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Histopathological effects of Bacterial and Fungal Species on some organs in female Rats *(Rattus rattus)*

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Abstract **- A laboratory study was conducted to evaluate the histological effects of vaginal infections caused by different types of bacteria and fungi using female rats. The rats were divided into four main groups: a control group (uninfected), two groups infected with *Staphylococcus aureus* and *Enterobacter cloacae*, and a fourth group infected with the fungus *Candida albicans*. Histological sections taken from the vagina and uterus after injecting 0.1 ml of each microbial suspension into the vaginal canal of the rats showed histological changes characterized by congested capillaries and infiltration of neutrophils and lymphocytes in the submucosal areas. This leads to mild focal vaginitis, endometritis, moderate neutrophilic infiltration, and congested blood vessels in the ovarian cortex. Blood analyses from the experimentally infected rats showed some abnormalities resulting from the dynamics of infection and immune responses. Bacterial infections stimulated a neutrophilic and monocytic response accompanied by a compensatory increase in platelet count, while fungal infections led to disruption in hematopoiesis (myelopoiesis) and exacerbated general cytopenia. The type of infection is a decisive factor in determining hematological patterns.**

Keywords **-** Bacterial vaginosis, *Candida albicans, Enterobacter cloacae, Staphylococcus aureus.*

INTRODUCTION

Bacterial vaginosis (BV) is defined as an imbalance in the vaginal microbiota, characterized by a decrease in the number of *Lactobacilli*. Most cases of infection begin with the formation of a biofilm, which facilitates the growth and proliferation of opportunistic bacteria (1). The vaginal mucosa serves as an important barrier against microbial invasion and maintains its balance through the interaction between the normal flora of the vagina and cervix and the host's immune mechanisms (2). Any disruption in this balance makes the tissues susceptible to infection by pathogenic bacteria and fungi. *C.albicans* is a common cause of vaginal infections, and some Gram-negative and Gram-positive bacteria, such as *Entero.cloacae* and *Staph.aureus*, also play a significant role in vaginal and cervical infections. These infections can be particularly severe in immunocompromised individuals.

Animal models, especially rats, are essential tools for understanding the mechanisms of vaginal infections and their tissue-related impacts. By inoculating the vagina with different species of microorganisms and examining the resulting pathological histological changes, the relative effects of bacterial and fungal infections on the health of the mucosal membranes and internal organs can be assessed (3). Cervicitis can result from the overgrowth of bacteria and fungi, leading to increased vaginal discharge, often with an amine or fishy odor, and the discharge may be white or gray in color. It may also cause burning during urination, itching, and inflammation of the cervix. Cervicitis increases the risk of preterm birth in pregnant women (4).

Women between the ages of 18 and 50 are most commonly affected, as cervicitis is more prevalent among women of reproductive age. However, cervicitis can also affect postmenopausal women, and iron deficiency may be associated with bacterial cervicitis in the early stages of pregnancy (5).

The aim of this study is to compare the vaginal histopathological changes caused by different bacterial and fungal infections in rats.

MATERIALS AND METHOD:

In this study, 20 adult female Wistar rats (*Rattus norvegicus*), each weighing approximately 250 g,

were used. The animals were obtained from the Animal Breeding Unit at the College of Veterinary Medicine – University of Tikrit. Their ages ranged between 16 and 18 weeks, and they were housed in the animal facility of the same college. Vaginal inoculation was carried out using a standardized aqueous suspension containing one of the selected microorganisms. The animals were kept in standard laboratory cages under controlled environmental conditions (12-hour light/dark cycle, temperature of 22 ± 2 °C, and relative humidity of 50–60%) with continuous access to a specialized rodent diet and clean water. All procedures adhered to institutional ethical standards and were approved by the Institutional Animal Care and Use Committee (6).

Experimental Design

The experimental animals were divided into four groups as follows:

Group 1: Control group
Group 2: Infected with *Enterobacter cloacae*
Group 3: Infected with *Staphylococcus aureus*
Group 4: Infected with *Candida albicans*

The microbial concentrations were standardized to 0.08 colony-forming units (CFU)/mL using sterile, pH-balanced phosphate-buffered saline (PBS). Under light anesthesia, 0.1 mL of each microbial suspension was injected into the vaginal canal of the rats using a sterile micropipette. The animals were monitored for any signs of infection or shock after administration.

Euthanasia was performed 72 hours post-injection using an overdose of an anesthetic agent **(such as ketamine/xylazine), followed by cervical dislocation. All rats weighed approximately 250 g at the time of euthanasia.**

Within one minute of euthanasia, a midline abdominal incision was made under sterile conditions. The vagina was carefully dissected and rinsed by immersion in cold PBS to remove free blood, then immediately fixed in 10% neutral buffered formalin for histopathological examination (7).

RESULTS AND DISCUSSION:

Group 1: Control Group

The sections taken from the vagina and uterus of the control group showed normal vaginal epithelium and an active, non-pregnant uterus with a normal submucosal layer, uterine glands, congested blood vessels, and normal uterine muscle, as shown in Figures (1, 2). The fallopian tube appeared normal, with intact mucosal, submucosal, and muscular layers, and normal glands containing a typical amount of mucus along with some desquamated cells, without any evidence of lesions,as seen in Figure(3) . Ovarian sections revealed a normal ovarian cortex containing normal primary follicles, mature secondary follicles, and a normal corpus luteum, as shown in Figure (4).

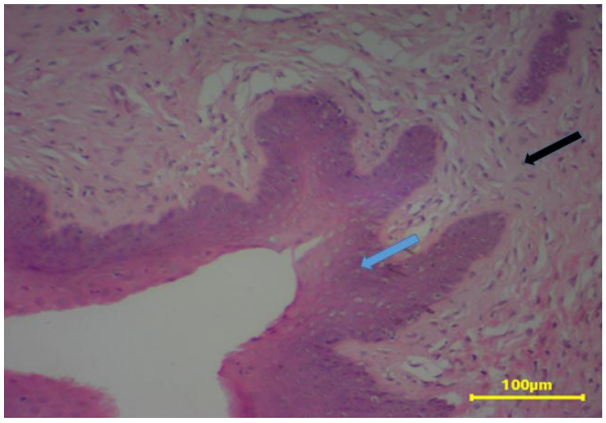

Figure 1. Cross section of (Blue arrow) .Normal submucosa (Black arrow) of the rat vagina from control group. Staining H&E. 100X. Scale bar 100 µm.

Group2: Infected with *Entero.cloacae*

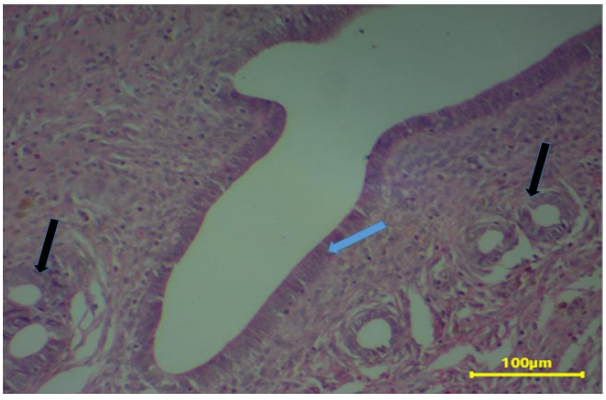

Figure 2. Cross section of epithelium (Blue arrow) . Normal submucosa and normal endometrial glands (Black arrow) of the rat uterus from control group. Staining H&E. 100X. Scale bar 100 µm.

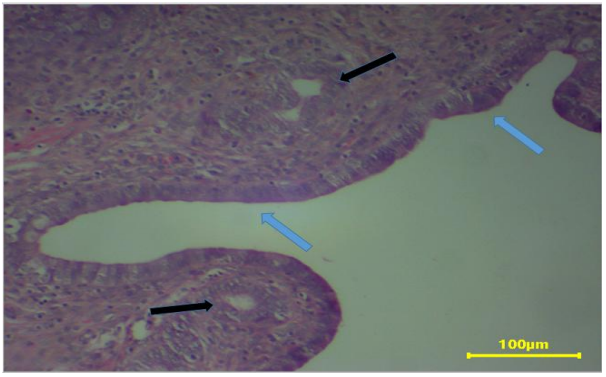

Figure 3. Cross section of oviduct of rat from control group showing normal oviduct, normal mucosa containing ciliated columinar epithelium (Blue arrow), normal submucosa and muscularis, normal mucin glands (Black arrow). Staining H&E. 100X. Scale bar 100 µm

Following the injection of 0.1 mL of an *Entero. cloacae* suspension into the vaginal canal of the rats using a sterile micropipette, the histological sections of the vagina and uterus revealed that the bacteria were introduced into previously healthy vaginal tissue and an active, non-quiescent uterus with normal vaginal and uterine glands and standard secretions. Despite this, focal areas of mild to moderate acute inflammatory responses were observed, characterized by congested capillaries and infiltration of neutrophils and lymphocytes in the submucosal areas, which may indicate focal mild vaginitis and endometritis,as shown in Figures (5, 6). No pathological changes were observed in the fallopian tubes or ovaries.

The presence of *Entero. cloacae* promotes tissue colonization through virulence factors, including the *rpoS* gene, which enhances the bacterial survival within the uterus and stimulates biofilm formation, leading to chronic inflammation and damage to the endometrial lining (8). The *slt* gene triggers an excessive immune response, resulting in bacterial cell wall lysis and subsequent inflammation of endometrial cells.(9)

Additionally, this bacterium produces iron-binding molecules known as siderophores, which help it acquire iron from the host, thereby supporting its growth (10). Some strains may also release toxins that cause tissue damage (11).

Entero. cloacae can stimulate a localized immune response, resulting in the release of pro-inflammatory cytokines such as TNF-α and IL-6, which contribute to damage and erosion of the endometrial epithelium (12). In chronic cases, this may lead to the formation of intrauterine scarring or fibrosis (Asherman's syndrome), potentially affecting embryo implantation or leading to infertility in some cases (13).

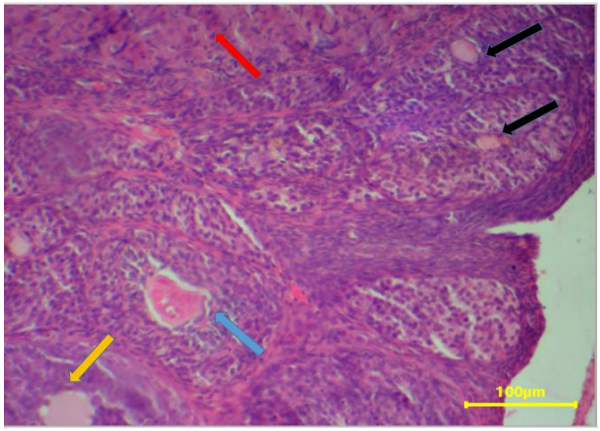

Figure 4. Cross section of the ovary of a rat from control group showing Normal primordial follicles (Black arrow) .Normal secondary follicle. Normal tertiary Graffian follicle (Yellow arrow) and normal corpus luteum (Red arrow). Staining H&E. 100X. Scale bar 100 µm.

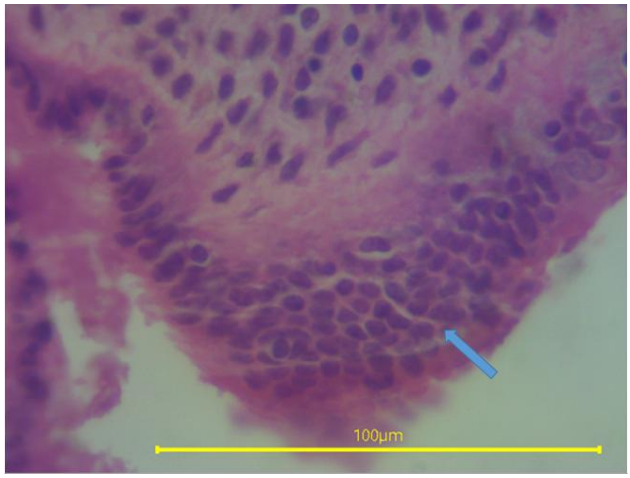

Figure 5. Cross section of uterus of a rat from the *Enterobacter cloacae* showing focal endometrial hyperplasia (Blue arrow). Staining H&E. 400X. Scale bar 100 µm

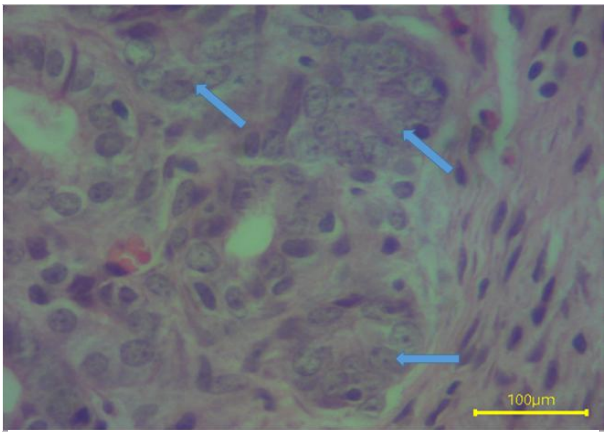

Figure 6. Cross section of in uterus of a rat from the *Entero. cloacae* showing hyperplasia of the uterine glands (Blue arrow). Staining H&E. 400X. Scale bar 100 µm.

Group 3: Infected with *Staph. aureus*

Following the injection of 0.1 mL of a *Staph.aureus* suspension into the vaginal canal of rats using a sterile micropipette, histological sections of the vagina and uterus revealed that the bacteria were introduced into tissue initially characterized by a healthy and intact vaginal epithelium and normal vaginal glands. However, mild hyperplasia of the vaginal glands was observed, along with hyperplasia of the endometrial lining and uterine glands, which also showed normal acidic secretions. Some glands appeared dilated and cystic, and there was mild vascular congestion along with marked neutrophilic infiltration between the muscle bundles of the uterine wall (14). A moderate inflammatory infiltration was noted in both vaginal and uterine sections, as shown in Figures (7, 8).

This bacterium can cause infections such as folliculitis or painful abscesses in the vulvovaginal area, conditions more commonly seen in immunocompromised women or following the use of contaminated instruments (15).

Staph. aureus adheres to vaginal epithelial cells through the *FnbA* gene, which facilitates resistance to vaginal secretions and urinary flow(16). It also produces toxins like α-hemolysin via the hla gene, which forms pores in host cell membranes, leading to cell lysis and death. This contributes to tissue destruction in both the vagina and uterus, promoting bacterial spread throughout the reproductive tract (17).

Additionally, the bacterium secretes immune-inhibitory factors such as protein A, which binds to the Fc region of antibodies, thereby impairing phagocytosis. It also releases factors that modulate immune signaling, helping it persist longer within the vaginal environment (18).

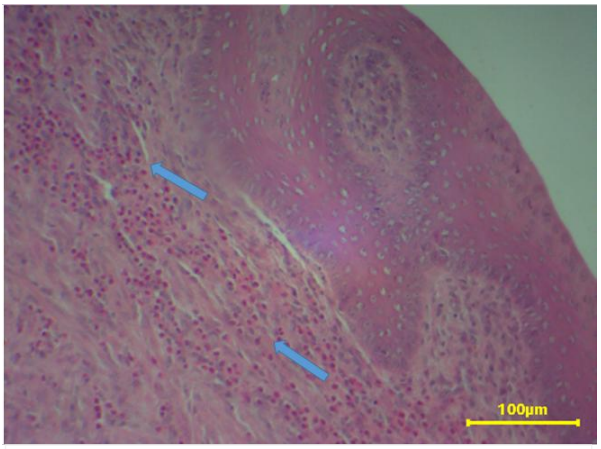

Figure 7. Cross section of in the vagina of a rat from 3rd group showing moderate infiltration of neutrophils at sub mucosa and between muscle fibers (Blue arrow). Staining H&E. 100X. Scale bar 100 µm.

As a result, *Staph. aureus* can cause scarring of vaginal tissues and its presence in the female reproductive tract may lead to chronic uterine inflammation, negatively affecting fertility, causing menstrual irregularities, and potentially leading to pregnancy complications such as preeclampsia (19).

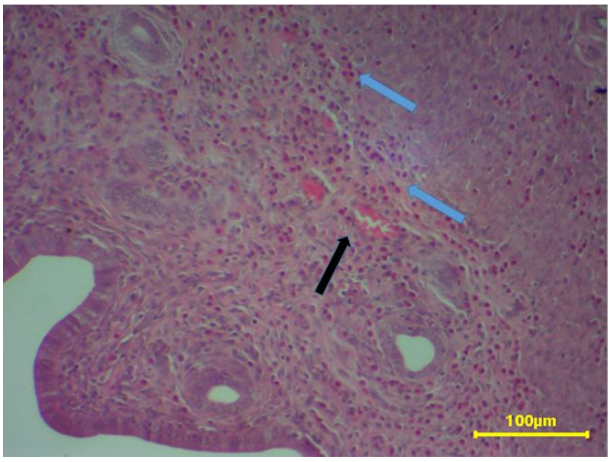

Figure 8. Cross section of in the uterus of a rat from the 3rd group showing a moderate infiltration of neutrophils (Blue arrow) and hyperemic blood vessels (Black arrow). Staining H&E. 100X. Scale bar 100 µm.

Group 4: Infected with *Candida albicans Full name*

Following the injection of 0.1 mL of a *C. albicans* suspension, histological sections revealed multiple focal areas of hyperplasia in the vaginal and uterine

epithelium, as well as hyperplasia in some vaginal and uterine glands. Congested blood vessels and moderate neutrophilic infiltration were observed, along with vascular congestion in the ovarian cortex, as shown in Figures (9, 10).

C. albicans has the ability to migrate from the vagina to the uterus and ovaries, causing inflammation in the tissues of these organs (20). A defensive tissue response occurs as a result of continuous stimulation from the presence of *C. albicans*, which is the body's attempt to repair damage and cope with chronic irritation caused by the infection (21).

Candida causes direct tissue damage and invasion, particularly in the vagina and uterus, through the release of enzymes. The hyperactivity observed in uterine glands likely represents an effort to clear the pathogen(22). The transition of *Candida* from its yeast form to the hyphal form enhances its ability to invade and penetrate host tissues (23).

At least 75% of women experience a yeast vaginal infection at least once during their reproductive years, and between 5% and 9% suffer from recurrent vulvovaginal candidiasis (RVVC), which is diagnosed when a woman has four or more symptomatic infections per year(24). This condition can lead to necrosis and fibrosis in vaginal and uterine tissues (25).

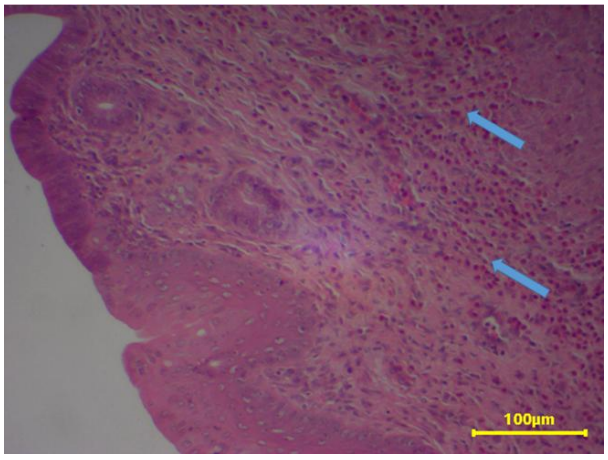

Figure 9. Cross section of in the vagina of a rat from the 4th group showing moderate infiltration of neutrophils (Blue arrow). Staining H&E. 100X. Scale bar 100 µm.

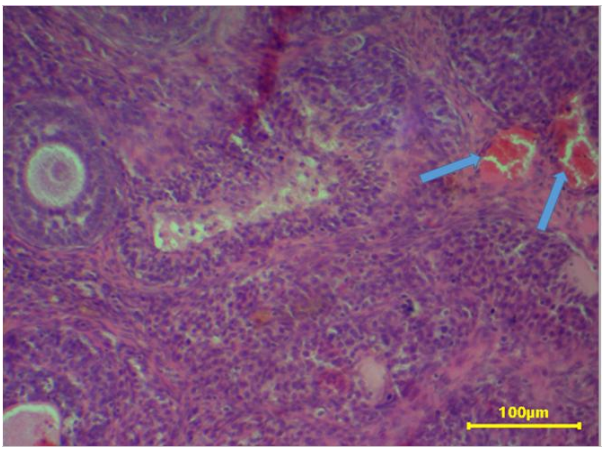

Figure 10. Cross section of ovary of a rat from the 4th group showing congested blood vessels at ovarian cortex (Blue arrow). Staining H&E. 100X. Scale bar 100 µm.

Conclusions

This study indicates that both bacterial and fungal infections have distinct histopathological effects on the vagina, uterus, and the female reproductive system as a whole. Bacterial infections are characterized by intense inflammation and immune responses, whereas fungal infections cause direct damage to the tissue structure.

The importance of histopathological diagnosis is emphasized to differentiate between these two types of infections for appropriate treatment selection. Vaginal infections caused by bacteria or fungi lead to clear histological and immune changes in the vagina, which may extend to involve the uterus and ovaries in chronic or severe cases.

Bacterial infections cause inflammation of the vaginal epithelium with loss of cellular cohesion and superficial ulcers, accompanied by dense cellular infiltration mainly of inflammatory cells (especially neutrophils). In contrast, fungal infections show histological changes including hyperkeratosis, inflammatory infiltration with the presence of fungal hyphae within the epithelial tissue, and the potential for ulceration and chronic inflammation.

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