ISOLATION, IDENTIFICATION, AND ANTIFUNGAL RESISTANCE OF

CANDIDA SPP. IN CATS FROM KOTA BHARU, KELANTAN

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CERTIFICATION

This is to certify that we have read this research paper entitled **'Isolation, identification and antifungal resistance of *Candida* spp. in cats from Kota Bharu, Kelantan'** by Nur Farahana Binti Zamri. In our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 5436 – Research Project.

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ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 5436 – Research Project

The vast majority of fungal infections that occur worldwide are caused by yeast. The virulence of yeast isolates can vary widely depending on the species. Many yeast isolates are harmless commensals or endosymbionts of their hosts, including humans. This research aimed to determine the antifungal resistance pattern of the *Candida* species isolated from cats in Kota Bharu, Kelantan, using systematic mycology and molecular identification techniques. Additionally, the study intends to identify the dominant *Candida* species isolated from cats in Kota Bharu, Kelantan. Swab samples were taken from the rectal area, the ear, the mouth, and the nasal of 60 cats in Kota Bharu, Kelantan, some of which exhibited abnormalities related to clinical signs of fungal infections and others that did not. Isolation and molecular identification were performed to determine which yeast isolates belonged to which species of *Candida*. As for the result, out of 60 ear swab samples, 60 rectal swab samples, 60 oral swab samples, and 60 nasal swab samples, 9 (15%) ear swab samples, 34 (56.7%) rectal swab samples, 21 (35%) oral swab samples and 21 (35%) nasal swab samples fungal isolates were identified as yeast species by isolation and microscopic examination. In the meantime, all of the results from the molecular detection were negative for *Candida auris* and the *Candida auris* resistant gene.

Keywords: *Candida species, Candida auris, fungal infection, yeast*

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 5436 – Projek Penyelidikan.

Sebilangan besar jangkitan kulat yang berlaku di seluruh dunia disebabkan oleh yis. Keganasan yis berbeza-beza bergantung kepada spesies. Banyak pengasingan yis adalah komensal yang tidak berbahaya atau endosimbionts, yang boleh termasuk manusia. Tujuan penyelidikan ini adalah untuk menentukan corak rintangan antikulat spesies *Candida* yang diasingkan daripada kucing di Kota Bharu, Kelantan, menggunakan teknik mycology rutin dan pengecaman molekul. Selain itu, kajian ini bertujuan untuk mengenal pasti spesies *Candida* yang dominan yang diasingkan daripada kucing di Kota Bharu, Kelantan. Sampel swab diambil dari kawasan rektum, telinga, mulut, dan hidung 60 kucing di Kota Bharu, Kelantan. Beberapa di antaranya menunjukkan keabnormalan yang berkaitan dengan tanda-tanda klinikal jangkitan kulat dan lain-lain yang tidak. Pengasingan dan pengenalan molekul dilakukan untuk menentukan yis mana yang mengasingkan kepunyaan spesies *Candida*. Sebagai keputusan, daripada 60 sampel swab telinga, 60 sampel swab rektum, 60 sampel swab oral dan 60 sampel swab hidung, 9 (15%) sampel swab telinga, 34 (56.7%) sampel swab rektum, 21 (35%) sampel swab oral dan 21 (35%) sampel swab hidung kulat diasingkan dikenal pasti sebagai spesies yis secara berasingan dan pemeriksaan mikroskopik. Sementara itu, semua hasil dari deteksi molekul kembali negatif untuk *Candida auris* dan *Candida auris* tahan gen.

Keywords : *Spesies Candida, Candida auris, jangkitan kulat, yis*

1.0 Introduction

Yeast is responsible for the majority of fungal infections globally. Yeast isolates vary in virulence depending on the species; many are innocuous commensals or endosymbionts of hosts, including humans (Ciurea *et al*., 2020). Given their innately variable antifungal susceptibility profiles, yeast isolates from clinical samples must be identified quickly. Still, the growing number of emerging pathogenic species that are not included in the repertoires of commercially available conventional identification kits complicates matters. In the identification, taxonomy, and epidemiological analysis of fungal diseases, modern molecular approaches based on PCR amplification of conserved sections of the genome and sequencing of the PCR results are becoming increasingly important. (Borman, Linton, *et al*., 2008).

For a positive patient outcome, all significant fungal infections require appropriate antifungal medication. Because there are only a few antifungal drug classes available, the emergence of resistance to single drug classes, and increasingly multidrug resistance, has a significant impact on patient management (Perlin *et al.*, 2017). The development of less toxic medicines that can be used safely in a wide range of patients suffering from various illnesses has contributed to an increase in antifungal use for prophylactic, empirical, and directed therapy over the last decade, leading to increased drug resistance. Environmental reservoirs for drug-resistant pathogens have developed as a result of agricultural use of medically related antifungal drugs (Perlin *et al.*, 2017).

Candida albicans is the most widely known yeast responsible for invasive fungal infections in humans and animals. (Horn *et al.*, 2009). While *Candida albicans* is the most common cause of invasive fungal infections in hospitals, infections caused by non-albicans *Candida* species are becoming more widely recognised as a significant source of infection. (Miceli *et al.*, 2011).

Candida glabrata and *Candida parapsilosis* are among the most common species of *Candida* that can also cause invasive fungal infections. *Candida parapsilosis* and *Candida tropicalis* have become more common in many countries, as have rarer species like *Candida famata, Candida kefyr, Candida pelliculosa, Candida rugosa*, and *Candida guilliermondii*. (Miceli *et al.*, 2011).

Candida parapsilosis is one of the most common causes of invasive candidiasis. Infants and patients in intensive care units are the most vulnerable to severe infection. In patients undergoing bone marrow or stem-cell transplantation, *Candida krusei* and *Candida tropicalis* are important sources of invasive fungal infections (Leung *et al.*, 2002; Trifilio *et al.*, 2007).

Apart from that, *Candida auris* which is one of the most important *Candida* species was first discovered in Japan in 2009 and has since become an increasingly common cause of illness (Satoh *et al.*, 2009). This yeast species infections are becoming increasingly common in countries across five continents, making this a global health issue (Ruiz-Gaitán *et al.*, 2018).

Candida auris is frequently misidentified as phylogenetically related species such as *Candida krusei, Candida haemulonii, Candida lusitaniae* and other species by

commercial identification techniques in clinical laboratories. Therefore, accurate molecular techniques must be used to confirm the species identification of any suspicious colony. To ensure proper treatment and disease control, early detection of new *Candida auris* cases is critical, no matter what measures are taken to prevent its spread (Mizusawa *et al*., 2017; Ruiz Gaitán *et al.,* 2017).

Y132F mutations in ERG11 relating with azole resistance gene and S639P mutations in FKS1 for echinocandin resistance have been reported to differ between clades. Fluconazole resistance has been linked to copy number variations in ERG11, which are most prevalent in clade III (Acosta-altamirano & Mart, 2020). An understanding of how *Candida auris* infection presents its multi-resistance must be obtained in order to improve health and control measures.

Non-albicans *Candida* isolates' resistance to existing antifungal medications is a significant barrier for future empirical therapeutic and preventive methods. *Candida krusei, Candida glabrata*, and other rare species may face azole resistance. *Candida guilliermondii* has a low susceptibility to fluconazole (75%) but a high susceptibility to voriconazole (91%). Fluconazole inhibits 40.5% of *Candida rugosa* isolates and Voriconazole inhibits 61.4% (Pfaller, Diekema, et al., 2006). Secondary Amphotericin resistance can develop in *Candida lusitaniae* (Hawkins & Baddour, 2003), and *Candida dubliniensis* can develop consistent Fluconazole resistance, particularly in Aids patients. (Martinez *et al*., 2002).

In clinical isolates of *Candida nivariensis*, Azole resistance has been observed. (Borman, Petch, *et al.*, 2008). Despite this, nearly all clinical isolates of *Candida* species from around the world are susceptible to echinocandins. Even so, some findings of decreased susceptibility or resistance to these antifungals have been made in the context of recurrent *candida*emia, severe immunosuppression, and prolonged

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echinocandin exposure. (Perlin, 2014; Pfaller et al., 2006). *Candida lipolytic, Candida parapsilosis, Candida lusitaniae, Candida glabrata*, and *Candida tropicalis* can cause breakthrough mycoses despite prophylactic or therapeutic echinocandin use. *Candida parapsilosis* is usually susceptible to echinocandins in the clinic, but it has a higher minimum inhibitory concentration for caspofungin, resulting in therapeutic failure. (Moudgal *et al*., 2005).

Candida guilliermondii was the second most abundant fungus detected in National Wildlife Rescue Centre (NWRC) swab samples from the enclosure's wall, floor, and enrichment, according to Malaysian wildlife research. This fungus is found naturally in the flora of human skin and mucous membranes, and it appears to have been the least pathogenic of the *Candida* species studied. (Pasqualotto *et al*., 2006). The author classified *Candida* species into three virulence groups, with *C. guilliermondii* being placed in the third category with the slightest pathogenic species. The circulation and contact of personnel with animals and the environment is greater in NWRC facilities, implying a higher prevalence of this fungus. Yeast is a common human resident, but when the immune system is compromised, it becomes an opportunistic pathogen, resulting in disease formation. (Seyedmousavi *et al*., 2015). Infection with the yeast species has been recorded in dogs whose natural protective barrier has been disrupted (Mueller *et al.*, 2002).

A limited number of studies show the prevalence of antifungal resistant yeast species found in cats and dogs in Malaysia in general and Kota Bharu, Kelantan in particular. This study aims to investigate antifungal resistant *Candida* spp. commonly found in cats and dogs in Kota Bharu, Kelantan.

2.0 Research problem

Yeast infection is a multidrug-resistant disease that can cause invasive infections linked to a high mortality in domestic animals (Miceli *et al*., 2011). Several commercial technologies have been developed that can identify these infections in 4 to 72 hours. Etest and Sensititre YeastOne are two examples of commercial technologies, while Vitek is an automated system. The results of these tests for *Candida* spp. are generally consistent with those obtained using reference methods. Although these methods can correctly identify clinically relevant yeast strains, they may fail to remember certain novel and emerging yeast strains, resulting in inadequate or erroneous identification.

The usage of medically related antifungal medications in agriculture has led to drug-resistant microorganisms accumulating in the environment (citation). Due to the few treatment options, the formation of drug resistance to a particular therapeutic class significantly limits therapy against this disease. Multidrug resistance can altogether remove treatment alternatives, resulting in a disastrous outcome for patients. Antifungal resistance in common yeast species, mainly *Candida* spp. among companion animals, on the other hand, is not commonly reported in Kelantan, particularly in the Kota Bharu district.

3.0 Research questions

3.1 What is the common *Candida* species in cats in Kota Bharu, Kelantan?

3.2 What is the antifungal resistance of *Candida* species isolated from the cats in Kota Bharu, Kelantan?

4.0 Research hypothesis

4.1 *Candida* species are the dominant *Candida* species present in cats in Kota Bharu, Kelantan.

4.2 *Candida* species isolated and identified in cats in Kota Bharu, Kelantan show antifungal resistance towards commonly used antifungal drugs.

5.0 Objectives

5.1 To identify the dominant *Candida* species from companion animals in Kota Bharu, Kelantan, by using systematic mycology and molecular identification techniques.

5.2 To determine the antifungal resistance of the *Candida* species isolated to certain antifungal drugs.

6.0 Literature review

6.1 Characteristics of Pathogenic Yeast

Yeasts are single-celled fungi that reproduce asexually by producing blastospores (budding) or sexually by producing asco- or basidiospores. They are not a formal taxonomic unit but rather a growth form shared by various unrelated fungus, including filamentous forms in some cases as part of their life cycle or under specific environmental conditions. There are over 80 genera and nearly 600 species known. *Candida albicans, Cryptococcus neoformans*, *Malassezia pachydermatis, Geotrichum candidum*, and *Trichosporon species* are the most critical veterinary pathogens. Dimorphic fungi, such as *Sporothrix schenckii, Blastomyces dermatitidis, Histoplasma capsulatum, Histoplasma farciminosum,* and *Coccidioides immitis*, look

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as yeasts in host tissue but thrive as molds in the environment. (Hörmansdorfer & Bauer, 2000).

6.2 Candida species in companion animals

Moisture-rich regions such as mucous membranes, mucocutaneous junctions, skinfolds, interdigital areas, and ears are ideal for yeast colonization. *Candida* species are common organisms that live almost entirely as commensal organisms and only rarely become harmful. A variety of skin microenvironmental parameters, such as bacterial microbiota, pH, salts, immunological responses, biochemistry, and physiology, may influence yeast adherence and growth, favouring different genotypes depending on the geographical location and skin sites. The outcomes of scientific studies on *Candida* yeasts in animals vary (Sihelská *et al*., 2017).

The *Candida* genus is thought to be a part of a dog's microbiome (Cleff *et al*., 2005). According to Sihelska, (2017), *Candida* spp. is not found in the typical skin flora. *Candida* yeasts generally colonize mucosal membranes, and their appearance on the skin is unusual and may be linked to the onset of a disease. Finally, *Candida* spp. does not generally appear on the skin of healthy dogs. This yeast's existence is more closely related to the mucosae (Sihelská *et al*., 2017).

6.3 Factors driving emergence of antifungal resistance

Therapeutic failure occurs when a medication given at a regular dose has no effect on a patient. This failure is caused by a combination of host, pharmacological, and microbial factors. It is more likely that patients with a weak immune system will not respond to antifungal therapy because the antifungal medicine is able to fight the illness even without help of a potent immunity because drug-impermeable microbes

colonise the surfaces of indwelling catheters and mechanical heart valves. Given the fact that prescription drug penetration at infection sites is poorly understood, it is known that drug delivery within the host contributes to treatment failure and an intra-abdominal abscess can seed drug resistance by exposing fungus to inadequate therapeutic doses (Perlin *et al.*, 2017).

It is possible that noncompliance with medication regimens may lead to suboptimal drug exposure in patients with chronic infections. Prophylactic, repeated, or long-term drug exposure is linked to the development of resistance. In the same way, agricultural fungicides with equivalent molecular targets to those of systemic antifungal drugs have seeded reservoirs of resistant organisms in the environment. It is possible for a bacterium's drug resistance to be either inherent (intrinsic) or external (acquired). Sometimes the same process as acquired resistance is involved in finding primary drug resistance in fungi which have never been subjected to drugs before, but unknown mechanisms can also be at play here (Perlin *et al.*, 2017).

6.4 Molecular identification of Candida species

The goal of molecular identification is to determine which species of *Candida* are present in samples from companion animals. Polymerase Chain Reaction (PCR) is a precise molecular detection method for distinguishing different *Candida* species isolated from materials.

The *ITS* region has been proposed as the universal fungus barcode sequence since it is the most commonly sequenced DNA region in fungi molecular ecology (Schoch *et al*., 2012). It has been most useful for molecular systematics at the species level, and even within species, in the past. Variation among individual rDNA repeats can sometimes be found within both the *ITS* and *IGS* regions, due to its higher degree of variation than other genetic sections of rDNA (for small- and large-subunit rRNA).

Turenne et al., (1999) found that changes in the diameters of fungal *ITS2* sections were helpful in identifying clinically significant fungi quickly. Several taxon-specific primers have been described that allow selective amplification of fungal sequences in addition to the usual *ITS1+ITS4* primers used by most labs (White *et al.*, 1990).

7.0 Materials and methods

7.1 Sample collection in Kota Bharu and Universiti Malaysia Kelantan Veterinary Clinic

Complete physical examination of the cats was done prior to taking the samples and relevant history was recorded. Patients' information including age and sex of the animals are recorded. Any abnormal findings such as presence of interdigital erythema, salivary staining on the fur, pustules, hyperpigmentation, and gross skin lesions are also recorded for further discussions. Swab samples are collected from 60 cats which consist of 30 owned cats and 30 stray cats in Kota Bharu and Universiti Malaysia Kelantan Veterinary Clinic, Kelantan with or without abnormalities relating to clinical signs of fungal infections.

Swab with transport media were used to collect the samples. Rectal swab, ear swab, nasal swab and oral swab from each animal are collected and transported in an icebox (chilled) to bacteriology and mycology laboratory at Faculty of Veterinary Medicine, Universiti Malaysia Kelantan. The samples are processed immediately or stored at 4°C chiller to be processed the next day.

7.2 Isolation and identification of *Candida* spp.

The swab samples taken are inoculated on Sterilized Sabouroud Dextrose Agar (SDA) and incubated at different temperature ranging from 37°C for *Candida* species to grow. No antibiotic added to prevent bacterial growth. After 48 to 72 hours, the colonies form on the SDA Agar was observed. The colonies of *Candida* spp appear cream colored, elevated, whole, smooth and butyrous. The colonies may produce small striations or outgrowths known as "feet", which are suggestive of some *Candida* spp (Ali, 2015).

7.3 Polymerase chain reaction

7.3.1 DNA Extraction

In this DNA extraction procedure, a combination of freezing process and DNA extraction kit was used. In freezing process, the cells were suspended in the 1000 µl distilled water and heated at 95°C for 15 minutes and submitted to freezing at -70°C. the samples were thawed at room temperature and centrifuged to remove the cell debris.

Next, for pre-lysing step, 1000µl of the yeast was resuspended in 240 µl Buffer T1 plus 30 µl Proteinase K solution. The mixture was vortex vigorously and incubated at 60°C for 3 hours or overnight. As to lyse the sample, the samples were vortex and 200µl Buffer B3. It was then vigorously vortex and incubated at 70°C for 10 min. The samples were again vortex briefly. 210µl Ethanol (96-100%) was added to the samples and were vortex vigorously. For each sample, one Nucleospin Tissue Column into a collection tube. The sample was applied into the column and centrifuged at 11 000 x g for 1 minute.

Next, the column was centrifuged for 1 minute at 11 000 x g and residual ethanol was removed to dry the silica membrane. The last step in DNA extraction procedure is to elute highly pure DNA. The Nucleospin Tissue column was placed into a 1.5 ml microcentrifuge and 100µl Buffer BE was added. It was then incubated at room temperature for 1 min and centrifuge for another 1 min at 11 000 x g. The tissue column was discarded and the microtubes was stored for Molecular identification procedure.

7.3.2 Amplification of *ITS2* gene amplification

Candida auris specific *ITS2* and *ERG11* genes were used as primers. 3µl of samples were added to 12.5 µl of Mastermix consisting of 7.5 µl Nuclease free water, 1 µl for both CauF and CauR was added into the mixture. The PCR amplification was done using the following protocols: initial denaturation at 95°C for 3 min, followed by 30 amplification cycles consisting of denaturation at 95°C for 15s, annealing at 68°C for 20s, extension at 72°C for 20s and final extension at 72°C for 5 min. The reaction yields PCR fragments of 163 base pairs (bp).

7.3.3 Amplification of Azole-resistance encoding gene (*ERG11*)

Azole resistant *Candida auris* specific *ERG11*F and *ERG11*R genes were used as primers. 3µl of samples were added to 12.5 µl of Mastermix consisting of 7.5 µl Nuclease free water, 1 µl for both Cau*ERG11*F and Cau*ERG11*R was added into the mixture. The PCR protocol was: initial denaturation 5 min at 95°C followed by 34 cycles of denaturation 30s at 95°C, annealing 30s at 59°C, extension 180s at 72°C and final extension 3 min at 72°C. The final PCR products yield 1500 base pairs (bp).

Table 1 : Primer used in this study

Primer	Target gene	Primer sequence	Amplification products (bp)	Reference
Cau	*ITS2*	F 5'CGCACATTGCGCCTTGGG-GTA-3' R 5'GTAGTCCTACCTGATTTG-AGGCGAC-3'	163	(Kordalewska *et al*., 2017)
CauErg11	*ERG11*	F 5'-GTGCCCATCGTCTACAA-CCT-3' R 5'-TCTCCCACTCGATTTCTG-CT-3'	1500	(Chowdhary *et al*., 2018)

7.4 Gel electrophoresis

The PCR products were added to the well on 2.0% agarose gel. Electrophoresis was carried out for 40 minutes at 100 volts and the gel was visualized using Gel Doc™ EZ Imager.

8.0 Results

7.1.1 Isolation, identification and confirmation of *Candida auris*

To isolate *C. auris*, the samples from rectal, oral, nasal and ear swabs were cultured Saboroud Dextrose Agar as mentioned above. *Candida auris* appeared as creamy, pasty, smooth and mucoid colonies. Gram staining and lactophenol cotton blue staining was done to observe the morphology of the *Candida* spp under microscope. Figure 1 and 2 represent the cultural characteristics of yeast species. Out

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of 60 ear swab samples, 60 rectal swab samples, 60 oral swab samples and 60 nasal

swab samples, 9 (15%) ear swab samples, 34 (56.7%) rectal swab samples, 21 (35%)

oral swab samples and 21 (35%) nasal swab samples fungal isolates were identified as

yeast species by isolation and microscopic examination.

Figure 1 : Gram-staining of the colonies shows gram positive, small, oval and budding colonies represents Yeast species (100x magnification)
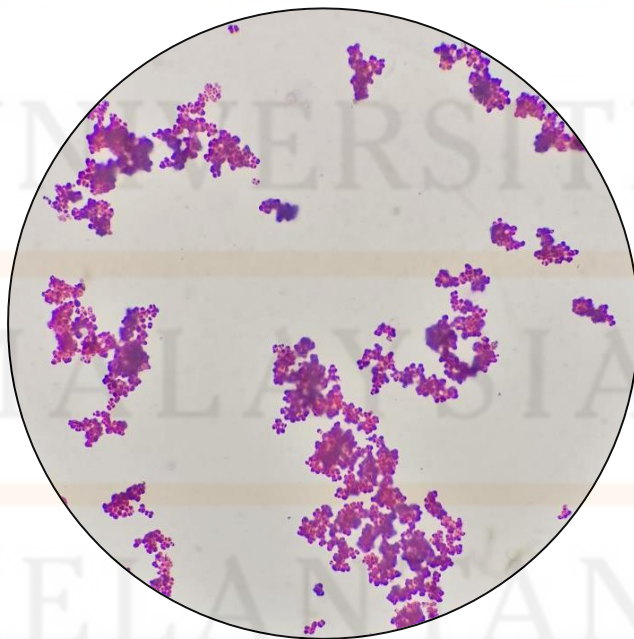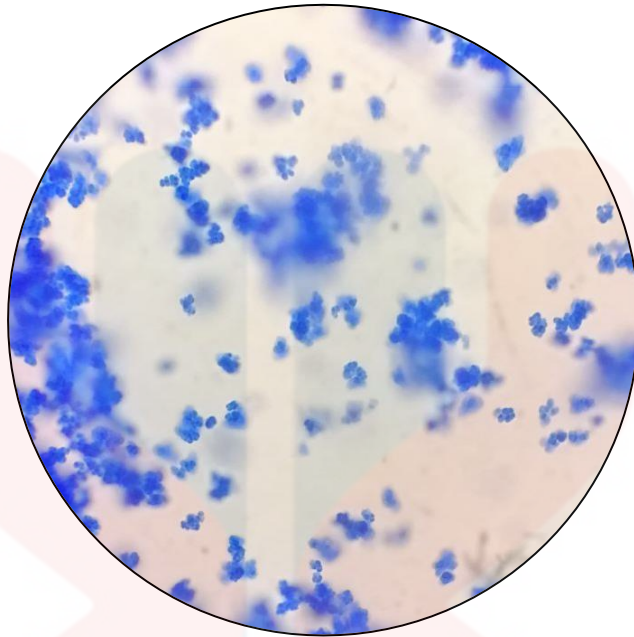

Figure 2 : Gram-staining of the colonies shows gram positive, small, oval and budding colonies represents Yeast species (100x magnification)

Figure 3 : Lactophenol cotton blue colonies shows small, oval, budding colonies represents Yeast species (100x magnification)

7.1.2 Molecular detection of *Candida auris*

Among isolated and identified yeast species, some samples shows no growth on secondary culture. Hence, to detect *Candida auris*, 67 samples isolated and identified to be yeast colonies which consist of 15 oral swab samples, 17 nasal swab samples, 29 rectal swab samples and 6 ear swab samples were subjected to PCR analysis for detection of *ITS2* gene. *ITS2* gene is a specific gene used for confirmation of *Candida auris* species (Kordalewska *et al*., 2017). All confirmed isolates of yeast species on SDA agar were used to detect *Candida auris* using *ITS2* gene primer. Figure 3 is the representative of gel electrophoresis image conducted to analyze the PCR product for *Candida auris* specific gene with the expected band of 163 bp. Unfortunately, all of the samples does not show any positive results.
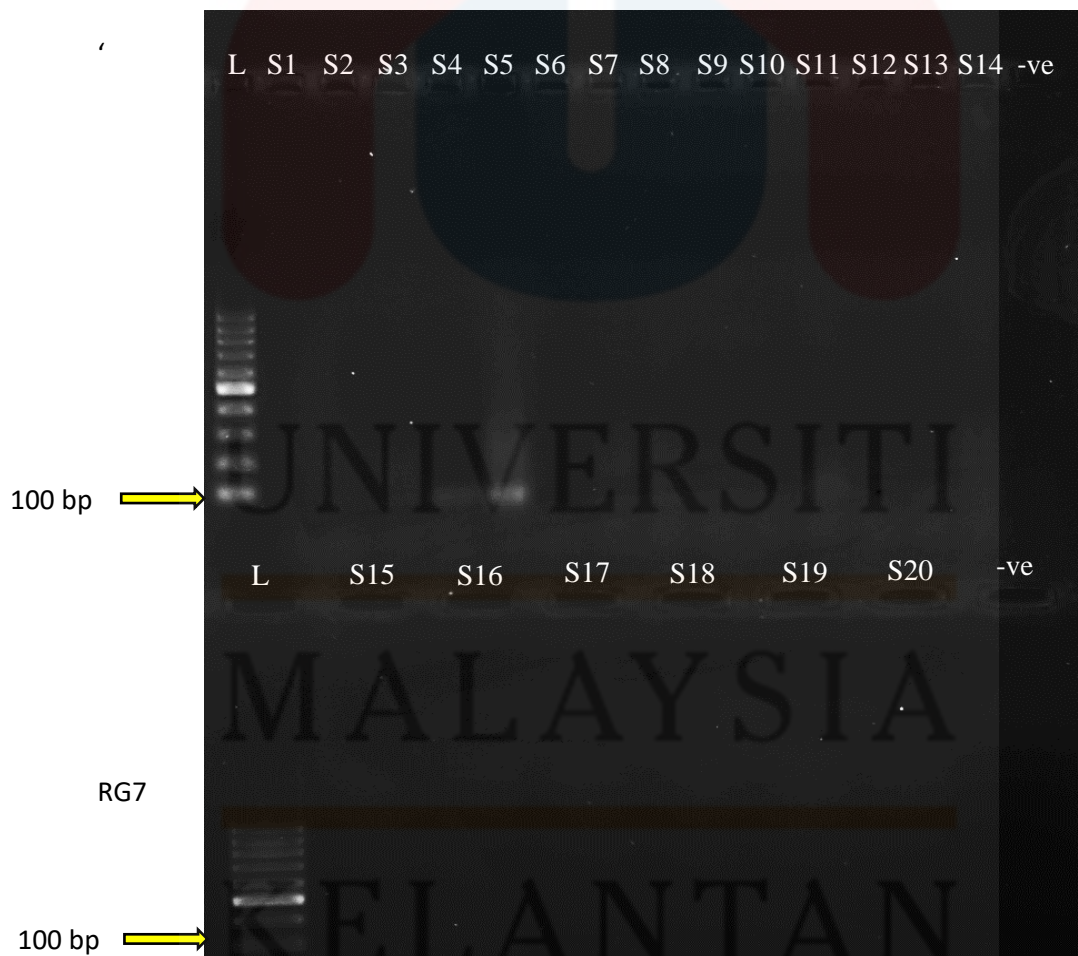
Figure 3 : Lane L : DNA ladder, Lane S1-S20 : Swab samples, Lane -ve : Negative control

7.1.3 Molecular detection of *Candida auris ERG11* resistance gene

To detect *Candida auris ERG11* resistance gene, 67 samples which consist of 15 oral swab samples, 17 nasal swab samples, 29 rectal swab samples and 6 ear swab were analyse by using PCR for detection of *ERG11* resistance gene. Figure 4 shows the gel electrophoresis image taken to analyse the PCR product for *Candida auris ERG11* resistance gene with the expected band of 1500bp. The result was unfortunately negative for all isolates.
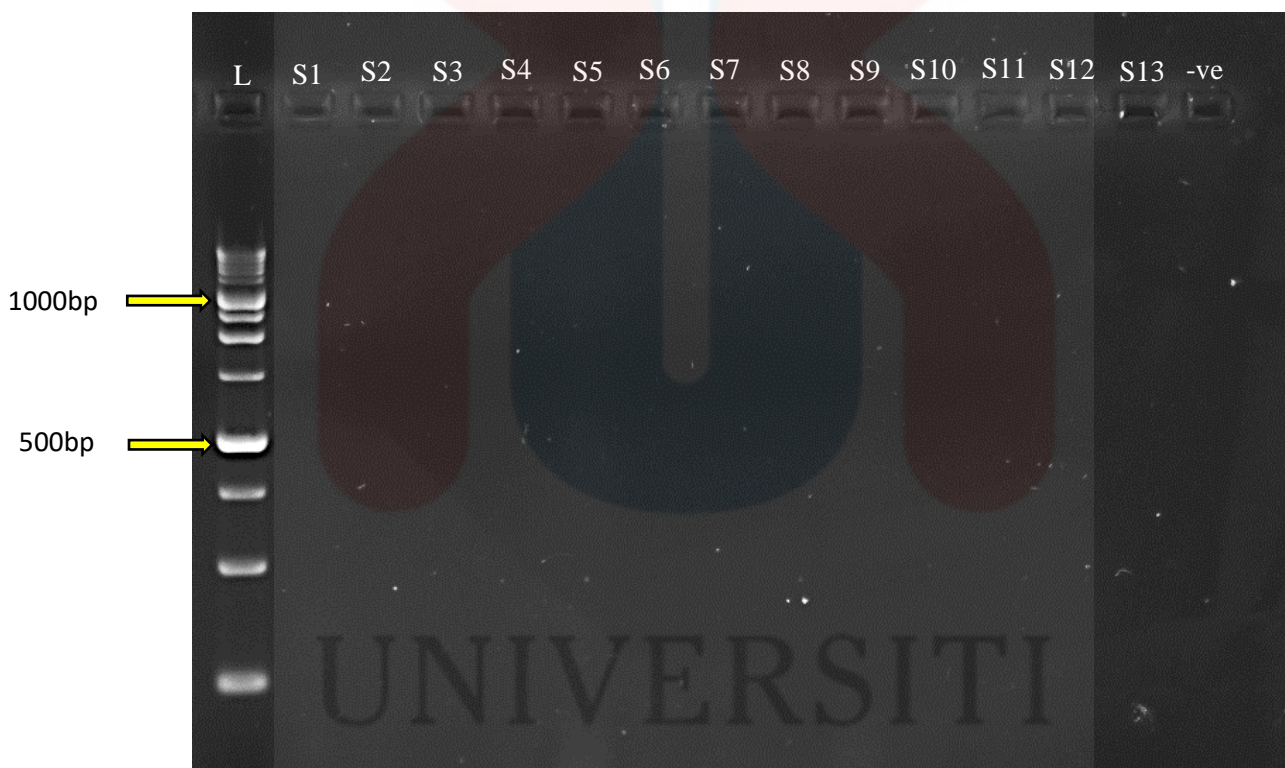
Figure 3 : Lane L : DNA ladder, Lane S1-S13 : Swab samples, Lane -ve : Negative control

9.0 Discussion

In this study, 240 swab samples were collected. In the process of identifying yeast isolates from the swab samples, 9 (15%) ear swab samples, 34 (56.7%) rectal swab samples, 21 (35 %) oral swab samples, and 21 (35%) nasal swab samples were examined. It was determined that the isolated fungi were species of yeast. The percentage of yeast isolates found in rectal swab samples is the highest, followed by those found in oral, nasal, and ear swab samples. *Candida* species are frequently found in the digestive tract and the genital tract of healthy hosts (Miceli *et al*., 2011). This is one of the possible factors that contributes to the high percentage of yeast isolates that can be found in rectal swabs.

Only 67 of the original 85 yeast isolates were successful in having their DNA extracted during the process of molecular testing. This is as a result of the lack of growth in the subculture that was intended to be further identified for molecular testing. The molecular detection of *Candida auris* and the resistance gene both show that there are no expected bands on the gel, but upon visualisation, the gel appears to have smearing on it. This particular type of visualisation may be the result of a number of different types of errors, such as low DNA quality or insufficient primer optimization. Modulating the stringency of a reaction in such a way that the specificity is adjusted is possible. This is accomplished by modifying variables such as reagent concentrations and cycling conditions, both of which have an effect on the outcome of the amplicon profile. The absence of PCR products is most likely due to overly demanding reaction conditions. Primer dimers and smearing that form with the primers or in the denaturized template DNA may also prevent amplification of PCR products because these molecules may no longer base pair with the desired DNA

counterpart. Primer dimers and smearing can form when either the primers or the template DNA are denatured (Lorenz, 2012)

In addition to this, there is the possibility that technical errors occurred during the DNA extraction process, which led to the residual ethanol on the silica membrane not being completely removed during the drying steps. In addition, the DNA extraction procedure that was carried out in this research was carried out utilising a combination of the freezing process and a DNA extraction kit. When extracting purified DNA from a variety of samples, one of the most effective methods is to use a DNA extraction kit. In addition, the quality of the DNA may be low because it has degraded as a result of contamination in the laboratory or because it was stored for an extended period of time at room temperature (Lorenz, 2012).

It is also possible that the *Candida auris* species were not discovered because the swab samples did not contain any of the species. This would explain why the species has not been discovered. The United States of America, Latin America, Europe, and Asia were the regions in which *Candida albicans* was found to be the most prevalent, despite the fact that this species was responsible for fewer than half of the total cases reported worldwide. There were substantial variations in the manner in which pathogens were dispersed across the various regions of the world. In comparison, *Candida parapsilosis* and *Candida tropicalis* only made up 11% and 14% of the isolates found in the United States, respectively. This was the case in Latin America, where they accounted for 30% and 25% of the total. *Candida glabrata* was found to be isolated in the United States at a rate of 18%, which was significantly higher than the rates found in Latin America (3%) or Europe (8%). *Candida parapsilosis*, which was found at a frequency of 19% in Latin America, was the second

most common of the pathogens isolated in this study worldwide after *Candida albicans*, which was found at a frequency of 45% (Colombo *et al*., 2003). According to these reports, these species of *Candida* are the most common species that have been identified, which raises the possibility that the molecular detection of *Candida auris* will produce a negative result.

In addition, the possibility exists that the false negative result in this study was caused by human error during the sampling procedure. Inadequate storage or sampling methods could be the factor for the absence of organisms in samples where they should have been present. Additionally, the presence of multiple strains of the *Candida auris* species could be one of the factors that contribute to a negative molecular detection of *Candida auris* using Polymerase Chain Reaction (PCR)

10.0 Conclusion

The isolation and identification of *Candida* species from cats in Kota Bharu, Kelantan is the first report on the detection of these fungus in Malaysia. It is anticipated that this information will serve as the preliminary data to initiate comprehensive and large-scale research on this fungus throughout the country. On the basis of the findings obtained from the identification of yeast isolates, one can draw the conclusion that yeast species are prevalent in the mucocutaneous regions of cats. According to the highest number of yeast isolates found in a rectal swab sample, it appears to be found most frequently in the gastrointestinal tract. In spite of this, the molecular detection of these yeast isolates came back negative for *Candida auris*. This was likely the result of a combination of factors, including a lack of time and human errors. With the help of the findings from this study, the researchers who are relevant

to the topic can develop appropriate guidelines for continuing the study and coming up with positive results.

11.0 Recommendations and future work

A larger number of animals, including dogs, can be used in this studies in the future, which will help improve the quality of the studies. This will lead to more varied findings, which can then be used to draw broader conclusions about the study. Secondly, in order to reach conclusions that can be relied upon, this research could benefit from the addition of additional primers specific to other *Candida* species. More accurate identifications can be made, and the goals of the study can be accomplished with greater ease if different primers are used for each species of *Candida*.

Appendix A
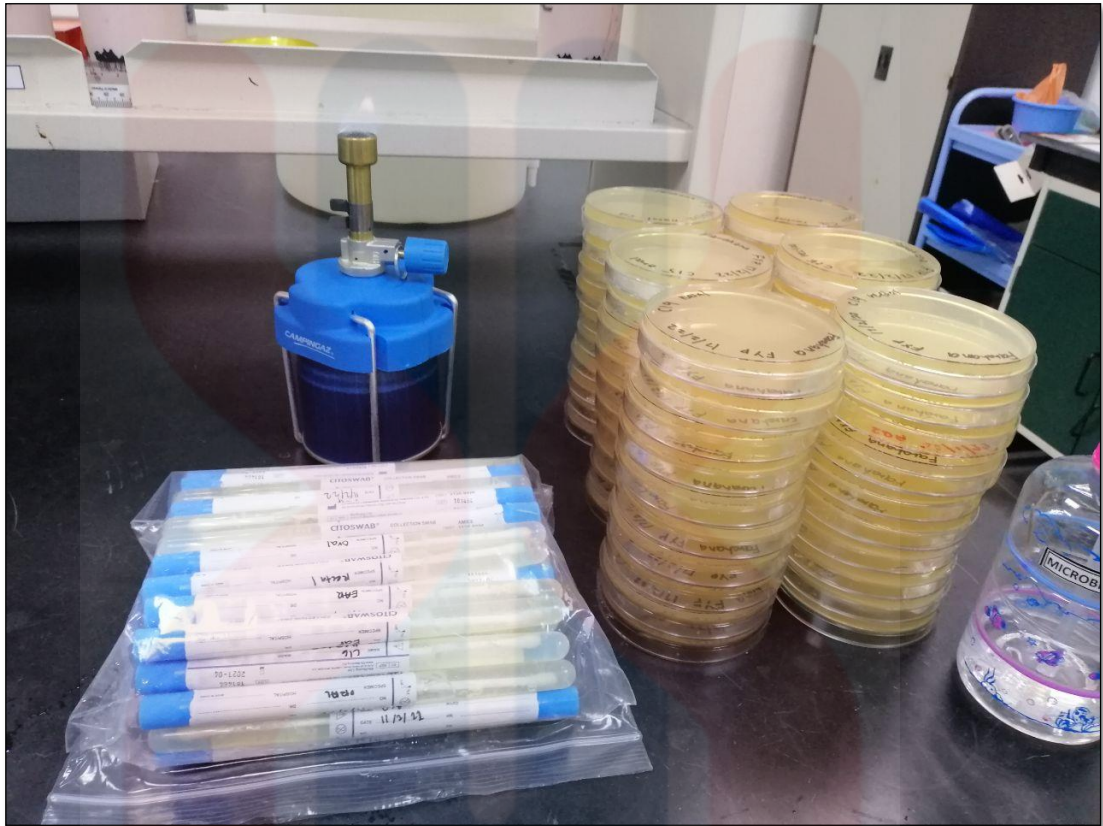
Appendix A. 1 : Swab samples with Saboroud Dextrose Agar (SDA)

Appendix A. 2 : Fungal isolates on Saboroud Dextrose Agar (SDA)
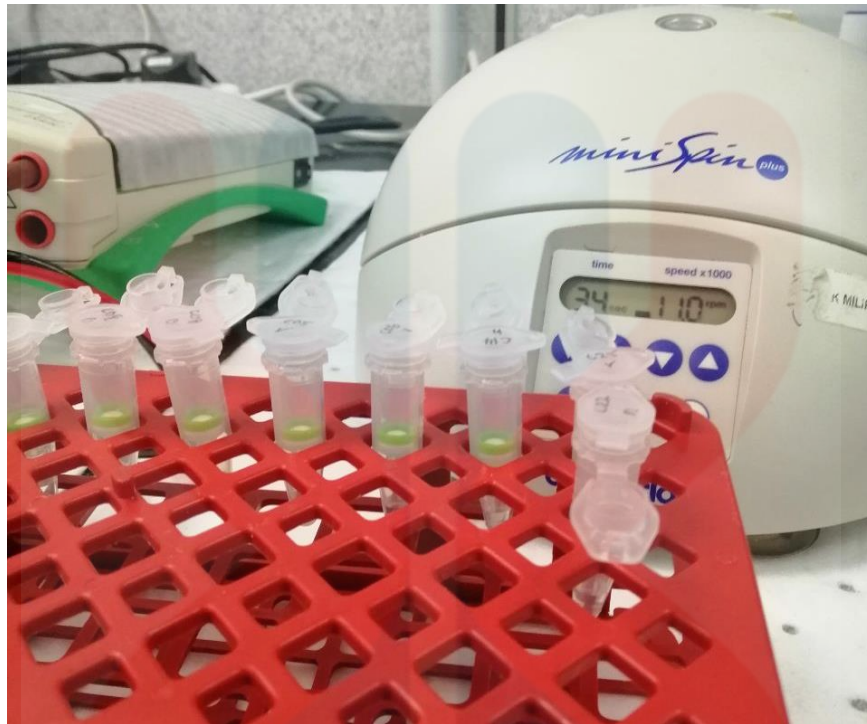
Appendix A. 4 : Microtubes with Nucleospin Tissue Column and Eppendorf Centrifuge Minispin machine

Appendix A. 3 : PCR tube for molecular detection

Appendix B

Appendix B. 1 : Consent form to conduct the study

IACUC UMK/ CONFIDENTIAL

Universiti Malaysia KELANTAN

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
UNIVERSITI MALAYSIA KELANTAN

Application for Approval
of a Project Involving the Use and Care of Animals

NOTE:

1. Please complete the application form in accordance to the Animal Ethics Committee Guidelines. Incomplete application will result in the return of the application and delay in the granting of the approval.

2. Attach a copy of the proposal (research / elective / teaching / other).

3. Application must be word-processed or typewritten and forwarded to the Chairperson, Animal Ethics Committee of the Faculties/Centres (ACUC PTJ), Universiti Malaysia Kelantan (UMK).

TYPE OF APPLICATION: [Please tick (/)]

RESEARCH (/) / ELECTIVE (   ) / TEACHING (   ) / OTHER (   ) Please specify: _____

If teaching / elective project, state course name and code:
FINAL YEAR PROJECT (DVT 5463)

NAME OF PRINCIPAL INVESTIGATOR / CO-ORDINATOR / SUPERVISOR / CHAIRPERSON:

DR. ERKIHUN AKLILU WOLDEGIORGIS
DR. GOH SOON HENG
NUR FARAHANA BINTI ZAMRI

FACULTY / CENTRE:
FACULTY OF VETERINARY MEDICINE

PROJECT TITLE:
ISOLATION, MOLECULAR IDENTIFICATION AND ANTIFUNGAL RESISTANCE OF *CANDIDA SPP.* FROM CATS AND DOGS IN KOTA BHARU, KELANTAN

Received by Secretary, Animal Ethics Committee

ACUC PTJ File No: Date:

Appendix B. 2 : Ethic Approval form prior to random sampling

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