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ISOLATION AND MOLECULAR DIAGNOSIS OF SOME TYPES OF PATHOGENS ASSOCIATED WITH GENITAL SECRETIONS IN MARRIED WOMEN

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Abstract

We conducted a study aimed at determining the prevalence of Microorganism vaginosis. Microorganism-associated with genital secretions in married women in The Adwaniyah city, Iraq. We collected 60 samples from women with genital secretions in married women. Vaginal swabs were collected BV-associated bacteria (*Gardnerella vaginalis*, anaerobic bacteria, *staphylococcus aurus*, *E. coli*, *N. gonorrhoeae* and *Candida albicans*) cultures; and swabs were collected for culture and *Staphylococcus aurus* PCR. The results of the present study showed that the highest percentage of *Staph. aurus* bacteria was (13.3%) of (8) isolates, followed by *G. vaginalis* with (10%) of (6) isolates, *E. coli* and *N. gonorrhoeas* with (6.67%) each of (4) isolates. The species then remained at (5%) of the (3) isolates. The results of the present study showed that after fungal samples, the highest percentage of *Candida albicans* was recorded, with (31.67%) of (19) samples were isolated and another species (26.67%) of the (16) isolates were fungal isolates.

Key words : Genital secretions, Fungal samples, Vaginal swabs, Anaerobic bacteria.

Introduction

Bacteria, parasites, fungi and viruses form the group of organisms that cause genital infection in women in general (Navratil, 2002; Nandan *et al*., 2001; Coach *et al*., 2001). Vaginitis is one of the most common genital infections. Studies show that 75% of women in Germany and 66% in the United States have at least one vaginal infection (Wenisch *et al*., 1993; McCormack *et al*., 1993). The incidence of this infection is linked to factors related to the reproductive system of the woman and her immune defenses with factors related to the pathogen itself, as well as to infectious factors that help the infection. Recall the most important factors that predispose the injury to the sexual partner (Hellberg *et al*., 1994). And anatomical factors and finally the presence of foreign bodies within the reproductive methods (Hodoglugil *et al*., 2000). Also play life pressure (Harville *et al*., 2005) and the incidence of some diseases a major role in the incidence of infections of genital infection such as diabetes or immunosuppression (Minkoff *et al*., 1999). The treatment of some drugs also plays an important role in the incidence of infection such as the treatment of antibiotics and hormonal drugs (Opaneye, 1999; Kurowski *et al*., 2000). Due to the importance of infection, vaginal infection and its health and social impact and the high cost of treatment and the absence of a study on the different types of microorganisms responsible for genital infection and the impact of different factors on infection in women 12. So we did this study.

Materials and Methods

Samples collection

Our study included 60 married women aged between 19-45 years. Women were randomly selected from women who reviewed Childbirth Hospital in Qadisiyah Governorate.

Diagnosis of samples

1. Diagnosis of fungi

A. Growth was measured on the soubroudagar, incubated at 37°C for 3-5 days.

B. A gramstain was applied to the larvae from the

center of the plant to observe the yeast shape of the fungus, which is characterized by its oval shape.

2. Diagnosis of bacteria

A. The bacteria were isolated by transplantation of swabs taken on blood agar and incubated at 37°C for 24 hours.

B. Make a gramstainfor swabs taken from the agar to observe the macrophage.

Bacterial genomic DNA extraction

Bacterial genomic DNA was extracted from *Staphylococcus* isolates by using (Presto™ Mini gDNA Bacteria Kit Geneaid, USA). One ml of overnight bacterial growth was added to 1.5 ml of BHI broth in microcentrifuge tubes and centrifuged at 10000 rpm for 1 minute. Then after the supernatant was discarded and bacterial cells pellets were used in genomic DNA extraction and the extraction was done according to company instruction. After that, the extracted gDNA was checked by Nanodrop spectrophotometer, then store at -20ºC until performing PCR assay.

Polymerase chain reaction (PCR) (*Staphylococcus*)

PCR was performed for detection of staphylococcus bacterium based on amplification of 16SrRNA gene. The 16SrRNA gene primes were designed in this study using NCBI-GenBank deposited sequence Staphylococcus sp. partial 16S rRNA gene, isolate BA-141 (GenBank: HF947327.1) by using primer3 plus design online. The primers were used to amplify a 410bp using 16S rRNA-F primer (ATGGATCCGCGCCGTATTAG) and 16S rRNA-R primer (AATGAC CCTCCACGGTTGAG) were provided by (Macrogen company, Korea). Then PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 2µl of purified genomic DNA and 1µl of 10pmole of forward primer and 1µl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (T100 Thermal cycler. Biorad/ USA) by set up the following thermocycler conditions; initial denaturation temperature of 95°C for 5 min; followed by 30 cycles at denaturation 95°C for 30 s, annealing 60°C for 30 s, and extension 72°C for 1min and then final extension at 72°C for 10 min. The PCR products were examined by electrophoresis in a 1% agarose gel, stained with ethidium bromide and visualized under UV transilluminator.

Results

The results of the present study showed that the highest percentage of *Staph. aurus* bacteria was (13.3%) of (8) isolates, followed by *G. vaginalis* with (10%) of (6) isolates, *E. coli* and *N. gonorrhoeas* with (6.67%) each of (4) isolates The species then remained at (5%) of the (3) isolates. The results of the present study showed that after fungal samples, the highest percentage of *Candida albicans* was recorded, with (31.67%) of (19) samples were isolated and another species (26.67%) of the (16) isolates were fungal isolates as shown in the table 1.

Molecular profile

In the present study, speciûcally active members of the married women microbiota were identified by comparing rDNA.

Detection by PCR

Preliminary results show that of bacteria are found in the married women samples are *Staphylococcus* which have been diagnosed according to the standard methods as well as PCR technique. The isolates were confirmed through the interaction of polymerization series and using special primers in each type of germs as well as through the use of electrophoresis method for each sample.

Detection of *staphylococcus* by PCR

PCR was performed for detection *Staphylococcus bacterium* based on amplification of 16SrRNA gene using 16S rRNA-F primer (ATGGATCCGCGCCGTATTAG) and 16S rRNA-R primer (AATGAC CCTCCACGGTTGAG). As detected by agarose gel electrophoresis image that shown the PCR product of 16S rRNA gene in Staphylococcus sp., where M: Marker (1500-100bp), lane (1-5 and 7-9) positive PCR amplification at (410bp) PCR product size (fig. 1).

Discussion

The main objective of this study was to determine the prevalence of microorganism from discharges from married women socio-economic factors were confounding these associations (Alkarim *et al*., ...). Lifestyle practices such as vaginal douching have also been associated with an increased prevalence of microorganism (Alkarim *et al*., ...; Holzman *et al*., 2001; Martino *et al*., 2002), although, the direction of causalities again uncertain, since

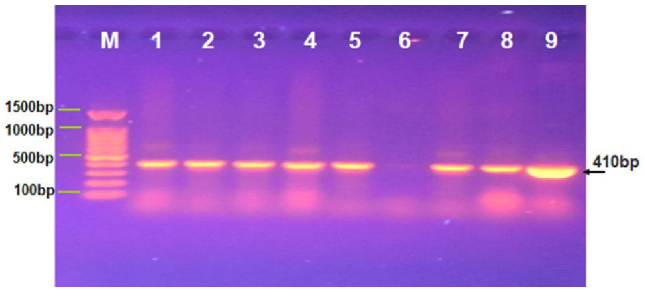

Fig. 1 : Agarose gel electrophoresis image shows the PCR product of 16S rRNA gene in *Staphylococcus* sp. isolates. Where, M: Marker (1500-100bp), lane (1-5 and 7-9) positive PCR amplification at (410bp) PCR product size.

Table 1 : Represents the numbers and percentages of isolated microorganisms.

Pathogens	Micro-organism	No. samples	Percentage (%)
Bacteria	*Staph. aurus*	8	13.3
	G. vaginalis	6	10
	E. coli	4	6.67
	N. gonorrhoeas	4	6.67
	Another species	3	5
Total		25	41.67
Fungi	*Candida albicans*	19	31.67
	Another species	16	26.67
Total		35	58.33
Total		60	100

most studies have been of cross-sectional nature and many potentially confounding factors such as educational, socio-economical and behavioral factors have not always been entirely controlled for. We did not find any association between BV(bacteria) or vaginal micro-organisms and vaginal hygiene practices such as douching before or after sex, the nature of douching compounds used, the source of the water, or with menstrual sanitary protection. This finding perhaps owes to the fact that a very large proportion of women reported these practices, thus any relatively small association with BV would be hard to find with our sample size. In our study, a large proportion of The results of the present study showed that the highest percentage of *Staph. aurus* bacteria was (13.3%) of (8) isolates, followed by *G. vaginalis* with (10%) of (6) isolates, *E. coli* and *N. gonorrhoeas* with (6.67%) each of (4) isolates The species then remained at (5%) of the (3) isolates. The results of the present study showed that after fungal samples, As for fungi, the results of our study showed that the fungus *Candida albicans* was the largest share of the insulation as shown in table 1 the results and this is consistent with many previous studies (Fonck *et al*., 2001). The highest percentage of *Candida albicans* was recorded, with (31.67%) of (19) samples were isolated and another species (26.67%) of the (16) isolates were fungal isolates, his is different from the findings observed in a study conducted by Hillier *et al*. (2001). Most of the staphylococci isolates were coagulase-negative, which are perceived to be normal commensal organisms.

Conclusion

In this population, BV prevalence was higher than in corresponding populations in Low culture and health. Further studies on the public health significance of microorganism (Bacteria & Fungi) in this kind of setting are needed to determine future strategies for intervention.

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