



THE IMPACT OF THE PROBIOTIC BACTERIA ON THE CONTAMINATED MICROBIAL OF THE FEMALE GENITAL TRACT AND ITS EFFECT ON THE IVF OUTCOME

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Hagar E. Ali¹; K.A. El-Dougdoug²; M.Y.Soliman³; M.M. Hazaa¹
and M.M. Sowelam¹

1- Botany Dept., Fac. of Sci., Benha Univ., Benha, Egypt

2- Agric. Microbiology Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt

3- OBS/GYN Dept., Fac. of Medicine, Ain Shams Univ., Cairo, Egypt

Keywords: Probiotic bacteria, female genital tract, ivf outcome, *Klebsilla sp.*, *E. coli*, *Candida sp.*, *Lactobacillus sp.*

ABSTRACT

The microbial contamination of the female genital tract has effect on the ivf outcome and negatively effect on fertility, fertilization and implantation rate. *Lactobacillus sp.* is the dominant organism of the vaginal flora that have an important role in the inhibition of pathogen. 48 patients were selected for this study aged between 20-40 years, During embryo transfer, 3 samples were collected from each patient from vagina, end cervical canal and the tip of the catheter. The samples were assayed microbiology. The microbial culture contained natural flora and pathogen organism .The isolated pathogens were identified as *Klebsilla pneumonia*, *Escherichia coli*, *Candida albicans*, *C. tropicalis*, *C. famata*, *C. parapsilosis*, *C. catenulate* according to Bergey's manual and confirmed by VITEK System. *Candida sp* found to be the most frequent in female genital tract. The probiotic (*Lactobacillus acidophilus*) and its metabolites (bacteriocin) were inhibited growth of isolated pathogenic microbial *in vitro*. The result showed that *L. acidophilus* and their metabolite bacteriocin have antagonistic activity against pathogenic microbes.

Recommendation: Oral or transvaginal administration of *Lactobacillus* or bacteriocin antiseptics; disinfected; maintain a health vagina and prevent the infection of the reproductive tract.

INTRODUCTION

The bacterial contamination has negatively effect on fertility, fertilization and implantation rate

(Selman, et al 2007). During embryo transfer techniques, embryos are loaded into a transfer catheter that passes through vagina and cervix into the uterus that may lead to possible microbial contamination of the uterine cavity that may alter the susceptibility of the endometrium and embryo implantation (Aboul fotouh, et al 2008). There are many factors that affect on implantation rates such as patient's age, endometrial receptivity and embryo quality (Egbase PE; et al 1999). The presence of pathogenic bacteria such as *E.coli* and *streptococcus viridians* decreased live birth rate (Eckert, et al 2003). Disequilibrium in microbiota promote the growth of opportunist microorganisms and the development of pathologies. *Vulvovaginal candidiasis* (VVC) is overgrowth of *candida spp* due to the disruption of the vaginal microbiota may be due to pregnancy, antibiotic therapy, use of contraceptives and spermicide, frequent sexual intercourse, diabetes and immunosuppression (Rathod, et al 2014 and Sobel, 2007). *Candida albicans* is the major causal of yeast *Vaginitis* but there are another species *Candida glabrata*, *Candida krusei* and *Candida tropicalis* infect the host.

The development of VVC has been associated with the lack of H₂O₂-producing *lactobacillus* species (vitali, et al 2007 and Chakoosari, 2013).

The normal vaginal flora of the human play an important role in preventing colonization of the pathogen including those responsible for bacterial vaginosis, yeast infections, sexually transmitted disease and urinary tract infection (Zhou, et al 2004, Burton, et al 2003). The lactobacillus especially *L. acidophilus*, *L. crispatus*, *L. jensenii* and *L. iners* is the dominant organism of the vaginal flora that have an important role in the inhibition of pathogen (Cannon, 2005 and Hoore, et al 2000).

(Received 28 November, 2017)

(Revised 24 December, 2017)

(Accepted 3 January, 2018)

Lactobacillus can inhibit the growth of pathogens by two mechanisms: (a) by production of antimicrobial factors that have adverse effect of pathogens such as lactic acid, hydrogen peroxide, bacteriocin and bacteriocin like substance such as *nisin*, *lactobrevin*, *acidophilin*, *acidolin*, *lactobacillin*, *lactocidin* and *lactolin* (Chakoosari, 2013 and Pendharkar et al 2015). (b) by adherence and competition. The mechanism of defence of *Lactobacillus* against candida colonization is the ability of *lactobacilli* to adhere and compete for receptors sites on vaginal epithelial cells (Simonetti, et al 2007). This study aims to application *Lactobacillus* or bacteriocin antiseptics; disinfected to maintain a health vagina and prevent the infection of the reproductive tract.

MATERIALS AND METHODS

The study took place from April 2015 to December 2016. 48 patients were selected for this study who attending Queens Hospital, suffering from 1st infertility, Cairo, Egypt and undergoing ivf treatment patient's age range between 20-40 years. patients undergoing long protocol treatment. Patients were selected with at least two good quality embryos available for embryo transfer. Three specimens were taken by nutrient broth swabs from each of tip of catheter, vagina and endocervical canal at the time of embryo transfer for each patient and send to the central laboratory, Kasr El-Ainin, Cairo University.

The swabs were cultured on blood agar for isolation, purification of contaminated microbes, The culture colonies were inoculated on MacConkey agar for gram negative bacteria, chocolate for anaerobic bacteria and Sabouraud dextrose agar for yeast (Oxoid, Cairo, Egypt). The isolated bacterial and *Candida* were identified accordance with Bergey's manual of determinative bacteriology (Holt, et al 1994). The identification of isolates were confirmed by VITEK 2 system using the colorimetric reagent cards that can measure various metabolic activities such as acidification, alkalization, enzyme hydrolysis and growth in the presence of inhibitory substance. After identification of each isolate, The VITEK2 system was used to detect the antibiotic sensitivity test and determine the minimum inhibitory concentration.

Antibacterial activity of *Lactobacillus acidophilus* CL1285: *L. acidophilus* CL1285 used in this study was obtained from Egypt Microbiology

Culture Collection, Cairo MIRCEN, Fac. of Agric., Ain Shams Univ., Cairo, Egypt. The inhibitory spectrum of *Lb. acidophilus* CL1285 cells (2×10^6) and cells free supernatants (CFS) were studied against isolated pathogen in this study (*Klebsiella pneumoniae*, *Escherichia coli*, *Candida albicans*, *C. tropicalis*, *C. famata*, *C. parapsilosis* and *C. catenulate*) *in vitro*. *Lactobacillus* inoculated to MRS agar media and incubated for 24 hrs at 30°C then divided as disc, the antagonist activity of *Lactobacillus acidophilus* was studied against pathogenic isolates using agar well diffusion. The CFS was obtained by centrifuging MRS broth cultures incubated for 24 hrs at 30°C at (6,000 rpm for 10 minutes at 4°C). The CFS was filtered through syringe filter (0.45 µm, Millipore). The inhibitory activity of CFS was tested using agar well diffusion method. (Cheesbrough, 2000).

Purification of bacteriocin: The partially purified bacteriocin was described by (Ouda, et al 2014 and Enan, et al 2013). One arbitrary unit (AU mL⁻¹) of crude bacteriocin preparation was defined as 5 µL of the highest dilution of PPE yielding a definite zone of inhibition of growth in the lawn of indicator organism. The highest dilution was multiplied by 200 µL (1 mL/5 µL) to obtain the arbitrary units per milliliter (AU mL⁻¹). CFS from *Lb. acidophilus* was adjusted at pH 6.5 and was treated with solid ammonium sulfate till 50% saturation level. The mixtures were stirred for 12 h at 4°C and centrifuged at (14,000 rpm for 1 hr at 4°C). The pellets were resuspended in 1 mM potassium phosphate buffer, pH 6.5 and dialyzed against the same buffer for 24 hrs at 4°C in dialysis tubing. This partially purified bacteriocin was sterilized by filtration through syringe filter (Amicon 0.45 µm, Millipore).

Amino acid composition: Amino Acids were determined by (Csomos, et al 2002). 200 µl of purified bacteriocin was hydrolyzed with 6N HCl in sealed tube, heated in oven at 100°C for 24 hrs, to evaporate HCl. The residue was dissolved in diluting citrate buffer (pH 6.5). HPLC was performed with an AAA 400 amino acid analyzer (Ingos Ltd., Czech Republic) equipped with an Ostion LG ANB ion exchange column. Free amino acids were separated by stepwise gradient elution using Na/K-citric buffer system (Ingos Ltd., Czech Republic). Post-column derivatization with minhydrin reagent and spectrophotometric measurement was used for determination of amino acids and biogenic amines.

RESULTS

Identification of bacterial & candidal isolates

Pathological *bacteria* and *Candida* isolated from vagina, endocervical canal and tip of catheter were identified according to Bergey's Manual of Determinative Bacteriology (Holt, et al 1994) and confirmed by VITEK 2 system as *K. pneumonia*, *E.coli*, *C. albicans*, *C. tropicalis*, *C. famata*, *C. parapsilosis* and *C. catenulate*.

The relation between pregnancy and presence of pathogen in the vagina, endocervix and tip of the embryo transfer catheter. The pregnancy rate is lower in the patients with *K. pneumonia* (6.25 %) compared to with negative microbial growth (93.75%). The pregnancy rate is lower in the patients with *E. coli* (0 %) compared to those with negative microbial growth (100 %). The pregnancy rate is lower in the patients with *Candida albicans* (18.75%) compared to those with negative microbial growth (81.25%) in (Table 1).

Table 1. Organism isolated from vaginal, endocervical and tip of embryo transfer catheter swaps and its relation on pregnancy outcome

Microbial Isolates	Cases		Pregnant (n=16)				Non Pregnant (n=32)			
			Microbial growth		No. microbial growth		Microbial growth		No. microbial growth	
	No.	%	No.	%	No.	%	No.	%		
<i>K. pneumonia</i>	1/16	6.25	15/16	93.75	3/32	9.37	29/32	90.62		
<i>E. coli</i>	0/16	0	16/16	100	2/32	6.25	30/32	93.75		
<i>Candida albicans</i>	2/16	12.5	14/16	87.5	12/32	37.5	20/32	62.5		
Other <i>Candida spp.</i>	3/16	18.75	13/16	81.25	3/32	9.37	29/32	90.62		

Antibiotics sensitivity of isolated microbes

K. pneumonia is sensitive to Minocycline, Colistin and Ritampian but it resistant to other antibiotics Ticarcillin, Piperacillin, Piperacillin/Tazobactam,

Ceftazidime, Cefepime, Aztreonam, Imipenem, Meropenem, Amikacin, Gentamycin, Tobramycin, Ciprofloxacin, Pefloxacin and Trimethoprim/ sulfamethoxazole (Table 2).

Table 2. Antibiotics sensitivity of *K. pneumonia* determined Interpretation by MIC.

Antibiotics	MIC	Interpretation	Antibiotics	MIC	Interpretation
<i>Ticarcillin</i>	>=128	R	<i>Amikacin</i>	>=64	R
<i>Ticarcillin/Clavulanic Acid</i>	>=16	R	<i>Gentamycin</i>	>=16	R
<i>Piperacillin</i>	>=128	R	<i>Tobramycin</i>	>=16	R
<i>Piperacillin/Tazobactam</i>	>=128	R	<i>Ciprofloxacin</i>	>=4	R
<i>Ceftazidime</i>	>=64	R	<i>Pefloxacin</i>	>=64	R
<i>Cefepime</i>	>=64	R	<i>Minocycline</i>	<=1	S
<i>Aztreonam</i>	>=64	R	<i>Colistin</i>	<=0.5	S
<i>Imipenem</i>	>=16	R	<i>Rifampicin</i>	<.0.5	S
<i>Meropenem</i>	>=16	R	<i>Trimethoprim/sulfamethoxazole</i>	>=320	R

E. coli is antibiotics sensitive to Piperacillin/Tazobactam, Meropenem, Amikacin and Nitrofurantoin. *E. coli* is resistant to Ampicillin, Ampicillin/Sulbactam, Cefazolin, Ceftazidime, Ceftriaxone,

Cefepime, Gentamycin, Tobramycin, Ciprofloxacin, Levofloxacin and Trimethoprim/sulfamethoxazole (Table 3).

Table 3. Antibiotics sensitivity of *E.coli* determined Interpretation by MIC.

Antibiotics	MIC	Interpretation	Antibiotics	MIC	Interpretation
ESBL	Pos	+	Meropenem	≤ 0.25	S
Ampicillin	≥ 32	R	Amikacin	8	S
Ampicillin/Sulbactam	≥ 32	R	Gentamycin	≥ 16	R
Piperacillin/Tazobactam	8	S	Tobramycin	≥ 16	R
Cefazolin	≥ 64	R	Ciprofloxacin	≥ 4	R
Cefoxitin	16	I	Levofloxacin	≥ 8	R
Ceftazidime	16	R	Nitrofurantoin	≤ 16	S
Ceftriaxone	≥ 64	R	Trimethoprim/sulfamethoxazole	≥ 320	R
Cefepime	2	+R			

+ = Deduced drug Pos = Positive

Candida species are sensitive to Fluconazole, Voriconazole, Caspofungin, Micafungin,

Amphotericin B and Flucytosine. The minimum inhibitory concentration for antibiotic also detected as: (Fluconazole) ≤ 1 , (Voriconazole) ≤ 0.12 , (Caspofungin) ≤ 0.25 , (Micafungin) ≤ 0.06 , (Amphotericin B) ≤ 0.5 , (Flucytosine) ≤ 1 .

Antibacterial activity of bacteriocin

Antimicrobial activity of bacteriocin was tested against multidrug resistance bacteria (*K. pneumonia* and *E. coli*) and *Candida* spp. (*C. albicans*, *C. tropicalis*, *C. famata*, *C. parapsilosis*, *C. catenulata*).

Bacteriocin composition: The amino acid of the purified bacteriocin of *Lactobacillus acidophilus* which was pooled from ion exchange chromatography are recorded in Table (4) seventeen amino acids were detected with different amount in the purified bacteriocin. Aspartic (ASP), Glutamic (GLU), Glycine (GLY), Alanine (ALA), Proline (PRO) present in the higher amount compared to Therionine (THR), Serine (SER), Valine (VAL), Isoleucine (ILE), Leucine (LEU), Tyrosine (TYR), Phenylalanine (PHE), Histidine (HIS), Lysine (LYS), Arginine (ARG), Cystine (CYS), Methionine.

Table 4. Amino acid composition of Bacteriocine produced by *L. acidophilus*

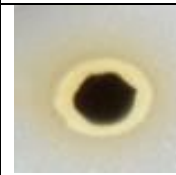
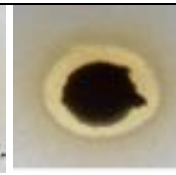

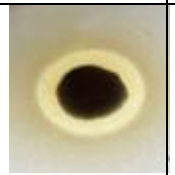
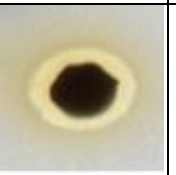







Amino acids	Amount g/100 ml	Amino acids	Amount g/100 ml
Aspartic (ASP)	0.10	Tyrosine (TYR)	0.03
Therionine (THR)	0.01	Phenylalanine (PHE)	0.04
Serine (SER)	0.02	Histidine (HIS)	0.01
Glutamic (GLU)	0.19	Lysine (LYS)	0.06
Glycine (GLY)	0.33	Arginine (ARG)	0.05
Alanine (ALA)	0.16	Proline (PRO)	0.19
Valine (VAL)	0.07	Cystine (CYS)	0.005
Isoleucine (ILE)	0.02	Methionine	0.01
Leucine (LEU)	0.05		

The agar diffusion method was used to study the effect of antimicrobial activity of *Lactobacillus acidophilus* which was mixed with *E. coli*, *K. pneumonia*, *C. albicans*, *C. tropicalis*, *C. famata*, *C. parapsilosis*, *C. catenulate*,

Table (5) demonstrate that the antimicrobial activity of *Lactobacillus acidophilus* against pathogenic isolates, the highest inhibition zone diameter was detected with *E. coli* 13.38±0.46, *C. albicans*

isolates showed inhibition zone diameter range (6.7±0.46, 7.16±0.46, 14.5±0.92, 11.9±0.72, 13.33±0.66 and 11.4±0.83), *C. parapsilosis* showed inhibition zone diameter 12.16±0.96, *C. tropicalis* showed inhibition zone diameter 11.06±0.66, *C. famata* showed inhibition zone diameter 3.16±0.46 while *K. pneumonia* showed inhibition zone 9.33±0.66. *C. catenulata* exhibited resistance to bacteriocin.

Table 5. The antibacterial activity of *Lactobacillus growth* (1 mm /disc) based on the growth inhibition of clinical microbes isolates by disc diffusion method

<i>C.albicans</i>	<i>C.albicans</i>	<i>C.albicans</i>	<i>C.albicans</i>	<i>C.albicans</i>	<i>C.albicans</i>
					
6.7±0.46	7.16±0.46	14.5±0.92	11.9±0.72	13.33±0.66	11.4±0.83
<i>C.catenulata</i>	<i>C.famata</i>	<i>C.parapsilosis</i>	<i>C.tropicalis</i>	<i>E.coli</i>	<i>K.peniamina</i>
					
0.00	3.16±0.46	12.16±0.96	11.06±0.66	13.38±0.46	9.33±0.66

Each value is mean of 3 replicates ± standard error Inhibition zones diameter (mm).

The inhibition zone of clinical microbe isolates: *Lactobacillus* cell culture was tested against pathogenic bacteria *E. coli*, *K. pneumonia*, *C. albicans*, *C. tropicalis*, *C. famata*, *C. parapsilosis*, *C. catenulate* by agar well diffusion method. The inhibition zone is increased with increase bacteriocin concentration. The inhibition zone of *C. albicans* in the concentration 100% is 11.9±0.72 but in the concentration 25% is 5.95±0.27. The inhibition zone of *C. tropicalis* in the concentration 100% is 7.16±0.46 but in the concentration 25% is 2.69±0.49. The inhibition zone of *C. famata* in the concentration 100% is 3.16±0.46 but in the concentration 25% is 0. The inhibition zone of *C. parapsilosis* in the concentration 100% is 12.16±0.96 but in the concentration 25% is 6.49±0.76. The

inhibition zone of *C. catenulata* in the concentration 100% is 0 but in the concentration 25% is 0. The inhibition zone of *E. coli* in the concentration 100% is 13.38±0.46 but in the concentration 25% is 4.76±0.67. The inhibition zone of *K. peniamina* in the concentration 100% is 9.33±0.66 but in the concentration 25% is 4.45±0.58. **(Table 6).**

Antimicrobial activity of bacteriocin produced by *L. acidophilus* in PPE was studied against sensitive bacterial and *Candida* by critical dilution assays. Results are given in **Table 7** by titration of PPE, about (4.00 to 7.00); (5.00 to 3.15); (8.00 to 5.45) and (6.00 to 347) CFU mL⁻¹ were obtained with *K penumina*; *Candida.alb -1*; *Candida.alb -2* and *E. coli*, respectively.

Table 6. Antimicrobial activity of bacteriocin against clinical microbes determined by the agar well diffusion and critical dilution assays

Clinical microbes	Bacteriocin concentration			
	25%	50%	75%	100
<i>C.albicans</i>	5.95±0.27	8.74±0.19	10.25±0.98	11.9±0.72
<i>C.tropicalis</i>	2.69±0.49	4.65±0.72	5.65±0.76	7.16±0.46
<i>C.famata</i>	0.00	0.00	0.00	3.16±0.46
<i>C.parapsilosis</i>	6.49±0.76	8.12±0.59	9.58±0.82	12.16±0.96
<i>C.catenulata</i>	0.00	0.00	0.00	0.00
<i>E.coli</i>	4.76±0.67	8.75±0.91	12.15±0.69	13.38±0.46
<i>K.peniamina</i>	4.45±0.58	6.25±0.76	8.13±0.65	9.33±0.66

*Inhibition zone diameter =(mm) calculated from three replicates for each concentration

Table 7. Growth of isolated clinical microbes in nutrient broth medium with or without partially purified bacteriocin

Time (min.)	Log (CFUml ⁻¹)							
	K pneumonia.		Candida.alb -1		Candida.alb -2		E.coli	
	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.
0	7.00	7.00	5.00	5.00	8.00	8.00	6.00	6.00
5	7.10	6.90	5.17	4.95	8.15	7.85	6.15	5.85
10	7.36	6.73	5.63	4.75	8.45	7.72	6.45	5.62
15	7.58	6.58	5.85	4.55	8.67	7.61	6.67	5.51
20	7.63	6.19	6.15	4.39	8.82	7.50	6.82	5.30
25	7.79	5.85	6.25	4.25	9.05	7.22	7.05	5.12
30	7.92	5.26	6.75	4.10	9.18	6.75	7.18	4.95
35	8.14	5.00	7.12	3.90	9.23	6.52	7.32	4.72
40	8.35	4.85	7.23	3.85	9.59	6.39	7.59	4.49
45	8.58	4.65	7.35	3.75	9.75	6.25	7.75	4.25
50	8.62	4.37	7.50	3.67	9.95	6.10	8.05	4.00
55	8.75	4.23	7.75	3.43	10.15	5.65	8.25	3.75
60	9.00	4.00	8.00	3.15	10.38	5.45	8.58	3.47

Cont. = Control without treatment

Treat.= Treatment

The broth media of pathogenic tested microorganisms without and with bacteriocin (control and treated groups respectively) to test antimicrobial activity against *K pneumonia*, *Candida.alb -1*, *Candida.alb -2* and *E.coli* as pathogenic clinical isolates, using well –defined cell concentration standardized, the concentration of bacterial cells was determined by standard of CFU counting (Schellenberg, et al 2006) (Table 7). The data show that *Lactobacillus acidophilus* produced the antibacterial compounds reducing the number of

pathogenic cells 4 log 10CFU of *K pneumonia* , 3.15, 5.45 log 10CFU of *Candida.alb -1*, *Candida.alb -2* respectively and 3.47 log 10CFU of *E.coli*.

DISCUSSION

Pathological bacteria and candida isolated from vagina, endocervical canal and tip of catheter were identified according to Bergey's Manual of Determinative Bacteriology (Holt, et al 1994) and con-

firmed by VITEK 2 system as *Klebsilla pneumonia*, *E.coli*, *C. albicans*, *C. tropicalis*, *C. famata*, *C. parapsilosis* and *C. catenulate* (Selman, et al 2007).

The vitek analyser was found to be valuable for the speed and accuracy, there are separate cards for Gram positive, Gram negative and yeasts - yeast like organisms, also contain antimicrobial susceptibility test that can determine the minimum inhibitory concentration (MIC) against organism. (Shetty, et al 1998 and Funke, et al 1998).

The pregnancy rate is lower in presence of pathogenic bacteria (*K. pneumonia*, *E. coli*) and *Candida* spp., comparable with those with negative microbial growth with agreement with (Eskandar, et al 2007).

The bacterial contamination has negatively effect on fertility, fertilization and implantation rate (Selman, et al 2007). During embryo transfer techniques, embryos are loaded into a transfer catheter that passes through vagina and cervix into the uterus that may lead to possible microbial contamination of the uterine cavity that may alter the susceptibility of the endometrium and embryo implantation (Aboul fotouh, et al 2008). There are many factors that affect on implantation rates such as patient's age, endometrial receptivity and embryo quality (Egbase, et al 1996). The presence of pathogenic bacteria such as *E.coli* and streptococcus viridians decreased live birth rate (Eckert, et al 2003). The enterobacteriaceae represent the most pathogenic bacteria affect the genital tract, *Escherichia coli* considered the most isolated species followed by *klebsilla* (Selman, et al 2007). Bacterial vaginosis represents a common imbalance of the vaginal bacterial flora that have pathogenic effects including increased rate of late miscarriage, premature rupture of the membrane, endometritis, to preterm labour and delivery (Liversedge, 1999). Disequilibrium in microbiota can favor the growth of opportunist microorganisms and the development of pathologies, such as candidosis, an infection caused by yeasts of the *Candida* genus. Vulvovaginal candidiasis (VVC) is overgrowth of *candida* spp. due to the disruption of the vaginal microbiota may be due to pregnancy, antibiotic therapy, use of contraceptives and spermicide, frequent sexual intercourse, diabetes and immunosuppression (Rathod, et al 2014 and sobel, 2007). *Candida albicans* is the commensal yeast of the oral, gastrointestinal and vagina mucosa in a healthy woman, but the overgrowth of these organism may change from commensal to pathogenic organism (Chakoosari, 2013). *Candida albicans* is the major causal of yeast Vaginitis but there are another

species *Candida glabrata*, *Candida krusei* and *Candida tropicalis* infect the host. The development of VVC has been associated with the lack of H₂O₂-producing lactobacillus species (Vitali, et al 2007). The normal vaginal flora of the human play an important role in preventing colonization of the pathogen including those responsible for bacterial vaginosis, yeast infections, sexually transmitted disease and urinary tract infection (Zhou, et al 2004 and Burton, et al 2003). The microflora of a health human vagina include 50 species of organisms which differ in composition according to several factors such as age, menarche, time in the menstrual cycle, pregnancy and infection (Reid, 2001 and Zhou et al 2004). *Lactobacillus* is the dominant organism of the vaginal flora that have an important role in the inhibition of pathogen (Cannon, 2005).

In this study, *Lactobacillus acidophilus* and its metabolites bacteriocin were tested against pathogenic isolates. The results detected that *L. acidophilus* and bacteriocin have inhibitory effect against pathogens.

Lactobacilli especially *L. acidophilus*, *L. cripatus*, *L. jensenii* and *L. iners* are considered the most dominant microorganisms in the vagina of healthy premenopausal women (Cannon, 2005). *Lactobacillus* can inhibit the growth of pathogens by two mechanisms : (a) by production of antimicrobial factors that have adverse effect of pathogens such as lactic acid, hydrogen peroxide, bacteriocin and bacteriocin like substance such as *nisin*, *lactobrevin*, *acidophilin*, *acidolin*, *lactobacillin*, *lactocidin* and *lactolin* (Chakoosari, 2013 and Pendharkar, et al 2015). (b) by adherence and competition. The mechanism of defence of *lactobacillus* against *candida* colonization is the ability of lactobacilli to adhere and compete for receptors sites on vaginal epithelial cells (Simonetti, et al 2007). Biasoli and Magaró, (2003). observed that the introduction of *L. casei* and *L. acidophilus* in vitro inhibit the adhesion of *candida albicans*. Lactobacilli are able to inhibit histone deacetylases (HDACs) that affect morphogenesis and virulence factor that lead to reduce the adhesion of *candida* sp. to vaginal mucosa. Butyrate and lactate are known as HDACs inhibitors. HDACs inhibitors improve sensitivity of *candida albicans* to azole drugs (Nguyen, et al 2011 and latham, et al 2012).

CONCLUSION

The presence of pathogenic bacteria (*Klebsilla pneumonia* and *E.coli*) and *C. albicans*, *C. tropicalis*

is, *C. famata*, *C. parapsilosis* and *C. catenulate*) have adverse effect on implantation rate and pregnancy outcome. *Candida albicans* is the commensal yeast of the oral, gastrointestinal and vagina mucosa in a healthy woman, but the overgrowth of these organism may change from commensal to pathogenic organism. *Lactobacillus* has antagonist activity against pathogen. This study detected that the use of *Lactobacillus* cells or its metabolites bacteriocin can inhibit the pathogens. This study aim to application *Lactobacillus* or bacteriocin anti-septics; disinfected to maintain a health vagina and prevent the infection of the reproductive tract.

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