

Characterization of Pathogens Community in Women with Vaginal Infections

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Abstract—Vagina organ of women is colonized by a variety of pathogenic and non-pathogenic microorganisms. Bacteria and fungi were the main microbial species isolated from vaginal infections. This study aimed to evaluate the microbial community in patients with vaginal infections and test their resistance to locally used antibiotics. 100 vaginal swabs were collected from married and non-pregnant women suffering from vaginitis who attended to Bent El-Huda Children Teaching Hospital at Thi-Qar province, Iraq. The collection period was from March to October 2019. The patients ages ranged between (15-55) years. Swabs were transported to the laboratory, and directly cultured on specific Culture media. All microbial species were identified using Traditional morphological and biochemical identification tests as well as Vitek 2. Microbial resistance was tested against 15 antibiotics. Number of infected women have increased in age 15 to 40 years, and then decreased from 41 years. A total of 84 bacterial isolate (gram positive and negative) and 33 *Candida* spp. were identified and tested against 15 antibiotics to show their resistance profiles. High diversity and abundance of bacterial species were observed, some of them were resistant to locally used antibiotics. *Candida* species were also resistant to fluconazole. In concluded, the prevalence of bacterial and yeast pathogens with antibiotics and antifungal resistance poses a health problem among married women. Therefore, Commitment to hygiene and avoid indiscriminate use of antibiotics are very necessary.

Keywords— vaginal infections, *S. aureus*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, *C. glabrata*

I. INTRODUCTION

The Vagina is one of the body sites that Colonized by variety of microorganisms (van de Wijgert *et al.*, 2014). The vaginal microbiome has changed over the past years (Kumar & Kumar, 2015). These microorganisms that inhabit the vagina are affected by various internal factors in women of reproductive age due to changes in the ecosystem and physiological system of vagina (Priestley *et al.*, 1997). There are also many external factors may affect the composite of the microbiota including the use of personal hygiene products or medicine (Fashemi *et al.*, 2013). An alteration in the vaginal microbiome may lead to

recurrent vaginal infections which Include; bacterial vaginosis (BV), aerobic vaginitis (AV), and vaginal candidiasis (Ofori-Amoah *et al.*, 2016). Bacterial vaginosis is an infection occur due to overgrowth of bacteria naturally found in the vagina, Particularly in women of reproductive age with depletion of lactobacilli level (Mitchell & Marrazzo, 2014). *Gardnerella vaginalis* and anaerobic bacteria such as *Peptostreptococci*, *Prevotella* spp, and *Mobiluncus* spp are a common organisms implicated in bacterial vaginosis (Bhat, Devi, & Shenoy, 2012; Kaambo *et al.*, 2018). (AV) is a vaginal infections caused by aerobic bacteria *Streptococci* and *E. coli* accompanied with an immunological disorder (Donders *et al.*, 2002; Mendling, 2016). Vaginal candidiasis is the most common yeast infection in women caused by candida species (Vazquez & Sobel, 2011) especially, *C. albicans* which cause about 90% of cases followed by *C. glabrata*. Vaginal candidiasis occur when mucosal membranes of vagina penetrated by candida species causing inflammatory response (Jeanmonod & Jeanmonod, 2019).

Vaginal infections with long term of treatments may turn the vaginal reservoir of multi-drug resistance pathogens (Beigi *et al.*, 2004) Antibiotics resistance was observed in bacteria and candida isolated from vaginal infections (Mn, 2018; Ofori-Amoah *et al.*, 2016).

This study aimed to screen the pathogenic species (bacteria and yeast) in vaginal of infected women and determining the its antibiotics resistance profile .

II. METHODS

A. Samples collection

Vaginal swabs were collected from 100 infected women with different ages attending Bent El-Huda Children Teaching Hospital in Al-Nasiriyah City Thi-Qar province South of Iraq. Infected women were tested on the basis of the presence of symptoms of vaginal infections identified by the gynecologist. The swabs were immediately inserted into a tube containing transport media, and then transported to laboratory. The collected vaginal swabs were directly

Streaked into several petri dish contains blood agar, chocolate agar and MacConkey agar and incubated at 37 °C for 48h. *Candida* samples were cultured on Sabouraud Dextrose agar (SDA), then was incubated aerobically at 37°C for 24-48hrs (Byadarahally *et al.*, 2011).

B. Isolation and Identification

Bacterial species and yeast are identified depending on the morphological features on culture medium and microscopic examination (Benson, 2002). Colonies were diagnosed initially according to its morphological characteristic when grow on media include colony shape, texture, color and edge as well as other properties (Holt *et al.*, 1994).

The biochemical tests were conducted: Coagulase test, Catalase test, Oxidase test, Urease test, Kligler iron (KI), Lactose fermentation, Indole test, Citrate utilization test. API 20 was used for confirmation the identification of *p. vulgaris*, *p. aeruginosa*, *E. coli*, and *k. pneumonia*. For yeast identification, different characteristics of the growing colonies were observed and recorded as well as germ tube test was done to determine albicans and non-albicans species (Sood, 2005).

C. Antibiotics susceptibility test

Susceptibility test was performed on all bacterial and yeast isolates using a panel of antibiotics. Kirby-Bauer disk diffusion susceptibility test protocol (Hudzicki, 2009). The following antimicrobials were used: Levofloxacin (5 µg), Azithromycin (15µg), Rifampin(5µg), Nitrofurantoin(30µg), Tetracycline(30µg), Amikacin(10µg), Imipenem(10µg), Ciprofcin(10µg),PencilinG(10µg),Amoxicillin-clavulanicacid(30µg),Tobramycin (10 µg), Ceftriaxone (10 µg), Gentamycin (30 µg),Trimethoprime-sufamethoxazole(5 µg) and Fluconazole(30 µg). The Sensitivity and resistance were determined by the criteria of the Clinical and Laboratory Standard Institute (CLSI, 2017).

D. Statistical analysis

Data were exported to Excel 2010 sheet to compute and analysis some parameters. Study Population was stratified according to age. We also computed the diversity (Shannon index) and frequency of pathogen species per sample using the percentages. The result of antibiotics susceptibility test was illustrated by computing Chi square test and *p*-value at level 0.05 to show the significant difference between different categorical variables.

III. RESULTS

A. Swab Sampling and relationship of vaginal infections to age categories

Vaginal swabs were collected from 100 infected patient women. The age of patients included in this study ranged from 15 to 55 years. Patients ages were divided into categories (Fig.1). The highest number of patients 24 patients was observed within the age category 21-25 years, following by age categories 26-30 and 31-36 years in number 22 and 21 patients respectively. Other patients

distributed on other ages categories.

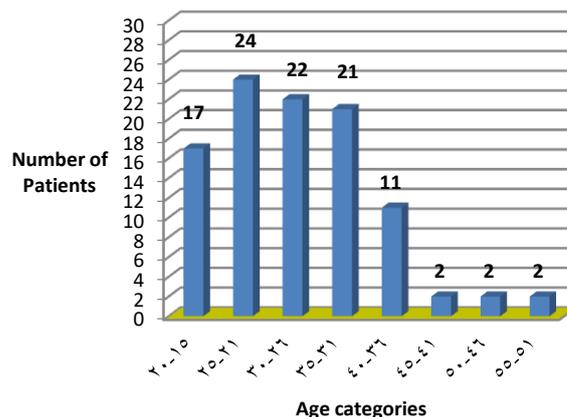


Fig.1: The number of patients according to age categories.

B. Bacterial Identification with Diversity and richness of microbial species

Bacterial species were isolated from vaginal swabs, which were collected from reproductive tract of patients. The frequency of *E. coli* (gram negative group) was the highest (31.6%) followed by *Candida* species (28.2 %). Presence of *Candida* species in such site is not insignificant compared with other bacteria species as *S. aureus* (16.2 %) and *G. vaginalis* (8.5%), which are from gram positive group. The number of other isolates bacterial species varied as shown in Fig.(2). The structure of microbial communities isolated from vaginal infections characterized with high diversity and richness in study samples. Shannon index value (1.7) confirmed the biodiversity although the highest percentage of samples was with one species. On the other hand, the number of microbial species (species abundance) is increasing per sample (four species per sample), indicating that richness also increased (Fig.3).

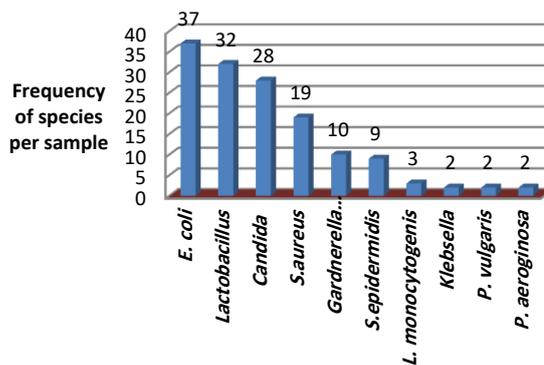


Fig.2: Frequency of microbial species per sample depending on the number of species isolates

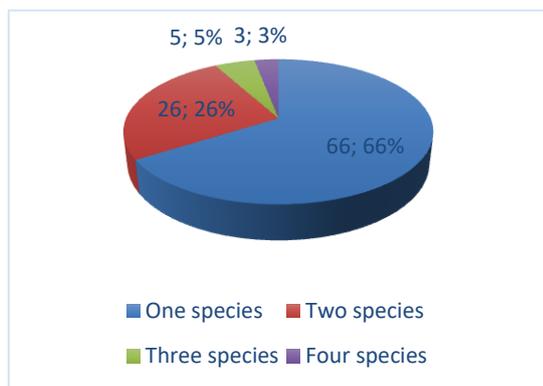


Fig.3: Diversity and richness of microbial species per sample (Shannon index = 1.9 (Typical value = 1.5 – 3.5).

C. Antimicrobial Susceptibility Test of bacteria

The highest percentage of resistance was shown by of *E. coli* (24.6%) followed by *S. aureus* (22.94%) (Table 1). Antibiotic resistance was reported at least by one bacterial isolate from all bacterial species.

Several *Candida* species including *C. albicans* were identified from vaginal swabs. The highest resistant percentage against fluconazole was observed in *Candida non albicans* (54.55%) followed by *Candida albicans* (21.21%). Both Table (2) and (3) show the effect of the types of antibiotics used on the isolated bacterial species.

TABLE1. The percentage of bacterial activity avgainst all antibiotics

Bacterial group	Resistant %	Sensitive %	Intermediate
Gram-Positive (p-value=0.002)			
<i>Listeria monocytogenes</i>	6.45	3.23	0
<i>Staphylococcus aureus</i>	22.94	24.73	13.62
<i>Staphylococcus epidermidis</i>	7.89	15.41	5.73
Gram-negative (p-value=0.009)			
<i>Escherichia coli</i>	24.63	40	20.73
<i>klebsiella</i>	2.93	1.22	0.73
<i>Proteus vulgaris</i>	1.95	2.20	0.73
<i>Pseudomonas aeruginosa</i>	2.44	0.73	1.71
Yeast (p-value=0.003)			
<i>Candida albicans</i>	21.21	21.21	0
<i>Candida non-albicans</i>	54.55	3.03	0

TABLE2. Antimicrobial susceptibility test of most predominant gram positive bacteria isolated from vaginal infections.

Species G+	LVX	%	AZM	%	CIP	%	RIF	%	GEN	%	NIT	%	TE	%	AMK	%	PEN	%	
<i>Listeria monocytogenes</i>	S	0	0	3.23	3	9.68	0	0	2	6.45	0	0	3	9.68	0	0	0	0	
	R	3	9.68	2	6.45	0	0.00	3	9.68	1	3.23	3	9.68	0	3	9.68	3	9.68	
	I	0	0	0.00	0	0.00	0	0	0	0.00	0	0	0	0	0	0	0	0	
<i>Staphylococcus aureus</i>	S	9	29.03	6	19.35	8	25.81	5	16.13	9	29.03	8	25.81	12	38.71	6	19.35	6	19.35
	R	4	12.90	9	29.03	2	6.45	13	41.94	8	25.81	1	3.23	6	19.35	10	32.26	11	35.48
	I	6	19.35	4	12.90	9	29.03	1	3.23	2	6.45	10	32.26	1	3.23	3	9.68	2	6.45
<i>Staphylococcus epidermidis</i>	S	8	25.81	5	16.13	4	12.90	3	9.68	5	16.13	5	16.13	5	16.13	3	9.68	5	16.13
	R	0	0	0.00	2	6.45	3	9.68	4	12.90	2	6.45	4	12.90	4	12.90	3	9.68	
	I	1	3.23	4	12.90	3	9.68	3	9.68	0	0.00	2	6.45	0	2	6.45	1	3.23	

TABLE3. Antimicrobial susceptibility test of most predominant gram negative bacteria isolated from vagina.

Species G-	LVX	%	CI	%	GE	%	T	%	AM	%	IP	%	TO	%	AM	%	CR	%	TS	%	
	X		P		M		E		K		M		B		C		O				
<i>Escherichia coli</i>	S	17	41.46	20	48.8	22	53.7	14	34.1	21	51.2	11	26.8	14	34.15	10	24.39	18	43.9	17	41.46
	R	12	29.27	8	19.5	9	22	12	29.3	7	17.1	3	7.32	19	46.34	19	46.34	5	12.2	7	17.07
	I	6	14.63	7	17.1	4	9.76	9	22	7	17.1	21	51.2	2	4.88	6	14.63	12	29.27	11	26.83
<i>Klebsiella pneumoniae</i>	S	2	4.88	1	2.44	1	2.44	0	0	0	0	1	2.44	0	0	0	0	0	0	0	0
	R	0	0	0	0	1	2.44	2	4.88	2	4.88	0	0	2	4.88	2	4.88	2	4.88	1	2.44
	I	0	0	1	2.44	0	0	0	0	0	0	1	2.44	0	0	0	0	0	0	1	2.44
<i>Proteus vulgaris</i>	S	2	4.88	1	2.44	1	2.44	0	0	0	0	1	2.44	2	4.88	0	0	2	4.88	0	0
	R	0	0	0	0	0	0	2	4.88	2	4.88	0	0	0	0	2	4.88	0	0	2	4.88
	I	0	0	1	2.44	1	2.44	0	0	0	0	1	2.44	0	0	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	S	2	4.88	1	2.44	1	2.44	0	0	1	2.44	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	2	4.88	1	2.44	0	0	2	4.88	2	4.88	1	2.44	2	4.88
	I	0	0	1	2.44	1	2.44	0	0	0	0	2	4.88	0	0	0	1	2.44	0	0	0

IV. DISCUSSION

Genital Tract Infection (GTI) is one of the major health problems associated with complications of gynecological diseases. GTI are caused by some bacterial or fungal pathogens, which are highly variable among

women (Go *et. al.*, 2006). Our study confirms that the community of vaginal microbiota contained several types of microorganism could cause bacterial vaginosis or candidiasis. There was a clear relationship between patient age and GTI. The high incidences of bacterial vaginosis or vaginal candidiasis were found among

women of childbearing age. Particular, high infection rates were reported in three age groups (21-25, 25-30, 31-35). These may be due to ages represent the early years of marriage in which sexual activity increases, in addition to arrival of reproductive hormones to the highest levels. Similar results with respect to age were seen in this study of (Khan & Khan, 2004, and (Krishnasamy, L., Saikumar, C., & Kumaramanickavel, 2019) .

The findings of this study indicate that the vaginal microbiota characterized with high diversity and richness overall community. These results were in agreement with those from Alioua et al., (2016). However, we found that vagina mostly colonized by *E. coli* followed by *Candida species*. Similar result was observed in a study done by Holland et al., 2003, which reported that the most commonly isolated bacteria from the vaginal women with vaginitis was *E. coli*. The occurrence of *E. coli* as member of vaginal communities is indicating that there is a proper receptors, nutrients and oxygen tension for these organisms to grow Also fecal contamination which related to personal hygiene (Cribby et al., 2008). The colonization of *Candida species* in particular *Candida albicans* the mucosal membrane in vagina have been demonstrated in previously studies (Merenstein et al., 2013; Pirota & Garland, 2006). The presence of fungal species may due to several factors e.g: the immunity of patients, age, infection with other diseases, prolonged antibacterial therapy, corticosteroid use, surgical procedures, poor nutritional status (Rodrigues et al., 2019). The presence of both *E. coli* and *C. albicans* are used as indicator.

High antibiotics resistance was observed by bacteria and *Candida species* isolated in our study. Bacterial resistance to some antibiotics may be due to several reasons, including the occurrence a mutation in the gene responsible for the private site of the antibiotic binding, or a change in the resistance mechanisms of these bacteria (Daoud, 2002).

Presence of resistance to fungal species *C. albicans* and non-*albicans Candida spp* have significant clinical relevance This may be due to fluconazole is considered the primary treatment option for treating these infections, this resistance comes by increasing the drug flow and changing or increasing the goal of the drug (Berkow & Lockhart, 2017). Vaginal infections by *Candida* was treated using azole antifungal agents and might due to prolonged exposure to such therapy can emergence novel drug-resist *Candida* (Mathema et al., 2001).

V. CONCLUSION

Many pathogenic bacteria and yeast colonize infected women with vaginitis. Most of these pathogenic agents have been shown high antibiotic resistance.

REFERENCES

Alioua, S., Abdi, A., Fhoula, I., Bringel, F., Boudabous, A., & Ouzari, I. (2016). Diversity of vaginal lactic acid bacterial microbiota in 15 Algerian pregnant women with and without bacterial vaginosis by using culture independent method. *Journal of Clinical and*

Diagnostic Research, 10(9): DC23–DC27. <https://doi.org/10.7860/JCDR/2016/21621.8546>

Beigi, R. H.; Austin, M. N.; Meyn, L. A., Krohn, M. A., & Hillier, S. L. (2004). Antimicrobial resistance associated with the treatment of bacterial vaginosis. *American Journal of Obstetrics and Gynecology*, 191(4), 1124–1129. <https://doi.org/10.1016/j.ajog.2004.05.033>

Benson, H. J. (2002). *Microbiological applications: laboratory manual in general microbiology*. McGraw-Hill.

Berkow, E. L., & Lockhart, S. R. (2017). Fluconazole resistance in *Candida species*: a current perspective. *Infection and Drug Resistance*, 10, 237.

Bhat, S., Devi, N., & Shenoy, S. (2012). Microbiological Profile of Vaginal Swabs. *Journal of Evolution of Medical and Dental Sciences*, 1(4), 509–513. <https://doi.org/10.14260/jemds/80>

Byadarahally Raju, S., & Rajappa, S. (2011). Isolation and identification of *Candida* from the oral cavity. *ISRN Dentistry*, 2011.

Cribby, S., Taylor, M., & Reid, G. (2008). Vaginal Microbiota and the Use of Probiotics. *Interdisciplinary Perspectives on Infectious Diseases*, 2008, 1–9. <https://doi.org/10.1155/2008/256490>

DAOUD, A. M. (2002). ESCHERICHIA COLIAS A CAUSE OF MORTALITIES AMONG. *Egyptian Journal of Agricultural Research*, 80, 1879.

Donders, G. G. G., Vereecken, A., Bosmans, E., Dekeersmaecker, A., Salembier, G., & Spitz, B. (2002). Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: Aerobic vaginitis. *BJOG: An International Journal of Obstetrics and Gynaecology*, 109(1), 34–43. <https://doi.org/10.1111/j.1471-0528.2002.00432.x>

Fashemi, B., Delaney, M. L., Onderdonk, A. B., & Fichorova, R. N. (2013). Effects of feminine hygiene products on the vaginal mucosal biome. *Microbial Ecology in Health and Disease*, 24(1), 19703.

Go, V. F., Quan, V. M., Celentano, D. D., Moulton, L. H., & Zenilman, J. M. (2006). Prevalence and risk factors for reproductive tract infections among women in rural Vietnam. *Southeast Asian Journal of Tropical Medicine and Public Health*, 37(1), 185.

Holland, J., Young, M. L., Lee, O., & Chen, S. C. A. (2003). Vulvovaginal carriage of yeasts other than *Candida albicans*. *Sexually Transmitted Infections*, 79(3), 249–250.

Holt, J. G., Krieg, N. R., & Sneath, P. H. A. (1994). Bergey's manual of determinative bacterology.

Hudzicki, J. (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. *American Society For Microbiology*, 1–13. Retrieved from <https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro>

Jeanmonod, R., & Jeanmonod, D. (2019). *Vaginal Candidiasis (Vulvovaginal Candidiasis)*. StatPearls Publishing.

Kaambo, E., Africa, C., Chambuso, R., & Passmore, J.-A. S. (2018). Vaginal Microbiomes Associated With Aerobic Vaginitis and Bacterial Vaginosis. *Frontiers in Public Health*, 6(March), 1–6. <https://doi.org/10.3389/fpubh.2018.00078>

Khan, I., & Khan, U. A. (2004). A hospital based study of frequency of aerobic pathogens in vaginal infections. *J Rawal Med Coll*, 29(1), 22–25.

Krishnasamy, L., Saikumar, C., & Kumaramanickavel, G. (2019). Aerobic Bacterial Pathogens Causing Vaginitis in Patients Attending A Tertiary Care Hospital and their Antibiotic Susceptibility Pattern. *J Pure Appl Microbiol*.

Kumar, A., & Kumar, D. (2015). Characterization of Lactobacillus isolated from dairy samples for probiotic properties. *Anaerobe*, 33, 117–123.

Mathema, B., Cross, E., Dun, E., Park, S., Bedell, J., Slade, B., ... Perlin, D. S. (2001). Prevalence of Vaginal Colonization by Drug-Resistant Candida Species in College-Age Women with Previous Exposure to Over-the-Counter Azole Antifungals. *Clinical Infectious Diseases*, 33(5), e23–e27. <https://doi.org/10.1086/322600>

Mendling, W. (2016). Vaginal microbiota. In *Advances in Experimental Medicine and Biology* (Vol. 902, pp. 83–93). Springer New York LLC. https://doi.org/10.1007/978-3-319-31248-4_6

Merenstein, D., Hu, H., Wang, C., Hamilton, P., Blackmon, M., Chen, H., ... Li, D. (2013). Colonization by Candida species of the oral and vaginal mucosa in HIV-infected and noninfected women. *AIDS Research and Human Retroviruses*, 29(1), 30–34. <https://doi.org/10.1089/aid.2012.0269>

Mitchell, C., & Marrazzo, J. (2014). Bacterial vaginosis and the cervicovaginal immune response. *American Journal of Reproductive Immunology*, 71(6), 555–563.

Mn, B. (2018). Antimicrobial Resistance Patterns in Acquired Urinary and Genital Tract Infections. *Journal of Microbiology & Experimentation Antimicrobial*, 6(1), 15–21. <https://doi.org/10.15406/jmen.2018.06.00181>

Ofori-Amoah, J., Mahama, F., Mensah, E. K., Orish, V. N., François, M., & Silverius, B. K. (2016). Microbial and Antibiotic Sensitivity Pattern of High Vaginal Swab Culture Results in Sekondi-Takoradi Metropolis of the Western Region of Ghana: Retrospective Study. *European Journal of Clinical and Biomedical Sciences*, 2(5), 45–50. <https://doi.org/10.11648/j.ejcb.20160205.13>

Pirotta, M. V., & Garland, S. M. (2006). Genital candida species detected in samples from women in Melbourne, Australia, before and after treatment with antibiotics. *Journal of Clinical Microbiology*, 44(9), 3213–3217. <https://doi.org/10.1128/JCM.00218-06>

Priestley, C. J., Jones, B. M., Dhar, J., & Goodwin, L. (1997). What is normal vaginal flora? *Sexually Transmitted Infections*, 73(1), 23–28.

Rodrigues, C. F., Rodrigues, M. E., & Henriques, M. (2019). Candida sp. Infections in patients with diabetes mellitus. *Journal of Clinical Medicine*, 8(1), 76.

Sood, R. (2005). *Medical Laboratory Technology*. JAYPEE BROTHERS PUBLISHERS.

van de Wijgert, J. H. H. M., Borgdorff, H., Verhelst, R., Crucitti, T., Francis, S., Verstraelen, H., & Jaspers, V. (2014). The vaginal microbiota: what have we learned after a decade of molecular characterization? *PloS One*, 9(8), e105998.

Vazquez, J. A., & Sobel, J. D. (2011). Candidiasis.