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Histopathological changes of Serratia rubidea isolated from cattle in mice

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ABSTRACT

The research conducted to isolate and identify the opportunistic pathogen *Serratia rubidaea* from other similar species using mice models for *S. rubidaea* infections. 100 fecal samples were taken from cattle in Baghdad City which were identified by biochemical tests, Vitek 2 compact, and PCR analysis, then sequencing for 16S rRNA. Twenty-four albino mice of both genders were divided into three groups: infectious and control. The first group infected with 1.5×10^8 cfu. (0.1ml\orally), the second group infected with 3.0×10^8cfu (0.1ml\orally), and the third group as negative control were injected with 0.1ml PBS. To characterize infection in mice numerous criteria were employed including clinical signs, mortality, and histological changes. From the collected fecal samples, 12 (12%) of the Serratia isolates were isolated using culture media, the isolates were positively identified as 99% *S.rubidea* using Vitke 2, and 98% by sequencing when matching with GenBank references was found registered the bacterial isolate with accession No. (OR757107.1). The histopathological changes of the intestine showed degenerative changes in the submucosal glands, cystic dilation, and inflammatory cell infiltration. while kidney exhibited vascular congestion, dilation of vessels, cellular swelling of the tubular epithelial lining, interstitial hemorrhage, and glomerular capillary congestion, and liver showed inflammatory cell infiltration around hepatic vessels, narrowing of hepatic sinusoids, focal hepatic necrosis with mild inflammation, perivascular inflammation, portal vein dilation, and periductal inflammatory infiltration. Finally, spleen displayed congestion in the red pulp, lymphoid depletion in the white pulp and splenic follicles, and scattered megakaryocytes. Some splenic follicles showed variation in size.

1. Introduction

Serratia is a genus of Gram-negative bacteria that currently consists of ten distinct species*: S. Marcescens, S. Liquefaciens, S. Proteomaculans, S. Grimesii, S. Plymuthica, S. Rubidaea, S. Odorifera, S. Ficaria, S. Fonticola, and S. Entomophila* (Grimont, *et al.*,1988; Jassim,2020). *Serratia rubidaea (S.rubidea*) is an opportunistic and zoonotic bacterium that is poorly understood in both of veterinary and human medicine. It is frequently found in natural environments (water, soil, and vegetables) and occurrences of isolation are infrequent according to (Al-Saphar and Al-Faragi, 2014). Infections are typically associated with clinically disabled people or develop as a result of invasive procedures and protracted broad-spectrum antibiotic therapy. In humans can isolated from the respiratory tract, feces, bile, and wounds, and blood. *S. rubidaea* can be responsible for infection, especially in debilitated patients and particularly immunocompromised ones, receiving broad-spectrum antibiotics or after undergoing invasive procedures or extensive surgery (Oufaska et al.,2023). This agent has also been reported to be present in wound infections in animals and intestinal content (Vijayakrishnan *et al*., 2010).

Innate immune recognition of bacteria is mediated by Toll-like receptors (TLR's). TLR-2 recognition of peptidoglycan and lipoteichoic acid and TLR-4 recognition of bacterial lipopolysaccharide (LPS) (Abdullah *et al*.,2017). It is possible that Serratia living in a human body will limit prodigiosin synthesis and so evade detection by the host's immune system. Prodigiosin can trigger a body's immune system (antibodies and T cells). Numerous strains seem to no longer be able to generate it at all (Ikram and Roa'a, 2022). Prodigiosin is characterized by apoptotic activity, and morphological examination of those cells showed that Prodigiosin caused chromatin condensation and shrinkage (Mahmoud and Yonis, 2015). Numerous other features of Serratia spp. pathogenicity and virulence have been investigated, such as adhesion, hydrophobicity, LPS, and extracellular products. It appears that this bacterium has ways of adhering to the surface of the host epithelial cell. Serratia spp had a reputation for having minimal intrinsic pathogenicity, which was consistent with its function as an opportunistic infection agent, even though nearly all isolates produce extracellular substances such DNase, chitinase, lecithinase, lipase, gelatinase, and siderophores, it appears that in Serratia spp these substances do not function as strong virulence factors (Hyyawi, and Nadhom, 2020). The aim of study the histopathological change of this bacteria on vital organs in mice.

MATERIAL AND METHODS

Samples collection:

One hundred cow excrement samples were gathered from different areas of Baghdad City and transported to the laboratory in less than 2hrs (Quinn *et al*.,2011).

Isolation and identification:

One gram of each fecal sample was put into a sterile test tube with 10 ml of normal saline and 0.1 ml of each sample suspension was inoculated on MacConkey agar or chrome agar, which was then incubated for 24 to 48 hours at 37 °C, the suspected colonies inoculated on nutrient agar are then incubated at 28°C (Quinn *et al*.,2011). By employing the vitek2 compact system conventional morphological and biochemical tests, as well as an amplification of the 16S rRNA gene using a PCR, the isolates of *S. rubidea* were identified down to the species level. To identify the bacterial isolates, the Vitek 2 system (bioMérieux, Lyon, France) was employed in accordance with the manufacturer's instructions. The Presto Mini g DNA Bacteria Kit was used to extract DNA in accordance with the manufacturer's instructions (Geneaid, KOBA). Thermo Scientific Inc., USA's NANODROP-2000 spectrophotometer was used to determine the concentration of DNA.16SrRNAPrimer:3'R5'TACGGTTACCTTGTTACGACTT3';F5'AGAGTTTGATCCTGGCTCAG amplification size 1500bp (Ahmed and Al-Samarraae, 2021; Kashash and Abdul-Kareem,2022).

A total volume of 25µl was used for the PCR amplification, which included 12.5µl of the Promega Master Mix, 1µl each of the Forward and Reverse Primers, 8.5µl of nuclease-free water, and 2µl of DNA template. The PCR condition protocol involved five minutes of initial denaturation at 95°C, thirty seconds of denaturation at 95°C, thirty seconds of annealing at 60°C, one minute of 72°C extension, and seven minutes of final extension at 72°C. DNA sequencing was done on the PCR product tubes containing the sample and the forward and reverse

primers of 16S rRNA and only one isolate was genetically sequenced and submitted to GenBank.

Experimental design:

The strain was cultured on nutrient agar incubated at 37C° for 24hrs. Then the culture was washed with PBS (pH=7.2) three times and two dilutions were prepared as infective doses (1.5×10^8, 3.0×10^8) cfu/ml according McFarland tube (Cockerill *et al*.,2012). During three days following the infection clinical signs and mortality rate were observed. Twenty-four healthy Swiss mice of both sexes aged between 7-8 weeks, weighted 13-16g were divided into three groups (each group 6 mice). Group1st infected with 1.5×10^8 cfu (0.1ml) (orally), group 2nd infected with 3.0×10^8cfu (0.1ml) (orally), and group 3rd control groups were injected with 0.1ml PBS by the same routes as infected groups. The mice were sacrificed, liver, kidney, heart and spleen tissues were harvested and washed with PBS then preserved in 10% formaldehyde solution for 24hrs. After removing excess fixative through washing, tissue samples were dehydrated in graded series of ethanol, cleaned with xylene and encapsulated in paraffin wax. For histological investigation, sections were stained with hematoxylin and eosin (H&E) (Bancroft and Gamble 2008). Specimens were taken from the liver, kidney, spleen, and intestine. The tissues were fixed in 10% formalin solution immediately after removal. (Luna,1968). All procedures carried out in this study were reviewed and accepted in compliance with the ethical principles of animal handling (Number P-G\651,24\3\2024) which submitted by the Scientific Committee at the College of Veterinary Medicine, University of Baghdad.

RESULTS AND DISSCUSION

Isolation and identification:

Out of 100 fecal samples, 5 (42%) and 7 (58%) showed normal feces and diarrhea, respectively (see Table 1).

Table 1: Source and Isolate Rates of *Serratia isolates*.

Studies \months	No. of examined samples	Type and source of sample	No.of examined isolates	Positive percentage %
September to December 2023	100	Normal feces	5	42%
		diarrhea	7	58%
Total	100		12	12%

Identification of *S. rubidea* was done by study colonial morphology on chrome agar, MacConkey agar and nutrient agar, all isolates showed similar appearance on chrome agar as small or large dark pink colonies (see Figure 1A, B). *S.rubidea* isolates were appeared as pink colonies on MacConkey agar, while on nutrient agar appeared as red colonies (see Figure 1C).

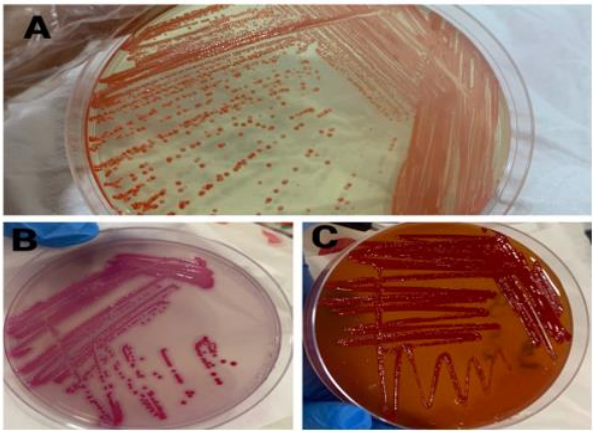

Figure 1. *Serratia rubidea* on (A)Nutrient agar (B) Chrome agar (C) MacConkey agar.

All isolates and standard strain examined by biochemical tests (see Table 2).

Table (2): Biochemical test of *Serratia isolates.*

Test	Result
Lactose fermentation	+
Chrome agar	+dark pink colony
Gram stain	-
Oxidase	-
Catalase	+

To confirm the identification of Serratia spp. Vitek 2 compact system was depended and the result showed that the isolated bacteria in this

study was *Serratia* and the species *rubidea* (see Table 3).

Table (3): Vitek 2 Compact System.

Identification Information					**Analysis Time:** 4.08 hours				**Status:** Final							
Selected Organism					99% Probability **Serratia rubidaea**											
Biochemical Details																
2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	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	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

It was determined that the isolate possessed 16 s rRNA. Therefore, the isolate tested positive for the 16S rRNA gene by 1.5% agarose gel electrophoresis and monoplex PCR amplification. (See Figure 2).

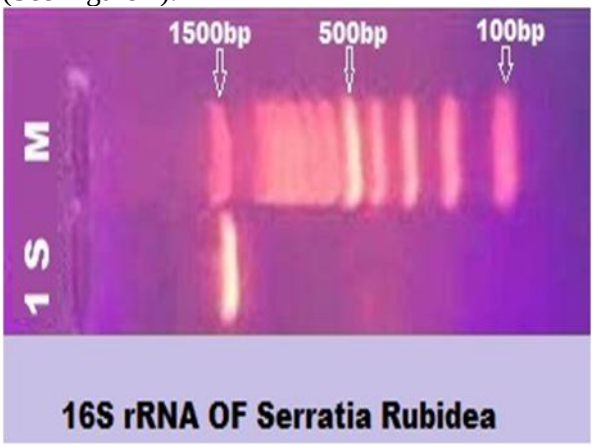

Figure 2. The 16S rRNA gene's amplified PCR products (1500 bp): Agarose gel electrophoresis, with 1.5% agarose, ethedium bromide staining, and a 2-hour electrophoresis at 75 volts, followed by a UV transilluminator light. M: The DNA molecular weight marker (100 bp ladder), and 1S: The *Serratia rubidea* 16SrRNA amplified PCR product.

The National Center for Biotechnology Information (NCBI) website's Basic Local Alignment Search Tool (BLAST) was used to examine the sequences (http://www.ncbi.nlm.nih.gov). The *Serratia rubidea* isolation and reference strains in GenBank had 98% homology (Accession No. ON678200.1). The GenBank registered the *S. rubidea* isolate with (Accession No. OR757107.1)

Infectious dose:

The clinical signs were observed in the challenged groups. After 2 days, all groups showed abnormal activity and dullness, less in appetite, diarrhea, whereas negative control group appeared to be unaffected.

Histopathological examination

Histopathological examination after 7-day post challenge showed that all examined group were affected with variable histopathological changes: The initial group results of the histopathology analysis of mice infected with $1.5x10^{8}$CFU/ml of *S. rubidea* revealed mild to moderate histological alterations. The intestine (colon) showed mild degenerative changes of submucosal gland (See Figure 3).

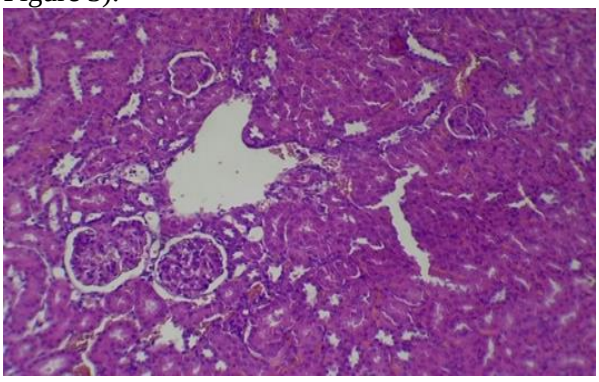

Figure 3. Histopathological section of colon (Group 1) post challenged mice showed various degrees of lymphoid depletion mild degenerative changes of submucosal gland (H&E stain 40X)**.**

The kidney showed moderate vascular congestion and dilation of kidney vessels (See Figure 4).

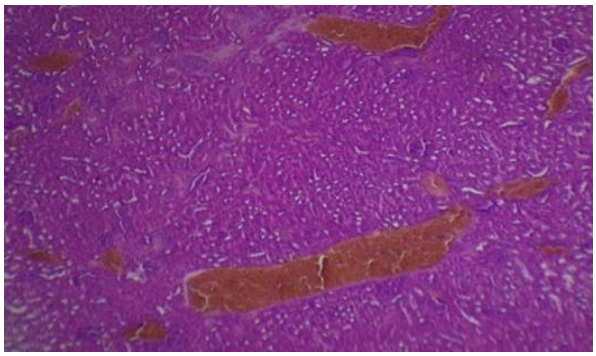

Figure 4. Histopathological section of kidney (Group 1) post challenged mice showed moderate vascular congestion and dilation of kidney vessels (H&E stain 40X)**.**

The majority of renal tubules showed cellular swelling of tubular epithelial lining with slight interstitial hemorrhage and capillary congestion of glomerular tuft (See Figure 5).

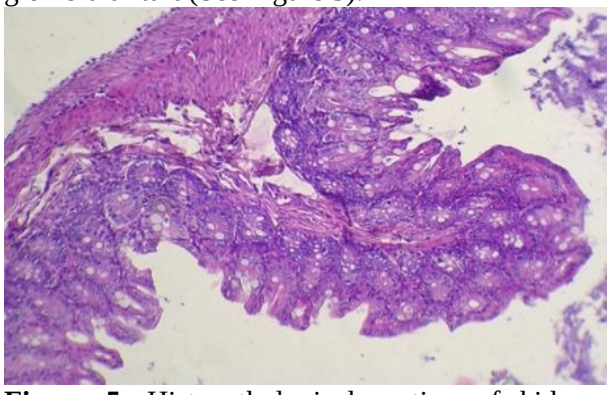

Figure 5. Histopathological section of kidney (Group 1) post challenged mice showed cellular swelling of tubular epithelial lining with slight interstitial hemorrhage and capillary congestion of glomerular tuft (H&E stain 40X)**.**

The liver showed moderate inflammatory cell infiltration composed of mononuclear cells (MNCs) and neutrophils mainly around hepatic vessels with complete narrowing of hepatic sinusoids due to marked hepatic swollen, other findings showed focal hepatic necrosis accompanied with mild inflammatory response in adjacent parenchyma (See Figure 6).

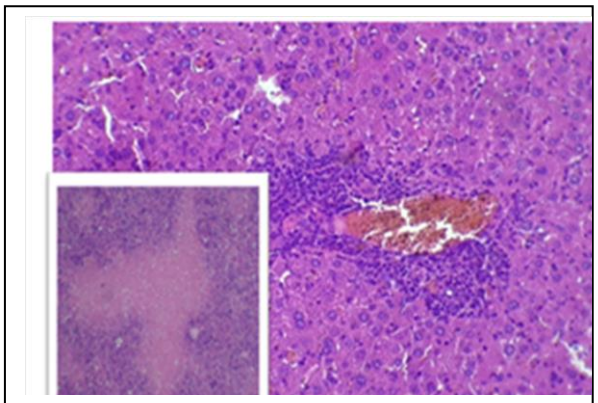

Figure6. Histopathological section of liver (Group 1) post challenged mice showed neutrophils mainly around hepatic vessels with complete narrowing of hepatic sinusoids and focal hepatic necrosis accompanied with mild inflammatory response (H&E stain 40X)**.**

The spleen showed main splenic manifestation showed red pulp congestion with slight lymphoid depletion of some splenic follicle (See Figure 7).

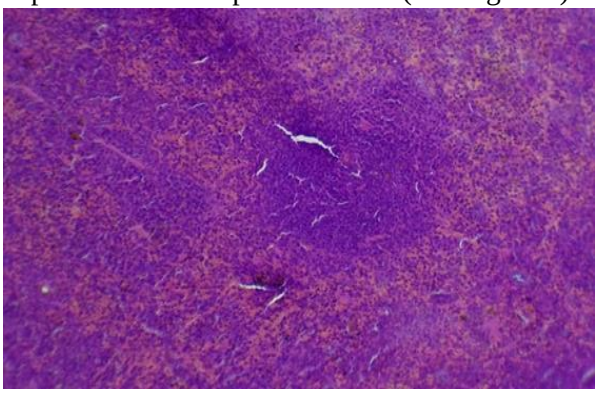

Figure 7. Histopathological section of spleen (Group 1) post challenged mice showed red pulp congestion with slight lymphoid depletion of some splenic follicle (H&E stain 40X)**.** While other manifestations showed various sizes of splenic follicle (See Figure 8).

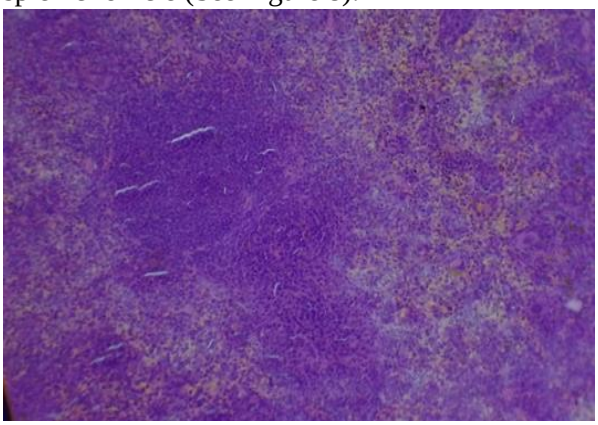

Figure 8. Histopathological section of spleen (Group 1) post challenged mice showed various sized of splenic follicle (H&E stain 40X)**.**

The histopathology study of mice infected with $3.0x10^{8}$CFU/ml of *S. rubidea* produced moderate histological changes in the second group of results. The intestine (colon) showed moderate

degenerative changes of submucosal gland with cystic dilation associated with vascular congestion and mild MNCs infiltration (See Figure 9).

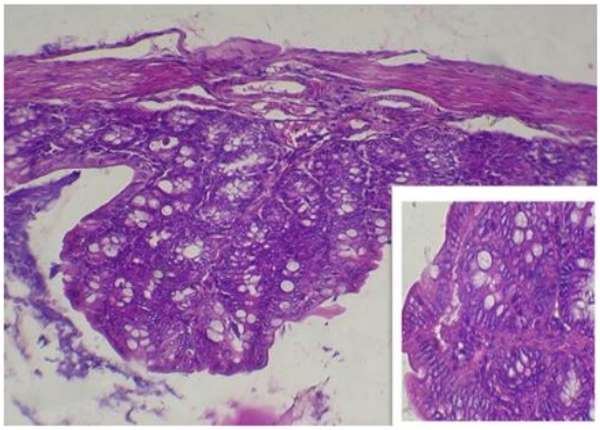

Figure 9. Histopathological section of colon (Group 2) post challenged mice showed moderate degenerative changes of submucosal gland with cystic dilation associated with vascular congestion and mild MNCs infiltration (H&E stain 40X)**.**

The kidney showed moderate cellular swelling of tubular epithelial lining of centrical of tubules with multiple interstitial hemorrhage (See Figure 10).

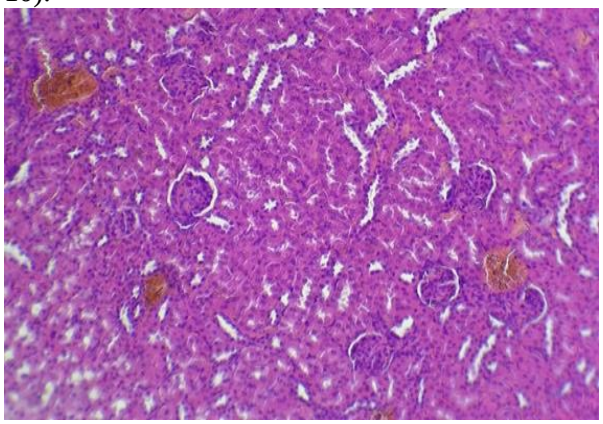

Figure 10. Histopathological section of kidney (Group 2) post challenged mice showed mild to moderate cellular swelling of tubular epithelial lining of centrical of tubules with multiple interstitial hemorrhage (H&E stain 40X)**.**

The liver showed main hepatic findings characterized by perivascular inflammatory cells infiltration with marked hepatic vessel congestion, other finding showed portal enlargement due to portal vein dilation and periductal MNCs infiltration vinous (See Figure 11).

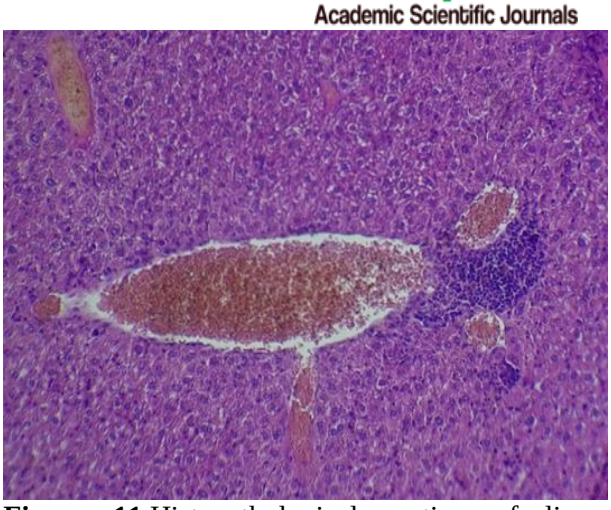

Figure 11.Histopathological section of liver (Group 2) post challenged mice showed perivascular inflammatory cells infiltration with marked hepatic vessels congestion (H&E stain 40X)**.**

The main splenic findings showed lymphoid depletion of white pulp with scattered megakaryocytes (See Figure 12,13).

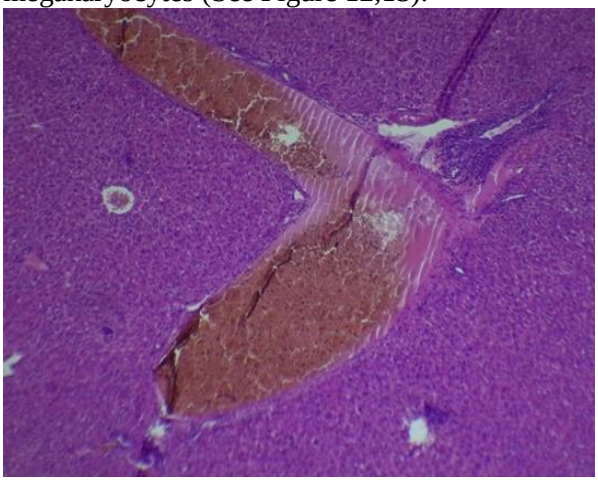

Figure 12. Histopathological section of liver (Group 2) post challenged mice showed finding showed portal enlargement due to portal vein dilation (H&E stain 40X).

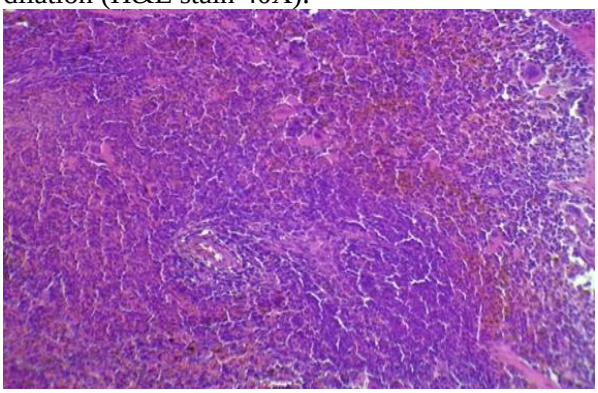

Figure 13. Histopathological section of spleen (Group 2) post challenged mice showed lymphoid

depletion of white pulp with scattered megakaryocytes (H&E stain 40X).

Discussion:

Serratia species infections are mostly restricted to the Serratia liquefaciens complex and Serratia marcescens. Because the other Serratia species are rarely isolated from clinical samples, there is a seldom of information on the diseases they cause. In this research, out of 100 stool samples (both normal and diarrheal), the isolation of *S. rubidea* yielded positive results in 12 percent of the cases from cattle. These results were in agreement with (RADIF and ALBAAYİT, 2019), which isolated two multi-drug resistance strains of *S. rubidaea* were found in the mouth of two healthy horses. Also, according to (Litterio *et al*.,2012), it was identified as a mixed wound infection brought on by a horse bite, and the authors concluded that grasses might be the infection's source. while (Khudhair *et al*.,2011) describe the first instance of thoracic empyema, which was caused by *S. rubidaea* that was found in the pleural fluid of an immunocompetent patient. The identification results of the Citrobacter isolate by PCR analysis, the isolate was examined to present 16 s rRNA. Hence the isolate was positive for 16SrRNA gene amplification at 1500bp. Our findings concurred with (Abboodi *et al*.,2021). Through PCR analysis, the presence of the 16 s rRNA gene, which amplified at 1500 pb, was used to identify the source of *S. rubidea*. Also, our findings are comparable to those reported in reference (Pereira *et al*.,2023), which revealed that the isolated Over 99.7% of the strain's sequences match those of several *S. rubidea*. Sequencing of *S. rubidea* 16S rRNA and BLAST analysis of the consensus sequence. The use of PCR-based tests for detecting and identifying Serratia spp. has grown due to its accuracy, sensitivity, speed, and capacity to be utilized DNA rather than highly infectious live cultures (Ahmed and Al-Samarraae,2021). The results of histopathological changes of both groups showed similar effect degree mild to moderate, the colon of both groups showed moderate degenerative changes of submucosal gland with cystic dilation associated with vascular congestion and mild MNCs infiltration, other findings goblet cells hypertrophy The minor lesion could be caused by colonic epithelial cells that can express and release particular cytokines in response to an invasive bacterial infection, like The interleukin -8 and monocyte chemotactic protein -1, and NFα(Ochiengetal, 2014). The result of kidney of both group cellular swelling of tubular epithelial lining with slight interstitial hemorrhage. while, the liver showed portal enlargement due to portal vein dilation and periductal MNCs infiltration vinous and focal hepatic necrosis accompanied with mild inflammatory response.in addition, the spleen showed lymphoid depletion of white pulp with scattered megakaryocytes. Our results agree with (Ahmed,2021) who pointed that the histopathological changes after challenge with infectious dose($8x10^6$CFU/ml) of Serratia marcescens the liver showed infiltration of many inflammatory cells in portal area mainly around congested blood vessels and the kidney showed hypercellularity of glomerular due to proliferation of mesenchymal cells, inflammatory cells infiltration and congested blood vessels (Mohammed, 2019). Also, the results agreement with (Yuri *et al*.,2012) which study the histopathological changes after immunization with antigens of *Serratia marcescens*, the liver showed congested of dilated sinusoids and mononuclear cells aggregation in portal area around blood vessels, while the kidney showed depletion of white pulp. Serratia spp. directly reproduce in the lymphoid tissue associated with the mucosa before spreading to the liver and spleen through the mesenteric lymph nodes. This causes a wide immunological response that triggers cell-mediated immunity, humoral immunity , and antibody response by secretory immunoglobulin A (IgA) (Marieke ,2012). The presence of *S. marcescens* in the infected macrophages leads to the activation of Th2 cells specific to *S. marcescens* and the activation of humoral immunity. IFN has increased the number of cells in Peyer's patches that secrete IL-4, IL-5, and IL-6 (Ye *et al*.,2014). The typical intestine entrance pathway begins with M cells, which allow reach to B cells rich Peyer's patches. Once the B cells recognize the particular Ig receptors (BCR), the B cells phagocytose *S. marcescens* (Zainab ,2012). The most common cause of splenomegaly is systemic *S. marcescens* infection. Despite the fact that leukocyte recruitment is frequently the cause of this increase in splenic cellularity (Nagata and Nishiyama ,2021). It is said that CD 4 T cells are S. marcescens-specific lymphocytes. These cells are localized in the small intestine's peyers patches (Marieke, 2012).

CONCLUSION

Serratia rubidea was successfully isolated from 12% of cattle fecal samples in Baghdad using a combination of culture, biochemical testing, and molecular methods. Critically, 16S rRNA genome

sequencing and PCR amplification provided definitive molecular identification that corroborated biochemical identification by Vitek 2. An experimental mouse infection model was then established using two infectious doses of *S. rubidea* ($1.5x10^8$ and $3.0x10^8$ CFU/ml). Histopathological examination after 7 days revealed consistent, multisystemic lesions in infected mice across both dose groups, with lesion severities ranging from mild to moderate compared to uninfected controls. Intestinal lesions included degeneration and cystic dilation of submucosal glands, goblet cell hypertrophy, vascular congestion, and inflammatory infiltrates. Kidney lesions featured vascular congestion, tubular epithelial swelling, interstitial hemorrhage, and glomerular capillary congestion. In the liver, inflammatory cell infiltrates surrounded hepatic vessels, with sinusoidal narrowing due to hepatocellular swelling, focal necrosis, and portal vein dilation. Splenic lesions consisted of red pulp congestion, lymphoid depletion, and scattered megakaryocytes. Collectively, these findings provide compelling evidence that S. rubidea possesses significant pathogenic potential, capable of inducing multisystemic inflammatory and degenerative lesions in an animal model even at the lower infectious dose tested. The characterized mouse model using higher bacterial inoculums appears highly useful for further interrogating this understudied bacterium's virulence attributes and pathogenesis mechanisms. Overall, this integrative study highlights the power of combining molecular diagnostic approaches with histopathological analysis for characterizing newly emerged or rarely reported bacterial pathogens of potential clinical and veterinary significance like *S. rubidea*.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors have no conflicts of interest to disclose related to the research and findings reported in this study.

ACKNOWLEDGMENTS

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التغيرات النسيجية المرضية لبكتريا *Serratia Rubidea* المعزوله من الأبقار في الفئران

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الملخص

تم إجراء البحث لعزل وتحديد العامل الممرض الانتهازي لبكتريا *Serratia Rubidaea* من الأنواع الأخرى المماثلة باستخدام نماذج الفئران لعدوى *S. Rubidaea*. تم أخذ 100 عينة براز من الأبقار في مدينة بغداد والتي تم تشخيصها عن طريق الاختبارات الكيموحيوية، Vitek 2 Compact، وتحليل PCR، ثم تحليل تسلسل الحامض النووي الرايبوزي S16. تم تقسيم أربعة وعشرون فأراً ألبينو من كلا الجنسين إلى ثلاث مجموعات: المعدية والسيطرة. المجموعة الأولى مصابة بـ 1.5×10^{8} cfu (0.1مل/فمويا)، المجموعة الثانية المصابة بـ 3.0×10^{8}cfu (0.1مل/فمويا)، والمجموعة الثالثة كمجموعة سيطرة سلبية تم حقنها بـ 0.1مل PBS. لتوصيف العدوى في الفئران تم استخدام معايير عديدة بما في ذلك العلامات السريرية، والوفيات، والتغيرات النسيجية. من عينات البراز التي تم جمعها، تم عزل 12 (12٪) من عزلات البكتريا باستخدام الأوساط الزرعية، وتم تحديد العزلات بشكل إيجابي على أنها *S.rubidea* 99٪ باستخدام Vitke 2، و 98٪ عن طريق التسلسل عند المطابقة مع مراجع البنك الجيني تم العثور على العزلة البكتيرية بالرقم (OR757107.1). أظهرت التغيرات النسيجية المرضية للأمعاء تغيرات تنكسية في الغدد تحت المخاطية وتوسع الكيس وارتشاح الخلايا الالتهابية. بينما أظهرت الكلى احتقان الأوعية الدموية، وتوسع الأوعية، والتورم الخلوي للبطانة الظهارية الأنبوبية، والنزيف الخلالي، واحتقان الشعيرات الدموية الكبيبي، وأظهر الكبد تسلل الخلايا الالتهابية حول الأوعية الكبدية، وتضييق الجيوب الكبدية، ونخر كبدي بؤري مع التهاب خفيف، والتهاب حول الأوعية الدموية. وتوسع الوريد البابي والارتشاح الالتهابي حول القناة. أخيرًا، أظهر الطحال احتقانًا في اللب الأحمر، ونضوبًا لمفاويًا في اللب الأبيض وبصيلات الطحال، وخلايا نواة متناثرة. أظهرت بعض بصيلات الطحال تباينًا في الحجم.

الكلمات المفتاحية: تسلسل الحامض النووي الرايبوزي S16 ، فحص تفاعل سلسلة البوليمر ، فحص فايتك.