INVESTIGATION OF THE PRESENCE OF BACTERIA ON SWAB SAMPLES TAKEN FROM ASEPTIC SURGICAL SITE IN DIFFERENT SURGERY DURATION AT VETERINARY MEDICINE TEACHING HOSPITAL UNIVERSITI MALAYSIA KELANTAN

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(D19B0037)

DOCTOR OF VETERINARY MEDICINE

Investigation of the Presence of Bacteria on Swab Samples Taken from Aseptic Surgical Site in Different Surgery Duration at Veterinary Medicine Teaching Hospital Universiti Malaysia Kelantan

By

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A research project submitted to the Faculty of Veterinary Medicine Universiti Malaysia Kelantan in partial fulfillment of the requirements for the Degree of Doctor of Veterinary Medicine

Faculty of Veterinary Medicine
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INVESTIGATION OF THE PRESENCE OF BACTERIA ON SWAB SAMPLES TAKEN FROM ASEPTIC SURGICAL SITE IN DIFFERENT SURGERY DURATION AT VETERINARY MEDICINE TEACHING HOSPITAL UNIVERSITI MALAYSIA KELANTAN

ABSTRACT

Endogenous and exogenous sources can cause bacterial contamination during surgery. The aim of this study was to investigate the presence of bacteria on skin swab samples taken from surgical sites of cat species in different surgery duration associated with various risk factors at Veterinary Medicine Teaching Hospital Universiti Malaysia Kelantan (HPVUMK). A total of 105 skin swabs samples were collected from selected 32 felines that are involved in reproductive, abdominal and orthopedic surgery at HPVUMK. The sample was taken before skin preparation with antiseptic, after skin preparation, and every 30 minutes of surgery until the surgery finished. Bacteria culture and isolation was performed and statistical analysis data was analysed using Pearson Chi Square test or Fisher's exact test by using SPSS 27 statistical software. From the bacteria culture, the sample taken before skin preparation procedure showed nine cats (28%) without any growth, while 23 cats (72%) with different numbers of colony growth where *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp., Corynebacterium spp.*, and *Bacillus spp.* were isolated and overall, only one sample had bacteria growth during the surgery which is at 1 hour 30 minutes of the surgery procedure. Statistical analysis data revealed the presence of bacteria colonies on the skin of a cat before undergoing skin preparation with antiseptic solution was significantly associated ($P<0.05$) with the management of the cat whether they were kept indoor, outdoor or semi-roamer and the result obtained from this study showed bacteria on the skin before skin preparation can be successfully be eliminated after antiseptic was applied. Besides, there is also data obtained from this study that revealed the presence of bacterial colonies during surgery was significantly associated ($P<0.05$) with the type of surgical procedure and the total number of people in the operation room at one time. Thus, the findings of this study prove that an aseptic technique practiced at HPVUMK was effective in eliminating the bacteria colony on the surgical site that has been prepared aseptically as the bacteria was not be able to be isolated in almost all of the surgical site after scrubbing with antiseptic and as only one out of 32 procedures got contaminated by the bacteria during the surgical procedure at HPVUMK, we can conclude that even in a proper practice of aseptic technique throughout the surgery procedure, however the chances for contamination to occur on the surgical site that has been prepared aseptically is still present.

Keywords: Bacteria, feline, skin swab, aseptic, surgical site.

KAJIAN KEHADIRAN BAKTERIA PADA SAMPEL SAPU YANG DIAMBIL DARI KAWASAN ASEPTIK PEMBEDAHAN DALAM TEMPOH PEMBEDAHAN BERBEZA DI HOSPITAL PENGAJAR PERUBATAN VETERINAR UNIVERSITI MALAYSIA KELANTAN

ABSTRAK

Sumber endogen dan eksogen boleh menyebabkan pencemaran bakteria semasa pembedahan. Matlamat kajian ini adalah untuk menyiasat kehadiran bakteria pada sampel sapuan kulit yang diambil dari kawasan pembedahan daripada spesies kucing dalam tempoh pembedahan berbeza yang dikaitkan dengan pelbagai faktor risiko di Hospital Pengajar Perubatan Veterinar Universiti Malaysia Kelantan (HPVUMK). Sebanyak 105 sampel sapuan kulit telah dikumpul daripada 32 ekor kucing terpilih yang terlibat dalam pembedahan reproduktif, abdomen dan ortopedik di HPVUMK. Sampel diambil sebelum penyediaan kulit dengan antiseptik, selepas penyediaan kulit, dan setiap 30 minit pembedahan sehingga pembedahan selesai. Bakteria kultur dan pengasingan bakteria telah dilakukan dan data analisis statistik dianalisis menggunakan ujian Pearson Chi Square atau ujian tepat Fisher dengan menggunakan perisian statistik SPSS 27. Daripada keputusan bakteria kultur, sampel yang diambil sebelum prosedur penyediaan kulit menunjukkan sembilan ekor kucing (28%) tanpa sebarang pertumbuhan, manakala 23 ekor kucing (72%) dengan jumlah dan jenis pertumbuhan koloni yang berbeza di mana *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp.*, *Corynebacterium spp*., dan *Bacillus spp*. telah didapati pada kultur media dan secara keseluruhannya, hanya terdapat satu sampel yang mempunyai pertumbuhan bakteria pada 1 jam 30 minit prosedur pembedahan. Dapatan itu mendedahkan kehadiran *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp.*, *Corynebacterium spp*., dan *Bacillus spp*. yang diketahui sebagai flora normal pada kulit kucing yang sihat dan didapati di persekitaran. Analisis data statistik menunjukkan kehadiran koloni bakteria pada kulit kucing sebelum menjalani prosedur penyediaan kulit dengan larutan antiseptik adalah signifikan ($P<0.05$) dikaitkan dengan pengurusan kucing sama ada ia dijaga di dalam rumah, bebas di luar kawasan rumah atau keduanya. Selain itu, terdapat juga data yang diperoleh dari kajian ini mendedahkan kehadiran koloni bakteria semasa pembedahan adalah signifikan ($P<0.05$) dikaitkan dengan beberapa faktor risiko termasuk jenis prosedur pembedahan dan jumlah bilangan orang di dalam bilik bedah pada suatu masa. Justeru, dapatan dari kajian ini membuktikan teknik aseptik yang diamalkan di HPVUMK adalah berkesan dalam menghapuskan koloni bakteria pada kawasan pembedahan yang telah disediakan secara aseptik kerana bakteria tidak dapat dijumpai di kebanyakan kawasan pembedahan yang telah dicuci dengan antiseptik dan kerana hanya satu daripada 32 prosedur sahaja telah dicemari oleh bakteria semasa prosedur pembedahan di HPVUMK, kita boleh membuat kesimpulan bahawa walaupun pelaksanaan teknik aseptik yang betul dilakukan sepanjang prosedur pembedahan, tetapi peluang untuk pencemaran berlaku di tapak pembedahan yang telah disediakan secara aseptik masih ada.

Kata kunci: Bakteria, kucing, swab kulit, aseptik, kawasan pembedahan.

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DEDICATION

First and foremost, I dedicate this project to Allah S.W.T for the strength and protection given to complete this project in fulfilling the course DVT 55204 Research Project.

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NO.	
No.	Number
spp.	Species
et al.	et alia (Latin term); and others
HPVUMK	Veterinary Medicine Teaching Hospital Universiti Malaysia Kelantan
BCE	Before Common Era
CE	Common Era or Current Era
DSH	Domestic Shorthair
DLH	Domestic Longhair
x2-test	Pearson Chi Square test
SPSS	Statistical Package for the Social Sciences

LIST OF SYMBOLS

NO.	
&	And
<	Less than
>	Greater than
%	Percentage
P-value	Probability value
n	Number of subsamples
°C	Degree Celsius
mg	Milligram
kg	Kilogram
ml	Milliliter
cm^2	Square centimeter

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CHAPTER 1

INTRODUCTION

Asepsis is a state in which there are no active pathogenic germs which can refer to all the measures taken, such as the use of sterile gloves, sterile drapes, and sterile instruments, to lower the possibility of bacterial, fungal, or viral contamination which also includes every preventive measure, operational procedure, and behavioural pattern that can be used to keep microbes out of the patient's body and the surgery site (Dockery, 2012). Preventing contamination is the aim of asepsis to reduce the number of germs in the surgical environment and avoid cross-contamination during surgery (DiGangi, 2019). Sustaining asepsis is thought to be the gold standard for surgical sterilisation and directly affects the course of treatment for patients (Association of Operating Room Nurses, 2006; Griffin *et al,.* 2016). To achieve asepsis, aseptic techniques need to be maintained and when applied properly, it can minimise the number of germs to a level that has minimal consequences (Block, 2001).

Preoperative surgical preparation is a crucial procedure of surgical asepsis, ensuring the surgical site is as free from contaminants as possible to minimise the risk of infections. A previous study involving horses revealed that a basic non-mechanical preparation method was just as effective in reducing skin bacterial counts as a mechanical method (Davids *et al.,* 2015). In line with these findings, the Veterinary Medicine Teaching Hospital Universiti Malaysia Kelantan (HPVUMK) follows a standard operating procedure that employs a mechanical technique for aseptic preparation. This protocol, based on the study by Fossum *et al.,* (1998), is likely designed to balance effectiveness with practicality and resource considerations.

The importance of maintaining aseptic technique throughout the entirety of a surgical procedure due to the fact that aseptic technique is a fundamental practice in surgery aimed at preventing infections by minimising the introduction of microorganisms into the surgical site (Cherney, 2018). Loftus *et al.* (2011) point out that a substantial number of infections arise from invasive clinical procedures contaminated with microorganisms when there is a breakdown in aseptic technique. The maintenance of aseptic technique provides a higher level of protection within the sterile field that is crucial for preventing postoperative infections, as the sterile field serves as a barrier against the introduction of harmful microorganisms and infections that occur after surgery can lead to prolonged recovery times, increased healthcare costs, and, in severe cases, life-threatening complications (Zabaglo & Sharman, 2023).

In the operating room, the most common cause of iatrogenic microbiological contamination is breaks in sterile protocols (Gaines *et al.,* 2017). There are few factors that can contribute towards the introduction of organisms during surgery. According to Mangram *et al.,* (1999), the risk of surgical site infection that cause by the external risk factors include the type and duration of the surgical procedure, surgeon's skill, the standard quality of preoperative skin preparation, adequacy, correct and timing of antimicrobial prophylaxis, insertion of any foreign material or implants like surgical screw and plate, insufficient sterilisation of surgical instruments.

Hence, this study was carried out to investigate the presence of bacteria on an aseptic surgical site in different surgery durations and to determine its predominant species. Moreover, to evaluate the effectiveness of aseptic techniques practised in HPVUMK in order to minimise postoperative infection. Furthermore, the data collected will serve as a reference for further comprehensive studies in the future in comparison with different environmental conditions especially in large animal surgery which required an on-field

procedure to be done and as a guidance for post-surgery management in order to tackle the critical aspect during post-operation.

1.1 Research problem statement

Aseptic technique has been widely practised by the surgery team who was conducting the surgical procedure in the operating room setup. There are several studies being reported on post-operative surgical site infection especially in the human medical field (Owens & Stoessel, 2008; Manian, 2014; Sattar *et al*, 2019). Therefore, it supported that even if the surgical site has been prepared aseptically, there are still risk factors that may contribute towards the presence of bacteria in the surgical site. This could be due to break in sterile procedures which are the greatest source of iatrogenic microbiological contamination in the operating room. Nevertheless, there was not enough information present and less study has been conducted to look for the presence of bacteria on an aseptic surgical site itself in different duration of surgical procedure specifically in animals, including veterinary institutions and clinics or hospitals especially in Malaysia.

1.2 Research questions

1. What are the common skin microflora species that can be found on the feline skin?
2. Do types of surgery procedure influence the surgery duration thus increasing the possibility for the presence of bacteria on an aseptic surgical site?
3. What are the risk factors contributing towards the presence of bacteria in the surgical site that has been prepared aseptically in Veterinary Medicine Teaching Hospital, Universiti Malaysia Kelantan?

1.3 Research hypothesis

1. The common skin microflora that can be isolated on feline skin was aerobic bacteria species such as *Staphylococcus spp., Streptococcus spp., Corynebacterium spp.,* and *Bacillus spp.*
2. Type of surgery procedure may influence the surgery duration thus increasing the possibility for the presence of bacteria on an aseptic surgical site.
3. Risk factors that can be contributed towards contamination are type and duration of the surgical procedure, surgeon's skill in manipulating and managing the instrument, the standard quality of preoperative skin preparation practised by the surgery team and total number of people in the surgery room at one time.

1.4 Research objectives

1. To determine the species of normal microflora present on feline skin.
2. To detect types of bacteria that may present on an aseptic surgical site in different types of surgery procedure and surgery duration.
3. To identify the risk factors contributing towards the presence of bacteria in the surgical site that has been prepared aseptically in Veterinary Medicine Teaching Hospital, Universiti Malaysia Kelantan.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Veterinary surgery and aseptic technique

The history of veterinary surgery and aseptic technique is diverse, with roots tracing back to ancient times. Aristotle, who studied anatomical similarities and differences between animals, was known as the early father of comparative medicine as his studies involved detailed examinations of anatomical similarities and differences among various animals (Tobias & Johnston, 2012). His contributions laid the basis for understanding surgical interventions in veterinary medicine. During the development of modern surgery, English surgeon Joseph Lister (1827–1912) demonstrated attempts to use antisepsis to manage surgical infection. The results of French chemist Louis Pasteur (1822–1895), who investigated fermentation and demonstrated that it was brought about by the growth of living things, served as the foundation for Lister's investigation. Lister believed that surgical patients' inability to heal their wounds was due to these invisible germs. He suggested that suppuration may be avoided by cleaning a wound with an antibacterial solution and covering it with a dressing soaked in the same germicidal liquid (Rutkow, 2010).

2.2 Importance of aseptic technique in veterinary surgery

The importance of aseptic technique is to ensure there is little chance of microbial contamination during surgery (Cherney, 2018). Any wound open to the atmosphere will become contaminated and with time those contaminant organisms may colonise the wound and cause an infection (Bowler *et al.*, 2001). Transient or contaminating bacteria do not normally colonise skin as they are usually acquired by contact with animals or the

environment. While resident or colonising bacteria live on a normal healthy skin and help to protect the skin from invasion by pathogenic species. Although they are not particularly pathogenic, they might spread infection after surgery or other invasive operations. However, both have the potential to be the sources of microbial contamination at the surgical site (Hopper & Moss, 2010).

2.3 Animal preparation and surgery area

In veterinary surgery, the surgical area and the animal preparation area should be close by but separated. According to the National Institute of Health/ Office of Animal care and Use (2016), different requirements will be applicable depending on the procedures and the species used. For the rodents and nonmammals, they can be prepped in the same room as the surgery, provided that the preparation area is located separately and at a suitable distance from the surgical space in order to prevent hairs and dander contamination while large animal preparation must be done outside of the operating room. Besides, all survival procedures have to be performed in approved work rooms or in specially designed surgical suites under aseptic conditions where confined sterile surgery area maintenance should involve routine cleaning and disinfection of the entire area (Clevenger *et al.,* 2018). While surgery that is non-survival, minor, emergency, or involving rodents or nonmammals, the procedure can be completed at the area that has been separated from other activities or can just be carried out in a workspace (Bernal *et al.,* 2009).

2.4 Factors contributes towards failure of aseptic technique

Maintaining an aseptic technique is a critical standard in healthcare settings. However, breaks in sterile procedures are identified as the primary source of iatrogenic microbiological contamination in the operating room (Gaines *et al.*, 2017). Identifying and correcting sterile technique errors that arise before, during, and following a surgical procedure is one of the primary responsibilities of the surgical team (Hopper & Moss, 2010). While for the external risk factors that have an important influence on the risk of infections at the surgical site include the type and length of the surgical procedure being performed, the surgeon's skill and experience, the standard of preoperative skin preparation, the adequacy, appropriateness, and timing of antimicrobial agent prophylaxis, as well as the insertion of internal fixation materials (Dharan & Pittet, 2022). Besides, other potential risk factors may come from the airborne contamination which is influenced by a number of sources including the number of people in the operating room and their activity level, the air quality and the rate of air exchange and the cleaning procedure's quality of the room environment (Roy, 2018).

2.5 Etiological agent of common microflora on a healthy skin

There are a variety of microorganisms colonising the body. These microbes may be beneficial, boosting immunity and preventing the proliferation of harmful microorganisms or they may be pathogenic, causing infection at the affected tissues (Grice *et al.,* 2008). The microbial populations present in and on the body vary with different anatomical locations (Grice & Segre, 2011). Several studies have been conducted to describe the microbiota of the human skin and other body parts (Grice & Segre, 2011; Hoffmann *et al.,* 2015). In normal healthy skin, although the skin microbiota of humans and dogs has been extensively studied, little was known about the skin microbiome of felines. However, the most current

study on the skin microbiota of cats has now focused on Staphylococcus due to its involvement in skin diseases (Lu & McEwan, 2007). There was a study that used a culture-based approach to characterise the skin microbiota of cats discovered that *Micrococcus, Acinetobacter, Streptococci*, and *Staphylococci* predominated in the sites that were sampled. Nevertheless, the bacteria could not be isolated from half of the samples, which the authors attributed to cats' natural grooming habits (Krogh & Kristensen, 1976).

2.6 Post-operative management

Post-operative management is a critical phase of patient care that plays a pivotal role in preventing complications and promoting a smooth recovery following surgery where the nature and extent of post-surgery care are influenced by various factors, including the type of surgery performed and the patient's medical history and its condition (Horn & Kramer, 2022). After the procedure, postoperative care continues in the recovery area, in the hospital, and during the period after discharge with extra attention needed for several surgical procedures (Mohabir & Coombs, 2022) Generally, depending on the degree of tissue damage and suffering, patients should get pain medication for three to 14 days following surgery with the same parameters that are tracked to evaluate if a patient needs analgesia during the perioperative phase are tracked to evaluate how well a treatment is working (Fossum *et al.,* 1998). However, preventive antibiotics given within an hour following surgical incision and continued usage during the early postoperative period have shown to be among the most effective approaches to prevent infection (Van Kasteren *et al.,* 2005; Prokuski, 2008).

CHAPTER 3

MATERIALS AND METHOD

3.1 Animal Ethic and Owner Consent

This study has been approved by the Animal Ethics Committee of Universiti Malaysia Kelantan (UMK/FPV/ACUE/FYP/001/2023) before the commencement of the fieldworks (Appendix A). In addition, the animals' owners were given a consent form of Animal Acceptance and Release before their animals can undergo surgery at Veterinary Medicine Teaching Hospital Universiti Malaysia Kelantan (Appendix B). Therefore, all patients that were selected in this study underwent the surgery procedure with the consent from the owner.

3.2 Study Design

The study was conducted at Veterinary Medicine Teaching Hospital Universiti Malaysia Kelantan (HPVUMK). The samples were collected from 32 selected cats that had a surgical procedure between 1st September 2023 until 25th October 2023. In this study, the sample interventions only involve cats that have an intact skin that requires at least 30 minutes of surgery duration and underwent a certain surgery procedure that consist of reproductive, abdominal or orthopedic procedure which include ovariohysterectomy, hindlimb amputation, intramedullary pinning, coxofemoral disarticulation, and diaphragmatic hernia repair without involvement of any open wound prior surgery. While cats with pre-existing open wounds prior to surgery were explicitly excluded from the study. This criterion was established to maintain a homogeneous sample and focus on surgeries without the complicating factor of existing wounds.

3.3 Surgical Preparation

The same aseptic preparation protocol was used for all patients. Induction and skin preparation was done at the skin preparation room which was located next to the operating room. After induction with gas isoflurane 5%, surgical sites were clipped and gauze soaked with chlorhexidine gluconate 4% was used to scrubbed in a back-and-forth motion starting from the centre where an incision line will be made followed by periphery area. Then, a tissue paper was used to wipe the area and gauze soaked with alcohol 70% was swabbed in the same manner before 10% povidone iodine detergent was applied. After that, the patient was moved from the skin preparation room to the operating room, and the final skin preparation was done at the surgical site by applying 10% povidone iodine solution just before draping. Patients had surgery in one of two surgical suites where both suites were equipped with a single surgery table and rebreathing anaesthetic machine. Interventions not requiring entirely sterile conditions were occasionally performed in the induction room. Intraoperative medication (Clavamox 17 mg/kg, Meloxicam 0.3 mg/kg and Vitamin B12 1ml) were administered at the skin preparation room. Preparation of the surgeon includes scrubbing with sterile iodine detergent and wearing sterile gowns, latex surgical gloves, masks, and caps. All surgery team personnel wore masks all the time.

3.4 Sampling Method

In this study, the sample was in the form of a swab that was taken on the skin at the area where the incision was made. The skin swab sample was taken at different points of time, which is before scrubbing right after clipping of hairs, after scrubbing with antiseptic solution, the first 30 minutes of surgery procedure and the next 30 minutes until finish the surgery using Levine sampling technique. To perform Levine technique, the sterile cotton swab can be moistened by aseptically pouring a small amount (1-2 drops) of sterile water

for injection only if the skin was too dry. Then, the swab needs to be placed over a 1 cm^2 area of intact and viable tissue on the skin bed while rotated for 5 to 10 seconds over that particular area and at the same time gently applying pressure downward with enough force to express fluid from the wound surface (British Columbia Provincial Nursing Skin & Wound Committee). After the specimen has been collected, it should be placed immediately into a sterile test tube with Amies transport medium and can be stored either at 4°C or at 21°C for one to four days (Rosa-Fraile *et al.,* 2005) before proceeding with bacteria culture.

In addition, another sample that was taken was the environmental sample from the faculty laboratory, skin preparation room and operation room using plate exposure method. The method was, a plate with nutrient agar needed to be left for a few hours in the environment before it was incubated for 24 to 48 hours at 37°C. The principle of this method was the physical settling of airborne contaminants from the surrounding environment onto the surface of nutrient agar. The microorganisms in the air will fall onto the agar surface as air passes over the plate. To promote microbial growth, the plate needs to be incubated for 24 to 48 hours at a temperature that would allow the expected microorganisms to develop, usually 37°C (Sharma, 2023). However, due to the hot weather during that time, the agar plate can't be left outside for too long because the agar becomes dried quickly. Therefore, the alternative done to get the sample was I left a sterile petri dish in each of the rooms for a day before performing the bacteria culture. To culture it, 3 ml of sterile water for injection was put inside the petri dish that had been left for a day and then the water was poured onto the nutrient agar before being incubated for one to two days at 37°C.

3.5 Data Collection for Risk Factors

Recorded animal-related data were age, breed, sex, and management. Surgeons' data recorded was their working experience and the total number or surgery involvement throughout the study. Surgical procedure data recorded were type of surgical procedure, surgical site where the samples were taken, duration of surgery interval between 1st skin incision and final closure and the total number of people present in the surgical room at one time. Finally, is the environment which includes the skin preparation room, operation room and the faculty laboratory.

3.5.1 Animal

Cats (n: 32; 6 months - 4 years old; 29 Domestic shorthair: 3 Domestic longhair; 5 males: 27 females; 8 indoor cats: 18 outdoor cats: 6 semi-roamer cats). The diverse demographics of the cat sample make it representative of the general feline population. This enhances the external validity of the study, allowing for more generalizable findings that can be applied to a broader range of cats.

3.5.1.1 *Age*

The sample included a diverse age range of cat patients from 6 months to 4 years old. This age diversity is important as it allows for the examination of surgical outcomes across various life stages. According to the Cat Care for Life chart, there are six feline life stages which are kitten (birth - 6 months), junior (7 months - 2 years), adult (3 - 6 years), mature (7-10 years) and senior (11 - 14 years) and super senior (>15 years) (Appendix C). Therefore, in this study, only three life stages were participated which were 3 kittens, 22 juniors with 7 adult cats.

3.5.1.2 *Breed*

Two breeds of cat were involved in this study which were local Domestic shorthair (DSH) and Domestic longhair (DLH) cat. Out of the total of 32 cats, the majority were DSH (29 cats), and the remaining three were DLH. According to Merriam-Webster, domestic cats can be a purebred, mixed breed, living with humans in the house, or living as a stray or wild cat in a neighbourhood. The reason for selection of these two breeds was because they were the one that came for surgery during the sample taking time frame.

3.5.1.3 *Sex*

The gender distribution in the sample showed 5 male cats and 27 female cats. This gender disparity may be relevant for surgeries with gender-specific considerations. As most of the cat patients that came to HPVUMK during these 2 months of sample taking time frame underwent ovariohysterectomy procedure, therefore the sample in terms of gender distribution will be more female compared to male.

3.5.1.4 *Management*

The categorization of cats into indoor (8 cats), outdoor (18 cats), and semi-roamer (6 cats) groups provides valuable information about their living environments. This classification considers the potential impact on the possibilities for the presence of normal flora if they were in contact with the bacteria that are present in the environment and the way of the owner taking care of the hygiene especially the places where the pets were kept. The justification is that cats classified as indoor dwellers are more likely to have limited exposure to outdoor environments and cats that are outdoor residents have a greater exposure to external environments. While semi-roamer cats fall between indoor and outdoor categories where they all have a different potential in transferring microorganisms

between the environment especially if they encounter with soil, plants, or other animals, leading to a different microbial exposure compared to fully indoor cats with more confined surroundings.

3.5.2 Surgeon

The sampling was done from 1st September 2023 until 25th October 2023. Therefore, there are five surgeons involved and participating in the surgery. As surgeons encounter various cases and challenges, they can adapt and refine their approaches, contributing to an environment of ongoing learning and enhancement. Acknowledging the individual contributions of each surgeon is crucial. Different experience levels and areas of expertise may give comprehensive details to the study. However, in this study the only criteria that was evaluated was based on their working experience involved with clinical practice especially in surgery.

3.5.2.1 *Working experience*

The varying levels of experience among the surgeons allows for an exploration of potential correlations between their expertise and surgical outcomes. It is believed that surgeons with more experience may have encountered a variety of situations and complications which they faced before, that made them to be more precautious in everything they did throughout the surgery procedure in comparison to a new graduate veterinarian that just started working. In this study, there were different total number of surgeries involved by each of the surgeons. Different years of working experiences by the surgery team and the total number of surgeries they involved was tabulated in the result (Table 4.6).

3.5.3 Surgery

In this study, cats that came to HPVUMK for certain surgery procedures will be chosen according to our target criteria which include only cat patients that were having an intact skin without involvement of any open wound prior surgery. Besides, all surgeries were performed at the operating room of the Veterinary Medicine Teaching Hospital, Universiti Malaysia Kelantan. The constant in the surgical setting is to ensure uniformity in the environment for each procedure.

3.5.3.1 *Type of procedure*

The selection of the type of surgery procedure in this study was not set in the first place. As long as the criteria of the sample unit is suitable for it to be selected, then it will be taken to be part of the study. Throughout the sample taking time frame, there are five surgeries included in this study such as ovariohysterectomy, limb amputation, coxofemoral disarticulation, intramedullary pinning and diaphragmatic hernia repair. All the procedures are classified based on the type of surgery which are categorized under abdominal surgery, orthopaedic surgery and reproductive surgery.

3.5.3.2 *Surgical site*

In this study, samples were taken from different surgery procedures which include ovariohysterectomy, hindlimb amputation, intramedullary pinning, coxofemoral disarticulation, and diaphragmatic hernia repair. Therefore, the surgical site will be different depending on the types of the procedure and the location where the incision line was made. The illustration was shown in the appendix (Appendix D, Appendix E, Appendix F, Appendix G).

3.5.3.3 *Duration of surgery procedure*

In this study, the surgery duration was categorised into two; surgery procedure that is less than 30 minutes (<30m) and surgery procedure that is more than 30 minutes (>30m). The minimum number of skin swab samples collected per cat was three samples which consist of a swab from the skin before being prepared aseptically using antiseptic solution, a sample after the surgical site was prepared aseptically and a sample of the first 30 minutes of the surgery. While the maximum number of samples per cat that has been collected in this study was five which involved surgery that took more than 1 hour upon skin incision until skin closure. In overall, the total number of skin swab samples collected from 32 cats with different duration of procedure was 105 skin swabs. For surgery finished within 30 minutes, the total number of swab samples taken was three. While surgery finishes after 1 hour or more, the total number of swab samples will be four or five respectively.

3.5.3.4 *Total number of people in the surgery room at one time*

As Veterinary Medicine Teaching hospital Universiti Malaysia Kelantan is an institution, there will be involvement of students during the study. The first one month of the sample collection period was during the semester break therefore, no students were involved during the surgery. The lowest number of people in the surgery room at one time during sample collection was three people which only involves the surgeon, anaesthetist and scrub nurse while the highest was 6 people which included the surgeon, assistant surgeon, anaesthetist and scrub nurses.

3.5.4 Environment

There were three different environments involved in this study which include skin preparation room, operating room and also bacteriology laboratory at the Faculty of Veterinary Medicine Universiti Malaysia Kelantan. Environmental samples were taken from all three areas to identify the possible microorganisms that are present in all those areas which may interfere with the finding of this study. The reason samples were collected was because those locations were the area where the swab samples were collected and processed. Based on the environmental samples from all those three environments, types of bacteria species that have been isolated on bacteria culture are *Staphylococcus spp., Streptococcus spp.,* and *Enterococcus spp.*

3.6 Bacterial Isolation

Bacteria culture was done which enables the controlled growth of bacterial cells in or on a culture medium in a lab setting. The common medium used at the Faculty of Veterinary Medicine Laboratory was nutrient agar and MacConkey agar. The primary culture was performed on nutrient agar and was incubated at 37°C for 24 hours. Then, the secondary culture was done by streaking a single colony on the nutrient agar and MacConkey before being incubated at 37°C for 24 hours.

Gram Staining

Gram staining was performed to identify the type of bacteria whether it is a gram-positive or gram-negative bacteria. The process of gram staining procedure done was first, a single bacteria colony was applied to a slide and then it was passed over a flame to ensure it stays on the slide. Then, crystal violet dye was applied, which stains all the bacteria to a purple colour. Next, iodine was then applied, which helps the dye bind to the

peptidoglycan layer of the cell wall, and this is followed by acetone, which washes away the dye. So, the purple dye will stay on gram positive bacteria because of the strong bond between the bacteria and a thick peptidoglycan layer, but in gram-negative bacteria it will be washed away, due to the thin peptidoglycan layer. Lastly, safranin dye was applied, which will stain gram-negative bacteria into pink colour.

Biochemical, oxidase and catalase tests

Biochemical tests are used to identify distinct species of bacteria by separating bacterial species according to their biochemical activities. There are 5 types of biochemistry test done in this study which is triple sugar iron (TSI) test, citrate test, urease test, indole and motility (SIM) test and methyl red-Voges-Proskauer (MR-VP) test.

For TSI, citrate and urease test, the steps involved were first, the bacteria sample was taken using a straight inoculation needle by touching the top of a well-isolated colony. After that, the colony was inoculated by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant. Then, the cap was left loose and incubated the tube at 37°C for 24 to 48 hours before the reaction of the medium can be examined. While for the SIM test, the isolated colony was stabbed once to a depth of 1/3 to ½ inch in the middle of the tube by using a straight inoculation needle and incubated the tube at 37°C for 24 to 48 hours before examining the tube.

For the MR-VP test, the MR-VP broth was inoculated with bacteria by aseptically transferred with a loop. Then, the inoculated tube was incubated at 37°C for 24 hours. After incubation, pipette was used to transfer half of the inoculated MR-VP broth to a clean test tube and labeled as “VP” and the other half of the inoculated broth was left in the original MR-VP broth test tube and labeled as “MR”. Next, for the methyl red test procedure, 4

drops of methyl red reagent were added to the “MR” tube and observed for the color of the medium immediately; positive reaction showed a distinct red colour while negative reaction showed a yellow color change. In the methyl red test (MR test), the test bacteria is grown in a broth medium containing glucose. When methyl red is introduced to a broth culture, its yellow colour changes to red if the bacteria can use the glucose to produce a stable acid. While for Voges-Proskauer test, the steps were four drops of Barritt’s A reagent (alpha-napththol) and four drops of Barritt’s B reagent (40% KOH) were added to the "VP" tube. Then, the test tube was held and mixed well. After that, the test tube was left undisturbed in a test tube rack for 30 minutes before the result could be obtained; positive reaction showed a pink-red color at the broth surface while negative reaction lacked a pink-red color on the broth surface.

Besides, oxidase and catalase were also done as an indicator of aerobic metabolism. For oxidase test, impregnated oxidase strip method was used where one drop of oxidase reagent was pipetted on a filter paper and then, fresh growth of bacteria colony was scraped using a stick and pressure was gently applied against the paper at the spot with oxidase reagent. After that, color changes were observed within five to 10 seconds. A positive result may appear deep purple blue color while a negative reaction appears no color change. While for the catalase test, one drop of hydrogen peroxide solution was put onto a glass slide. After that, a sterile wooden stick was used to take the bacteria colonies and immerse in the hydrogen peroxide solution. Then, the presence of bubbles was observed immediately. Bubbling indicates the sample was positive for catalase test.

Selective Media Bacterial Culture

The samples were also cultured on selective media such as mannitol salt agar (MSA) for gram positive bacteria and xylose lysine deoxycholate agar (XLD) and eosin methylene blue (EMB)

for gram negative bacteria.

MSA agar was performed to isolate and identify *Staphylococcus aureus* from clinical and non-clinical material. It is a selective and differential medium specifically for isolation of *Staphylococcus species.* While XLD and EMB agar was performed to isolate gram negative bacteria such as *Salmonella spp., Shigella spp., Escherichia coli, Proteus spp., Enteropbacter spp.,* and *Pseudomonas spp*. XLD medium was designed to boost the frequency of development of the more fastidious pathogens and the pathogens are distinguished from several non-pathogens that do not ferment lactose or sucrose, as well to the non-pathogenic lactose fermenters. On the other hand, EMB medium consists of two dyes which are methylene blue and eosin, that prevent the development of gram-positive bacteria. The capacity of EMB agar to ferment lactose allows it to distinguish between various gram-negative bacterial species.

Statistical Analysis

In this study, all data was analysed by using the Pearson Chi Square test (x2-test) or Fisher's exact test and the statistical comparisons were carried out using SPSS 27 statistical software. The association between all the data collected which include animals, surgeon and surgery related were tested with the possibility for the presence of bacteria growth throughout the surgery. The test is used to determine if there's a relationship between those variables, especially the risk factors involved with the possibility of contamination occurring during the surgery.

CHAPTER 4

RESULTS

Data was systematically collected from 32 cats that underwent different surgery procedures at HPVUMK. The result obtained were divided into few sections which include the types of bacteria species that been isolated from the skin swab samples throughout the study, data of the patients, data of the surgeons, the correlation between all the risk factors involved in this study with the number of bacteria colony growth and also the prevalence and the association of all the risk factors that can contribute to the presence of bacteria during the surgery procedure.

4.1 Types of Bacteria Species

Analysis of skin swab samples collected from 32 cats species (*Felis catus)* by using bacterial culture yielded a great number of positive results in this study. Skin swab samples taken before scrubbing with antiseptic solution of 23 cats were having growth for the skin sample taken before the scrubbing procedure. While the other 9 out of 32 cats do not have any bacterial colony growth during the primary culture. The isolated bacteria obtained were identified as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp., Corynebacterium spp., and Bacillus spp. Staphylococcus aureus* which was detected in almost all samples taken before skin preparation was done and one sample during 1 hour 30 minutes of surgery procedure with some of the cats possessing 2 different species of bacteria. The type of bacteria species from the highest to the lowest number of species isolated was 18 of 28 colonies with *Staphylococcus aureus* (63.4%) followed by four of 28 colonies was *Staphylococcus epidermidis* (14.3%), three of 28 colonies was *Bacillus spp.*

(10.7%), two of 28 colonies had *Streptococcus spp.* (7.1%), and only one with *Corynebacterium spp.* (3.6%) (Figure 4.1).

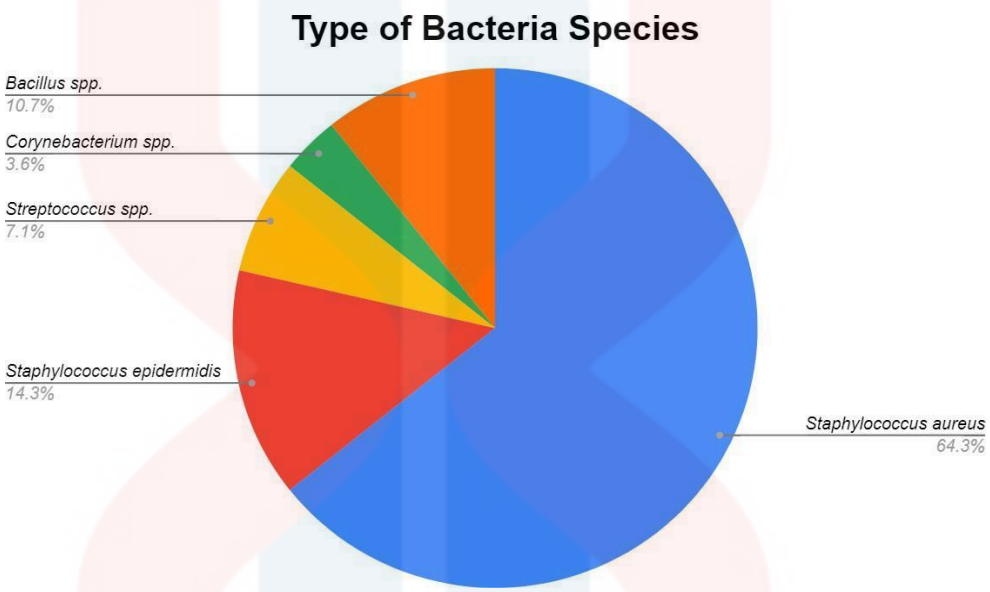


Figure 4.1: Type of bacterial species isolated from the skin swab sample.

4.2 Data of the Cat Patient

A total of 105 skin swab samples were successfully collected from 32 cats in this study. The highest number of cat life stages based on age involved were the junior cat with 22 cats, followed by 11 adult cats and three kittens (Figure 4.2). While for the breed, 29 cats were DSH and the other three were DLH (Figure 4.3) and based on the sex of the cat, most of them were female and 5 out of 32 were male cats (Figure 4.4). In terms of management, the highest number is the semi-roamer cat, followed by 8 indoor and 6 outdoor cats (Figure 4.5).

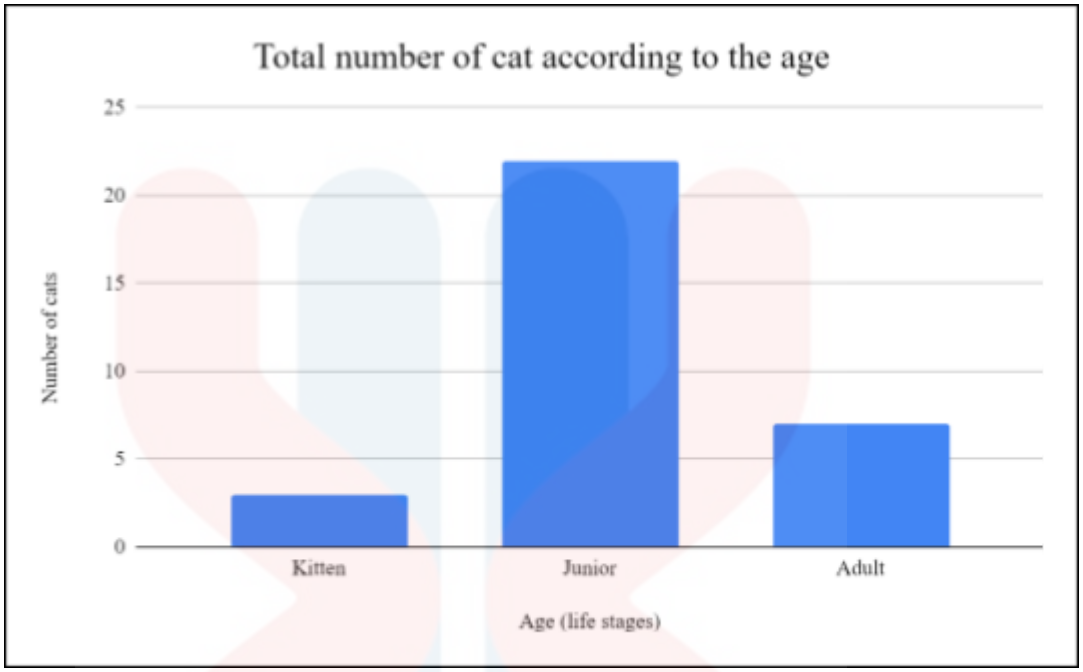


Figure 4.2: Total number of cat patient according to their life stage

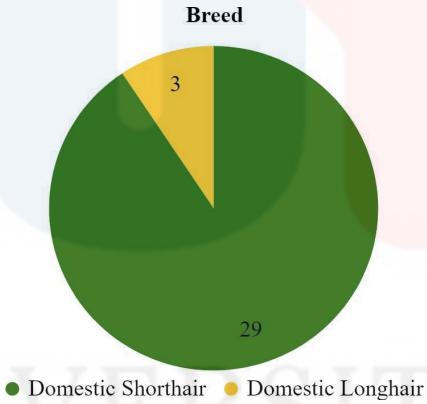


Figure 4.3: Total number of cat patient according to the breed

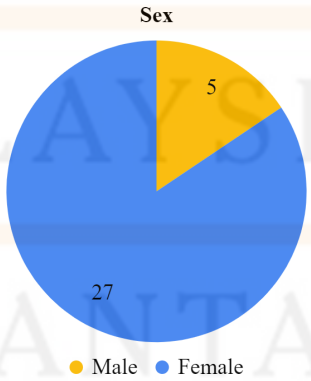


Figure 4.4: Total number of cat patient according to the sex

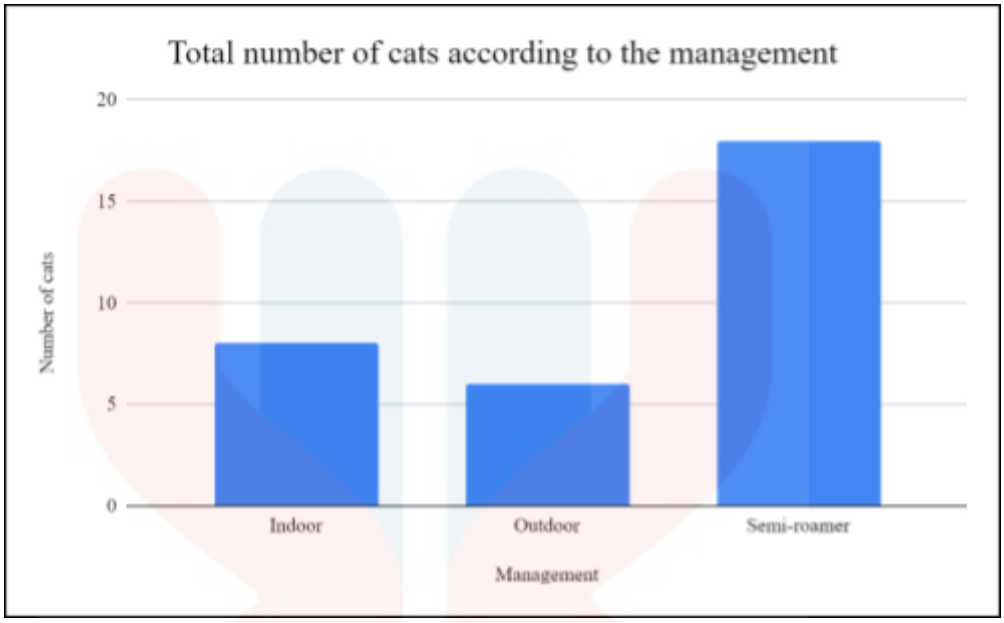


Figure 4.5: Total number of cat patient according to the management

4.3 Data of the Surgeon

Surgeons involved in this study were all certified veterinarians that graduated in different years from local universities. Their working experience with the total number of surgery involvement was presented in the figure 4.6. As mentioned in the data collection, the surgery procedures that take place in this study with the total number of procedures were shown in figure 4.7. In addition, for the duration of the surgery procedure, most of the procedures were finished within 30 minutes with the total of 26 out of 32 cats, followed by four cats that the surgery finished within 1 hour and two cats at 1hour 30mins (Fig. 4.8).

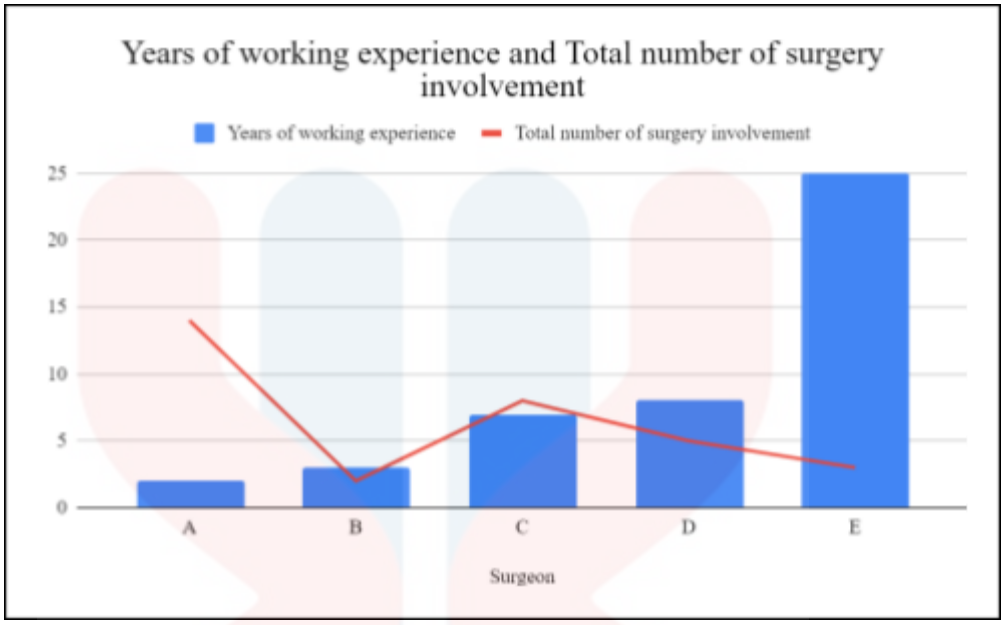


Figure 4.6: Surgery teams' years of working experience with their total number of surgeries involvement.

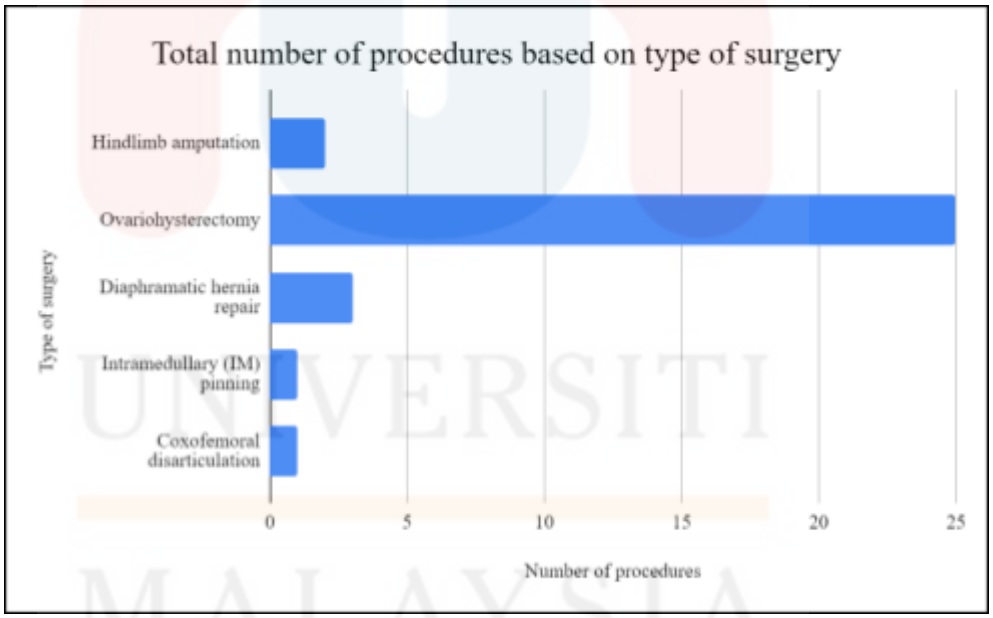


Figure 4.7: Total number of procedures based on types of surgery involved in this study.

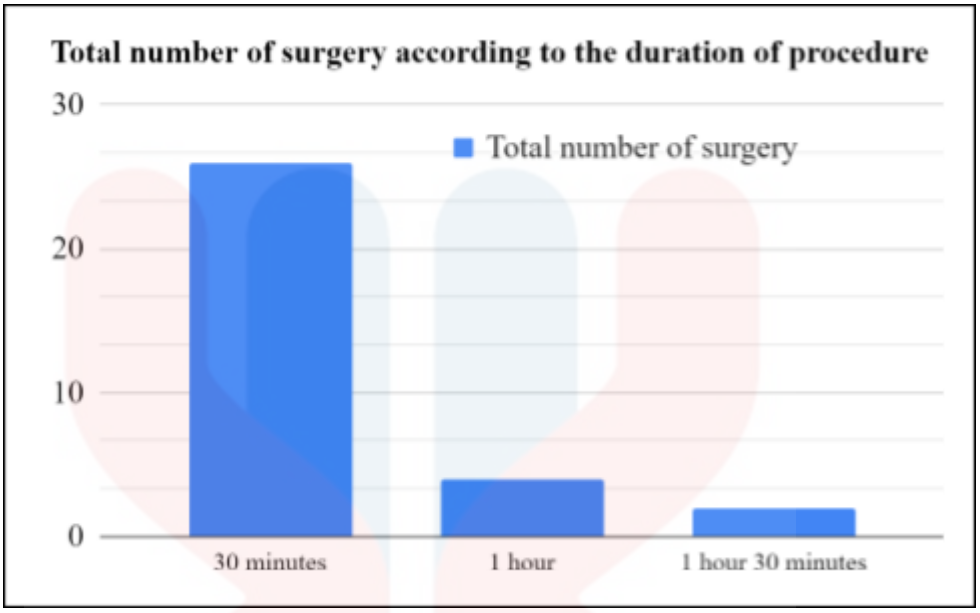


Figure 4.8: Total number of surgeries according to the duration of procedure.

4.4 Bacteria Isolated According to Different Sampling Duration

In this study, sampling of the skin swab was taken before skin preparation with an antiseptic solution right after the surgical site was shaved, after skin preparation where antiseptic was applied on the surgical site, and every 30 minutes of the surgical procedure until the surgery finished. Figure 4.9 below showed a bar chart of the total number of samples with bacteria isolated according to the surgery duration taken before skin preparation until surgery finished.

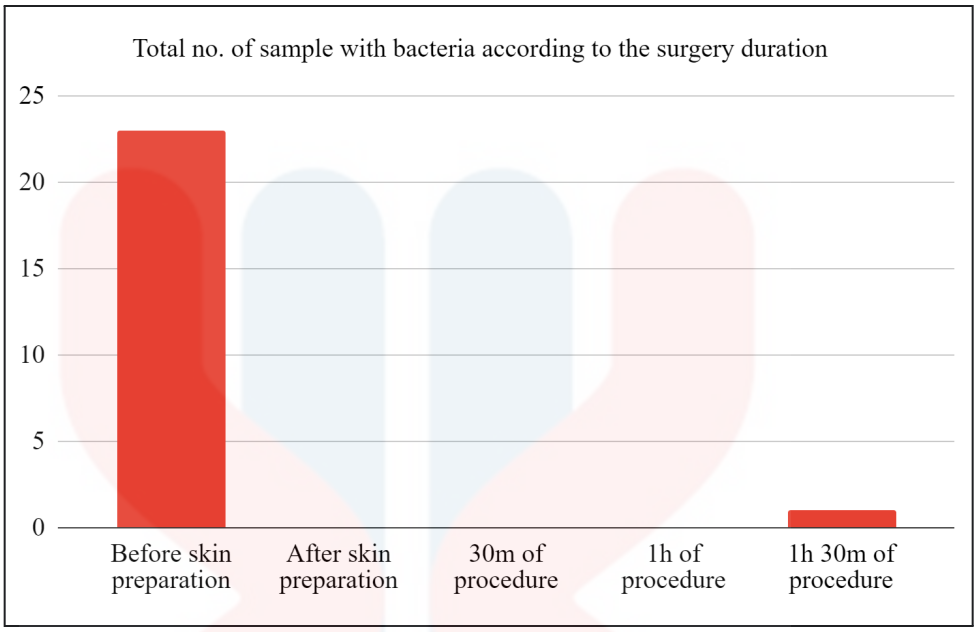


Figure 4.9: Total number of samples with bacteria isolated according to the surgery duration.

Based on the result from Figure 4.9, bacteria colonies were isolated from 23 out of 32 cats skin swab samples before skin preparation was done and all samples taken after skin preparation with antiseptic solution and up till 1 hour of procedure were free from bacteria. However, one of the samples taken at 1 hour 30 minutes of the surgery procedure got contaminated where bacteria was managed to be isolated during bacteria culture.

4.5 Correlation Between the Risk Factors and the Bacteria Colony Growth

The risk factors such as type of surgery, number of animals involved in each of the procedures, surgeon in charge the surgery procedure based on years of working experience, surgery duration with number of samples involved were tabulated with the aim to evaluate the correlation between those factors with the total number of bacteria colony growth during the surgery. Based on table 4.1, only one sample had contamination during the surgery procedure where we managed to isolate *Staphylococcus aureus* at 1 hour 30 minutes in one of the orthopaedic surgery procedures done by a surgeon with <10 years of

working experience.

Table 4.1: Number of samples with bacteria colony during the surgery based on different risk factors; type of surgery, no. of animals involved, surgeon in charge based on working experience, surgery duration with number of samples involved.

Type of surgery	No. of animal	Surgeon in charge (working experience; years)	Surgery duration	No. of sample	Sample with bacteria growth
Abdominal	3	<10	30 mins	2	0
			1 hour	1	
Orthopaedic	4	<10	1 hour	2	0
			1 hour 30 mins	2	1
Reproductive	25	<10	30 mins	22	0
		>10		3	

The other factor that plays a role in the possibility for the bacteria growth during surgery procedure is the total number of people in the operating room at one time. From table 4.2, we can see different numbers of people in the operating room contribute to the enhancement of the chances for contamination during the surgery procedure to occur even though in this study only one procedure had a total of 6 people during the procedure. In addition, based on the finding of the sample with bacteria growth during the surgery, for the first 1 hour of the procedure, there was no bacteria colony isolated and it was free from bacteria. However, the contamination happened at 1 hour 30 minutes after the procedure where we managed to isolate *Staphylococcus aureus* from the swab sample taken at the surgical site.

Table 4.2: Number of samples with bacteria colony during the surgery based on the number of people in the operating room at one time

No. of people in the operating room	No. of cat patient underwent surgery	Sample with bacteria growth during surgery		
		30 minutes	1 hour	1 hour 30 minutes
3	14	0	0	0
4	17	0	0	0
6	1	0	0	1

4.6 Prevalence and the Association of the Risk Factors

Statistical analyses of variables were conducted using descriptive statistics to determine the association of risk factors related to the presence of bacteria in different durations of surgery procedure. The data was analysed by using the Pearson Chi Square test (x2-test) or Fisher's exact test. Statistical comparisons were carried out using SPSS 27 statistical software. The x2-test was considered as statistically significant when the probability (P) was lower than 0.05 ($p<0.05$). The risk factors of cats associated with the presence of bacteria before skin preparation with antiseptic solution were recorded including their age, breed, sex, and management. The details of variables used for each factor are provided in (Table 4.3). Based on the result, the management of the cat has a significant association with the presence of bacteria colonies before the skin preparation was performed.

Table 4.3: Prevalence of the presence of bacteria colony before skin preparation and associated risk factors on cats (n=32) in HPVUMK

Variables		Total sample with bacteria colony		Prevalence %	*p*-value
		Present	Absent		
Age					0.078
	Kitten	1	2	33.3	
	Junior	15	7	68.2	
	Adult	7	0	100	
Breed					0.184
	DSH	22	7	75.9	
	DLH	1	2	33.3	
Sex					1.000
	Male	4	1	80	
	Female	19	8	70.4	
Management					<0.001
	Indoor	1	7	12.5	
	Outdoor	17	1	94.4	
	Semi-roamer	5	1	83.3	

Based on the result, there was a significant association between the management of the cat with the presence of bacteria colonies on the skin of the cat before being prepared aseptically right after the fur was shaved ($p = <0.001$). While the other factors were not. However, in this study, the prevalence for the presence of bacteria colonies was more than 90% in an adult cat (100% prevalence rate) and for outdoor cats with the prevalence of 94.4%.

The risk factors associated with the presence of bacteria during the surgery were recorded which include type and duration of surgery procedure, surgeons working experience, and total number of people in the surgery room at one time. The details of variables used for each factor are provided in (Table 4.4).

Table 4.4: Prevalence of the presence of bacteria colony during the surgery and associated risk factors on cats (n=32) in HPVUMK

Variables		Total sample with bacteria colony		Prevalence %	*p*-value
		Present	Absent		
Type of surgery					0.027
	Abdominal	0	3	0.0	
	Orthopaedic	1	3	25.0	
	Reproductive	0	25	0.0	
Duration					0.188
	<30 minutes	0	26	0.0	
	>30 minutes	1	5	16.7	
Surgeon's working experience					1.000
	<10 years	1	28	3.4	
	>10 years	0	3	0.0	
No. of people in room at one time					<0.001
	3 people	0	14	0.0	
	4 people	0	17	0.0	
	6 people	1	0	100	

The result showed there is a significant association between the type of surgery and number of people in the room at one time with the presence of bacteria colonies on the skin of the cat during the surgery ($p = <0.05$). While the duration and surgeon's working experience were not associated with the presence of bacteria during the surgery.

CHAPTER 5

DISCUSSION

5.1 Isolated Bacterial Species

There are numerous bacteria that colonise the body which can be pathogenic, causing illness in the tissues they contact, or they may be helpful, boosting immunity and preventing the proliferation of harmful microorganisms (Grice *et al.,* 2008; Grice & Segre, 2011). Different anatomical regions correspond to diverse communities of bacteria within the body and they vary from individual to another as a result of external and internal variables, such as surroundings and immunological condition, respectively (Grice & Segre, 2011; Zeeuwen *et al.*, 2017). Based on the current finding, the bacteria species that were isolated from the skin swab sample were identified as *Staphylococcus spp., Streptococcus spp., Corynebacterium spp., and Bacillus spp.* with *Staphylococcus aureus* has a highest number isolated from the sample and it was detected in almost all samples taken before skin preparation which almost similar with the findings from the most earliest research used culture-based methods done by Krogh & Kristensen (1976) where from their study the samples were dominated by *Micrococcus, Acinetobacter, Streptococci*, and *Staphylococci*. In addition, another study done in 2005 also found that one of the most isolated genera of opportunistic microbes from both humans and animals is *Staphylococcus* because mammals' skin, throat, and nasal mucous membranes are the primary physiological habitats for staphylococci (Wertheim *et al.,* 2005). Other than *Staphylococcus spp., Bacillus spp.* and *Corynebacterium spp.* were also managed to be isolated from the sample. These bacteria are common microflora that may be present in the environment with *Bacillus* species are widely distributed in nature, existing in diverse terrestrial and aquatic habitats

(Ravine, 2019). However, *Corynebacterium spp.* is particularly abundant found on human skin. Therefore, it can be isolated from the skin of cats due to their closer interaction with human microbiota (Ross *et al.,* 2018).

Based on this study, we can see that aseptic technique practiced in HPVUMK was successful in eliminating bacteria at the surgical site where from the result of bacteria culture from the skin swab samples, all samples were free from bacteria after skin preparation with antiseptic solution up to 1 hour of surgery procedure.

5.2 Association for the Presence of Bacteria Based on Cat's Related Data

This study provides new points of view on the association for the presence of bacteria on the skin of cats among different age groups. So, based on the finding, the age was not significantly associated with the presence of bacteria colonies on the skin ($p = 0.078$). Due to the limited number of studies reported previously on the bacterial prevalence in cats and age association, it is difficult to compare the present findings with other reports (Moon *et al.,* 2022). Other factors which are the breed and sex of the cat also were not having association with the bacteria presence. This is because studies assessing the cutaneous microbiota of different animal species including cats are still in their early stages and more research is needed to determine the effects of a few unknown elements on the microbiota on animal skin (Older *et al.,* 2019).

On the other hand, based on the finding, only management of the cats has a significant association with the presence of bacteria on skin with a probability less than 0.05 ($p < 0.001$). There are multiple interrelated aspects that can be responsible for the association between the management of cats and the development of bacteria on their skin. First and foremost, because they are allowed to roam around in a variety of settings, outdoor cats are continuously exposed to a wide range of diseases that can be found in soil

and on different surfaces (Older *et al.*, 2017). The complex network of relationships that cats create in their surroundings increases the likelihood of bacterial transmission by touch or contaminants in the environment (Tan *et al.*, 2020). This is supported by the statistical result from this study where outdoor and semi-roamer cats have a high prevalence rate for the presence of bacteria colonies before skin preparation which is more than 80% (prevalence of outdoor cat; 94.4% and prevalence of semi-roamer cat; 83.3%).

5.3 The Association Between the Risk Factors and the Possibility of Contamination During the Surgery Procedure

Based on the statistical analysis findings from this study, two risk factors that significantly influence the presence of bacteria colonies on the skin of cats during surgery are the type of surgery and the number of people in the surgery room at one time with the probability value of $p = 0.02$ and $p<0.001$ respectively. From the finding, the contamination occurs during the orthopaedic surgery at 1 hour 30 minutes of procedure with only one out of four procedures having bacteria isolation from the swab sample. However, the duration for all four orthopaedic procedures were different; two procedures finished within 1 hour while the other two were at 1 hour 30 minutes. Due to the fundamental differences in surgical techniques, instrument manipulation between procedures by different surgeons with different levels of working experience, the correlation between the type of surgery and the presence of bacteria has been observed. As in this study, only one of the orthopaedic surgeries got contaminated at 1 hour 30 minutes of the procedure, it is believed that even if the same procedure was done by different surgeons with different levels of working experience, it will influence the outcome of the study because even though the surgery can take longer than expected time in certain situations, when it was performed by a skilled surgeon, it can actually reduce the time allocated for that procedure. This could make sense

because the surgeon is skilled enough to do the surgery quickly and at the same time, it may reduce the chances for the surgical area to be exposed for too long. Thus, this helps in minimising the chance of contamination. Notably, as only one got contamination out of 32 procedures with the combination of all the surgeons' related risk factors, it suggests that there is possibility for the contamination of the surgical site to occur especially if there is breaking in any part of the sterility measure throughout the procedure.

While for the number of people in the operating room, it has a correlation with the presence of bacteria during the procedure in the operating room as from this study finding, one procedure with six people involved at one time had a contamination occur where *Staphylococcus aureus* was isolated during the surgery at 1 hour 30 minutes of procedure. The result from this study that was analysed using Fisher's exact test also revealed a significant association between the number of people in the operating room at one time with the presence of bacteria growth from the skin swab sample taken at the surgical site that was aseptically prepared ($p<0.001$). This was supported by other studies that have noted that inexpensive and straightforward actions, such limiting the number of individuals in the operating room and minimising their movements, can help to lessen the number of microorganisms that are dispersed throughout the air (Fitzgerald, 1979; Brandt *et al.,* 2008). And the occurrence of surgical wound infections is significantly impacted by the quality of the operating room which can be determined by the structural aspects of the facility and its systems, as well as the management and action of people that influence movement of airborne particles originating from operating room and act as primary sources of contamination (Cristina *et al.,* 2012).

CHAPTER 6

CONCLUSION

In conclusion, all the bacteria species which are *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp., Corynebacterium spp., and Bacillus spp.* that been isolated from the cats' skin swab samples before being prepared aseptically were a common microflora that can be found in healthy skin of cat and in the environment. They may be beneficial to the host. Through this current study, we know that an aseptic operating procedure practiced in HPVUMK was successful in eliminating bacteria after skin preparation was done using antiseptic solution. However, breaking in sterility during surgical procedure may cause them to cause infection towards the surgical site if the bacteria managed to enter through the skin barrier that has been incised.

In terms of types of surgery procedure and surgery duration towards the possibility for the presence of bacteria on an aseptic surgical site, both have a correlation between each other. This is because the surgeons who performed the procedure may influence the duration of the procedure. Therefore, even if the same procedure was done, the duration for the procedure to be finished will differ among each of the individuals who performed it. Thus, this increases the possibilities for the isolation of bacteria on the surgical site if contamination happened during the surgical procedure.

Generally, as only one out of 32 procedures got contaminated by the bacteria during the surgical procedure at HPVUMK, we can conclude that even in a proper practice of aseptic technique throughout the surgery procedure, the chances for contamination to occur on the surgical site that has been prepared aseptically is still present, due to various factors especially when involving surgery that can be considered as a major procedure and also influenced by the number of people in the operating room at one time.

6.1 Recommendation

As for future research, it is recommended that the number of animal sample sizes should be increased. Having a larger sample size will increase the study's reliability, enabling more trustworthy and broadly applicable findings. It also would give a greater degree of precision of the outcome, lower the error margin, and strengthen appreciation for the study's conclusions. However, due to time constraints, the number of patients selected in the study was only 32. Increasing the sample size to more than 50 can further increase the validity of the result. Therefore, it is also recommended that the study period should be increased to at least 3 months in order to achieve the quantity of sample size. Besides, in order to have a more thorough comprehension of the variables impacting sterility during surgery, the study's criteria had to cover a variety of surgical procedures. This expansion would allow researchers to investigate differences in contamination risk between various surgical procedures done at HPVUMK.

REFERENCES

Dockery, G. D. (2012). *Aseptic techniques. Lower Extremity Soft Tissue & Cutaneous Plastic Surgery, 53–68.* https://doi.org/10.1016/b978-0-7020-3136-6.00007-2

DiGangi, B. A. (2019). *Asepsis. High‐Quality, High‐Volume Spay and Neuter and Other Shelter Surgeries, 65–88.* https://doi.org/10.1002/9781119646006.ch4

Association of Operating Room Nurses (2006). *Recommended practices for sterilization in the perioperative practice setting. Association of periOperative Registered Nurses (AORN) Journal. 83 (3): 700–722.*

Griffin et al., (2016). *The Association of Shelter Veterinarians' 2016 Veterinary Medical Care Guidelines for Spay-Neuter Programs. Journal of the American Veterinary Medical Association, 249(2),165-188.* https://doi.org/10.2460/javma.249.2.165

Burgess, B. A. (2019). *Prevention and surveillance of surgical infections: A review. Veterinary Surgery.* https://doi.org/10.1111/vsu.13176

Alexander, J. W. (1985). *The Contributions of Infection Control to a Century of Surgical Progress. Annals of Surgery, 201(4), 423-428.* https://doi.org/10.1097/00000658-198504000-00004

Block S. S. (2001). Infection Control Hospital Epidemiology. *Definition of terms. In: Block SS, ed. Disinfection, Sterilization, and Preservation* (5th ed.). Philadelphia, PA: Lippincott, Williams & Wilkins; 2001:19–28.

Davids, B. I., Davidson, M. J., TenBroeck, S. H., Colahan, P. T., & Oli, M. W. (2015). *Efficacy of Mechanical versus Non-Mechanical Sterile Preoperative Skin Preparation With Chlorhexidine Gluconate 4% Solution. Veterinary Surgery, 44(5), 648–652.* https://doi.org/10.1111/vsu.12335

Bourel, C., Buczinski, S., Desrochers, A., & Harvey, D. (2013). *Comparison of Two Surgical Site Protocols for Cattle in a Field Setting. Veterinary Surgery, 42(2), 223–228.* https://doi.org/10.1111/j.1532-950x.2013.01089.x

Fossum T. W, Seim, H. B, & Colville T. P. (1998). Principles of surgical nursing, in: Pratt PW : *Principles and practice of veterinary technology, (4th ed.) (pp 383-41)*.

Rowley S, & Clare S. (2011). ANTT: a standard approach to aseptic technique. *Nursing Times; 107: 36, 12-14*. https://www.nursingtimes.net/clinical-archive/infection-control/antt -a-standard- approach-to-aseptic-technique/5034771.article

Cherney, K., (2018, September 29). *Aseptic Technique.* Healthline.

https://www.healthline.com/health/aseptic-technique

Loftus, R. W., Muffly, M. K., Brown, J. R., Beach, M. L., Koff, M. D., Corwin, H. L., & Yeager, M. P. (2011). *Hand Contamination of Anesthesia Providers Is an Important Risk Factor for Intraoperative Bacterial Transmission. Anesthesia & Analgesia, 112(1), 98–105.* https://doi.org/10.1213/ANE.0b013e3181e7ce18

Zabaglo M., & Sharman T., (2023, July 3). *Postoperative Wound Infection*. National Library of Medicine (NIH). National for Biotechnology Information. https://www.ncbi.nlm.nih.gov/books/NBK560533/

Gaines, S., Luo, J. N., Gilbert, J., Zaborina, O., & Alverdy, J. C. (2017). *Optimum Operating Room Environment for the Prevention of Surgical Site Infections. Surgical infections*, *18*(4), 503–507. https://doi.org/10.1089/sur.2017.020

Mangram, A. J., Horan, T. C., Pearson, M. L., Silver, L. C., & Jarvis, W. R. (1999). *Guideline for Prevention of Surgical Site Infection, 1999. Infection Control & Hospital Epidemiology, 20(04), 247–280.* https://www.cambridge.org/core/journals/infection-control-and-hospital-epidemiology/article/abs/guideline-for-prevention-of-surgical-site-infection-1999/31585A523649826D6497F019D0907A60

Owens, C. D., & Stoessel, K. (2008). *Surgical site infections: epidemiology, microbiology and prevention. Journal of Hospital Infection, 70, 3–10.* https://doi.org/10.1016/s0195-6701(08)60017-1

Manian, F. A. (2014). *The Role of Postoperative Factors in Surgical Site Infections: Time to Take Notice. Clinical Infectious Diseases, 59(9), 1272–1276.* https://doi.org/10.1093/cid/ciu552

Sattar, F., Sattar, Z., Zaman, M., & Akbar, S. (2019). *Frequency of Post-operative Surgical Site Infections in a Tertiary Care Hospital in Abbottabad, Pakistan. Cureus, 11(3), e4243*. https://doi.org/10.7759/cureus.4243

Tobias, K. M., & Johnston, S. A. (2012). *Veterinary Surgery: Small Animal*. Canada: Elsevier Saunders.

Rutkow I., (2010). *The Rise of Modern Surgery: An Overview.* (20th Ed.). Philadelphia: Elsevier.

Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical microbiology reviews*, *14*(2), 244–269. https://doi.org/10.1128/CMR.14.2.244-269.2001

Hopper, W. R., & Moss, R. (2010). *Common Breaks in Sterile Technique: Clinical Perspectives and Perioperative Implications. Association of periOperative Registered Nurses (AORN) Journal, 91(3), 350–367.* https://doi.org/10.1016/j.aorn.2009.09.027

NIH/OACU (National Institutes of Health/Office of Animal Care and Use) (2016). *Guidelines for survival rodent surgery. Animal Research Advisory Committee Guidelines.*Bethesda,MA: NIH/OACU. https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_surgery.pdf

Clevenger R. R., Bernal J, Talcott M., Gleason, T. R., Rindfield, T., and Robert F., & Hoyt, Jr. (2018) *Surgery. In: Weichbrod RH, Thompson GAH, Norton JN, editors. Management of Animal Care and Use Programs in Research, Education, and Testing.* (2nd edition). Chapter 34. https://doi.org/10.1201/9781315152189-34

Bernal, J., Baldwin, M., Gleason, T., Kuhlman, S., Moore, G., & Talcott, M. (2009). *Guidelines for Rodent Survival Surgery. Journal of Investigative Surgery, 22(6), 445–451.* https://doi.org/10.3109/08941930903396412

Dharan, S., & Pittet, D., (2022). *Environmental controls in operating theaters. Journal of Hospital Infection, 51(2), 79-84.* https://doi.org/10.1053/jhin.2002.1217

Roy, M-C., (2018). *Guide to Infection Control in the Healthcare Setting: Surgical Site Infections in the Operating Room.* International Society for Infectious Disease (ISID). https://isid.org/wp-content/uploads/2019/06/ISID_GUIDE_THE_OPERATING_ROOM.pdf

Grice E. A., Kong H. H., Renaud G., Young A. C., NISC Comparative Sequencing Program, Bouffard, G. G., Blakesley, R. W., Wolfsberg, T. G., Turner, M. L., & Segre, J. A. (2008). *A diversity profile of the human skin microbiota. Genome Research Institute. 18(7):1043–5*0. https://doi.org/10.1101/gr.075549.107

Grice, E. A., & Segre, J. A. (2011). *The skin microbiome. Nature Reviews Microbiology, 9(4), 244–253.* https://doi.org/10.1038/nrmicro2537

Hoffmann, A. R., Proctor, L. M., Surette, M. G., & Suchodolski, J. S. (2015). *The Microbiome. Veterinary Pathology, 53(1), 10–21.* https://doi.org/10.1177/0300985815595517

Lu, Y.-F., & McEwan, N. A. (2007). *Staphylococcal and micrococcal adherence to canine and feline corneocytes: quantification using a simple adhesion assay. Veterinary Dermatology, 18(1), 29–35.* https://doi.org/10.1111/ j.1365- 3164.2007.00567.x

Krogh, H. V., & Kristensen, S. (1976). *A study of skin diseases in dogs and cats. II. Microflora of the normal skin of dogs and cats. Nordisk veterinary medicine, 28(9), 459–463*.

Horn, R., & Kramer, J. (2022, September 19). Postoperative Pain Control. https://www.ncbi.nlm.nih.gov/books/NBK544298/

Mohabir P. K., & Coombs, A. v., (2022, September) Post-operative Care. MSD Manual. https://www.msdmanuals.com/professional/special-subjects/care-of-the-surgical-patient/postoperative-care

Van Kasteren et al., (2005). *Quality improvement of surgical prophylaxis in Dutch hospitals: evaluation of a multi-site intervention by time series analysis. Journal of Antimicrobial Chemotherapy, 56(6), 1094–1102.* https://doi.org/10.1093/jac/dki374

Prokuski, L., (2008, May). *Prophylactic Antibiotics in Orthopaedic Surgery.* Journal of the American Academy of Orthopaedic Surgeons 16(5):p 283-293. https://journals.lww.com/jaaos/abstract/2008/05000/prophylactic_antibiotics_in_orthopaedic_surgery.7.aspx

British Columbia Provincial Nursing Skin & Wound Committee. *Procedure: Swab for Culture & Susceptibility in Suspected Wound Infection* https://www.clwk.ca/get-resource/swab-for -culture-susceptibility-cs-for-suspected-wound-infection-procedure/

Rosa-Fraile, M., Camacho-Muñoz, E., Rodríguez-Granger, J., & Liébana-Martos, C. (2005). Specimen storage in transport medium and detection of group B streptococci by culture. *Journal of clinical microbiology*, *43*(2), 928–930. https://doi.org/10.1128/JCM.43.2.928-930.2005

Sharma, R. (2023, May 30). *Plate Exposure Method: Principle and Procedure*. ACME Research Solution. https://acmeresearchlabs.in/2023/05/30/plate-exposure-method-principle-and-procedure/

Zeeuwen *et al*., (2017). Gram-positive anaerobe cocci are underrepresented in the microbiome of filaggrin-deficient human skin. Journal of Allergy and Clinical Immunology, 139(4), 1368–1371. https://doi.org/10.1016/j.jaci.2016.09.017

Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen, W., van Belkum, A., Verbrugh, H. A., & Nouwen, J. L. (2005). *The role of nasal carriage in Staphylococcus aureus infections. The Lancet Infectious Diseases, 5(12), 751–762.* https://doi.org/10.1016/S1473-3099(05)70295-4

Ravine T. J. (2019). Bacillus: An Environmental Contaminant or Misunderstood Pathogen?. Journal of Bacteriology and Mycology.; 6(6): 1117. https://austinpublishinggroup.com/bacteriology/fulltext/bacteriology-v6-id1117.pdf

Ross, A. A., Müller, K. M., Weese, J. S., & Neufeld, J. D. (2018). *Comprehensive skin microbiome analysis reveals the uniqueness of human skin and evidence for phylosymbiosis within the class Mammalia. Proceedings of the National Academy of Sciences, 115(25), E5786–E5795.* https://doi.org/10.1073/pnas.1801302115

Moon, D. C., Choi, J. H., Boby, N., Kim, S. J., Song, H. J., Park, H. S., Gil, M. C., Yoon, S. S., & Lim, S. K. (2022). Prevalence of Bacterial Species in Skin, Urine, Diarrheal Stool, and Respiratory Samples in Cats. *Pathogens (Basel, Switzerland)*, *11*(3), 324. https://doi.org/10.3390/pathogens11030324

Older, C. E., Diesel, A. B., Lawhon, S. D., Queiroz, C. R. R., Henker, L. C., & Hoffmann A. R. (2019). *The feline cutaneous and oral microbiota are influenced by breed and environment. Plos One.* https://doi.org/10.1371/journal.pone.0220463

Older, C. E., Diesel, A., Patterson, A. P., Meason-Smith, C., Johnson, T. J., Mansell, J.,Suchodolski J. S., & Hoffmann, A. R. (2017). *The feline skin microbiota: The bacteria inhabiting the skin of healthy and allergic cats. PLOS ONE, 12(6), e0178555.* https://doi.org/10.1371/journal.pone.0178555

Tan, S. M. L., Stellato, A. C., & Niel, L. (2020). *Uncontrolled Outdoor Access for Cats: An Assessment of Risks and Benefits. Animals : an open access journal from MDPI, 10(2), 258*. https://doi.org/10.3390/ani10020258

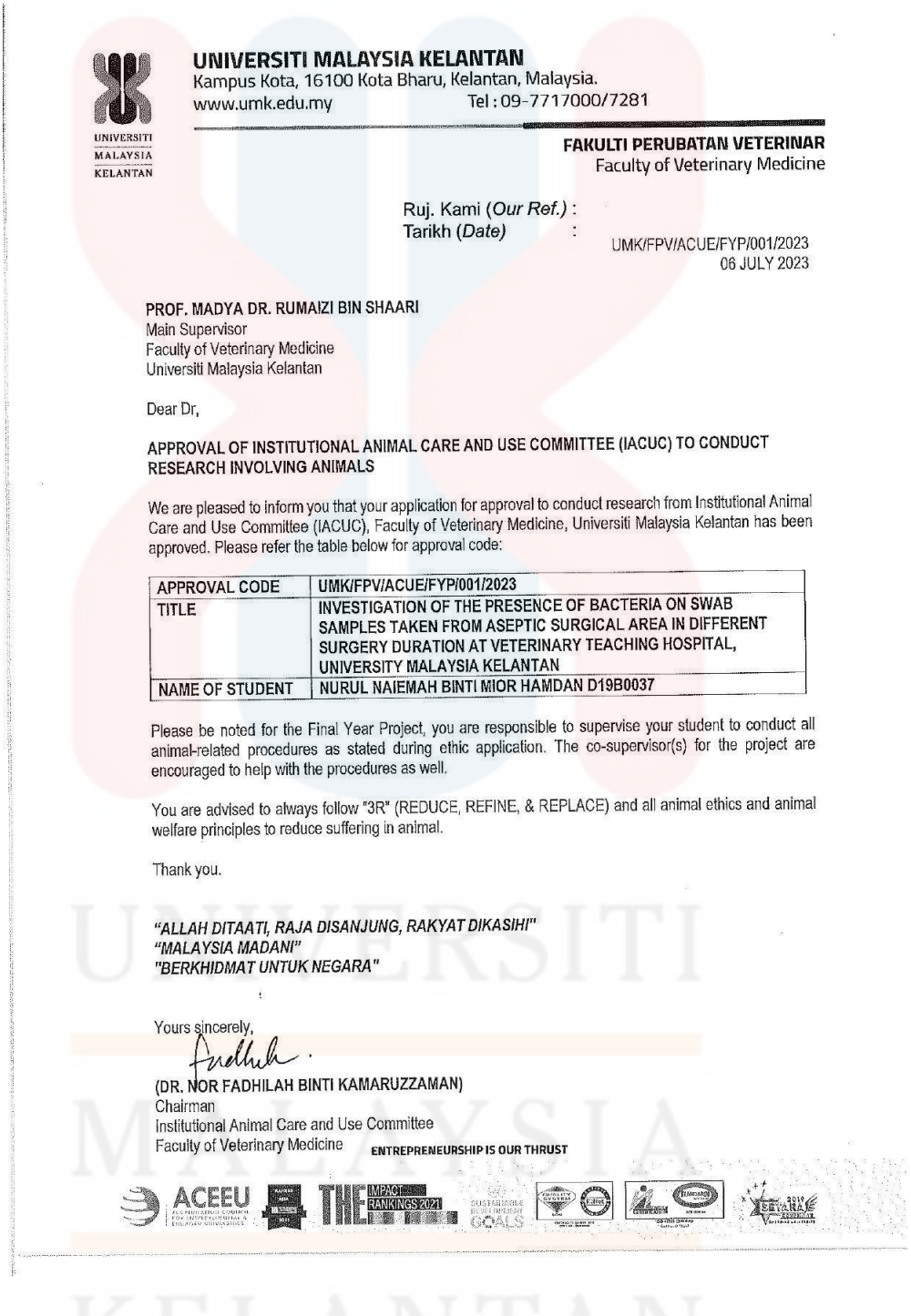
Fitzgerald, R. H. (1979). *Microbiologic Environment of the Conventional Operating Room. Archives of Surgery, 114(7), 772.* https://doi.org/10.1001/archsurg.1979.01370310014003

Brandt, C., Hott, U., Sohr, D., Daschner, F., Gastmeier, P., & Ruden, H. (2008). *Operating Room Ventilation With Laminar Airflow Shows No Protective Effect on the Surgical Site Infection Rate in Orthopedic and Abdominal Surgery. Annals of Surgery, 248(5), 695-700.* https://doi.org/10.1097/sla.0b013e31818b757d

Cristina, M. L., Spagnolo, A. M., Sartini, M., Panatto, D., Gasparini, R., Orlando, P., Otria, G., & Perdelli, F., (2012). *Can Particulate Air Sampling Predict Microbial Load in Operating Theatres for Arthroplasty? PLoS ONE, 7(12), e52809.* https://doi.org/10.1371/journal.pone.0052809

APPENDICES

Appendix A



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Kampus Kota, 16100 Kota Bharu, Kelantan, Malaysia.
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UNIVERSITI MALAYSIA KELANTAN

FAKULTI PERUBATAN VETERINAR
Faculty of Veterinary Medicine

Ruj. Kami (*Our Ref.*) : UMK/FPV/ACUE/FYP/001/2023
Tarikh (*Date*) : 06 JULY 2023

PROF. MADYA DR. RUMAIZI BIN SHAARI
Main Supervisor
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan

Dear Dr,

APPROVAL OF INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) TO CONDUCT RESEARCH INVOLVING ANIMALS

We are pleased to inform you that your application for approval to conduct research from Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan has been approved. Please refer the table below for approval code:

APPROVAL CODE	UMK/FPV/ACUE/FYP/001/2023
TITLE	INVESTIGATION OF THE PRESENCE OF BACTERIA ON SWAB SAMPLES TAKEN FROM ASEPTIC SURGICAL AREA IN DIFFERENT SURGERY DURATION AT VETERINARY TEACHING HOSPITAL, UNIVERSITY MALAYSIA KELANTAN
NAME OF STUDENT	NURUL NAIEMAH BINTI MIOR HAMDAN D19B0037

Please be noted for the Final Year Project, you are responsible to supervise your student to conduct all animal-related procedures as stated during ethic application. The co-supervisor(s) for the project are encouraged to help with the procedures as well.

You are advised to always follow "3R" (REDUCE, REFINE, & REPLACE) and all animal ethics and animal welfare principles to reduce suffering in animal.

Thank you.

"ALLAH DITAATI, RAJA DISANJUNG, RAKYAT DIKASIHI"
"MALAYSIA MADANI"
"BERKHIDMAT UNTUK NEGARA"

Yours sincerely,

(DR. NOR FADHILAH BINTI KAMARUZZAMAN)
Chairman
Institutional Animal Care and Use Committee
Faculty of Veterinary Medicine

ENTREPRENEURSHIP IS OUR THRUST

Animal Ethic Approval letter

Appendix B

	FAKULTI PERUBATAN VETERINAR UNIVERSITI MALAYSIA KELANTAN	No. Semakan: 02 No. Isu: 03
	KLINIK VETERINAR UMK PERJANJIAN PENERIMAAN & PELEPASAN HAIWAN UMKFPV/KVUMK/BR017	Tarikh Berkuatkuasa: 01/01/2021

SEKSYEN A (SECTION A)
SYARAT-SYARAT PENERIMAAN HAIWAN UNTUK DIMASUKKAN KE KVUMK
(CONDITIONS FOR ACCEPTANCE OF ANIMALS INTO KVUMK)

Tuanpunya, wakil atau agen:
(The owner, representative or agent)

a) MEMBERI KUASA kepada Klinik Veterinar UMK untuk menjalankan apa jua cara bius, prosedur pembedahan dan/atau rawatan kepada haiwan tersebut (seksyen B), sepertimana mereka memutuskan perlu untuk menyelamatkan haiwan tersebut BERSETUJU dan TAHU risiko ubat bius dan risiko prosedur atau pembedahan. (*AUTHORIZES the Klinik Veterinar UMK to carry out such anaesthetic procedures, surgical procedures and/or medical treatments of the above animal as they may decide necessary to preserve the life of the said animal and AGREES and AWARE of the anaesthetic risk and surgical procedure risk*).

b) BERSETUJU untuk membayar kesemua bayaran mengikut kadar bayaran terkini yang telah ditetapkan oleh UMK.
(*AGREES to settle all payments according to the latest fee rate fixed by UMK*).

c) BERSETUJU untuk megambil haiwan/ karkas tersebut selepas diberitahu secara lisan atau dengan komunikasi secara elektronik tidak lewat 7 hari bekerja selepas tarikh pemberitahuan. Selepas masa tersebut Klinik Veterinar UMK boleh melupuskan haiwan tersebut sebagaimana yang patut dan tiada ganti rugi akan dibayar.
(*AGREES to collect the animal/ carcass after being informed verbally or via electronic communication not later than 7 working days from date of posting; after which the Klinik Veterinar UMK may dispose such animal as appropriate and no compensation shall be payable*).

d) BERSETUJU menyelesaikan keseluruhan kos (seperti yang telah dipersetujui) yang terlibat dalam rawatan, prosedur, diagnostik dan prosedur kecemasan yang telah dilakukan sepanjang tempoh haiwan berada di klinik (hidup atau mati). Jikalau gagal, tindakan undang-undang akan diambil.
(*AGREES to settle all the cost (as per agreed) involved in the treatment, procedures, diagnostics and emergency procedures that has been done to the animal during hospitalisation in the clinic (alive or dead). Legal action will be taken if fail to do so*).

e) BERSETUJU untuk Klinik Veterinar UMK mengambil tindakan yang perlu bagi menyelamatkan haiwan dalam keadaan kecemasan atau mengambil tindakan yang berpatutan bagi memenuhi Akta Doktor Veterinar 1974.
(*AGREES to Klinik Veterinar UMK taking necessary actions to safeguard the animal in emergency situations or act accordingly as per stated in the Veterinary Surgeon Act 1974*).

f) Postmortem akan dilakukan ke atas semua haiwan mati, atas kebenaran/ permintaan pemilik kacuali tidak dibenarkan. Postmortem dengan permintaan akan dikenakan bayaran mengikut

kadar semasa Makmal Patologi. Karkas akan dilupuskan oleh pihak klinik dengan permohonan pemilik. Karkas hanya akan diserahkan kepada tuanpunnya jika diminta.
(*Postmortem will be performed on all dead animals, with owner's permission/ request unless otherwise. The postmortem charges will be bare by the owner upon request according to the Pathology Laboratory charges rate. The carcass will be returned to the owner only on request*).

g) BERSETUJU untuk membenarkan pengambilan sampel tambahan seperti darah, air kencing, tisu dan lain-lain prosedur diagnostik untuk penyelidikan penyakit haiwan/ pembelajaran pelajar. Maklum bahawa haiwan dan data klinikal yang diperoleh akan digunakan bagi tujuan penyelidikan, pengajaran dan pembelajaran pelajar UMK. (potong bahagian ini dan tandatangan jika tidak bersetuju).
(*AGREES to allow additional sample such as blood, urine, tissue or others may be taken during a diagnostic procedure to support research on diseases in animals/ student learning. These procedures will only be performed if they do not pose additional risks to the animals as determined by the clinicians. No additional cost will be incurred with the procurement of these samples. Aware that animal and clinical data gathered will be involved in the research, teaching and learning process of UMK's students). (Strike through and initial if declined*).

Saya telah memahami dan menerima syarat-syarat yang telah ditetapkan di atas untuk haiwan saya seperti ternyata di Seksyen B.
(*I understand and accept the terms as stated above for my animal as stated in section B*).

Nama tuanpunya/ wakil/ agen: _____
Name of owner/ representative/ agent
Tandatangan: _____
Signature
No kad pengenalan: _____
I/C No
Tarikh: _____
Date

Nama Staf Klinik Veterinar UMK: _____
Name of Klinik Veterinar UMK's staff
Tandatangan: _____
Signature

Consent Form of Animal Acceptance and Release

	FAKULTI PERUBATAN VETERINAR UNIVERSITI MALAYSIA KELANTAN	No. Semakan: 02 No. Isu: 03
	KLINIK VETERINAR UMK PERJANJIAN PENERIMAAN & PELEPASAN HAIWAN UMKFPV/KVUMK/BR017	Tarikh Berkuatkuasa: 01/01/2021

SEKSYEN/ *SECTION* B

DATA HAIWAN (*ANIMAL DATA*)

ID:	No Kes/ case No:	Spesis/ Species:
Baka/ Breed:	Umur/ Age:	Jantina/ Sex:
Warna/ Marking:	Tempoh tinggal/ Staying period:	
	Dari/ From:	Ke/ To:

SEKSYEN C

Saya mengesahkan penerimaan balik haiwan saya seperti ternyata di Seksyen B pada masa discaj dari Klinik Veterinar UMK.
I acknowledge the receipt of my animal as stated in Section B at the time of discharge from Klinik Veterinar UMK.

Nama tuanpunya/ agen: _____
Name of owner/ agent
No K/Pengenalan: _____
I/C No.
Tandatangan: _____
Signature
Tarikh: _____
Date

Status haiwan semasa discaj:
Animal Status at discharge

Hidup/ Alive Mati/ Dead
PM + Pelupusan/ *Disposal*
PM + Pemulangan karkas/ *Return Carcass*
Pelupusan Sahaja/ *Disposal only*
Bawa pulang karkas/ *Take home carcass*

Discaj oleh staf Klinik Veterinar UMK:
Discharge by Klinik Veterinar UMK's staff:

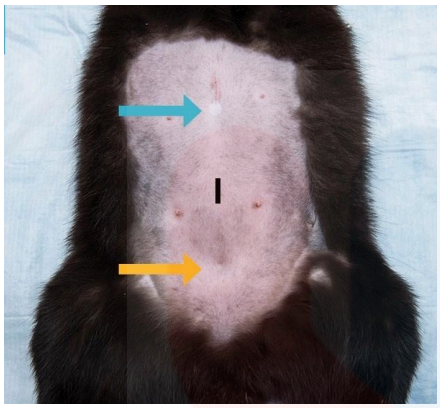
Nama: _____
Name
Tandatangan: _____
Signature

Consent Form of Animal Acceptance and Release

Appendix C

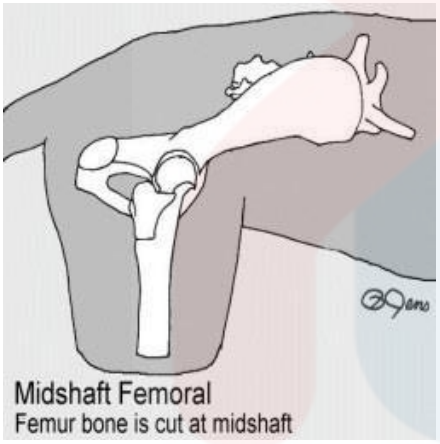
Cat life stage chart by Cat Care for Life

Appendix D



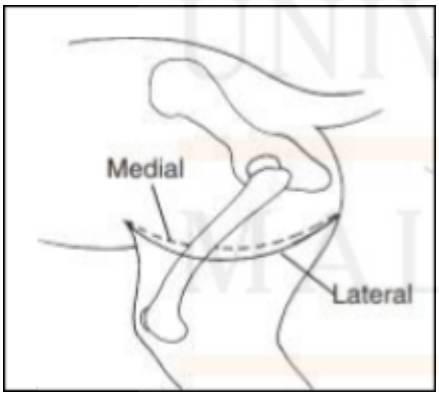
Incision line area for ovariohysterectomy procedure in a cat. Incision (black line); umbilicus (blue arrow); cranial brim of the pubis (yellow arrow).

Appendix E



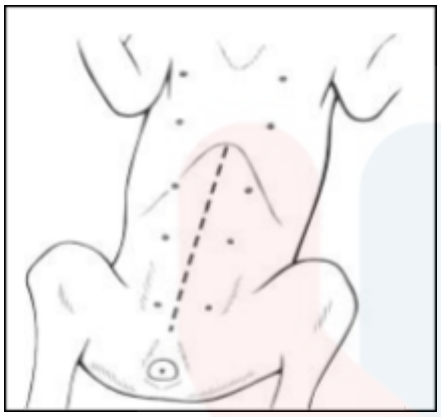
Incision line area for hindlimb amputation procedure in a cat (depends on the area intended to be removed).

Appendix F



Incision line area for coxofemoral disarticulation procedure in a cat. Skin incision was made around the rear limb at the level of the middle third of the femur.

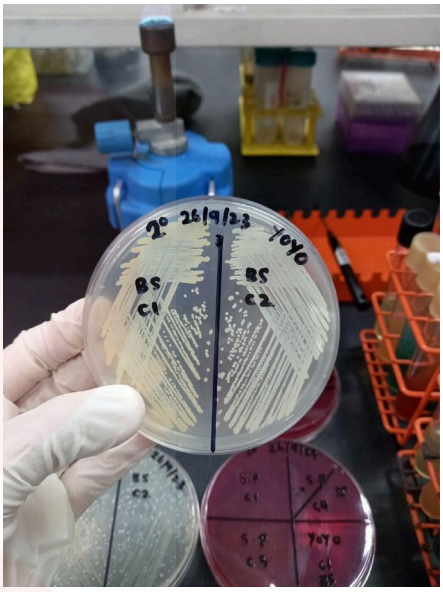
Appendix G



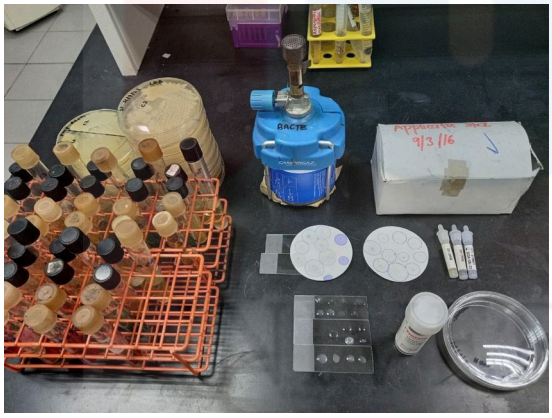
Incision line area for diaphragmatic hernia repair in a cat. Skin incision was made at the ventral midline beginning near the xiphoid process and extending caudally to the pubis (depending on the condition of herniation).

Appendix H Pictures of sample processing

Preparation of culture media



Secondary culture of pure colonies from one of the cat swab samples



Biochemical test, oxidase test and catalase test using bacteria colonies from secondary culture

Bacteria culture on selective media; Mannitol salt agar, Eosin Methylene-Blue agar, and Xylose Lysine Deoxycholate agar