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GENOTYPING OF VAGINOLYSIN GENE OF GARDNERELLA *VAGINALIS* ISOLATED FROM PRETERM LABOR PATIENTS IN HILLA CITY

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ABSTRACT

The present study aims to detection Genotyping of vaginolysin gene for *Gardnerella vaginalis* which isolated from preterm labor patients by PCR-RFLP in Hilla cityIn this study, 150 clinical sample were collected from patients with preterm labor submitted to Al-Hilla Teaching Hospital and Babylon Maternity and pediatric hospital. After extraction of DNA from high vaginal swabs, the DNA was subjected to PCR- RFLP method for identification of *G.vginalis*. results show It was found only (20%) of bacteria was give positive result. However, vagainolysin were carried out using specific primer and the result shows that 12 samples were positive amplification 1551bp then products were digested with *EcoR* I the results showed that vagainolysin exhibited three fragment 1000, 717, 100 bp respectively, assertion that *G.vaginalis* have four Genotyping this improving that it is had more virulent .

Key words: *G.vaginalis* , cpn60 , vaginolysin, PCR-RFLPs, preterm labor

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 (Brotman, 2011). The causes of PTB in most cases have not been established although several risk factors have been identified. 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 (Dimetry *et al*., 2007).

Gardnerella vaginalis: is the single individual from the sort *Gardnerella*, which is belonged to the family Bifidobacteriaceae in phylum Actinobacteria (Gravett *et al.*, 1986). It is at first named *Haemophilus vaginalis* by pioneers, the microorganism was later referred to as *Corynebacterium vaginale* and systematically considered as *G. vaginalis* (Forbes *et al.*,2007).

It is Fastidious, facultative anaerobic, non-motile and don't have flagella, endospores, or regular cases. In vaginal discharge varied into Gram response of *G. vaginalis* may shift from positive to negative. The second diagnostic feature of a Gram-positive cell wall variety due of di-amino pimelic acid and lipopolysaccharide disappear in the cell wall (Catlin 1992; Piot *et al*., 1980). It is maltose, Glucose and starch fermenter without gas, non-nitrate production, has unable to esculin hydrolysis. The studies concentrated and focused on the genetic primer and found that *G. vaginalis* detection could be achieved with varying degree of success with primers specific for universal cpn60 target of *G. vaginalis* was applied in a prospective study of the vaginal microbiota of women with preterm premature rupture of membranes (Darwish *et al.*,2007)

G. vaginalis produces cytotoxin, which its enable fuse into lipid layers. It is known that morphotypes of bacteria excrete enzyme, for example mucinase, sialidase and IgA protease. These enzymes are factors of virulence since they destroy mucines, which play a significant role in functioning of female reproductive tract, and they also facilitate adherence of bacteria to epithelial cells of urogenital tract. These catalysts are variables of destructive since they possess mucines, which assumed a noteworthy part in working of female gential tract, and they likewise encourage adherence of organisms to epithelial cells of urogenital tract.

vaginolysin: a cholesterol-dependent cytolysin, The Cholesterol Dependent Cytolysin family is made up of more than 15-protein produced by distinct group of gram-positive genera, it is species specific for human cells and encodes a pore-forming toxin (Gelber *et al*., 2009).

MATERIALS AND METHODS

Sample collection: One hundred fifty high vaginal swabs samples of preterm labor were recovered All samples or individual were admitted to Al-Hilla surgical teaching hospital and Maternariey and pediatric hospital in Al-Hilla city/ Iraq.

DNA Extraction: DNA was extracted from high vagina swab according to the kit (genaid U.S.A.)

Detection of specific gene markers by PCR: Primer and PCR conditions were used to detect

gene of *G. vaginalis* are present in table (1). However, each 25µl of PCR consist of each upstream and downstream primer (2.5 µl), free nuclease water (2.5 µl), DNA extraction in concentration 0.1µg/ml (5µl), and master mix (12.5 µl). The polymerase chain reaction amplicon was detected by gel electrophoresis on 1.5% agarose gels for 40 min at 70 V.

Genes	sequence of primer	Amplicon (bp)	PCR condition	Reference
Vly	F-5'CCGTCACAGGCTGAACAGT3' F-5'TTACTGGTGTATCACTGTAA3'	1551	94ºC 10min 1x 94ºC 2min 52ºC 1min 40x 72ºC 1min 72ºC 10min 1x	Gelber *et al.*, 2008
Cpn60	F-5'CGCATCTGCTAAGGATGTTG3' R-5'CAGCAATCTTTTCGCCAACT3'	615	94ºC 10min 1x 94ºC 1min 62-66C1min 35x 72ºC 1min 72ºC 10min 1x	In this study procedure designed by Optimize Protocol Writer online

Genotyping by Restriction-Endonuclease Digestion (RFLP-PCR): After PCR were done to the vaginolysin gene and visualized under UV-trans illuminator the (5um) from the product were digested with (20 U) EcoRI restriction enzyme with 5 u free nuclease water then incubated for 3 hours in 42°C, the final product was detected by gel electrophoresis 3% agarose. It's used for both distinguishing isolates and genotyping for bacterial isolate as shown in table 2 and 3.

Table 2: Protocols of RFLP mixture volumes

NO	Content s of reaction mixtures	Volume
1	deionized Sterile water	16.3 µl
2	Buffer, RE 10X	2 µl
3	Acetylated BSA 10µg/µl	0.2µl
4	Deoxy Nucleic Acid template	1 µl
5	Restriction enzyme10u/µl	0.5 µl
Final volume		20 µl

Table 3: cutting sites and Restriction Enzymes mentioned in this study.

Restriction Enzyme	Primer Set	Cutting Sites	References
EcoR *I*	Asp299Gly *G/A*	5'G AATT3' 3'CTTA G5'	9

RESULTS

The bacteria was diagnosed as *G.vaginalis* depending on Chaperonin protein 60 was used gave 615 bp the result shown that out of 150 samples only 30 (20%) isolates were positive results as showed in figure 1.

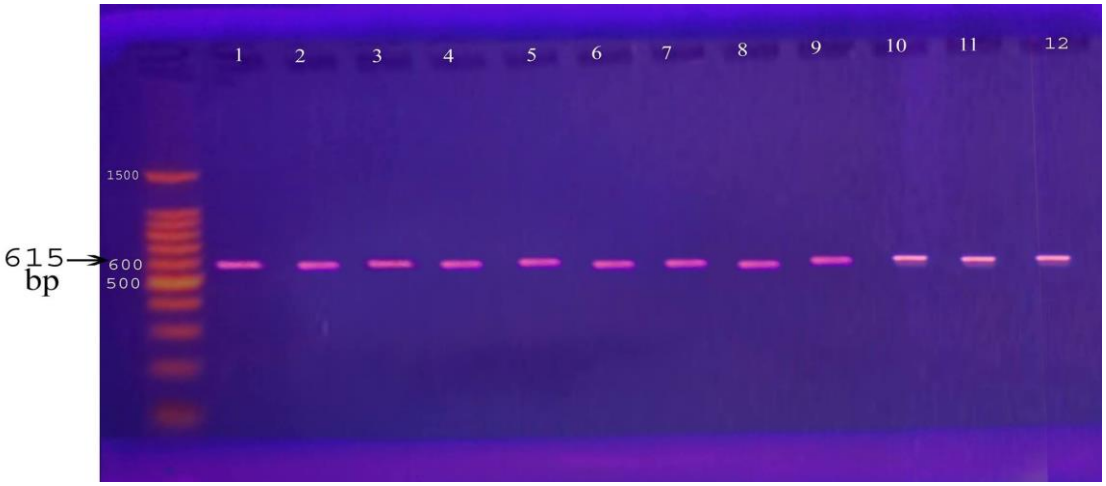

Figure 1: Gel electrophoresis of a 641 bp specific for detecting Cpn60 gene of *G.vaginalis* PCR product were separated by electrophoresis in an 1% agarose gel, at 70 volt for 30 min. Lane1: 1 adder, lane:2, 3,4,5,6,7,8,9,10,11,12 positive results as *G.vaginalis* 1500 bp marker (Ladder).

Molecular detection of vaginolysin gene (VLY) was done for all *G. vaginalis* isolates. The results showed that 12 samples (36.7%) wer positive results for this virulence the positive results were detected by the presence of 1551 bp band when compared with ladder as shown figure 2.

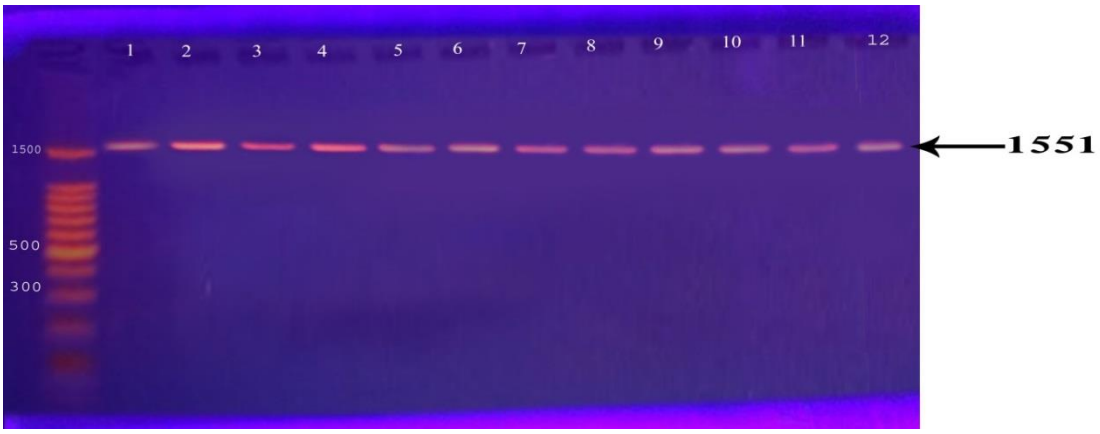

Figure 2 :1% Agarose gel electrophoresis at 70 volt for 50 min for *Vly* PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bp ladder; lane (1-12) were positive for this gene, The size of product is 1551 bp.

On the other hand, amplification of vly gene was digested using EcoRI enzyme provided by Biolab Company, the result shown that three fragments were present 110, 717, 1000 respectively as shown in figure 3.

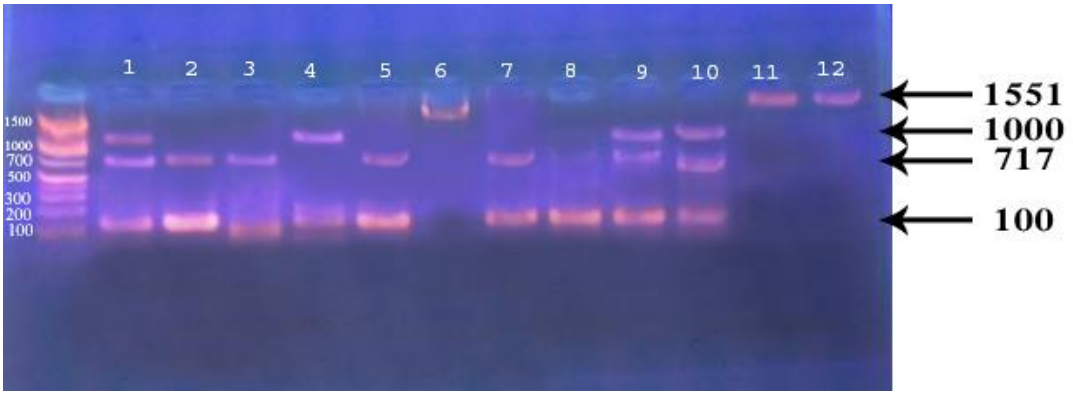

Figure 3: 2% Agarose gel electrophoresis at 70 volt for 50 min for *genotyping vly* . L: 1500 bp ladder; lane (1-12) were positive for this gene, the size of product is (1551) uncut Vly (100-1000-717) bp.

DISCUSSION

G. vaginalis was isolated from patients with preterm labor from, the percentage of this result is disagreement to that results obtained by Jousimies-Somer *et al*., (2002), which referred that the percentage to isolate this bacterium was 40.8% in Nigeria whereas the reproductive age play role when it isolated in America and Europe as mentioned by Karatan *et al*., (2009). This variation in isolation rate may be due to nature and size of the habit to the area that sample were taken, or due to the antibiotic usage by the patient.

The chaperon sequence has been employed as a target for detection and identification of organism which is considered a universal target

Maraffini *et al* (2006) which was said that bacteria adhere to the epithelial cell in urine with large quantity in cohesive shape especially at pregnancy period.

The isolation rate of this bacteria from aborted women are belong to estrogen abnormality during the pregnancy period which convert the Glycogen to lactic acid made vagina environment acidic that give chance to *G.vaginalis* to grow this acidity are responsible to prevent *lactobacillus spp*. grow and produced hydrogen peroxide , this bacteria adhere and evade the uterine endothelial made it evade the embryo tissue or chorioamnotic membrane area which responsible for tropical secretion of phospholipase A_2 and prostaglandin release which accountable on early delivery , abortion and broken immature tissue .

However, vaginolysin gene was present in only 12 isolates, the absent of gene in other isolates that will give an interpretation that (vly) may be encoded by more than one genetic loci may have a role in vaginolysin production and may be other bacterial exotoxins have the ability to direct lysis of cells and ultimately help with microbial spread through tissues by causing instant damage to the extracellular matrix or the plasma membrane of eukaryotic cells (Marrazo *et al*., 2009)

It is generally considered as an important virulence specific factor a cholesterol-dependent cytolysin which increases the availability of the cellular contents, like a substrate to bacterial growth (Patterson *et al*., 2010).

VLy is a cytolisin is a pore-forming protein and utilizes the complement regulatory molecule CD59 to activate, on human epithelial cells, the epithelial p38-mitogen-actived protein kinase, leading to the cell death.

Genotyping of *G.vaginalis* by PCR-RFLP of Vaginolysin Gene: Molecular detection of vaginolysin gene (VLY) was done for 12 *G. vaginalis* samples. The positive results for *vly* virulence were detected by the presence of 1551 bp band compared with allelic ladder after that digested with *EcoR* I enzyme after digestion three fragments were present 110, 717, 1000 respectively. The *EcoR* I restriction enzyme cutting of the amplicon *vly* product generated different restriction pattern with the number of fragments varying from one to three as shown in figure 3.

The different size of fragments product of *vly* gene might be referred to polymorphism between strains. The *vly* gene and PCR-RFLP of the vly genotyping is an important method for infectious disease. However, there is no previous study was done in Iraq about genotyping of vaginolysin gene isolated from preterm labor women.

Vly gene is polymorphic and molecularly changeable and this polymorphism because of variation in allelic types on the 3' end of the gene which differ in their arrangement and, variation in size of product refers to *vaginolysin* gene comprise three distinguished sites (i) the N-terminal site, (ii) a central highly conserved region, and (iii) a C-terminal region which contain-tandem repeat (Teenus et al., 2015).

when genotyping 100 appeared indicate that its carrying proline residues in this site the proline loop, whereas the 717 bp was meant its varied in carry (Glycine and cysteine), the last 1000 bp carried Aspartic acid called (Asparagine) when the sample carried at least two fragments mean its virulent when carried three fragment its more virulent as mentioned by Tenke *et al*., (2012)

in some negative culture cases, this facilitate treatment target to be given early instead of waiting of the delay about 30-72 h for culture report, with cost consumable and equipment of achieved bacteria (Teenus *et al*., 2015)

The recombinant *N*-terminally-hexahistidine-tagged VLY lacking the putative signal sequence (amino acid) (AA) residues is play a crucial role in enzyme cut when VLY mutants were carrying the VLY-coding gene lacking AA as a template for PCR-mediated, site-directed mutagenesis targeted to the whole genome.

Conclusion

G.vaginalis considered one of the causative agent that lead to preterm delivery , Cpn60 is more specific for detection this bacterium, vaginolysin is main virulence factor for its pathogenesis, Genotyping of it showed that have been more pathogenic when have multiple band .

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REFERENCES

Brotman R.M., Vaginal microbiome and sexually transmitted infections: an epidemiologic perspective. J Clin Invest. 121: 4610-7 (2011).

Catlin B.W., Gardnerella vaginalis: Characteristics, Clinical Considerations, and Controversies. Clin Microbiol Rev. 5(3): 213-237 (1992).

Darwish A., Elnshar E.M., Hamadeh S.M. and M.H. Makarem, Treatment options for bacterial vaginosis in patients at high risk of preterm labor and premature rupture of membranes. J Obstet. Gynaecol. Res. 33: 781e7 (2007).

Dimetry S.R., El-Tokhy H.M., Abdo N.M., Ebrahim M.A. and M. Eissa, Urinary tract infection and adverse outcome of pregnancy. J. Egypt Public Health Assoc. 82: 203 e18 (2007).

Forbes B.A., Sahm D.F. and A.S. Weissfeld, Bailey and Scott′s Diagnostic Microbiology. 12th ed. Mosby, USA (2007).

Gelber, S., EAguilar J.L., Lewis K.L. and A.J. Ratner, Functional and phylogenetic characterization of vaginolysin the human-specific cytolysin from Gardnerella vaginalis. J Bacteriol. 190: 3896–903 (2008)

Gravett, M.G., Hummel, D. and D.A. Eschenbach, Preterm labor associated with sub clinical Amniotic fluid. Infection and with bacterial vaginosis. Obstet. Gyneco. l67: 229-237 (1986).

Jousimies-Somer, H.R., Summanen, P., Citron, D. M., Baron, E., Wexler, H.M. and S.M. Finegold, Wadsworth-KTL Anaerobic Bacteriology Manual. Sixth Edition. Star Publishing Co. Belmont, CA 94002 (2002).

Karatan, E. and P. Watnick, Signals, regulatory networks and material that build and break bacterial biofilm. Microbial snd Molecular Biology 73(2): 310-347 (2009).

Maraffini, L.A., Dedent A.C. and O. Scheewind, Sortase and the art of anchoring proteins to the envelopes of Gram–positive bacteria. Microbiol. Mol. Biol. Rev. 70: 192-221 (2006).

Marrazo, J.M., Antonio, M., Agnew, K. and S.L. Hiller, Distribution of genital Lactobacillus strains shared by female sex partners. J. Infect. Dis. 199(5):680-683 (2009).

Patterson, J.L., Stull-Lane, A., Girerd, P.H. and K. K Jefferson, Analysis of adherence, biofilm formation and cytotoxicity suggests a greater virulence potential of *Gardnerella vaginalis* relative to other bacterial-vaginosis-associated anaerobes. Microbiology 156:392–399 (2010).

Piot, P., Van Duck, E., Peeters, M., Hale, J., Totten, P.A. and K.K. Holmes, Biotypes of *Gardnerella vaginalis*. J. Clin. Microbiol. 20: 677–679 (1984).

Teenus Parame, Jayaprakash, John J. Schellenberg and Janet E. Hill, Resolution and Characterization of Distinct Subgroups of Gardnerella vaginalis in the cpn60-Based Vaginal Microbiota Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan. (2015).

Tenke, P., Köves, B., Károly, N., Hultgren, S.J., Mendling, W., Wullt, B., Grabe, M., Wagenlehner, F.M., Cek, M., Pickard, R., Botto, H., Naber, K.G. and T.E. Johansen, Update on biofilm infections in the urinary tract. World J. Urol. 30: 51–57 (2012)