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Detection of R plasmid with assessment of drug resistant genes in Bacterial vaginitis at labor

A Thesis

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By

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1. Introduction

Genital tract infection (GTI) is a global health problem which affects men, women, families and communities. These infections threaten the women's health and may have severe consequences such as infertility, ectopic pregnancy, chronic pelvic pain, abortion and an increased risk of HIV transmission. Therefore, proper prevention and treatment of these diseases are of great importance genital tract infection (Masoumeh *et al.*, 2019).

Infectious and inflammatory disease of the upper genital tract (endometrium, fallopian tubes, ovaries, and pelvic peritoneum) can cause infertility, ectopic pregnancy, and persistent pelvic pain (Ruggeri *et al.*, 2016). Inflammation of the placenta and amniotic membranes marks the start of BV in labor, which appears to be caused by both maternal and fetal causes. Preterm labor can be caused by an inflammatory response, which is associated with significant morbidity and mortality despite medical intervention (Goldenberg *et al.*, 2008).

Inflammation of the placenta and amniotic membranes called chorioamnionitis is an ascending infection that originates in the lower urogenital tract and migrates into the amniotic cavity. Chorioamnionitis correlates to preterm delivery prior to 37 weeks of gestation. In most studies, proxies of intrauterine infection (clinical chorioamnionitis, histological chorioamnionitis, intra-amniotic increase in cytokines) are associated with acute neonatal morbidity and mortality and, at least to some degree, with neurological impairments (periventricular leukomalacia, intraventricular hemorrhage, cerebral palsy, polymicrogyria), chronic lung disease, and involution of the thymus in the preterm infant (Peng *et al.*, 2018).

Bacterial Vaginitis (BV) has been reported in a third of women worldwide at various stages of life, due to the complex balance of the vaginal microbiota. It is a common cause of abnormal vaginal discharge and is associated with other health problems. Since the first description of anaerobic microbes associated with Bacterial Vaginitis such as *Gardnerella vaginalis* in the 1960s, researchers have stepped up the game by incorporating advanced molecular tools to monitor and evaluate the extent of dysbiosis within the vaginal microbiome, particularly on how specific microbial population changes compared to a healthy state (Sujit *et al.*, 2011).

Many researches on BV in Iraqi populations have been published; the prevalence of BV in Kirkuk ranges from 4 to 64%. depending on the study population's racial, regional, and clinical factors. The prevalence among asymptomatic women ranges from 12 to 20% and similar percentages were observed in pregnant women (Nejad and Shahla, 2008).

While in Baghdad the BV rate of the cases was 71% (22 % of the swabs was present in gram negative bacteria, while gram-positive bacterial swabs was present in 19 % of the cases). According to studies on bacterial vaginitis in Diyala/Al-Khalis City, pregnant women had a greater number and proportion of bacterial vaginitis 60% and non-pregnant women 9%. *Gardnerella vaginalis* produced the most infections, according to bacteriological tests of the isolates 51,0 %, followed by *Staphylococcus aureus* 22,0%, and *Klebsiella* 10%. (Hameed, 2019).

Multidrug resistant (MDR) is a vexed term, from 1980 it was used to mean “resistant to multiple agents” without the number or types of agents being specified, more recently, the European Centre for Disease Prevention and Control

(ECDC) has attempted to formalize the term as “resistant to three or more antibiotic classes” (Hawkey *et al.*, 2018).

Antibiotic Resistance is an urgent problem in clinical settings because they sharply increase mortality risk in critically ill patients. The horizontal spread of antibiotic resistance genes among bacteria is driven by bacterial plasmids, promoting the evolution of resistance. Bacteria acquire resistance to antibiotics through two principal routes: chromosomal mutation and the acquisition of mobile genetic elements such as plasmids by horizontal gene transfer (Aleksun and Levy, 2007). Plasmids are circular DNA molecules that replicate independently of the chromosome and are able to transfer horizontally between bacteria by conjugation. Plasmids play a key role in the evolution of bacterial Antibiotic Resistance, disseminating resistance genes among the most worrisome clinical pathogens conjugative plasmids are certainly the most important drivers of AR spread in bacterial families such as Enterobacteriaceae and Enterococcaceae, which include some of the most important nosocomial infections (Millan, 2018).

This study is aimed to:

- 1-Detect the clinical bacterial isolates that cause infections of the female genital tract in labor.
- 2-Study the resistance to antibiotics pattern of the isolated bacteria.
- 3- Determine the MDR isolates.
- 4-Detection the presence of R plasmid in the resistances isolates.
- 5-Find the correlation between R plasmid and bacterial resistance.

antibiotics out of the total number of total 14 antibiotics, *S. aureus* was the most MDR bacteria isolated which was 23.9% (14 isolate) while *S. epidermidis* consist 12.6% (9 isolates) of MDR isolates. While in gram negative bacteria *E. coli* was the most MDR bacteria isolated which was 22.0% (16 isolate) followed by 9.8% (7 isolates) of *K. pneumonia* and 4.2% (3 isolates) of *P. aeruginosa*.

Activity detection of efflux pumps showed that Gram-negative bacteria had a stronger efflux pump activity than Gram-positive bacteria, the highest activity found in *K. pneumonia* at 100% in concentrations 0 of ethidium bromide, comparatively, *P. aeruginosa* show the effectiveness of efflux pumps at high concentrations (100,00) mg/ml, and this may be due to its possession of the efficacy of purines that prevent the entry of ethidium bromide that it is driven by flow pumps.

Ten of MDR *E. coli* isolates were selected and subjected to a plasmids curing of. The results showed that curing plasmids of MDR *E. coli* isolates did not contribute to canceling the resistance of a high percentage of drugs, which indicates that resistance to only some of these drugs are of plasmid origin, including Trimethoprim/Sulfamethoxazole and some others are chromosomal, including Cefixime and Piperacillin antagonists.

PCR technique was performed on all previous MDR isolates of *E. coli* for the detection of *bla*_{CTX}, *bla*_{TEM}, *bla*_{OXA} and *bla*_{SHV} genes in order to determine their ability to produce broad-spectrum β -lactamase enzymes. The present results showed the presence of *bla*_{CTX}, *bla*_{TEM}, *bla*_{OXA} and *bla*_{SHV} genes in the studied isolates in varying proportions. The study showed that 0/10 (0%) of the isolates contain the *bla*_{CTX} gene and 4/10 (40%) of the isolates contain the *bla*_{TEM} gene. for gene *bla*_{OXA} 3/10 (30%) of the isolates contained it, while the *bla*_{SHV} gene was the least prevalent of the previous genes with 2/10 (20%) of the isolates contained it.