ISOLATION AND IDENTIFICATION OF ENTERIC BACTERIAL PATHOGENS IN LOCAL CHICKEN AND DUCK EGGS IN KOTA BHARU, KELANTAN

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CERTIFICATION

This is to certify that we have read this research paper entitled **'Isolation and Identification of Enteric Pathogens in Local Chicken and Duck Eggs in Kota Bharu, Kelantan'** by Iman Natasha Sofea Binti Mohd Jafri, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 5436 – Research Project.

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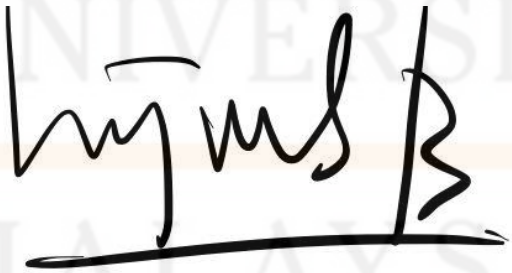

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Thank You

DEDICATIONS

I dedicate my work to the most important person and my life's greatest blessings, my parents, Siti Nor Azrin Binti Sheikh Mohamed Al-Bajerai and Mohd jafri Bin Adam, for their endless support. Not to forget, my lovely family, Kakak, Bro-Haziq, Ferhat, and Jibrail for the love and support. My nephew, Mikhail and my niece, Khadeeja. My parents and my family are the main reason I keep going. Thank you.

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Table of Contents

List of tables

List of appendices

ABSTRACT

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

Food borne diseases are one of the most concerning health issues globally and also locally, the source of the foodborne disease can be from the food for example from eggs. That can include chicken and duck eggs. The aim of this study is to isolate and detect the enteric bacterial pathogens in local chicken and duck eggs in Kota Bharu, Kelantan. A total of 30 chicken eggs and 30 duck eggs were collected from six wet markets around Kota Bharu, Kelantan region and they were all being isolated on Xylose Lysine Deoxycholate. The suspected growth on XLD agar was then being proceeded to biochemical tests for further identification. From the results, a total of 60 samples from chicken and duck eggs showed mix colonies and mix results were shown from the biochemical tests. The main objective of this study is to identify the contamination of chicken and duck eggs from wet markets across Kota Bharu, Kelantan. Based on the results from morphological colonies and biochemicals were *Shigella* spp., *Escherichia coli* and *Salmonella* spp were isolated. In conclusion, there are presence of enteric bacterial pathogens in chicken and duck eggs that could be a potential zoonotic disease of public health concerns.

Keywords: *Enteric pathogens, Public health, chicken eggs, duck eggs, food safety*

ABSTRAK

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

Penyakit bawaan makanan adalah antara isu yang membimbangkan, punca utama isu penyakit bawaan makanan adalah daripada makanan dan ini juga termasuk daripada sumber telur ayam dan juga telur itik. Tujuan utama kajian ini adalah untuk mengkaji jenis bakteria yang terdapat di dalam telur ayam dan telur itik di sekitar Kota Bharu, Kelantan. Jumlah keseluruhan 30 telur ayam dan 30 telur itik dikumpulkan dari 6 pasar di sekitar Kota Bharu, Kelantan. 60 telur ayam dan telur itik kemudiannya ditumbuhkan di atas agar Xylose Lactose Deoxycholate agar. Bakteria yang tumbuh di atas agar XLD ini kemudiannya akan digunakan untuk ujian seterusnya iaitu ujian biokimia untuk menentukan jenis bakteria yang terdapat di permukaan serta di dalam telur ayam dan juga telur itik. Keputusan daripada ujian tersebut menunjukkan koloni campuran serta campuran keputusan daripada ujian biokimia yang meunjukkan bahawa terdapat lebih daripada satu jenis bakteria. Objektif utama kajian ini adalah untuk mengkaji kadar pencemaran yang terdapat di dalam telur ayam dan juga telur itik di sekitar Kota Bharu, Kelantan. Berdasarkan keputusan ujian, bakteria yang ditemui adalah *Shigella* spp., *Escherichia coli* dan *Salmonella* spp. Kesimpulannya, terdapat bakteria enterik yang boleh menyebabkan terjadi nya penyakit zoonosis kepada orang awam dan boleh menjejaskan kesihatan awam.

Kata kunci: *Enterik patogen, Kesihatan awam, Keselamatan makanan, Telur ayam, Telur itik*

1.0 Introduction

Foodborne disease is one of the growing infectious diseases worldwide. According to The Centres for Disease Control and Prevention (CDC), there are estimated about 48 million cases reported in the United States annually, and the diseases are commonly caused by wide arrays of enteric pathogens which include *Salmonella spp*. (CDC, 2021). *Salmonella* spp. the most common bacteria species found in chicken and duck eggs has an abundance of serovars, and the most common serovars found in chicken and duck eggs are *Salmonella enteritidis (SE) and Salmonella typhimurium (ST).* Both serovars can cause salmonellosis in humans and are routinely isolated from food samples (Popoff et al., 2003). *Salmonella* spp. are commensal in the intestinal tract of ducks and chickens. Based on a study, about 75% of salmonellosis infections in humans are mainly derived from poultry and eggs by products (Hald et al., 2004). Aside from salmonellosis, other enteric bacterial pathogens may cause food-borne illnesses such as *Clostridium botulinum, C. perfringens, Bacillus subtilus, Bacillus cereus* (Wiley & Sons 2003).

In Malaysia, foodborne diseases are one of the most common issues in medical facilities. Though case fatalities were rarely reported, the cases were mostly reported in children below 15 years old (45%) followed by adults between 16-25 years old (42%) (KKM, 2006). This statistic shows that young people are vulnerable for foodborne infections and therefore this needs to be prevented through public health awareness and education. Food safety plays a major role for the prevention of foodborne pathogens to humans. Thus, this study aims to identify the presence of enteric bacterial pathogens in common food (chicken and duck eggs) in a selected

market in Kota Bharu, Kelantan through isolation and biochemical identification. The findings from this study would determine the contamination level as well as encouraging food safety practice during preparation and cooking to ensure the safety of food before consumption and therefore further minimise the risk of getting food-borne illnesses.

2.0 Research problem

Enteric bacteria are the most common bacteria that can lead to food-borne disease in humans. However, studies on enteric bacterial pathogens in chicken and duck eggs in Kota Bharu, Kelantan are limited.

3.0 Research questions

3.1 Are the eggs in Kota Bharu wet markets free from enteric bacterial pathogens?

3.1 What are the common enteric bacteria found in chicken eggs and duck eggs that can cause food-borne diseases?

4.0 Research hypothesis

4.1 Majority of the chicken and duck eggs in the market are contaminated with enteric bacterial pathogens.

4.2 *Escherichia coli* and *Salmonella* spp. are the most common enteric pathogens found in chicken and duck eggs.

5.0 Objectives

5.1 To isolate enteric bacterial pathogens from chicken and duck eggs.

5.2 To identify enteric bacterial pathogens from chicken and duck eggs through biochemical assay

6.0 Literature review

The most common enteric bacteria that can be found in chicken and duck eggs are *Escherichia coli, Salmonella* spp., and *Shigella* spp. All of this bacteria can be the source of food borne disease that are potential zoonotic disease to public health and it is a very serious issue related to food safety. Based on research, there was a prominent increase in the number of children getting infected and some are dying due to food illness. Also, WHO has stated that most of the cases, 75% are caused by bacteria. The main source of the contamination can be from the farm to fork. In the case of food borne disease, normally the clinical signs of vomiting diarrhoea, nausea, abdominal cramp and high fever will be shown if we are being infected by the pathogenic bacteria (Kavita Arumugam, Sunarjati Sudigdoadi, Gaga Irawan et al., 2015).

6.1 Common bacteria in chicken and duck eggs

The most common bacteria in chicken and duck eggs are *Salmonella* spp., *Shigella* spp., *and E.coli*. The most common and reported cases of *Salmonella* spp. are *S. enteritidis* and *S. typhimurium.* (Baggesen et al., 2002; Aktas et al., 2007). Salmonellosis is a food-borne disease that could be pathogenic to humans and possibly lead to severe illness and death. (D'Aoust, 1994; Parry et al., 2002; Dimitrov et al., 2007) For example, *Salmonella spp*. that can be found in chicken and duck's eggs are *S. enteritidis* and *S. typhimurium* (Rajashekara et al., 2000; Thorns, 2000; Foley et al., 2011) Based in study, *S. enteritidis and S. enteritidis* are the main cause of salmonellosis infection in human. The transmission of the *Salmonella* spp. to humans is through the

consumption of chicken and duck's eggs and eggs are the main sources of this salmonellosis food-borne disease. Physiologically, *Salmonella* spp. is a normal flora found in the intestinal tract of chicken and duck. And *Salmonella* spp. are common bacteria found on the chicken and duck's eggs (Baggesen et al., 2002; Aktas et al., 2007).

The other common enteric pathogens are *E. coli,* and *Shigella* spp. Based on the study, most of the foodborne illness cases among the public are from these bacteria. For *E.coli*, this bacteria will produce enterotoxin, a type of toxin will colonise and cause degeneration of the intestinal mucosa that can lead to severe diarrhea. Prolonged diarrhea could lead to dehydration and in severe cases, this could lead to death. The contamination of this bacteria can be from vertical or horizontal. Vertically, it can be transmitted from the infected reproductive tissue straight to the eggs and for horizontal transmission, it can be transmitted from the outside environment such that this bacteria could gain its entry to the eggs through the cracked egg shells. (Kavita Arumugam, Sunarjati Sudigdoadi, Gaga Irawan et al., 2015).

7.0 Materials and methods

7.1 Chicken and duck eggs sampling

A total of 60 eggs (30 chickens, 30 ducks) were collected from five selected markets in Kota Bharu, Kelantan. Individual eggs were sampled randomly and carefully kept in the sterile zip lock bags with stuffed cotton to prevent shell damage. A total of 5-10 eggs were collected from each market and properly labelled. Upon collection, the eggs were stored in the refrigerator at 4°C before processing.

7.2 Isolation and identification of enteric bacteria from eggs

The egg's shell was wiped with 70% alcohol prior to cracking to minimise external contamination. A total of 5-7 eggs-each representing each market was cracked open and pooled together in the sterile zip lock bag and mixed thoroughly. And the sample was being placed in the universal tube that contained Buffered Peptone Water.

Next, 100 ml of the pooled sample was pipetted and directly dispersed onto the Xylose Lysine Deoxycholate agar (XLD) surface using a routine streaking method. The XLD agar (selective media) was used in this study to target the enteric bacteria for rapid identification. The inoculated agar was incubated at 42°C for 24-48 hours. The colonies were selected randomly based on the shape, size, colour and consistency, and secondary culture was performed on the nutrient agar with similar incubation conditions to obtain individual colonies. Negative samples of which no growth present on the plate was discarded after 48 hours.

7.3 Identification of enteric bacteria

To identify enteric bacteria, various biochemical tests were carried out to obtain biochemical profile for genus identification. The set of biochemical tests used in this study was citrate, urease, triple sugar iron (TSI), sulphide, indole, motility test (SIM) and Methyl Red and Voges-Proskauer (MRVP), respectively.

8.0 Results

Table 8.1, and 8.2 shows the result of the morphological colonies on XLD agar from both chicken and duck eggs.

Table 8.1: Morphological results for primary culture from duck eggs

Markets	Primary culture		
	Swab Samples	**Egg 1**	**Egg 2**
Market A, Stall 1	-	Black and glistening colonies	-
Market B, Stall 1	-	-	-
Market B, Stall 2	-	Black, glistening and whitish colonies	-
Market C, Stall 1	-	Black and glistening colonies	Black and glistening colonies
Market C, Stall 2	Black and glistening colonies. Whitish colonies	-	-
Market C, Stall 3	-	-	-

Table 8.2: Morphological results for primary culture from chicken eggs

Markets	Primary culture		
	Swab Samples	**Egg 1**	**Egg 2**
Market A, Stall 1	-	-	-
Market A, Stall 2	Black and glistening colonies	-	Black and glistening colonies
Market B, Stall 1	-	Black, glistening and whitish colonies	Black and glistening colonies
Market B, Stall 2	Black and glistening colonies	-	-
Market C, Stall 1	-	-	-
Market C, Stall 2	-	-	-

From the morphological colonies results shown from primary culture results, the positive results indicate the growth of bacteria, and the most abundance colonies that were present on XLD agar are black and glistening colonies, and whitish colonies. Secondary culture is preceded in order to obtain pure colonies and biochemical tests were preceded in order to identify the bacteria at genus level. Negative results from

the primary culture were contaminated. For duck eggs samples, Market A, Stall 1 (Swab samples and Egg 2), Market B, Stall 1(Swab samples, Egg 1 and Egg 2), Market B, Stall 2(Swab samples and Egg 2), Market C, Stall 1(Swab samples), Market C, Stall 2(Egg 1 and Egg 2) and Market C, Stall 3 (Swab samples, Egg 1 and Egg 2). For chicken egg samples, Market A, Stall 1(swab samples, Egg 1 and Egg 2), Market A. Stall 2 (Egg 1), Market B, Stall 1(Swab samples). Market B, Stall 2(Egg 1 and Egg 2), Market C, Stall 1(Swab samples, Egg 1 and Egg 2), and Market C, Stall 2(Swab samples, Egg 1 and Egg 2). All of the negative results indicate contamination.

Table 8.5 Biochemical results from duck egg samples

Markets	Citrate	SIM	TSI	MRVP	Urease	Suspected bacteria
Market A, Stall 1, Egg(1), Colony III	+	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: -	-	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market B, Stall 2, Egg(1), Colony I	-	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	-	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market B, Stall 2, Egg(1), Colony II	+	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	+	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market B, Stall 2, Egg(1), Colony III	-	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	-	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market C, Stall 1, Egg(1), Colony II	-	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	+	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market C, Stall 2, Swab Samples, Colony I	-	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: + VP: -	-	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market C, Stall 2, Swab Samples, Colony II	+	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: + VP: -	+	*Salmonella* spp., *E.coli* and *Shigella* spp.

Table 8.6 Biochemical results from chicken egg samples

Markets	Citrate	SIM	TSI	MRVP	Urease	Suspected bacteria
Market A, Stall 1, Egg(1), Colony I	-	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	-	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market A, Stall 1, Egg(1), Colony II	+	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	+	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market A, Stall 1, Egg(2), Colony I	+	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: + VP: -	+	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market A, Stall 1, Egg(2), Colony II	+	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	+	*Salmonella* spp., *E.coli* and *Shigella spp.*
Market A, Stall 1, Egg(2), Colony III	-	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	-	*Salmonella* spp., *E.coli* and *Shigella spp.*
Market A, Stall 2, Swab Samples, Colony I	+	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	-	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market A, Stall 2, Swab Samples, Colony II	+	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	-	*Salmonella* spp., *E.coli* and *Shigella spp.*
Market A, Stall 2, Swab Samples, Colony III	+	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: -	-	*Salmonella* spp., *E.coli* and *Shigella* spp.
Market B, Stall 1, Swab Samples, Colony III	-	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	-	*Salmonella* spp., *E.coli* and *Shigella spp.*
Market B, Stall 1, Egg(2), Colony II	-	S: + I: - M: -	Acid/Acid Hydrogen sulfide: + Gas Production: +	MR: - VP: +	-	*Salmonella* spp., *E.coli* and *Shigella* spp.

9.0 Discussion

Food-borne disease can be caused by different types of enteric bacteria, for example *Salmonella* spp., *E.coli, Shigella* spp,. and *Klebsiella* spp,. Eggs are one of the most common enteric bacteria that can be found to cause food borne disease that has the potential of zoonotic disease to the public and this is a very serious concerning issue related to food safety (Kavita Arumugam, Sunarjati Sudigdoadi, Gaga Irawan et al., (2015). In order to figure out the potential zoonotic risk from chicken and duck eggs, a study was carried out to detect the potential enteric bacteria from the eggs.

XLD agar is a selective media and normally is used to isolate *Enterobacteriaceae* bacteria especially *Salmonella* spp. and *Shigella* spp. Other Gram-negative bacteria such as *Proteus* spp. and *Klebsiella* spp. can also grow on XLD agar (Marshall et al., 2009). In XLD agar there are compositions of yeast extract as a source of nutrients and vitamins specifically for the growth of Gram-negative bacteria. Gram-positive bacteria can never growth on XLD agar due to the deoxycholate content as a selective component in XLD agar, and this act as inhibitory to the gram positive bacteria (Marshall et al., 2009) As mentioned previously, XLD agar can only grow the gram negative bacteria as such *Salmonella* spp., *Shigella* spp., *Klebsiella* spp., *E.coli* and *Proteus* spp. Therefore, the expected results are being narrowed down to these four types of bacteria.

From the result of 60 eggs (30 chicken eggs and 30 duck eggs), it was found that the samples have mixed reactions that are highly suggestive for either *E. coli, Salmonella* spp and *Shigella* spp. All of these three bacteria are common contaminants of the eggs that may originally contact with fecal materials during collection. Most of the market has the same result, this may indicate that there was a high possibility of common

contaminants found on the eggs. However, all of these isolated bacteria could lead to zoonotic potential (Kavita Arumugam, Sunarjati Sudigdoadi, Gaga Irawan et al., 2015)

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Salmonella spp. can be found in chicken and duck's eggs are *S. enteritidis* and *S. typhimurium* (Rajashekara et al., 2000; Thorns, 2000; Foley et al., 2011) Based in study, *S. enteritidis* and *S. enteritidis* the main causes of foodborne illness in human and it has one of the main reported case that causes foodborne disease in human. Based on a CDC report on the year between 2009 to 2015, the FDOSS received reports of 5,760 outbreaks that were the result of 100, 939 illnesses and 5, 699 of hospitalization cases and with a total of 145 deaths. These are the reports for foodborne illnesses. About 896 or 30% outbreaks were from *Salmonella spp*. infection. And the source of infection from chicken was about 12% (CDC, 2013). From the report, *Salmonella spp.* infection is one of the most major concerns in human health, this infection could lead to death. And based in report, around 168, 000 cases of human that were being infected in European Union and approximately around 1.4 million cases were reported in the US (WHO, 2005; EFSA, 2008; 2010; 2011) Common *Salmonella* spp. that affects humans are *S. enteritidis* and *S. typhimurium* could lead to salmonellosis that could shows symptoms of nausea, vomiting, diarrhoea and cramps on the abdominal region. Other than that, it could lead to enteric fever that includes typhoid fever and paratyphoid fever (CDC, 2013). From the result obtained from the study, surprisingly, *Salmonella* spp., was not found in this study. Several important species of *Salmonella* spp. such as *S. enteritidis* and *S. typhimurium* are commonly isolated bacteria in eggs (Popoff et al., 2003). However, we believe that there is a possibility of sampling error during processing (prolong eggs processing) and as a result, dominant contaminant bacteria such as *E.coli and Shigella* spp might surpass the growth *Salmonella* spp

resulting in false negative results. Further investigation should be conducted to isolate *Salmonella* spp using eggs samples

Another isolated bacteria found in chicken and duck eggs is *E. coli,* this type of bacteria is one of the most common bacteria that could lead to zoonotic disease that can affect the public health. The most common species of *E. coli* from egg sources are APEC. *E.coli* are a toxin producing bacteria. Normally, these toxins will cause damage or degeneration of the intestinal mucosal layer that could lead to diarrhea, prolonged diarrhea could lead to dehydration and in severe cases, the infection of this bacteria could lead to death. It will normally colonise in the gastrointestinal tract of humans (Kaper et al., 2004). The clinical signs of this bacteria are abdominal cramp, severe diarrhea, vomiting, and high fever (Kavita Arumugam, Sunarjati Sudigdoadi, Gaga Irawan et al., 2015). The type of *E.coli* can be determined by using the phenotyping method (Caglar *et al.*, 2017), the most common type of *E.coli* that can cause foodborne disease are enteropathogenic *E.coli* or EPEC, and Shiga-toxin producing *E.coli* or STEC (Fang *et al.*, 2017)

Shigella spp. is a gram negative and facultative anaerobic bacteria. And Shigella can grow on XLD agar, for *Shigella spp.* normally the colony morphology would be whitish to yellowish colony on XLD agar. Shigella were being isolated from this study and note that shigella can cause food borne illness in humans that may affect the health of humans. Shigellosis can lead to acute infection that can cause mild diarrhea, vomiting and nausea. Based on research, about 18% of people with shigellosis are admitted to hospital due to severe infection in the USA (Cambridge et al., 2008). The type of *Shigella* spp. can be determined by performing a phenotyping method.

Based on the results from the colony morphology on XLD agar, and also biochemical test, they were not only highly suggestive for one type of bacteria, it could be a mixture of bacteria. This might be for several reasons and conditions. The first possibility that needs to be included is the storage of the sample, and the pure culture was failed to be obtained. The storage of the sample is very important, the temperature of the storage also needs to be taken into consideration. The bacteria need to be isolated as soon as we collect them, this is to avoid any contaminations of the bacteria. Mixed culture of bacteria on XLD agar could be the result of contamination. And the delay of the isolation process from the samples could highly lead to the overgrowth of the bacteria that might result in overgrowth of the bacteria and hence lead to a mix of colonies that will result in the mixture of the biochemical test results. The unbalanced temperature on the chiller could be one of the reasons for mix colonies. For example, *Salmonella* spp. need to be stored in a temperature of 4℃, in order for it to be isolated (Gantois et al., 2008)

The most prominent and dominant colonies on XLD agar and based on biochemical test results, it is highly suggestive that the bacteria that were being isolated are *Salmonella* spp, *E.coli* and *Shigella* spp.

10.0 Conclusion

In conclusion, several enteric bacteria were successfully isolated and identified from chicken and duck eggs from a wet market in Kota Bharu, Kelantan. All of these bacteria are able to cause foodborne illnesses to humans, therefore raising public health concern among consumers in Kota Bharu, Kelantan.

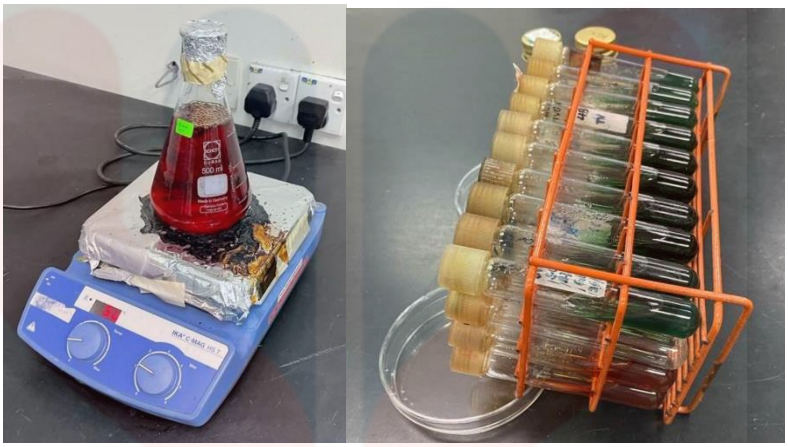
11.0 Recommendations and future work

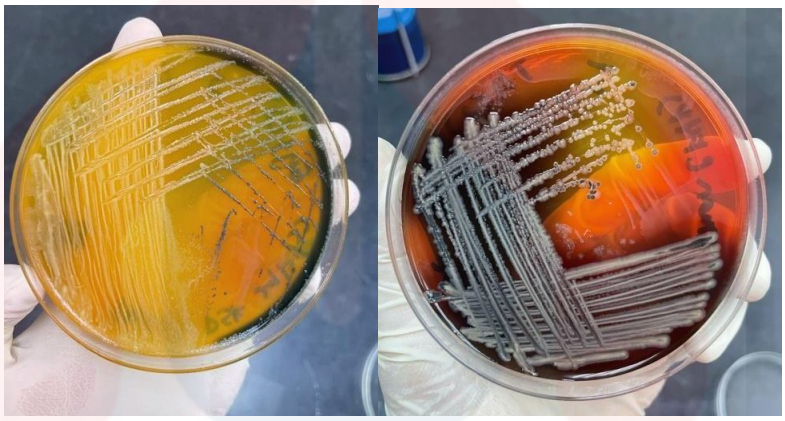
As for the recommendation and future work, it can be improved by increasing the number of samples as well as increasing the target market. So, the target and the samples of the chicken and duck eggs can be broadened. The other improvement that can be done is by isolating fresh samples, after collecting the samples from the targeted market, an isolation process needs to be directly isolated on XLD agar. The other improvement for future work that can be done is by isolating the bacteria on other agar, as we know that XLD agar is mainly for gram negative bacteria to grow as it is a selective type of media. The bacteria can be isolated on different types of agar such as blood agar, and nutrient agar, in order to broaden up the isolated bacteria. The other recommendation for future work, is by using methods of molecular or serology to further identify the bacteria for example serology for *Salmonella* spp. and *E.coli.*

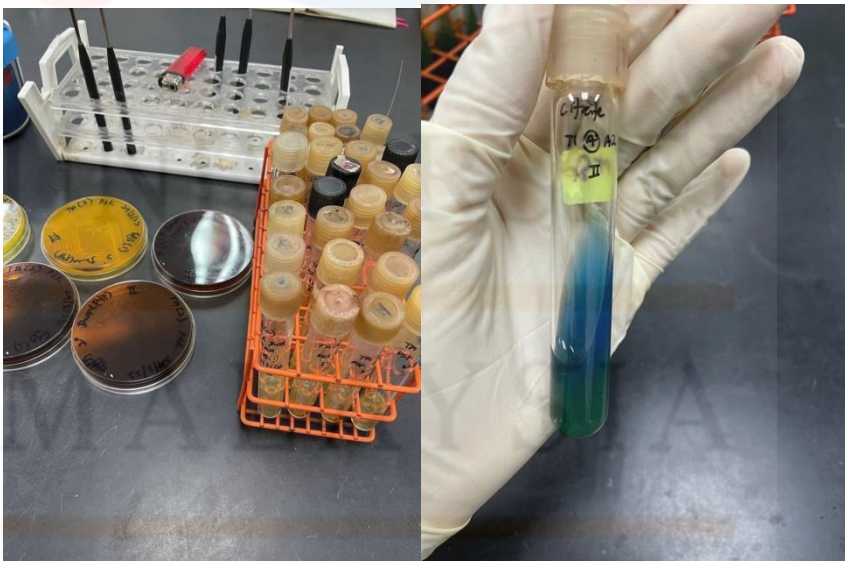
Appendix A

Appendix A: Chicken and duck eggs sampling from selected wet market

Appendix B







Sample collections, agar preparation and colonies observation and biochemical test results

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