UNIVERSITI PUTRA MALAYSIA

BACTERIOLOGICAL EVALUATION OF COMMERCIAL BIOLOGICALLY APPROPRIATE RAW FOOD (BARF) FOR PET IN MALAYSIA

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BACTERIOLOGICAL EVALUATION OF COMMERCIAL BIOLOGICALLY APPROPRIATE RAW FOOD (BARF) FOR PET IN MALAYSIA

LEW LI RONG

A project paper submitted to the
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It is hereby certified that we have read this project paper entitled ‘Bacteriological evaluation of commercial biologically appropriate raw food (BARF) for pet in Malaysia’, by Lew Li Rong and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

I dedicated this thesis to both greatest supporters in my life, my parents,

(Lew Toh Chin and Lee Shiau Ling),

My siblings,

(Cian, Wei, Shuang, Jun)

My mental mentor,

(Wong Yu Hao)

Supervisor, co-supervisor, staff of FPV, UPM and my beloved friends, who have endlessly supported me throughout this journey.

This work is a testament to the love, faith, and friendship that have sustained me. Without you I would not be able to be this far.

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LISTS OF ABBREVIATIONS

BARF	Biologically Appropriate Raw Food
PFMA	Pet Food Manufacturers' Association
SPC	Standard Plate Count
AMR	Antimicrobial Resistance
MDR	Multidrug Resistance
HACCP	Hazard Analysis Critical Control Point
GMP	Good Manufacturing Practice
DVS	Department of Veterinary Service

ABSTRAK

Abstrak daripada kertas project yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Tahun Akhir.

BACTERIOLOGICAL EVALUATION OF COMMERCIAL BIOLOGICALLY APPROPRIATE RAW FOOD (BARF) FOR PET IN MALAYSIA

Oleh

LEW LI RONG

OKTOBER 2023

PENYELIA: PROF. MADYA DR. SITI KHAIRANI BEJO

PENYELIA BERSAMA: DR. NUR INDAH AHMAD

Peningkatan pilihan pemilik haiwan peliharaan terhadap diet Makanan Mentah Sesuai secara Biologi (Biologically Appropriate Raw Food atau BARF) merupakan satu cabaran penting kepada veterinar, kerana ia membawa potensi risiko kepada kesihatan haiwan dan kesihatan awam. Kajian ini bertujuan untuk menilai komposisi bakteriologi produk BARF komersial di Malaysia. Pada bulan Ogos 2023, 30 sampel dikumpulkan dari enam pengeluar tempatan yang terletak di kawasan Kuala Lumpur. Analisis Bilangan Plate Piawai (Standard Plate Count atau SPC) dan kultur bakteria dijalankan untuk menentukan kandungan dan tahap mikrobiologi. Keputusan menunjukkan bahawa tahap kebersihan semua sampel berada dalam julat yang dapat diterima,

kerana tiada nilai SPC melebihi ambang had yang ditetapkan untuk daging mentah yang ditujukan untuk masakan manusia (5.0×10^6 cfu/g). Walau bagaimanapun, dapatan kami mendedahkan pencemaran dengan patogen peluang, termasuk spesies *Salmonelle* spesies *Staphylococcus* spesies dan terutamanya bakteria dari keluarga *Enterobacteriaceae* (contohnya *Escherichia coli, Klebsiella pneumonia, dan Proteus vulgaris*). Secara keseluruhan, penilaian terhadap produk BARF menunjukkan bahawa mereka mengekalkan tahap kebersihan yang selamat sesuai untuk pengambilan haiwan peliharaan. Namun demikian, potensi risiko yang timbul daripada pencemaran dengan bakteria berjangkit menunjukkan perlunya meningkatkan kesedaran pemilik haiwan peliharaan tentang penularan penyakit zoonotik dan amalan kebersihan peribadi di Malaysia.

Kata Kunci: Diet berasaskan daging mentah, kucing dan anjing, penilaian mikrobiologi

ABSTRACT

An abstract of the project presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Final Year Project.

BACTERIOLOGICAL EVALUATION OF COMMERCIAL BIOLOGICALLY APPROPRIATE RAW FOOD (BARF) FOR PET IN MALAYSIA

by

LEW LI RONG

OCTOBER 2023

SUPERVISOR: ASSOC. PROF. DR. SITI KHAIRANI BEJO

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The increasing preference among pet owners for Biologically Appropriate Raw Food (BARF) diets poses a significant challenge to veterinarians, as it carries potential risks to both animal and public health. This study aimed to assess the bacteriological composition of commercial BARF products in Malaysia. Thirty samples were collected from six local manufacturers located in Kuala Lumpur. Standard Plate Count (SPC) and bacterial culture analyses were conducted to determine the microbiological content and levels, respectively. The results indicate that the hygiene levels of all the samples were within an acceptable range, as none of the SPC values exceeded the established threshold for raw meat intended for human cooking (5.0×10^6 cfu/g). However, the

findings revealed contamination with opportunistic pathogens, including *Salmonella* species, *Staphylococcus* species, and bacteria from the *Enterobacteriaceae* family (e.g., *Escherichia coli, Klebsiella pneumonia, and Proteus vulgaris*). Overall, the assessment of BARF products suggests a safe hygiene level suitable for pet consumption was maintained. Nonetheless, the potential risk arising from contamination with infectious bacteria suggested the need to enhance pet owners' awareness of zoonotic disease transmission and personal hygiene practices in Malaysia.

Keyword: raw meat-based diet, cats and dogs, microbiological evaluation

1.0 INTRODUCTION

Recently, a new trend in pet nutrition, known as Biologically Appropriate Raw Food (BARF), has gained popularity in Malaysia. According to Bulochova and Evans (2021), BARF involves feeding pets unprocessed animal products or by-products from various animal species, without any thermal processing. This feeding practice was first introduced by an Australian veterinarian Dr. Ian Billinghurst in 1993, who was impressed by the healing potential of raw food diets (Billinghurst, 1993). In Malaysia, the first commercial BARF manufacturer emerged in 2008, marking the beginning of the BARF industry in the region. Since then, this industry has grown significantly, with over 17 establishments operating in 2023. Similarly, the United Kingdom witnessed a notable increase in BARF suppliers, rising from seven registered companies in 2007 to more than 80 in 2018. This surge in production capacity aligns with the heightened popularity and demand for this dietary choice (Withenshaw *et al*., 2020). Moreover, it is important to note that commercial BARF products typically come with a higher price compared to traditional dry kibble and wet canned pet foods.

Additionally, Ahmed *et al.* (2021) found that many pet owners believe BARF diets offer a natural and healthy approach, resembling ancestral pet diets that primarily consisted of uncooked foods. However, it is important to note that empirical evidence supporting the potential health benefits of BARF diets for pets is lacking. Moreover, the increase in commercial BARF pet products has raised public health concerns regarding possible transmission of zoonotic bacteria from pets to their human owners. For instance, a feline tuberculosis outbreak in England in 2020 was suspected to be linked to BARF feeding practices (Halloran *et al.*, 2021). Drawing from a study by Bottari *et al.* (2020), it has been observed that a majority of BARF samples in Italy show elevated total bacterial counts, suggesting contamination. Nevertheless, comprehensive data regarding the bacteriological status of BARF products in Malaysia remain notably limited.

Therefore, the objectives of this project were to analyse the level of bacterial contamination and to isolate and identifie bacteria in commercial BARF samples.

2.0 LITERATURE REVIEW

2.1 Raw Ingredients

Research reported that BARF diets consist a diverse spectrum of animal protein sources, including poultry (such as chicken and turkey), waterfowl (such as duck), aquatic species (such as fish and mussel) and livestock (such as beef, lamb and venison) (Kiprotich *et al.*, 2023). In accordance with Brozic *et al.* findings in 2020, BARF aimed to replicate the nutritional profile of prey animals found in their natural habitat. This involves maintaining a proportional composition of approximately 80% muscle tissue, 10% bone content, 5% secretory organs, and an additional 5% from others. Therefore, these diets also include organ meats (e.g., heart, liver, and rumen), bones and a source of fiber in the forms of vegetables or fruits. Furthermore, it is noteworthy that BARF formulations may include essential vitamins and trace minerals to ensure a comprehensive and balanced nutritional profile for pets.

2.2 The Manufacturer

The majority of BARF products are marketed in a frozen state primarily to extend their shelf life. Additionally, this freezing process serves to reduce or eliminate potential contamination by pathogenic microorganisms (Davies *et al.,* 2019). Subsequently, pet owners have easy access to BARF manufacturers to purchase raw food products. In 2019, the Pet Food Manufacturers' Association (PFMA) highlighted various distribution channels available for procuring pet raw food products, including online pet food manufacturers, traditional pet retail stores, and exhibition fairs. Moreover, most of the BARF manufacturers provide comprehensive ingredient lists for their products, along with instructions for pet owners who are feeding their pets with this raw dietary regimen by printing them on the package. In addition, it is notable that some of the manufacturers convinced their clients regarding freezing can eliminate and destroy the bacteria contained in the BARF.

2.3 Benefits of BARF

In a study conducted by Viegas *et al.* in 2020, about 69% of pet owners selecting BARF diets for their pets believed that this dietary choice aligns with animals' carnivorous nature. This finding is consistent with survey-based research like that of Bulochova and Evans (2021), where 93% of owners choose BARF diets due to their perception of being more natural and species-appropriate. Moreover, they believed that BARF is a more natural way of feeding and allows the pets to express their natural behaviors by eating raw, as their ancestors (wild animals). Other than that, pet owners often prefer BARF diets over traditional pet foods to eliminate processed foods. To further explain, these are some of the main reasons behind it which 21% of pet owners feeding BARF diets mentioned having bad experience with traditional pet food because it had caused issues in the past. Furthermore, 19% expressed a lack of trust in it and 6% reported that their pets refused to consume the traditional pet food (Morelli *et al.*, 2019).

Pet owners commonly believe that BARF diets can enhance their pets' health (Morgan *et al.*, 2017). Their research indicates that the BARF dietary concept has a positive impact on various aspects of pet health, encompassing immunological, dermatological, gastrointestinal, and periodontal. Furthermore, Anderson *et al.* (2018) found that BARF diets often downregulate pro-inflammatory cytokine genes, thus helping in chronic inflammatory conditions. Allergenicity is minimized because BARF usually is made of a single protein source, potentially benefiting pets with skin or gastrointestinal allergies (Brozic *et al.*, 2020). It is also suggested that chewing raw bones can be advantageous for oral hygiene in raw pet diet feeding (O'Halloran, 2020). However, the study emphasized the necessity for systematic studies to conclusively exclude alternative explanations for these observed enhancements.

2.4 Potential Risks of BARF

Concerns about nutritional imbalances on BARF diets for pets especially after consuming BARF for a long period of time were raised (Anon, 2020). Kohler *et al.* (2012) warned pet owners about potential dietary hyperthyroidism and thyrotoxicosis in animals fed BARF diets due to the ingestion of raw thyroid gland tissue. These tissues were not effectively broken down by gastric acid, leading to absorption in the body. Additionally, BARF diets, especially those heavy in raw fish, carried a risk of thiaminase presence, an enzyme that can degrade thiamine and cause thiamine deficiency in animals, resulting in neurological issues (Bischoff and Rumbeiha, 2018; Bettendorff and Wins, 2021). Moreover, consuming excessive quantities of beef liver can elevate the risk of hypervitaminosis A, leading to osteopathic manifestations, particularly impacting the axial skeleton in small animal species (Polizopoulou, 2005).

In the context of BARF diets for pets, safety concerns are paramount due to the potential health risks associated with raw meat consumption. Raw meat ingestion in dogs and cats can lead to foodborne illnesses, by where 45% of BARF-fed pets experienced gastrointestinal issues like diarrhea, constipation, and vomiting (Morelli *et al.*, 2019). Additionally, pets on a BARF diet are at an increased risk of transmitting infectious diseases through their dietary choices. A report suggested a possible link between BARF feeding and a feline tuberculosis outbreak in England (O'Halloran's 2020). Furthermore, it is essential to recognize that raw meat can harbor parasitic organisms. According to Ahmed *et al.* (2021), raw meat may contain parasite larvae, which can complete their life cycle and mature into adult-stage parasites in small animals.

2.5 Public Health

Transmission of pathogens from raw meat-based food can occur through both direct and indirect routes. Indirect transmission occurs when pet owners engage in inadequate hygiene practices, leading to cross-contamination between their own food and pet food. Bulochova and Evans (2021) have elucidated that indirect contact can arise when pet owners share utensils and equipment with their pets during the preparation of BARF, potentially transferring pathogens to surfaces and infecting the owners. A survey revealed that approximately three-quarters of BARF-feeding pet owners prepare raw pet food in the same location as their own food, such as the kitchen. Moreover, most of these owners do not separate chopping boards or utensils for pet food preparation (Morgan *et al.* in 2022). Notably, in 2018, three cases of Shiga toxin-producing *E. coli* infections in the United Kingdom were attributed to the handling of contaminated raw pet food and close contact with pets that had consumed it (Trier *et al*., 2021).

Raw meat in BARF products has been identified as a significant source of human exposure to antimicrobial-resistant (AMR) bacteria (O'Halloran, 2020). Nuesch-Inderbinen *et al.* (2019) highlighted that BARF feeding has been recognized as a risk factor for the shedding of antimicrobial-resistant *Enterobacteriaceae* family by pets. The study documented the presence of 3.9% colistin-resistant and 2% aminoglycoside-resistant bacteria of this particular family. Furthermore, there was an outbreak of multidrug-resistant *Salmonella sp.* that spanned several states within the United States, leading to the onset of illness in a total of five pet owners, tragically resulting in one fatality (Nichols *et al.*, 2022). An epidemiological inquiry established a significant association between this outbreak and the pet owners feeding their small animals with the contaminated raw turkey and chicken products (Hassan *et al.,* 2019). Furthermore, the outbreak of human campylobacteriosis cases can be linked to close contact with domestic pets. This is supported by instances of zoonotic transmission observed in cases where puppies, fed raw diets

containing chicken by-products, transmitted the pathogen to their owners (Campagnolo *et al.*, 2018).

2.6 Pet Food Safety Requirements

The regulations pertaining to the safety of pet food in Europe, which are congruent with those governing animal feed and animal by-products (ABP), are principally under the purview of Regulation (EC) No. 2073/2005. This regulation prescribes microbiological standards for minced meat and mechanically separated meat (MSM), specifying a minimum colony count of 5.0×10^5 cfu/g and a maximum threshold of 5×10^6 cfu/g. It is noteworthy that this regulation was used in previous studies, conducted by van Bree *et al.* in 2018, which investigated the extent of bacterial contamination in BARF products in the Netherlands, as well as in the research conducted by Brozic *et al.* in 2020, which assessed the quality of BARF products in Italy. Notably, in accordance with the European Union's (EU) regulatory framework, the SPC ranges in both countries have been categorized as marginally acceptable, as they fall within the spectrum bounded by the minimum and maximum values.

2.7 Bacteria content in BARF

Bacteria belonging to the *Enterobacteriaceae* family have consistently been identified as the most frequently encountered microorganisms within BARF (Davies *et al.,* 2019). The bacteria were ubiquitous in all samples examined including *Escherichia coli, Hafnia* sp., *Klebsiella* sp., *Pantoea* sp., and *Serratia* sp. (Hellgren *et al.*, 2019). *Escherichia coli* was isolated from a substantial 98% of the samples (Bacci *et al.*, 2019), whilst the prevalence of *Klebsiella* sp in the raw meat was 4.76% (Zhang *et al.*, 2018). Meanwhile, *Pantoea* sp. exhibits an average relative prevalence exceeding 5% (Wang *et al.,* 2022). Bottari et al. (2020) identified that 71% of the samples were contaminated by *Salmonella* sp. Other than gram negative bacteria, gram positive bacteria were found in BARF too such as *Bacillus* sp., *Staphylococcus* sp. and *Streptococcus* sp.

3.0 MATERIALS AND METHODS

3.1 Sample Collection

Thirty raw BARF samples were collected from six manufacturers in Kuala Lumpur, with each manufacturer providing five different products for the BARF sample set. These samples were stored frozen at -18°C in their original packaging at the Bacteriology Laboratory of the Faculty of Veterinary Medicine, University of Putra Malaysia. The frozen samples were thawed in a chiller set at 2 to 4°C for 24 hours before analysed.

3.2 Standard Plate Count (SPC)

The estimation of bacterial populations within the BARF samples was carried out through the application of the Standard Plate Count (SPC) methodology. This technique involved the initial dilution of a gram of each sample with 9.0 ml sterile distilled water, resulting in the generation of a sequence of dilutions ranging from 10^{-1} to 10^{-10}. Next, 0.1 ml of each dilution was spread on nutrient agar plates using a sterile spreader and incubated at 37°C for 24 hours. Then, only the plates with a cell count range of 25 to 250 cells were chosen as suggested by the Food and Drug Administration in the US (2001). In fact, Colony Forming Units (CFU) were subsequently calculated according to the provided formula.

$$Cfu/ml = \frac{Number\ of\ colonies \times Dilution\ factor}{Volume\ of\ culture\ plate\ (ml)}$$

3.3 Bacteria Isolation and Identification

3.3.1 Isolation of Bacteria

The swab sample from BARF was inoculated into 9 ml of sterile buffered peptone water and incubated at 37°C for 18 to 24 hours for isolation of Salmonella. One milliliter (1.0 ml) of the mixture was transferred into 9.0 ml of Rappaport-Vassiliadis (RV) for further incubation. Afterwards, 100 µl of RV inoculum was inoculated onto XLD agar. The BARF sample was also inoculated onto blood agar for isolation of other bacteria. Both inoculated XLD and blood agar were incubated at 37°C for 18 to 24 hours.

3.3.2 Identification of bacteria

A pure culture of bacteria isolated was obtained by inoculating a single colony from the blood agar and XLD agar onto nutrient agar. Incubation was carried out at 37°C for 18 to 24 hours.

Gram Staining

Gram staining was performed on all bacteria isolated for identification. The process details were described below. One drop of normal saline was placed on a glass slide and mixed with an isolated colony. The smear was air-dried and stained with Crystal Violet. The smear was washed with tap water after one minute. The procedure was repeated with Lugol's Iodine. Next, the smear was rinsed with acetone for 2-3 seconds. Lastly, the smear was stained with Carbol Fuchsin and washed with tap water after one minute. The slide was then ready to observe under a microscope at 100x magnification.

Oxidase Test

The isolated colony was placed on the deionised water droplets on filter paper. The positive result was obtained when the colour changes to blue within 30 seconds.

Catalase Test

The isolated colony was mixed with hydrogen peroxide (H_2O_2) droplets on a clean glass slide. The positive result was obtained when there was production of gas bubbles. This is because catalase is an enzymatic component responsible for catalyzing the decomposition of hydrogen peroxide into water (H_2O) and molecular oxygen (O_2).

Coagulase Test

The isolated colony was mixed with normal saline droplets on a clean glass slide. Then, a drop of rabbit plasma was added and the glass slide was swirled. The positive result was obtained when there was a rocky appearance in the suspension indicating clumping occurred.

Triple Sugar Iron (TSI) Test

The isolated colony was inserted into the central region of the agar medium, reaching the tube's base, and then streaked across the surface of the agar slant. The positive result was obtained when there were changes in the coloration of both the agar slant and butt, the presence of air bubbles and the manifestation of black pigment.

Sulfide Indole Motility (SIM) Test

The isolated colony was stabbed into the agar medium. Kovac's reagent was subsequently added. The positive result was obtained when there was presence of black pigment, increased turbidity and colour transition from yellow to red.

Urease Broth Test

The isolated colony was streaked on the agar surface. The positive result was obtained when the colour changes from yellow to pink.

Citrate Test

The isolated colony was streaked on the agar surface. The positive result was obtained when the colour changes from green to blue.

3.4 Statistical Analysis

The statistical analysis of SPC data in BARF research involved several key steps. First, we assessed data normality using the Shapiro-Wilk test since the sample size was less than 50 while the homoscedasticity was tested with the Levene test. Then, we used Kruskal Wallis Test to compare the SPC level between brands as well as between protein sources. Significance was set at a 5% threshold, and all analyses were conducted using the SPSS Statistics software for robust statistical evaluation.

3.5 Evaluation of Information Provided by Manufacturer

In the context of BARF consumption, a comprehensive evaluation encompassed a range of factors pertaining to the information disseminated by manufacturers across different platforms, including packaging, websites, and social media channels. This evaluation considered multiple dimensions, which included benefits of consuming BARF, potential risks related to bacteria, safe storage method, safe preparation before intake, safe handling of leftovers, abnormal findings after consumption and the owner's personal hygiene. This multifaceted assessment of manufacturer-provided information ensured a comprehensive understanding of the guidance offered to consumers in relation to BARF diets, encompassing safety considerations associated.

4.0 RESULTS

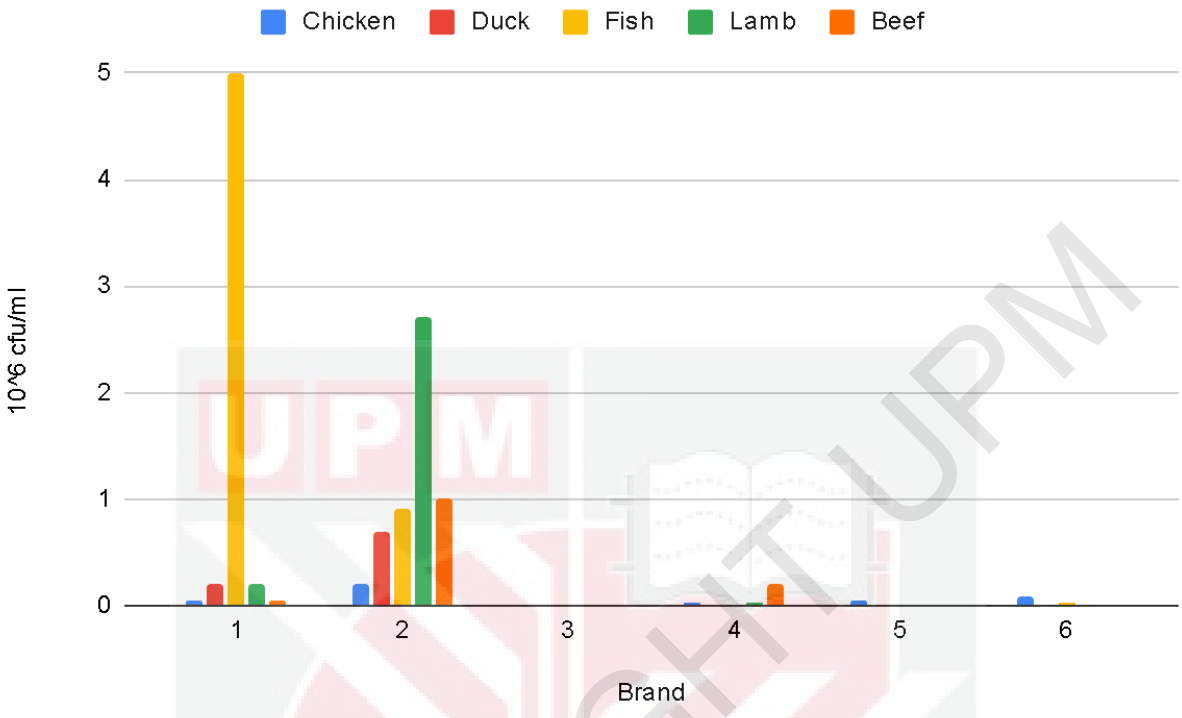
4.1 Standard Plate Count (SPC)

Chicken samples showed Brand 3 with the lowest (1.3×10^3 cfu/ml) and Brand 2 with the highest (2.1×10^5 cfu/ml). Duck samples ranged from Brand 4 (3.7 × 10^3 cfu/ml) to Brand 2 (6.5 × 10^5cfu/ml). Fish samples from Brand 3 had the lowest SPC (3.0×10^2 cfu/ml), while Brand 1 had the highest (5.0×10^6 cfu/ml). Lamb samples had Brand 3 at the lowest (1.0×10^3 cfu/ml) and Brand 2 at the highest (2.7×10^5 cfu/ml). Beef samples had Brand 3 at the lowest (6.0×10^2 cfu/ml) and Brand 2 at the highest (1.0×10^6 cfu/ml). In summary, Brand 3 fish samples had the lowest SPC, while Brand 1 had the highest, highlighting significant contamination differences among brands, emphasizing the need for robust quality control in BARF product production.

Table I: Result of Standard Plate Count (SPC) in 10^3 cfu/ml

	Chicken	**Duck**	**Fish**	**Lamb**	**Beef**
Brand 1	41	178	5000	247	49
Brand 2	211	650	850	2690	1020
Brand 3	1.3	4.4	0.3	1	0.6
Brand 4	17	3.7	7.1	22	163
Brand 5	37	5.7	2.8	5	13
Brand 6	81	3.9	15.4	7.4	5.2

Figure I: Result of Standard Plate Count (SPC)



4.2 Isolation and Identification of Bacteria

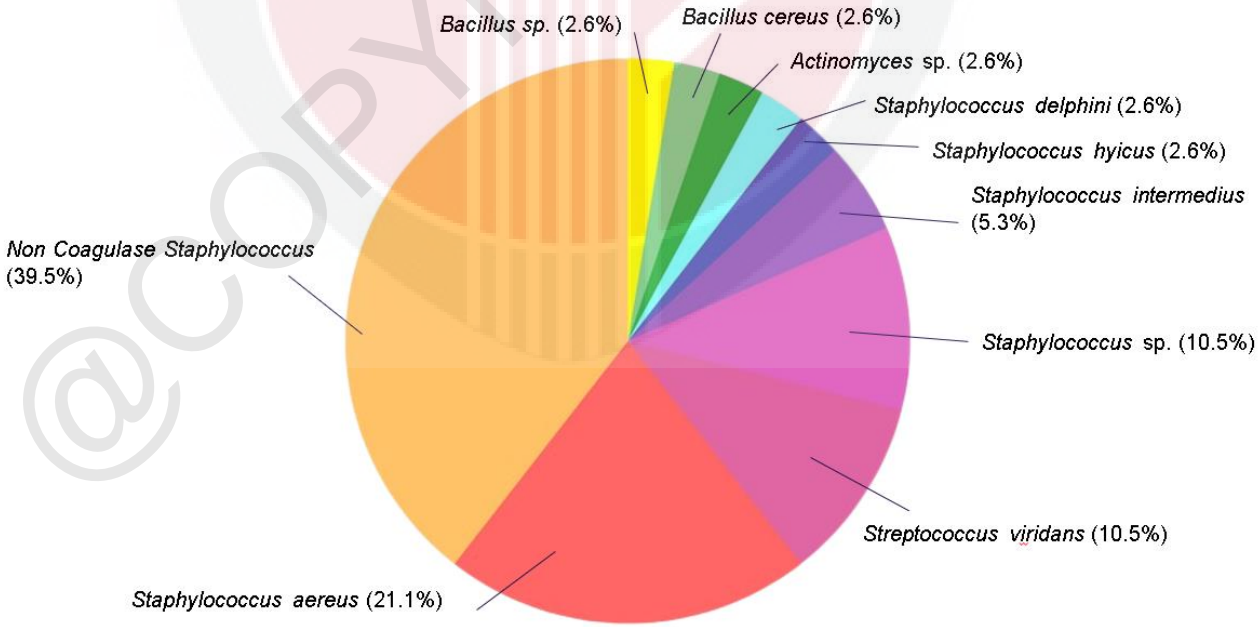
4.2.1 Gram Positive Bacteria

The microbiological analysis of the specimen has revealed a total of 38 discrete bacterial colonies, encompassing a diverse spectrum of species spanning the genera *Bacillus*, *Actinomyces*, *Staphylococcus*, and *Streptococcus*. Specifically, the colony distribution includes single occurrences of *Bacillus* sp*., Bacillus cereus, Actinomyces* sp*., Staphylococcus delphini,* and *Staphylococcus hyicus*, collectively accounting for a minor proportion of 7.89% of the total colonies. Furthermore, *Staphylococcus intermedius* constitutes 5.26% of the cumulative colony count. Significantly, both *Staphylococcus* sp. and *Streptococcus viridans* each contribute 10.53% to the overall colony composition. Noteworthy is the prominence of *Staphylococcus aureus*, comprising approximately 21.06% of the entire colony population, while *Non-coagulase staphylococcus* predominates with a representation of 39.47%.

Table II: Gram positive bacteria isolated from the sample

Bacteria	**Sample Amount**	**Percentage (%)**
Bacillus sp.	1	7.89
Bacillus cereus	1	7.89
Actinomyces sp.	1	7.89
Staphylococcus delphini	1	2.63
Staphylococcus hyicus	1	2.63
Staphylococcus intermedius	2	5.26
Staphylococcus sp.	4	10.53
Streptococcus viridans	4	10.53
Staphylococcus aureus	8	21.06
Non-coagulase staphylococcus	15	39.47
Total	**38**	**100.00**

Figure II: Gram positive bacteria isolated from the sample



4.2.2 Gram Negative Bacteria

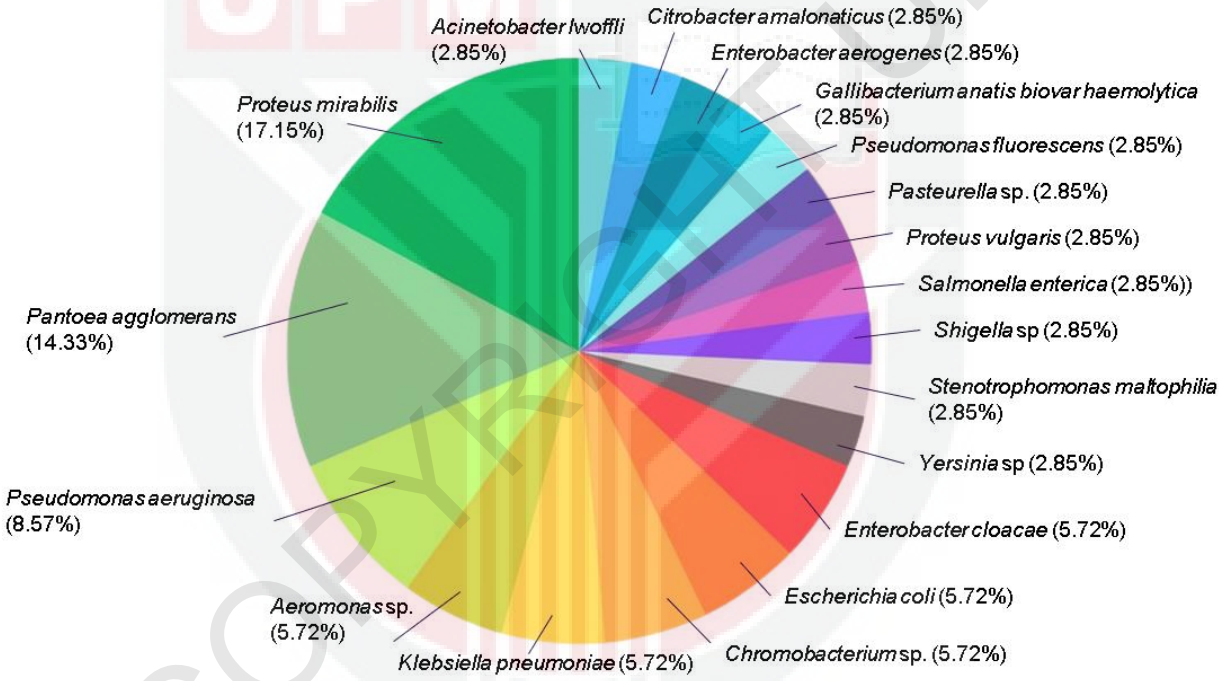
There are 35 colonies isolated from all the BARF sample. For example, *Actinobacter lwoffii, Citrobacter amalonaticus, Enterobacter aerogenes, Gallibacterium anatis bv. Haemolytica, Pseudomonas fluorescens, Proteus vulgaris, Pasteurella* sp.*, Salmonella Enterica, Shigella* sp.*, Stenotrophomonas maltophilia* and *Yersinia kristensenii* were identified, each constituting 2.86% of the total population. Furthermore, *Enterobacter cloacae, Escherichia coli, Chromobacterium* sp.*, Klebsiella pneumonia, Aeromonas sp.,* and *Pseudomonas aeruginosa* were each contribute 5.71%. Notably, *Pantoea agglomerans* was isolated five time, accounting for 14.29% of the population. The highest representation was observed for *Proteus mirabilis*, with six colonies, constituting 17.14% of the total colonies.

Table III: Gram negative bacteria isolated from the sample

Bacteria	**Sample Amount**	**Percentage (%)**
Actinobacter lwoffli	1	2.86
Citrobacter amalonaticus	1	2.86
Enterobacter aerogenes	1	2.86
Gallibacterium anatis bv. Haemolytica	1	2.86
Pseudomonas fluorescens	1	2.86
Proteus vulgaris	1	2.86
Pasteurella sp.	1	2.86
Salmonella enterica	1	2.86
Shigella sp.	1	2.86
Stenotrophomonas maltophilia	1	2.86
Yersinia kristensenii	1	2.86
Enterobacter cloacae	2	5.71
Escherichia coli	2	5.71
Chromobacterium sp.	2	5.71

Klebsiella pneumonia	2	5.71
Aeromonas sp.	2	5.71
Pseudomonas aeruginosa	3	8.57
Pantoea agglomerans	5	14.28
Proteus mirabilis	6	17.14
Total	**35**	**100.00**

Figure III: Gram negative bacteria isolated from the sample



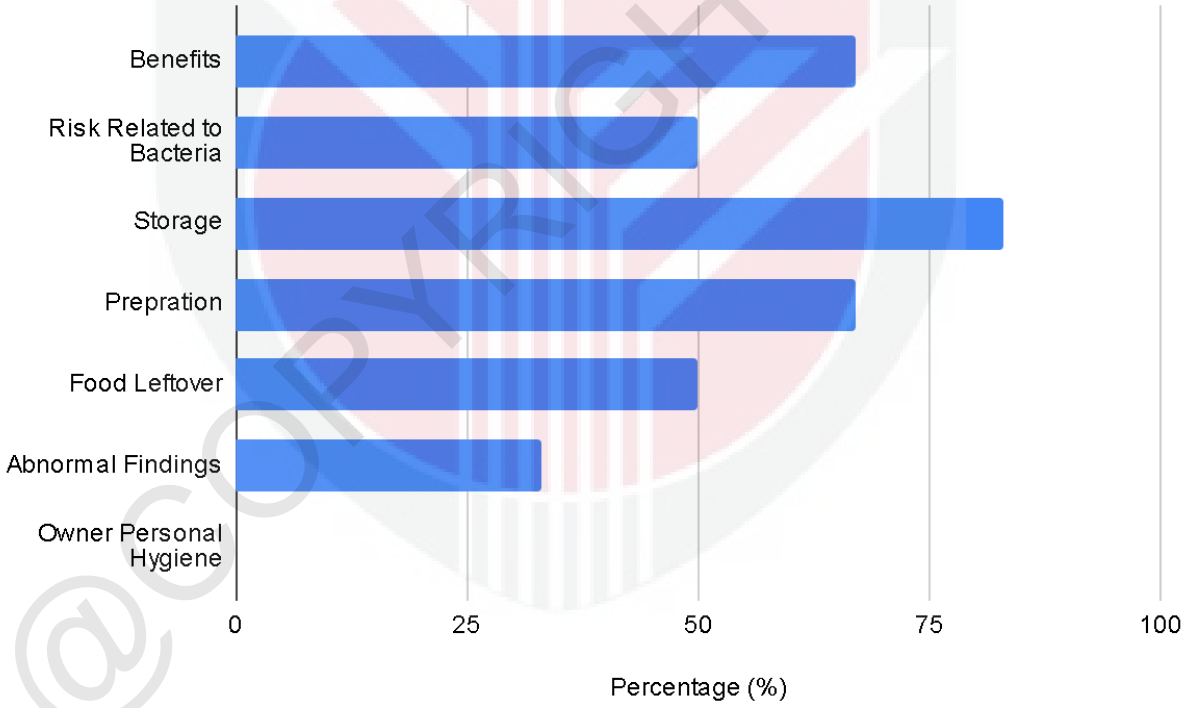
4.3 Information Provided by Manufacturers

The microbiological criteria related to BARF, as articulated by individual manufacturers, have been systematically documented in Table IV. It is noteworthy that a substantial majority of these manufacturers, specifically 83% (5 out of 6), have addressed the topic of safe storage methods for BARF on their respective websites, elucidating key aspects such as the recommended refrigerator temperature for optimal preservation. Furthermore, a notable proportion, 67% (4 out of 6) of manufacturers, have provided information regarding both the benefits of BARF consumption and the safe procedures for its preparation prior to feeding to pets. Additionally, half of the manufacturers, accounting for 50% (3 out of 6), have proactively addressed the potential risks associated with the consumption of BARF. In contrast, a more limited segment, constituting 33% (2 out of 6) of manufacturers, have raised awareness regarding the identification and management of abnormal findings post-consumption of BARF. However, it is notable that none of the manufacturers have broached the subject of pet owner personal hygiene in relation to BARF feeding practices.

Table IV: Information provided by manufactureres

	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6
Benefits		/		/	/	/
Risks related to bacteria		/	/	/		
Storage method	/	/		/	/	/
Preparation process	/	/	/	/		
Handling leftover	/			/		/
Abnormal findings	/	/				
Owner personal hygiene						

Table IV: Information provided by manufacturers.



5.0 DISCUSSION

In the present study, the sample with the highest SPC level is the fish product from Brand 1, having a count as high as 5.0×10^6 cfu/ml. This observation can be attributed to the potential unreliability of meat sources used by Brand 1, which the manufacturer has purchased raw fish meat from a questionable source, as suggested by the information contained in the Food Microbiology 3rd Edition (2008). Conversely, the sample exhibiting the lowest SPC level (3.0×10^3 cfu/ml) also belongs to fish products but is manufactured by Brand 3. Furthermore, the same brand has an average low SPC level for all products. Consequently, it can be concluded that Brand 3 has the highest level of product quality among the six different manufacturers producing BARF because it is able to control the microbiological level in the products throughout the processing. Indeed, the present investigation has shown a noteworthy variation in SPC levels among different through statistical analysis conducted using the SPSS software. The Kruskal-Wallis test used in this analysis has discerned significant variations among the brands, particularly in the context of comparisons between Brand 1 and Brand 3, as well as between Brand 2 and Brand 3.

In the current study, the researcher conducted a comparative analysis of the microbial contamination levels using SPC, across various protein sources, including chicken, duck, fish, lamb, and beef. It was observed that Brand 2 exhibited consistently elevated SPC levels across all protein sources, except for fish products, where Brand 3 has the highest contamination levels, as previously noted. In accordance with the findings presented in the 3rd Edition of "Food Microbiology" published in 2008, the high level of bacterial contamination in Brand 2's products may be due to various factors. These factors include potential issues related to the reliability of meat source, the hygienic conditions within the processing plant, the stability of storage temperatures, as well as potential complications arising during transportation. Furthermore, the

statistical analysis using SPSS software yielded results indicating that there were no statistically significant differences in SPC levels observed between protein sources.

The analysis of the SPC in this study reveals a range from 3.0×10^2 cfu/ml to 5.0×10^6 cfu/ml. Consequently, the SPC range observed in the current investigation falls within a marginally acceptable range, similar with the results obtained in prior research conducted in the Netherlands and Italy. This SPC analysis aligns with the outcomes of a study by van Bree *et al.* (2018), which examined the presence of microbiological pathogens in commercial BARF products in the Netherlands, yielding SPC values ranging from 7.9×10^2 cfu/ml to 5.0×10^6 cfu/ml. In contrast, the SPC levels in Italy are considerably lower than those observed in Malaysia, from 4.2×10^4 cfu/ml to 3.8×10^6 cfu/ml (Brozic *et al.*, 2020). Consequently, it can be inferred that the quality of BARF products is superior in Italy compared to both the Netherlands and Malaysia, while the quality levels in Malaysia and Italy are the same.

Moreover, the study assessed the bacterial contamination levels in raw pet food in comparison to dry and wet pet food. Surprisingly, dry pet food exhibits the highest proportion of samples with a Standard Plate Count (SPC) exceeding 10^6 cfu/ml. In the present investigation, merely 3.33% of raw pet food samples (1 out of 30) were identified as having SPC levels at an unacceptable borderline. In contrast, a concerning 7% of dry pet food samples (11 out of 150) in Lebanon were found to exceed the SPC borderline. Serhan *et al.* (2023) explained that this may be due to the absence of microbiological standards governing the permissible microbial load in pet food, resulting in inadequate quality control measures.

Other than that, there are several bacteria found in the BARF sample. Firstly, *Bacillus* sp. constituted a relatively minor fraction, with a prevalence of 5.3% observed among the 38 isolates examined. This occurrence represents a substantial deviation from the prevalence rate of 48.7% reported by Osman *et al.* in their study conducted in 2018. It is essential to note that *Bacillus* sp. poses a significant health risk, given its capacity to induce emetic and diarrheal syndromes in

humans, resulting in symptoms such as vomiting and abdominal pain, respectively (McDowell *et al*., 2023).

Additionally, a distinct occurrence of *Streptococcus* sp. was observed, with a prevalence of 10.5% detected among the 38 isolates under investigation. This finding deviates significantly from the extensive prevalence of this bacterial species, which has been reported to reach as high as 85% in research conducted by Boonyong *et al.* in 2019. According to The Center for Food Security & Public Health (2020), this bacterial infection becomes obvious over the course of several days to a week, while toxic shock-like syndromes can rapidly progress into severe illnesses within a matter of hours.

Furthermore, the isolation of *Staphylococcus* species, encompassing *Staphylococcus aureus, Staphylococcus hyicus*, and *Non-coagulase Staphylococcus*, was identified in approximately 39.5% of the total isolates (15 out of 38). This prevalence closely aligns with the findings from a prior study conducted by van Bree *et al.* in 2018, which reported a comparable incidence of approximately 35.0%. It is important to note that this category of bacteria has the potential to induce skin diseases in animals, including conditions such as mastitis, exudative epidermitis and pyoderma, as documented by Quinn *et al.* (2011).

The findings regarding *Salmonella* sp. align with Fredriksson-Ahomaee *et al.*'s (2017) research, which reported a 3.33% (1/30) prevalence of *Salmonella* sp. contamination in raw pet diets. However, our study reveals a notably lower prevalence compared to Bottari *et al.* (2020), where *Salmonella* sp. contamination rates reached 71%. However, it remains a noteworthy discovery due to the intrinsic pathogenicity of *Salmonella enterica*, which has the potential to induce zoonotic outbreaks with implications for both animal and human well-being.

In contrast to Hellgren *et al.* 's 2019 study, our research found only 57.9% (17/30) of the samples contaminated with *Enterobacter* family bacteria, whereas they reported the presence of *Enterobacteriaceae* bacteria in all their samples. Hellgren *et al.* (2019) suggested that these microorganisms are part of the normal intestinal microbiota and can easily spread during the

slaughtering process. Thus, the presence of these bacteria in our tested pet food samples was expected, as BARF production does not involve measures to eliminate them.

Moreover, within the context of the present investigation, *Escherichia coli* was detected in 2 out of 30 samples, representing a percentage of 6.66%. This demonstrates a notable divergence from the findings of van Bree *et al.* (2018), whose research indicated an approximate 23% incidence of this particular bacterium. The presence of *Escherichia coli* serves as an indicator of potential fecal contamination in BARF products, thereby giving rise to pertinent concerns regarding the adherence to hygienic standards during the phases of food handling and processing.

Our current investigation reveals a notably lower prevalence of 6.67% (2/30) for *Klebsiella pneumonia.* In sharp contrast, a recent study by Theocharidi *et al*. (2022) reported the detection of this bacterial strain in a substantial 81.8% of the meat samples they examined. It is a notable pathogen since *Klebsiella pneumoniae* has the potential to instigate respiratory infections in both the animal and human populations. In 2020, Hu *et al.* reported the emergence of multidrug resistant (MDR) in this bacterium, linked to various plasmids carrying antimicrobial resistance (AMR) genes.

It is noteworthy to observe that none of the manufacturers mentioned the owner's personal hygiene on their websites, social media platforms and even on the packaging. This warns of the necessity of public awareness regarding this issue because BARF products potentially serve as vectors for zoonotic diseases. Furthermore, another intriguing finding is the information provided related to bacteria. Brands 1, 2, and 4 claimed that gastric acid can eliminate and eradicate bacteria present in BARF. However, it is essential to note that extant literature has documented instances where certain pathogens, such as *E. coli, Salmonella* sp., and *Listeria* sp., have demonstrated the ability to survive exposure to hydrochloric acid (Takumi *et al.*, 2001; Bernardeau *et al*., 2017; Akritidou *et al.*, 2022)

In summary, it can be said that the BARF products industry in Malaysia currently considered as a grey zone area due to lack of official information, support, and evidential backing from regulatory authorities. To enhance the quality of BARF products, it is recommended that manufacturers apply for certification in food hygiene and safety standards such as Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Practices (GMP) protocols. Additionally, the Department of Veterinary Services (DVS) should conduct routine microbiological assessments of pet food products and, if deemed necessary, implement measures including product recalls to ensure the safety of pet food in Malaysia.

6.0 CONCLUSION AND RECOMMENDATION

This study has determined that the BARF product hygiene level, as assessed by microbiological criteria, meets the acceptable standards for raw meat intended for human consumption prior to cooking. Furthermore, the bacterial colonies isolated during this study show a variety of opportunistic pathogens, posing a potential risk to vulnerable populations such as immunosuppressed individuals, pediatric patients, and geriatric individuals among both pets and humans. Additionally, this research suggests enhancing public awareness regarding health risks associated with contaminated BARF products because the majority of them harbor zoonotic pathogens capable of infecting both pets and their owners.

For future investigations, it is recommended to expand the scope of the study to encompass homemade BARF products that would provide valuable insights. Moreover, the study of BARF products can be approached from various perspectives. For instance, conducting questionnaire surveys to analyze owners' perspectives on this emerging feeding trend would provide valuable data for a comprehensive understanding.

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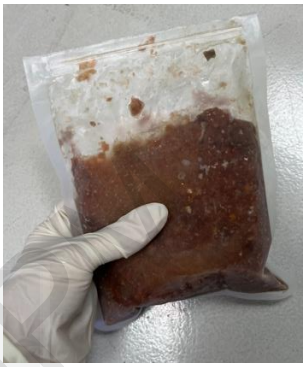
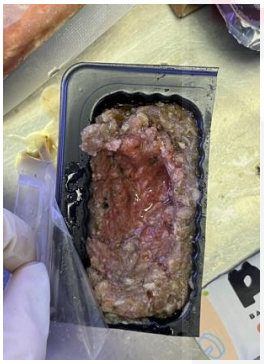
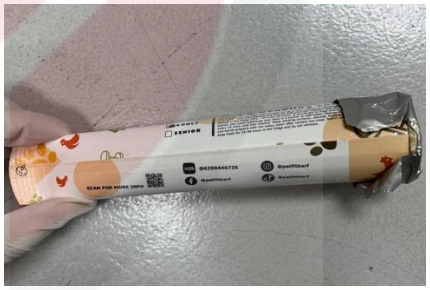
APPENDICES

Appendix A: Example of BARF sample



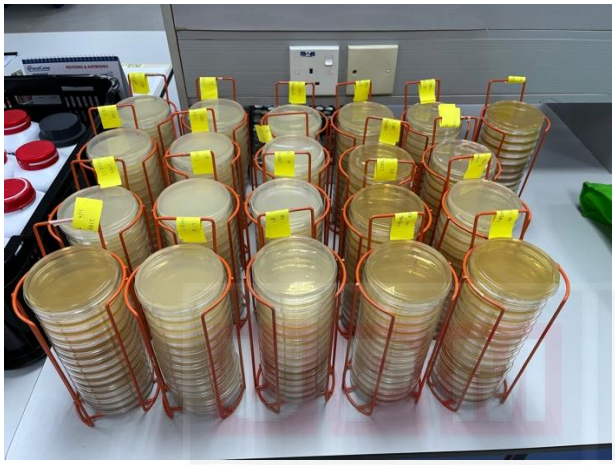






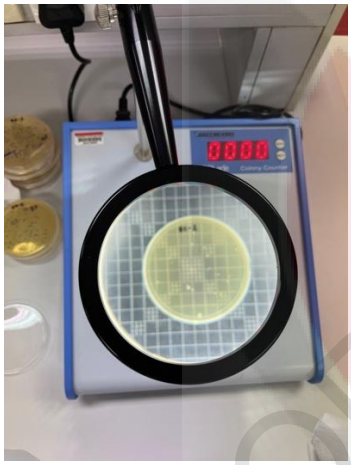



Appendix B: Standard Plate Count

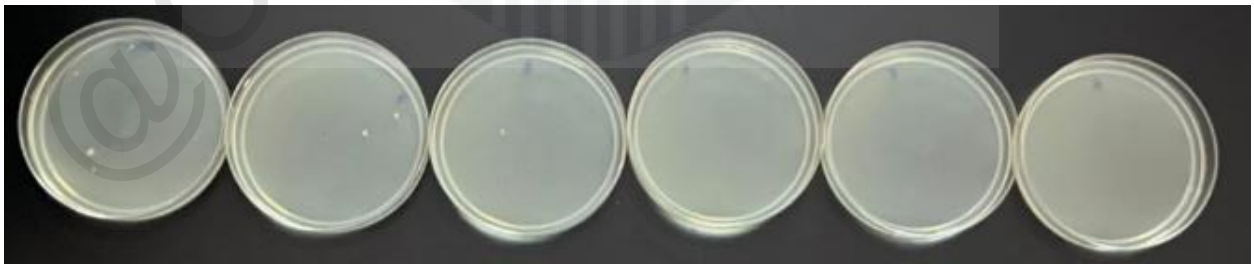
Inoculation on Nutrient Agar



Calculation of Colony Formed



Result of SPC

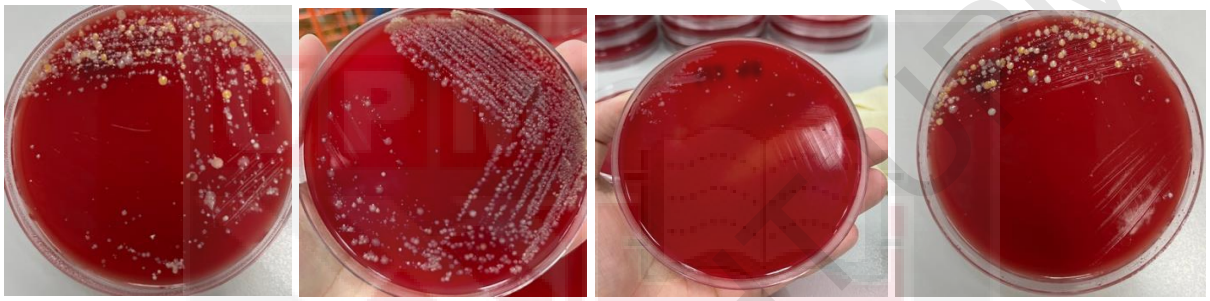


Appendix C: Colony Morphology and Cell Morphology

Colony Morphology

Blood agar is used as the primary bacteria culture medium to isolate all species. Then, XLD agar is used to isolate specific bacteria such as *Salmonella* sp. Also, pure culture is done using nutrient agar.

Blood Agar



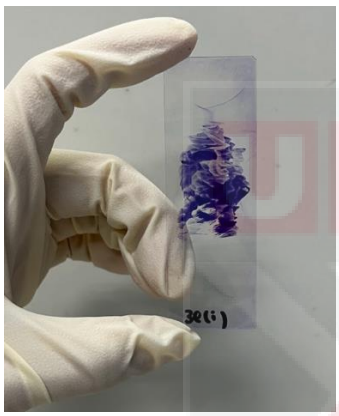
XLD Agar

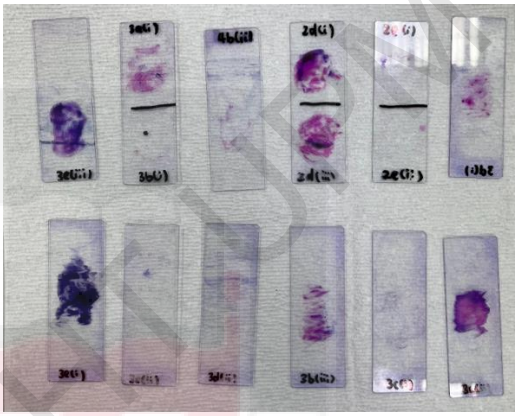
Nutrient Agar

Cell Morphology

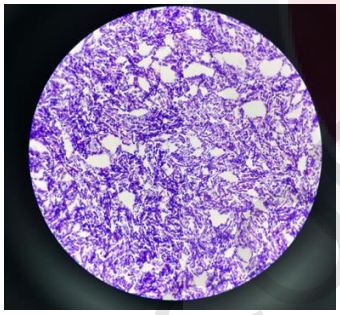
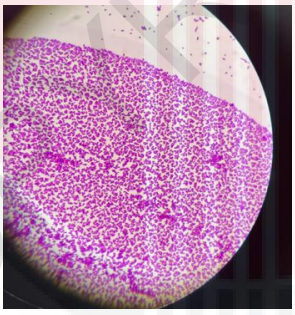
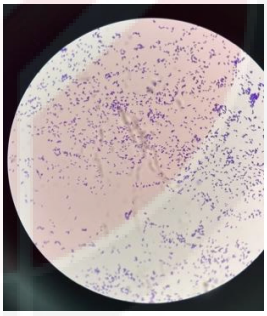
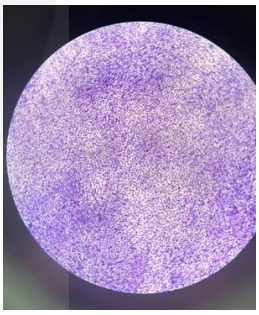
To appreciate cell morphology, the isolated colony is stained with Gram staining and observed under microscope.

Stained Slide





Microscopic morphology

Appendix D: Biochemical tests used in the experiment