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## DETECTION OF PATHOGENIC BACTERIA AND MIXED INFECTIONS WITH YEASTS WHICH CAUSE VAGINITIS AND IT'S RELATIONSHIP WITH THE AGE IN IRAQI WOMEN

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#### Abstract

This study aims to isolate the bacterial pathogens and mixed infections with yeasts which cause vaginitis in Iraqi women and it's relationship with the age .The result revealed that the bacterial pathogens which isolated from genital tract identified as: Escherichia coli, Pseudomonas aeruginosa, Gardnerella viginals ,Klebsiella oxytoca ,Staphylococcus aureus and Proteus mirabilis. The results of bacterial susceptibility tests showed that all isolates were resistant to Cefalexin, Tetracycline, Streptomycin and Rifampicin, as well as, all isolates were sensitive to Chloramphenicol, but these were different in sensitivity for other antibiotics. The results of mixed infection of bacteria with yeasts revealed that Candida albicans was co-existing with S.aureus in 14 vaginal swabs, E.coli in 6 vaginal swabs and with two swabs of each Proteus mirabilis and K.oxytoca, while no mixed infection was observed neither with P. aeruginosa nor with G. viginals and the results of the relationship of bacterial vaginitis with the age showed that incidence of infection was high among females age group( 40-67 years).

Staphlococcus aureus ,Escherichia coli,Psudomonas aeruginosa,Gardnerella viginals ,Klebsiella oxytoca .Proteus mirabilis

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Rifampicin, Streptomycin Tetracycline Cefalexin,

, Chloramphenicol

Candida albicans

.mirabilis	6 E.coli	14 S.aureus
P. aeruginosa	G. viginals	K. oxytoca P
	.( 67-40)	

#### Introduction

Bacterial vaginitis (BV) is an infection of the lower female genital tract that occurs predominantly in women after marriage (1, 2). It is caused by an alteration in the normal vaginal flora in which the normally predominant Lactobacilli are replaced by pathogenic bacteria likeEscherichia coli, Pseudomonas aeruginosa, Gardnerella viginals and Proteus mirabilis (3,4). The mixed infection means the coexistence of yeasts with bacteria, it's considered to be the most important vaginal infection due to its association with upper urogenital tract infections and complications during pregnancy (5, 6). Due to the importance of this infection this study was designed to isolate and identify the bacteria from genital tract, evaluate the mixed infection of different kinds of bacteria with yeasts and estimate the relationship of bacterial vaginitis with the age.

### Methods

Two vaginal swabs from 250 female patients who attended to the obstetric and gynecology clinics in Fatima AL-Zahra hospital in Baghdad during a period from the beginning of July 2006 to the end of March 2007. Twenty five of control samples were collected from healthy women using sterile speculum and swabs by gynecologists. The swabs were transported to the laboratory by inoculating the swab into a sterile tube containing 3.0 ml of saturate transport medium (Brain heart infusion broth). One of the swabs was directly inoculated onto Blood agar. MacConkev agar for microbiological investigation, the other swab was used for direct examination by wet mounted film and Gram stained for detection of bacteria, inoculated culture plates were incubated at 37°C for 24-72 hr. The isolated colonies were identified by morphological feature, biochemical tests beside motility test. Stock culture was made by inoculating single colony of the isolated bacteria into a slant of Nutrient agar and kept in the refrigerator at 4 °C.

### **Identification of bacteria**

The isolated bacteria were identified according to (7, 8, 9 and 10) as follows:-1-Gram stain 2-Biochemical tests: Included: - oxidase, indole, catalase, urea hydrolysis, gelatinase, kliglar iron agar, coagulase, phenylalnine deaminase, motility and Whiff test for detection of *G. viginal*.

#### Antibiotic susceptibility Test

Disk agar diffusion method was carried out according to (11; 12 and 13).

#### **Identification of yeasts**

The isolated yeasts were identified as described by (14) as follows:-

1-Gram stain according to (15).

**2-Production of chlamydospores** according to (16).

**3-Production of germ tube** according to (17).

**4-Biochemical tests** according to (18) which included.

A- Sugar fermentation test.

B- Carbohydrate assimilation test.

#### **Results and Discussion**

The bacterial identification was confirmed according to(19) by routine biochemical tests in addition to cultural and microscopic tests The results show that from 250examined vaginal swabs, 31 isolates were positive to *S*. *aureus* was the mainly widespread bacterial pathogen of vagina and followed by *E.coli* 21 isolates, then *P.aeruginosa* 18 isolates, *Klebsiella oxytoca* 11 isolates, *G. viginals* 10 isolates and *Proteus mirabilis* 6 isolates as shown in table (1).

Table 1: The different bacterial pathogens from	
vaginal swabs ( patient and control samples).	

Microorganism	isolates\p	isolates\c	
Witcioorganishi	atients	ontrol	
Staphylococcus aureus	31	2	
Escherichia coli	21	3	
Pseudomonas aeruginosa	18	2	
Klebsiella oxytoca	11	0	
Gardnerella viginals	10	2	
Proteus mirabilis.	6	3	
Mixed infection (bacteria with yeasts)	24	0	
Total positive samples	121	12	
Total Samples	250	25	

The antibiotic susceptibility was determined for selected strains of different vaginal pathogens. The results revealed that all selected pathogens were resistant to Cefalexin, Rifampicin, Streptomycin and Tetracycline; on the contrary, all of them were sensitive to Chloramphenicol, while they were variable in their sensitivity to other antibiotics as shown in table (2).

Isolate number	Gentamicin	Rifampicin	Co-trimoxazole	Ceftriaxon	Cefalexin	Linkomycin	Chloramphenicol	Erythromycin	Tetracycli	Neomycin	Ampicillin	Trimethoprime	Streptomycin
Escherichi a coli NO6	R	R	S	S	R	R	S	R	R	S	R	S	R
Pseudomonas aeruginosa NO 3	R	R	R	R	R	R	S	R	R	R	R	R	R
K. oxytoca NO 5	R	R	R	R	R	S	S	S	R	R	R	R	R
Proteus mirabilis NO 3	S	R	S	R	R	S	S	S	R	R	S	R	R
Staphylococcus aureus NO 4	R	R	S	R	R	S	S	S	R	R	R	R	R
Gardnerella viginals NO 10	R	R	S	R	R	S	S	S	R	R	R	R	R

Table 2: Susceptibility of randomly chosen bacterial isolates to different antibiotics.

R= resistant, S= sensitive

It was clear that the drug of choice for treatment of urogenital infection was Chloramphenicol which showed (100 %) sensitivity in this study. This result closely resembled most of the studies in our country and the world (20, 21; 22) but we must know the risk when this antibiotic was used because it effected negatively on blood and kidneys (23). Therefore the patients are experimenting more and more with alternative natural remedies such as yoghurt which appears to contain compounds that are active against urogenetal infection and can help to prevent this infection (24).

The relationship of vaginal infection with the age was investigated in this study as shown in table(3) it was clear that incidence of infection was high among females age group( 40-67 years). It seems that bacterial infection increases with age that is caused by an alteration in the normal vaginal flora in which the normally predominant Lactobacilli are replaced by pathogenic bacteria.

Table 3: The relationship of bacterial vaginitiswith the age.

Age range of the patients (year)	Bacteria-from- vaginal swab
19-29	19
30-39	18
40-49	33
50-67	27
total	97

The incidence of mixed infection of bacteria with *Candida spp.* was investigated in the current study and revealed that *C. albicans* was co-existing with S.aureuse in 14 vaginal swabs, *E.coli in* 6 vaginal swabs and with two swabs of each *P. mirabilis* and *K. oxytoca* while no mixed infection was observed neither with *P. aeruginosa* nor with *G. viginals*, as shown in table (4).

Microorganism	NO.	%
Staphylococcus aureus	14	58.3
Escherichia coli	6	14.5
Proteus mirabilis	2	8.3
Klebsiella oxytoca	2	8.3
Total	24	100

# Table 4: Mixed infection of Candida albicanswith bacteria in vaginal swabs.

The co-existence of *Candida* with bacteria may arise from competition for nutrients, contamination from other sites, or co-existing as secondary pathogens, it was found that the highest incidence of combined infection came with *S.aureus*, this was in agreement with(25) who suggested that *C.albicans* stimulate the growth of *Staphylococci*. The other bacteria like *E.coli*, *P. mirabilis* and *K. oxytoca* showed lower incidence of combined infection with *Candida*.

Our findings confirmed that there was no combined infection neither with *P. aeruginosa* nor with *G. viginals*. The absence of combination with *P. aeruginosa* may be due to substance produced by *P. aeruginosa* that inhibits the growth of *Candida*, the mentioned findings was also reported by (26) who demonstrated an inhibitory effect produced by *P. aeruginosa* on *C.albicans* and suggested that, this inhibitory effect may be due to the pyocin pigment produced by these bacteria.

Similarly there was no case of combined infection between *C.albicans* and *G. viginals*, and that may be due to the possibility of production of an inhibitory material by *C.albicans* against *G. viginals*, an observation which meet with the observation of other workers(27 and 28) who noticed that no combined infection between *C.albicans* and *P.aeruginosa* or *G. viginals*.

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