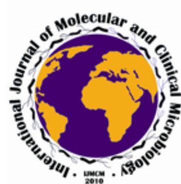

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Comparison of Culture and Molecular Techniques for Identifying *Gardenerlla vaginalis* in Urinary Tract Infection

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ABSTRACT

Urinary tract infection is the most prevalent infection in the human societies. This study aims to identify *G. vaginalis* in the urinary samples of the patients suffered from UTI by culture and PCR techniques. 200 patients with urinary tract infection signs have entered in this study. urine sample was cultured in the blood agar medium, a small quantity of the sample used for the purpose of molecular technique. In order to analyze the data, SPSS software and chi square (x^2) test has been used. Of 200 studied patients, *G. vaginalis* was isolated and identified in 6 (3%) and 29 (14.5%) patients with the culture and PCR techniques, respectively. The sequencing of the samples confirmed phenotyping identification. On the basis of the results obtained from both two techniques, it can be expressed that PCR technique is more appropriate to identify *G. vaginalis*. The achieved results show that *G. vaginalis* is regarded as one the agents causing the occurrence of urine infection. Yet, in many medical diagnosis laboratories, the bacterium is undetectable due to hardly growth. Also, considering the conducted techniques, PCR method is of a higher accuracy and speed.

1. Introduction

Urinary Tract Infection (UTI) is accumulation of the bacteria around the urinary tracts and, then, their climb to other members of the urinary system (Mims, 2004). Urinary tract infections are the most common outpatient infections, but predicting the probability of UTI through symptoms and test results can be complex (Christine et al., 2018). UTI consists of infection of kidneys, bladder and urinary tracts. This infection is the most prevalent infection in all age groups which it's lack of diagnosis and treatment can cause acute complications, including disorders related to urinary system, blood pressure, uremia and, in the pregnant women, lead to premature delivery and, even, abortion. Increase of risk of urinary infection in the infants, Pregnant women, patients with injured spinal cord, diabetic individuals and sclerosis, following the usage of catheters, has been reported. The rates of infection in women is higher than men, and ratio of affliction of the women with this disease has been reported up to three times as much as the men (Mims, 2004; Nicole et al., 2019). This infection is especially important in children, as it may lead to chronic infection, fever, nonspecific symptoms or childhood impairment (Baily, 1987; Christine et al., 2018).

The studies carried out in various societies show that most of the etiologic factors of the urinary infection are intestinal bacteria belonging to Entrobacteriaceae family among

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which *Escherichia coli* is more prevalent. In addition, many other bacteria such as gram positive species and anaerobic bacteria, viruses and fungi are capable of playing a role among them (Freedman, 2005). Out of the bacteria being causative factor of genital infection and infertility, it can be referred to *Chlamydia*, *Mycoplasma*, *Ureaplasma*, *Streptococcus, Gardenerlla* and etc (Solimani et al., 2007; Omar et al., 2017; Migle et al., 2018).

Gardnerella is a small (1.0–1.5 μm in diameter), non-spore-forming, non-motile, facultative anaerobic, and Gram-variable-staining bacteria. They are variable in the 8 to 12 hours and are positive for the appropriate medium, but they become gram negative soon; furthermore, they lack capsules, spore and flagellum. They need very rich environments and do not need x and v factors. An appropriate environment for them is a blood-agar containing 10% of the sheep's blood. After 48 hours of incubation in anaerobic jar and a temperature of 37°C, transparent, colorless and similar to the head clip colonies are appeared (Zinsser & Jaklik, 2008).

It has been proven that *G. vaginalis* is a dominant microorganism in 95% of women with clinical signs of vaginosis even though it is isolated from vaginal discharge of 40 to 50% of healthy women (Numanović et al., 2008). In human, it generates vaginitis, non- specific-urethritis, infection of urinary system, fever following the delivery, infection of neonates and abortion. Secretion in vaginitis is not high and give off a smell allocating to the rotten fish. Secretion is grey, viscous and elastic, and its pH is in a range from 5 to 5.5 approximately.

Usually, conditions of culturing the anaerobic bacteria, as *Gardenerlla* are not provided in the medical diagnosis laboratories and required a special medium and condition. In this research, prevalence of the *G. vaginalis* is introduced by the culture and PCR techniques.

2. Materials and Methods

Research methodology was descriptive–analytical type based on questionnaire which information, including age, gender, marital status, job, residence place, education level, history of prior infection, background disease, precedence of abortion and antibiotic consumption in the recent three months were stipulated. Also, the total urine analysis factors, following the performance of test, were received (taken) and analyzed, and urine culture and PCR tests was conducted on the samples.

In this investigation, by prescription of the curer physician and possession of the urinary infection signs, 200 patients referred to Farabi laboratory of Tonekabon in north of Iran were placed under analysis test and urine culture by standard conditions. Also, a little amount of each sample was used for the purpose of molecular technique. collection of the urine samples was in such a way that, for women, genital organ was washed by water firstly; then, the first sample of urine was poured away and collected from middle of urine in the dish allocating to culture of the sterile urine. For men, the first part of urine was poured a way firstly and middle part of urine collected in the dish allocating to culture of the urine.

2.1. Culture and identification

Following the collection, the samples were transferred to the microbiology section of laboratory. After sampling by calibrated loop, samples were inoculated in to nutrient broth medium along the sheep blood and, then, added onto the sterile paraffin for anaerobic conditions and incubated in the 37°c for 24 hours. After incubation time, a little amount of the sample from nutrient broth transport to in the blood agar, and the culture medium was placed into anaerobic jar along with a gas pack A (Merck-Germany); and incubated in 37°c for 48 hours (Collee et al., 1996). After passage of 48 hours from time of incubation, colonies of *G. vaginalis* were tested for final confirmation in terms of gram stain, catalase, oxidase, hydrolysis of starch, urease, Indole and metyl red (MR) tests.

2.2. Molecular identification

Considering that PCR method is specific and fast completely for the purpose of isolation of factor, the taken samples, in this study, were not enriched, and they were evaluated by PCR method directly. After taking of sediment from the principal samples, extraction of DNA was implemented according to the instructions of the manufacturing company (Qiagene, Lot No: 11872534, Cat No: 51306). In order to perform PCR, specific primer made by TAG Company (Copenhagen-Denmark) was used (Table 1).

Table 1. The specific primers were used

Gene name	Primers	5'→3'	length	References
16S rRNA	F	CTCTTGGAAACGGGTGGTAA	280 bp	Henriques et al., 2012
	R	TTGCTCCCTCAAAAGCGGTT		

Each reaction was performed in a total volume of 25 µl, which contained 12.5 µl of master mix (Amplicon USA), 6 µl of sterile distilled water, 1 µl of 10 pmol of forward and reverse primers (table 1), and 4.5 µl of the extracted DNA were transferred to the sterile 0.2 micro tube (Henriques et al., 2012). The negative control tube contained the same PCR reagents as above but had 4.5 µl of water substituted for the DNA template. Then, the samples were spin and vortexed, respectively.

In order to begin process of polymerization process, Thermal cycle apparatus was regulated in 95°c for 5 minutes aiming at initial denaturation. Subsequently, 40 cycles of PCR was conducted in form of 94°c for 30 seconds, 60°c for 30 seconds and 72°c for 60 seconds, and final extension was enforced in 72°c for 10 minutes.

An aliquot of all PCR products was run on a 1.5% (w/v) agarose gels with a 100 bp DNA ladder (Fermentas-Russia) and electrophoresed at 75 V for 40 min. The bands were visualized using ethidium bromide staining and photographed after UV treatment by a trans illuminator (UV doc, England).

Positive cases of *G. vaginalis* were sent to the Macrogene company in the southern Korea for the purpose of sequence determination. Using SPSS software, the data collected in two descriptive and analytical sections have been analyzed by use of chi-square (x^2) test.

3. Results

Out of 200 samples of the cultured urine, 6 strains (3%) were isolated and identified considering phenotyping tests. Also, *G. vaginalis* was identified in 29 patients (14.5%) with the PCR technique. Amplification of 16SrRNA gene of *G. vaginalis* has been observed in fragment 280 bp (figure 1). Alignment of sequences and it's comparison in the gene bank showed that the obtained isolates, in terms of sequence of 16SrRNA gene are *G. vaginalis.*

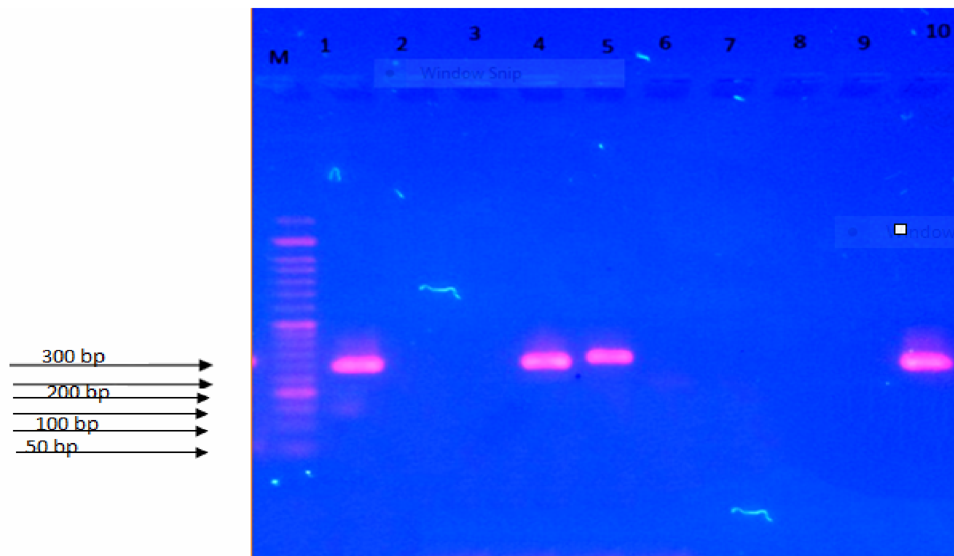

Figure1. amplification of 16SrRNA gene of *G. vaginalis* has been observed in fragment 280 bp.: M size marker 50 bp (Fermentase-Germany), No 10 positive control of the *G. vaginalis*, No 2 of negative control, No 4, 5, 10, positive samples of *G. vaginalis* and the No 3, 6, 7, 8 and 9 of negative samples.

Considering data of table 2 and p-value which is less than 0.05, a significant positive relationship has been observed between quantity WBC and bacteria in sample in the culture technique. Also there exists a significant positive relationship between of gender and quantity of epithelial in the PCR technique too.

Considering data of table 3 and chi-square test and significance level of less than 0.05 with 95% confidence it can be said both culture and PCR techniques to detect *G. vaginalis* is a good way. Since the percentage of patients identified using PCR techniques is more than culture. So can qualify concluded that PCR technique to identify *G. vaginalis* is a more appropriate method.

Table 2. A study of chi-square (x^2) test among variables of the research

Index demographic	Inconstant	Number	Culture +		P Value	PCR+		P Value
			Frequency	Valid percent		Frequency	Valid percent	
Gender	Men	51	1	0.5	0.151	2	1	0.02
	Women	149	5	2.5		27	13.5	
Marital status	Single	45	2	1	0.519	2	1	0.20
	Married	155	4	2		27	13.5	
WBC	0-1	42	0	0	.001	2	1	.190
	2-3	70	0	0		8	4	
	4-5	28	0	0		5	2.5	
	6-7	19	1	0.5		4	2	
	8-9	12	1	0.5		2	1	
	>10	15	3	1.5		4	2	
	Many	14	1	0.5		4	2	
Epithelial cell	0-1	41	0	0	.071	3	1.5	.003
	2-3	79	2	1		10	5	
	4-5	43	2	1		8	4	
	6-7	14	0	0		0	0	
	8-9	11	0	0		3	1.5	
	>10	8	1	0.5		4	2	
	Many	4	1	0.5		1	0.5	
Bacteria in urine	Negative	49	0	0	.002	2	1	.033
	Few	90	0	0		13	6.5	
	Moderate	48	5	2.5		11	5.5	
	Many	13	1	0.5		3	1.5	

Table 3: A study of chi-square (x^2) test a mong variables of the research

	Positive		Negative		Total		P value
	Nomber	%	Nomber	%	Nomber	%	
Culture	6	3	194	97	200	100	.022
PCR	29	14.5	171	85.5	200	100	.034

4. Discussion

UTI is the most prevalent infection in the human societies (Efstathion et al., 2003).

More than 150 million individuals are infected with this group of diseases throughout the world annually which lack of diagnosis and treatment of them (can cause intensive complications, including urinary system disorders, blood pressure, pyelonephritis, septicemia, uremia and, in the pregnant women, lead to premature delivery and even, abortion) (Batra et al., 2000; Sharifi yazdi & Soltan dalal, 2011).

In this research carried out on the 200 patients with UTI, presence of *G. vaginalis* was studied with the aid of culture and PCR technique. Of 200 studied patients, *G. vaginalis* was isolated and identified in 6 patients (3%) within their urinary infection assisted by the culture technique.

The studies conducted by the researchers in various parts of the world reported outbreak of this bacterium, with culture technique, less than 1% to 25% for example; woolefty et al., 1996; Lam et al., 1998; Brantonin, 2005; Weels et al., 2012 and Kock, 2010 reported prevalence of this bacterium to be 2.3%, 25%, 0.89%, 13.5%, 4.18% and 5%, respectively.

In addition to culture technique, molecular technique was used in this research in order to identify *G. vaginalis*. The obtained result showed that this bacterium was identified in 29 patients with frequency of 14.5% the obtained results suggest higher PCR positive cases compared to the culture technique. 6 patients who had become positive in the culture technique become positive in the PCR technique in terms of *G. vaginalis* as well.

The studies carried out regarding comparison of culture and PCR in identification of *G. vaginalis* in patients with the bacterial vaginosis (Vaginitis) Suggest existence of a difference between two techniques. For example, gergvoa et al (2013) reported that the prevalence of this bacterium was 26.29% in the culture technique and 58.22 in PCR technique (Gergova et al., 2013), Alla et al., (2001) reported its prevalence as 26.4% in culture technique and 44% in PCR technique (Alla et al., 2001), Rita et al., (2004) as 4.1% in culture technique and 26.16% in PCR technique (Rita et al., 2004), Gamale gad et al., (2014) as 32% in culture technique and 45% in PCR technique reported (Gamale gad et al., 2014). The results are in line with the results of other researchers.

Conclusion

Totally, in the patients with UTI, *G. vaginalis* was identified by phenotyping and molecular method. On the basis of the obtained results, a noticeable percentage of the patients have been infected with this bacterium, and considering hazardous consequences of this infection, on time diagnosis of this bacterium in the patents lacking clinical signs seems to be necessary.

Refereces

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