Faculty of Resource Science and Technology

Evaluation of Microbial Water Quality of Selected Swimming Pools in Kuching, Sarawak

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DECLARATION

I hereby declare that this thesis entitled "Evaluation of Microbial Water Quality of Swimming Pool in Kuching Area" is the result of my own effort and work. It has not been submitted anywhere for any award. No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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List of Abbreviation

cm	Centimeter
CFU	Colony-formimg unit
EMB	Eosin Methylene Blue
TCBS	Thiosulfate-Citrate-Bile-Salts-Sucrose
g	Gram
h	Hour
H_2S	hydrogen sulfide
L	Liter
m	Meter
mL	Milliliter
MPN	most portable number
psi	Pound per Square Inch
pH	Hydrogen ion logarithm
rpm	Revolutions per minute
sp	Species
SIM	Sulfide, Indole, Motility
UV	Ultraviolet
µL	Microliter
°C	Degree Celsius
%	Percent
CFU	Colony Forming Unit
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid

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Evaluation of microbial water quality of swimming pools in Kuching area

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ABSTRACT

Swimming pool has a high probability of becoming a transmitter of foodborne or waterborne diseases among users. Microbial water quality is assigned as monitor to the safety of consumers using the pool with the lowest chance of infection. UNIMAS Competition Pool, Boulevard Kids Recreational Pool and MBKS Public Swimming Pools were selected as study site for the microbial water quality whereas *Escherichia coli* and *Vibrio cholera* are selected as contamination indicator of swimming pool water. Spread plate technique was used to enumerate the microbial distribution. Biochemical tests were also conducted to confirm the presence of the indicator bacteria. PCR was conducted for confirmation test of *V. cholerae*. *E. coli* was found to be positive in 3.57% of water samples from UNIMAS with 1.888×10^4 CFU/mL, 3.33% of water samples from Boulevard with 1.718×10^8 CFU/ mL and 2.78% from MBKS with 7.900×10^8 CFU/mL. However, the result does not affect the safety of all the swimming pools. There was no *V. cholerae* found in all water samples obtained in Kuching. Physiological parameters did not showed significant relationship with the microbial indicators distribution in water samples.

Key words: Swimming pool water quality, spread plate techniques, biochemical test, PCR, physiological parameters

ABSTRAK

Kolam renang merupakan satu alat perebak penyakit antara satu pengguna sama lain. Kualiti air mikrobial telah dianggap satu keadaan keselamatan untuk pengguna kolam renam dengan peluang jangkitan yang paling rendah. Kolam Renang UNIMAS, Kolam Rekreasi Kanak-kanak Boulevard, dan Kolam Renang Awam MBKS telah dipilih sebagai lokasi penyelidikan untuk kualiti air mikrobial manakala Escherichia coli dan Vibrio cholerae dipilih sebagai penunjuk pencemaran mikrobial dalam kolam renam. Teknik plat sebaran telah diguna untuk menghitung pengedaran mikrobial. Ujian biokimia juga dijalankan untuk memastikan kewujudan penunjuk bakteria. PCR telah dijalankan untuk ujian pemastian V. cholerae. E. coli telah didapati positif dalam 3.57% sampel air dari UNIMAS dengan 1.888×10^4 *CFU/mL, 3.33% dari Boulevard dengan* 1.718×10^8 *CFU/ mL dan 2.78% dari MBKS dengan* 7.900×10^8 *CFU/mL. Walaubagaimanapun, keputusan tersebut tidak mempengaruhi tahap keselamatan ketiga-tiga kolam renang. V. cholerae tidak dijumpa dalam semua sampel air yang diambil dari semua kolam renang di kawasan Kuching. Parameter fisiologi menunjukkan tiada kaitan dengan pengedaran penunjuk mikrobial dalam sampel air.*

Kata kunci: Kualiti air kolam renang, teknik plat sebaran, ujian biokimia, PCR, parameter fisiologi

Section 1: Introduction

Swimming pools is usually opened for public usage where it can accommodate more than hundreds people used a pool for water based-recreational activities. Therefore, water in the pool is a good transmission tool for infectious diseases among human population throughout the world (Atallah *et al*, 2008). Contamination of water can lead to spreading of diseases from those infected to thousands of healthy individual. Disinfectant such as chlorine powder or chlorine gas is added into the pool to keep the water sanitary in standard condition. However, there are standards to monitor the halogen-based disinfectant in the water regularly to avoid the development of evasive ability of germs against disinfectant (WHO, 2006). Cost saving procedure is the most important fact of water quality maintenance of the public pools in developing countries (Issam and Samir, 2004). Although monitoring is done in schedule, there is still leakage of the monitor procedure which could easily ignore the possibility of improper sanitization of the water and lead to contamination such as Cryptosporidium and Giardia contamination of swimming pools as reported by Schets *et al* (2004) in Netherlands. Another case reported in Spain that adenovirus type 4 outbreak which was detected to be related to swimming pool (Artieda *et al*, 2009). The situation consequences increased the transmission rate and waterborne diseases outbreak in swimming pool.

Therefore, it is necessary to have monitors the level of water quality in swimming pool to determine microbiological quality as it is the most important parameter for the safety of swimming pool water. It is also reported some parameter have the probability to affect the microbial population in water sample, for example pH and temperature (Erdinger *et al*, 1997). Water quality monitoring criteria included total coliform count, presence or absence detection and membrane filtration (CDC, 2010). Spread plate technique is a

conventional bacterial enumeration technique which taking advantage of rapid and cost saving than other techniques (Munsch-Alatossava *et al*, 2007). Therefore, spread plate qualtification test was used as water quality monitoring procedure in this study.

The main objective of this study:

1. To enumerate the microbial indicator of water quality of 3 selected swimming pools in Kuching area.
2. To detect the presence of *Vibrio cholera* and *Escherichia coli* as fecal indicators in water samples from 3 swimming pools in Kuching area.
3. To determine the physiochemical condition of selected swimming pools and associates that with *V. cholerae* and *E. coli* distribution.

Section 2: Literature Review

2.1. Pathogens in Swimming Pool

According to Kathy Pond (2005a), there are several pathogens had included as indicator of water safety for monitoring the water quality. *Campylobacter jejuni*, *Escherichia coli O157:H7, Helicobacter pulori, Legionella sp., Mycobacterium avium complex, Shigella sp.,* and *Vibrio sp.* are common fresh water bacterial pathogens that can be found in most water recreation and some are lethal to human (Barrell *et al*, 2000). The pathogenic protozoa such as *Cryptosporidium, Giardia, Microsporidia,* and *Naegeria fowleri* can be commonly found in swimming pool (Kathy, 2005a). Some of these protozoa are resistance to chlorine disinfection such as *Microsporidia* and *Cryptosporidium*. *Schistosoma sp.* is the pathogenic nematode which can survive under chlorine disinfectant in the swimming pool (Franca *et al*, 2002). Viruses such as adenovirus, coxsackievirus, echovirus, Hepatitis A virus and Hepatitis B virus should also be considered as one of the disease transmission factor in the public swimming pool. These organisms are highly contagious in little dose through their point of entry on human body (Kathy, 2005b). Kathy (2005b) also stated that consumer can be infected with waterborne diseases in contact of pathogen-containing water pools, however, the infection is depending on the dose and physical condition of the consumer at the time of exposure.

2.1.1. Factors Associated with Bacterial Contamination in Swimming Pool

Microorganisms which are continuously introduced into a well-maintained swimming pool should be kept lower than threshold level which compliance with standard regulation (Atallah, 2008). The nitrogen-based nutrient such as urea and phosphate-based nutrient is excreted from the human body and the water of the swimming pool washed them away from skin due to the movement of the body in the water.

Apart from that, fecal contamination from enteric system can lead to distribution of carbon sources into the pool water. Some heavy metals or dust from atmosphere can also contaminate the water of the public outdoor swimming pools (Erdinger *et al*, 1997). In addition, the heat energy from sunlight warms up the water surface, causing the rise of water temperature which provide the optimum temperature for the microbes to grow actively. Phytoplankton in the water is able also absorb sunlight and provides carbon sources to the bacteria in the water.

2.1.2. Transmission of pathogenic microorganism

Pathogenic microorganisms are transmitted to a human host through route of entry which may invade the human host to continue their survival (Gerald *et al*, 2007). Transmission of pathogens from swimming pool water to human host is commonly through respiratory tract, skin and gastrointestinal tract. Air-transmitted microorganism can transmit to host through the aerosol which released from swimmers to the inhaler. Gastrointestinal tract is also a favourite route for microorganism such as *E.coli* as fecal coliform to infect a host in

swimming pool water (Issam and Samir, 2003). Fecal coliform are able to survive in the human bacteriostatic barrier in gastrointestinal tract such as acidic HCl and bile salt (Gerald *et al*, 2007).

2.2. Evidence of Microbiological Contaminations Related to Swimming Pool

According to Crone (1974), from a total of 227 total of water samples collected from 20 swimming pools in London, *Staphylococcus aureus* was present as 67% in water samples with concentration of which less than 100 CFU/ 100 mL (43 %) and more than 100 CFU/ 100 mL (53 %). The prevalence of *S. aureus* indicated the ability to survive and grow in swimming pool and cause *staphylococcus* related outbreak.

From the annual report concluded by Michael from CDC in 1985, two issues which related to swimming pool had been highlighted in the report. Giardiasis outbreak was cause by the lack of pool water filtering system to filter out giardia cysts from the water (Michael, 1988). Apart from that, *Pseudomonas folicultis* outbreak which first reported in 1975 was majorly related to swimming pool due to inadequate pH level with free residue chlorine level.

Issam and Samir (2003) had investigated the swimming pool water samples which collected from at West Bank of Palestine and found that most of swimming pool water samples were not complied with Palestinian and WHO standards. There were about 91.3% of swimming pools which were contaminated with *Salmonella sp*. and about 40% of the samples from Total Bacterial Count test did not compliance to the standard. These results

had concluded that the developing countries have poor swimming pool water quality maintaining system.

In 2004, Vermont Department of Health (VDH) announced the outbreak of Norovirus gastroenteritis due to swimming club in Vermont. The malfunctioned chlorine feeding system had leaded to the outbreak due to decreasing of free residue chlorine level which to have decreased disinfection effect against the pathogen in the swimming pool (*Vermont Department of Health*, 2004).

In the same year, *Cryptosporidium* and *Giardia* present was reported to be found on 7 swimming pool filters in Netherlands (Schets *et al*, 2004). In the report, 11.8% of swimming pool water found to be positive which 4.6 % was *Cryptosporidium*, 5.9 % was *Giardia* and 1.3 % of both *Cryptosporidium* and *Giardia*. *Cryptosporidium* oocysts and *Giardia* cysts were detected from one toddler pool and a learner pool water samples collected. The risk assessment of infection was indicated as one infection per 10,000 persons per year (Schets *et al*, 2004).

In 2006, the report of *E. coli* strain O157 outbreak had been summarized. The Outbreak of *E. coli* O157 was reported related with swimming pool (Verma *et al*., 2006). The infected persons are frequently been found at the group of age less than 5 years old. There are 106 cases reported regarding to the outbreak in England and Wales in between 1995 and 2000. However, as the technology becomes advanced, the maintained free residue chlorine at the adequate level had showed the decreasing of susceptible cases of hemorrhagic *E. coli* strain outbreak in United Kingdom.

There was microbiological contamination investigated at Tirana and Durres region of Albania in 2008. According to the investigation done by Mirela *et al* (2008), 6 pools were selected and monitored twice a month from May to September in 2008. A total of 260

water samples were collected from the pools and analyzed. From the result obtained, most samples had standard Total Coliform Count. However, the fecal indicators exceeded the referenced value. Apart from laboratory investigation, 826 patients who suffered the waterborne illness symptoms were interviewed. From the total, 72 cases were positive with both *Chlamydia trachomatis* and *Candida albicans* infection, 72 cases were positive of only *C. trachomatis* infection and 448 cases were positive of only *C. albicans* infection. The rest of 234 cases were negative from the infection. Not only that, from 122 staphylococcus infection cases, 39% were infected with *Staphylococcus aureus* and 5% were *Staphylococcus epidermis* positive. The rest of the cases were negative from both species infection.

Lastly, pharyngoconjunctival fever outbreak was reported in July 2008 at Spain. From the report published by Artieda (2009), 59 children were affected by the outbreak resulted from in contacted with swimming pool water. Approximately 5 out of 6 pharyngeal swab samples Adenovirus type 4 were found to be positive which obtained from the affected children. From the interview, 73% of primary cases were confirmed contacted with municipal swimming pool. 25% of secondary cases were confirmed contacted with primary cases.

From the case study above, it became a necessary reason to conduct the investigation of microbial water quality of swimming pool in Kuching. The sanitary condition of public swimming pool water should be studied to ensure the safety of pool user and also to reduce the risk of waterborne-illness which related to swimming pool.

2.3. Guidelines of Water Quality Monitoring for Swimming Pools

A well-organized pool had monitoring procedure guideline to maintain the water quality of the pool. The following is the guideline from World Health Organization (2006) used to standardize the local swimming pool water quality.

For a well-maintained pool, the turn over period should be between three to four hours for competitive pools which is 50 m long as shown in Table 1 in Section Appendix and the disinfectant dosing and filter operation should be 24 hours continuously operated (WHO, 2004). For the microbiological criteria, there had been separated into few type of microorganisms: heterotropic colony count, thermotolerant (fecal) coliform or *Escherichia coli*, *Pseudomonas auruginosa, staphylococcus aureus,* and *giardia* and *cryptosporidium.*

Heterotropic colony count (HPC) is the enumeration test which is culture-based intended to grow microorganism found in water samples (WHO, 2003). The test should be conducted at 35°C-37°C, and the colony counted should be less than 200CFU/mL of water samples (WHO, 2006). As long as no coliform or *E.coli* present in the water samples collected and condition of pool maintenance is within satisfactory level, the incidentally higher count is still acceptable (Queensland, 2004). However, the sudden rise of the count should raise a concern immediately. If the high count is still persisting, the result can be concluded that the pool maintenance operating system is unsatisfied and immediate investigation of the system is required (Queensland, 2004).

Another criterion is the thermotolerant coliform or *E.coli*. In laboratory method, coliforms are defined as lactose fermenter and gas production group of microorganism including *Escherichia coli* (CDC, 2010). The concentration of thermotolerant coliform or *E.*coli should be less than 1/100 mL within operational level (WHO, 2006). It is acceptable

of the count of coliform is less than 10 per 100 mL of water sample. However, if it persist to be more than 10 counts per 100mL of water samples, investigation is necessary be performed (Barrell *et al*, 2000). The presence of the coliform or *E.coli* indicates that possibility of fecal contamination (Queensland, 2004). It can also indicated that the failure of treatment process to maintain sanitary of the pool at the time of sampling. Any count in 100 mL required concerns and repetition of test shall be conducted (Queensland, 2004).

Apart from that, *Pseudomonas auruginosa* is also an indicator of microbiological criteria. *P. auruginosa* is a waterborne pathogen which causes gastrointestinal infection to the consumer (WHO, 2003). It shall not be present in a well-maintained pool. The presence of *P. auruginosa* indicates that the possible of other environmental pathogens such as *legionella sp.* present in the water (Queensland, 2004). Therefore, if *P.auruginosa* count more than 100/100mL is detected in water samples, it is recommended to repeat the test after turbidity, disinfectant residual and pH is re-measured and one turnover is passed (WHO, 2006).

Staphylococcus aureus is another criterion of microbiological monitoring. However, it is seldom to have a routine test for *S.aureus* which is not always necessary to be tested (Queensland, 2004). According to CDU of Queensland government (2004), when there is any link between disease outbreak and pool suspected, the test for presence of *S.aureus* should be included as a part of water investigation as monitoring water quality. Well-maintained pools and proper sample collection normally does not result in detectable of *S.aureus* in 100mL of water samples (Queensland, 2004). However, if *S.aureus* is detected, the count should be less than 100 mL (WHO, 2006).

Giardia and *Cryptosporidium* are pathogenic microorganism which can be transmitted through contaminated water such as swimming pools. It is necessary not to

detect on the presence of *Giardia* and *Crytosporidium* in water samples (Queensland, 2004). CDU of Queensland (2004) stated that if the adult or cyst of either *Giardia* or *Cryptosporidium* or both detected in the water samples, however, it can be indicated that there are particular problem present in the operating and maintaining system of the pools.

Lastly, there is also possibility of unsatisfactory microbiological results as a criterion of microbiological monitoring. Any unsatisfactory results obtained from the laboratory investigation, resampling for microbiological testing should be conducted to increase the effectiveness of the corrective actions (Queensland, 2004). However, if the problem is persisted, failure of pool maintaining and operating system may be indicated.

2.4. Spread Plate Technique

Spread plate technique is developed since the technique used by Robert Koch to fix the microbiological organism on the microscopic slides in 1877 which utilized the gelatin and microscopic slide in order to visualize the disease causing microorganism (Brock, 1988). Afterwards, spread plate technique is then widely used recently for the bacterial quantification steps. Spread plate technique is a technique which spreads the bacterial suspension evenly over the agar plate surface (Wise, 2006). By using a small amount of bacterial suspension, the bacterial is spread by sterile bent glass rod so that the growth of colony can be enumerated subsequently during incubation. This technique is mostly used for the aerobic culture of microorganism (Jaisai, 2010). The single colony formed on the agar represents one colony forming unit which might cause by the single cell

multiplication or growth. The acceptable colony count is within the range of 30 and 300 colonies. The colony count less than 30 CFU is considered as too few to count because it will be statistically unreliable. The colony count more than 300 CFU is considered as too numerous to count because it may cause counting errors. The advantage of spread plate techniques is that spread plate method can have a low colony count (CFU/mL) which disinfectant treated swimming pool water may have extremely low amount of bacteria compared to MPN method which gives higher colony count (Hunsinger *et al*, 2005.).

2.5. Polymerase Chain Reaction

Presumptive test alone is insignificant to prove that there is any specific species of microorganism in the swimming pool which should be reported back to the pool manager. Because of most the biochemical metabolism of microorganism shared among species, it makes us difficult to make a significant result as detection of some pathogenic microorganism which could harm the pool user. Molecular identification is necessary for confirming the existence of the specific species. Polymerized Chain Reaction (PCR) is one of the methods to molecularly detect the specific gene which only present in the specific species, which can used to differentiate the species from whole cloud of the microbial population (Sharbatkhori *et al*, 2009). The process amplifies the specific gene using primer to tag the location of gene where it can be amplified into thousands of copies so that we can easily detect the presence of the gene using gel electrophoresis. OmpW is the gene which presence in *Vibrio cholerae*. OmpW encodes the outer membrane protein of the *V.cholerae* which makes it specific nutrient utilization and metabolism processes in environmental change adaptation (Nandi *et al*, 2005).

Section 3: Materials and Methods

3.1. Sample Preparation

3.1.1. Sample Collection

Sample collection was performed with reference of WHO sampling techniques (WHO, 1997). Falcon tube and 1% sodium thiosulfate were pre-sterilized through autoclaving before use. Then swimming pool water was collected at 20 cm from water surface. Then 450 µl of 1% sodium thiosulfate solution was added to the water samples to deactivate the residue chlorine in the water samples to prevent further disinfectant effects on microbiological population. Then the water samples collected were preserved in ice. The water samples were processed within 2 hours after sample collected. The temperature and pH of the water samples were measured and recorded.

3.1.2 Enumeration by Spread Plate Method

About 10 ml of water samples were transferred to conical flasks containing 90 ml of lauryl tryptose broth, LTB (ratio 1:10) and incubated in shaking incubator at 37°C for 16-24 hours. The steps were repeated for the enrichment for *Vibrio cholera* in water sample by using alkaline peptone water, (pH pre-adjusted to 8.6). After incubation, the enriched samples were proceeded for serial dilution of 10^{-1}, 10^{-2} and until 10^{-7}. Figure 3.1 showed the summary of the serial dilution on enumerating bacterial concentration in water samples:

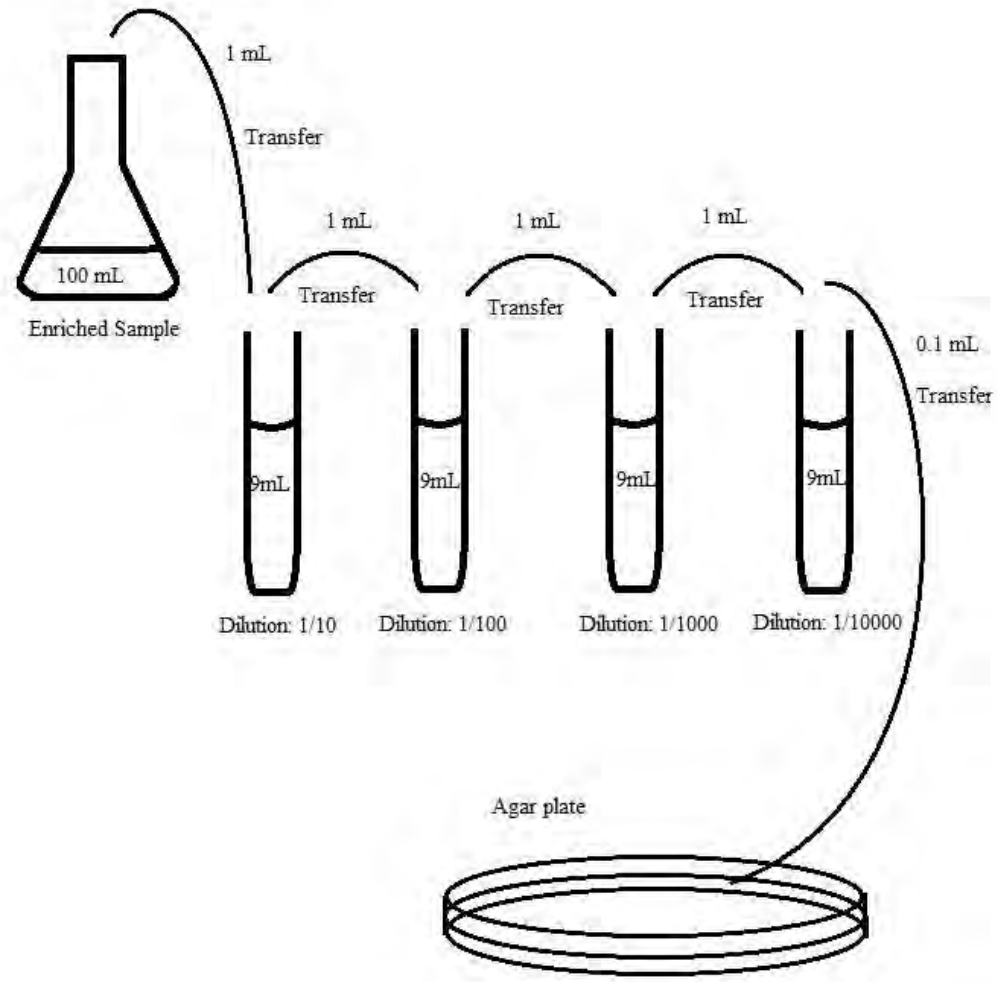

Figure 3.1: Summary of serial dilution

After dilution, the diluted samples were transferred to agar plate for microbial indicator detection. Aseptically, 100 µl of enriched sample from LTB flask is pipetted to eosin methylene blue agar (EMB agar). Then, the sample was spread through the surface of the agar by using a sterile glass hockey stick. The agar containing the sample was incubated at 37°C for 24 hours for reading of CFU. The spread plate technique was applied for different dilution of samples and each dilution was made with a replicates. The spread plate technique was repeated for enriched samples incubated in APW containing flask. The

thiosulfate-citrate-bile-salts-sucrose, TCBS agar used for APW enriched samples and each dilution was also proceeded for a two replicate. The agar plates were incubated at 29°C for 24 hours. Colonies formed on the surface of the agar plates were observed, counted and recorded to calculate the colony forming unit, CFU in the samples. However, only desired colony and number of colony formed on the agar plates were counted and recorded as a measurement. The following is the CFU per mL calculation formula:

$$\mathrm{CFU/mL} = \text{No. of colonies} \times \frac{1}{\text{dilution factor}} \times \frac{1}{\text{volume used in mL}}$$

3.2. Biochemical detection

3.2.1 Isolate Purification

Once the desired colony on agar plates such as metallic green colonies on EMB agar and yellow colonies on TCBS agar were counted and recorded from the agar plates, positive colony on the agar plates was isolated using streak plate technique for obtaining the pure culture from the massive mixture of the bacterial population on the agar plate. By using a sterile inoculating loop, a metallic green colony grew on the EMB agar was streaked into a new sterile EMB agar through streak plate method. Then the agar plate was incubated at 37°C for 24 hours for the further test. For colony grew on the TCBS agar, the yellow colony formed on the TCBS agar was inoculated using sterile inoculating loop to a new sterile TCBS agar plate through streak plate technique aseptically. Then the agar plate was

incubated at 29°C for 24 hours. After the pure culture on the plates was isolated, the positive colony formed on EMB agar and TCBS agar was further isolated in Nutrient agar, NA for further test.

3.2.2 Sulfide, Indole and Motility Test

SIM test is used complete the detection of the presence of *Escherichia coli* found on the EMB agar. Using a sterile inoculating stab, a metallic green colony isolated on EMB agar was swabbed and then stabbed into a tube containing 10 ml of SIM medium. Then the tube was incubated in incubator at 37°C for 24 hours. After incubation, the medium was added a few drops of Kovach's reagent for indole test (MacWilliams, 2009). Indole positive appears to change the colourless reagent to pink. Motility positive shows increased in turbidity of the medium whereas hydrogen sulfide production positive turns the medium black. A positive result for *E.coli* in SIM medium supposed to be indole and motility positive but sulfide negative.

3.2.3 Voges-Proskauer Test

Voges-Proskauer, VP test was used to complete the detection of *E.coli* and *V.cholerae* from the isolates of different agar plates. Before the test ran, the testing culture was first incubated in MR-VP broth. One colony from NA culture isolated from previous agar plate