

MICROBIOLOGICAL SCREENING FOR VAGINAL HEALTH: DETECTING *Candida albicans* AND *Staphylococcus aureus* IN FEMALE STUDENT'S URINE

Nasywaa Amalia¹, Maya Sari^{1, □}, M. Arief Fadillah¹, RA. Dewi Maria Yuliani²

¹Medical Laboratory Technology Study Program, Health Polytechnic of The Ministry of Health Banten

²Tigaraksa Regional General Hospital

*Corresponding author: mayasari6996@gmail.com

ABSTRACT

Vaginal discharge (leucorrhea) is a common condition in women and may be physiological or pathological. Pathological leucorrhea often results from infections caused by microorganisms such as fungi and bacteria, associated with poor genital hygiene. This study aimed to isolate and identify the microorganisms causing leucorrhea symptoms in the urine of female students at Poltekkes Kemenkes Banten, emphasizing its relevance to preventive health efforts through cross-professional collaboration in microbiology and reproductive health. Twenty urine samples from second-year students showing premenstrual leucorrhea symptoms were collected using purposive sampling. Laboratory analyses included fungal culture on Sabouraud Dextrose Agar (SDA), germ tube testing for *Candida albicans*, bacterial culture on Nutrient Agar (NA), catalase testing, and Gram staining. The results showed that 7 samples (35%) were positive for *Candida albicans* and 2 samples (10%) were positive for *Staphylococcus aureus*. Both organisms are recognized as causative agents of pathological leucorrhea and reproductive tract infections. The findings highlight the importance of maintaining genital hygiene and the integration of microbiological screening into health education to prevent infections related to women's reproductive health. This study underscores the role of interprofessional collaboration in laboratory science and health education for early detection and management of infection-related conditions, contributing to the prevention of non-communicable complications through integrated health services.

Keywords: *Candida albicans*; *Staphylococcus aureus*; pathological leucorrhea; personal hygiene

INTRODUCTION

Reproductive health refers to the overall physical, mental, and social well-being of individuals in all aspects related to reproductive function and processes. For women, maintaining reproductive health is essential, particularly through proper personal hygiene to prevent infections caused by bacteria, fungi, and parasites, which can lead to vaginal discharge or *leucorrhea* (Utami et al., 2024).

Vaginal discharge, also known as *fluor albus* or *leukorrhea*, can be classified as physiological or pathological. Physiological discharge is normal, clear, odorless, and occurs as part of hormonal changes during the menstrual cycle. In contrast, pathological discharge often results from infections caused by microorganisms such as *Candida albicans*, *Trichomonas vaginalis*, or *Staphylococcus aureus*. *Candida albicans* infection is one of the most common causes of abnormal vaginal discharge. Globally, about 75% of women experience vaginal discharge at least once in their lifetime, and nearly half experience recurrent episodes (Zuhdy et al., 2018). Poor genital hygiene, high humidity, and unsterile water used for cleansing can promote microbial growth, including fungi and bacteria, increasing the risk of genital irritation and infection (Napitupulu et al., 2023). Under favorable conditions, pathogens such as *Candida* spp. and *Staphylococcus* spp. may colonize and multiply rapidly in urine samples (Utami et al., 2024).

A preliminary survey conducted at Poltekkes Kemenkes Banten in October 2024 revealed that 98% of second-year female students had experienced vaginal discharge, and 60.7% had symptoms within the past

month. These findings indicate that vaginal discharge remains a common health concern among students, highlighting the need for further investigation. Therefore, this study aims to isolate and identify microorganisms causing vaginal discharge symptoms in urine samples of female students at Poltekkes Kemenkes Banten.

This study aims to isolate and identify microorganisms responsible for leucorrhea symptoms in premenstrual urine samples from female students of Poltekkes Kemenkes Banten. The findings are expected to provide scientific evidence on the types of microorganisms involved and to support reproductive health education for young women.

MATERIALS AND METHODS

This research used a descriptive design to isolate and identify microorganisms associated with leucorrhea symptoms in urine samples of female students at Poltekkes Kemenkes Banten. The population consisted of 196 second-year female students. Twenty urine samples were collected purposively from students who experienced premenstrual leucorrhea symptoms.

Inclusion Criteria

Participants were selected based on the following criteria: Female students in their second year of the Medical Laboratory Technology Department, Poltekkes Kemenkes Banten, academic year 2024/2025, who voluntarily agreed to participate in the study, aged 17–22 years and experiencing vaginal discharge symptoms (leucorrhea) occurring 2–3 days before menstruation, which represents the ideal sampling period.

Exclusion Criteria

The exclusion criteria included: Individuals who did not consent to participate in the study and participants who were currently menstruating at the time of sample collection.

Specimen Sampling

All instruments and materials were prepared prior to sample collection. Before collecting urine samples, respondents were instructed to wash their hands thoroughly with soap and dry them with tissue paper. The external genital area, specifically the labia and vulva, was cleaned from front to back to minimize contamination. Midstream urine samples were collected by discarding the first and last portions of the urine stream, ensuring that only the middle portion was captured in sterile urine containers. Each container was immediately sealed tightly and the specimens were promptly processed for analysis.

Cultivation of Fungi and Bacteria

Urine samples were centrifuged at 1500 rpm for 10 minutes. The supernatant was discarded, and the sediment was collected for inoculation. Using a sterile inoculating loop, the sediment was streaked onto Sabouraud Dextrose Agar (SDA) for fungal growth and Nutrient Agar (NA) for bacterial growth using the streak plate method. The SDA plates were incubated at 37°C for 3–5 days to allow fungal colonies to develop, while NA plates were incubated at 37°C for 24 hours for bacterial growth.

Catalase Test

A drop of 3% hydrogen peroxide (H₂O₂) was placed on a clean glass slide. A small portion of bacterial colony from the NA plate was transferred onto the drop and gently mixed. The presence of bubble formation indicated a positive catalase reaction, confirming that the bacteria produced the catalase enzyme.

Gram Staining

Gram staining was performed to differentiate bacterial isolates. After preparing the materials, a bacterial smear was fixed on a clean glass slide using physiological saline and heat fixation over a Bunsen flame. The smear was then stained with crystal violet for 1 minute, followed by Gram's iodine for 1 minute as a mordant. Decolorization was carried out with 90% ethanol for 30 seconds, after which the slide was counterstained with safranin for 1 minute. The stained preparations were examined under a light microscope to observe bacterial morphology and Gram reaction.

Germ Tube Test

For the Germ tube test, all materials were sterilized prior to use. One milliliter of serum was placed into a sterile test tube. A colony of *Candida* grown on SDA+ was inoculated into the serum and mixed until

the clumps dispersed evenly. The culture was then incubated at 37°C for 2–3 hours. After incubation, a drop of the culture was placed on a clean glass slide, covered with a cover slip, and examined microscopically. The formation of Germ tubes, observed as elongated tube-like outgrowths from yeast cells, indicated a positive result for *Candida albicans*.

RESULTS AND DISCUSSION

Isolation of *Candida albicans*

Based on macroscopic observations, *Candida* colonies showed clear and typical characteristics after 3–5 days of incubation on Sabouraud Dextrose Agar (SDA). As seen in Figure 1, the colonies were round, smooth, and white to cream in color, with a soft texture and a distinct yeast-like smell. From 20 urine samples that were cultured, seven samples (S1, S4, S8, S14, S15, S19, and S20) showed colony growth identified as *Candida*, giving a prevalence rate of 35% (Table 1).

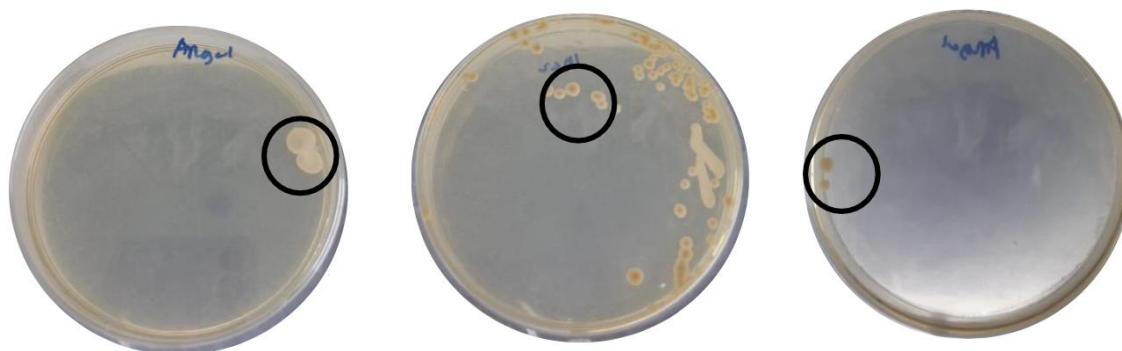


Figure 1. Macroscopic growth of *Candida albicans* on Sabouraud Dextrose Agar (SDA)

Table 1. Results of fungal culture and germ tube test for *Candida albicans*

Sample	Culture Result	Germ Tube Test
S1	+	+
S2	-	-
S3	-	-
S4	+	+
S5	-	-
S6	-	-
S7	-	-
S8	+	+
S9	-	-
S10	-	-
S11	-	-
S12	-	-
S13	-	-
S14	+	+
S15	+	+
S16	-	-
S17	-	-
18	-	-
S19	+	+
S20	+	+

To confirm the identification, a germ tube test was performed, as shown in Figure 2. The microscopic observation showed long tube-like structures growing from the yeast cells, called germ tubes. These tubes had no separation between the parent cell and the tube, which is a special feature of *Candida albicans*. The presence of these germ tubes confirmed that the positive samples contained *Candida albicans*.

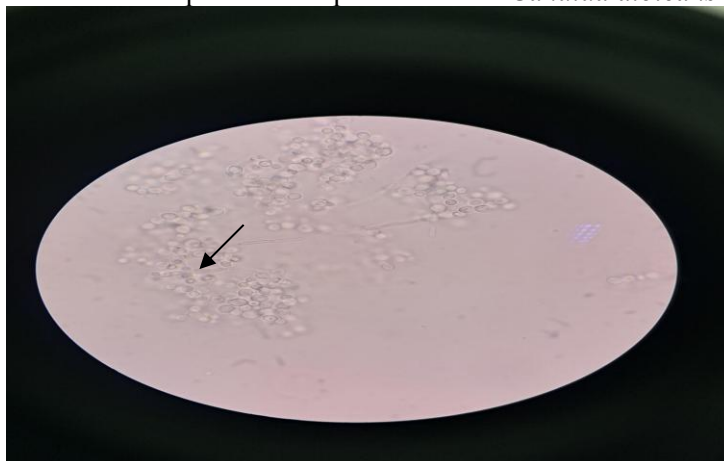


Figure 2. Germ tube test

Isolation of *Staphylococcus aureus*

From a total of 20 urine samples inoculated on Nutrient Agar, 17 samples showed bacterial growth, and after catalase testing revealed that 7 of these samples were catalase positive that the positive reaction was indicated by the formation of gas bubbles, confirming the presence of the catalase enzyme (Table 2). Bacterial colonies identified as *Staphylococcus* were observed. The colonies appeared dense and evenly distributed across the agar surface, indicating good bacterial growth on NA medium. The colonies exhibited typical characteristics of *Staphylococcus*, including a round shape, smooth surface, golden-cream coloration, and regular margins.

Figure 4 showed the results of the Gram staining test performed on samples that previously tested positive in the catalase assay. In figure (2a) the bacterial was Gram positive with colonies appeared as purple, grape-like clusters of cocci, which are characteristic of *Staphylococcus* spp. In contrast, image (b) was the bacterial was Gram negative that showed red-colored bacteria, indicating that their cell walls did not retain crystal violet and instead absorbed the safranin counterstain. Among the seven samples tested with both catalase and Gram staining, two samples (S14 and S20) were Gram positive, indicating the presence of *Staphylococcus aureus* Table 3.

Table 2. Results of bacterial culture and catalase test

Sample	Culture Result	Catalase Test
S1	+	+
S2	+	-
S3	+	-
S4	+	-
S5	+	-
S6	+	-
S7	-	-
S8	+	+
S9	+	-
S10	+	-
S11	+	+
S12	+	-
S13	-	-
S14	+	+
S15	+	+

Sample	Culture Result	Catalase Test
S16	+	-
S17	+	-
18	-	-
S19	+	+
S20	+	+

Table 3. Gram Staining of Catalase-Positive Samples

Sample	Gram Staining
S1	Gram Negatif
S8	Gram Negatif
S11	Gram Negatif
S14	Gram Positif
S15	Gram Negatif
S19	Gram Negatif
S20	Gram Positif

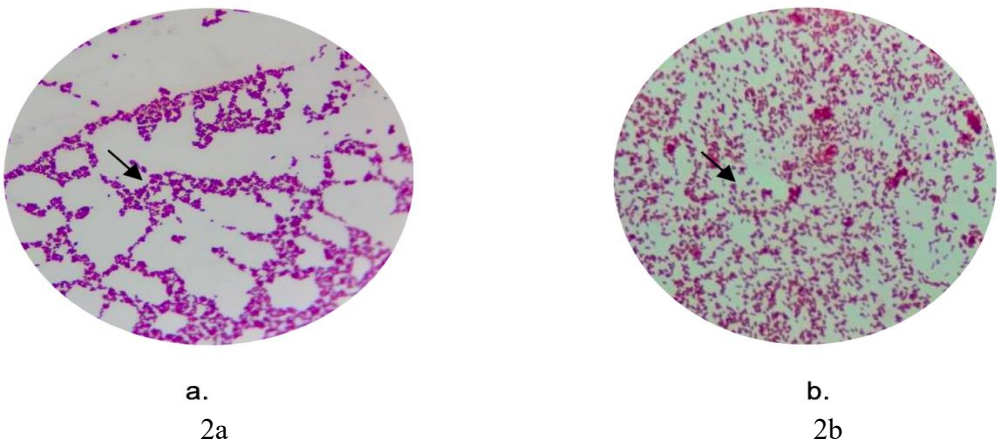


Figure 4. Gram staining

The presence of *Candida albicans* in urine samples was likely associated with poor personal hygiene. Approximately 33.3% of respondents reported rarely drying the genital area after urination. This condition promotes fungal growth since *C. albicans* thrives in warm and moist environments (Hamida et al., 2024). In contrast, negative results may be attributed to good hygiene practices, such as changing underwear at least twice daily to prevent excessive vaginal moisture. The incidence of infections caused by dermatophytes and *Candida albicans* has increased across various regions. Contributing factors include poor toilet hygiene, unhealthy dietary habits, risky sexual behavior, and lack of cleanliness awareness in public facilities such as schools, workplaces, and public toilets. Hafizah et al. (2022) reported the presence of *Candida* spp. in environmental water sources, including school and public toilets, swimming pools, and other facilities. Furthermore, Indonesia’s tropical climate provides favorable conditions for the growth and proliferation of fungi, bacteria, and viruses, contributing to the high prevalence of vaginal discharge among adolescent females. Microscopic examination of *Candida albicans* was performed using the germ tube test. Positive fungal isolates exhibited germinating yeast cells with elongated, tube-like outgrowths resembling a “paddle” shape, consistent with the typical morphology of *C. albicans*. *Candida albicans* cells are oval, reproduce by budding, and form germ tubes. A positive germ tube reaction is indicated by the presence of these elongated hyphal structures without septa between the mother and daughter cells (Afif et al., 2023).

Catalase testing was performed to differentiate *Staphylococcus* from *Streptococcus* species. The test determines the presence of the catalase enzyme, which breaks down hydrogen peroxide (H₂O₂) into oxygen and water. The procedure involved adding two drops of 3% H₂O₂ onto a bacterial smear; the formation of gas bubbles indicated a positive reaction and confirmed the presence of catalase (Fadilah and Mayasari, 2022). Further confirmation was conducted through Gram staining of the seven catalase-positive isolates. Two isolates (S14 and S20) were Gram-positive cocci arranged in grape-like clusters, consistent with *Staphylococcus aureus*. Gram-positive bacteria retain crystal violet and appear purple under the microscope, a key characteristic of *S. aureus* (Madigan et al., 2021). The remaining five isolates were Gram-negative, indicating that they were likely other bacterial species such as *Bacillus* spp. or environmental contaminants. Only 2 out of 20 premenstrual urine samples (10%) were conclusively identified as *Staphylococcus aureus*, showing typical NA colony morphology, positive catalase reactions, and Gram-positive cocci under microscopic observation. Although the prevalence of *S. aureus* was relatively low, its presence remains clinically significant, particularly among women presenting with mild urinary tract symptoms or asymptomatic infections.

CONCLUSION

Microorganisms, including fungi and bacteria, were successfully isolated from the urine samples of female students exhibiting symptoms of leucorrhea. *Candida albicans* (35%) and *Staphylococcus aureus* (10%) were identified as the predominant microorganisms, suggesting that inadequate personal hygiene practices may play a significant role in the development of pathological leucorrhea. These findings underscore the importance of reproductive health education and the enhancement of personal hygiene awareness among young women to prevent infection-related reproductive disorders.

REFERENCES

- Afif, N.H., Septiana, W, dan Astiandari. 2023. Diagnostic and Management of Sexually Transmitted Infections in Daily Practice. Unpri Press Surabaya.
- Fadilah, W.R., & Mayasari, U. 2022. Isolasi Dan Karakterisasi Bakteri Heterotrofik Pada Kawasan Perairan Pantai Indah Kalangan Tapanuli Tengah. *Metamorfosa: Journal of Biological Sciences*. 9(2): 306.
- Hafizah, Y. 2022. Gambaran *Candida albicans* Pada Urin Remaja Di Panti Asuhan X Banjarmasin.
- Hamida, I. 2022. Hubungan *Personal Hygiene* Dan Keberadaan *Candida albicans* Dengan Gejala Keputihan Pada Remaja. *Jurnal Aisyiyah Medika*. 1(2).
- Madigan, M., Blender, K. dan Buckley, D. 2021. Edition Microorganisms. *Sixteenth Edition*. 1129.
- Napitupulu, L., Sipahatur, D. M., & Pasaribu, S. M. (2023). *Identification of Trichomonas vaginalis in urine samples causing leucorrhea among women in River Park residential area*. *Health and Indonesian Journal of Pharmacy*, 15, 2016–2021.
- Utami, L. 2024. Hubungan Personal Hygiene Terhadap Keberadaan Jamur *Candida Albicans* Penyebab Gejala Keputihan Pada Urin Mahasiswi di Perguruan Tinggi XXXX Tahun 2024. *Ranah Research : Journal of Multidisciplinary Research and Development*. 6(4): 1002–1013.
- Zuhdy, N., Hana, C., & Widyasih, H. (2022). *Psychosocial stress and pathological leucorrhea incidence among female students*. *Jurnal Forum Kesehatan*, 8(1), 9–14.