Baghdad governorates, Iraq , Iraqi Journal of Veterinary Sciences, Vol. 36, No. 4, 2022 (895-903).

Yang, C.; Li, H. ; Zhang, T. ; chu,Y.; Zuo, J. and chen, D.(2020). Study on antibiotic Susceptibility of *Salmonella typhimurium* from to the third and forth generation Cephalosporins. *Sci. Reports* , 10:3042.

Luk , C. H. ; Valenzuela, C. ; Gil, M. ; Swistak, L.; Bomme, P.; Chang, Y.Y.; Mallet, A. ; Enninga, J. (2021). *Salmonella* enters a dormant state within human epithelial cells for persistent infection. *Plos pathog* 17(u): e 10095

Jassim, Muhannad Jassam Muhammad (2018). Isolation, identification and pathogenicity of *Salmonella* bacteria in goats in some areas of Salah al-Din Governorate. Master Thesis, College of Education for Women, Tikrit University, Iraq.

Al-Nuaimi, Bayader Jalal Abdul-Hamid. (2014). The relationship of some risk factors to the spread of salmonella bacteria isolated from humans in Salah al-Din Governorate. Master Thesis, College of Education for Women, Tikrit University, Iraq.

Al-Shaik bzainy, H. A. O.; Zaman, N. A. ; Mohammed, P. L. (2021). Detection of *Salmonella*, *Shigella* and *Candida spp.* in stool from diarrheal children and evaluation the heating effect on *Salmonella* phage in Kirkuk city. *Tikrit Journal of Pure Science* Vol. 26 (4).

Al-Ahbabi, Kaiser Diab Ahmed (2019). Isolation and Identification of *Salmonella* Germs in Domestic and Wild Pigeons, Master Thesis, College of Education for Girls, University of Tikrit, Iraq.

Schutze , G. E. ; Kirby, R.S.; Flick, E. L. ; Stefanova, R. ; Eisenach, K. D. and Cave, M.D.(1998). Epidemiology and molecular Identification of *Salmonella* infection in children . *Arch.pediat . Adolesc . Med.*152:659-664.