UNIVERSITI PUTRA MALAYSIA

MICROBIOLOGICAL QUALITY OF *Cerithidea obtusa* AND THE ANTIBIOTIC SENSITIVITY OF SELECTED BACTERIA

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "**Microbiological Quality of *Cerithidea obtusa* and the Antibiotic Sensitivity of Selected Bacteria**", by Aina Liyana binti Hazri and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement of the course VPD4999 – Final Year Project.

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DEDICATION

Specially dedicated to my parents, family, friends and all siput sedut lovers….

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ABSTRAK

Abstrak daripada kertas projek yang dikemukan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

KUALITI MIKROBIOLOGI *Cerithidea obtusa*

DAN KERENTANAN BAKTERIA TERHADAP ANTIBIOTIK

Oleh

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Cerithidea obtusa merupakan salah satu hidangan makanan rakyat Malaysia dan dikenali sebagai siput sedut. *Cerithidea obtusa* adalah pemakan penuras, oleh itu, ia berkemungkinan mempunyai kualiti mikrobiologi yang rendah berdasarkan persekitaran yang didiami. Kualiti mikrobiologi *Cerithidea obtusa* yang terdapat di pasar borong masih belum dinilai. Justeru, kajian ini dijalankan untuk menilai kualiti mikrobiologi didalam *Cerithidea obtusa*, mengenalpasti patogen seperti *E.coli*, *Salmonella* dan *Vibrio* spp. dan kerentanan bakteria tersebut terhadap antibiotik.

Empat belas sampel terkumpul (*pooled*) siput sedut (*Cerithidea obtusa*) yang diperoleh dari pasar basah di kawasan Selangor dan Kuala Lumpur telah diperiksa untuk kualiti mikrobiologi, kehadiran *E.coli*, *Salmonella* dan *Vibrio* spp. dan untuk menentukan

kerentanan bakteria tersebut terhadap antibiotik. Sumber *Cerithidea obtusa* yang diperoleh untuk kajian ini adalah dari Malaysia (14.3%) dan Indonesia (85.7%).

Lebih 28% daripada jumlah sampel diuji untuk kiraan plat piawai (Standard Plate Count) mempunyai min jumlah bakteria melebihi lingkungan yang dibenarkan (>5,000,000 CFU/g) yang telah ditetapkan oleh *Microbiological Reference Criteria for Food*, October 1995 (Food Administration Manual, 1995) manakala, kesemua sampel diuji untuk koliform plat piawai (Coliform Plate Count) menpunyai min bilangan dalam lingkungan yang tidak dibenarkan (> 1,000 CFU/g). Analisis stastistik menunjukkan tiada perbezaan ($p>0.05$) dalam kualiti mikrobiologi *Cerithidea obtusa* dari Indonesia dan Malaysia. Oleh itu, kualiti mikrobiologi *Cerithidea obtusa* tidak dipengaruhi oleh sumber yang diperoleh.

Dalam kajian ini, 21.4% daripada sampel adalah positif untuk *E.coli*, 14.2% adalah positif untuk *Salmonella* spp. dan 7.21% adalah positif untuk *Vibrio* spp.

Kesemua isolat *E.coli* dan *Salmonella* spp. rentan terhadap oxytetracycline. 66.7% isolat *E.coli* rentan terhadap trimethoprim/sulfamethoxazole, florfenicol, azithromycin, ciprofloxacin dan ceftriaxone. 50% isolat *Salmonella* spp. rentan terhadap trimethoprim/sulfamethoxazole, florfenicol, amoxycillin, ciprofloxacin dan ceftriaxone. Manakala, isolat *Vibrio parahaemolyticus* rentan terhadap erythromycin sahaja.

Kesimpulannya, kajian ini menunjukkan bahawa *Cerithidea obtusa* mempunyai kualiti mikrobiologi yang sangat rendah dan ia mengandungi patogen yang mempunyai daya kerentanan terhadap pelbagai antibiotic. Oleh itu, kajian berterusan perlu dilakukan untuk menyiasat pencemaran terhadap kualiti air dan cara pengendalian *Cerithidea obtusa.*

Kata kunci : *Cerithidea obtusa*, *E.coli, Salmonella, Vibrio*, kerentanan, antibiotik

ABSTRACT

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

MICROBIOLOGICAL QUALITY OF *Cerithidea obtusa*
AND THE ANTIBIOTIC SENSITIVITY OF SELECTED BACTERIA

by

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2016

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Cerithidea obtusa is a local delicacy also known as *siput sedut*. *Cerithidea obtusa* is a filter-feeder therefore may have poor microbiological quality depending on the hygiene and cleanliness of the water environment where they are derived. The microbiological quality of the *Cerithidea obtusa* available in the local market have not been evaluated. This study was performed to assess the microbiological quality of *Cerithidea obtusa*, to detect the presence of selected pathogen such as *E.coli*, *Salmonella* and *Vibrio* spp. in *Cerithidea obtusa* and their antibiotic susceptibility pattern.

Fourteen pooled sample of *Cerithidea obtusa* was purchased from 10 wet markets around the areas of Kuala Lumpur and Selangor and were examined for the microbiological quality, occurrence of *E.coli*, *Salmonella* and *Vibrio* spp. and to determine the antibiotic susceptibility of the isolates. The information on the shellfish origin and source was supplied

by the vendor and it was revealed that 14.3% of the shellfish was from Malaysia and 85.7% was from Indonesia.

The microbiological analysis revealed that 28.6% of the total sample tested for Standard Plate Count (SPC) of *Cerithidea obtusa* had mean count that were beyond the limit (>5,000,000 CFU/g) set by *Microbiological Reference Criteria for Food*, October 1995 (Food Administration Manual, 1995) for shellfish, while all of the samples tested for Coliform Plate Count (CPC) had mean count of more than 1,000 CFU/g. There was no significant difference in the microbiological quality of *Cerithidea obtusa* from Malaysia and Indonesia. This indicate that the origin of *Cerithidea obtusa* may not be associated with the microbiology quality of the produce.

E.coli was detected in 21.4% of samples, while 14.2% and 7.21% were positive for *Salmonella* spp. and *Vibrio* spp, respectively. All *E.coli* and *Salmonella* spp. isolates were resistant to oxytetracycline. More than half (66.7%) of *E. coli* isolates were resistant to trimethoprim/sulfamethoxazole, florfenicol, azithromycin, ciprofloxacin and ceftriaxone. Half (50%) of the *Salmonella* spp. isolates were resistant to trimethoprim/sulfamethoxazole, florfenicol, amoxycillin, ciprofloxacin and ceftriaxone. The isolated *Vibrio* spp. was resistant to erythromycin only.

In conclusion, the study suggests that the local *Siput sedut* have poor microbiological quality and harbour pathogens that exhibited multidrug resistant trait. More studies need to be performed to investigate the source of the contamination such as water quality and *Cerithidea obtusa* handling .

Keywords: *Cerithidea obtusa, E.coli, Salmonella, Vibrio*, antibiotic sensitivity

1.0 INTRODUCTION

Cerithidea obtusa is a marine gastropods from the class of Gastropoda orthogastropoda, order of Sorbeoconcha, super family of Cerithidea and from family of Potamididae and locally know as "siput sedut" in Malaysia (Sealifebase, 2012). It can be found in abundance in mangroves and estuarine mudflats in tropical and subtropical areas (Sealifebase.org, 2012). It is considered as one of the delicacies in Malaysia.

Sewage is generally believed to be the main source of river pollution in Malaysia (Azni & Abdullah, 2006). Therefore, foodborne bacteria such as *E.coli* (Feldhusen, 2000) and *Salmonella* can be present due to faecal contamination (Lyhs, 2009). In addition, consuming aquatic animals from estuarine exposes consumer to bacteria such as *Vibrio* spp. which are commonly found and considered as human pathogens (Feldhusen, 2000).

The judicious use of antibiotic in aquatic farming is a part of good veterinary practice (FDA, 2014). Exposure of antibiotic residue from aquaculture exposes estuarine aquatic animals and may cause antibiotic resistance and it should be considered a public health concern.

Cerithidea obtusa is a popular local delicacy in Malaysia, however little information exists on the microbiological quality and pathogens that could be present in this shellfish. *Cerithidea obtusa* is a filter-feeder therefore may have poor microbiological quality depending on the hygiene and cleanliness of the water environment where they are derived. Thus, this study was conducted to determine the "siput sedut" level of hygiene and potential public health significance that could arise from consuming the seafood.

The objectives of present work are:

1. To assess the microbiological quality of *Cerithidea obtusa*.
2. To detect the presence of selected pathogen such as *E.coli*, *Salmonella* spp and *Vibrio* spp.
3. To determine the antibiotic susceptibility pattern of *E.coli*, *Salmonella* spp and *Vibrio* spp.

The hypotheses of present work are:

1. The microbiological quality of gastropods is poor.
2. *E.coli*, *Salmonella* and *Vibrio* spp are able to be isolated.
3. Selected pathogens are resistant to various antimicrobials.

2.0 LITERATURE REVIEW

2.1 MICROBIOLOGY OF *E.coli, Salmonella* spp. AND *Vibrio* spp.

2.1.1 *E.coli*

E. coli are Gram-negative, rod-shaped bacteria (Hale, 2013), motile with petrichous flagella and non-motile (Farmer *et al*., 2007). They are oxidase negative, ferment glucose and indole positive (produces indole from trytophan) (Sahm *et al*., 2002). They are normally acetate utilizer as the only source of carbon, citrate negative and appear metallic green sheen on EMB agar (Acharya, 2013). *E.coli* are found in large numbers in the intestines of humans and warm-blooded animals and may contaminate aquatic environment from sewage or untreated water. Thus, filter feeder such as *Cerithidea obtusa* concentrates these bacteria in the gut. *E.coli* are also used as indicator of fecal contamination (Feldhusen, 2000). There are 6 pathotypes of *E.coli* associated with diarrhea, Shiga toxin-producing *E. coli* (STEC), Enterotoxigenic *E.coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Diffusely adherent *E. coli* (DAEC) and STEC is associated with foodborne outbreak (CDC, 2015)

2.1.2 *Salmonella* spp

Salmonella are Gram-negative rod shaped (Olgunoğlu, 2012). They produce typical colonies of glassy black centers and in circumstances colonies may appear completely black (Wallace *et al*., 2014). They are urease-negative because they produces enzyme urease, which hydrolyzes urea, have the ability to utilizes glucose and lactose/sucrose fermentatively, forms H_2S and CO_2 thus showing presence of bubbles or cracks in the agar with blackening of butt, (Sahm *et al*., 2002) do not deamines lysine causing the agar slant to remain purple and it

decarboxylase thus causing the fermentation of the butt to revert back to purple (Sahm *et al*., 2002). They agglutinate with Polyvalent O Serum (Andrews *et al.,* 2014) They can be isolated from seafoods harvested from contaminated inland waters (Nickelson *et al*., 2001). These are the types *Salmonella* spp that are known to cause disease in humans, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Virchow, *Salmonella* Hadar, *Salmonella* Heidelberg, *Salmonella* Newport, *Salmonella* Infantis, *Salmonella* Agona, *Salmonella* Paratyphi A, *Salmonella* Paratyphi B, *Salmonella* Paratyphi C, *Salmonella* Typhi (CDC, 2015).

2.1.3 *Vibrio* spp

Vibrio are Gram-negative, curved or straight rod and non-spore forming (Price & Tom, 2000). *Vibrio* spp. are very common in estuarine and coastal environments (Feldhusen, 2000). It grows on Thiosulfate-Citrate Bile TCBS agar with appearance of green and yellow colonies, whereby green is presumptively known as *Vibrio parahaemolyticus* or *Vibrio vulnificus*, yellow colonies are presumptively known as *Vibrio cholerae* or *Vibrio alginolyticus* (Sahm *et al*., 2002). They are all oxidase positive except for *Vibrio metschnikovii* (Price & Tom, 2000). *Vibrio* spp. ferment glucose and produce glucose oxidatively, therefore it shows positive results in oxidative-fermentation test (Price & Tom, 2000). API 20E is considered the best identification kit to detect for speciation.

2.2 BIOLOGY OF *Cerithidea obtusa*

Cerithidea obtusa are from the class of Gastropoda, order of Sorbeoconcha, super family of Cerithidea, family of Potamididae and known as Siput sedut locally (Sealifebase, 2012). It is distributed along the mangroves and estuarine mudflats in tropical and subtropical areas of Indo-West Pacific (Sealifebase, 2012). It is distributed throughout 11 countries in

Indo-West Pacific and one of the countries is Malaysia (Sealifebase, 2012). It has a shell medium in sized of maximum shell length of 6 cm and high conical spire and broad rounded whorl base (Sealifebase, 2012). They feed on algae and debris using their tiny teeth called radula (Nordsieck, 1998)

2.3 SIGNIFICANCE OF PUBLIC HEALTH

2.3.1 *E.coli*

E.coli is a bacteria that normally live in the intestines of people and animals. It can be used as indicator of faecal contamination (Feldhusen, 2000). Pauziah, 2006 reported that sewage treatment plants recorded the highest number of *E. coli* in 2004, and is the major source for water pollution in Malaysia. The bacteria may cause symptoms such as watery or bloody diarrhea, abdominal cramps, with or without fever. Pathogenic type of *E.coli* that causes diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons (CDC, 2015)

2.3.2 *Salmonella* spp

Fish, crustaceans and molluscs were implicated as vehicles of many cases of foodborne outbreaks (Bujjamma *et al*., 2015). The source of aquatic environment polluted by *Salmonella* could be due to poor water quality, fecal contamination from wild animals or livestock (Hazzah *et al.*, 2011). With incubation period between 12-72 hours, symptoms will develop such as diarrhea, fever, and abdominal cramps (CDC, 2015). *Salmonella* spp causes the highest amount of estimated annual death of 378 people from 2000-2008 in United States (Scallan, *et al*., 2011). The occurrence of Salmonella spp. in seafood has been reported in Vietnam, India, Sri Lanka, Thailand, Taiwan and Japan (Ponce *et al*., 2007).

2.3.3. *Vibrio* spp

Vibrio are causing gastroenteritis to human via consumption of raw or under cooked shellfish (Raghunath *et al*., 2008) In the United States, transmission of Vibrio infections is primarily through the consumption of raw or under cooked shellfish (Gomathi *et al*., 2013). In Malaysia, *Vibrio cholera* is transmitted via contaminated drinking water, cooked food and raw or undercooked seafood (Lim, 2001). With an incubation period of 1-7 days for *Vibrio vulnificus* and 2-48 hours for *Vibrio parahaemolyticus,* they are capable of causing diarrhea and death in immunocompromised person (FDA, 2015). A cholera outbreak in Terengganu, Malaysia causes 400 humans to be hospitalized for acute diarrhea and complications during the outbreak in November, 2009 (Suhaili, *et al*., 2012). *Vibrio* not only causes gastroenteritis but it will also cause skin infection when open wound are exposed to contaminated water (CDC, 2013)

3.0 MATERIALS AND METHODS

3.1 Sampling method

A total of 14 pooled "siput sedut" samples were purchased from 10 markets in separate locations of Selangor for a period of 2 weeks. The samples were placed in an ice boxes and sent to Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for isolation within 24 hours.

3.2 MICROBIOLOGICAL QUALITY

The shell of the *Cerithidea obtusa* was cleaned using 70% alcohol. The meat of *Cerithidea obtusa* was removed from the shells and the entire organism were pooled into portions of 10g and then homogenized with 90mL of peptone water (Oxoid CM0009B) using stomacher for 2minutes (10^{-1}).

3.2.1 Dilution of Sample

Dilution procedure of 10 fold was performed as described by (Maturin & Peeler, 2001).

3.2.2. Plating of Dilutions

a. Standard Plate Count

Preparation of Standard Plate agar (APHA) (CM 0463) was done as in Appendix 1. A total of 0.1mL of aliquot from 10^{-3} - 10^{-8} dilution is pipetted onto the surface of SPC agar (APHA) (CM 0463). The inoculum was then spread out over the entire surface of the agar plate by using L-shaped spreader and incubated at 37° C for 48 hours under aerobic condition.

b. Coliform Plate Count

One mL of aliquot from 10^{-3} - 10^{-8} dilution was pipetted onto the petrifilm (3M™ Petrifilm™ Coliform Plate Count) and incubated at 37° C for 24 hours under aerobic condition.

3.2.3 Selecting Plates and Counting Colonies

Plates are selected and counted based on (Maturin & Peeler, 2001)

3.3 ISOLATION

3.3.1 Selective enrichment procedure

E.coli

The shell of the *Cerithidea obtusa* was cleaned using 70% alcohol. The meat of *Cerithidea obtusa* was removed from the shells were pooled into portions of 3g and then homogenized with 27mL of peptone water (Oxoid CM0009B) using stomacher for 2 minutes.

Salmonella

The shell of the *Cerithidea obtusa* was cleaned using 70% alcohol. The meat of *Cerithidea obtusa* was removed from the shells and were pooled into portions of 3g and then homogenized with 27mL of peptone water (Oxoid CM0009B) using stomacher for 2 minutes. One mL of homogenized sample were transferred into 9 mL of buffered peptone water (Oxoid CM0509T). Pre-enriched samples were incubated at 37° C for 24 hours under aerobic condition. 0.1mL of each pre-enriched samples was transferred into Rappaport-Vassilidiasis broth (Oxoid CM0866B) and incubated at 42° C for 24 hours under aerobic condition.

Vibrio

The shell of the *Cerithidea obtusa* was cleaned using 70% alcohol. The meat of *Cerithidea obtusa* was removed from the shells and were pooled into portions of 3g and then homogenized with 27mL of alkaline peptone water (Oxoid CM1028B) using stomacher for 2 minutes. The homogenized sample was incubated at 30° C for 24 hours under aerobic condition.

3.3.2 Culture of enriched samples on selective media

E.coli. Preparation of Eosin Methylene Blue (EMB) agar (Oxoid CM0069B) was done as in Appendix 2. After homogenization using stomacher, 3 loopfuls of each homogenized sample was streaked onto EMB agar. Then the plates were incubated at 37° C for 24 hours under aerobic condition.

Salmonella. Preparation of Xylose Lysine Deoxycholate (XLD) agar (Oxoid CM0469B) and Brilliant Green (BGA) agar (Oxoid CM0263) was done as in Appendix 3. After incubation, 3 loopfuls of each enriched sample was streaked onto XLD agar and BGA agar .Then, then the plates were incubated at 37° C for 24 hours under aerobic condition.

***Vibrio*.** Preparation of Thiosulfate-Citrate Bile Salt (TCBS) agar (CONDA CAT N º 1074) with 2% NaCl was done as in Appendix 4. After incubation, 3 loopfuls of each enriched sample was streaked onto TCBS with 2% NaCl. Then, the plates were incubated at 30° C for 24 hours under aerobic condition.

3.4 IDENTIFICATION PROCEDURES

E.coli.

Typical *E.coli* colony on EMB agar appear as black colonies with green metalic sheen. Single colonies from EMB agar was picked and Gram-staining was performed. Typical colonies from EMB agar was subcultured onto nutrient agar (Oxoid CM0003) to obtain sufficient pure colonies for identification and confirmation by biochemical test (Sulfide Indole Motility-SIM). After 24 hours, 1 drop of Kovacs reagent (Remel #R21227) was drop into the tube to detect for ability of the bacteria to produce indole from the tryptophan.

***Salmonella* spp.**

Typical *Salmonella* spp. colony appear as glossy colonies with black centres or completely black colonies on XLD agar and pink colonies on BGA agar. Single colonies from XLD and BGA agar were picked and Gram-staining was done. Then, typical colonies from XLD and BGA agar were subcultured onto nutrient agar in order to obtain sufficient pure colonies for identification and confirmation by biochemical test (Triple Sugar Iron-TSI, Lysine Iron Agar-LIA, Sulfide Indoe Motility-SIM, Citrate test and Urease test and serological test using Salmonella Polyvalent Agglutination Serum. Salmonella isolates were sent to Veterinary Research Institute, Ipoh for serotyping.

***Vibrio* spp.**

Typical colonies are green and yellow colonies on TCBS agar. Single colonies was picked and Gram-staining was done. Then typical colonies from TCBS agar were subcultured onto Tryptic Soy (TSA) agar (BD #221283) in order to obtain sufficient pure colonies for

identification and confirmation by Oxidase test and biochemical test (Triple Sugar Iron-TSI and oxidative fermentation test). For, species identification, API 20E was proceed.

3.5 ANTIBIOTIC SUSCEPTIBILITY TEST

Antibiotic susceptibility test was performed using a standard agar disc diffusion method.

3.5.1 Agar Disk Diffusion Procedure

Preparation of Mueller-Hinton agar (Oxoid CM0337) was done as in Appendix 5. Isolated colonies from non-selective agar was transferred into a test tube containing Tryptic Soy Broth (Oxoid CM0129) and vortex thoroughly in order to make a bacterial suspension. The suspension was incubated at 37° C for 2-6 hours under aerobic condition. The agar used for *E.coli* and *Salmonella* isolates are nutrient agar whereas agar used for *Vibrio* isolates was TSA agar After incubation, the turbidity of bacterial suspension was adjusted to 0.5 McFarland standard. A sterile swab was dipped into the bacterial suspension and then streaked over the entire surface of Mueller-Hinton agar. Antibiotic disc used in this study were based selected based on clinical and veterinary significance.

Antibiotic disc used in this procedure were from Oxoid and were applied onto the plate using mechanical dispensing apparatus. *E.coli* were tested against oxytetracycline (30µg), trimethoprim/Sulfamethoxazole (25µg), florfenicol (30µg), azithromycin (30µg), ciprofloxacin (5µg) and ceftriaxone (30µg). *Salmonella* were tested against oxytetracycline (30µg), trimethoprim/Sulfamethoxazole (25µg), florfenicol (30µg), amoxycillin (10µg), ciprofloxacin (5µg) and ceftriaxone (30µg). *Vibrio* were tested against oxytetracycline (30µg), trimethoprim/Sulfamethoxazole (25µg), florfenicol (30µg), doxycycline (30µg), azithromycin

(30µg) and erythromycin (15µg). The plates were inverted and incubated at 37° C for 24 hours under aerobic condition. After incubation, the zone of complete inhibition, was measured using caliper. The growth inhibition zone was compared with zone-size interpretative table as in (CLSI , 2010).

4.0 RESULTS

MICROBIOLOGICAL QUALITY OF *Cerithidea obtusa* AND THE ANTIBIOTIC SENSITIVITY OF SELECTED BACTERIA

Fourteen pooled samples of *Cerithidea obtusa* were purchased from different vendors from 10 wet markets in Kuala Lumpur and Selangor and were examined for the microbiological quality, occurrence of *E.coli*, *Salmonella* dan *Vibrio* spp. and to determine the antibiotic susceptibility of the isolates. The information on the shellfish origin and source was supplied by the vendor and it was revealed that 14.3% of the shellfish was from Malaysia and 85.7% was from Indonesia (Appendix 6).

The microbiological analysis revealed that 28.6% of the total sample tested for SPC of *Cerithidea obtusa* had mean count (30,809,000 CFU/g) that were beyond the limit (>5,000,000 CFU/g) set by the Microbiological Reference Criteria for Food, October 1995 (Food Administration Manual, 1995), while all of the samples tested for CPC had mean count (1,026,428 CFU/g) of more that 1,000 CFU/g. Statistical analysis was done to determine the relationship between the SPC and CPC of each sample with the origin of *Cerithidea obtusa.* There was no significant difference (P>0.05) for both SPC and CPC between *Cerithidea obtusa* from Malaysia and Indonesia.

Three pooled samples (21.4%) of *Cerithidea obtusa* were tested positive for *E.coli*. Two (66.7%) of *E.coli* isolates which originated from Indonesia and one *E.coli* isolate which originated from Malaysia showed similar resistance towards oxytetracycline (30µg) as in Table 1 & 2. Only one isolate (P7) showed susceptibility towards

trimethoprim/sulfamethoxazole (25µg), florfenicol (30µg), azithromycin (30µg), ciprofloxacin (5µg) and ceftriaxone(30µg).

Two pooled samples (14.3%) of *Cerithidea obtusa* were tested positive for *Salmonella* spp. which originated from Indonesia. The isolates showed resistance towards oxytetracycline (30µg) as in Table 3 & 4.

One sample (7.12%) of *Cerithidea obtusa* which originated from Indonesia were presumptively tested positive for *Vibrio* spp. API 20 E was done for further identification and revealed that it was *Vibrio parahaemolyticus* and antibiotic sensitivity results showed that it was resistant to erythromycin (15 µg).

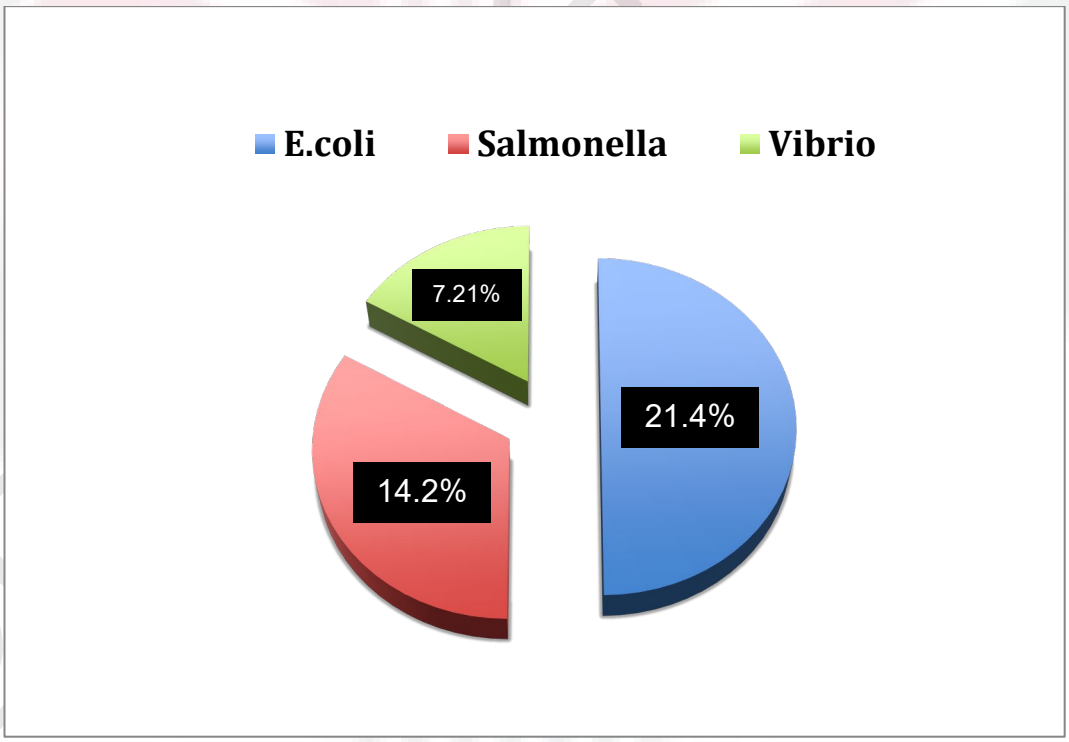

Figure 1: Bacteria isolated from *Cerithidea obtusa*.

Table 1: Antibiotic susceptibility pattern of *E.coli* isolated from *Cerithidea obtusa*

Antibiotic	E. coli (n=3)	
	Resistant	Susceptible
Trimethoprim/sulfamethoxazole (25µg)	2 (66.7%)	1 (33.3%)
Oxytetracycline (30µg)	3 (100%)	0
Florfenicol (30µg)	2 (66.7%)	1 (33.3%)
Ceftriaxone (30µg)	2 (66.7%)	1 (33.3%)
Ciprofloxacin (5µg)	2 (66.7%)	1 (33.3%)
Azithromycin (30µg)	2 (66.7%)	1 (33.3%)

Table 2: Antibiotic susceptibility pattern of *E.coli* isolated from *Cerithidea obtusa* from Indonesia and Malaysia

Sample	Trimetoprim/ sulfamethaxazole	Oxytetraycline	Florfenicol	Ceftriaxone	Ciprofloxacin	Azithromycin	Origin
P4(c)	R	R	R	R	R	R	Indonesia
P5	R	R	R	R	R	R	Indonesia
P7	S	R	S	S	S	S	Malaysia

Legend : R : Resistant, S : Susceptible

Table 3: Antibiotic susceptibility pattern of *Salmonella* spp. isolated from *Cerithidea obtusa*

Antibiotic	Salmonella (n=2)	
	Resistant	Susceptible
Trimethoprim/sulfamethoxazole (25µg)	1(50%)	1(50%)
Oxytetracycline (30µg)	2(100%)	0
Florfenicol (30µg)	1(50%)	1(50%)
Ceftriaxone (30µg)	1(50%)	1(50%)
Ciprofloxacin (5µg)	1(50%)	1(50%)
Amoxycillin (10µg)	1(50%)	1(50%)

Table 4: Antibiotic susceptibility pattern of *Salmonella* spp. isolated from *Cerithidea obtusa* from Indonesia

Sample	Trimetoprim/ sulfamethaxazole	Oxytetraycline	Florfenicol	Ceftriaxone	Ciprofloxacin	Amoxycillin	Origin
P 1	S	R	R	R	S	R	Indonesia
P 4 (a)	R	R	S	S	R	S	Indonesia

Legend : R : Resistant, S : Susceptible

5.0 DISCUSSION

This study revealed that pathogens of public health concerns such as *E.coli, Salmonella* and *Vibrio* spp were present in the local "siput sedut". Seafood are generally prone to bacterial contamination, especially filter-feeders such as mussels and oysters, which concentrate these bacteria in their filtration systems (Hazzah *et al.*, 2011). *E.coli,* which was present at a rather high prevalence, can be used as indicator of faecal contamination (Feldhusen, 2000). Pauziah (2006) reported high level of *E.coli* in sewage treatment plants and linked this to be the major sources for water pollution in Malaysia. Seafood are able to accumulate and concentrate pathogenic microorganisms that are naturally present in harvest waters due to their nature of filter feeding (Iwamoto et al., 2010)

E.coli and *Salmonella* isolates show 100% resistance towards oxytretracycline. This could be due to the usage of oxtetracycline in packing water to preserve the seafood especially when produce are imported from other countries (Shariff *et al*., 2000). This finding is in agreement (Donea & Halden, 2015) who documented that oxytetracycline to be the most commonly detected antibiotic compound in farmed fish and wild shrimp. Isolate of *Vibrio parahaemolyticus* were resistant to erythromycin and this were also reported by Noorlis *et al*., 2011 in freshwater fish at retail market in Selangor, Malaysia.

Two out of three *E.coli* isolates, all of *Salmonella* spp isolates and *V. parahaemolyticus* isolated were from "siput sedut" that originated from Indonesia. According to Supriyadi & Rukyani, 2000, oxytetracycline and erythromycin are the antibiotics normally used in aquaculture in Indonesia. Oxytetracycline are also used widely for treatment of bacterial diseases in fish and shrimp (Supriyadi & Rukyani, 2000). While application of erythromycin at 4 ppm by bath is practiced by Indonesian farmers to control bacterial disease

of shrimp (Supriyadi & Rukyani, 2000). Antibiotics are mostly given as medicated food pellets and food surplus not eaten by the fish, together with drug excreted, will eventually reach the bottom (Serrano, 2005). Previous research indicates that 70-80 percent of the drug used (oxytetracycline) were found in the sediment underneath the fish farm (Samuelsen *et al.,* 1992). The negative effects of administration of oxytetracycline have resulted in emergence of drug-resistant bacteria, and detection of antibiotic residues in exported fish products has resulted in rejection for the Japanese market (Serrano, 2005).

There are two factors that contributed to the emergence of resistance bacteria, namely contamination of swamps and estuaries with greywater or sullage (wastewater from households without faecal contamination) and uncontrolled usage of antibiotics in aquaculture. According to Azni & Abdullah, 2006, sullage may contain untreated organic material, household chemicals and pathogens similar to sewage water. Thus it may pollute the environment of *Cerithidea obtusa* giving rise to resistant pathogens ofa public health concern (Cook *et al*., 2001). Shariff *et al*., 2000 reported that uncontrolled usage of antibiotics in aquaculture contributes to the emergence of resistant bacteria. Since most prescribing in aquaculture sector is done by untrained salespersons of little knowledge regarding disease, the products prescribed may have little to do with the disease (Shariff *et al*., 2000) and led to the emergence of resistant bacteria. into the environment such as the swamps and estuaries. Resistant bacteria carried by food-producing animals can spread to people, mainly via the consumption of inadequately cooked food; handling of raw food or cross-contamination with other foods; but also directly from the environment (Health Action International Asia Pacific, 2013).

Health implications of consuming food contaminated with resistant bacteria are the transfer of resistance gene to human pathogens (Heuer *et al*., 2016). According to Heuer *et al*., 2016, the implications of antimicrobial resistance are increased number of infections, increased frequency of treatment failure and increased severity of infections. Humans exposed to antibiotic resistant bacteria have higher chances of getting infection from bacteria that are resistant (Heuer *et al*., 2016) Due to the ability of bacteria to survive with the presence of antibiotic, it would take a longer time to treat the infections as it takes time to find the suitable antibiotics that is able to kill the agent.

6.0 CONCLUSION AND RECOMMENDATIONS

As a conclusion, the study suggests that *Cerithidea obtusa* have poor microbiological quality and harbour pathogens such as *E.coli*, *Salmonella spp.* and *Vibrio parahaemolyticus* that exhibited multidrug resistant trait. *E.coli* and *Salmonella* spp isolates showed 100% resistance towards oxytetracycline. While *V. parahaemolyticus* showed resistance towards erythromycin.

As a recommendation, I suggest extension and expansion of the present study to a larger population in order to develop better estimates of *E.coli*, *Salmonella* and *Vibrio* spp. in *Cerithidea obtusa.* As *Cerithidea obtusa* is considered a delicacy in Malaysia, it is important to create awareness to the public regarding the pathogens that *Cerithidea obtusa* may harbour and the health implications of it.

Lastly, the ecosystem and water quality from where the *Cerithidea obtusa* originated must be studied in order to correlate the findings to the presence of bacteria it harbour.

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APPENDIX 1

MEDIA PREPARATION FOR ISOLATION OF MICROBIOLOGICAL QUALITY

Standard Plate Count Agar

23.5g + 1 litre of distilled water

↓

Autoclaving at 121°C for 15 minutes

↓

Pour into plates

APPENDIX 2

MEDIA PREPARATION FOR ISOLATION OF *E.coli*

Eosin Methylene Blue Agar

37.5g + 1 litre of distilled water

↓

Autoclaving at 121°C for 15 minutes

↓

Cool to 60°C and shake the medium in order to oxidise the methylene blue (i.e. restore its blue colour) and to suspend the precipitate

↓

Pour into plates

APPENDIX 3

MEDIA PREPARATION FOR ISOLATION OF *Salmonella* spp.

Pre-enrichment broth (BPW)

20g + 1 litre of distilled water

↓

Dispense 9 mL into screw-capped bottles

↓

Autoclaving at 121°C for 15 minutes

Enrichment broth (RVS broth)

26.75g + 1 litre of distilled water

↓

Dispense 10mL volumes into screw-capped bottles

↓

Autoclaving at 115°C for 15 minutes.

Plating media

XLD agar

53g + 1 litre of distilled water

↓

Heat with frequent agitation until the medium boils

↓

Cool to 50°C in water bath

↓

Pour into plates

BGA agar

Suspend 50g in 1 litre of distilled water.

↓

Autoclaving at 121°C for 15 minutes

↓

Pour into plates

APPENDIX 4

MEDIA PREPARATION FOR ISOLATION OF *Vibrio* spp.

Enrichment broth (APW)

30g + 1 litre of distilled water

↓

Autoclaving at 121°C for 15 minutes

Plating media

TCBS agar

88g + 1 litre of distilled water

↓

Add in 2% of NaCl

↓

Heat with frequent agitation until the medium boils

↓

Cool to 50°C in water bath

↓

Pour into plates

APPENDIX 5

MEDIA PREPARATION FOR ANTIBIOTIC SENSITIVITY TEST

Plating agar

Mueller-Hinton Agar

38g + 1 litre of distilled water

↓

Autoclaving at 121°C for 15 minutes.

↓

Pour into plates

APPENDIX 6

MICROBIOLOGICAL QUALITY OF *Cerithidea obtusa*

No	CPC (CFU/g)	SPC (CFU/g)	Origin
1	$<25 \times 10^5$	$<25 \times 10^5$	Indonesia
2(a)	$<25 \times 10^5$	$<25 \times 10^5$	Indonesia
2(b)	$<25 \times 10^5$	$<25 \times 10^5$	Indonesia
2(c)	$<25 \times 10^5$	$<25 \times 10^5$	Indonesia
3	$<25 \times 10^5$	$<25 \times 10^5$	Malaysia
4(a)	$<25 \times 10^4$	3,300,000	Indonesia
4(b)	$<25 \times 10^4$	8,400,000	Indonesia
4(c)	770,000	27,000,000	Indonesia
5	$<25 \times 10^4$	420,000,000	Indonesia
6	$<25 \times 10^4$	380,000,000	Indonesia
7	$<25 \times 10^3$	$<25 \times 10^3$	Malaysia
8	$<25 \times 10^3$	$<25 \times 10^3$	Indonesia
9	$<25 \times 10^3$	$<25 \times 10^3$	Indonesia
10	$<25 \times 10^3$	51,000	Indonesia

Legend: SPC : Standard Plate Count, CPC : Coliform Plate Count.