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ISOLATION OF THE FUNGI *Candida albicans* IN TEENAGE GIRLS URINE USING SABOURAUD DEXTROSE AGAR (SDA) MEDIA

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ABSTRACT

Candida albicans is a species of pathogenic fungus that causes candidiasis. Candidiasis in women can occur due to poor personal hygiene such as moist reproductive organs, excessive use of antiseptics, rarely changing sanitary napkins, frequent use of tight underwear and inappropriate vaginal washing movements. The aim of the research was to determine whether or not the fungus *Candida albicans* was present in the urine of teenage girls isolated on *Sabouraud Dextrose Agar* (SDA) media. The research is descriptive in nature, carried out in June 2024, starting with urine sample collection, followed by sample examination in the microbiology laboratory of the Medical Laboratory Technology Department. The research population, namely teenage girl at the Al-Manar Modern Islamic Boarding School, Aceh Besar, grades 7 and 8, consisted of 60 respondents. The research sample was 20 respondents taken using purposive sampling technique. The results of research on 20 urine samples were positive for *Candida albicans* in 7 urine samples of young women at the Al-Manar Modern Islamic Boarding School. In positive samples for *Candida albicans*, there was the growth of round-shaped colonies, smooth surfaces, yellowish white in color, with a yeasty smell on *Saboraud Dextrose Agar* (SDA) media. Microscopic observation found blastospores in preparations using 10% KOH and pseudohyphae were found in the Germ Tube test. It can be concluded that the results of the isolation of *Candida albicans* in the urine of teenage girls obtained positive results in 7 samples (35%) and negative results in 13 samples (65%) on SDA media.

Keywords: *Candida albicans, Urine, Teenage girl, Saboraud Dextrose Agar*

INTRODUCTION

Candida albicans, a fungal pathogen in humans, can cause various kinds of mucosal and systemic infections, especially in individuals with immune disorders (Berman, 2012; Kados D. 2019; Macias, 2023). *Candida albicans* is one of the Candida species that is responsible for the majority of candidiasis cases (McManus, 2014). *Candida albicans* can cause two main types of infections in humans, namely

superficial infections, such as oral or vaginal candidiasis, and life-threatening systemic infections (Mayer et al, 2013; Wang, 2015; Sadeghi et al, 2020).

Candidiasis vaginalis is a common vaginal infection caused by candida species, which affects 70% of women (Kaur, 2021). Although not a fatal disease, the quality of life of adolescents in their most socially and economically productive periods can be destroyed (Cassone, 2016). This infection usually attacks sufferers with weak levels of immunity, impaired immunity and organ dysfunction (Rustam, 2019). And several predisposing factors for vulvovaginal candidiasis include diabetes mellitus (DM), increased endogenous estrogen (due to pregnancy or obesity), immunosuppression (i.e., transplant patients, antimetabolite or chemotherapy treatment, or HIV infection), use of broad-spectrum antibiotics, and environmental factors. others (David and Solomon, 2023).

Candidiasis in the vagina can be caused by moist reproductive organs, excessive use of antiseptics, rarely changing sanitary napkins, frequently wearing tight clothing, inappropriate vaginal washing movements, using puddles of water to wash the vagina and not drying it after urinating (BAK) and Defecation (BAB) (Arizki et al., 2021). Vaginal discharge is the most frequently experienced problem and the highest risk for young women (Pradnyandari et al., 2019). Adolescent girls are susceptible to candidiasis due to poor personal hygiene and environmental sanitation which supports the growth of the *Candida albicans* fungus. According to research conducted by Zakiyyah on 40 urine samples of female students at the Salafiyah Hidayatul Qomariyah Islamic Boarding School, Bengkulu City, 6 samples showed positive results, these results indicate a tendency for *Candida albicans* infection in young women living in Islamic boarding schools (Zakiyyah, 2022).

Another research conducted by Ratna on environmental sanitation on 11 samples of Surabaya Islamic Boarding School bathroom water also found positive results for Candida in 3 samples (Ratna, 2019). This shows that environments that have poor sanitation, such as bathrooms in Islamic boarding schools, can also be a source of growth of Candida *albicans* which can threaten the overall health of young women. In research conducted by Sari regarding the relationship between vaginal hygiene and the incidence of candidiasis, it was found that 34 respondents (81.0%) experienced candidiasis vaginalis, while 8 respondents (19.0%) did not experience candidiasis vaginalis, so it can be stated that there is a relationship between vaginal hygiene and the incidence of candidiasis. vaginalis (Sari, 2019). Therefore, it is important to increase awareness of the importance of maintaining genital health and environmental sanitation, especially in Islamic boarding school environments to reduce the risk of developing fungal infections such as candidiasis.

The Al-Manar Modern Islamic Boarding School, which was founded in 2001, is located in the Aceh Besar region and has an integrated formal education institution where its students live in dormitories. Based on the results of the initial survey, it was discovered that some female students at the Islamic boarding school experienced complaints of vaginal discharge accompanied by itching in the reproductive organs, as well as indications of poor personal hygiene, such as not changing underwear regularly. Environmental factors also play a role, where the Al-Manar Modern Islamic Boarding School still relies on drilled well water and has a shared bath. Some of the things mentioned above can increase the risk of candidiasis, considering that an environment that lacks sanitation, such as using shared baths and using drilled well water, can support the growth of *Candida albicans*.

Based on the above, it is necessary to carry out research to see whether or not *Candida albicans* is present in the urine of teenage girls at the Al-Manar Modern Islamic Boarding School, Aceh Besar using *Sabouraud Dextrose Agar* (SDA) isolation media. SDA (*Sabouraud Dextrose Agar*) is one of the media most commonly used in laboratories to see the growth of fungi, especially *Candida albicans* because it has a pH variation of 4.5-6.5 and the optimum temperature for growth is around 280C-370C (Getas et al., 2014).

METHODS

Research methods

This type of research is a descriptive laboratory observational study, aimed at identifying Candida albicans in the urine of young women at the Al-Manar Modern Islamic Boarding School on *Sabaroud Dextrose Agar* (SDA) media. The population in this study were young women at the Al-Manar Aceh Besar Modern Islamic Boarding School in grades 7 and 8, totaling 60 respondents. And the sample was 20 respondents using a purposive sampling technique. The inclusion criteria were: 13-14 years old, had symptoms of candidiasis, willing to be a respondent. The sample examination location was carried out at the Microbiology Laboratory, Medical Laboratory Technology Department, Health Polytechnic, Ministry of Health, Aceh on June 2024.

Inspection Procedure:

1. Pre Analytics

The tools used are oven, autoclave, hot plate, microscope, centrifuger, Erlenmeyer flask, object glass, cover glass, measuring cup, incubator, petri dish, dropper pipette, urine pot, test tube, tube rack, stirrer, spoon, tube. round, spirit lamp, scales, permanent marker, matches, paper lens, sterile cotton swab, wrapping paper, and corn string. The materials used in this research were urine, Sabouraud Dextrose Agar (SDA) media, distilled water, KOH 10% and the antibiotic Chlorampenicol. Preparation for sterilization of tools, namely glass-based tools such as Erlenmeyer flasks, test tubes and petri dishes. The tool is wrapped in wrapping paper and then placed in the oven for 1 hour at a temperature of 160ºC (Damayanti, 2020).

2. Analytics

Making Sabouraud Dextrose Agar (SDA) Media according to Bastian (2022), the way to make Sabouraud Dextrose Agar (SDA) media is: Prepare the tools needed. Weigh 26 grams of SDA media into the Erlenmeyer flask. Then, add 400 mL of distilled water and homogenize on a hot plate/stove. Add the antibiotic Chlorampenicol after the medium is warm. Then, pour about half or a quarter of the media into the petri dish. Wait until the media is hard.

Urine Specimens

The urine sample was put into a clean and dry test tube, then centrifuged at a speed of 3000 rpm for 15 minutes. Next, the supernatant is discarded and the precipitate/sediment is taken (Fahmi, 2023). Next, inoculation was carried out on the media by taking the sediment from the urine from which the supernatant had been removed. After that, put 2 ml of 0.85% NaCI into a test tube and mix with the urine sediment using a sterile cotton swab, mix until the solution becomes cloudy. Then, the suspension was inoculated zig zag on Sabouraud Dextrose Agar (SDA) media using a sterile cotton swab. Incubate in an incubator at 37°C for 72 hours for approximately 3 days and observe every day to see whether there is colony growth or not.

Candida albicans examination

Macroscopic examination of the *Candida albicans* fungus on *Sabouraud Dextrose Agar* (SDA) media, namely looking at or observing the shape of the colony, surface, size, color and smell (assessment is carried out every day to observe the development of fungal growth during the incubation period). Microscopic examination with 10% KOH solution was observed under a microscope at initial magnification of 10x and magnification of 40x. Observe parts such as blastospores then carry out a Germ tube test to confirm the results (Fahmi, 2023). The Germ Tube Test is carried out by taking *Candida albicans* colonies and placing them in a test tube containing 1 mL of blood serum and then incubating them in an incubator for 3 hours. After that, 1 drop of the colony was taken and observed with a microscope at 40x magnification to differentiate between *Candida albicans* and non-*Candida albicans* fungi (Ekawati, 2023).

Data analysis

Data analysis will be carried out in this study by calculating the percentage of positive and negative *Candida albicans* in the urine of adolescent girls. The research results obtained were calculated using the relative frequency formula (Fr), namely the number of samples examined multiplied by the number of percent (%).

RESULTS AND DISCUSSION

Based on the results of research on the identification of *Candida albicans* in the urine of teenage girls at the Al-Manar Modern Islamic Boarding School, Aceh Besar, the results obtained are as shown in table 1 below.

Table 1. Results of examination of the *Candida albicans* fungus on SDA media

Sample on SDA media	*Results*	*Fungal Species*
Sample1	Negative(-)	No mold growth
Sample 2	Negative(-)	No mold growth
Sample 3	Negative(-)	No mold growth
Sample 4	Negative (-)	No mold growth
Sample 5	Negative (-)	No mold growth
Sample 6	Positive (+)	*Candida albicans*
Sample 7	Negative (-)	No mold growth
Sample 8	Positive (+)	*Candida albicans*

Sample 9	Positive(+)	*Candida albicans*
Sample10	Negative (-)	No mold growth
Sample11	Positive (+)	*Candida albicans*
Sample12	Negative (-)	No mold growth
Sample13	Negative (-)	No mold growth
Sample14	Negative (-)	No mold growth
Sample15	Negative (-)	No mold growth
Sample16	Negative (-)	No mold growth
Sample 17	Positive (+)	*Candida albicans*
Sample 18	Positive (+)	*Candida albicans*
Sample 19	Negative (-)	No mold growth
Sample 20	Positive (+)	*Candida albicans*

Based on Table 1. The results of the inoculation examination of urine samples on SDA media showed that samples 6, 8, 9, 11, 17, 18 and 20 were positive for *Candida albicans*, while the rest were negative *for Candida albicans.*

Table 2. Percentage of *Candida albicans* Fungus Inoculation Results in the Urine of Adolescent Girls at the Al-Manar Modern Islamic Boarding School, Aceh Besar using SDA media

No.	*Inoculation Results*	*Amount*	*Percentage (%)*
1.	Positive *Candida albicans*	7	35
2.	Negative *Candida albicans*	13	65
Amount		20	100

Based on table 2, the percentage of *Candida albicans* inoculation results in the urine of teenage girls at the Al-Manar Aceh Besar modern Islamic boarding school using SDA media, namely 7 samples (35%) were positive for *Candida albicans* and 13 samples (65%) were negative and can be seen in figure 1.

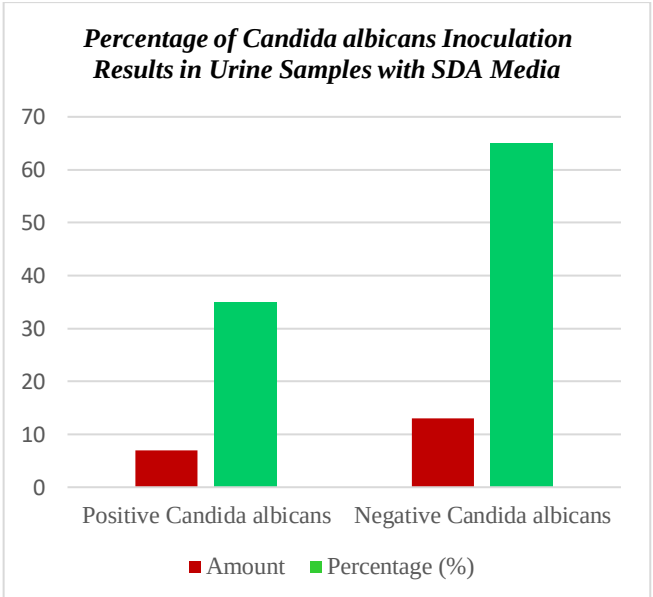

Figure 1. Percentage of *Candida albicans* inoculation results in urine samples

using SDA media

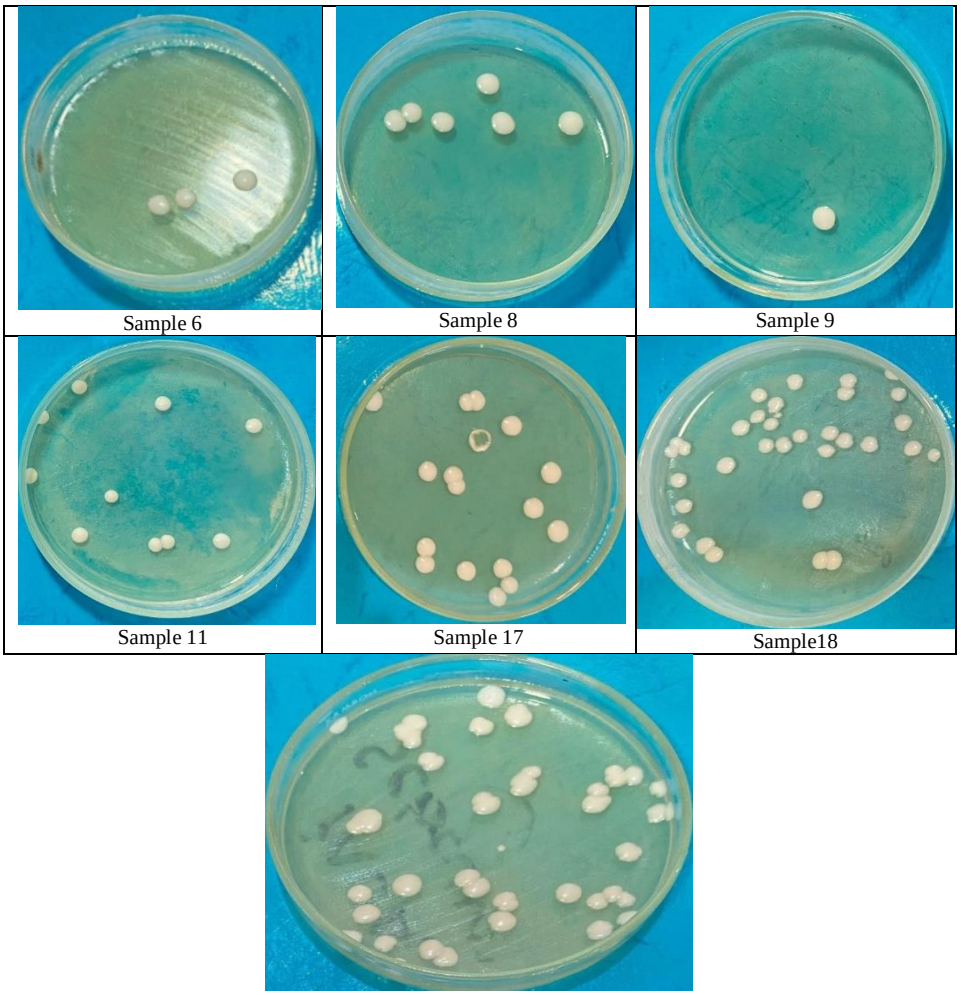

Figure 2. Results of growth of *Candida albicans* fungus colonies in urine samples using SDA (*Sabaroud Dextrose Agar*) media

The results of the growth of the *Candida albicans* fungus on SDA media were followed by microscopic observation with the addition of 10% KOH to observe blastospores, namely a specific form of yeast cells which can be seen in Figure 3a with an objective magnification of 10x10 to find the field of view, then continued with a magnification of 10x40 to clarify the observation. To find the Candida albicans species, a confirmatory test is needed, namely a Germ tube test with the addition of 1 ml of serum and incubation in an incubator. In this test, pseudohyphae or round sprouts with long septa will be found. The positive results of the *Candida albicans* examination can be seen in figure 3b.

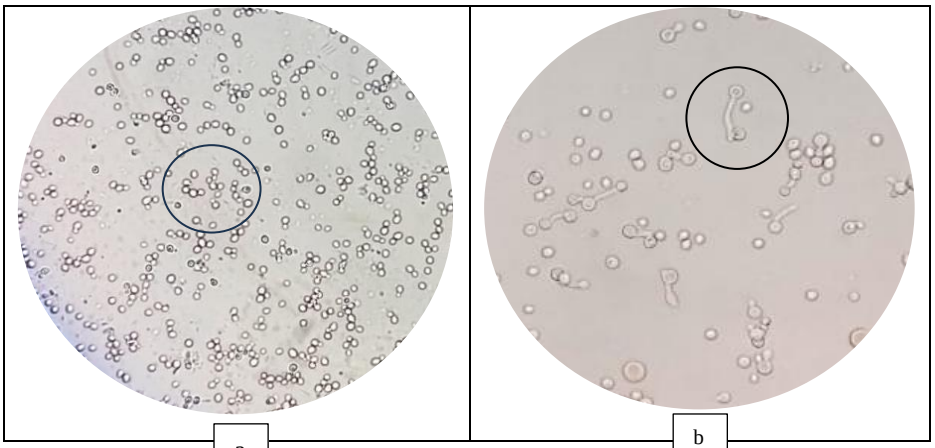

Figure 3. Microscopic Observation Results of Colonies Growing on SDA media and Germ tube test (a). *Blastospores* (b). *Pseudohypha*

Candida albicans is an opportunistic fungal pathogen in humans that can cause mucosal and systemic infections (Frazer et al., 2019; Jain et al., 2022). *Candida albicans* is a fungus that can cause candidiasis, this fungus can live as a saprophyte or vice versa. As normal flora in the body, *Candida albicans* usually lives in the digestive, respiratory and vaginal organs. However, under certain conditions this fungus can become a pathogen and cause candidiasis, such as in hot climates which make the vagina moist, immune problems, physiological changes in the body and lack of personal hygiene (Berlian, 2020).

After conducting research on urine samples from young women at the Al-Manar Modern Islamic Boarding School, Aceh Besar, it was found that the results of inoculation with SDA media were positive for *Candida albicans* in 7 samples (35%) and negative in 13 samples (65%). In this study, *Candida albicans* infection in young women at the Al-Manar Modern Islamic Boarding School, Aceh Besar occurred due to a lack of knowledge and awareness of personal and environmental hygiene. Therefore, it is important to increase awareness and knowledge about how to maintain healthy genetics to avoid *Candida albicans* fungal infections. According to Ekawati (2023), another factor that influences *Candida albicans* infection in young women is polluted or less clean water in daily activities such as bathing and washing. The *Candida albicans* fungus can contaminate water from air, dust, attached moss and from contaminated microorganisms so that well water or uncovered tanks are more likely to be contaminated with *Candida albicans* fungus.

On macroscopic examination of positive samples for *Candida albicans*, there were round-shaped colonies with a smooth surface, yellowish-white in color and smelled of yeast on *Sabaroud Dextrose Agar* (SDA) media. This is in line with the research of Rahmayanti, et al, (2022) which stated that the *Candida albicans* fungus grew very well. both on SDA media, the colonies are round, have a smooth surface, are yellowish white and have a yeasty smell. In microscopic observation of Candida albicans blastospores were found in preparations using 10% Potassium Hydroxide (KOH). According to Husni (2018) a 10% KOH solution can lyse skin, nails and hair so that fungal structures can be seen under a microscope like blastospores or hyphae.

On microscopic examination of the Germ tube test, *pseudohyphae* were found in the second 30 minutes to the fourth 30 minutes. Ekawati (2023) said that identification of the *Candida albicans* fungus was carried out using the Germ tube test to determine the ability of the fungus to grow sprouts on materials containing protein such as serum and plasma. The presence of blastospores or yeast cells that form sprouts (*pseudohyphae*) in the Germ tube test indicates that the identification of the *Candida albicans* species was successful.

This research is in accordance with research conducted by Syarifah Zakkiyah (2022) at the Salafiyah Hidayatul Qomariyah Islamic Boarding School, Bengkulu City. In 2022, out of 40 samples, there were 6 positive samples for *Candida albicans*. Research conducted by Aprillia Tri Putri (2019) at the Fatmawati Orphanage, Palembang City, also found the presence of *Candida albicans* fungus in the urine of 60 foster children, there were 30 samples (50%) that were positive for *Candida albicans*, while 30 samples were also negative (50%), so it is known that young women are susceptible to candidiasis, especially those living in Islamic boarding schools.

Candida albicans is a dimorphic fungal species that can change shape according to environmental conditions (Afrina, 2018). The fungus *Candida albicans* has three striking biological characteristics, namely its ability to grow in the form of yeast, pseudohyphae, and hyphae (Lu & Liu, 2014; McBride, 2017; Chen et al., 2020; Wakade & Krysan, 2021). In the case of the pathogen *Candida albicans* changing shape from blastospores to pseudohyphae, this change in shape can help the fungus avoid phagocytosis of macrophages or immune cells which play a role in defense against infection by swallowing, capturing and breaking down foreign particles such as bacteria and fungi thereby increasing the chance of the fungus infect the host. The development of pseudohyphae into hyphae indicates a serious infection. Hyphae are the most important phase in understanding the disease process which can cause damage to the mucosal epithelial tissue and then cause infection (Chen et al, 2020).

Sabaroud Dextrose Agar (SDA) is a peptone supplement medium with dextrose which is used to help fungal growth. Peptone as a nitrogen source used in this medium is dextrose which will be an energy source for fungal growth (Tominik, 2020). Afrina (2018) said that the morphology of *Candida albicans* is influenced by growth media, temperature, nutrient concentration, pH and osmotic pressure. *Candida albicans* grows on *Sabaroud Dextrose Agar* (SDA) media at a temperature of 28-37°C for 72 hours, approximately 3 days at pH.

CONCLUSION

Based on the results of *Candida albicans* isolation in the urine of young women at the Al-Manar Modern Islamic Boarding School, Aceh Besar using Sabaroud Dextrose Agar (SDA) media, the results were positive for *Candida albicans* in 7 samples (35%) and negative for *Candida albicans* in 13 samples (65%).

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