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Isolation of *Candida* spp. from Women with Yeast Vaginitis and Study the Effect of Different Types of Douching on *Candida* spp.

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Abstract: The interest of current research was to determine the effect of different types of vaginal douching (which are commercially available in pharmacies and legally prescribed by gynecologist to female patients with vaginal yeast candidiasis on *Candida* species). From ninety seven vaginal swab samples which were collected in the presence of specialized Gynecologist, sixty seven samples were positive, while thirty samples were negative. *Candida* isolates includes different species as follows, *Candida albicans* 46 isolates (68.65 %), *C. tropicales* 11 isolates (16.41 %), *C. parasilopses* 7 (10.44 %), and *C. kyfer* 3 isolates (4.47 %). The inhibitory effect (inhibition diameter) of douches was ranged from 50 mm in Gyno Baking Soda to 5 mm in Women Care, while some types of douches had no effect at all. Finally, it was concluded that despite the inhibitory effect for many types of douching on yeast isolates, such douches were cannot be used as a treatment for yeast vaginitis since they may increase the risk of yeast vaginal infection.

Key words: vaginal douching, gynecologist, vaginal candidiasis, vaginal discharge, bacterial vaginosis and trichomoniasis.

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Introduction:

Vaginitis (inflammation of the vagina) is the most common gynecologic condition registered in the clinic. It's clinically diagnosis based on the presence of symptoms of abnormal discharge, vulvovaginal discomfort, or both. Discharge flows from the vagina daily as the body's way of maintaining a normal healthy environment. Normal discharge is usually clear or milky with no malodor (1). The most common causes of vaginitis in symptomatic women are bacterial vaginosis (40-50%), vaginal candidiasis (20-25%), and trichomoniasis (15-20%); yet 7-72% of women with vaginitis may remain undiagnosed (1, 2). Any change in the amount, color, smell, irritation, itching or burning could be due to an imbalance of healthy bacteria in the vagina, leading to Bacterial Vaginosis (BV) which is a very common condition and characterized by alterations of the vaginal flora (3- 5). Accurate diagnosis may be exclusive, and care must be taken to distinguish these conditions from other infectious and noninfectious causes (1).

A complex and intricate balance of microorganisms maintains the normal vaginal flora, these organisms include lactobacilli, Corynebacteria, and yeast (6).

The age of the patient affects the anatomy and physiology of the vagina. For example in prepubertal children vaginal pH is more alkaline, the vaginal mucosa is columnar epithelium, vaginal mucous glands are absent, and the normal vaginal flora is similar to that of postmenopausal women (eg, gram-positive cocci and anaerobic gram-negatives are more common), than do in pubertal and postpubertal adolescents and women.

While in pubertal and postpubertal adolescents and women vaginal pH is more acidic, the vaginal mucosa is stratified squamous, vaginal mucous glands, and the normal vaginal flora is dominated by lactobacilli, and hypertrophied hymens and vaginal walls (7, 8).

The second most common cause of vaginitis is vaginal candidiasis in which the yeast species *C. albicans* is the most common causative agent (9). Some of frequent risk factors that leading to such infection are, oral contraceptive use, young age at first intercourse, increased frequency of intercourse, diabetes, HIV or other immunocompromised states, long-term antibiotic use, wet vulva due to tight clothing, diet favoring refined sugar and yeast, and pregnancy (1, 10).

On the other hand*;* the third type of vaginitis, is trichomoniasis which caused by *Trichomonads* to initiate *T. vaginalis* infection.

T. vaginalis is an oval-shaped or fusiform-shaped flagellated protozoan that is 15 μm long (the size of a leukocyte). These organisms primarily infect vaginal epithelium; less commonly, they infect the endocervix, urethra, and Bartholin and Skene glands. *Trichomonads* are transmitted sexually and can be identified in as many as 80% of male partners of infected women. Risk factors include tobacco use, unprotected intercourse (11). The other type of vaginitis is the non-infectious vaginitis, is usually occurs due to allergic reaction or irritation, another cause is atrophic vaginitis due to estrogen deficiency. Tight-fitting clothing, scented detergents, soaps, feminine sprays, and poor hygiene are common causes of candidal vaginitis (1).

As for vaginal yeast infection; the symptoms can include, Itching or burning, soreness, thick, white, vaginal discharge that looks like cottage cheese and may smell like yeast/bread, though usually odorless, burning during urination and intercourse, and swelling, soreness, or rash on the outer lips of the vagina (12). Furthermore such infection may have no Symptoms at all (13). Woman may has the ability to transmit a yeast infection to a male sex partner, even though yeast infection is not considered to be a true sexually-transmitted disease (STD) because it can occur in healthy women as well as in women who are not sexually active. Treatment of yeast infection in men, like in women, involves antifungal medications (13, 14). Examples of available antifungal medications are: fluconazole, terconazole (Terazol), clotrimazole (Gyne-Lotrimin), miconazole (Monistat), butoconazole (Gynazole), and Nystatin. The mode of action of such drugs is to inhibit the ability of fungus to multiply and form new cell membranes (15, 16).

Mild or moderate infections can sometimes be treated with a single dose of oral antifungal medication. These types of medications usually work to cure the infection (80% to 90% success rate), but some people may have recurrent or resistant infections (15).

However, many women find that home care strategies can help control unpleasant symptoms. These include

allowing air to circulate around the vagina when possible by wearing loose, cotton undergarments and clothing. Removing undergarments at night may also be helpful (14). Furthermore treatment of vaginitis may include sitz baths and instruction regarding proper toilet and hygiene techniques (1).

Women who experience recurrent vaginal yeast infections, or yeast infections that do not clear up with treatment, should immediately contact a healthcare provider for professional diagnosis and management. If a woman has more than four episodes of vulvovaginal candidiasis (VVC) in a year, she is deemed to have recurrent vulvovaginal candidiasis (16- 18).

The aim behind this work was to study the inhibitory effect of over- the counter douching on the isolated *Candida* spp (by using well diffusion method) , and the ability or possibility of these douches to treat yeast candidiasis beside the usual antifungal treatments.

Material and methods:

Collection of specimens

Vaginal swab specimens were collected from ninety seven women that suspected to have yeast vaginitis, from October 2015 to February 2016. All specimens were collected from patients in the presence of specialized Gynecologist in Gynecology clinic. The patients were with different ages that ranging from (26 to 60) years old.

Mycological investigation was carried out for all specimens. The investigation was formed by taking specimens (vaginal swabs) by using sterilized tools. The collection involves specimens of vaginal discharge that should be taken by using a sterile transport media cotton swab, then subsequently inoculating in a primary isolation medium such as sabouraud's Dextrose Agar medium for 2-3 days at 37 ºC.

Diagnoses of *Candida* spp:

There are different methods or steps for the diagnoses of vaginitis, according of the severity of infection.

I. Primary diagnoses of *Candida* spp. for instance, the symptoms of thick, cruddy discharge, itch and irritation are classic for yeast infection.

II. Conformation diagnoses includes,

A. Culture of spaceman's:

Specimens of vaginal discharge should be taken by a swab, and then will be examined under light microscope for the presence of yeast, showing yeast under microscope confirm the diagnoses, in some cases culture is necessary, especially when a woman have a complicated and repeated infection, this method is more accurate than the examination under light microscope, and it takes a few more days to show the results (13- 14, 19).

B. Biochemical tests:

Identifying yeasts to the species level in the clinical microbiology laboratory rely on criteria such as morphology, growth characteristics and carbon source assimilation or fermentation, as well as appearance on differential isolation media (20- 21). Isolates of *C. albicans* were typically identified by their ability to form germ tubes (GT) or chlamydospores under appropriate conditions (22).

C. API 20 C test kit:

The identification of other species of *Candida*, was take place by commercial carbohydrate assimilation systems, such as the API 20 C test kit (23).

The commercially available samples of douching that were used in current work are listed in (Table 1). The experiment was done by using Well

Diffusion Method, and the inhibition zone (if present) was measured by mm.

Agar disk-diffusion testing developed in 1940 (24), is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing (25). In this well-known procedure, agar plates were inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (or wells by cork borer) (about 6 mm in diameter), containing the test compound at a desired concentration, were placed on the agar surface. The petri dishes were incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured, (Figure 1, 2).

Table (1): Douche type according to the using method and pH.

No.	Douche	pH	Type of use	Manufacturer company	Country
1	Backing Soda	8- 9	Internal use	PHARMA LIFE	Syria
2	ELMA (Backing Soda)	8- 9	Internal use	Cosmo Active	Turkey
3	Women Care	8- 9	Internal use	Omega pharma	Lebanon
4	Femme Care (Backing Soda)	8- 9	Internal use	United International Pharmaceutical Co.	Jordan
5	Gyno (Backing Soda)	8- 9	Internal use	turkuaz	Turkey
6	Femme Care Vinegar	2.8-3.5	Internal use	United International Pharmaceutical Co.	Jordan
7	ELMA Povidone Iodine	5.5	Internal use	Cosmo Active	Turkey
8	Germ X	5.5	Internal use	turkuaz	Turkey
9	Gyno FOAM	4.2	External use	turkuaz	Turkey
10	Backing Soda	8- 9	External use	PHARMA LIFE	Syria
11	ELMA Vinegar	3- 5	External use	Cosmo Active	Turkey

Results and Discussion:

From ninety seven vaginal swab samples that were collected from patients with vaginitis, sixty seven samples were positive and thirty samples were negative, as well as the frequency of isolates according to women's age was described briefly in (Table 2).

Table (2): The frequency of patients age and isolates.

Age	No. of samples	Ve (+)	Ve (-)
26- 30	15	9	6
31-35	17	11	4
36- 40	12	8	4
41- 45	20	14	4
46- 50	15	10	5
51- 55	8	6	4
56- 60	10	9	3
Total	**97**	**67**	**30**

Ve (+): positive result means present of yeast growth
Ve (-): negative result means absent of yeast growth

The identification tests for all isolates reproach the following species; *C. albicans* 46 isolates (68.65 %), *C. tropicales* 11 isolates (16.41 %), *C. parasilopses* 7 isolates (10.44 %), and *C. kyfer* 3 isolates (4.47 %) respectively. Statistical analyses for age groups (26-30, 31-35, 41- 45) showed non-significant differences between means at ($p \leq 0.05$), (Table 3).

Table (3): Statistical analyses showing the relation among age groups and the frequency of *Candida* isolate.

Age groups	Mean		±SD
26- 30	6.00	c	2.00
31-35	7.67	c	2.52
36- 40	12.33	bc	3.79
41- 45	6.00	c	2.00
46- 50	12.00	bc	3.61
51- 55	19.00	b	3.00
56- 60	28.67	a	6.43
LSD		**7.144**	

Small letters (a, b, c, bc) indicate to comparison between means in column, similar letters are non-significantly differences between means at ($p \leq 0.05$), Using (LSD test).

It was obvious that's the majority of isolates were belong to the genus *Candida albicans* since the major causative agent in 85-90% of cases for vaginal yeast infection is *Candida albicans* (9, 26), then followed by *C. tropicales*, *C. parasilopses*, and *C. kyfer* respectively, hens the less frequent yeast species are *Candida glabrata* (*Tropicales*) and *C. parapsilopsis* in 5-10% of cases for vaginal yeast infection which may cause infection that cannot be treated with usual treatments (13-14). The inhibitory effect of eleven douche types on all *Candida* isolates was as follows; some of douches gave negative results (no effect), while the most douche types gave positive results as illustrated in (Table 4), (Figure 1, 2).

Table (4): The inhibitory effect (mm) of eleven Douching types on *Candida* isolates.

No.	Douche	Diameter of inhibition zone (mm)
1	Backing Soda [internal]	No effect for *C. albicance* **, Ve (-)** 25 mm for other *Candida* isolates **, Ve (+)**
2	ELMA (Backing Soda)	No effect for *C. albicance* **, Ve (-)** 15 mm for other *Candida* isolates **, Ve (+)**
3	Women Care	5 mm **, Ve (+)**
4	Femme Care (Backing Soda)	No effect **, Ve (-)**
5	Gyno (Backing Soda)	50 mm **, Ve (+)**
6	Femme Care Vinegar	15 mm **, Ve (+)**
7	ELMA Povidone Iodine	7 mm **, Ve (+)**
8	Germ X	No effect **, Ve (-)**
9	Gyno FOAM	8-10 mm **, Ve (+)**
10	Backing Soda [external]	No effect**, Ve (-)**
11	ELMA Vinegar	20 mm **, Ve (+)**

Ve (+): positive result means douche cause inhibitory effect on *Candida* isolates
Ve (-): negative result means douche not cause inhibitory effect on *Candida* isolates

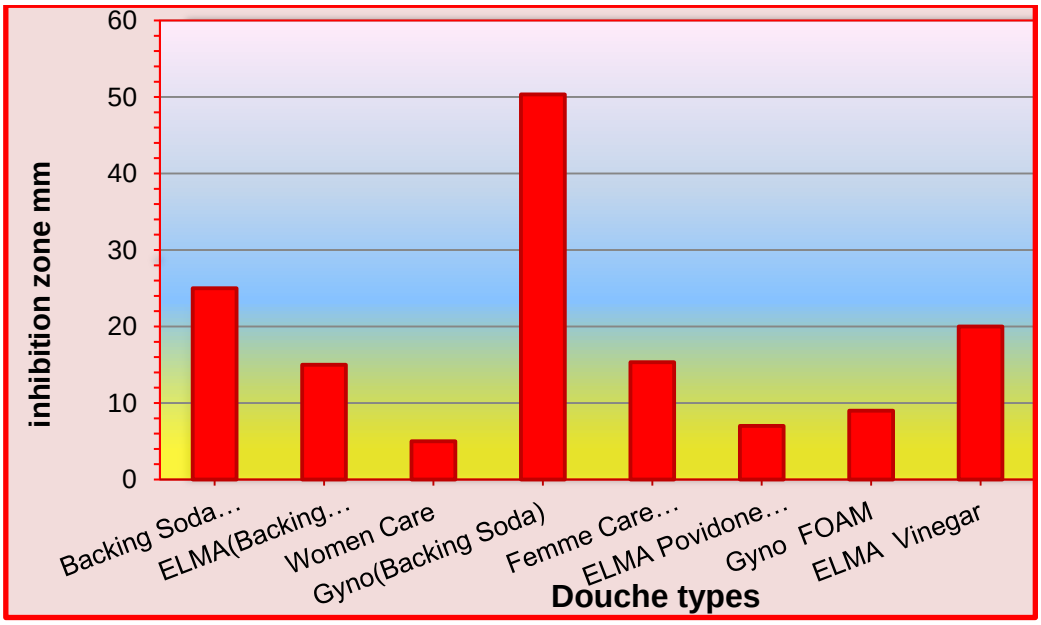

Figure (1): The inhibitory effect of douche types (mm) on *Candida* isolates.

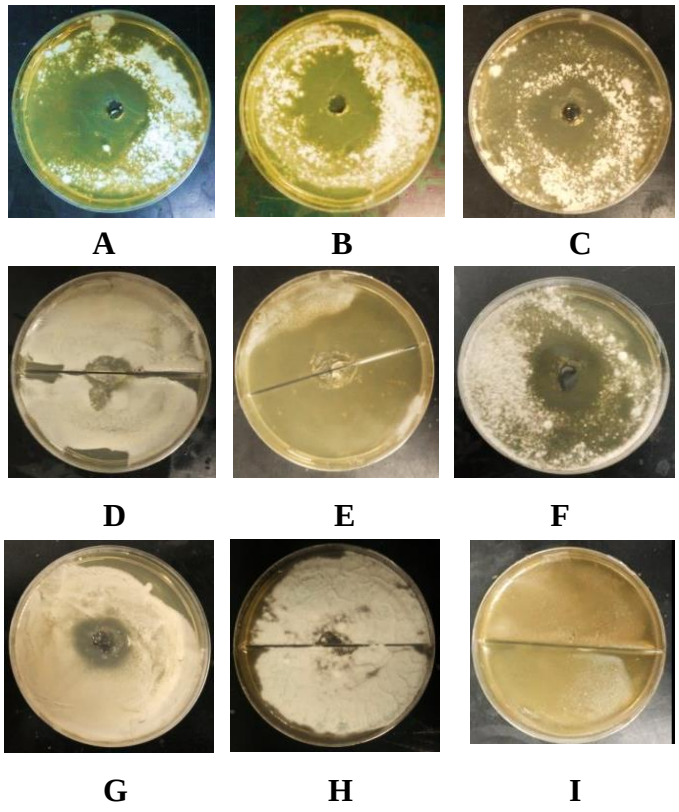

Figure (2): The inhibitory effect of some douche types (inhibition zone measured by mm) on *Candida* isolates in SDA medium, at 37º C, for 2- 3 days.A, Gyno (Backing Soda) - B, ELMA (Backing Soda) - C, ELMA (Povidone Iodine) - D, Femme Care (Backing Soda) - E, Gyno (Backing Soda) - F, Femme Care (Vinegar) - G, Women Care - H, Backing Soda – I, Control (D.W). Note: The inhibition zone in mm (when present) for each douching types is illustrated in (Table 4).

Statistical analyses for douche types (ELMA Backing Soda and Femme Care Vinegar) showed non-significant differences between means at ($p \leq 0.05$). (Table 5).

Table (5): Statistical analysis of the inhibitory effect of douche types (mm) on *Candida* isolates.

Douche type	Mean		±SD
Backing Soda (internal)	25.00	b	3.00
ELMA (Backing Soda)	15.00	d	1.00
Women Care	5.00	f	1.00
Gyno (Backing Soda)	50.33	a	2.31
Femme Care Vinegar	15.33	d	0.58
ELMA Povidone Iodine	7.00	ef	1.73
Gyno FOAM	9.00	e	2.00
ELMA Vinegar	20.00	c	3.61
LSD		**3.706**	

Small letters (a, b, c, d, e, f, ef) indicate to comparison between means in column, similar letters are non-significantly differences between means at ($p \leq 0.05$), Using (LSD test).

The most significance cause for the normal organism disturbance is the common use of Douching which affect the normal environment of the vagina, causing an inflammation and further imbalance of the organisms which normally present in the vaginal canal. This may cause symptoms such as chronic vaginal discharge and discomfort (14). Furthermore douching is associated with a number of health issues such as (cervical malignancy, pelvic inflammatory disease, endometrites, high risk of sexually transmitted infections , irritation, and bacterial vaginosis) (27, 28). Also, frequent use of douching with water may results in an imbalance of the pH of the vagina, and this may put women at risk for certain vaginal infections, particularly yeast infections (29). The normal premenopausal vaginal pH is 3.8- 4.2, at this pH, the growth of pathogenic organisms is usually inhibited, while pubertal and post pubertal adolescents and women have a more acidic vaginal pH (8). A number of factors can change or alter the vaginal environment and vaginal flora and could raise the chance of a woman's to get yeast vaginitis include, feminine hygiene products, immunologic status and underlying skin diseases, pregnancy, diabetes, douching, taking oral contraceptives, vaginal medications, antibiotics, sexually transmitted diseases (STDs), weekend immune system, sexual intercourse, and stress (1, 13- 14, 30). Besides, antiseptics could result in an imbalance of the natural bacteria in the vagina, leading to increase the chance of infection.(31). Furthermore, unclean douching tools can also introduce undesirable foreign bodies into the vagina. For these reasons, the routine of douching is strongly discouraged these days except when ordered by a physician for specific medical reasons (31). Douching may also move bacteria

into the uterus and Fallopian tubes, causing fecundity problems (32). In recommendation Patients must be noticed or reminded that douching may spread the vaginal or cervical infection into the uterus, increasing the opportunity or incidence of PID (pelvic inflammatory diseases) and they need to be educated about using topical creams for vaginitis treatment (candidal and bacterial) as necessary instead of douches (1, 32).

Conclusions: despite the inhibitory effect for many types of douching on yeast colonies or yeast growth, investigations profound that the irritant chemicals, method of use of such component, can actually increase the problem of vaginitis instead of decrease or eliminate it.

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