DOI: 10.24996/ijs.2022.63.8.8

ISSN: 0067-2904

The Correlation Between *Salmonella typhi* Associated Gallstone Formation and Gallbladder Cancer

Sahira I.H. Al-Sanjary*, Amera Mahmood M. Al-Rawi
Department of Biology, College of sceince, University of Mosul, Mosul, Iraq

Received: 31/8/2021 Accepted: 19/12/2021 Published: 30/8/2022

Abstract

The current research focused on the detection of *Salmonella typhi* and its relationship with the formation of gallstone and gall bladder cancer. Samples were collected from patients aged between 32-67 years (males and females) in Mosul city hospitals. The samples included 30gallbladder fresh tissues from patients suffering from gallstone and 20 formalinfixed-paraffin embedded (FFPE) gallbladder tissue from patients confirmed with gallbladder cancer. The results showed that 33% *S. typhi* isolates were diagnosed from the tissue samples using conventional methods, biochemical tests and Vitek2. All fresh tissues samples gave positive PCR results for the presence of *FliC-d* and *CdtB* genes and 46% positivity for *S. typhi* compared with conventional methods, 64% for females, 35% for males and 40 % positive in the gallbladder cancer of which 62% were females and 37%males.

Keywords: *S.typhi*, Gallstone, Cancer, *FliC-d*, *CdtB*.

العلاقة بين تكون حصى المرارة المرتبطة بجرثومة *Salmonella typhi* وسرطان المرارة

ساهرة ادريس حميد السنجري* ,أميرة محمود محمد الراوي

قسم علوم الحياة, كلية العلوم, جامعة الموصل, موصل, العراق

الخلاصة

اولى البحث الحالي اهتماماً بالتحري عن جرثومة *Salmonella typhi (S.typhi)* وعلاقتها بتكون حصى وسرطان المرارة، جُمعت النماذج من المرضى الراقدين في مستشفيات مدينة الموصل والذين تراوحت اعمارهم من 32−67 عاماً ومن كلا الجنسين، شملت النماذج 30 عينة من نسيج المرارة Fresh tissue من مرضى يعانون من حصى المرارة و20 عينة من نسيج المرارة المثبت بالفورمالين والمحفوظ بشمع البارافين من المرضى المؤكد اصابتهم بسرطان المرارة. شُخصت جرثومة *S. typhi* وبنسبة 33%من عينات نسيج المرارة باستخدام طرق الزرع الروتينية والاختبارات الكيموحيوية فضلا عن تقنية الفايتك 2. تم التحري عن الجينات *FliC* و *CdtB* باستخدام تقنية تفاعل البلمرة المتسلسلPCR واعطت جميع عزلات*S.typhi*نتيجة ايجابية. كما استخدمت تقنية تفاعل البلمرة المتسلسل لتأكيد انتشار جرثومة *S. typhi* بين المرضى المصابين بحصى وسرطان المرارة، واعتماداً على نتائج تقنية PCR بلغت نسبة عزل جرثومة *S. typhi* من المرضى المصابين بحصى المرارة 46% مقارنة بنتائج الزرع الروتينية وبنسبة 64% للاناث و35% للذكور، فيما بلغت نسبة عزل الجرثومة المرضى المصابين بسرطان المرارة 40%منها 62% للاناث و37% للذكور.

*Email: asahera34@gmail.com

Introduction

Gall bladder cancer (GBC) is considered one of the most common cancers of the bile duct in the world [1]. It has been described as the most serious as it is usually without symptoms and cannot be detected in early stages due to the absence of indicators of the disease [2]. High rates of incidence were recorded in several countries in the world including Chile, Bolivia, Japan, Korea and China [3]. India recorded the highest rates of incidence as gallbladder cancer represented one of the most common tumours in north India, particularly in females [4]. Since the onset of the nineteenth century, it was observed that gallstone is related to the gallbladder cancer and is considered one of the risk factors which is accompanied with chronic cholecystitis [5, 6]. Gallbladder stone disease has recently increased among the Iraqi society due to many risk factors such as bacterial infections and some HLA class II antigens [7]. Moreover, patients who are carriers of *S. typhi* and *H. pylori* are more vulnerable to the gallbladder cancer as the *S. typhi* and the formation of the gallstone have been described as the most important risk factors for gallbladder cancer [8].

Salmonella typhi is considered as one of the pathogens that possess many virulence factors that enhance its pathogenicity such as cytolethal distending toxins (CDT), flagella, fimbria, in addition to pathogenicity islands as one of the distinguished genetic elements in the bacterial chromosome [9, 10]. The external part of the flagella is filament shaped and consists of 20,000 protein units of the flagellin protein that give flagella the hardness and ability to move. It has an important role in the incidence of infecting the host cells. It also helps the bacteria to escape from certain locations inside the cell [11, 12]. S. *typhi* infection not only causes chronic inflammation and damage to the mucosa, but also produces toxins like cytolethal distending toxins (CDT) which contribute to DNA damage and induce cell-cycle arrest.CDT consists of three subunits: *CdtA, CdtB* and *CdtC. CdtB* possesses enzymatic activity DNase I, whereas *CdtA* and *CdtC* contribute in attaching the holotoxin with the plasma membrane of the host cell. *CdtB* activity depends on the gene expression of two genes, pertussis like toxins A (*pltA*) and B (*pltB*) [13]. The triple complex *pltA, pltB* and *CdtB* make changes in the cell by destroying the DNA, arresting the cell cycle and affecting the paths of signal transduction. In addition to that, the occurrence of genotoxicity that is caused by the bacteria toxins,enhances the incidence of gallbladder cancer [8].

Due to the importance of confirming the relationship between gallstone formation and its development into gallbladder cancer, which are related to the infection with *S. typhi*, the current research focused on the detection of the *S. typhi* in addition to detecting some of its virulence factors that play an important role in gallbladder cancer.

Materials and Methods

Samples: Fifty samples were obtained from patients aged between 32-67 years (males and females) and were subjected to gallbladder cholecystectomy at Alzahrawi Teaching Hospital and Alzahrawi Private Hospital in Mosul City. The samples included:

Thirty samples of gallbladder fresh tissue collected from gallstone patients who had undergone gallbladder cholecystectomy. The samples were immediately taken to laboratory where the gallstones were photographed. Then a section of the tissue was placed in sterilized plane tubes containing 5 ml of BHI broth and incubated at 37°C for 24 hours. Larger tissue samples were cut into smaller pieces prior to being cultured. After overnight incubation, they were cultured on MacConkey agar, Salmonella-Shigella (SS agar) and Xylose Lysine Deoxycholate agar (XLD agar) and then incubated at 37°C for 24 hours [14]. Colonies were identified by biochemical tests confirmed using Vitek2 compact system.

Molecular Detection of *S.typhi*

Depending on the DNA extraction from fresh tissues, *Salmonella typhi* DNA was extracted using Geneaid Genomic DNA Purification kit for the detection of *FliC* and *CdtB* genes.

Samples were then homogenized and 25 mg of tissue was combined with 1 ml of the of BHI broth and centrifuged at 12000rpm for 2 min [3].

The 20 Formalin Fixed-Paraffin Embedded (FFPE) gallbladder tissue samples confirmed gallbladder cancer patients who had undergone gallbladder cholecystectomy due to cancer, 15 samples had both cancer and gallstones and 5 samples had gallbladder cancer only. FFPE tissue was subjected to many processes before extracting the genomic DNA [15]. *S. enterica* serovar typhi (ATCC: 14028) strain was used as positive control.

Extraction and Purification of Genomic DNA: The DNA was extracted from cultured tissue (n=10), fresh tissues (n=30) and FFPE tissue (n=20) using Geneaid Genomic DNA Purification kit. The concentration of the DNA was measured using the Nanodrop spectrophotometer and the purity was measured by taking the absorption reading at 280/260 nm.

Amplification of the Two Genes (*Flic-d* and *CdtB*): This was performed using the polymerase chain reaction technique (PCR). Specific primers were used to amplify a 763bp fragment *Flic-d*- Forward 5′- ACTCAGGCTTCCCGTAACGC -3′ and *FliC-d*– Reverse 5′- GGCTAGTATTGTCCTTATCGG-3′enzymatic amplification was performed by using 35 cycles of 5.30 min at 94ºC, 30 sec at 55ºC and 6 min at 72ºC,and specific primers were used to amplify a 508 bp fragment *CdtB*- Forward 5′- TAAGTGGTACTGCCGGTGTG -3′ and *CdtB*- Reverse 5′- GTAGGTGCGAGTACGGCTAC -3′ enzymatic amplification was performed by using 35 cycles of 3.30 min at 95ºC, 45 sec at 59ºC and 8 min at 72ºC.

Gel Electrophoresis Analysis: The amplification results were analysed using the gel electrophoresis device as the mixture was loaded in agarose gel well prepared with a concentration of 1.5% using the buffer solution (1XTBE). The voltages were stabilized at 90 volt/cm for 75 minutes and then the results were photographed by a digital camera.

Results

Direct examination of the gallbladder demonstrated various sizes of the gallstones ranging from 2 mm - 4 cm and their numbers varied between 1 - 50 stones, as shown in Figure 1

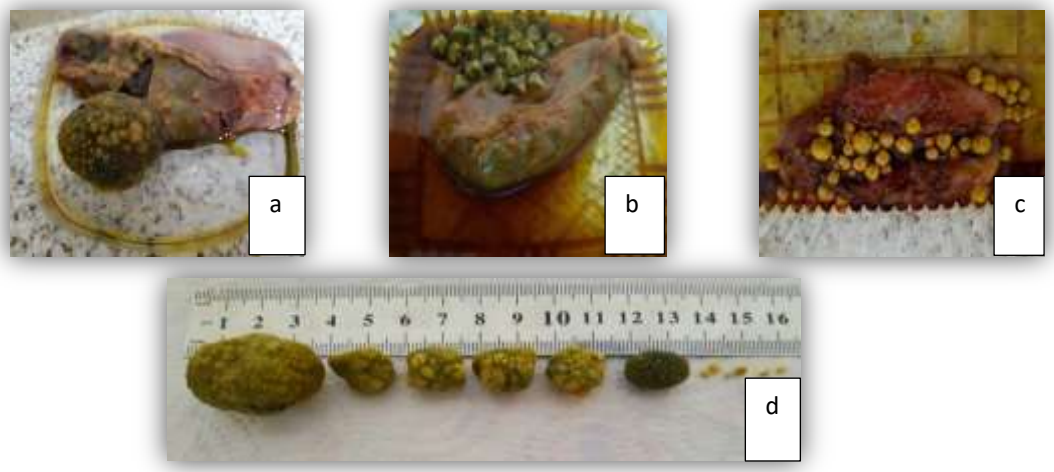

Figure 1- The gallstones,a: single and large gallstones, b, c: numbers varied between 1 - 50 stones and d: the gallstones differ in sizesranging from 2 mm - 4 cm.

Results of *S. typhi* isolated culture from fresh tissues revealed that 10 (33%) had positive results due to the appearance of pale colonies on MacConkey agar, pale colonies with black centres on SS agar and red colonies with black centres on XLD agar due to the production of H_2S (Figure 2).

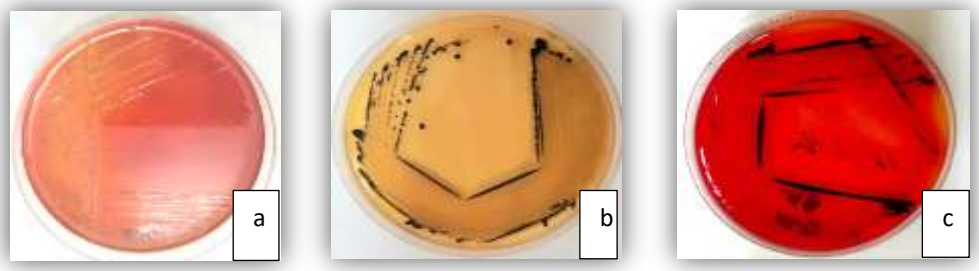

Figure 2- Representative *Salmonella typhi* colonies on :(a) MacConkey agar, (b) S S agar, (c) XLD agar.

Biochemical test results were shown to be positive for catalase enzyme and Methyl red test. While they were negative for indole production test, Voges-Proskauer, Simmons citrate production, urease and oxidase enzymes. The results of the diagnosis were confirmed using Vitek2 compact system as shown in Figure 3.

ASCo

bioMérieux Customer: **Microbiology Chart Report** Printed Jun 19, 2020 18:02 CDT

Patient Name: Sahera, 1 Patient ID: 394
Location: Physician:
Lab ID: 394 Isolate Number: 1

Organism Quantity:
Selected Organism : Salmonella ser.Typhi

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.88 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Salmonella ser.Typhi** 0005610440004210		
ID Analysis Messages	Confirm by serological tests			

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	-			

Figure 3- *Salmonella typhi* diagnostic results using Vitek2 compact system.

Salmonella typhi were detected by PCR technique for amplification. The results revealed two bands,763bp and 508bp, for flagellin *FliC-d* and toxins (*CdtB*) genes respectively. The results showed that 10 (33%) isolates of *S. typhi* were diagnosed from the tissue samples using conventional methods, biochemical tests and Vitek2. All fresh tissues samples gave positive PCR results for the presence of *FliC-d* and *CdtB* genes, and 14 (46%) positivity for *S. typhi* compared with conventional methods, 9 (64%) for females, 5 (35%) for males. Moreover, the percentage of *S. typhi* infection in gallbladder cancer was 8 (40%) from FFPE samples, 5 (62%) and 3 (37%) from females and males respectively, and negative result from 5 samples infected with gallbladder cancer only as shown in Figures4 and 5**.**

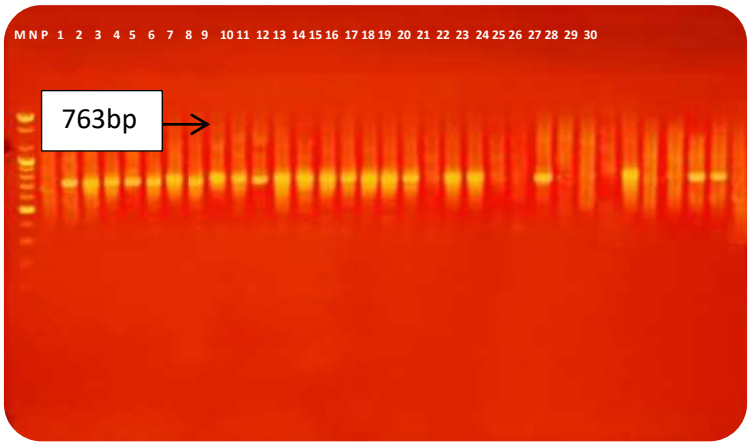

Figure 4-PCR products of the *FliC-d* gene of *S. typhi*. The size of the PCR product is 763 bp, (M) DNA ladder, (N) negative Control ,(P) positive control, 1-14 gallstone samples,15-30 gallbladder cancer samples.

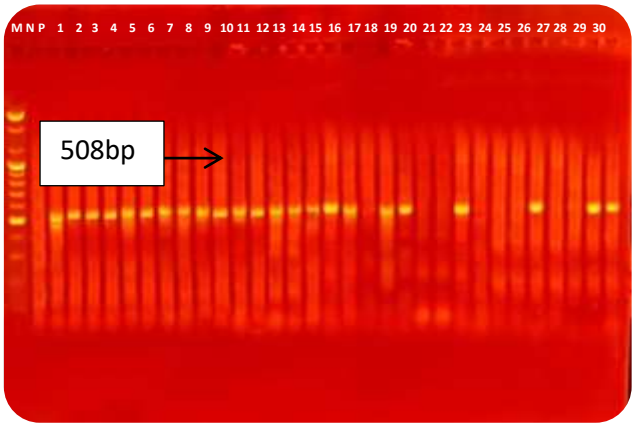

Figure 5-PCR products of the *CdtB* gene of *S. typhi*, the size of the PCR product is 508 bp, (M) DNA ladder, (N) Negative Control, (P) positive control, 1-14 gallstone samples,15-30 gallbladder cancer samples.

Discussion

Cancers are related to several factors including genetic and the type of life. However, the infection with *S. typhi* and stone formation represent the most important risk factors as with the increase in stone size, the risk of infection with the chronic bile inflammation increases especially when the size of the stone exceeds 3 cm where it is regarded as the most serious with ten folds compared to the small stone [16]. Many studies demonstrated the existence of gallstone in 70-90% of patients infected with gallbladder cancer as the stones cause direct mechanical infection of the bile membrane during the contraction of the gallbladder, especially when the stone is big, of irregular shape and occupies a big area of the gallbladder cavity [17]. Stones increase the surface area of the microbes colonization which becomes too difficult to be eliminated by traditional antibiotics and also the gallstone might cause a mechanical infection consequently causing chronic inflammations [5].

The present study showed that the average infection with gallbladder cancer increases with age as it was clear that most of the cases of gallbladder cancer and gallstone were for ages over 30 years while the maximum was for 65 years of age. This study agrees with Shukla *et al*. [18] who reported that the average infection with gallbladder cancer increases after the age of 45 years and the maximum age for the infection is 6) years. The study of Lyer and his colleagues [19] indicated that there is a correlation between gallbladder cancer and females

specifically in the developing countries that are characterized with high incidence of *S. typhi*. It was observed that infections in females were 2-3 times more than the infections in males because the estrogen hormone causes an increase in the saturation of gallbladder with cholesterol, which in turn, contributes to form gallstones that enhance the incidence of gallbladder cancer.

Gallstone is considered as one of the main risk factor of gallbladder cancer as reports indicate that the highest average of gallbladder cancer was recorded in Chile (12.8 for each 100,000 for females and 6.3 for each 100,000 for males). Also, gallbladder cancer is considered as the second main reason of death in females in Chile [3]. In Iraq, as reports indicate, the prevalence of *Salmonella Typhi* among patients infected with cancer, cholecystitis, gallstones and all toxin genes that were isolated from *S. typhi* of patients with gallstones, while none *CdtB* was isolated from bacteria in patients with cholecystitis and 84.6% of *S. typhi* contained *pltA* and *pltB* genes [20]. *S. typhi* plays an important role in the incidence of several digestive system diseases, including its relationship with cancer. It is characterized by its ability of using various mechanisms including the destruction of DNA, activating the paths that cause tumours, producing carcinogen compounds, stimulating the chronic inflammations or inhibiting the immunity [21]. The current research focused on the detection of *S. typhi* and studying its relationship with the formation of gallstone and gallbladder cancer. Chronic carriers of this pathogen have an approximately eightfold excess risk of developing gallbladder carcinoma than non-carriers and an approximately 200-fold excess risk of developing hepatobiliary carcinoma compared with people who have had acute typhoid and have cleared the infection [22, 23]. In addition, epidemical studies demonstrated that *S. typhi* carriers are more likely to be infected with gallbladder cancer compared to the uninfected ones [17].

Isolation of *S. typhi* from the cultured tissues was in conformity with the results of [24]. The diagnosis of the *S. typhi* by Vitek2 compact system showed 99% conformity as this system is considered one of the rapid and accurate technique in terms of diagnosing bacteria. In the present study, molecular methods were employed to detect the gene *CdtB* which is considered one of the most important virulent factors that have an impact on the development of the infection that leads to cancer, in addition to *Flic-d* which encodes the construction of flagellin protein because it contains specific and qualitative sequences for *S. typhi.* Researchers Hartl and Sigal [25] indicated that the virulence genes *CdtB* and *Flic-d* can be employed as marker genes to detect the *S. typhi* in the various biological samples including that of gallbladder cancer. Dutta and his colleagues [26] emphasized the use of the sensitive and qualitative techniques to diagnose *S. typhi* from the gall bladder samples.

Mittal and his colleagues [15] obtained an isolation percentage of 56% using the gene *Flic-d* from chronic cholecystitis and gallstones cases and achieved positive results using the same gene from the gallbladder cancer with a percentage of 59%). *S. typhi* is related to the gallstones due to their ability to form the biofilm on the stone surfaces [9]. In addition, *S. typhi* has the ability to change the gene expression when they are within the biofilm [3, 27]. Studies have demonstrated that *S. typhi* also has the ability to suppress the immune response in gallbladder mucosa or reduce the level of infection in the gallbladder and regulate the immune environment which in turn may cause changes in the mucous membrane. These changes can develop and lead to carcinogenic changes in the gallbladder [28]. The *CdtB* gene toxins of *S. typhi* were described as the most important as they cause genetic instability of the infected gallbladder cells which expose these cells to the risk of malignant changes [8].

Conclusion:

In this study, the prevalence of *S. Typhi* among patients infected with gallbladder cancer and gallstones, the results of polymerase chain reaction (PCR) showed 46% positivity for *S. typhi*

in the gallstone and 40% positive in the gallbladder cancer. All samples gave positive PCR assay results for the presence of *FliC* and *CdtB* genes.

Disclosure and Conflict of Interest

Conflict of Interest: The authors declare that they have no conflicts of interest.

References

[1] R. Shukla, P. Shukla, A. Behari, D. Khetan, R.K. Chaudhary, Y. Tsuchiya, T. Ikoma, T. Asai, K.Nakamura, V.Kapoor." Roles of *Salmonella typhi* and *Salmonella paratyphi* in Gallbladder Cancer Development". *Asian Pacific Journal of Cancer Prevention*, vol. 22, no. 2,pp. 509-516,2021.

[2] R. Kanthan, J.L. Senger, S. Ahmed, S.C. Kanthan, "Gallbladder Cancer in the 21st Century". *Journal of Oncology*, vol .10, pp.26, 2015.

[3] J. Koshiol, A. Wozniak, P. Cook, C. Adaniel, J. Acevedo, L. Azocar, A.W. Hsing, J.C. Roa, M.F. Pasetti, J.F. Miquel, M.M. Levine , C. Ferreccio , "Salmonella enterica serovar Typhi and gallbladder cancer: A case-control study and meta-analysis". *Cancer Medicie,* vol.5, no.11, pp.3310-3235, 2016.

[4] R.K. Malhotra , N. Manoharan , N.K. Shukla, G.K. Rath, "Gallbladder cancer incidence in Delhi urban: A 25-year trend analysis". *Indian Journal Cancer*, vol.54, no.4, pp,673-7,2017.

[5] U. Dutta, N. Bush, D. Kalsi, P. Popli, V.K. Kapoor, "Epidemiology of gallbladder cancer in India". *Chinese Clinical Oncology* , vol. 8, no.4, pp. 33-20, 2019.

[6] V. Jayanthi, S. Sarika, J. Varghese, V. Vaithiswaran, M. Sharma, M.S. Reddy, V. Srinivasan , G.M.M. Reddy , M. Rela , S. Kalkura , "Composition of gallbladder bile in healthy individuals and patients with gallstone disease from north and South India*".* *Indian Journal of Gastroenterology*, vol.35, no.5, pp.347-53, 2016.

[7] S.A. Jassam, W.A. Hadi, B. Hameed, B.H. Abdullah, "Gallbladder stone formation in Iraqi patients is associated with bacterial infection and HLA class II-DRB1 antigens". *Indian Journal of Pathology and Microbiology* , vol.63, no. 4,p p.570,2020

[8] L.P. Sepe, K. Hartl, A. Iftekhar, H. Berger, N. Kumar, C. Goosmann, S. Chopra, S.C. Schmidt, R.K. Gurumurthy, T.F. Meyer, F. Boccellato, "Genotoxic Effect of *Salmonella* Paratyphi A Infection on Human Primary Gallbladder Cells".*ASM Journals*, vol.11,no.5,pp. 01911-20, 2020.

[9] E.G. Di Domenico, I. Cavallo, M. Pontone, L. Toma, F. Ensoli, "Biofilm Producing Salmonella Typhi: Chronic Colonization and Development of Gallbladder Cancer". *International Journal of Molecular Sciences*, vol. 18, no.9, pp. 1887, 2017.

[10] N.A. Rajab, and A.M. Turki, "Assessment of Histopathological change in the liver and spleen of mice infected with *Salmonella typhi* following treatment with Ciprofloxacin". *Iraqi Journal of Science*", vol 62,no.3, pp. 761- 768,2021.

[11] G.N. Chang, C.S. Chen, K. Chieh Ho, "Molecular cloning and expression of a phase I flagellin gene from *Salmonella*entericaserovar Cholerasuis". *Taiwania*, vol. 53,no.3, pp. 201- 209, 2007.

[12] J.A. Ibarra, and O. Steele-Mortimer, "Salmonella –the ultimate insider. Salmonella virulence factors that modulate intracellular survival". *Cell Microbiol*, vol.11, no.11, pp.1579-86, 2009.

[13] L. Zha, S. Garrett , J. Sun , *"Salmonella* Infection in Chronic Inflammation and Gastrointestinal Cancer". *Diseases*, vol.7, no.1, pp.28, 2019.

[14] J.M. Willey, L.M. Sherwood, C.J.Woolverton,*"*Prescott'sMicrobiology",10th edition. McGraw-Hill Education, Inc: USA. 2017.

[15] V. Mittal, S. Kumar, A. Pandey, S. Singh," Is Salmonella enterica serovar Typhi associated with carcinoma gall bladder". *Biomedical Research*, vol. 29, no.8,pp. 1617-1620, 2018.

[16] V. Nagaraja and G.D. Eslick, "Systematic review with meta-analysis: the relationship between chronic Salmonella typhi carrier status and gall-bladder cancer". *Alimentary Pharmacology Therapeutics*, vol.39, no.8, pp.745-50, 2014.

[17] U. Dutta, "Gallbladder cancer: Can newer insights improve the outcome: Gallbladder cancer: Newer insights". *Journal of Gastroenterology and Hepatology*, vol. 27, no.4, pp.642-53, 2012.

[18] V.K. Shukla, H. Singh, M. Pandey, S.K. Upadhyay, G. Nath, "Carcinoma of the gallbladder–is it a sequel of typhoid". *Digestive Diseases Sciences*, vol. 45, no.5, pp.900-3, 2000.

[19] P. Iyer, S.G. Barreto, B. Sahoo, P. Chandrani, M.R. Ramadwar, S.V. Shrikhande, A. Dutt, "Non-

typhoidal Salmonella DNA traces in gallbladder cancer". *Infectious. Agents Cancer*, vol. 11, no. 1, pp.12, 2016.

[20] Z.K. Hanan, E.H. Mezal, M.B. Saleh, H.S. Saleh, "Histopathological study of Gallbladder diseases and Molecular Detection of genotoxins of Salmonellaand Typhi from Gallbladder of Patients Undergoing to Gallbladder Cholecystectomy in Thi-Qar province/Iraq*".European Journal of Molecular &Clinical*, vol.7, no.6, pp.56-66. 2020.

[21] L. Elsalem, A.A. Jum'ah, M.A. Alfaqih, O.Aloudat, "The Bacterial Microbiota of Gastrointestinal Cancers: Role in Cancer Pathogenesis and Therapeutic Perspectives". *Clinical Experimental Gastroenterology*, vol. 6, no.13, pp.151-185, 2020.

[22] V. Samaras, P.I. Rafailidis, E.G. Mourtzoukou, G. Peppas, M.E. Falagas, "Chronic bacterial and parasitic infections and cancer". *Journal of Infection in Developing Countries*, vol.4, no.5, pp.267-281, 2010.

[23] C.P. Caygill, M. Braddick, M. J. Hill, R.L. Knowles, J.C. Sharp, "The association between typhoid carriage, typhoid infection and subsequent cancer at a number of sites". *Europe Journal Cancer Prevention*, vol. 4, no.2, pp. 187-93. 1995.

[24] A.A. Njum, R.N. Hassan, and J.A. Alwan, "Identification of Antibiotic- Resistant in *Salmonella typhi* Isolated from Typhoid Patient in Samawa". *Iraqi Journal of Science*", vol.60, no.5, pp.980-984.2019.

[25] K. Hartl and M. Sigal, "Microbe-Driven Genotoxicity in Gastrointestinal Carcinogenesis". *International Journal of Molecular Sciences*, vol. 21, no.20, pp.7439. 2020.

[26] U. Dutta, P.K. Garg, R. Kumar, R.K. Tandon, "Typhoid carriers among patients with gallstones are at increased risk for carcinoma of the gallbladder". *American Journal Gastroenterology*,vol. 95, no. 3, pp.784-7, 2000.

[27] R.W. Crawford, R. Rosales-Reyes, L. Ramirez-Aguilar Mde, O. Chapa-Azuela, C. Alpuche-Aranda, J.S. Gunn, "Gallstones play a significant role in *Salmonella* spp. gallbladder colonization and carriage". *Proceedings of the National Academy of Sciences of USA*, vol.107, no.9, pp. 4353–4358, 2010.

[28] M. Diez-Garcia, R. Capita, C. Alonso-Calleja, "Influence of serotype on the growth kinetics and the ability to form biofilms of Salmonella isolates from poultry". *Food Microbial*, vol.31, no. 2, pp.173-80, 2012.