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EFFECT OF METRONIDAZOLE ON ADHERENCE PROPERTY OF *GARDNERELLA VAGINALIS* ISOLATED FROM PRETERM LABOR PATIENTS IN AL-HILLA CITY, IRAQ

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ABSTRACT

One hundred fifty clinical sample were collected from preterm labor patients with (bacterial vaginosis, urinary tract infection and aborted women) 80, 15, 55 respectively, admitted to Babylon Maternity and pediatric hospital and Al-Hilla Teaching Hospital, at the period from February to October 2016. These high vaginal samples were subjected to different methods of identification of *G.vginalis* mainly traditional bacteriological methods. It was found that 6 (4%) *G.vaginalis* isolates were recovered by using selective media and Viteck 2 system where 30 (20%) isolates were recovered dependent on direct extraction to high vaginal swab on molecular level. The screening of the adherence property were carried out to all (6) isolates and showed that all of it were capable to adhere on the epithelial cell then subjected to Metranidazole reduce it. Finally in vitro anti-adherence activity to Metronidazole were studied and the results revealed that all isolates were inhibited by aqueous solution at concentration (5%) which gave results (100%) very effective to eradication adherence, the results proved that is effective on disrupted adherence in the first step.

KEYWORDS: *G.vaginalis*, Viteck 2, adherence, preterm labor, Metranidazole.

INTRODUCTION

Preterm labor (PTL) is labor which occurs before 37 completed weeks of gestation and can lead to preterm birth (PTB). PTB causes most of neonatal deaths and different forms of neonatal morbidities[1], The causes of PTB in most cases have not been established although several risk factors have been identified.[2] Because many of these infections are

asymptomatic, underestimation of their importance may have been occurred Furthermore, few studies focusing on these infection, and they investigated only one infection in relation to PTB, such as chlamydia, bacterial vaginosis, or urinary tract infection.[3,8]

Gardnerella vaginalis is Fastidious, facultative anaerobic, small, pleomorphic rods that are non-motile and do not possess flagella, endospores, or typical capsules In vaginal fluid smears the Gram reaction of *G. vaginalis* may vary from positive to negative It is non-motile and do not possess flagella, endospores, or typical capsules In vaginal fluid smears the Gram reaction of *G. vaginalis* may vary from positive to negative.[4] Another characteristic feature of a Gram positive cell wall exhibited due to is the absence of di-amino pimelic acid and lipopolysaccharide in the cell wall.[9] *G. vaginalis* is able to form an adherent biofilm on the vaginal epithelium of women with BV. The biofilm incorporates other bacterial groups into its layers, suggesting that it may enable other anaerobes to colonize the vagina. G. vaginalis also produces the toxin vaginolysin.[10]

Adhesion factor: Adhesion is an important virulence factor for pathogens considered first step in pathogenesis in all pathogenic organism have ability to adherence to the host tissue and resist the flashing action and evade the host defense this include pili, filamentous networks, fimbriae, flagella and lipopolysaccharide(LPS)especially O-antigen found to play roles in bacterial adhesion.[11] The clue cell is a squamous epithelial cell which is covered by bacteria. First step of infection progress is adhesion of bacteria to epithelial cell through the adhesion molecules. The most important adhesion molecules of epithelium are E-cadherins, fibronectins, Toll like receptors and carbohydrates. In bacteria, pilis, lipopolysaccharide and biofilm have primary importance.[12] Pili and other adherent molecules are cell associated structures often involved in adhesion and some of which act as hemagglutinins, Morphology of pili appear as a thin and long flexible structure, usually present in small numbers (type –L) and a more numerous, shorter,thicker and straight pilus (S-pili). It is surface appendix have the diameter(3-7.5) um that facilitate the adherence on specific receptor on the virginal epithelial cell.[13]

MATERIALS AND METHODS

Sample collection

A total of 150 patients diagnosed as preterm labor by the physician who were admitted to Babylon Maternity and pediatric hospital and Al-Hill Teaching Hospital, at the period from February to October 2016**.** After obtaining the permission from the subjects for examination

and sampling, high vaginal, two cotton swabs were used for each patient; as the specimens were collected.

Culture characterstic: isolates have been cultured on Colombia agar supplemented with 5%fresh blood with the addition (Naldixic acid – Gentamycin and Nystatin) diagnosed according to.[14]

detection by viteck 2 system: according to cassatte manufacture**.**

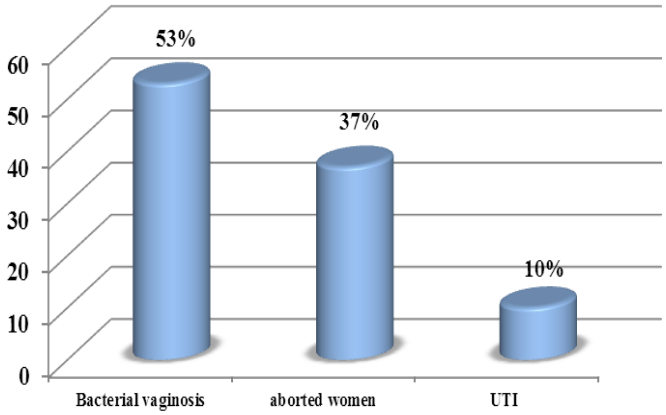
Adherence Test

The ability of *G. vaginalis* to adhere to epithelial cells one of the important virulence factors of these bacteria and detected as following steps.

1- The brain heart infusion broth (bacterial broth) were prepared, inoculated then incubated under anaerobic conditions for 72 hrs.
2- Dilution of bacterial broth was prepared by use phosphate buffer saline (PBS) and 1.5× 108 cell/ml were taken.
3- The urinary tract epithelial cells were prepared by centrifugation of 5ml urine sample from healthy female at 5000 rpm for 10 minutes and washing with PBS for three times.
4- Epithelial cells were filtered by filter paper, then placed the epithelial cells on cover slides by press the cover on surface of filter paper then lifted to be dry.
5- The cover slides were placed on sterile glass plate then add 5 ml of previously prepared bacterial broth, and placed the plate contains the epithelial cells with bacterial broth in incubator for 1 hr. at 37°C.
6- The cover slides were washed by PBS to remove non adherent bacteria. Then the epithelial cells were fixed by ethanol for 15 minutes. After that the slide was stained with Giemsa stain (30%) for 20 minutes and washed by DW then lifted to dry by air.
7- The cover slides were placed on glass slides by inverted position, and tested under light microscope.[15]

RESULTS AND DISCUSSION

*Depending on (culture c*haracteristic, biochemical test and viteck 2 system) detection 6 isolates of bacteria were obtained during this study belonging to different clinical manufactures as shown in figure (1) and confirmed by molecular identification. On the other hand, the result was showed that from 80 patients with bacterial vaginosisonly[3] isolates were obtained which gave a percentage (53%) agree with.[17,16]



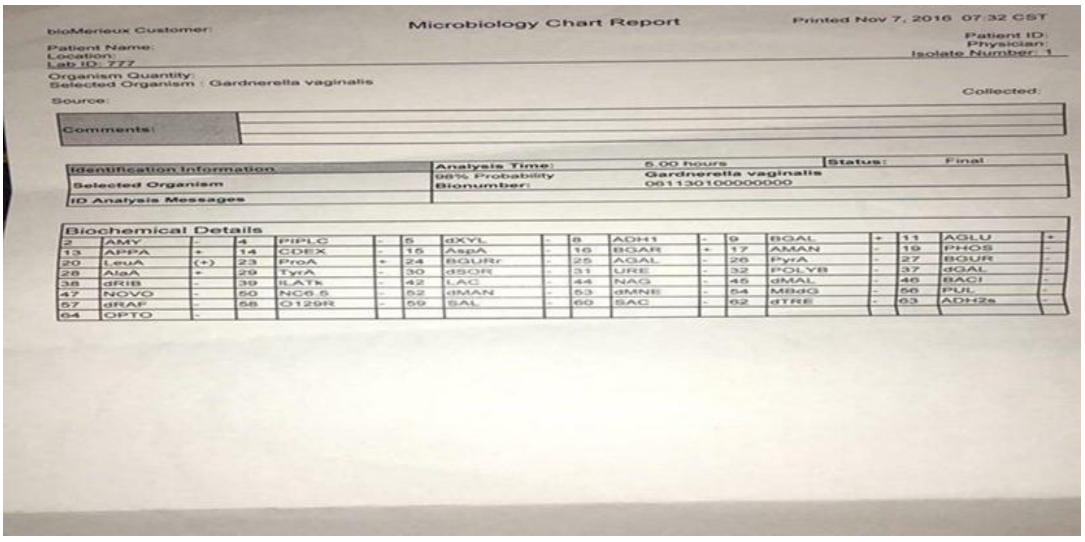
*Positive results obtained only from 6 patients (3 bacterial vaginosis, 1 UTI and 2aborted women).

Figure 1: Distribution of patients according to clinical diagnosis.

Identification of *G. vaginalis* by Vitek2 system

To confirm the isolates of *G. vaginalis* was used automated VITEK 2 system using GN-ID cards which contained (64) biochemical tests as shown in Table (3-4). The results demonstrate that all (6) isolates were confirmed with ID message confidence level ranging excellent (Probability percentage from 94 to 99.7%). This technique is characterized by fast detection of bacteria without need for many of culture media as well as reduces cultural contamination.

Table (1): Biochemical testing of *G. vaginalis* isolates (No. 6) using Vitek$_2$ system.



Detection adherence ability of *G. vaginalis* to vaginal Epithelial Cells

Adherence of *G. vaginalis* to urogenital tract epithelial cells is the first step in the pathogenesis of *G. vaginalis* infection and is facilitated by the action of several adhesions located on the surface of bacteria small fimbriae.

The result showed that all *G. vaginalis* isolates (6) have ability to adhere to epithelial cells as shown in figure (2).

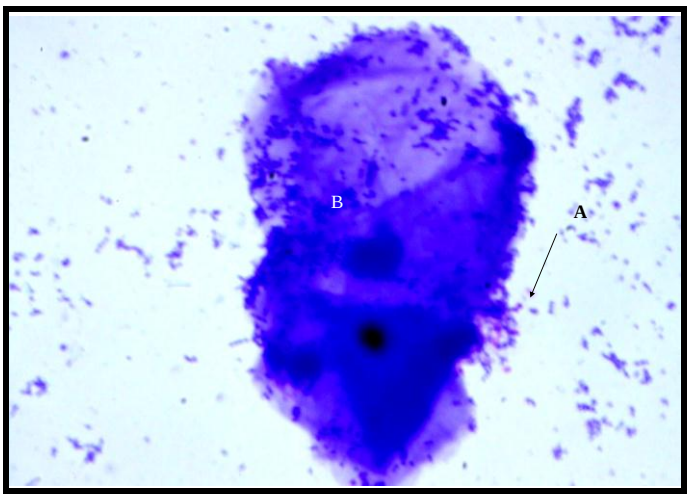


Figure (2) Adherence ability of *G. vaginalis* to epithelial cells under (1000X) magnification power.

A: *G.vaginalis* B: epithelial cell

The high adherence ability correlated with study done by[18] they focused on the most essential role of pili in the binding capacity to host cells including epithelial cells.

The binding activity to epithelial cells can be the critical step in its invasion and survival in host tissues and thus contribute to enhance the pathogenicity of this organism. Because of surface components of bacteria were potentially important in the occurrence of various vaginal infections.[19][20] show that *G. vaginalis* have the ability to adhere the vaginal epithelial cell. This bacteria is just opportunistic pathogens, it may be deduced that adherence is an important virulence factor.

Bacteria adhere to epithelial cell by recognizing these molecules and they cause infection after a couple of years N-acytlgalactoseamine and D-galactoseamine receptors were found on *G. vaginalis* cell wall so it was suggested carbohydrates of epithelial cell can involve in adhesion by recognizing these receptors. Also they suggested that other mechanisms can take part in adhesion of *G. vaginalis* to epithelial cells. Studies on *G. vaginalis* pili demonstrated

the importance of it in mediation of interaction of this bacterial organism with vaginal tissues and interrupt the cellular signaling via extracellular matrix proteins/ integrin's in genital regions.[21,22]

This pathogen expresses two distinct fimbria-molecules on its cell surface, one of which is composed of a subunit protein (named *FimA*) encoded by the *fimA* gene, and termed long fimbriae, while the other consists of a subunit Mfa protein encoded by the *mfa1* gene and termed short fimbriae.[23]

In addition, the critical role of fimbriae in adhesive and invasive events of this bacterial organism to epithelial cells it is have proteinase's catalytic activity which important for effective adherence of *G. vaginalis* to genitourinary tract.

3.2.2.1. Effect of Metronidazole on adherence

We were used Metronidazole (flagyl) solution 500mg/100ml as a diluted antibiotic for screening of anti-adhesion activity was carried out by adding it to the epithelial cell with addition of *G. vaginalis* as shown in Figure (3)

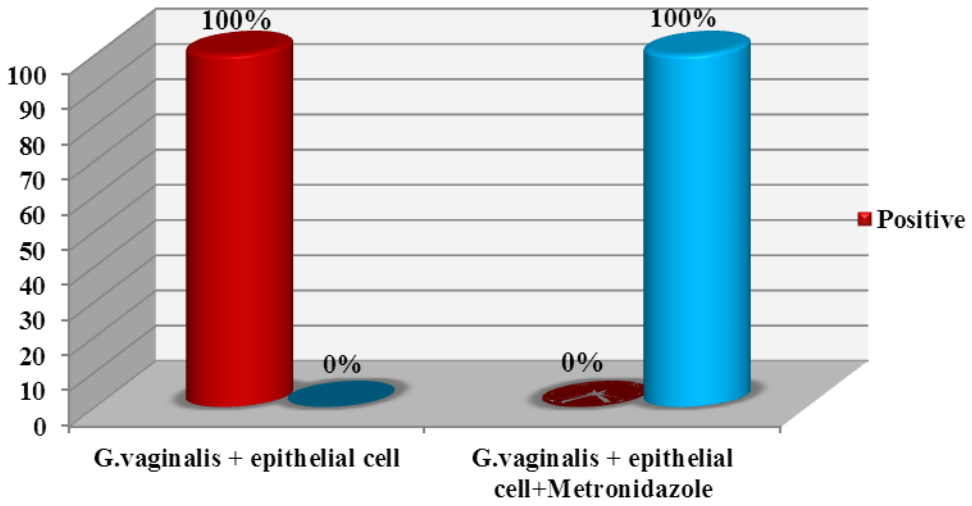


Figure (3): Effect of metronidazole on adhesive property of

G. vaginalis

These results showed that the metronidazole produced the highest inhibition activity against the adhering activity which inhibited the adherence in (100%) percentage. This high activity related to metronidazole is especially effective against anaerobic infections, such as *G. vaginalis* infections because in anaerobic conditions, the metronidazole molecule changes the

environmental conditions leading to oxidative stress so lead to inhibit the DNA repair enzymes leading to death of anaerobic bacteria, but having no effect on aerobic tissues.[24]

Metronidazole can be considered an important antibiotic for anaerobic infection.[25] Since then, this compound has also played an important role in treating anaerobe related infection in the vagina this contribute to its biological mechanism of action and it is believed to involve in production cytotoxic redox metabolite which believed to be the key component of microorganism killing by metronidazole.

This result was agreement with those results obtained by[26] which found that metronidazole is effective to inhibited the adherence activity in (95%) percentage, While this results is disagreement with results obtained by[27],[28] who were found that the effect of metronidazole on adherence at rate 44%, 30% respectively . In a study, after treatment of clue cell with sodium-meta-periodate which destroys the C-C bond between hdyroxl groups of carbohydrates, adhesion of *G. vaginalis* to epithelial cell was inhibited.[29][30] who were found a metronidazole comprising a NO_2 group in a reduced form, so linked via a chemical linkage bond to a adhesion molecule. So the latter is linked to the metronidazole by a propionic acid group.

This chemical linkage leads to selective inhibition and adhesion property. This indicate why this bacterium is difficult to be obtained from vagina and also sub MIC concentration of some antibiotic will assist in losing the bacteria their adhesive characteristics in the vagina and that also explain why this bacteria are isolated from epithelial cell.[31]

CONCLUSION

All isolates are capable to adhere and causes infection, in this research we demonstrated that Metronidazole is effective to eradicated the adherence.

Ethical Approval

A valid consent was achieved from hospitals administration and from each female (patients and controls) before their inclusion in the study. For every female or her followers, the procedure had been informed before the samples were collected, making absolutely sure that they understood the procedure that was to be carried out. The subjects were sentient that they had the right to reject to be included in the study without any detrimental effects.

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