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BIOLOGICAL ACTIVITY OF SOME *LACTOBACILLUS* SPP. IN COMBINATION WITH CONVENTIONAL ANTIBIOTIC AGAINST SPECIES ISOLATED FROM CATTLE WITH GENITAL TRACT INFECTION

A Thesis

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1.1 Introduction

High reproductive performance is a factor that affects the productivity of dairy and beef cows and is essential to keep farms economically viable. Inadequate nutrition, ineffective reproductive management, and a poor energy balance during the postpartum period are some of the multifactorial causes of poor reproductive efficiency. These factors might negatively affect the immune system and enhance the invasion of pathogens. Clinical and Subclinical Endometritis (SEM) in cows is the key factor contributing to its adverse impact on fertility. A higher rate of infertility and the prevalence of (SEM) harm cows (Ricci *et al*., 2017).

Involution includes both the significant endometrial healing that is essential and the typical inflammation of the uterus. Innate immune defense, which serve as the uterus' primary form of defense, may be defeated by the proliferation of pathogenic bacteria, or the intensity or length of inflammation may worsen rather than improve fertility (Overton and Fetrow, 2008).

LeBlanc, (2012) has analyzed current theories of uterine inflammation and defense as well as the process of natural involution In most cows, this process results in the bacterial infection being cleared up and eventually epithelium being repaired, at which point inflammation is controlled. The effects of clinical endometritis, a frequent reproductive condition in female domestic animals, range from no influence on fertility to pregnant sterility. Animals' general health is affected, and it has a negative effect on how well they reproduce (Amiridis *et al*.,2003).

One of the most significant causes of reproductive insufficienty is a disease of ganitelia that afflict cows during the postpartum period. Up to half of dairy cows may experience at least one of Metritis, purulent vaginal discharge, Endometritis, or Cervicitis during the postpartum period. Inadequate immunological response to

bacterial infection, failure to eliminate harmful germs from the genital tract, or persistent inflammation that impairs rather than enhances reproductive function are the causes of these diseases (LeBlanc, 2014).

Bacterial infection was developed through two to three weeks after calving. In the first two months following calving, reproductive disorders can range from severe clinical symptoms and illness to an imperceptible but considerable decline in fertility (Sheldon *et al*., 2009).

According to numerous studies, between 30 % and 50 % of cows have subclinical endometritis and / or cervix inflammation between four and eight weeks after giving birth, which is linked to decreased reproductive performance. These illnesses are expensive for individual cows because of the demonstrated direct and indirect effects, and they are expensive for herds and the industry because of the high incidence risks. Reduced an activity of innate immune system and poor management and control of inflammation from two weeks before to three weeks after the calving are partly to blame for the high prevalence of illness (Dubuc *et al.*, 2011).

Ovulation in cows is disrupted by uterine bacterial infection or bacterial products because they reduce pituitary LH output and affect postpartum ovarian follicle size and function (Opsomer *et al.*, 2000). Repeat breeding is the condition in which cows do not become pregnant after three inseminations, which prolongs the period of low milk production and low birth rates and causes higher financial losses to dairy companies (Singh *et al.*, 2008).

Numerous studies provide credence to the idea that the presence of bacteria in the reproductive tracts of dairy and beef cattle results not only from external colonization but also from a hematogenous transmission mechanism. Galvao and associates (Galvao *et al.*, 2019) additionally, they demonstrated in their investigations that the hematogenous route is an effective method of uterine

pathogen infection. There is a drive to create adaptable antimicrobial medicines with minimal resistance-inducing potential in addition to employing traditional antibiotics to treat these illnesses (Roope *et al*., 2019).

Other investigations have shown that *Lactobacillus*, which is a component of the healthy cows' vaginal microflora, acts as a strong microbiological barrier against genital pathogen infection by sticking to a certain type of epithelium and creating inhibitors (Niu *et al*.,2019)

Vaginal *Lactobacilli* prevent the growth of genitourinary pathogenic microorganisms through mechanisms of competitive exclusion of pathogens, stimulation of the host immune system, and production of specific antibacterial substances like acetic and lactic acids, hydrogen peroxide, and antimicrobial peptides (Charteris *et al*., 2001).

1.2 Aims of The study

A new approach to treat the genital infection in cows by using prpbiotics were used in this study in first tim in Iraq. therefore, the aims of this current study and their protocol was involved:

1- Isolation, characterization of bacterial isolates from cows genital tract with different clinical conditions
2- Determine the sensitivity of isolates to most common used antibiotics or antimicrobial agents which are used in clinical genitalia disorders
3- Evaluate the effectiveness of antimicrobial potential of some species of *Lactobacilli* against the bacterial species which isolated from genital tract infection in cows as synergistic factor to treat genitelia infection or to reduce virulancity of the bacteria isolated from genital tract infection .

Abstract

The reproductive system of cows is particularly susceptible to various infections, mostly, with bacteria that are resistant to many types of antibiotics. It is necessary to find solutions or alternative treatments to control this infection. This study aims to evaluate a new in vitro approach by using the potential antimicrobial metabolites of cell-free supernatant (CFS) of *Lactobacillus acidophillus* and *Lactobacillus plantarum* individually and as a mixture to control the bacterial species causing reproductive tract infections in cattle.

Vaginal swabs were collected from cows from different fields for large animals belonging to the city of Baquba and from several fields within the governorate during the period from mid-October 2021 until the end of January 2022, and (81) swabs were examined. Among (81) smears, (268) bacterial isolates appeared, all isolates were examined in general, and the highest bacterial isolates were:

Staphylococcus spp. (75/268) 28%), followed by *Pseudomonas* spp. 61(22.7%), Escherichia coli 49 (18.3%), *Sphingomonas* spp.46 (17.1)%, *Kocurea* spp. 23 (8.6%), and *Granulicatella* spp. 14) 5.2%). The antibiotic sensitivity and antibacterial abilities of cell free supernatant (CFS) extracts prepared from the tested lactobacilli were evaluated using disc diffusion, microplate diffusion and the broth microdilution technique.

Gentamicin and ampicillin were the highest sensitive antibiotic agent against all isolates, and there were differences in the sensitivity of bacterial isolates to other antibiotics selected for this study, showing: *E.coli* (type 3), *Staphylococcus* spp., *Pseudomonas*. *Sphingomonas* (Types 1 and 2), *Kocurea* spp. and

Granulicatella spp. were sensitive to oxytetracycline but *Escherichia coli* (Types 1,2 and 4) were resistant.

Escherichia coli (type 1,2,3 and 4), *Pseudomonas* spp. , *Sphingomonas* spp. (Type 1 and 2), *Kocurea* spp., and *Granulicatella* spp.. were sensitive to cefotaxime, while *Staphylococcus* spp.. were resistant. *E. coli* (type 1, 2, 3 and 4), *Shingomonas* (type 1), and *Kocurea* spp. , and Granulicatella spp. It was sensitive to Cefixime while *Staphylococcus* spp. It was resistance .*Shingomonas* (type 1), *Kocurea* spp., *Escherichia coli* (type 1, 2, 3 and 4) and *Granulicatella* spp. It was sensitive to cefixime, while *Staphylococcus* spp., *Pseudomonas* spp., and *Sphingomonas* spp. (Type 2) was resistant. *Staphylococcus* spp. , *Sphingomonas* spp (type 1 and 2), and *Granulicatella* spp. It was sensitive to clindamycin, while it was *Escherichia coli* (1, 2, 3, 4). *Pseudomonas* spp. and *Kocurea* spp. It was resistance. All isolates in this study were resistant to metronidazole and cloxacillin.

There were significant differences ($P<0.05$) in the minimum inhibitory concentration (MIC) of the studied groups of different dilutions of the test suspension (CFS) for all bacterial isolates (*Lactobacillus acidophillus*, *Lactobacillus Planterum* and *Lactobacillus acidophillus* mixed with *Lactobacillus planterum*). In addition, there were significant differences between different bacteria types at the 50% concentration of *Lactobacillus* spp. Compared with other concentrations of CFS used, which were (25%, 12.5%, and 6.25%)..

The CFS of *L.acidophillus* is more effective than other tested *Lactobacillus* spp. against *E. coli* spp. In addition, we found that CFS *L. acidophillus* and *L. plantarum* CFS were more effective if used as mixtures against isolates of *Staphylococcus* spp. and *Granulicatella* spp. and *Pseudomonas* spp. and *Kocurea* spp. While no inhibitory effect was observed even at high concentration of

Lactobacilli CFS (50%) against .*Sphinomonas* spp (type 1 and 2). However, the mean value ± standard deviation rate of the used concentrations of the CFS cell-free superfluid of *L. acidophillus* showed significant differences ($p < 0.05$) in the growth inhibition of pathogenic bacteria.

In result, *Lactobacillus* spp. is a good alternative therapy due to its composition of many natural antimicrobial molecules which makes it an attractive candidate to inhibit the growth of pathogens and this in turn is a suitable option for reducing antibiotic use in animals as part of the One World initiative, One Health) Globalism. It can be said that in this field of study it is possible to understand the relationship between the microbial composition of the endometrium and its role in fertility, and to understand the mechanism of action of bio-stimulants in different species and their positive role in reducing the incidence of pathogens that impede production and reproduction in farm animals.