

# Potential use of artificial intelligence for vaginal swab analysis in the assessment of common genital disorders: a pilot study

Claudio Foschi<sup>1,2</sup>, Monica Cricca<sup>1,2</sup>, Silvia Lafratta<sup>1</sup>, Giacomo Nigrisoli<sup>1</sup>, Michele Borghi<sup>1</sup>, Andrea Liberatore<sup>1</sup>, Gabriele Turello<sup>1</sup>, Tiziana Lazzarotto<sup>1,2</sup>, Simone Ambretti<sup>2</sup>

<sup>1</sup>Microbiology, DIMES, University of Bologna, Italy

<sup>2</sup>Microbiology Unit, IRCCS S. Orsola-Malpighi Hospital, Bologna, Italy

## SUMMARY

Genital disorders, such as vulvo-vaginal candidiasis (VVC), bacterial vaginosis (BV), and aerobic vaginitis (AV), are very common among fertile women and negatively impact their reproductive and relational life. Vaginal culture can help in the diagnostic workflow of these conditions. Recently, culture-based techniques have taken advantages of up-front specimen processing units, which also include a digital imaging system to record images of plates at programmable time points.

In this proof-of-concept study, we assessed the characteristics of digital plate images of vaginal swabs plated by WASPLab system into different media, in order to detect microbial growth morphotypes specific for each genital disorder.

A total of 104 vaginal specimens were included: 62 cases of normal lactobacilli-dominated flora, 12 of BV, 16 of VVC, and 14 of AV were analysed. Vaginal specimens were plated by WASPLab system into different chromogenic media and blood agar plates. Plate images were taken automatically by the digital imager at 38 h post-inoculation.

We found that each genital condition was characterized by specific morphotypes in terms of microbial growth and colony colour, thus allowing the potential use of artificial intelligence not only to assess the presence of specific microbial genera/species but also to 'categorize' peculiar clinical conditions.

Received March 30, 2022

Accepted September 14, 2022

## INTRODUCTION

Genital infections/disorders affect many reproductive-aged women with significant clinical, relational, and socio-economic impact (White *et al.*, 2011). The most common genital disorders in fertile women include vulvo-vaginal candidiasis, bacterial vaginosis, and aerobic vaginitis.

Bacterial vaginosis (BV) is a condition of dysbiosis characterized by a shift in microbial composition from the normally *Lactobacillus*-dominated vaginal environment to a high-complexity, polymicrobial community (Campisciano *et al.*, 2021). The most common microbial fingerprints of BV reside in the vaginal presence of higher loads of anaerobic bac-

teria, such as *Gardnerella vaginalis*, *Atopobium* spp., and *Prevotella* spp. (Ceccarani *et al.*, 2019). Although BV can be asymptomatic, the microbial changes are usually accompanied by vaginal symptoms, such as a thin, greyish-white discharge and a foul-smelling "fishy" vaginal odour (Ding *et al.*, 2021).

Vulvo-vaginal candidiasis (VVC) is caused by yeasts of *Candida* genus, which, in particular conditions, instead of being part of the normal vaginal microflora, become a robust opportunistic pathogen, with a tendency to overgrow (Denning *et al.*, 2018). *C. albicans* is responsible for 80-92% of VVC cases, even though non-*albicans* species are becoming more and more prevalent, with higher rates of recurrence and antimicrobial resistance (Denning *et al.*, 2018). The most common clinical characteristics of VVC include vaginal itching, burning, white or cottage cheese-like discharge, and pain during intercourse (Yano *et al.*, 2019).

Aerobic vaginitis (AV) is a vaginal infectious entity characterized by a dysbiotic vaginal microflora, with the reduction of lactobacilli and the abnormal presence of aerobic, mainly enteric bacteria such as

### Key words:

Vaginal disorders, PhenoMatrix, WaspLab, Vaginal swabs, Artificial intelligence.

### Corresponding author:

Claudio Foschi

E-mail: claudio.foschi2@unibo.it

*Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus* (Donders *et al.*, 2017). Usually, during AV, variable degrees of inflammation (i.e., leucocytes) are present, leading to various symptoms, as introital and vaginal redness, stinging and burning sensations, the presence of sticky, yellow vaginal discharge, and dyspareunia (Sonthalia *et al.*, 2020).

The diagnosis of these genital conditions relies on the integration of clinical signs/symptoms with microbiological data. Although several molecular techniques (i.e., NAAT) have recently been introduced in routine laboratory practice, microscopic and culture-based approaches remain the reference and most common methods for the diagnosis of genital dysbiosis/infections (Paladine and Desai, 2018; Richter *et al.*, 2019; Lamont *et al.*, 2020).

Recently, culture-based techniques have taken advantages of the introduction of an up-front specimen processing unit, where samples are plated on agar media and automatically moved from processing to incubation to bench (e.g., WASPLab system, Copan Diagnostics, Brescia, Italy). The system also includes a digital imaging system to record images of plates at programmable time points (Foschi *et al.*, 2020; Foschi *et al.*, 2021).

Moreover, an advanced artificial intelligence module (PhenoMatrix software, Copan Diagnostics) can develop algorithms able to pre-assess and pre-sort culture plates based on its 'reading' of digital plate images. The software can segregate culture plates according to the presence of bacterial growth, and the colour detection module can recognize and differentiate colony colours on chromogenic media (Van *et al.*, 2019; Faron *et al.*, 2016).

In this proof-of-concept study, we assessed the characteristics of digital plate images of vaginal swabs plated by WASPLab system into chromogenic media. To detect microbial growth morphotypes specific for each genital disorder, we included vaginal specimens from women with various clinical conditions. These data could help to develop advanced algorithms in PhenoMatrix software able to segregate culture plates based on 'genital conditions' (e.g., differentiate 'healthy subjects' from women suffering from VVC, BV, or AV).

## METHODS

### *Study setting and sample collection*

The study was performed at the Microbiology Unit of S. Orsola-Malpighi University Hospital of Bologna (Italy). Vaginal swabs (eSwab, Copan Diagnostics) submitted to the laboratory for routine diagnostic procedures from January to March 2021 were prospectively analysed.

Each vaginal sample underwent a Gram-stained microscopic evaluation, a Nugent score assessment (Zozaya-Hinchliffe *et al.*, 2010), as well as a micro-

bial culture on solid media (see specific paragraph below).

Based on clinical and microbiological data, vaginal swabs were categorized into various groups, each indicating a different clinical condition/genital disorder:

- 1) 'normal' vaginal environment (absence of symptoms, microscopic presence of a lactobacilli-dominated flora, Nugent score ranging from 0 to 3);
- 2) BV condition (reduction/absence of lactobacilli, 'clue cells' and presence of a mixed flora consisting of small and curved Gram-variable rods, Nugent score between 7 and 10) (Zozaya-Hinchliffe *et al.*, 2010);
- 3) VVC (suggestive symptoms, together with the microscopic presence of yeasts) (Baron *et al.*, 2013);
- 4) AV (suggestive symptoms, diminished/absent lactobacilli, microscopic presence of leukocytes, parabasal cells, small coliform bacilli, cocci or chains) (Donders *et al.*, 2017).

'Mixed' conditions (e.g., AV and VVC at the same time), as well as cases with a Nugent score between 4 and 6 ('intermediate' vaginal flora) were excluded from the study.

The required sample size of the study was evaluated according to the formula proposed by Viechtbauer *et al.* for pilot studies (Viechtbauer *et al.*, 2015). Since the genital disorders included in the study are very common among fertile women (e.g., AV prevalence: 8-15%, BV prevalence: 30%), we determined that an enrolment of about 15 women for each condition would be sufficient, assuming a confidence interval of 95% (Donders *et al.*, 2017; Javed *et al.*, 2019).

This study was conducted according to the regulations of the Ethical Committee of S. Orsola-Malpighi Hospital as well as the 1964 Helsinki Declaration and its later amendments. All samples remained anonymous throughout the duration of the study.

### *WaspLab workflow for vaginal swabs*

Microbial culture from vaginal swabs was performed as follows:

- 1) using WASPLab for processing, a volume of 10  $\mu$ L of the vaginal swab (eSwab) is automatically plated (four-streak method) onto three culture media, i.e., tryptic soy agar with 5% sheep blood, CHROMagar Orientation medium and CHROMagar Candida medium (Kima Meus, Padua, Italy). CHROMagar Orientation is a non-selective differentiated medium for the isolation, differentiation, and enumeration of bacteria, especially for genitourinary tract pathogens. Specific chromogens allow the development of colorimetric changes in bacterial colonies based on genus/species: for example, *Escherichia coli* colonies appear dark pink to reddish; colonies of *Klebsiella* spp., *Citrobacter* spp. and *Enterobacter* spp. are metallic blue; enterococci appear as small-sized blue-green colonies (Ohtaki *et al.*, 2020).

- CHROMagar Candida medium is a selective and differential medium for the isolation of fungi. With the inclusion of chromogenic substrates, the colonies of the most common species produce different colours on the isolation plate, thus allowing the direct identification of *C. albicans* (green colour) and *C. tropicalis* (blue colour), while other yeast species (*C. krusei*, *C. glabrata*, *S. cerevisiae*, etc.) cannot be discriminated, typically appearing with white/pink/purple colours (Ahn *et al.*, 2021).
- Inoculated plates are moved by a conveyor belt into the WASPLab incubator, where the plates are incubated at 35°C in an aerobic atmosphere. As described elsewhere (Faron *et al.*, 2016; Kirn, 2016), the WASPLab contains a digital imager to automatically take images of plates at programmable time points throughout incubation. In our workflow, plate images are taken at 38 h post-inoculation, defined as the final incubation time on WASPLab for the growth and detection of the commonest commensal and pathogenic bacteria of the genital tract.
  - CHROMagar Candida plates are automatically screened by PhenoMatrix software incorporated into WASPLab. This software analyses the plates to identify differences in growth and colony colour and is programmed to correspond specifically to the medium type used by the laboratory. By means of an internal algorithm, the software automatically separates 'negative' from 'non-negative' plates. In our protocol, plates with fungal growth are further subdivided by the software in '*C. albicans* complex', *C. tropicalis* and 'other' *Candida* species (i.e., needing species identification by other method), based on colony colour. Moreover,

PhenoMatrix gives a semiquantitative evaluation of the number of *Candida* colonies (e.g., low, intermediate, or large number of colonies).

- Digital images of tryptic soy agar and CHROMagar Orientation media are read by the operator and checked for the presence and type of microorganisms. When needed, microbial colonies underwent a species level identification by MALDI-TOF mass spectrometry (MALDI-TOF MS). Moreover, an antimicrobial susceptibility testing (AST) is performed for bacterial pathogens.

### Sample analyses

We assessed if the various genital conditions/disorders included in the study (i.e., 'healthy', BV, VVC, AV) were characterized by specific and recognizable microbial growth 'morphotypes'.

## RESULTS

A total of 104 vaginal samples were included in the study: 62 cases (59.6%) of normal vaginal flora, 12 of BV (11.5%), 16 of VVC (15.3%) and 14 of AV (13.4%) were analysed. *Table 1* shows the clinical/microscopic criteria used for the diagnosis of each genital disorder, as well as a summary of microbial culture characteristics. A detailed description of microbial growth phenotypes referred to each genital condition is reported below.

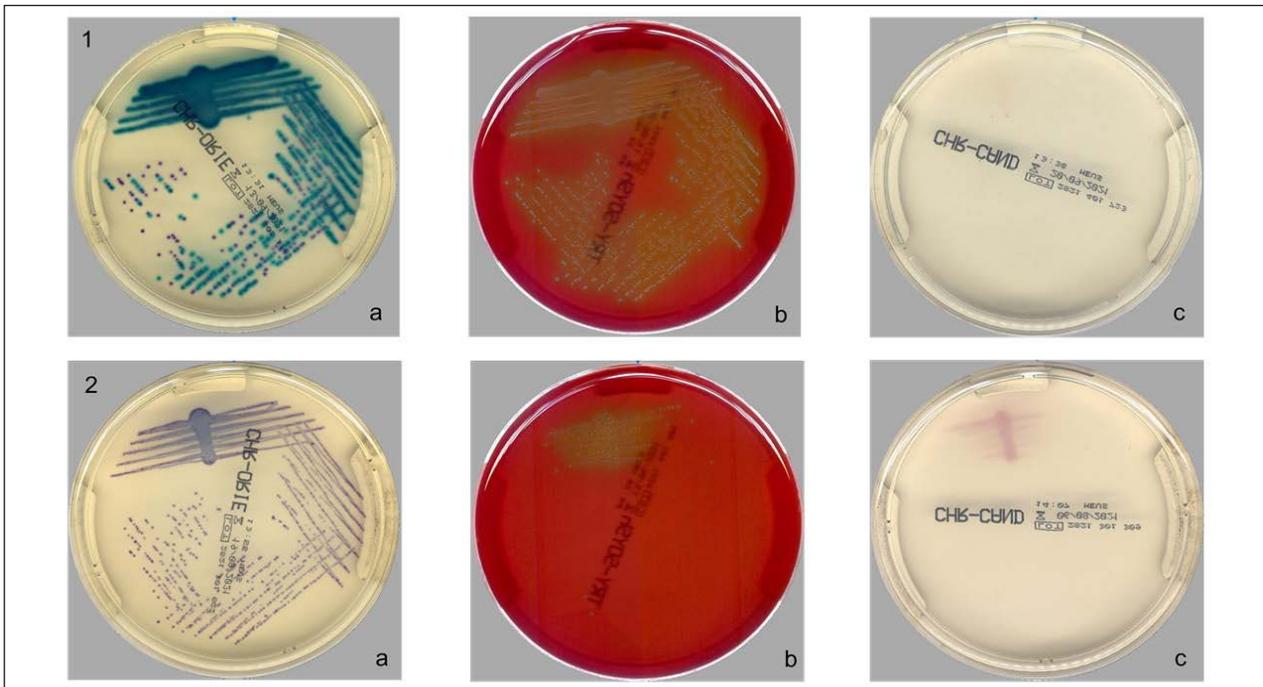
### Normal vaginal environment

About 70% of women with a normal vaginal flora showed a Nugent score of 0-1, whereas the remaining 30% of subjects showed a score of 2-3. Cultures of vaginal samples belonging to 'healthy' women were

**Table 1** - Microscopic and culture characteristics of the genital disorders analysed in the study.

| Vaginal condition             | Clinical/microscopic criteria   | Culture phenotype  | Concordance between microscopic diagnosis and culture |
|-------------------------------|---|--|---|
| <b>Normal flora</b><br>(n=62) | No symptoms; microscopic presence of gram-positive rods (lactobacilli-dominated flora); 70% Nugent score 0-1; 30% score 2-3       | Growth of lactobacilli (flat blue/violet colonies on CHROMagar Orientation; small grey colonies with a 'viridans' haemolysis on TSA-5% sheep blood).   | 93.5% (58/62)   |
| <b>BV</b><br>(n=12)           | Reduction/absence of lactobacilli, 'clue cells', mixed flora consisting of small and curved Gram-variable rods; Nugent score 8-10 | Absence/reduction of lactobacilli; presence of small grey translucent colonies on TSA-5% sheep blood (identified as <i>Gardnerella vaginalis</i> ).  | 91.6% (11/12)   |
| <b>VVC</b><br>(n=16)          | Suggestive symptoms; microscopic presence of yeasts (fungal spores/hyphae)  | Growth of lactobacilli; significant growth of <i>Candida</i> colonies on CHROMagar <i>Candida</i> plates. 87.5% <i>C. albicans</i> ; 12.5% <i>C. glabrata</i>  | 100% (16/16)  |
| <b>AV</b><br>(n=14)           | Inflammatory symptoms; diminished lactobacilli, presence of leukocytes, small coliform bacilli, cocci, or chains                  | Absence/reduction of lactobacilli; growth of commensal aerobic microorganisms, mainly of intestinal origin. 64% Enterobacterales ( <i>E. coli</i> , <i>K. pneumoniae</i> ), 12% enterococci, 7% <i>S. aureus</i> , 7% <i>S. agalactiae</i> . | 100% (14/14)  |

BV: bacterial vaginosis; VVC: vulvo-vaginal candidiasis; AV: aerobic vaginitis; TSA: tryptic soy agar.



**Figure 1** - Digital images of vaginal cultures from women with a normal lactobacilli-dominated flora. Vaginal swabs are plated onto CHROMagar Orientation medium (a), tryptic soy agar with 5% sheep blood (b), and CHROMagar Candida medium (c). On CHROMagar Orientation, lactobacilli appear as flat/submerged blue/violet colonies, whereas on tryptic soy agar with 5% sheep blood as small grey colonies with a 'viridans' haemolysis.

mainly characterized by the growth of lactobacilli. As shown in Figure 1, on CHROMagar Orientation, lactobacilli appear as small, flat/submerged colonies. Their colour can vary from blue/green to violet. On tryptic soy agar with 5% sheep blood, lactobacilli can grow as small grey colonies with a 'viridans' haemolysis. No microbial growth is present on CHROMagar Candida plates. In all analysed samples, lactobacilli growth reached the third/fourth quadrant of agar plates.

Concordance between microscopy and culture was 93.5%: in 4 cases the microscopic presence of gram-positive rods was accompanied by poor/absent growth of lactobacilli.

#### Bacterial vaginosis (BV)

When vaginal swabs from BV-affected women (Nugent score 8-10) were cultured, the main phenotypes of bacterial growth were as follows (Figure 2):

- 1) reduction (lactobacilli on only the first quadrant of agar plates) or absence of lactobacilli;
- 2) presence (growth from the second quadrant on) of small grey translucent non-haemolytic colonies on tryptic soy agar with 5% sheep blood.

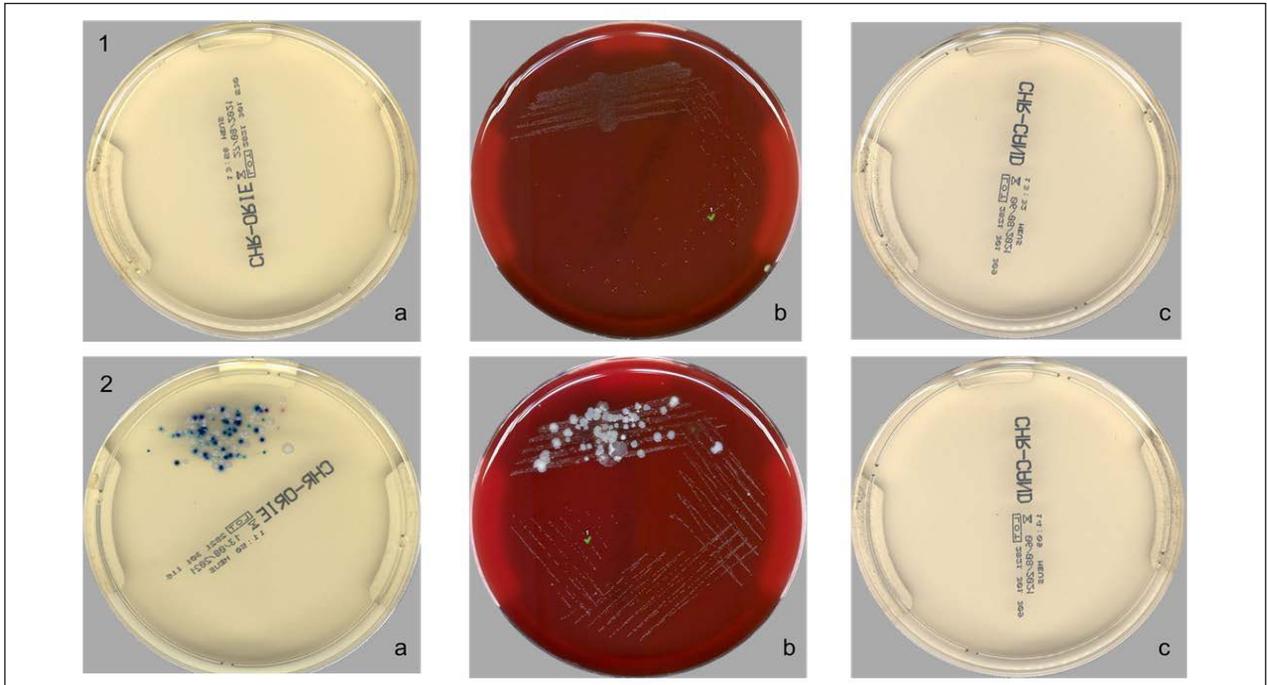
When subjected to MALDI-TOF MS, these colonies were identified as *Gardnerella vaginalis*. No microbial growth was present on CHROMagar Candida plates. In 1 case of BV, no growth of *G. vaginalis* was detected by culture (concordance between microscopy and culture: 91.6%).

#### Vulvo-vaginal candidiasis (VVC)

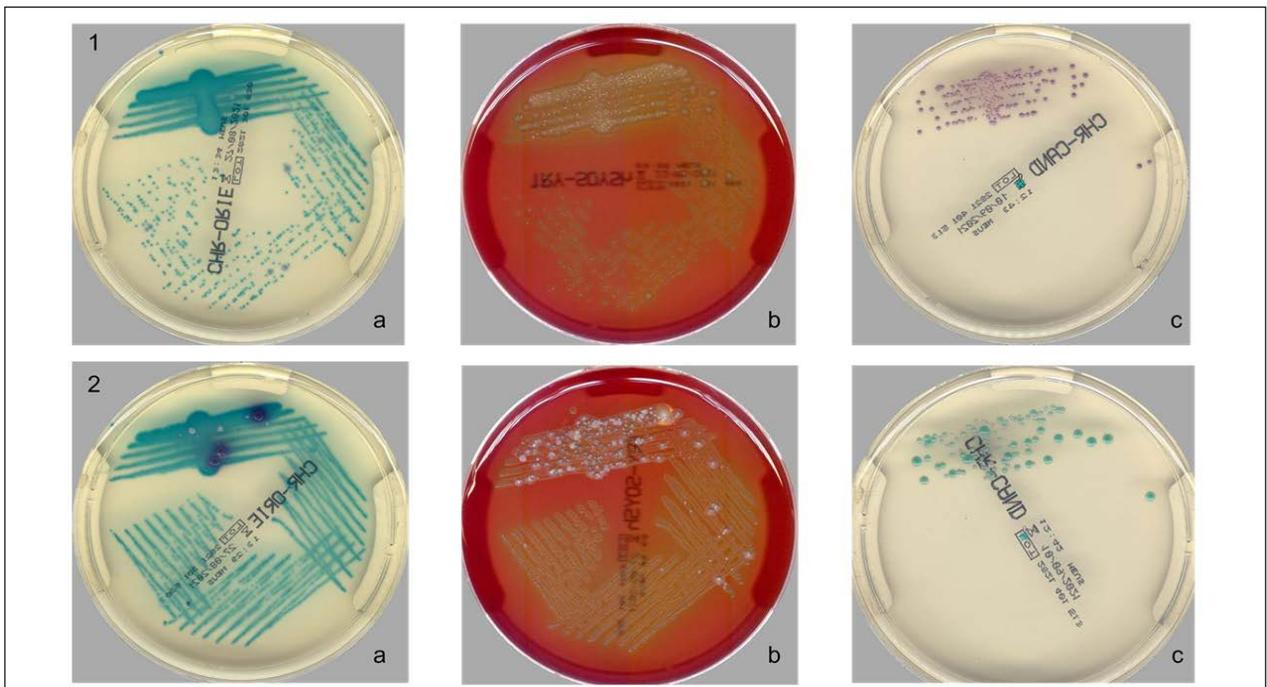
The cases of VVC were mainly characterized by a lactobacilli-dominated vaginal flora together with a significant growth of *Candida* colonies. As shown in Figure 3, *Candida* appears as big, opaque, wax-like colonies on CHROMagar Candida plates. Colony colour varies based on the species: for example, colonies of *C. albicans* complex (87.5% of cases found during the study) appear light/medium green, whereas *C. glabrata* colonies (12.5% of cases) are rose or light/dark mauve. In 10 samples (62.5%), the number of yeast colonies was 50 to 100, whereas in the remaining 6 cases (37.5%) *Candida* colonies exceeded 100. Concordance between microscopy and culture was 100%.

#### Aerobic vaginitis (AV)

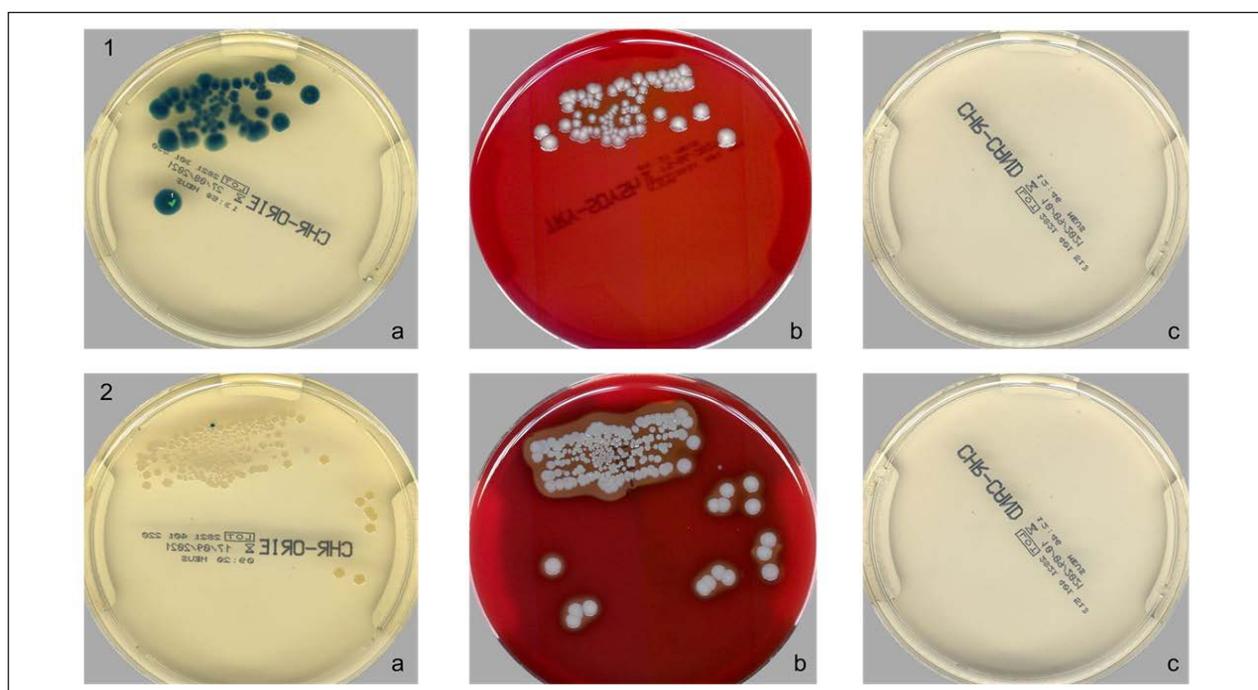
Cultures of all vaginal samples taken from women with AV showed the absence of lactobacilli and the presence of commensal aerobic microorganisms, mainly of intestinal origin. The most frequently encountered bacteria were Enterobacterales (64%) (e.g., *Escherichia coli*, *Klebsiella pneumoniae*) and enterococci (12%), followed by *Staphylococcus aureus* (7%) and *Streptococcus agalactiae* (7%). All AV cases were characterized by at least 50 bacterial colonies on agar plates. Figure 4 shows examples of digital images of vaginal cultures from women with AV. Concordance between microscopy and culture was 100%.



**Figure 2** - Digital images of vaginal cultures from BV-affected women. Vaginal swabs are plated onto CHROMagar Orientation medium (a), tryptic soy agar with 5% sheep blood (b), and CHROMagar Candida medium (c). It is possible to notice the absence (Panel 1) or the reduction (Panel 2) of lactobacilli, and the presence of small grey translucent colonies on tryptic soy agar with 5% sheep blood (identified as *Gardnerella vaginalis* by MALDI-TOF MS).



**Figure 3** - Digital images of vaginal cultures from VVC-positive women. Vaginal swabs are plated onto CHROMagar Orientation medium (a), tryptic soy agar with 5% sheep blood (b), and CHROMagar Candida medium (c). A lactobacilli-dominated vaginal flora (see lactobacilli on CHROMagar Orientation and tryptic soy agar) is accompanied by a significant growth of *Candida* colonies on CHROMagar Candida plates. *Candida* appears as, opaque, wax-like colonies with the colour depending on the species: colonies of *C. glabrata* are rose or light/dark mauve (Panel 1), whereas *C. albicans* complex colonies appear light/medium green (Panel 2).



**Figure 4** - Digital images of cultures of vaginal swabs from women with AV. Vaginal swabs are plated onto CHROMagar Orientation medium (a), tryptic soy agar with 5% sheep blood (b), and CHROMagar Candida medium (c). In a condition of dysbiosis (i.e., absence of lactobacilli), it is possible to notice the growth of commensal aerobic microorganisms, mainly of intestinal origin. Panel 1: colonies of *Klebsiella pneumoniae*, appearing metallic blue on CHROMagar Orientation medium; Panel 2: colonies of *Staphylococcus aureus*, appearing gold and opaque on CHROMagar Orientation medium.

## DISCUSSION

Genital disorders are very common among fertile women and can negatively impact their sexual, reproductive, and relational life.

BV is associated with poor reproductive outcomes such as miscarriage, preterm birth, and infectious diseases including chorioamnionitis, endometritis and pelvic inflammatory disease (Ding *et al.*, 2021). Moreover, in several epidemiological studies it has been reported that BV represents a risk factor for the acquisition of sexually transmitted infections (STIs) (Allsworth *et al.*, 2011).

Similarly, due to its dysbiotic and inflammatory nature, AV can also be linked to the increased risk of preterm pre-labour rupture of membranes, chorioamnionitis and preterm delivery, as well as to a greater likelihood of acquiring HIV and other STIs (Donders *et al.*, 2011).

VVC affects millions of women worldwide. Its frequent re-occurrence is a cause for high morbidity and strongly impacts women's quality of life (Faria-Gonçalves *et al.*, 2020). The increasing trend of reduced susceptibility to antifungals and the possible implications for foetal well-being are other major challenges of *Candida* vaginal infections (Holzer *et al.*, 2017; Moreira *et al.*, 2021).

Considering the clinical and socio-economic impor-

tance of these conditions, a reliable and affordable diagnosis of genital disorders/infections is crucial for correct management in terms of appropriate and tailored treatment.

Even though wet mount microscopy of vaginal secretions has excellent performance for the assessment of many vaginal conditions (Vieira-Baptista *et al.*, 2021), culture-based approaches can be helpful for different reasons. In the case of VVC, culture allows the identification of *Candida* species, necessary to set up a correct antifungal treatment (Ahn *et al.*, 2021). Due to the growing and alarming problem of antimicrobial resistance, culture can be useful for the isolation and AST of aerobic bacteria (e.g., Enterobacterales, enterococci, *S. aureus*) responsible for AV (Donders *et al.*, 2017).

Moreover, vaginal culture can be suitable for epidemiological purposes, as a confirmatory technique, or for obtaining viable microbial strains for further investigations (e.g., subtyping, pathogenicity assessment, evaluation of virulence traits and antimicrobial resistance determinants).

In this context, clinical microbiology laboratories can take advantage of highly efficient, modular, and customizable specimen processing, culture incubation and work-up systems. Specimens move from front-end processing to 'Smart Incubation' and 'Digital Microbiology' with artificial intelligence/interpretive

algorithms for automatic plate reading (WASPLab system, Copan Diagnostics) (Thomson and McElvina, 2019).

The artificial intelligence module (PhenoMatrix software, Copan Diagnostics) can develop algorithms able to pre-sort culture plates based on its 'reading' of digital plate images, segregating culture plates according to the presence of bacterial growth, and colony colour (Van *et al.*, 2019; Faron *et al.*, 2020).

In this work, we assessed whether various genital conditions (i.e., normal vaginal flora, BV, VVC, and AV) have recognizable and peculiar fingerprints when vaginal swabs were cultured by WASPLab system.

Vaginal swabs, plated onto two chromogenic media (CHROMagar Candida and CHROMagar Orientation) and tryptic soy agar with 5% sheep blood, were incubated in an aerobic atmosphere. Digital plate images were taken at 38 h post-incubation.

We found that each genital condition was characterized by specific morphotypes in terms of microbial growth and colony colour, thus allowing the potential use of artificial intelligence not only to assess the presence of specific microbial genera/species but also to 'categorize' specific clinical conditions.

In our workflow, PhenoMatrix system automatically screens CHROMagar Candida plates, sorting samples based on *Candida* species (i.e., *C. albicans* complex, *C. tropicalis*, other *Candida* species, distinguished by different colony colours).

PhenoMatrix algorithm can be modified/expanded, setting a deeper analysis of all media plates used for vaginal swab streaking. Combining the analyses of chromogenic media and blood agar, it would be possible to sort vaginal samples grouped in 'clinical categories', distinguishing lactobacilli-dominated vaginal samples from other genital disorders. Moreover, PhenoMatrix can provide a quantitative evaluation of bacterial/fungal colonies. Colony enumeration can be useful to differentiate a real infection from a mere bacterial colonization (e.g., *Candida* spp., Enterobacteriales), or to estimate the number of lactobacilli in the assessment of dysbiotic conditions (e.g., BV, AV). Recently, Dauwalder and colleagues demonstrated that PhenoMatrix software is able to efficiently analyse digital plate images from urine samples, providing standardized and reproducible results (Dauwalder *et al.*, 2021).

Here, to the best of our knowledge, for the first time, we introduced the possibility of an automated analysis of vaginal swabs, permitting quicker results and decreasing the laboratory technologist's workload.

Although our workflow seems promising, further studies, including a larger panel of samples, are needed to better evaluate the performance of WASPLab system. Moreover, as a preliminary proof of feasibility, we are planning to test whether a classification based on the data presented in this study would be

able to correctly classify a new set of vaginal samples. It should be taken into account that several cases of genital disorders can be characterized by an overlapping of different conditions (e.g., BV and AV), and that some microbial species/strains (e.g., some species of lactobacilli) may not grow well in an aerobic atmosphere, requiring more complex nutritional supplements or incubation conditions. Moreover, BV can be associated with anaerobic Gram-negative microorganisms, different from *Gardnerella vaginalis*; thus, the absence of *G. vaginalis* in culture cannot completely exclude BV diagnosis.

In conclusion, this study aimed to design a tailored workflow for vaginal swab cultures, in the perspective of using artificial intelligence for 'categorizing' genital conditions/disorders.

These preliminary data can help create a PhenoMatrix algorithm, including different types of analyses according to plate type and combining the individual result of each plate in a single sample result.

### Acknowledgments

We would like to thank all the technical staff of the Microbiology Unit for their excellent assistance during the study.

### References

- Allsworth J.E., Peipert J.F. (2011). Severity of bacterial vaginosis and the risk of sexually transmitted infection. *Am J Obstet Gynecol.* **205**, 113.e1-6.
- Anh D.N., Hung D.N., Tien T.V., Dinh V.N., Son V.T., Luong N.V., et al. (2021). Prevalence, species distribution and antifungal susceptibility of *Candida albicans* causing vaginal discharge among symptomatic non-pregnant women of reproductive age at a tertiary care hospital, Vietnam. *BMC Infect Dis.* **21** (1), 523.
- Baron E.J., Miller J.M., Weinstein M.P., Richter S.S., Gilligan P.H., Thomson R.B. Jr, et al. (2013). Executive summary: a guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)(a). *Clin Infect Dis.* **57** (4), 485-488.
- Campisciano G., Zanutta N., Petix V., Giangreco M., Ricci G., Maso G., et al. (2021). Vaginal Dysbiosis and Partial Bacterial Vaginosis: The Interpretation of the "Grey Zones" of Clinical Practice. *Diagnostics (Basel).* **11** (2), 191.
- Ceccarani C., Foschi C., Parolin C., D'Antuono A., Gaspari V., Consolandi C., et al. (2019). Diversity of vaginal microbiome and metabolome during genital infections. *Sci Rep.* **9** (1), 14095.
- Dauwalder O., Michel A., Eymard C., Santos K., Chanel L., Luzzati A., et al. (2021). Use of artificial intelligence for tailored routine urine analyses. *Clin Microbiol Infect.* **27** (8), 1168.e1-1168.e6.
- Denning D.W., Kneale M., Sobel J.D., Rautemaa-Richardson R. (2018). Global burden of recurrent vulvovaginal candidiasis: a systematic review. *Lancet Infect Dis.* **18**, e339-e347.
- Ding C., Yu Y., Zhou Q. (2021). Bacterial Vaginosis: Effects on reproduction and its therapeutics. *J Gynecol Obstet Hum Reprod.* **50**, 102174.
- Donders G.G.G., Bellen G., Grinceviciene S., Ruban K., Vieira-Baptista P. (2017). Aerobic vaginitis: no longer a stranger. *Res Microbiol.* **168**, 845-858.
- Donders G., Bellen G., Rezeberga D. (2011). Aerobic vaginitis in pregnancy. *BJOG.* **118**, 1163-1170.
- Faria-Gonçalves P., Rolo J., Gaspar C., Oliveira A.S., Pestana P.G., Palmeira-de-Oliveira R., et al. (2020). Recurrent vulvovaginal *Candida* spp isolates phenotypically express less virulence traits. *Microb Pathog.* **148**, 104471.
- Faron M.L., Buchan B.W., Relich R.F., Clark J., Ledebor N.A.

- (2020). Evaluation of the WASPLab Segregation Software To Automatically Analyze Urine Cultures Using Routine Blood and MacConkey Agars. *J Clin Microbiol.* **58**, e01683-19.
- Faron M.L., Buchan B.W., Vismara C., Lacchini C., Bielli A., Gesu G., et al. (2016). Automated Scoring of Chromogenic Media for Detection of Methicillin-Resistant Staphylococcus aureus by Use of WASPLab Image Analysis Software. *J Clin Microbiol.* **54** (3), 620-624.
- Foschi C., Turello G., Lazzarotto T., Ambretti S. (2021). Performance of PhenoMatrix for the detection of Group B Streptococcus from recto-vaginal swabs. *Diagn Microbiol Infect Dis.* **101**, 115427.
- Foschi C., Gaibani P., Lombardo D., Re M.C., Ambretti S. (2020). Rectal screening for carbapenemase-producing Enterobacteriaceae: a proposed workflow. *J Glob Antimicrob Resist.* **21**, 86-90.
- Holzer I., Farr A., Kiss H., Hagmann M., Petricevic L. (2017). The colonization with Candida species is more harmful in the second trimester of pregnancy. *Arch Gynecol Obstet.* **295**, 891-895.
- Javed A., Parvaiz F., Manzoor S. (2019). Bacterial vaginosis: An insight into the prevalence, alternative treatments regimen and it's associated resistance patterns. *Microb Pathog.* **127**, 21-30.
- Kirn T.J. (2016). Automatic Digital Plate Reading for Surveillance Cultures. *J Clin Microbiol.* **54**, 2424-2426.
- Lamont R.F., van den Munckhof E.H., Luef B.M., Vinter C.A., Jørgensen J.S. (2020). Recent advances in cultivation-independent molecular-based techniques for the characterization of vaginal eubiosis and dysbiosis. *Fac Rev.* **9**, 21.
- Moreira D., Ruiz L.S., Leite-Jr D.P., Auler M.E., Ramos R.T.B., Costa V.T., et al. (2021). Difference Between the Profiles Presented by Yeasts that Colonize the Vaginal Mucosa or Cause Primary or Recurrent Candidiasis. *Mycopathologia.* **186** (3), 411-421.
- Ohtaki H., Takahashi A., Niwa A., Yonetamari J., Nakayama A., Kuchibiro T., et al. (2020). Evaluation of presumptive identification of Enterobacterales using CHROMagar Orientation medium and rapid biochemical tests. *J Clin Lab Anal.* **34** (10), e23453.
- Paladine H.L., Desai U.A. (2018). Vaginitis: Diagnosis and Treatment. *Am Fam Physician.* **97**, 321-329.
- Peters B.M., Yano J., Noverr M.C., Fidel P.L. Jr. (2014). Candida vaginitis: when opportunism knocks, the host responds. *PLoS Pathog.* **10**, e1003965.
- Richter S.S., Otiso J., Goje O.J., Vogel S., Aebly J., Keller G., et al. (2019). Prospective Evaluation of Molecular Assays for Diagnosis of Vaginitis. *J Clin Microbiol.* **58** (1), e01264-19.
- Sonthalia S., Aggarwal P., Das S., Sharma P., Sharma R., Singh S. (2020). Aerobic vaginitis - An underdiagnosed cause of vaginal discharge - Narrative review. *Int J STD AIDS.* **31**, 1018-1027.
- Thomson R.B. Jr, McElvania E. (2019). Total Laboratory Automation: What Is Gained, What Is Lost, and Who Can Afford It? *Clin Lab Med.* **39**, 371-389.
- Van T.T., Mata K., Dien Bard J. (2019). Automated Detection of Streptococcus pyogenes Pharyngitis by Use of Colorex Strep A CHROMagar and WASPLab Artificial Intelligence Chromogenic Detection Module Software. *J Clin Microbiol.* **57**, e00811-19.
- Viechtbauer W., Smits L., Kotz D., Budé L., Spigt M., Serroyen J., Crutzen R. (2015). A simple formula for the calculation of sample size in pilot studies. *J Clin Epidemiol.* **68** (11), 1375-1379.
- Vieira-Baptista P., Grincevičienė Š., Oliveira C., Fonseca-Moutinho J., Cherey F., Stockdale C.K. (2021). The International Society for the Study of Vulvovaginal Disease Vaginal Wet Mount Microscopy Guidelines: How to Perform, Applications, and Interpretation. *J Low Genit Tract Dis.* **25** (2), 172-180.
- White B.A., Creedon D.J., Nelson K.E., Wilson B.A. (2011). The vaginal microbiome in health and disease. *Trends Endocrinol Metab.* **22**, 389-393.
- Yano J., Sobel J.D., Nyirjesy P., Sobel R., Williams V.L., Yu Q., Noverr M.C., Fidel P.L. Jr. (2019). Current patient perspectives of vulvovaginal candidiasis: incidence, symptoms, management and post-treatment outcomes. *BMC Womens Health.* **19**, 48.
- Zozaya-Hinchliffe M., Lillis R., Martin D.H., Ferris M.J. (2010). Quantitative PCR assessments of bacterial species in women with and without bacterial vaginosis. *J Clin Microbiol.* **48**, 1812-1819.