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Pathological Study of the Experimental Infection of Mice with *Morganella morganii* Urine Isolate of Women

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Abstract: ***Twenty eight BALB/C mice were divided into two groups. The first one infected with 8*10^{9}CFU Morganella morganiiintra peritoneally, and the second group served as a negative control group. The mice were sacrificed at day 1, 2, 3, 5, 8, 10, and 16 post inoculations. The bacteria were isolated from samples of liver and lung collected through day five of the infection while the bacteria were isolated consistently from the urinary tract. The pathological changes were mild to moderate inflammatory response in the heart, intestine, lung, and spleen, and a moderate to severe inflammatory reaction in the kidney. The heart showed mild myocarditis and pericarditis. There was hyperplasia in the gut associated lymphoid tissue with mild enteritis. There was moderate pneumonia and hemorrhage in the lung and moderate granulomatous hepatitis with micro abscess in the liver. There was severe nephritis in the kidney composed mainly of lymphocyte in the interstitial tissue, and suppurative ureteritis and cystitis. In conclusion, M. morganiicauses wide spread infection, and it has the ability to infect many tissues and organs. It causes a persistent renal infection with suppurative inflammation.***

Keywords: Pathological changes, *Morganella morganii*, Urinary tract infection, Mice

1. Introduction

Urinary tract infection is one of the most common bacterial infections; forty percent of women experienced it throughout their life (Sheerin, 2011).). The urinary tract infections are either uncomplicated when bacteria infect healthy women, or complicated infection in which anatomical and functional abnormalities will occur (Jasmine and Guy, 2007). Bacterial pathogens are considered the most common urinary infections, and Escherichia coli is the primary pathogen in uncomplicated infections followed by *Staphylococcus saprophyticus* (Allan , 2002). 3). *Morganella morganii* is an uncommon bacterial pathogen isolated from urinary tract infection (Sheung-Mei Lau *et al.,* 2004).). *Morganella morganii* is an opportunistic bacterium which first identified as uropathogen in Iraq in 2009 (Jamela and Ibtesam, 2009). It is a facultative anaerobic gram negative bacterium which is found normally in the environment and in the intestinal flora, and is often implicated in nosocomial urinary tract infections (Hung-Yang Chang *et al*., 2011). *M. morganii* was a common pathogen of the catheterized urinary tract, and it was isolated from some cases of pyelonephritis and cystitis (Julia *et al*., 2006). We aimed to study the tendency of the *M. morganii* to invade and maintain a urinary tract infection

2. Material and Methods

1) Twenty eight white BALB/C mice were obtained two weeks before the beginning of the study.
2) *Morganella morganii* was isolated in the Al-karama Teaching Hospital, Wassit, Iraq. M. morganii was isolated from a 32 year old female who suffered from urinary tract infection. The microorganism was first isolated on human blood agar (Himedia) and MacConkey agar (Oxoid), and then identified by API 20E (BioMerieuxVitek, Inc).
3) Experimental design: the mice were divided into two groups. The first (21) were injected intra peritoneally with 8X109 CFU of *Morganella morganii*, and the second group (7) of mice was injected with sterile phosphate buffer saline intra peritoneally to serve as negative control group.
4) Four mice were sacrificed at day 1, 2, 3, 5, 8, 10, and 16 post infection (three from the first group and one mouse from the second group). The exception was on day 16 when 2 mice were sacrificed from the infected group because one of the mice died on day 3 due to bacteremia. Samples were collected from liver, lung, heart, spleen, intestine, kidney, ureter, bladder, and brain for bacterial isolation and pathological examination. Samples for bacterial isolation (approximately 0.7-1cm, 0.1-0.15 gm.) except for the heart (half of it) were taken aseptically and homogenized in a 5ml sterile phosphate puffer saline. Then 6 drops (20 µl) from the concentrated and others from the first dilution were cultured on MacConkey agar and count. Samples for pathological examination were preserved in 10% neutral buffered formalin solution.
5) Bacterial identification was done on the bacteria isolated from the internal organs to guarantee the species of *Morganella morganii* .
6) Histopathological slides were done from the preserved samples according to Luna, (1968).

3. Results

Bacterial isolation

Result of *Morganella morganii* isolated from tissue samples collected from the infected mice are shown in table 1. The bacteria were isolated frequently in a heavy concentration from kidney and bladder of the infected group at all-time points, while the organism was isolated from liver and lung only in the first five days of the infection. Only one mouse had a sever bacteremia, and the *M. morganii* isolated from the brain and testes of this mouse in addition to the other organs.

Table 1: The isolation of *M. morganii* from different internal organs
+ Mild ++ moderate +++ heavy ++++ very heavy.

	liver	Lung	Kidney	Bladder
1st day	++	++	+	+
2nd day	+++	++	+++	++
3rd day	+++	++	++++	+++
5th day	+++	+	++++	++++
8th day	-	-	+++	+++
10th day	-	-	+++	+++
16th day	-	-	++	+++

Pathological study

The pathological changes in the internal organs of the infected mice ranged from acute inflammation composed mainly of edema and polymorphonuclear cells infiltration at the early stage of the infection to chronic pathological changes accompanied by mononuclear cells aggregation. The lesions were more sever in the urinary tract in contrast to other organs.

There was mild polymorphonuclear cells infiltration among the muscle bundles of the heart at the first day post infection which is replaced gradually with lymphocytes at the following days. Finally lymphocytes were the dominant inflammatory cells in the myocardium and pericardium. A similar reaction occurs in the intestine at the second day post infection when mild infiltration of neutrophils and lymphocytes in the mucosa and sub mucosa followed by hyperplasia of the gut associated lymphoid tissue at the fifth day post infection. At the sixteenth day post infection, infiltration of lymphocytes and macrophages was observed in the mesenteric adipose tissue.

In the spleen, mild white pulp hyperplasia was observed at the third day post infection while there was mild infiltration of lymphocytes and macrophages at the eighth day; there was only moderate hyperplasia of the white pulp at the sixteenth day post infection. The one severe bacteremic mouse showed meningitis composed mainly of neutrophils (Fig. 1A).

The lung had mild neutrophils infiltration with interalveolar wall thickening at the second day post infection, and then there was moderate lymphocyte infiltration around blood vessels and in the interstitial tissue at the third day. There was mild hemorrhage in the alveoli at the fifth day. Finally, there was lymphocyte aggregation around blood vessels and air ways in the lung parenchyma at the sixteenth day post infection (Fig. 1B). On the other hand, the liver had very mild neutrophil infiltration in the sinusoid at the first day post infection (Fig. 1C), and mild vacuolar degeneration of the hepatocytes with moderate aggregation of neutrophils in the liver parenchyma at the second day. There was severe hepatocyte vacuolation accompanied with neutrophils and lymphocytes aggregation in the liver parenchyma at the fifth day (Fig. 1D).

These findings persisted to the sixteenth day post infection, and revealed as multiple granulomatous lesions scattered in the liver parenchyma and mononuclear cell aggregation around blood vessels with micro abscesses.

The urinary tract had moderate to severe inflammatory response. There was acute cellular degeneration in the renal tubules with congested blood vessels, especially the glomerular capillaries, at the first day post infection, accompanied with moderate neutrophil infiltration around the glomeruli at the second day. The third day post infection revealed moderate aggregation of lymphocytes and neutrophils in the kidney parenchyma with hemorrhagic areas and mild endothelial proliferation (Fig. 1E).

Lymphocytes became the dominant inflammatory cell in the kidney at the fifth to tenth days post infection (Fig. 1F), and then at sixteenth day there was moderate mononuclear cells aggregation in kidney parenchyma especially around the glomeruli and blood vessels. The sub mucosa of the ureter infiltrated with neutrophils at the second day post infection, and then there were prevesicular lymphocytic aggregation and also in the wall of the ureter with vacuolation of the epithelial lining at third day post infection. There was exudate in the lumen of the ureter composed of inflammatory cells and sloughed epithelial lining and continued to the end of the experiment (Fig. 1G).

At the second day post infection the urinary bladder revealed neutrophils infiltration in the sub mucosa and continued to the eighth day but with mixed inflammatory cells neutrophils and lymphocyte (Fig. 1H). At sixteenth day post infection revealed only a lymphocyte infiltration with congested blood vessels and mononuclear cells infiltration in the lumen and wall of the bladder.

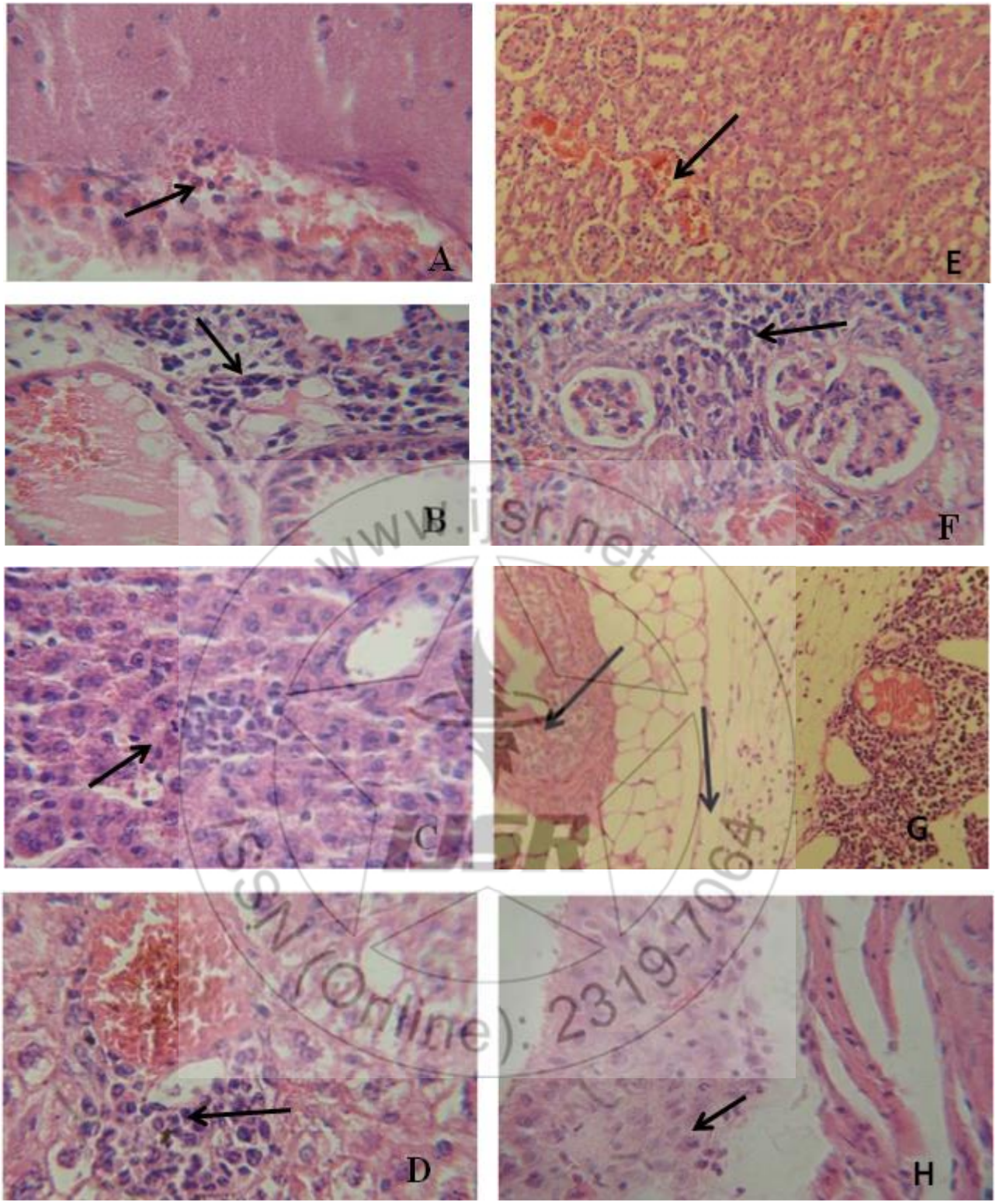

Figure 2: Hematoxylin and eosin stained representative images of histopathological changes in the mice. **A**: Infiltration of neutrophils in the congested meninges at the third day of infection (arrow) (X400). **B**. There were lymphocytes aggregations around blood vessel and air ways in the lung parenchyma at the sixteenth day of infection (arrows) (X400). **C:** Mild neutrophils aggregation in the liver parenchyma 24 hr. after the infection(arrows) (X 400). **D**: there were neutrophils and lymphocytes aggregations around blood vessel in the liver parenchyma with severe vacuolation of hepatocytes at fifth day of infection(arrows) (X400).**E**:Hemorrhagic area in the renal tissue at the third day of infection(arrows) (X100).**F:**There is lymphocytes aggregation in the interstitial tissue with hemorrhage(arrows) (X 400).**G:**There were neutrophils and lymphocytes infiltration in the wall of the ureter and in the adipose tissue surrounded it.(arrows) (X 100) .**H:**There were inflammatory cells infiltration mainly neutrophils in the mucosa and sub mucosa of the bladder (arrows).(X 100).

4. Discussion

The isolation of *M. morganii* from most of the internal organs of the infected mice indicated the invasion and transmission of the microorganism from the intra-peritoneum to the internal organs and that's may be via the hematogenous route. However, bacteremia is rarely caused by *M. morganii* (McDermott and Mylotte, 1984). Commonly bacteremia caused by *M. morganii* occurred secondarily to urinary or hepatobiliary tract infection with elevated mortality rate (Lee and Liu, 2006).*M. morganii* eliminated from most of the internal organs within eight days except for the urinary tract it stayed consistent to the end of the study.

The pathological changes in the internal organ supported the bacterial isolation. There was mild inflammatory response in the internal organs other than the urinary tract organs that revealed sever response and persist to the end of the study, there was mild acute inflammatory response at the first three days of infection in the lung, heart, intestine, and spleen, which replaced by the chronic inflammatory cells at the last period, and there was severe inflammation in the kidney, ureter, and urinary bladder, this broad reaction may be due to the reaction of the body to the bacteremia, the *M. morganii* was implicated in several bacterial sepsis elderly and immunocompromised patients (Samonis*et al*.,2001; Santaeugenia *et al.*,2002).

Many reports incriminate *M. morganii* for pericarditis in children with immunodeficiency (Yang *et al*., 2006; Young Kuk Cho., *et al*., 2010). Hyperplasia of the gut associated lymphoid tissue related to the rapid growth of *M. morganii* (Shroff *et al.*, 1995). There was acute inflammatory reaction in most of the internal organs at the first few days of infection composed mainly of polymorphonuclear cells that replaced by the chronic inflammatory cells lymphocytes and macrophages, *M. morganii*pleuropneumonia associated with macrophages and neutrophils infiltration in the alveoli with congestion and hemorrhage in the piglets (Ono *et al.,*2001).

M. morganii isolated from the kidney and urinary bladder almost all the study time, and severe chronic inflammatory response was seen in the kidney, ureter, and bladder indicate the persistent *M. morganii* infection in the urinary tract, studies indicated that *M. morganii* is not one of the common urinary tract contributor may be because of its slow growth rate in urine (Senior, 1983). Even though *M. morganii* is less frequently isolated from the urine samples, it is one of the bacterial causes of the urinary tract in (Laura *et al*., 2010;Tulin and Tuncay 2013).

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