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Original Article

Immunopathological Changes Post-Infection with *Enterobacter cloacae* in Rabbits

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Abstract

Nosocomial infections have serious effects on health conditions in humans and animals. The present study aimed to investigate the pathogenesis of *Enterobacter cloacae* post intraperitoneal inoculation in rabbits to investigate the immunological and possible pathological effects. A total of 42 rabbits were randomly divided into two equal groups (n=21). The first group was inoculated with 3×10^8 CFU/ml of the virulent isolate of *E. cloacae* intraperitoneally (IP), while the second group was injected IP with phosphate buffer saline and considered a control negative group. The animals were sacrificed at different time post-infection at 48/72 h, and at day 7 post-bacterial inoculation. The results revealed a significant increase in the concentration of TNF-α, especially in the infected groups. In addition, there were different pathological lesions in different organs of animals, mainly in the infected groups, which represents by vascular congestion and edema with polymorphoneutrophiles infiltration in the lungs, kidneys, and heart. This study is considered the first trial which aimed to observe the pathological changes of *E. cloacae* in vital organs in rabbits.

Keywords: *Enterobacter cloacae*, Histopathology, Immune responses, Pathogenesis

1. Introduction

Enterobacter is a Gram-negative, non-spore-forming, and rod-shaped bacterium. Moreover, this facultative anaerobic bacterium is related to the family *Enterobacteriaceae*. Two species of this bacterium are recognized as *Enterobacter cloacae* and *E. aerogenes* which are considered nosocomial pathogens and opportunistic bacteria. These species have been found in patients in intensive care units particularly in those who were put on mechanical ventilation. An *E. cloaca* is omnipresent in terrestrial and aquatic environments (sewage, food, water, and soil). Furthermore, these species are present as commensal microflora in the alimentary tracts of animals and humans. In addition, *E. cloacae* is regarded as a pathogen in insects and plants (1).

An *E. cloacae* is also recognized as a nosocomial bacterium contributing to septic arthritis, skin/soft tissue infections, bacteremia, lower respiratory tract and urinary tract infection, endocarditis, osteomyelitis, and intra-abdominal infections (2). *E. cloacae* contaminates different medical devices, such as intravenous fluids and other hospital devices (3). Moreover, *E. cloacae* was repeatedly reported and appeared as a nosocomial and opportunistic microorganism in the neonatal intensive care units (4). *E. cloacae* is also an opportunistic microorganism responsible for systemic and local infections in humans (5). *E. cloacae* infection in neonates hospitalized in intensive care unit caused septicemia (6). These bacteria can be transported to the neonates through total parenteral nutrition solutions, intravenous fluids, and

medical equipment. The general endogenous reservoirs of this bacterium in humans include the respiratory and urinary tracts of sick patients, gastrointestinal tract of healthy adults, pus, urine, and sputum (7, 8). This study aimed to investigate the pathogenesis of *E. cloacae* post intraperitoneal inoculation in rabbits to study the immunological and possible pathological effects.

2. Materials and Methods

2.1. Bacterial Isolation

Swabs were obtained from the erosion and ulceration of fans, skin, and the gills of the infected fish, mainly *Cyprinus carpio L*. These fish were reared in the Al-Hay district with floating cages. The swabs were cultured in Mac Conkey agar, followed by the identification and confirmation of the bacterial isolate by an API 20 NE system.

2.2. Animals and Experimental Design

A total of 42 local breed rabbits with a mean weight of 2-2.5 kg were used in the current experiment and housed in an animal house of Veterinary Medicine College, Waist University, Waist, Iraq, under typical environmental conditions (14:10 h light: dark cycle with 20±2°C). After two weeks of acclimation, the animals were divided into two groups. The rabbits in the first group (n=21) were inoculated 3×10^{8} CFU/ml intraperitoneally (IP) with virulent pathogens of *E. cloacae*, and the second group (n=21) was left as control and was injected IP with phosphate buffer saline. From each group, seven rabbits were sacrificed after 48 h, 72 h, and 7th days post-injection with a virulent strain of *E. cloacae*. At the end of each of the above-mentioned periods, the animals (n=7) were euthanized by the overdose of Ketamine-Xylazine mixture, and the tissue specimens from organs (heart, lung, and kidney) were then recovered and preserved directly in 10% neutral buffered formalin.

On the 7th day post-infection and before animal scarifying, the blood was collected from the marginal ear vein of rabbits with Venoject without any anticoagulants. Subsequently, the serum sample was separated to evaluate immune response (TNF-α). The immunological evaluation was conducted to estimate the levels of serum TNF-α by Sandwich ELISA kit according to manufacturers' instruction (SunLong Biotech, China).

2.3. Histopathological Examination

Tissue specimens about 1 cm^3 from the heart, lung, and kidney were preserved directly in 10% neutral buffered formalin to prepare paraffin wax blocks. Following that, hematoxylin and eosin staining was performed for histopathological evaluations (9).

2.4. Statistical Analysis

The data were analyzed using the least significant difference and a one-way ANOVA to make comparisons between the means of the groups. In addition, a *P*-value less than 0.05 was considered statistically significant (10).

3. Results

3.1. TNF-α

The concentrations of TNF-α in the serum samples of the experimental groups were measured, and the recorded data showed a significant increase ($P<0.05$) in group 1 infected with *E. cloacae*, compared to the control negative group in which they increased markedly at day 7 post-infection (Figure 1).

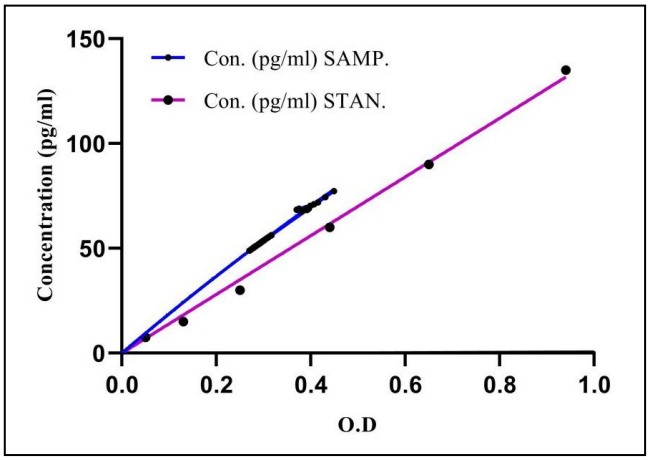

Figure 1. Concentrations of TNF-α in the infected animals, compared to standard values

3.2. Histopathology of the Heart, Lung, and Kidney

The cardiac sections obtained at 48 h post-infection revealed myocarditis, necrosis of cardiac muscles, infiltration of fibrinous exudate with inflammatory cells (mainly neutrophils), and congestion of blood vessels containing inflammatory cells (Figure 2a). Additionally, pulmonary edema filled the lumen of bronchioles and mild thickening of alveoli (Figure 2b). For the kidneys, the glomerular tufts increased their sizes as their capillaries were congested and infiltrated with polymorphoneutrophils (PMNs) (Figure 3a); moreover, swollen-degenerated lining tubular epithelium caused narrow lumen and pyelitis as an acute inflammatory reaction (Figure 3b).

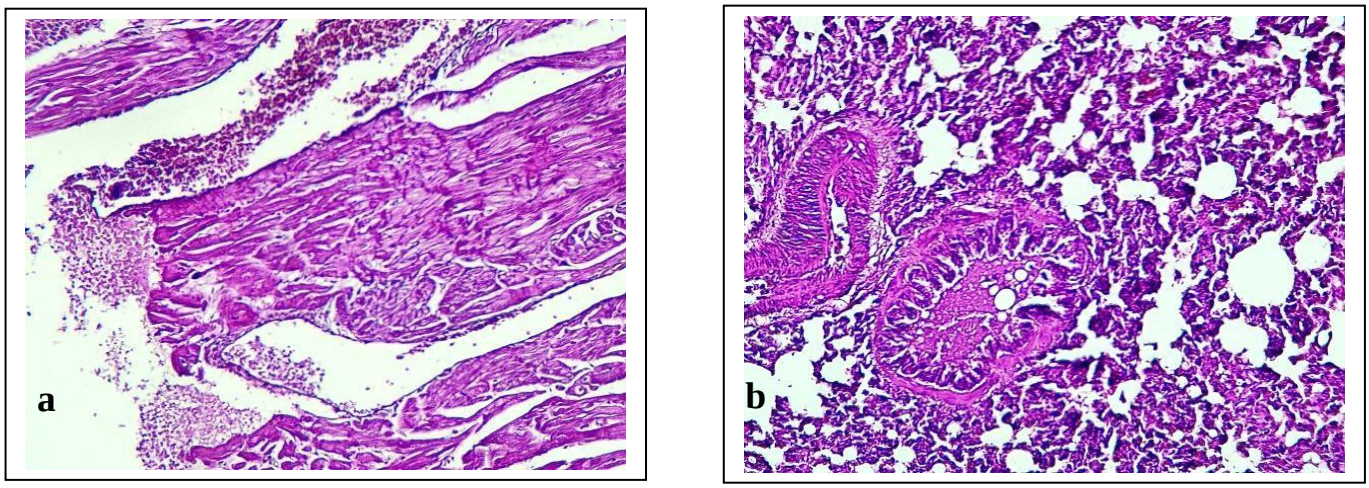

Figure 2. Histopathological section in the heart and lung of rabbits infected with *E. cloacae* at 48 h: **a**. necrotic endocarditis. **b**. Lung bronchial edematous fluid and thickening of alveolar walls. (H&E stain, 200×).

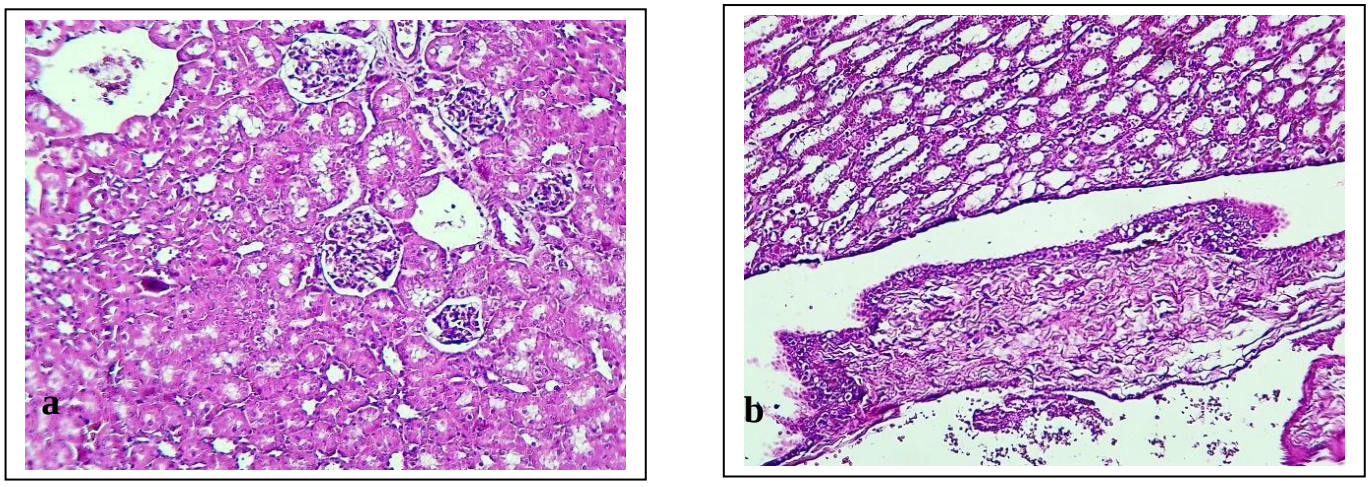

Figure 3. a. Histopathological section in the kidney 48 h post-infection with *E. cloacae*: swelling of glomeruli and dilation of renal blood vessels. **b**. Pyelitis (H&E stain, 200×).

On the 3rd day post-infection, the animals were sacrificed to investigate the histopathological changes of the heart, kidney, and lungs. The results of the heart histopathological evaluations showed epicarditis appeared as the thickening of epicardium due to congested blood supply and accumulation of edematous fluid with fibrinous exudate (Figure 4a). The necrosis of myocardial fibers appeared as swollen-eosinophilic (Figure 4b). In case of the renal lesions, microscopically evaluations revealed vacuolar degeneration of glomerular epithelium and cellular swelling of the tubular epithelium (Figure 5a). There was an acute pyelitis with inflammatory exudate in the lumen of the pelvic (Figure 5b).

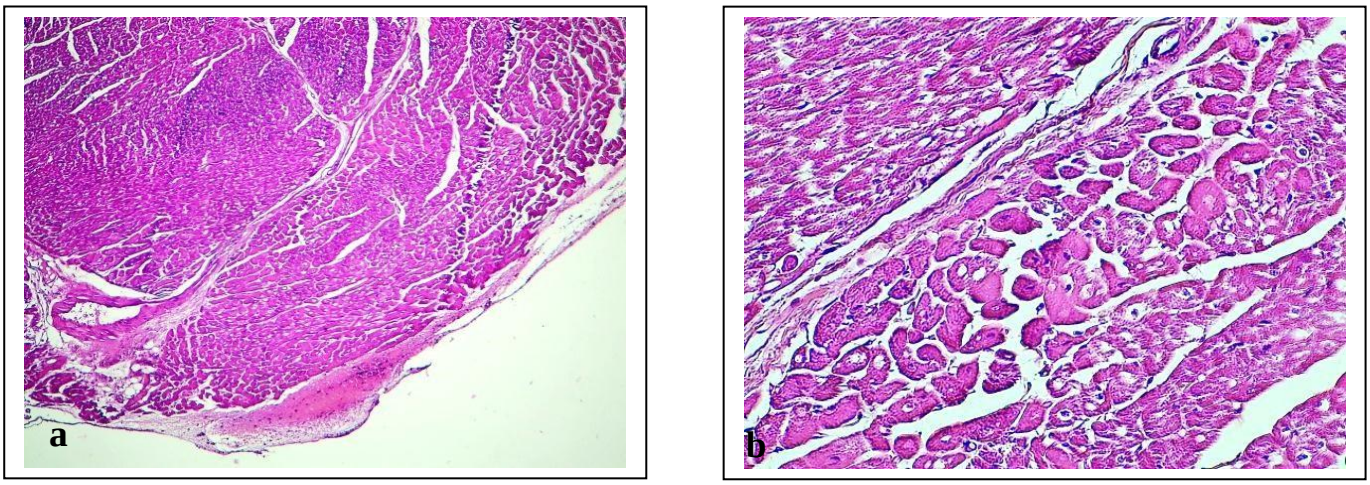

Figure 4. a. Histopathological section in the heart at day 3 post-infection with *E. cloacae* showing necrotic pericarditis. **b.** Necrotic myocarditis. (H&E stain, 40&200×).

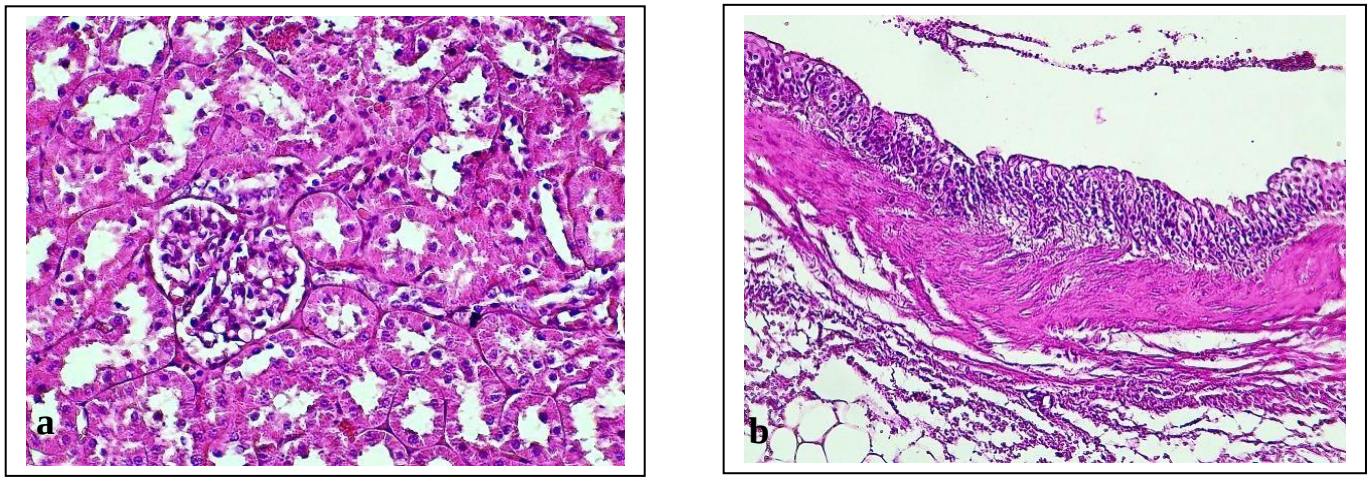

Figure 5. a. Histopathological section in the kidney at day 3 post-infection with *E. cloacae* revealing the vacuolar degeneration of glomerulus and cellular swelling of tubular epithelium. **b.** Acute pyelitis with inflammatory exudate in the lumen of the pelvic. (H&E stain, 100×).

On the 7th day post-infection, there was marked epicarditis characterized by the infiltration of fibrinous-suppurative exudate and necrosis of cardiac tissues (Figure 6), followed by the inflammatory exudate filling the cavity of the heart. The pulmonary sections showed acute interstitial pneumonitis, thickening of interalveolar septa, severe degeneration of bronchial epithelium, hypertrophy of bronchial smooth muscles, and the thickening of pleura (Figure 7).

The histopathological evaluations of the kidneys revealed a distention of glomeruli with congestion and vacuolar degeneration of endothelial cells of glomerular tufts, focal pyelonephritis, infiltration of mononuclear cells, degenerated renal tubule, and congestion of blood vessels (Figure 8). Large abscess formation with the infiltration of suppurative exudate in stromal tissues of the internal cavity surrounded by congested granulation tissue (Figure 9).

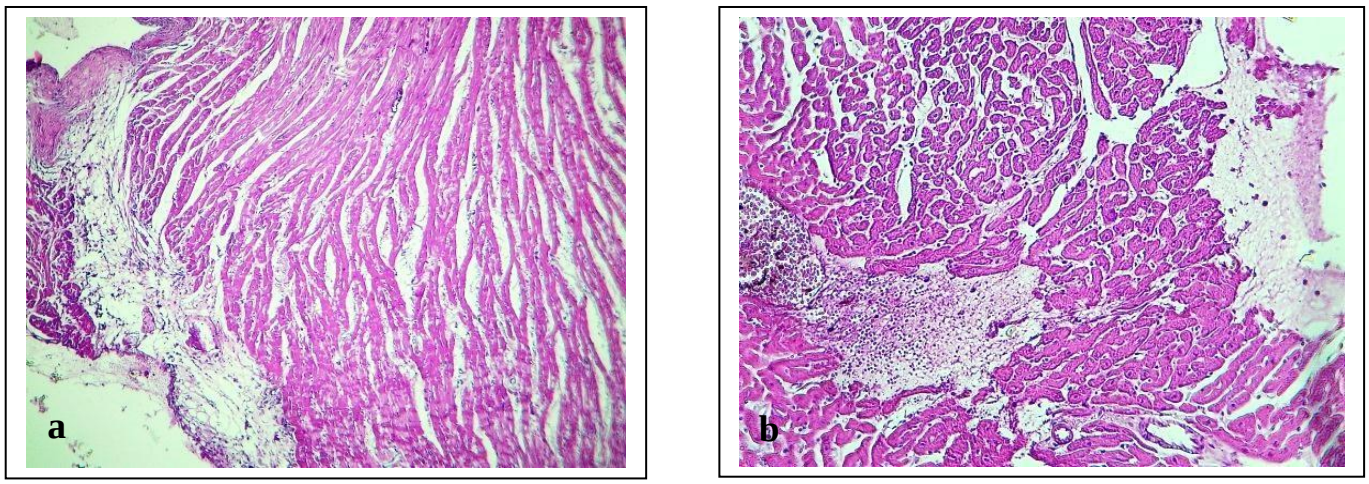

Figure 6. a. Histopathological section in the heart at day 7 post-infection with *E. cloacae* showing acute fibrinous-suppurative epicarditis. **b.** Presence of fibrinous-suppurative exudate in the valvular cavity. (H&E stain, 100×).

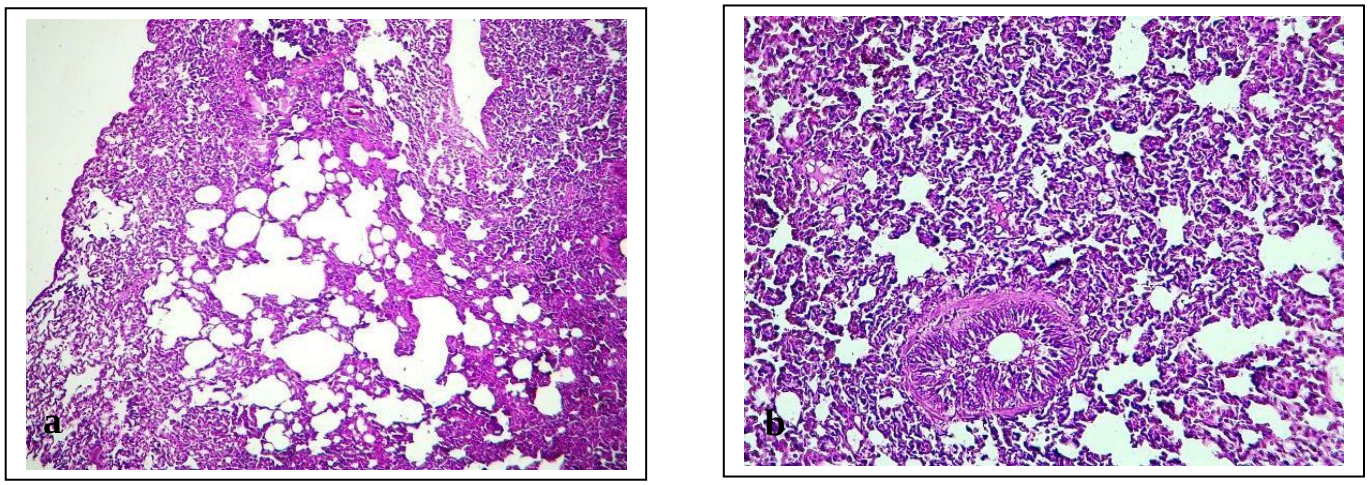

Figure 7. a. Histopathological section in the lung at day 7 post-infection with *E. cloacae* showing interstitial pneumonia and focal emphysematous area. **b.** Severe degeneration of bronchiolar epithelium and alveolar collapse. (H&E stain, 40&100×).

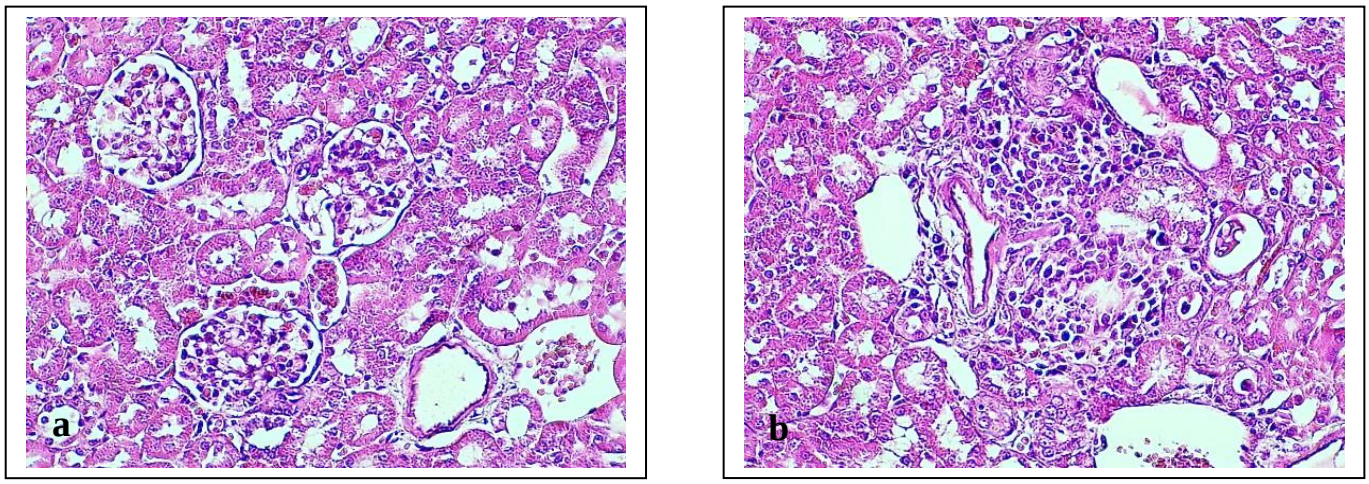

Figure 8. a. Histopathological section in the kidney at day 7 post-infection with *E. cloacae* showing vacuolar degeneration of endothelium glomeruli, inflammatory cells in tubules, and dilated blood vessels. **b.** Focal pyelonephritis. (H&E stain, 100×).

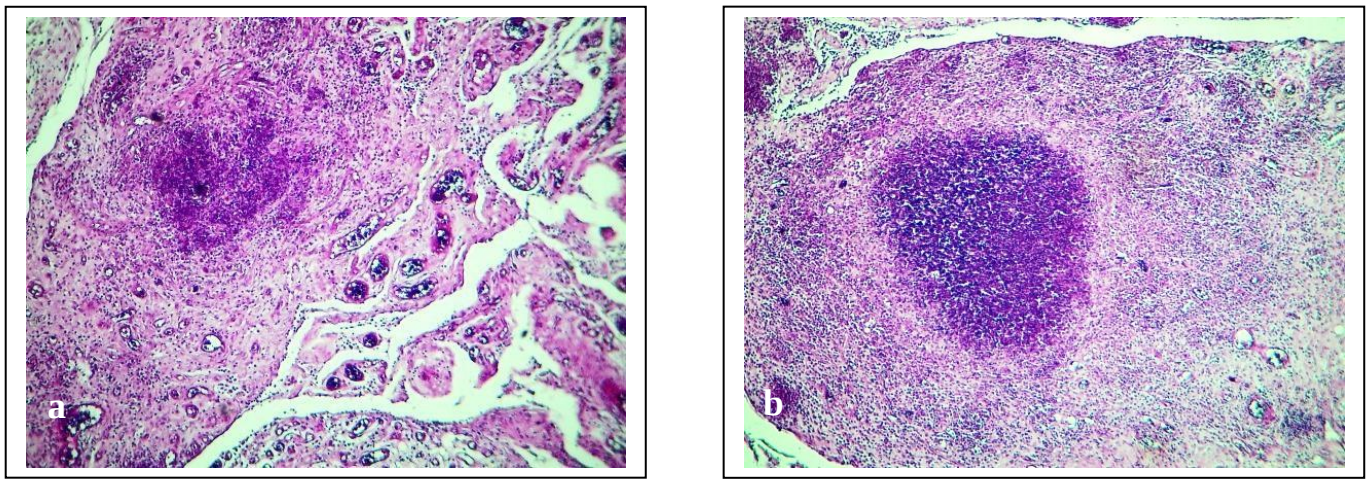

Figure 9. a. Histopathological section in supporting tissues at day 7 post-infection with *E. cloacae* showing suppurative exudate and proliferated congested-granulation tissue, **b.** Large abscess. (H&E stain, 100×).

4. Discussion

Enterobacter pathogens are among the nosocomial pathogens that may cause serious inflammatory problems under immunosuppressive states as occurred in the present experimental infection of rabbits with *E. cloacae* which infected various organs and caused histopathological changes, such as acute fibrinous exudate, and few PMNs were predominant at 48 h and 3 days post-infection. On day 7 post-infection, there were abscess formation and focal aggregation of inflammatory cells, vacuolar degeneration of lining epithelium, and endothelium. These findings may occur due to the biofilm production by these pathogens. Oleiwis, Najim (11) suggested the virulence activity of *E. cloacae* by the production of biofilms (extracellular matrix makes a three-dimensional system) in various environments causing opportunistic infections and colonization of medical instruments. This bacterium is considered one of the 10 greatest isolated hospital-acquired bacteria (12).

Kim, Lee (13) reported that curli fimbriae expression serves as an essential function in biofilm development in *E. cloacae*. Moreover, they revealed that *csgA* and *csgD* genes were found in 11 (78.6%) of the 14 isolates. In addition, Zogaj, Bokranz (14) suggested that biofilm formation was associated with cellulose and curli fimbriae expression.

One of the most necessary virulence factors of *E. cloacae* is curli fimbriae due to its action in biofilm formation. The pathogenesis of this bacterium depends essentially on its capacity to express and make curli fimbriae which is included in cells collection, substratum attachment, and production of biofilm, which is also considered an important agent that interact with host proteins. It is also reported to assist the spreading of microorganisms in the host. *E. cloacae* and produce biofilms in various environments causing opportunistic infections and colonization of medical instruments (15, 16).

A marked significant difference was noticed in the infected groups, compared to the control group, concerning an increase in the TNF-α concentration. Our data are consistent with the findings of another previous study which showed a significant increase in the concentration of TNF-α, especially in pediatric patients who were infected by Gram-negative bacteria which led to an increase in the levels of TNF-α (17).

Authors' Contribution

Study concept and design: S. R. S. A.
Acquisition of data: S. R. S. A., Z. I. I. and Z. J. M. J.
Analysis and interpretation of data: Z. J. M. J.
Drafting of the manuscript: S. R. S. A. and Z. I. I.
Critical revision of the manuscript for important intellectual content: S. R. S. A., Z. I. I. and Z. J. M. J.
Statistical analysis: Z. J. M. J
Administrative, technical, and material support: S. R. S. A., Z. I. I. and Z. J. M. J.

Ethics

The study protocol was confirmed and approved by the Council of Veterinary Medicine College, University of Wasit, Wasit, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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