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Research Report

Oral rinse as a potential method to culture *Candida* isolate from AIDS patients

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ABSTRACT

Background: *Candida isolate is easily sampled from oral cavity by swabbing directly on the candidiasis lesion, to be smeared onto slides for direct examination or cultured in a growth medium. This method is by far the gold standard for defining candidiasis diagnosis. However it is difficult to apply on sensitive patients and almost impossible on patients showing no clinical appearance of oral candidiasis. AIDS patients are very prone to candida infection and have a tendency of repetitive infection involving mixed species. As many candida species show different susceptibility to anti-fungal agents, it is necessary to identify the species causing the infection in the management of oral candidiasis. Oral rinse is a suggested method to obtain candida isolate to be cultured for further analysis such as species identification. This method is simple and less risky on infection transmission as less tools are required in the procedure.* ***Purpose:*** *This study aimed to assess the application of oral rinse as an alternative method to culture Candida isolate from AIDS patients.* ***Methods:*** *A cross-sectional observative study was conducted in HIV/AIDS in-Patient Facility of Intermediate Care Unit for Infection Disease, Dr. Soetomo Hospital Surabaya. Fourteen stadium 4 AIDS patients matching criteria were swabbed on 1/3-posterior of the tongue, and then given 10 ml phosphate buffer saline to rinse vigorously for 15 seconds. Both specimens were cultured on Sabouraud's dextrose agar and colony growth was observed.* ***Results:*** *Candida colonies were able to grow from all 14 isolates (100%) by both methods. Qualitatively, cultures from oral rinse specimens were more populated than cultures from swab specimens.* ***Conclusion:*** *Oral rinse is an applicable technique to obtain Candida species isolate. This technique is safe, easy, non-invasive, and needs less tools therefore less risky for HIV transmission.*

Key words: *HIV/AIDS, oral candidiasis, Candida species, methods for obtaining isolate*

ABSTRAK

Latar belakang: *Isolat Candida mudah diambil dengan cara mengusap lesi candidiasis, baik untuk dioleskan pada kaca preparat untuk pemeriksaan langsung maupun dikultur. Hingga kini, metode tersebut dinyatakan sebagai "standar emas" untuk menentukan diagnosis. Namun kekurangan metode ini yaitu berisiko merangsang muntah pada pasien sensitif, dan hanya bisa dilakukan bila tampak jelas ada lesi. Candidiasis adalah penyakit nosokomial yang sering terjadi. Pasien AIDS sangat rentan terhadap infeksi oportunis ini secara rekuren dan persisten. Identifikasi spesies penyebab harus dilakukan karena berbagai spesies Candida memiliki kerentanan yang berbeda terhadap berbagai jenis antifungal. Oral rinse adalah metode pengambilan isolat Candida yang non-invasif. Isolat yang didapat bisa dikultur dan diidentifikasi, selain itu bisa dilakukan pada pasien yang belum menunjukkan adanya lesi candidiasis untuk menentukan besar risiko pasien terkena candidiasis, sehingga dapat ditentukan perlu tidaknya pemberian profilaksis antifungal.* ***Tujuan:*** *Penelitian ini bertujuan mengamati efektivitas metode oral rinse untuk mengisolasi Candida dari rongga mulut pasien AIDS.* ***Metode:*** *Penelitian observasional dilakukan di bagian Rawat Inap Unit Perawatan Intermediate Penyakit Infeksi RSUD Dr. Soetomo. Swab pada 1/3 posterior lidah dilakukan pada 14 pasien yang memenuhi kriteria, kemudia pasien diberi 10 ml phosphate buffer saline untuk berkumur kuat-kuat selama 15 detik. Spesimen yang didapat melalui kedua metode dikultur pada medium Sabouraud untuk diamati.* ***Hasil:*** *Koloni Candida berhasil dikultur dari 14 spesimen (100 %) melalui kedua metode isolasi. Secara kualitiatif tampak bahwa hasil kultur dari oral rinse tampak lebih subur.* ***Kesimpulan:*** *Oral rinse adalah metode yang dapat diaplikasikan untuk*

mengisolasi Candida dari rongga mulut pasien. Tekniknya mudah, aman, non-onvasif, dan tidak memerlukan peralatan dan ketrampilan khusus, sehingga mengurangi risiko transmisi HIV.

Kata kunci: *HIV/AIDS, oral candidiasis, Candida species, metode isolasi spesimen*

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INTRODUCTION

Candida species (*Candida* spp.) is likely the most familiar cause of fungal infections to clinicians and patients.[1] It is reported to cause over 70% of invasive fungal infection in hospitalized patients. Beginning with contracting mucosal candidiasis, certain hospitalized patients are at risk of contracting nosocomial candidemia because of their underlying medical conditions, while medical interventions such as antibiotic use, the presence of central venous catheter, and hemodialysis further increase the risk of contracting candidemia.[2] Patients infected with HIV and AIDS are at the highest risk of candida infection. Oropharyngeal candidiasis may be seen even in patients with cluster of differentiation 4^+ ($CD4^+$) counts over 200/mm^3, and becomes more common when the $CD4^+$ count falls below 300/mm^3.[3,4]

Opportunistic infections are major factors contributing the progressivity of HIV infection as inflammation induces viral replication and eventually leads to $CD4^+$ depletion.[5] Oral candidiasis in HIV and AIDS patients must be managed urgently and thoroughly to delay the progresivity of AIDS. Preventive measures may be achieved by detecting the infection as early as possible in hospitalized HIV positive individuals. Management of oral candidiasis consists of correct diagnosis and proper choice of treatment. Defining the species causing the infection is very important, because every species of *Candida* has different sensitivity to anti-fungal. The specificity might not be as specific as bacteria against antibiotics, and most fungal species are sensitive to azoles. However, resistence to azole treatment has recently become widely reported. It is very important for clinicians to identify the microorganism causing the infection to be able to prescribe the most potent anti-fungal agent.[4]

The gold standart to obtain *Candida* isolate from any mucosal site is by swabbing the creamy-white pseudomembran directly from the oral candidiasis lesion. This allows the isolate to later be observed by direct microscopy and cultured in growth medium.[6] However this method is hardly applicable when obvious lesion is not present. Swab sticks, although simple and considered non-invasive, may provoke vomitting when swabbed in posterior areas, especially in sensitive patients. Therefore, an alternative method must be developed to obtain *Candida* isolate from patients without clear clinical presentation of oral candidiasis and to determine the possibility for the patient to contract oral candidiasis during his hospitalization. Collecting oral rinse, or oral lavage, is relatively easy to apply, without any special skill from the clinician and caretakers needed. Less tools are also needed when applying this method, and this might be the most beneficial point while working with HIV positive individuals. This study is aimed to assess the application of oral rinse as an alternative method to obtain candida isolate from HIV/AIDS patients, in the hope of setting up a better and safer protocols in the management of oral candidiasis in HIV/AIDS patients.

MATERIALS AND METHODS

This cross-sectional observative study was conducted in In-Patient Facility of Intermediate Care Unit for Infectious Disease (HIV and AIDS Facility) in Dr. Soetomo Hospital Surabaya. All stadium 4 AIDS patients in the facility were clinically examined. Fourteen met the sampling criteria, which included receiving no treatment for fungal infection. Swab specimen was taken from 1/3-posterior of tongue dorsum, then the swab stick was immersed in Sabouraud's broth and incubated for 24 hours in 37° C. From the same patient, oral rinse specimen was taken by telling the patient to rinse vigorously with 10 ml phosphate buffer saline (PBS) for 15 seconds. Oral rinse specimen was then centrifugated, and the harvested pellet was also immersed in the Sabouraud's broth and incubated for 24 hours in 37° C. Cotton swab stick was then used to stir the liquid medium, then spreaded onto Sabouraud's dextrose agar, and incubated for another 24 hours in 37° C. Agar mediums were then observed visually and qualitatively for colony growth comparison.

RESULTS

Out of 14 patients involved in the study, 12 were males and 2 were females. All of them diagnosed with stadium 4 AIDS. Oral examination showed that none of the patients had good oral health status. They all had one or overlapping oral mucosal lesions. Oral pseudomembranous candidiasis was clinically suspected in 6 patients. Atophic erythematous condition on tongue and buccal mucosa was present in 8 patients. Exfoliative cheilitis is generally common as patients tended to have dry mouth, but only 3 severe cases were noted. Two patients presented angular cheilitis and one oral hairy leukoplakia.

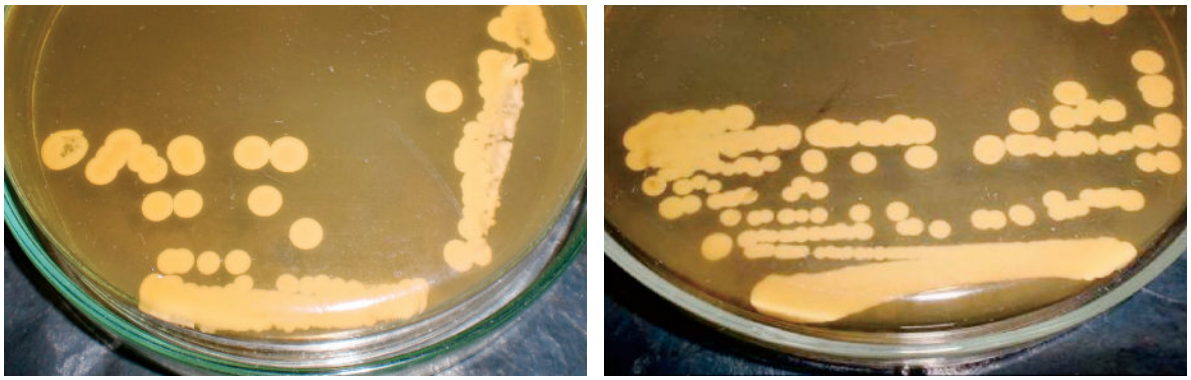

Figure 1. Colony growth from swab specimen.

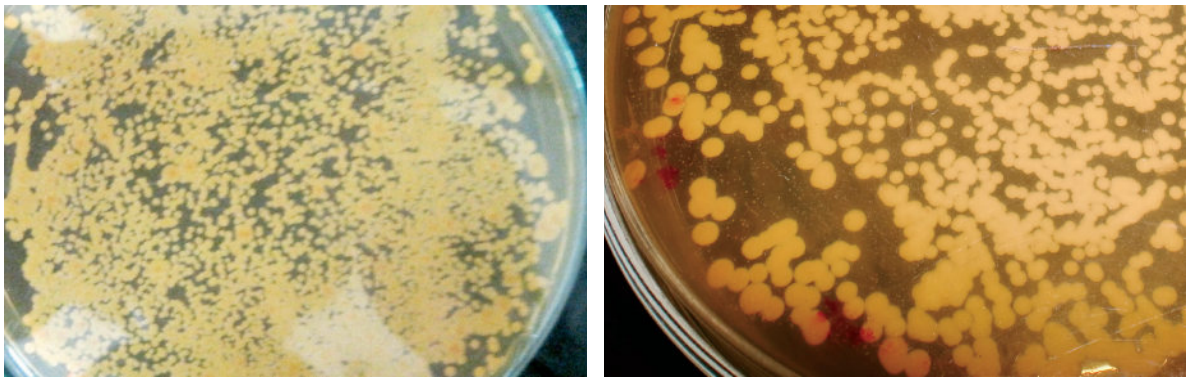

Figure 2. Colony growth from oral rinse specimen.

As shown in figure 1 and 2 *Candida* spp from 14 isolates (100%) taken by both methods were able to grow on the Sabouraud medium. Qualitatively, cultures from oral rinse specimens were more populated than cultures from swab specimens.

DISCUSSION

HIV-infected individuals are at the highest risk of candida infection. Oral candidiasis is associated to 90% of HIV/AIDS cases.[7] Opportunistic infections are the most contributing factor in the progressivity of HIV infection. Inflammation induces HIV replication and eventually leads to $CD4^+$ count depletion.[5] The severity of $CD4^+$ depletion is not the sole determinant of risk, however, as recent data suggest an independent, inverse relationship between plasma HIV viral load and the prevalence of oropharyngeal and oesophageal candidiasis.[4] Since the introduction of highly active antiretroviral therapy (HAART), the incidence and prevalence of opportunistic infections on HIV positive individuals has declined. However, in countries with limited settings where HAART is not readily available, Candidiasis still constitute a large disease burden.[3]

The most common species identified in human infections at any mucosal sites is *Candida albicans* (*C. albicans*), however additional species are increasing in frequency and include *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis* and *C. tropicalis*. *C. albicans* is isolated from the oropharynx of over 40% of normal individuals and is a standard commensal of the gastrointestinal tract , therefore the strain causing disease is derived from the patient's own flora.[1,4] *Candida* species are generally sensitive to broad spectrum antifungal agents eventhough reports suggest resistance to fluconazole by non-albicans species and a certain strain of *C. albicans*. This is the reason for clinicians to identify correctly the microorganism causing the disease.[8–10]

Eighteen patients recently admitted to the Intermediate Care Unit for Infectious Diseases were included in the study, however 4 of them must be excluded because 2 were barely conscious while the other 2 were not able to sit up. All 14, 12 males and 2 females, were diagnosed with stadium 4 AIDS, indicating $CD4^+$ counts below 200/mm^3. Most of them were totally unaware of their oral health condition and could not decide if they have ever contracted candidiasis on any mucosal part or if they have ever been given treatment for candidiasis before. Oral examination on those patients revealed that none of them had a good oral health condition. All of them had at least one or overlapping mucosal lesions which are closely-associated to HIV infection such as oral pseudomembranous candidiasis, oral hairy leukoplakia and linear gingival erythema, or lesions which are less associated with HIV infection such as mucosal erythema, angular cheilitis and exfoliative cheilitis.

Diagnosis of oral candidiasis is made based on clinical presentation and laboratory confirmation. There are three types of clinical presentations of oral candidiasis and the pseudomembranous type is probably the easiest one to recognize clinically.[4,6] Swabbing directly on the lesion is the easiest way to obtain the isolate for further laboratory examination. The isolate can then be smeared on a glass slide and stained with KOH or Gram preparation. Appearance of blastophores, pseudohyphae and true hyphae would confirm the diagnosis of oral candidiasis. The rest of the isolate can also be grown on to standard growth medium for candida species.[3,6,10] However, the gold method does not cover taking isolates from patients presenting less obvious form of candidiasis, such as the erythematous type. Patients with risk of contracting candidiasis, such as hospitalized patients and especially HIV positive ones, should receive early management for this debilitating opportunistic infection.[3,4,6] As swabbing can only be done on obvious lesion, a different approach should be developed to take isolates from patients who do not present clinical candidiasis yet but at risk of contracting one.

Oral rinse is very potential for these conditions. Rinsing movement allows dettachment of salivary mucin containing microorganisms. Phosphat buffer saline ensures the cells obtained are well preserved during transfer time to the laboratory.[8,10] By telling the patient to rinse his mouth, we also gather information about the patient's motoric and sensoric reflex. This will also be a consideration for clinician to prescribe topical antifungal and antiseptic in the form of rinse or gargle. The most beneficial point in this method is that it takes less tool and it does not require a special skill from the clinician. Swabbing method, eventhough considered non-invasive, requires a skillful dexterity to swab the cotton swab stick on the mucosal surface. If the lesion is situated in the posterior region, the movement would easily trigger the patient to vomit, and this will increase the risk of infection transmission.

In conclusion, as long as the patient is able to perform oral rinsing and has good gag reflex, oral rinse might stand as an applicable technique to obtain *Candida* species isolate for further laboratory assessment. This technique is safe, easy, non-invasive, and needs less tools therefore less risky in the sense of HIV transmission.

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