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MOLECULAR DETECTION OF ANTIBIOTICS PROFILE OF *GARDENERELLA VAGINALIS* WHICH ISOLATED FROM PRETERM LABOR

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Abstract

In this study, one hundred fifty samples were collected from patients with preterm labor (PTL), who have been attending to Babylon Maternity and pediatric hospital and Al-Hilla Teaching Hospital, at the period from February to October 2016. Two swabs were collected one for culturing and the other for direct extraction for isolation *Gardnerella vaginalis*. Out of the 150 samples only 6(4%) on culture and 30 (20%) on molecular level isolated from preterm labor caused by Bacterial vaginosis, Urinary tract infection and aborted women. The results shown that only 6 isolates belong to *G. vaginalis* confirmed by using Vitek 2 system and molecular detection by specific primers. Preterm birth is one of the most common causes of neonatal morbidity and mortality. Associated with sub sequent preterm labor in up to 40% of cases as shown in our results. Three Antibiotics were used at a molecular level (Tet b) were varied between resistance and sensitive to this gene (10) sample are resist,(RdxA) all sample are sensitive to its gene at percentage (100%), whereas (erna) gene were resist in all samples (100%).

Keywords : *Gardnerella vaginalis*, antibiotics pattern, Cpn60, Erna, Tetb, RdxA.

Introduction

Preterm labor (PTL) occurs before 37 completed weeks of gestation leading to preterm birth (PTB). Resulting in neonatal deaths and different forms of neonatal morbidities (Brotman, 2011). Several risk factors have been identified related to the causes of PTB in most cases because of, there is no specific effect (Bugs) have been established. Infection is asymptomatic, underestimation of their importance may have been occurred. However, no attention payed on these infection from the researcher, so they examined 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 related to the family Bifidobacteriaceae in phylum Actinobacteria (Gravett *et al*., 1986). The first named *Haemophilus vaginalis* by pioneers, however, this bacteria was later alluded to as *Corynebacterium vaginale* and systematically relegated as *G. vaginalis* (Forbes *et al*., 2007) .

Additionally, it is a Gram negative cell divider because of its amino corrosive creation, overlaid structure and low peptidoglycan content (Catlin 1992; Piot *et al*., 1980) it is Glucose, maltose and starch fermenter without gas, unable to esculin hydrolysis, non-nitrate decrease , however, ready to develop in high osmosity arrangement and produce acetic acid.

Materials and Methods

Sample Collection

The total number of samples were collected (150) 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:

kit (Genaid U.S.A.) was used in DNA extraction from bacterial isolate

Detection of specific gene markers by PCR: The Cpn60 primer was used to amplify chaperon protein a detection primer listed in Table (1).

Table 1 : Detection primer sequences with their amplicon size Base pair (bp) and condition

Genes	Primer sequence (5'-3')	Size (bp)	PCR condition	Reference
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 Optimise Protocol Writer online

Detection of *Gardnerella vaginalis* Antibiotics resistance by PCR

Nucleic acid (DNA) that extracted from bacterial cells, was used as a template in specific PCR for the detection of antibiotics resistance genes listed in Table (2). A single reaction mixture contained 2.5µl of upstream primer, 2.5µl of downstream primer, 5µl of extracted DNA, 12.5µl of master mix and 2.5µl of nuclease free water. The resulting PCR products were run in 1.5% agarose gel.

Table 2 : Antibiotics resistance primers sequences with their amplicon size Base pair (bp) and their condition.

Genes	Primer sequence (5'-3')	Size(bp)	PCR condition	Reference
Erna	F- 5'AACACCCTGAACCCAAGGGACG 3' R-5'CTTCACATCCGGATTCGCTCGA3'	405	94ºC 10min 1x 94ºC 2min 55ºC 1min 40x 72ºC 1min 72ºC 10min 1x	3
Rdx A	F-5' GCAGGAGCATCAGATAGTTCT 3' R-5' GGGATTTTATTGTATGCTACAA 3'	169	94ºC 10min 1x 94ºC 1min 62ºC 1min 35x 72ºC 1min 72ºC 10min 1x	9
Tet b	F-5AAAACTTATTATATTATAGTG3' R-5' TGGAGTATCAATAATATTCAC3'	315	94ºC 10min 1x	7

Primer and PCR conditions were used to detect antibiotics resistance gene of *G. vaginalis* are present in table (2). 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 detect by gel electrophoresis on 1.5% agarose gels for 40 min at 70 V.

Results

Isolation of *Gardnerella vaginalis*:

A total of 150 swabs samples were obtained from patients diagnosed as preterm labor by the physician who were admitted to Babylon Maternity and Pediatric Hospital and Al-Hillah Teaching Hospital, at the period from February to October 2016. Among 150 clinical samples, only 35 positive results on molecular level depending on 16SrRNA and 30 on Cpn60, and 6 showed positive results on culture and Vitek 2 system, as shown in Table (3-1).

This study focused on *G. vaginalis* because of the strong association of this organism with PT.

Table 3 : Number and Percentage of Bacteria isolated from swabs Samples of Patients with preterm labor.

No. of swab samples	On culture		On molecular			
	Positive results	Negative results	16SrRNA		Cpn60	
			Positive results	Negative results	Positive result	Negative result
150 samples	6(4%)	144	35(23.3%)	115	30(20%)	120

Molecular Antibiotics Profile:

G. vaginalis is typically treated with metronidazole and clindamycin together for prevent re-infection but only limited data are available with respect its resistance (Tomusiak *et al*., 2011). In recent study Tetracycline are variable between resistance and sensitive the percentage of resistance was 10 (66.7%) were gave 315 bp. when compared with allelic ladder.

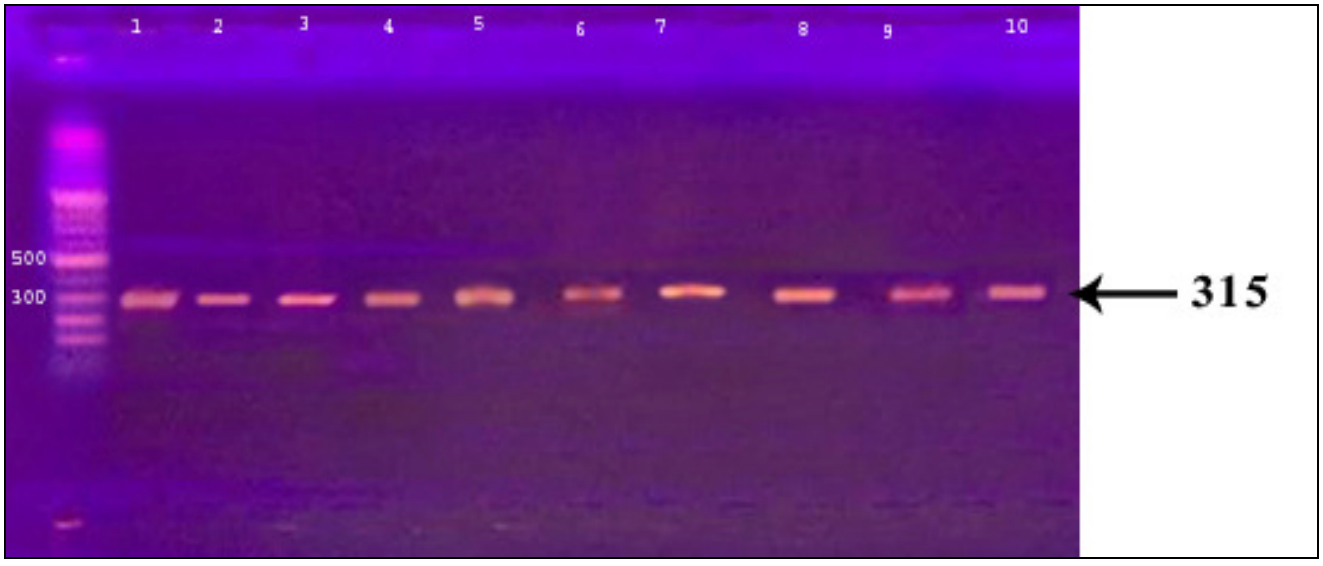

Fig. 3-20 :1% Agarose gel electrophoresis at 70 volt for 50 min for *Tet* B PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bp ladder; lane (1-10) were positive for this gene, the size of product is 315bp.

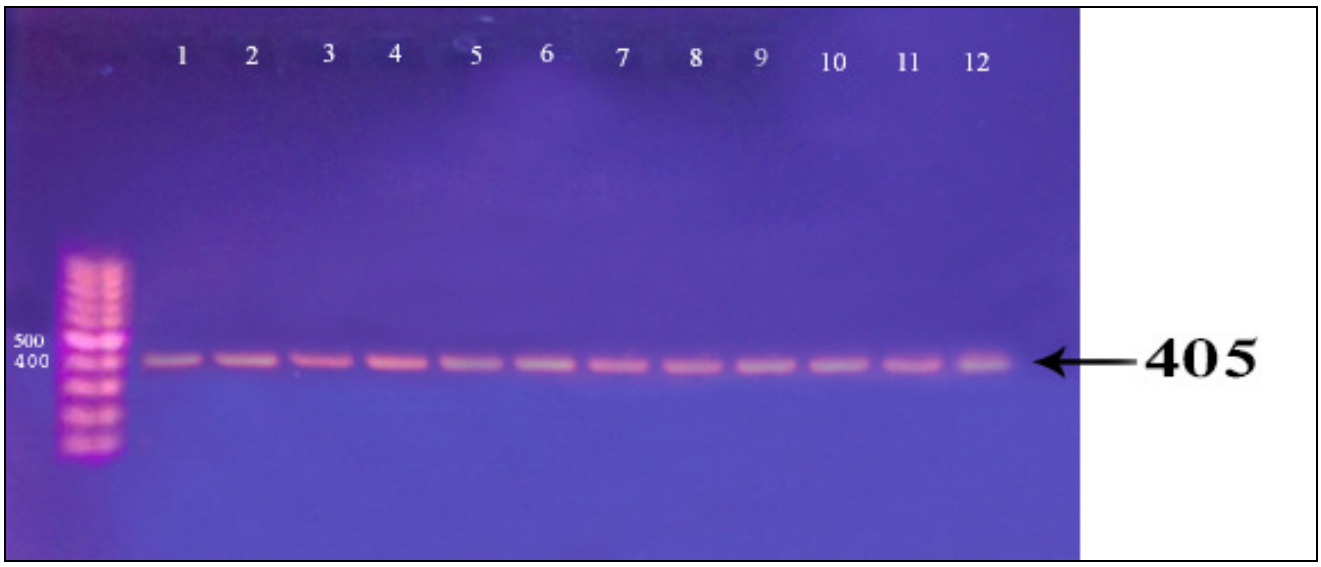

Fig. 3-21 :1% Agarose gel electrophoresis at 70 volt for 50 min for *erna* PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bpladder; lane (1-12) were positive for this gene, the size of product is 405 bp.

Fig. 3-22 :1% Agarose gel electrophoresis at 70 volt for 50 min for *Rdx*a PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bpladder; lane (1-12) were negative for this gene, the size of product is196 bp.

Discussion

The results of this study was in agreement with results obtained by (AlJummaly and Abdulla, 2008) who found that the prevalence of isolated was (4.4) diagnosed as Gram curved bacteria in Al –Mosul city, However Humadi (2010) only diagnosis the presence of clue cell as a diagnostic feature to *G. vaginalis* at percentage (19.6%) in Baghdad city while , the results of this study was in disagreement with the results obtained by Al-Alwani (2008) in Al-Ramadi city who found the prevalence was (27%) from the culture Al-Joboree, (1990) were found that *G. vaginalis* is the prevailing agent in preterm labour the percentage was (1.9%) in comparison with control in Al-Mosoul city, while the results of Al-Sultany (2012) was disagree with results obtained in this study ,She was found the frequency of *G. vaginalis* was (27.5%) percentage from the culture in Babylon city , Moreover Al-Dhalmi (2013) were found the isolated *G. vaginalis* at percentage (10%) in Al-Kufa city, whereas Abed Jabuk (2014) were found that there was no *G. vaginalis* in Babylon city on BV patient's. this variation may be due to the Geographic distribution and to type of sample and the Antibiotic's uptake. This results were in agreement with these results obtained by (Moulds and Jeyasingham, 2010). However, in agreement with those results obtained by which found all isolates were resist to Tetracycline due to the Antibiotics are unable to cross the cell wall.

Tetracycline antibiotics are protein synthesis inhibitors, inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. They do so mainly by binding to the 30S ribosomal subunit in the mRNA translation complex the putative mechanism for tetracycline resistance was the presence of the *tetM* gene which was carried on bacterial chromosome ,which was found in tetracycline-resistant strains of *G. vaginalis* this result were similar to those obtained by Harwich et al.(2010).

Resistance to tetracycline was due to the drug permeability cannot reach to targeted sites because of the cell wall play as a limited factor prevent the antibiotics entry to the cells as described by Al-Jobouri (1991).

The second antibiotics was clindamycin which is completely resistance to it (100%) gave 405 bp compared with allelic ladder This result was close to those result obtained by (Herfindal and Gourly, 1996). which found that all isolates are completely resist to this Antibiotics

Clindamycin has a primarily bacteriostatic effect. It is a bacterial protein synthesis inhibitor by inhibiting ribosomal translocation, in a similar way to macrolides. So binding to the 50S rRNA of the large bacterial ribosome subunit metronidazole has been found in some *G. vaginalis* strains (Löfmark *et al*., 2010).

The last choice is Metronidazole which is completely sensitive there was no band present as shown in figure (3-15).

Metronidazole has also been used in women to prevent preterm birth associated with bacterial vaginosis, amongst other risk factors including the presence of cervicovaginal fetal fibronectin (fFN). Metronidazole was ineffective in preventing preterm delivery in high-risk pregnant women (selected by history and a positive fFN test) and, conversely, the incidence of preterm delivery was found to be higher in women treated with metronidazole. The study was agreement with results obtained by (Fredricks *et al*., 2009; Bradshaw *et al*., 2006), the repressed rate of activation of drug inside the cell through its reduction, Increased activity of DNA repair systems, Increased activity of enzymes that consume oxygen (i.e. catalase, peroxidase, and superoxide reductase), Accelerated clearance of the drug from the cell by active efflux.

The well-characterized mechanism of resistance to metronidazole is the inactivation or deletion of genes with nitro reductase activity such as explained by (Dhand and Snydman, 2009).

Conclusion

Preterm birth is one of the most common causes of neonatal morbidity and mortality. Associated with sub sequent preterm labor in up to 40% of cases as shown in our results. Three Antibiotics were used at a molecular level (Tet b) were varied between resistance and sensitive to this gene (10) sample are resist, RdxA) all sample are sensitive to its gene at percentage (100%), whereas (erna)gene were resist in all samples (100%).

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