



Evaluation and antimicrobial susceptibility testing of *enterococcus faecalis* isolated from high vagina

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Abstract

The aim of the present study was to investigate the incidence of *Enterococcus faecalis* in high vaginal swabs and study its antibiotic resistance profile. In this prospective study 127 married women at reproductive age have been included their ages ranged between (17-55) years with mean age (29.25 ± 0.029). Vaginal swabs were inoculated on microbiological media including blood agar and chocolate agar, incubated at 37 C for 24-48 hrs. *Enterococcus faecalis* have been found in 57.5% of swabs showed positive results for growth as 42.5 % showed positive results for gram negative and gram positive other than *Enterococci* and bacterial growth appeared in only (47) HVS sample showed positive result for bacterial growth 37%. The highest rate of infection was among the age group of (26-35) years as 23 (48.9%). The antibacterial resistance of *Enterococci* to different antibiotics showed that the bacteria showed absolute resistance to the cephalixin and they were highly resistant to Ampiclox and cefixime 92.8% resistance for all of them and was highly resistant to cefotaxime and doxycycline, While it showed high sensitivity to amikacin, levofloxacin and nitrofurantoin (92.9 %, 81.9% and 74.1% for the three antibiotics respectively while the rest of the antibiotics showed moderate resistance.

Keywords: enterococcus fecalis, streptococcus faecalis, enterococci, vagina infection

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INTRODUCTION

Bacterial vaginosis is the most common infection in women of childbearing ages (Gingelmaier and Verner et al 2016). It is an uncommon health problem; vaginitis is the term used to denote the presence of inflammation and or infection in the vagina (Berg et al 1984; Chirenje et al 2018). The symptoms of vaginosis include itching and agray to watery discharge with a fishy smell if untreated lead to serious complication (Donders et al 2002; Wathne et al 1994). Vaginal infections can be caused by bacteria, fungi, parasite or viruses, these infections can cause symptoms such as soreness and itchiness around the vulva and unusual vaginal discharge. (Mancuso et al 2007). Bacterial vaginitis is sometimes caused by aerobic bacteria like *E coli*, *Staphylococcus aureus* and beta hemolytic *Streptococci* (Mumtaz et al 2008; Donders et al 2011) and bacterial vaginosis is the invasion of the vagina with aerobic bacteria, it occurs when there is alteration of the vaginal ecology with gross depletion of normal bacterial flora (Lactobacilli) with over

growth of anaerobic microorganisms (Nobel et al 2004 Demoba et al 2005; Lakshmi et al 2012).

The common organisms implicated in bacterial vaginosis include *Gardenerella Mycoplasma hominies* and anaerobic bacteria such as *Peptostreptococcus Prevotella* species and *Mobilinicus Spp.* (Hillier et al 1993). *Enterococcus* is a bacterium that is typically present in the bowel, the species *Enterococcus faecalis* was the most often associated with the presence of all three signs of bacterial vaginosis including PH>4 changed color of vaginal secretion and positive amino odor test (Bubu et al 2017, Romanik et al 2007). Until 1984 *E. faecalis* was known as *Streptococcus faecalis* previously categorized as part of the genus *Streptococcus*. According to the CDC 2020 *Enterococcus fecalis* is responsible for approximately 80 % of human infection, it can cause infection in people when it enters wounds,

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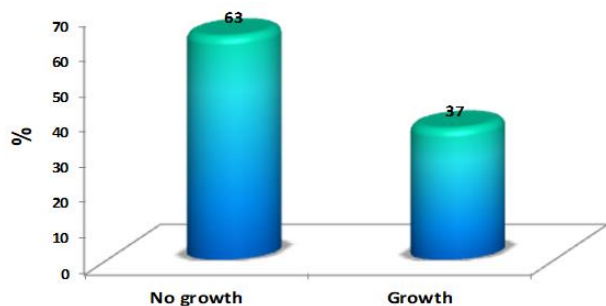


Fig. 1. Total number of the HVS samples growth

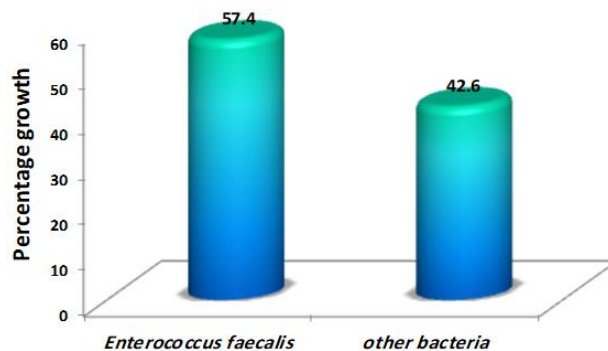


Fig. 2. Enterococcus faecalis percentage and other bacteria isolate

blood or urine of people especially those with weakened immune system (Owusu et al 2009; Daood et al 2020b).

E. faecalis infections is one of bacteria which is isolated from vaginal swabs. *Enterococcus faecalis*, it is associated with preterm birth, very low birth weight delivery and puerperal sepsis which causes substantial morbidity and mortality. It had been reported that *E. faecalis* is the most common etiological agent of aerobic. Vaginitis at a rate of 32.26% followed by *E. coli* 8-25% (Sangeetha et al 2015) While Ahmed et al 2018 isolated these bacteria (16.67%) from HVS, and it was isolated at 7.2% from HVS. of 250 women in Erbil by Ahmad et al 2015. *Enterococci* are usually non-hemolytic but occasionally alfa-hemolytic as 60% when human or rabbit was use (Ya suy Oshiike et al 1987).

AIM OF THE STUDY

The aim of the present study was to investigate the incidence of *Enterococcus faecalis* in vaginal swabs and determine its antimicrobial resistance to antibiotics.

MATERIALS AND METHODS

In this prospective study (127) married women at reproductive age have been included their ages ranged between (17-45) years with a mean age of (29.25±0.029). Attending private clinics HVS were taken from them and were transported under a septic conditions to the laboratory, the swabs were inoculated on blood and chocolate agar, incubated at 37 c for 24-48 hrs. under microaerophilic conditions in candle Jar , the result of the bacterial growth were identified according to the colony morphology depending on size, height and shape of the colonies (Verner et al 2016; Mancuso et al 2015) and the presence biochemical tests, presence or absence of hemolysis (hemolytic properties) and standard biochemical tests were done including its ability for growth on Bileesculin agar and its resistance to Azide and Bile salt.(Koneman et al 2017). Thin films were prepared and stained by gram staining method and examined for the cell shape and staining characteristic (Xu et al 2007) then the biochemical test including; catalase growth on 6.5% NaCl hydrolysis of Arginine and sugar fermentation test, gelatin hydrolysis.

Table 1. Percentage rate of infection among the age groups

Age group (Years)	No. and % of infection
1 15 - 25	9 (19.2%)
2 26 - 35	23 (48.9%)
3 36 - 45	9 (19.2%)
4 46 - 55	6 (12.7%)

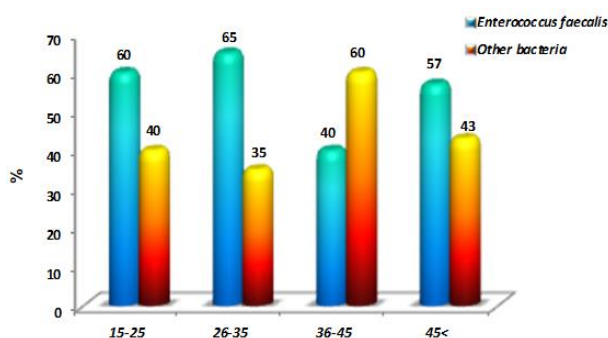


Fig. 3. Enterococcus faecalis predominate agent causing genital infection among age groups

Antibiotic sensitivity test: using the standard culture and sensitivity using the disk diffusion method (Kirby Bauer 1966) for the isolated bacteria and the standard antibiotic disk provided by the Bioanalyses and oxide company (Sobouti et al, 2020).

RESULTS

The total number of the HVS samples were (127) out of them 80 (63%) samples showed no growth while 47 (37.1%) showed positive result for bacterial growth as shown in **Fig. 1**.

Fig. 2 showed that *Enterococcus faecalis* forming 27 (21.2%) of the isolated bacteria (57.4%) of positive growth while other bacteria including Gram negative coccobacilli forming (20) HVS sample (15.7%) of the isolate.

Table 1 showed that the highest rate of infection was among the age group of (26- 35) years as 23 (48.9%) of the females included were infected followed by age group (15-25) and (36-45) at rate of 9(19.2 %) for both age groups, the age group of (46-55) years showed the lowest rate 6(12.7%) of infection.

Table 2. Percentage of resistance of isolates *Enterococcus faecalis* to different antibiotics

Antibiotic	Concnct.	Sensitivity %	Moderate %	Resistance %
Cephalexin	25mg	11.1	3.6	85.3
Amoxicillin	25mg	0	0	100
Ampiclox	25mg	3.6	3.6	92.3
Amoxi-clav	25mg	33.3	14.8	51.9
Cefixim	30mg	0	7.4	92.6
Cefotaxim	30mg	7.4	11.1	81.5
Ceftrixon	30mg	48.2	7.4	44.4
Tetracyclin	30mg	22.2	14.8	63
Gentamycin	30mg	63	14.8	22.2
Doxicilin	10mg	7.4	14.8	77.8
Nitrofuration	50mg	74.1	0	25.9
Naldic acid	30mg	14.8	11.1	47.1
Refampicin	5mg	55.5	18.6	25.9
Trimethoprim	25mg	3.7	7.4	88.9
Levofloxacin	5mg	81.9	11.1	7.5
Ciprofloxacin	30mg	55.6	18.5	25.9
Norfloxacin	30mg	55.6	7.4	37
Amikacin	30mg	92.5	3.7	3.7

Fig. 3 showed that *Enterococcus faecalis* was the predominate agent causing genital infection in females as it was isolated at rate of (67%), (60 %) and (57%) at age groups (26-35), (15-25) and (46-55) above respectively while it forms (40%) at age group (36-45) years.

Table 2 showed that the percentage of resistance of isolates *Enterococcus faecalis* to different antibiotics. *Enterococcus faecalis* showed the highest sensitivity to the antibiotic Amikacin 92.5% followed by Levofloxacin and Nitrofurantoin (81.9% and 74.1%) respectively, while it showed medium sensitivity to Gentamycin and ceftrixon (63% and 48%) respectively. The isolates showed absolute resistance to Amoxicillin (100%) and high resistance to Ampiclox and cefixim (92%) for all, and Trimethoprim cefotaxim cephalixin and Doxycillin (88.9%, 81.5%, 85.3% and 77.8%) respectively.

They showed moderate resistance to Tetracyclin 63%, Ceftrixon 44.4% and 18.6% of isolates showed moderate sensitivity to Refampicin and 14% to Amox-clav.

DISCUSSION

This study showed that the result of culture of HVS taken from women attending the private clinics in Mosul city during the period between October 2018 October 2019 showed that 80 samples (63%) of the swabs was negative for the bacterial growth while only 47 sample (37%) showed positive result vaginitis can be caused by different pathogens. Others can be caused by *Chlamydia trachomatis* (7%) *Neisseria gonorrhoeae* (1%) and *Trichomonas vaginalis* 0.5%. Other fastidious bacteria like *Gradnerella vaginalis* and *Prevotella species* causes 12% of the cases. Sugata et al 2016 and other bacterial vaginosis was diagnosed in 56.5 % and vaginal candidiasis in 32.8 % (Begaj et al 2017).

The result of **Fig. 1** showed that 27(21.2%) of the isolated strains belongs to *Enterococcus faecalis* while

20(15.7%) belongs of the total samples to gram negative coccobacilli and other cocci other than *Enterococci* **Fig. 2** showed that 57.5 % of the isolated bacteria belong to *Enterococcus faecalis* which were identified depending on biochemical characteristics, while the remaining 42.5% belongs to Gram negative *Coccobacilli* and other non-*Enterococcus* gram positive cocci.

Enterococcus accounts for 20 % of vaginal infection (sujata et al 2016) while Ghasemie et al 2016 isolated *Enterococci* at a rate of 8.14% from women with vaginal discharge *Enterococcus faecalis* vaginal discharge. *Enterococcus faecalis* forming 89.9 5% of the isolated and *Enterococci* were present in human intestine and can be transmitted to the vagina in the community of low socioeconomic status (Ghasemi et al 2016).

From **Table 1** appeared that the highest infection of *Enterococcus* was at age groups (26-35) forming 47.5% followed by age groups (36-45) and (15-25) years which was (19.6%) for both groups.

Infections vaginitis is the most common complain of women at reproductive age and sexually active age. (Yudkin G. Vaginal discharge) in Mepherson AA editor women problems.

Jacobsen et al (2008) stated that vaginal candidiasis is the most common among postmenopausal women. The disease is prevented in the united states as 29.2% of wome at age groups between 14-49 years (Mark et al 1998). Bacterial vaginosis that the highest rate of infection with bacterial vaginosis was among women of (30-40) years (8.8%), while the least were seen at age group 10-20 and 50-60 years (1.3%), while the highest rate of infection were at age group of 31-40 in nepal and the rate of infection were low below 20 years which was consistent with our result and may be due to high sexual exposure prevalence of bacterial vaginosis and its association with risk factor among nonpregnant women.

The results of **Table 2** showed that the prevalence of resistance among isolates to some of the antibiotics Amoxycillin, Cephalexin, and Ampiclox, while amikacin

and levofloxacin was highly effective against the isolates that result was in consistence with the result of Khan et al 1991 who stated that pencillins and tetracycline were among the lowest percentage sensitivity. High vaginal swab routin microscopy and culture sensitivity in diabetic and non-diabetic retrospective (Ateryichakvaborty et al 2015) reported that *E. faecalis* resistance to Ciprofloxacin (49.67 %) and Gentamicin (43.88%) antibiotic resistance pattern of *Enterococci* isolates from nosocomial infection in a tertiary care hospital in Eastern India (Atreyi et al 2004) found that *Enterococcus faecalis* showed (96 %) sensitivity to Tetracycline and from **Table 1** it appeared that the highest infection with *Enterococcus* was at age groups (26-45) (47.5 %) followed by age groups 36-45 and 5-25 which was 19.6% for both.

Infection vaginitis is the most common complain of women at reproductive and sexually active age (Sighn and Jyotsna Singh (study of risk factors for infection vaginitis in reproductive women (International J of contemporary medical research vol.5 (issue 12 December 2018) and it is the second most common problem after menstrual disorder . Vaginal discharge in McPherson AA. editor. Women problem in general practice 2nd ed Oxford. Oxford university press 1988 Jacobsen et al (2008) stated that vaginal candidiasis is more common among premenopausal women (Faiqah Umar et al., 2019).

The disease is prevalent in the United States as (29.2%) of women. age groups mostly at age between (14-49) years bacterial vaginosis (Mark et al 1998). Eliza Ranjit et al showed that the highest rate of infection with bacterial vaginosis was among women of (30-40) years (8.8 %) while the least were seen at age group (10-20) and (50-60) years (1.3%) while the highest infection rate were at age group of (31-40) Nepal and the infection rate

were low below 20 years which was consistent with our result which may be to high sexual exposure. Prevalence of bacterial vaginosis and its association with risk factors among non-pregnant women (Daood et al 2020a).

The result of **Table 2** showed the prevalence of resistance of the isolate to some of the antibiotic Cephalexin, Amoxicillin, Ampiclox while the antibiotics Amikacin and levofloxacin was highly infection against the isolates that result was in consistent with the result of (Khan et al 1991) who stated that Pencillins and Tetracycline were among the lowest percentage sensitivity (Khan et al,1991).

Atreyi et al (2015) reported that *E. faecalis* resistance to Ciprofoxacin (49.67 %) and to Gentamicin (43.88 %). Rudy et al. (2004) found that *Enterococcus faecalis* showed 96% sensitivity to Nitrofurantoin, 19% to Ciprofoxacin. and 28 % to Tetracycline.

Our result contrast the result of (Ghasemi et al) who showed that all isolated *Enterococci* are Amoxicillin and Gentamicin and the resistance to Ciprofloxacin.

CONCLUSION

Enterococcus faecalis was regarded an important cause of vaginal infection and common health problem; vaginitis which was resistant to antibiotics lead to highest infection of *Enterococcus* at age groups 26-35 forming women with vaginal discharge.

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