European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 02, 2020

Detection of Herpes Simplex Virus Type 2 and Some Bacterial Vaginosis Isolated from Women in Hilla City/ Iraq

Intisar H. Hadi*1, Hanan N. Hasan2

1,2Maternity and Pediatrics Hospital, Health Directorate of Babylon City, Babylon, MOH/Iraq

E-mail: Intisarhadi2007@gmail.com

Abstract:

Background:

Herpes simplex virus type 2 (HSV-2) and bacterial vaginosis are characterized by frequent reactivation and shedding of the virus and by the attendant risk of transmission to sexual partners.

Aims and objective: to detection herpes simplex virus type two and some bacterial vaginosis from women and inductions of these microbes with them due to chronic infection.

Patients and methods: This Cross Sectional study was carried out for a period of (1) year from July (2019) to July (2020). This study included 150 women were suffering from bacterial vaginosis the sample was high vaginal swabs from married female were admitted to the out-patient clinics of Gynecology and Babylon Hospital for Maternity and Pediatrics. End cervical swab or secretion and blood from each case were collected following standard procedure for microscopic examination and isolation of bacteria. All the isolates were screened and identified via the VITEK-2 System (BioMerieux). This is a phenotypic type of identification, which depends on biochemical reactions to identify the isolates. Herpes simplex virus was detected by immunofluorescence assay and qRCR.

Results:

In this study, 150 vaginal swab positive growth after culturing (aerobic and anaerobic) on different media and detected on GN-ID, GP-ID with VITEK-2 Compact were identified many types of isolated bacteria were found, these isolates were included 35(23.3%) *Streptococcus viridians*, 30(20%) *Gardnerella vaginitis*, 25(16.7%) *Lactobacillus*, 25(16.7%) *Listeria monocytogens*, 10(6.7%) *Bacteroides*, 10(6.7%) *Prevottella*, 8(5.3%) *Fusobacteriam* and 7(4.6%) *Enterococcus faecalis*. In this study revealed that the Immunofluorescence assay detect 55/150 (36.6%) of cases positive for Herpes simplex Virus type 2. Herpes simplex Virus type 2 was isolated from blood samples by qPCR technique. It was found that, from (150) samples of women vaginosis, 55(36.6%) isolated were related of Herpes simplex Virus type 2 at 272 bp.

Conclusions:

Herpes simplex virus type 2 (HSV-2) and bacterial vaginosis are two of the most common important infections of the female genital tract, both are associated with an increased risk for acquiring HIV infection, and they are associated with each other.

Keywords: Herpes simplex virus, Vaginosis, Bacterial infections, RCR, Genital tract.

Introduction:

Herpes simplex virus type 2 (HSV-2) and bacterial vaginosis (BV) are two of the most prevalent infections of the female genital tract [1]. Both infections are associated increased risk for HIV acquisition [2]. While HSV-2 and BV have been epidemiologically linked in many studies [3,4]. Bacterial vaginosis (BV) is a poly-microbial syndrome that represents the main cause of abnormal vaginal discharge worldwide, with prevalence in the general population ranging from 20 to 50% [5]. Several studies have suggested bilateral interactions between BV and HIV-2 [6,7]. Bacterial vaginosis (BV) is a poly-microbial condition characterized by depletion of hydrogen-peroxide producing vaginal lactobacilli and overgrowth of *Gardnerella vaginalis* and other anaerobic bacteria [8].

Although BV is the most common cause of abnormal vaginal discharge, 50%–75% of women with BV remain asymptomatic. Bacterial vaginosis is common worldwide among women of reproductive age [9]. Herpes simplex virus type-2 (HSV-2) is a common worldwide, and the leading cause of genital ulcer disease [10]. Most HSV-2 infections are asymptomatic, with >80% of HSV-2 seropositive individuals asymptomatically shedding virus [11].

Aim of study:

To detection herpes simplex virus type two and some bacterial vaginosis from women and inductions of these microbes with them due to chronic infection.

Materials and methods:

Patients and clinical specimens:

This Cross Sectional study was carried out for a period of (1) year from July (2019) to July (2020). 150 women were suffering from bacterial vaginosis, the sample include high vaginal swabs from married female were admitted to the out-patient clinics of Gynecology and Babylon Hospital for Maternity and Pediatrics. The sampling was carried out by specialized clinicians and all subjects underwent speculum examination. Each female were underwent-detailed history regarding age from (18 to 40 years). The swab was used for clinical diagnosis (Amsel's criteria and Nugent criteria), the swab was immediately placed into Amie's Transport Media to be used for the bacteriological diagnosis by culturing and identification of vaginal flora and other pathogenic bacteria. Five ml of blood samples were collected from the same women according to diagnosis of seiner doctors. The blood samples were divided in to two part, 2ml of blood were putted in EDTA tube, to obtain of whole blood, and 3ml was putted in disposable gel tube to separate serum used to detected the virus by immunofluorescence assay, the samples were stored in freezing at (-20^{o}C) until used.

Ethical Approval: A valid consent was achieved from each patients before their inclusion in the study.

Identification of bacteria:

Colonial morphology and microscopic examination:

A single colony from each primary positive culture (aerobic) on blood, MacConkey and nutrient agar and identify it depending on its morphological properties (colony shape, size, color, borders, and texture) and exam it by light microscope after being stained with Gram's stain. After examination it, biochemical tests were done on each isolates to complete the final identification according to [12, 13, 14]. In addition, culture of all the samples (swabs) was done on a blood agar, chocolate agar and (MRS) agar plates and incubated in microaerophlic condition (using a candle jar) at 37°C for 24 hrs (48 hrs for MRS agar). After overnight incubation, they were checked for bacterial growth.

GN-ID, GP-ID with VITEK-2 Compact:

This system consists of personal computer, reader/incubator made up of multiple internal components including: card cassette, card filler mechanism, cassette loading processing mechanism, card sealer, bar code reader, cassette carousel and incubator, in addition to transmittance optics, waste processing, instruments control electronics and firmware. The system was equipped with an extended identification database for all routine identification tests that provide an improved efficiency in microbial diagnosis, which reduces the need to perform any additional tests, so that safety for both test and user will be improved. All the following steps are prepared according to the manufacturer's instructions. Three ml of normal saline were placed in plane test tube and inoculated with a lopefull-isolated colony. Insert the test tube into dens check machine for standardization of colony to McFarland is standard solution (1.5 x 108 cell/ml). The standardized inoculums were placed into the cassette and a sample identification number entered into the computer software via barcode. The VITEK-2 card type then is read from barcode placed on the card during manufacture, and the card, thus, connected to the sample ID number. Then the cassette was placed in the filler module. When the cards were filled, the cassette was transferred to the reader/ incubator module. All subsequent steps were handled by the instrument, the instrument; controls the incubation temperature, optical reading of the cards and continually monitors and transfers test data to the computer for analysis.

Immunofluorescence assay for detection of microorganisms:

Indirect Immunofluorescence assay (IFA) Kit for the simultaneous diagnosis in human serum of IgM antibodies of the main etiological agents of infectious HsV2.

Molecular study:

Genomic viral DNA Extraction:

Genomic DNA from blood samples were extracted by using gSYAN DNA kit extraction kit (Frozen Blood protocol) Geneaid. USA.

Genomic DNA estimation by using Nano drop spectrophotometer

The extracted blood genomic DNA was checked by using Nano drop spectrophotometer (THERMO. USA), which measured DNA concentration (ng/μL) and check the DNA purity by reading the absorbance at (260 /280 nm).

Results and discussion:

In this study, 150 vaginal swab positive growth after culturing (aerobic and anaerobic) on different media and detected on GN-ID, GP-ID with VITEK-2 Compact were identified many isolated types of bacteria were found, these isolates were included 35(23.3%) *Streptococcus viridians*, 30(20%) *Gardnerella vaginitis*, 25(16.7%) *Lactobacillus*, 25(16.7%) *Listeria monocytogens*, 10(6.7%) *Bacteroides*, 10(6.7%) *Prevottella*, 8(5.3%) *Fusobacteriam* and 7(4.6%) *Enterococcus faecalis*. These results were shown in Table (1).

Table (1): different types of bacteria isolated from women suffering from vaginosis

No.	Type of bacteria	Number of isolates	%
1.	*Streptococcus viridians*	35	23.3%
2.	*Gardenerella vaginitis*	30	20%
3.	*Lactobacillus*	25	16.7%
4.	*Listeria monocytogens*	25	16.7%
5.	*Bacteroides*	10	6.7%
6.	*Prevottella*	10	6.7%
7.	*Fusobacteriam*	8	5.3%
8.	*Enterococcus faecalis*	7	4.6%
Total		**150**	**100%**

These results were agreement with results obtained by [15] who found that the prevalence of viridans group streptococci in the female genital tract were found in 25% were culture positive for viridans group streptococci. Viridans streptococci are normal inhabitants of mucous membrane-lined cavities of the animals and humans. They form part of the normal flora of the upper respiratory tract, all the regions of the gastrointestinal tract, the female genital tract and are most commonly found in the oral cavity [16]. A study of [17] found that *Gardenerella vaginalis* was isolated from vaginosis women in percentage (25.33%). The risk to get *G. vaginalis* resulted from their ability to biofilm formation and may be establish chronic persistent infection [18]. Biofilm formation make the infection hard to cured and this result from fact that biofilm producing bacteria need tenfold concentration of antibiotics to get rid when compared with itself but without biofilm [19]. In addition, the results were disagreement with results of [20] who found that the percentage of *Lactobacillus* spp was (40%), while [21] found that the percentage was (32.5%). The percentage of *Lactobacillus* spp. as the predominant in vaginal flora, is an indicator of healthy vagina but its presence in low amounts with a high proportion of other pathogens is pathogenic. They play an important role in protecting against pathogens invasion or overgrowth by production of hydrogen peroxide, bacteriocins, and lactic acid [22]. A study of [23] found that *Listeria* species were isolated from cervico-vaginal samples. Many women can carry *Listeria* asymptomatically in their GI tract or vagina [24]. *Listeria monocytogenes* are members of the normal microbes of the female genital tract Listeria *monocytogenes* may infect the new- borns, leading to neonatal sepsis and meningitis [25]. The results in this study were disagreement with results of [26, 27] who found that *Bacteroides* and *Prevottella* were isolated from vaginal infection in rate (15.33%) and (20%) respectively. The bacteria like Mobiluncus, Gardnerella, *Bacteroides* and *Prevottella* can be leads to vaginosis [28], and they are the major reason that associated with BV with preterm and abortion but this way for assay procedure are costly [29]. *Enterococcus faecalis* is one of the main constituents of the flora in human and animal intestinal tracts [30]. *E. faecalis* had an ability

to form biofilm in the vagina considering that the urinary tract abutted the genital tract, we speculated that *E. faecalis* in the vagina was probably derived from the intestinal tract [31, 32]. The sera were showed anti-IgM to Herpes simplex virus type 2, Immunofluorescence is the visualization of antigens using antibodies as fluorescent probes. The benefits of immunofluorescence are numerous, and the technique has proven to be a powerful tool for determining the cellular distribution of known antigens in the frozen tissues or in the localization of specific DNA sequences on chromosomes. The method has achieved the status of combining high sensitivity with high resolution in the visualization of antigens and will be a major tool for many years that any pathologist studying cells or molecules cannot afford to ignore. For a methodology article on immunofluorescence labeling of formalin-fixed, paraffin-embedded tissue or microbial antigens [33]. In this study revealed that the IFA detect 55/150 (36.6%) of cases positive for HsV2. The results were shown in Figure (1). This results were agreement with results of [34] who found that the direct immunofluorescence assay of specimen of vaginosis gave a sensitivity (38.6%) related to HsV2. In this study, Herpes simplex Virus type 2 was isolated from blood samples by qPCR technique. It was found that, from (150) samples of women vaginosis, 55(36.6%) isolated were related of HsV2 at 272 bp as shown in figure (2). The speed and sensitivity of polymerase chain reaction (PCR) have made it a popular method for the detection of microbiological agents in both research and clinical specimens [35]. For the detection and genotyping of herpes simplex virus (HSV) in clinical specimens, PCR has proven to be faster, more sensitive and safer than earlier methods which included isolation of the virus in cell culture followed by immunofluorescence microscopy. While PCR-based assays for HSV detection possess clear advantages over these earlier techniques [36]. In PCR, a certain kind of reagent (primers) is used to target a small but specific part of the virus-genome (deoxyribo-nucleic acid (DNA) or ribonucleic acid (RNA) in question, and with the help of an enzyme, this small genomic area is amplified over and over again if the target is present [37]. Our data are in accord with previous findings regarding other risk factors for BV. HIV infection has been shown to be a major risk for BV development [38].

Conclusions:

Herpes simplex virus type 2 (HSV-2) and bacterial vaginosis are two of the most common important infections of the female genital tract, both are associated with an increased risk for acquiring HIV infection, and they are associated with each other.

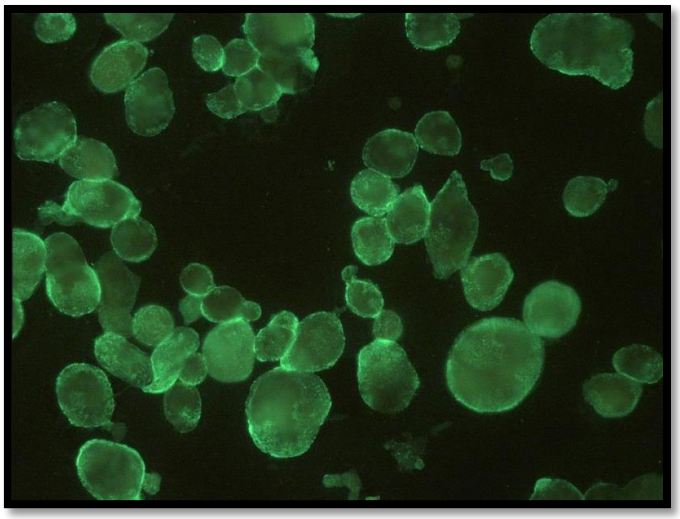

Figure (1): Detection of Herpes simplex virus type 2 by immunofluorescence assay

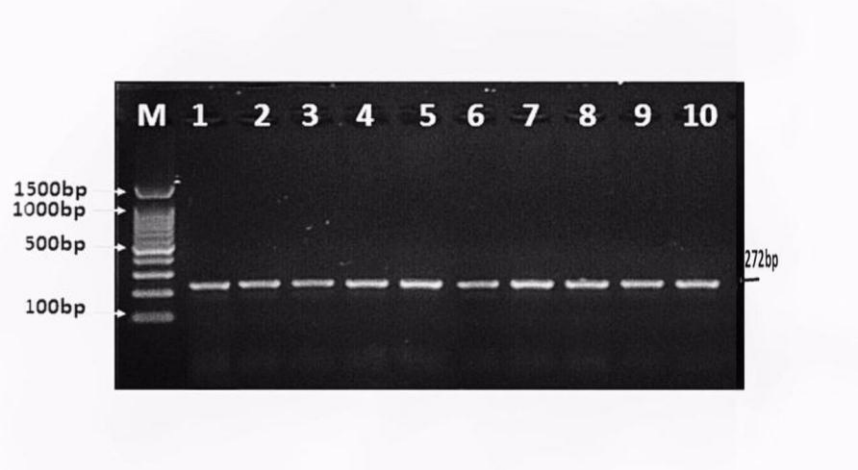

Figure (2): Agarose gel electrophoresis image that showed PCR product analysis of *HsV-2* gene virus in blood samples. M (Marker ladder 1500-100bp). Lane (1-10) some positive gene virus in at 272 bp product size

References:

1. Shannon, B., Gajer, P., Yi, T. J., Ma, B., Humphrys, M. S., Thomas-Pavanel, J., ... & Huibner, S. (2017). Distinct effects of the cervicovaginal microbiota and herpes simplex type 2 infection on female genital tract immunology. *The Journal of infectious diseases*, *215*(9), 1366-1375.
2. Looker, K. J., Elmes, J. A., Gottlieb, S. L., Schiffer, J. T., Vickerman, P., Turner, K. M., & Boily, M. C. (2017). Effect of HSV-2 infection on subsequent HIV acquisition: an updated systematic review and meta-analysis. *The Lancet infectious diseases*, *17*(12), 1303-1316.
3. Mehta, S. D., Nandi, D., Agingu, W., Green, S. J., Bhaumik, D. K., Bailey, R. C., & Otieno, F. (2020). Vaginal and Penile Microbiome Associations with HSV-2 in Women and their Male Sex Partners. *The Journal of Infectious Diseases*.
4. Looker, K. J., Welton, N. J., Sabin, K. M., Dalal, S., Vickerman, P., Turner, K. M., ... & Gottlieb, S. L. (2020). Global and regional estimates of the contribution of herpes simplex virus type 2 infection to HIV incidence: a population attributable fraction analysis using published epidemiological data. The Lancet Infectious Diseases, 20(2), 240-249.
5. Bertini, M., Serdaroglu, S., & Kutlubay, Z. (2017). Bacterial vaginosis and sexually transmitted diseases: relationship and management. *Fundamentals of Sexually Transmitted Infections*, 75.
6. Haddad, L. B., Wall, K. M., Kilembe, W., Vwalika, B., Khu, N. H., Brill, I., ... & Allen, S. (2018). Bacterial vaginosis modifies the association between hormonal contraception and HIV acquisition. *AIDS (London, England)*, *32*(5), 595.
7. Alizadehmohajer, N., Shojaeifar, S., Nedaeinia, R., Esparvarinha, M., Mohammadi, F., Ferns, G. A., ... & Balouchi, A. (2020). Association between the microbiota and women's cancers–Cause or consequences?. *Biomedicine & Pharmacotherapy*, *127*, 110203.
8. Ventolini, G., & Hamood, A. Current progresses on vaginal microbiome, bacterial vaginosis and biofilms.
9. Ranjit, E., Raghubanshi, B. R., Maskey, S., & Parajuli, P. (2018). Prevalence of bacterial vaginosis and its association with risk factors among nonpregnant women: A hospital based study. *International journal of microbiology*, *2018*.
10. Posavad, C. M., Zhao, L., Dong, L., Jin, L., Stevens, C. E., Magaret, A. S., ... & Koelle, D. M. (2017). Enrichment of herpes simplex virus type 2 (HSV-2) reactive mucosal T cells in the human female genital tract. Mucosal immunology, 10(5), 1259-1269.
11. Torcia, M. G. (2019). Interplay among vaginal microbiome, immune response and sexually transmitted viral infections. *International journal of molecular sciences*, *20*(2), 266.
12. McFadden, J. (2000). Biochemical Tests for the Identification of Aerobic Bacteria. *Clinical Microbiology Procedures Handbook, 3rd Edition*, 503–642.

13. Baron, E. J., Peterson, L. R. and Finegoldens, S. M. (1994). Bailey and Scotts diagnostic microbiology .9th Ed. Mosology. Co. USA.
14. Collee, J. G., Fraser, A. G., Marmion, B. P. and Simmon, A. (1996). Pactical Medical Microbiology. 14th Ed. Churchill Living Stone, USA.
15. Cybulski, Z., Urbański, B., Grabiec, A., Talaga, Z., Pawlak, M., Wareńczak-Florczak, Ż., & Roszak, A. (2019). The incidence of inflammation among patients suffering from cervix cancer with positive beta haemolytic streptococci cultures from genital tract. *Reports of Practical Oncology & Radiotherapy*, *24*(5), 428-431.
16. Bunyan, I. A., Umran, B. J., & Salman, Z. K. (2017). Analyzing the correlation of TLR-4 Asp299Gly and Thr399Ile polymorphism in women with Bacterial Vaginosis in Hillah city. *Research Journal of Pharmacy and Technology*, *10*(12), 4178-4182.
17. Bunyan, I. A., Gatea, A. K., & Hameed, A. K. (2020). Molecular identification and genotyping of Atopobium vaginae, 16s rRNA gene from Bacterial Vaginosis miscarriage women in AL-Hillah city. *Quality Assurance*, *11*(1), 124-130.
18. Bunyan, I. A., Gatea, A. K., & Hameed, A. K. MOLECULAR DETECTION OF BACTERIAL VAGINOSIS AND ITS ASSOCIATION WITH MISCARRIAGE IN AL-HILLAH CITY. Health, 23(5), 308.
19. Abedon, S. T. (2019). Phage-antibiotic combination treatments: Antagonistic impacts of antibiotics on the pharmacodynamics of phage therapy?. *Antibiotics*, *8*(4), 182.
20. Castellano, P., Ibarreche, M. P., Borges, L. L., Arias, F. N., Ross, G. R., & De Martinis, E. P. (2018). Lactobacillus spp. impair the ability of Listeria monocytogenes FBUNT to adhere to and invade Caco-2 cells. *Biotechnology letters*, *40*(8), 1237-1244.
21. Aslam, S., Sayeed Saraf, V., Saleem, S., Saeed, S., Javed, S., Janjua, M., & Bokhari, H. (2020). Lactobacillus species signature in association with term and preterm births from low-income group of Pakistan. *The Journal of Maternal-Fetal & Neonatal Medicine*, 1-10.
22. Kovachev, S. (2018). Defence factors of vaginal lactobacilli. *Critical reviews in microbiology*, *44*(1), 31-39.
23. Whitman, K. J., Bono, J. L., Clawson, M. L., Loy, J. D., Bosilevac, J. M., Arthur, T. M., & Ondrak, J. D. (2020). Genomic-based identification of environmental and clinical Listeria monocytogenes strains associated with an abortion outbreak in beef heifers. *BMC veterinary research*, *16*(1), 1-13.
24. Ghasemian, A., Nojoomi, F., Abhari, M. H., & Mehrabadi, J. F. (2018). A multiplex real-time PCR technique for simultaneous detection of Listeria monocytogenes and Streptococcus agalactiae among asymptomatic pregnant women. *Reviews in Medical Microbiology*, *29*(3), 136-139.
25. Anand, V., Holmen, J., Neely, M., Pannaraj, P. S., & Bard, J. D. (2016). The brief case: neonatal meningitis caused by Listeria monocytogenes diagnosed by multiplex molecular panel.
26. Chen, D., Chen, G., Chen, C., Zeng, X., & Ye, H. (2020). Prebiotics effects in vitro of polysaccharides from tea flowers on gut microbiota of healthy persons and patients with inflammatory bowel disease. *International Journal of Biological Macromolecules*.
27. Würstle, S., Hapfelmeier, A., Wöhrle, C., Dichtl, K., Lahmer, T., Rasch, S., ... & Pichler, M. (2019). Changes in pathogen spectrum and antimicrobial resistance development in the time-course of acute necrotizing pancreatitis. *Journal of Gastroenterology and Hepatology*, *34*(12), 2096-2103.
28. Scapaticci, M., Da Rin, G., & Bartolini, A. (2020). Comparison between a novel molecular tool and conventional methods for diagnostic classification of bacterial vaginosis: is integration of the two approaches necessary for a better evaluation?. *The new Microbiologica*, *43*(3).
29. Waiswa, P., Higgins, B. V., Mubiri, P., Kirumbi, L., Butrick, E., Merai, R., ... & Preterm Birth Initiative Kenya & Uganda Implementation Research Collaborative. (2020). Pregnancy outcomes in facility deliveries in Kenya and Uganda: A large cross-sectional analysis of maternity registers illuminating opportunities for mortality prevention. *Plos one*, *15*(6), e0233845.
30. Wang, K., Cao, G., Zhang, H., Li, Q., & Yang, C. (2019). Effects of Clostridium butyricum and Enterococcus faecalis on growth performance, immune function, intestinal morphology, volatile fatty acids, and intestinal flora in a piglet model. *Food & Function*, *10*(12), 7844-7854.

31. Liu, X. L., Xiao, Y., Zhang, H., & Wu, L. J. (2018). Comparison of vaginal flora in patients with spontaneous abortion and women with normal first-trimester. *Reproductive and Developmental Medicine*, *2*(3), 150.
32. Kao, P. H. N., & Kline, K. A. (2019). Dr. Jekyll and Mr. Hide: how Enterococcus faecalis subverts the host immune response to cause infection. *Journal of molecular biology*, *431*(16), 2932-2945.
33. Jamil, S. (2019). *Serological, Molecular Indentification of Cytomegalo, Herpes Simplex Viruses And Phylogenetic Analysis of Hsv1-Tk And Hsv-2-gD Gene in Pregnant Women in Peshawar, Pakistan* (Doctoral dissertation, university of Peshawar, Peshawar).
34. DANIEL, K. K. (2019). *SEROPROFILE, RISK FACTORS OF HEPATITIS B, C, SYPHILIS AND DISEASE STAGE AMONG HIV PATIENTS ATTENDING ENGINEER HOSPITAL, NYANDARUA COUNTY, KENYA* (Doctoral dissertation, Kenyatta University).
35. Babafemi, E. O., Cherian, B. P., Banting, L., Mills, G. A., & Ngianga, K. (2017). Effectiveness of real-time polymerase chain reaction assay for the detection of Mycobacterium tuberculosis in pathological samples: a systematic review and meta-analysis. *Systematic reviews*, *6*(1), 1-16.
36. Hayette, M. P., Seidel, L., Adjetey, C., Darfouf, R., Wéry, M., Boreux, R., ... & Arrese, J. (2019). Clinical evaluation of the DermaGenius® Nail real-time PCR assay for the detection of dermatophytes and Candida albicans in nails. *Medical mycology*, *57*(3), 277-283.
37. Rafique, S. (2018). *Seroprevalence and molecular characterization of Infectious bronchitis virus variants from poultry in Pakistan* (Doctoral dissertation, QUAID-I-AZAM UNIVERSITY ISLAMABAD).
38. Alcendor, D. J. (2016). Evaluation of Health Disparity in Bacterial Vaginosis and the Implications for HIV-1 Acquisition in African American Women. *American Journal of Reproductive Immunology*, *76*(2), 99-107.