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Rapid Detection of Microbial Profile among Women with Vaginitis in Hilla City, Iraq

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ABSTRACT

Bacterial vaginosis is a complex, polymicrobial infection ascribed to an overgrowth of strict or facultative anaerobic bacteria or unconventionally cultivate bacteria like *Mycoplasma hominis, Ureaplasma urealyticum.* It is the most common cause of vaginal discharge among women in reproductive age. The malodorous vaginal discharge resulted from production of amines like putrescine, cadaverine and trimethylamine due to bacterial deamination reactions. Forty vaginal swabs were collected from a marriage women with age (mean ± SD= 31.8±7.57) during the period from October 2014 to January 2015. All patients visit gynecology clinic in Al-Mahaweel hospital. All swabs immersed in physiological solution and then loaded to AF genital system plate. The results revealed that 22(55%) attributed to polymicrobial bacterial vaginosis, 1 (2.5%) for trichomoniasis and 2(5%) for candidiasis as a single agent vaginitis. Mix infection, double agent vaginitis were 13(32.5%). Low percent of multiple agent vaginosis were recorded 2 (5%) which include bacterial vaginosis, trichomoniasis and candidiasis at the same swab. *Staphylococcus aureus* compile 32(80%), *Enterococcus faecalis* 26(65%), *Streptococcus agalactiae* 12(30%), *Mycoplasma hominis* 10(25%), *Ureaplasma urealyticum*7(17.5%), *Gardnerella vaginalis* 10(25%), *Proteus spp./Providencia spp.* 8(20%), *E. coli* 6(15%) while *Neisseria gonorrhoeae* and *Psudomonas* spp. not detected while 3(12.5%) for *T. vaginalis* and 15(37.5%) for *Candida* spp. Antibiotic susceptibility results for *Mycoplasma hominis* and *Ureaplasma urealyticum* show total sensitivity to clindamycin and pefloxacin while resisted to the other in different percentage (5%-15%). This study conclude that, bacterial vaginosis compile high percentage among women with vaginal discharge. It can be present alone or along with candidiasis while rare cases record a combined infection of bacterial vaginosis along with trichomoniasis and both candidiasis and trichomoniasis may be as secondary infection that enhanced after bacterial vaginosis. Also conclude excellent response to treatment with tetracycline and new fluoroquinolones such as pefloxacin.

INTRODUCTION

Vaginitis is an inflammation of the vaginal lining. It can occur in females of any age accompanied with or without discharge (Carr *et al*., 1998). Vagina represent an ecological niche inhabited by numerous of aerobes and anaerobes coexist in a dynamic balance. It dynamically changed in structure and composition according to age, timing of menstrual cycle, infections, pregnancy, sexual status and personal hygiene (Lamont *et al*., 2011). Vaginitis includes a conditions that cause vaginal and occasionally vulvar symptoms, such as burning, irritation, itching, odor, and vaginal discharge. Infectious vaginitis includes the three vaginal infections bacterial vaginosis, trichomoniasis and candidiasis (Barry *et al*., 2011; Sobel, 2007). Vaginal discharge and bad odor can be found in about 30 percent of women and attributed mainly to bacterial vaginosis (Allsworth and Peipert, 2007). Vagina is normally in habited by *Lactobacillus* spp. The beneficial protective effects of lactobacilli resulted from their competition with undesirable bacteria to adhere to vagina lining, stimulation of mucosal immunity, production of lactic acid which leads to high acidic medium (pH = 3.8-4.2) of vagina and production of some bacteriocin and hydrogen peroxide as a

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defense mechanisms (Aroutcheva *et al*., 2001; Fredricks *et al*., 2005). The real explanation of vaginosis symptoms is troubling in the balance of normal flora of *Lactobacillus* spp. and replacing them with anaerobic bacteria which leads to decreasing in the levels of hydrogen peroxide and organic acids in the vagina. The main causes of bacterial vaginosis are vanished of the indigenous vaginal lactobacilli and over growth of the anaerobic bacteria of the vaginal mucosa on the other hand (Fredricks *et al*., 2005). Bacterial vaginosis is a complex, polymicrobial infection ascribed to an overgrowth of strict or facultative anaerobic bacteria such as *Gardnerella vaginalis, Prevotella* spp., *Mycoplasma hominis, Ureaplasma urealyticum Veillonella* spp and uropathogens like *Escherichia coli, Proteus* spp., *Klebsiella* spp., *Serratia* spp. (Lamont *et al*., 2011; Fredricks *et al*., 2005). Concern vulvovaginal candidiasis, about 75% of women will be involved at least once during life time (Sobel, 2003). The women at high risk to get candidiasis includes those with sexual activity, recent antibiotic use, pregnancy, and immunosuppression like diabetes (Ohmit *et al*., 2003; De Leon *et al*., 2002). The main causative agent is *Candida albicans* (other species can be also involved). The signs includes a thick cottage-cheese–like discharge with vaginal pruritus, pain, burning, erythema and edema (PHAC, 2010). Trichomoniasis is a sexually transmitted vaginal infection that is caused by a parasite *Trichomonas vaginalis*. Symptoms include a thin, frothy, yellow-green, foul-smelling vaginal discharge (Taylor-Robinson *et al*., 2006; Sutton *et al*., 2007)

As a multi-causes infection the gynecologist need to know the exact causative agent at least it is bacterial, yeast of parasite. The correct conventional diagnosis need to do vaginal swab culture for bacteria and yeast and microscopic examination for trichomoniasis to prescribe the correct medication. Unfortunately this procedure not performed by most of the laboratories due to their needs long time and costly. Moreover for bacterial vaginosis we need to know type of bacteria due to the treatment strategy for treatment of (CDC, 2006). The correct treatment depends on full correct diagnosis and theses are very important to prevent the post bacterial vaginosis complication like preterm birth (if the bacterial vaginosis occur during pregnancy), pelvic inflammatory disease (results from spread of infection to the uterus or fallopian tubes, chronic pelvic pain and tubal pregnancies and may leads to reproductive organs damage (OPA, 2012). The current aims to diagnosis and antibiotic susceptibility testing of vaginitis causative agent in single test well plate during 18-24 hours.

MATERIAL AND METHOD

Samples collection

Forty vaginal swabs were collected from a marriage women with age (mean ± SD= 31.8±7.57) during the period from October 2014 to January 2015. All patients visit gynecology clinic in Al-Mahaweel hospital and all of them have vaginal discharge and other symptoms like frequency, urgency, pruritus and itching. All swabs immersed in physiological solution and then loaded to AF genital system plate (Liofilchem/Italy).

Samples Inoculation

A.F. genital system (Liofilchem/Italy) is a 24-well system containing desiccated biochemical and antibiotic substrates for detection, presumptive identification and susceptibility test of microorganisms from urogenital specimens allows the detection, semi-quantitative count, presumptive identification and susceptibility test of *Mycoplasma hominis* and *Ureaplasma urealyticum*, the detection and presumptive identification of the microorganisms most frequently isolated from vaginal and urethral swabs and seminal fluid, such as: *Trichomonas vaginalis, Escherichia coli, Proteus* spp./*Providencia spp., Pseudomonas spp., Gardnerella vaginalis, Staphylococcus aureus, Enterococcus faecalis, Neisseria gonorrhoeae, Streptococcus agalactiae (Group B)* and *Candida sp.* The medium of the wells can be summarized as follow in table (1).

Table 1: Well content and results interpretation.

Well	Content	Results	
		+ve	-ve
1-GR+	Culture medium for growth of mycoplasmas Growth of mycoplasmas (102 < titer < 104 CFU/mL)	Red	Yellow
2-GR++	Culture medium for growth of mycoplasmas Growth of mycoplasmas (104 < titer < 105 CFU/mL)	Red	Yellow
3-GR+++	Culture medium for growth of mycoplasmas Growth of mycoplasmas (titer > 105 CFU/mL)	Red	Yellow
4-ADC	Culture medium with substrate for decarboxylation of arginine, Arginine Test: identifies *Mycoplasma hominis*	Red	Yellow
5-UR	Culture medium with substrate for hydrolysis of urea, Urea Test: identifies Ureaplasma urealyticum	Red	Yellow
6-TR/YE	Culture medium with substrate for isolation of Trichomonas vaginalis and *Candida* spp.		
7-TE	Culture medium containing Tetracycline 8µg/mL	Red (R)	Yellow (S)
8-PEF	Culture medium containing Pefloxacin 16 µg/mL	Red (R)	Yellow (S)
9-OFX	Culture medium containing Ofloxacin 4 µg/mL	Red (R)	Yellow (S)
10-DO	Culture medium containing Doxycycline 8µg/mL	Red (R)	Yellow (S)
11-E	Culture medium containing Erythromycin 16 µg/mL	Red (R)	Yellow (S)
12-CLA	Culture medium containing Claritromycin 16µg/mL	Red (R)	Yellow (S)
13-MN	Culture medium containing Minocycline 8 µg/mL	Red (R)	Yellow (S)
14-JOS	Culture medium containing Josamycin 8 µg/mL	Red (R)	Yellow (S)
15-CD	Culture medium containing Clindamycin 8 µg/mL	Red (R)	Yellow (S)
16-ESC	Culture medium with substrate for isolation of *Escherichia coli*	blue	grey-red
17-PRO	Culture medium with substrate for isolation of Proteus spp./Providencia spp.	brown-black	yellow
18-PSE	Culture medium with substrate for isolation of *Pseudomonas* spp.	turbid green	yellow-blue
19-GAR	Culture medium with substrate for isolation of *Gardnerella vaginalis*	yellow-orange	red
20-STF	Culture medium with substrate for isolation of *Staphylococcus aureus*	black ring	yellow
21-STR	Culture medium with substrate for isolation of *Enterococcus faecalis*	black	yellow
22-NES	Culture medium with substrate for isolation of *Neisseria gonorrhoeae*	blue	colourless
23-STG	Culture medium with chromogenic substrate for isolation of *Streptococcus agalactiae*	green	yellow
24-CAN	Culture medium with substrate for isolation of *Candida* spp.	turbid yellow	green

The samples were inoculated as follow:

1. All vaginal swabs Immersing in the vial of physiological solution and wait 5 minutes. Carefully squeeze the swab against the vial wall.
2. Transfering 0.2 mL of inoculated physiological solution into each well of the system.
3. Covering wells 1 to 5, 7 to 15, 19 and 24 with 1 drop of Vaseline oil (supplied with kit). Sealing the system with the lid provided.
4. Incubation at 36 ± 1°C for 18-24 hours.
5. Interpreting the results according to the manufacturer guidelines.

RESULTS

Among 40 vaginas swab collected from women with vaginitis 22(55%) attributed to polymicrobial bacterial vaginosis, 1 (2.5%) for trichomoniasis and 2(5%) for candidiasis as a single agent vaginitis. Mix infection, double agent vaginitis were 13(32.5%). Low percent of multiple agent vaginosis were recorded 2 (5%) which include bacterial vaginosis, trichomoniasis and candidiasis at the same swab table (2). The results of the current study were disagreed with Klufio *et. al.*(1995), who state that the combined infection (two or more causative agent at the same time) uncommon among women with vaginitis. The current study was in accordance with those founds the single infection account for 51% while mixed infection consist 19%. Also he found that 57.89% attributed to mixed bacterial and candidiasis, 26.32% for mixed candidiasis and trichomoniasis and then 15.79% for mixed bacterial and trichomoniasis (Lennox *et al*., 2013).

Table 2: Coexistence of Three Group of Vaginitis.

Vaginitis Causative Agents		*n*=40	Percentage	
Single agent	Bacterial	22	55%	62.5%
	Trichomonal	1	2.5%	
	Candidial	2	5%	
Double agent	Bacterial +Trichomonal	2	5%	32.5%
	Bacterial +Candidial	11	27.5%	
	Trichomonal+ Candidial	0	0%	
Multiple agent	Bacterial+Trichomonal+Candidial	2	5%	5%
Total		40	100%	

The percentage of each causative agent were 3(12.5%) for *T. vaginalis* and 15(37.5%) for *Candida* spp. Figure (1). This results in accordance with Klufio *et al.* (1995) who found that 19% and 23% of samples were positive for *T. vaginalis* and *Candida* albicans while another study state that C. albicans compile 12.5% (Akinbiyi *et al*., 2008). *Staphylococcus aureus* compile 32(80%), *Enterococcus faecalis* 26(65%), *Streptococcus agalactiae* 12(30%), *Mycoplasma hominis* 10(25%), *Ureaplasma urealyticum* 7(17.5%), *Gardnerella vaginalis* 10(25%), *Proteus* spp./Providencia spp. 8(20%), E. coli 6(15%) while *Neisseria gonorrhoeae* and *Psudomonas* spp. not detected in this study. The percentage of causative agent completely different in adolescence (15-19 years) as stated by Di Bartolomeo *et al*. (2002), who found that *M. hominis* consist 16.5% *U. urealyticum* 61.4%, *Candida* spp 17.8%, *T.vaginalis* 2.4% and *S. agalactiae* 5.6%.

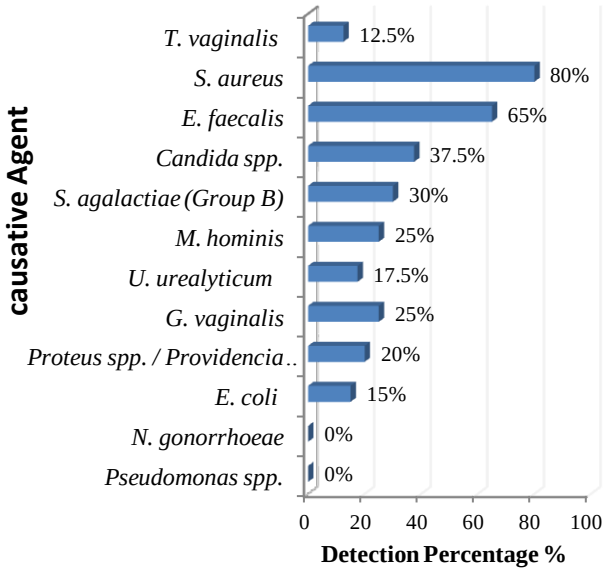

Fig. 1: Percentage of Vaginitis Causative Agents.

The results of *G. vaginalis* detection in accordance with other studies who state that *G. vaginalis* compile 17-30% of all vaginitis among married woman[A]. Figure (2) show the positive and negative result for *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*.

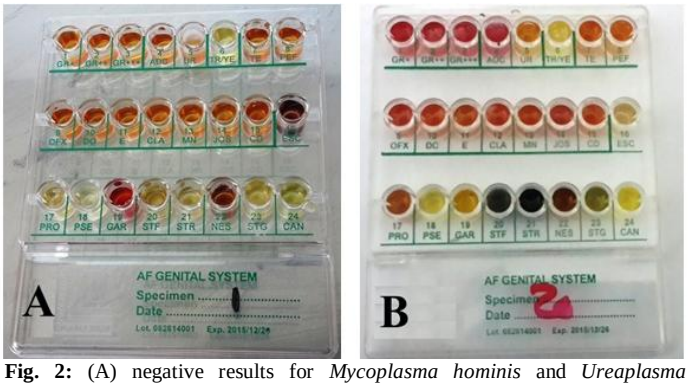

Fig. 2: (A) negative results for *Mycoplasma hominis* and *Ureaplasma urealyticum* while positive for *Gardnerella vaginalis*. (B) Positive results for *Mycoplasma hominis* and Ureaplasma urealyticum while negative for *Gardnerella vaginalis*

The results of antibiotics susceptibility revealed that all samples that give positive results for *Mycoplasma hominis* and *Ureaplasma urealyticum* were totally sensitive to clindamycin and pefloxacin while resisted to the other in different percentage (5%-15%) as shown in figure (3). Oral tetracyclines have historically been the drugs of choice for use against urogenital and systemic infections due to *M. hominis*and *Ureaplasma* spp. in adults. They are susceptible to tetracyclines but acquired resistance to tetracyclines has been reported (Waites *et al*., 2014).

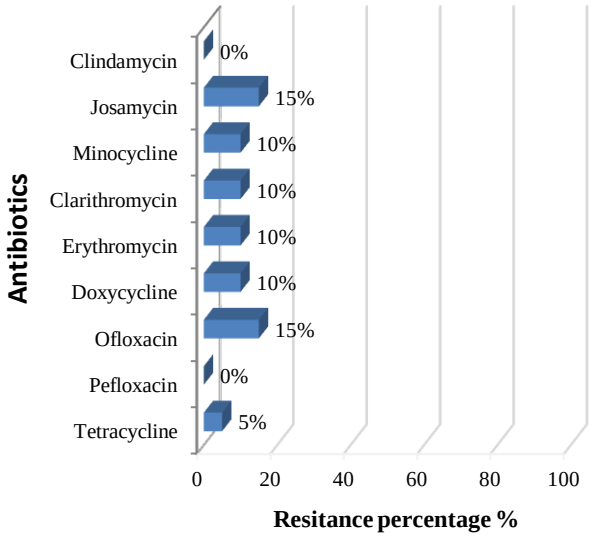

Fig. 3: Antibiotic Resistance of *Mycoplasma hominis* and *Ureaplasma urealyticum.*

DISSCUSION

Vaginitis can be assigned to bacterial vaginitis, candidiasis or trichomoniasis. Bacterial vaginosis is most frequent urogenital tract infection in women ages 15-44 years (CDC, 2014). It is the most common cause of vaginal discharge among women in reproductive age. The malodorous vaginal discharge resulted from production of amines like putrescine, cadaverine and trimethylamine as a result of anaerobes overgrowth. These amines become volatile at alkaline pH (like those after sexual intercourse and during the menstrual cycle) leads to malodorous of vaginal discharge[H]. Bacterial vaginosis is believed to be caused by proliferation of a number of organisms, including *Gardnerella vaginalis*, *Mobiluncus* species, *Mycoplasma hominis* and *Peptostreptococcus* species (Hill, 1993). The single-agent vaginitis can be easily diagnosed via the type and color of vaginal discharge but the multi-agent vaginitis hard to correctly diagnosed. A recent studies found more than one of the three pathogens in 22% of the vaginitis samples (Lowe *et al*., 2009).

The accurate diagnosis of vaginitis causative agent can reduce the threat of fetal loss or preterm birth during Pregnancy. This is may result from ascending infection when the bacteria stay long time without reliable treatment (Leitich and Kiss, 2007). The correct diagnosis also reduce the resolution periods and the cost of the reliable medication. The risk to get *G. vaginalis* resulted from their ability to biofilm formation and may be establish chronic persistent infection (Swidsinski *et al*., 2005; Swidsinski *et al*., 2010). Biofilm formation make the infection hard to cured and this result from fact that biofilm producing bacteria need tenfold concentration of antibiotics to get rid when compared with itself but without biofilm (Bunyan *et al*., 2013). Ureaplasmas and *M. hominis* are intrinsically susceptible to fluoroquinolones; however, newer fluoroquinolones such as pfloxacin and moxifloxacin are more active *in vitro* against human mycoplasmas than older ones such as ofloxacin and ciprofloxacin (Waites et al., 2014).

CONCLUSION

Bacterial vaginosis compile high percentage among women with vaginal discharge. It can be present alone or along with candidiasis while rare cases record a combined infection of bacterial vaginosis along with trichomoniasis and both candidiasis and trichomoniasis may be as secondary infection that enhanced after bacterial vaginosis. Also conclude excellent response to treatment with tetracycline and new fluoroquinolones such as pefloxacin.

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