# Isolation, identification and distribution of potentially pathogenic bacteria associated with selected water bodies and fish organs in different locations in Peninsular Malaysia

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## ABSTRACT:

The need for passive and periodic surveillance on the presence of potential pathogenic bacteria in water bodies, sediments and tissues of fish in Malaysian water bodies is imperative. This study aimed at determining the presence of potential pathogenic bacteria in water bodies, sediment and tissues of sea bass, snapper, grouper and tilapia which may be associated with disease outbreak in selected water bodies in Peninsular Malaysia. Water samples were collected from cage side, sea waters and sediments from Ketam, Kukup, Kuala Selangor, Kuala Lingii and Seri Serdang for bacterial isolation and identification. Fifty samples each were collected from the kidney, intestine, gill and skin of sea bass, snapper, grouper and tilapia from each of these water bodies mentioned above. The bacteria were isolated and identified using standard biochemical techniques. Several pathogenic bacteria were isolated and identified from the selected water bodies, sediments and tissues of sea bass, snapper, grouper and tilapia in Ketam, Kukup, Kuala Selangor, Kuala Lingii and Seri Serdang. In all the selected water bodies, sediments and the selected tissues of fish, bacterial isolates that predominated were *Vibrio* spp., *Aeromonas*spp,faecal coliforms and *E.coli*and the highest level of contamination were in Kuala Lingii and Seri Serdang. However, in all the 4 types of fish, *V. parahaemolyticus* and *A. hydrophilia* were the predominant isolate and the highest rate of isolation were from tilapia and sea bass. Based on the results of this study,*V. parahaemolyticus* and*A. hydrophila*were the predominant bacteria isolatesin these water bodies, sediments and fish tissues. The isolation and identification of multi-drug resistant bacteria in this study is an indication that animal and human activities may play vital roles which has not been previously reported in Malaysia. Periodic surveillance of water bodies, sediments and fish tissues for the detection of pathogenic bacteria especially in Malaysian water bodies is therefore a necessity.

## 1. INTRODUCTION

Fish is a vital source of food for people and contributes about 60% of global supply of proteins. About 60% of developing nations derive 30% of their yearly protein need and or requirement from fish [1]. It has been opined that the type of micro-organisms that are found in any group of fish in an environment depends on its habitat [2] . Bacteria pathogens that contaminate water bodies are broadly categorized into indigenous and non-indigenous. While the indigenous bacterial pathogens such as *Vibrio* species and *Aeromonas*species are usually found naturally living in the fish's habitat, the non-indigenous such as*Escherichia coli, Clostridium botulinum, Shigelladysenteriae, Staphylococcus aureus, Listeria monocytogens*and *Salmonella*contaminate either the fish or the habitat (or both in some instances) through different means[3].Both pathogenic and potentially pathogenic bacteria associated with diseases of fish and shellfish include *Vibrio* spp., *Aeromonas* spp., and others [4].

On the other hand, the microbial diversity of fresh water or rivers and lakes may consist of complex flora of microorganisms, including genuinely aquatic pathogens and other components introduced from human, animal and plant sources[5],[6]. The scale of human activities has been demonstrated to exert some detrimental effects on coastal waters. Since numerous shell fishes used food particles from large volume of waters, if these waters are contaminated with sewage, the risk that enteric pathogens from infected humans may be present is high and these contaminants may subsequently be concentrated by the filter feeding nature of the fish[5].

In order to enhance predictive capability for possible disease outbreaks in addition to provision of an opportunity for designing effective and efficient preventive management al strategy, information on the bacterial load and types of bacteria associated with different water bodies, sediments and organs of apparently healthy fishes in Malaysian water bodies is therefore necessary. Information on this vital aspect in different water bodies, sediments and tissues of apparently healthy fishes are limited. This study was conducted to determine the presence of potential pathogenic bacteria in water bodies, sediments and tissues of sea bass, snapper, grouper and tilapia which may be associated with disease outbreak in selected water bodies from Ketam, Kukup, Kuala Selangor, Kuala Lingii and Seri Serdng in the Peninsular Malaysia.

## 2 - MATERIALS AND METHODS:

### 2-1:Study area:

This study was conducted in Malaysia from four sites for breeding fish in cages .Pulau ketam which is a small island located off the cost of Klang away 50,2 KM south of Kuala Lumpur ,Kuala Selangor which located on long of Selangor river away 68,4 KM north of Kuala Lumpur, Kuala Lingii in Melaka away 145,2 KM south of Kuala Lumpur and the fourth site Pulau Kucup in Johor away 329,8 KM south of Kuala Lumpur. The fifth location that from pasar malam in Sri Serdang which is the local market in Selangor.

### 2-2:Sampling

#### 2-2-1:Water and sediment

Three stations were selected for water and sediments sampling and these stations were Ketam, Kukup and Kuala Lingi. In each of the three locations, samples were collected from both water from cage, sea water and sediments. The samples were taken to the laboratory for bacterial isolation, identification and biochemical analysis.

#### 2-2-2:Fish

For the fishes, sampling was done in 5 different stations viz; Ketam, kukup, Kuala Selangor, Kuala lingii and Sri Serdang. In each of the fish that were sampled, selected organs such as the kidney, intestine, gills and skin were aseptically collected. The samples were aseptically transported to the laboratory for isolation and identification of possible presence of pathogenic bacteria isolates.

### 2-3:Sample preparations for bacterial counts

#### 2-3-1:Fish :

One gram of the fish sample(10 samples sea bass,8 samples from other types of fish)(kidney, intestines, gills and skin)were dissected out, blended and mixed properly in a mortar. It was aseptically transferred to a sample bottle containing 9mls of 0.1% sterile peptone water. The

bottle was closed and shaken thoroughly for 10 minutes and allowed to stand for 20 minutes, after which a 10 fold serial dilution was carried out in duplicates and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37$^{o}$C for 48 hrs as described by[7]. Coliform organisms and gram negative enteric bacteria counts were determined using pour plate method with Mac Conkey agar, EMB Agar, respectively.

Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar were used for pathogenic Vibrio spp. The plates were incubated at 35$^{o}$C for 24 hrs. The observed colony growth were counted using Coulter Colony counter according to plate count method. The media mRS agar used for pathogenic Aeromonas spp. and Pseudomonas agar used to isolate Pseudomonas spp. Identification of the organisms was done using the phenotypic and biochemical characteristics as described previously[7].

**2-3-2:Water and sediment:**

Eight samples of water and 8 samples of sediments were also collected from sites. Water samples were collected in 100 ml of sterile Bijou bottles under the water surface with the depth of 10 to 15 cm in different location within the cage-cultured farm and ro outside of farm .All the samples were kept in the sterile container sand preserved in low temperature with icepack. The sediment was sampled by an Ekman grab (Wildco, USA) and a portion of the top 5 cm of sediment, was scooped into a 50 ml sterile bottle, after removing debris and shells. Filled sediment bottles were capped before they were placed together in a zip-lock poly ethylene bag according to station. All water and sediment samples were always kept in an ice filled chest, before their transfer to the laboratory. In the laboratory, all water and sediment samples were stored in a 4 C freezer until microbiological analyses .

**2-4:Preparation of serial dilution**

Nine milliliters of sterile water was poured aseptically into five tubes each and 1 ml of the original crushed fish sample was added to the first test tube and mixed thoroughly. Another 1 ml was taken from the first tube and added to the second test tube and mixed very well. From the second test tube, another 1 ml was taken and introduced into the third test tube and mixed very well. This procedure continued until the fifth test tube. The crushed sample was therefore diluted from 10 -1 to 10-5 for each fish sample .Fecal and total coliform counts were performed using

the standard membrane filtration technique. The 100 ml water sample was filtered using 0.45 mm pore size, 47 mm diameter filter membrane as described by APHA [8].

Multiple tube technique was used for the enumeration of Most Probable Number of coliform bacteria. Nutrient agar (NA) as a basal medium MacConkey agar as a differential medium and Blood agar as a special medium were used to determine enteric bacteria. Escherichia coli are isolated by inoculating the sample in Bismuth green bile broth. Enteric bacteria isolated on respective selective or differential media were identified on the basis of their colonial . For sediment samples, 1 g of sediments were homogenized in 9 ml of sterile 0.85% saline before5 ml of the homogenate was filtered through 0.45 mm nitrocellulose filters (47 mm).Serial dilutions of estuarine water and sediment (1 g in 9 ml saline) samples were prepared using sterile 0.85% saline and were spread plated in the agars. The inoculated plates were incubated at 37 C for 24–48 h and 30 C for vibrio and Aeromonas spp.mean was calculated as total plate count[9],[10].

**2-5:Inoculation of plates**

Duplicate plates of nutrient agar were inoculated with 0.1 ml of the diluted solution ($10^{-2}$ to $10^{-5}$) using glass spreader technique. All plates were incubated at a temperature of 37°C for 24 h before colony enumeration and isolation.

**2-6:Antibiotic resistance:**

The antibiotic susceptibility test was performed according to Kirby- Bauer disk diffusion method [11] using Mueller-Hinton (MH) agar (Oxoid, Basingstoke, U.K.). The inoculums was prepared in tryptone soy broth and the concentration of the bacterial cells were adjusted to a 106 colony forming unit using sterile physiological saline to correspond to 0.5 MacFarland standard. The inoculums was swabbed on the prepared MH agar. The antibiotics tested and their concentrations were Tetracycline (TE)-30 µg, Streptomycin(S)-20 µg, Chloramphenicol(c)-30µg, Bacitracin(B)-10 µg, Gentamicin(CN)-10 µg, Polymyxin B(PB)-10 µg, Penicillin G(P)-10µg, Methicillin(MT)-5µg, Ampicillin(AMP)-10µg, Nitrofurantoin(F)-200µg, Kanamycin(K)-30µg, Novobiocin(NV)- and Vacomycin(VA)-30µg. E. coli ATCC 25922 was included as control for the series of antibiotic susceptibility determinations. After incubation for 24 h, the zones of inhibition were interpreted according to the criteria recommended by[12].

### 2.7:Statistical analysis:

The data were elaborated statistically using the software package SPSS 18. In cases in which bacterial levels were below the detectable limit a value of zero was assigned for statistical analysis. The mean and standard deviation were calculated, and regression analyses were performed to analyze significant relationship. Analysis of variance (ANOVA) was carried out to determine if there were differences in pattern of bacterial population between stations

## 3. Result and discussion:

### 3.1:Number of bacteria isolated from various samples (CFU/mL)

The number of bacteria isolated (CFU/100 mL) from water samples both from the cage waters, sea water and sediments from Ketam, Kukup and Kualalingi, using TCBS are summarised and presented in Table 1. The samples cultured on TCBS revealed significantly higher number of *V. cholera* from water originating from cages in all the three locations compared to the number of *V. cholera*in samples from sea water and sediments from the three location. However, from the results obtained, cage water from Ketam is much more contaminated with *V. cholera* compared to cage water from Kukup and Kualalingi. This was followed by sediment in Ketam ($2.0 \times 10^3$) with sea water having the lowest number of *V. cholera.* Kukup and Kualalingii similarly followed the same pattern in which higher number of *V. cholera*were isolated from sediment as against sea water. It was observed that samples from Kukup had the lowest number of *V. cholera*isolated while Ketam had the highest number of bacteria (*V. cholera*)isolation.

For *V. parahaemolyticus* however, higher *V. parahaemolyticus*($2.9 \times 10^3$)were isolated from water sediments in Ketam compared to water from the cage and sea water in contrast to the case of *V. cholera*. This was followed by water samples from the cages and the lowest number of *V. parahaemolyticus*($0.4 \times 10^3$) was from sediment. For kukep, the highest level of *V. parahaemolyticus* contamination was found in water from the cage followed by sediments and sea water which had similar levels of *V. parahaemolyticus* contamination. In kualalingii, the highest level of *V. parahaemolyticus*($1.6 \times 10^4$) was obtained from sediment, followed by water from cage which had $0.88 \times 10^4$ and the least level of *V. parahaemolyticus* contamination was obtained from sea water which recorded $0.10 \times 10^4$. While levels of *V. parahaemolyticus* contamination in sea water was high in ketam, the level of *V. parahaemolyticus* contaminationin

kualalingii was significantly lower than even those from the cage and sediment. Generally, *V. parahaemolyticus* bacterial contamination level were highest in water from cage in Ketam and lowest in sea water and sediment from Kukup.

*Vibrio vulnificus* contamination vary between the locations and type of samples. In Ketam, highest level of bacterial contamination ($2.2 \times 10^4$) was obtained from cage water and the least was observed from water from the sediment. In Kukep, no bacterial contamination from both cage water and sediment were detected while $2.2 \times 10^2$ level of bacterial contamination were observed from seas water. In kualaLingii, level of *V. vulnificus* contamination were $4.9 \times 10^3$ and $3.8 \times 10^2$ at KualaLingii cage water and sediments respectively. The highest level of *V. vulnificus* was obtained in Ketam and the least level of contamination was obtained from Kukup.

For *Aeromonas spp*., the level of contaminations in Ketam were $1.4 \times 10^4$, $0.33 \times 10^3$ and $0.12 \times 10^3$ in cage water, sea water and sediments respectively while in Kukep, *Aeromonasspp* contamination were $0.3 \times 10^2$, $1.4 \times 10^2$ and $0.5 \times 10^2$ at cage water, sea water and sediments respectively. In KualaLingii, *Aeromonasspp* contamination were $3.7 \times 10^3$ and $2 \times 10^2$ from cage water and sediments respectively. Contamination with *Aeromonas spp*. appeared to be higher in cage water from Ketam and the least contamination was obtained from sediments from Ketam location. In this study, the highest level of V. cholorae contamination were obtained from water collected at cages in all the three locations and the highest was from Ketam. The possible reason for the higher level of V. cholorae is the association of the location with the urban settlement (Kuala Lumpur) which is likely to serve as source of contamination to the water[13]. This findings are in agreement with the findings reported in a previous related study who reported that organisms from infected individuals in the vicinity of the water body may be present in the water and could be concentrated via the filter feeding habits of the fish [5], [14]Additionally, fresh water or rivers and lakes usually have complex flora of microorganisms which could either be genuinely aquatic species or exogenous components introduced from terrestrial, animal and plant sources, thereby increasing the level of bacterial contamination of the water [5], [13].The high contamination level from water at cage sides compared to sea water and sediments could also be associated with human activities around the area in addition to the presence of the fish which will naturally attract disease pathogens. Other related studies have equally reported that level and extent of human activities could have a detrimental effect on coastal waters [5], [15].Although the level of V. cholorae contamination both from cage water and sediments in Kualalingii

(Melaka is a town far less inpopulation compared to Kuala Lumpur) were high, they were relatively less than those from Ketam. For both V. parahaemolyticus, V. vulnificus and A. hydrophilia, the level of contamination were consistently higher form cage waters from ketam compared to sea waters and sediments from these locations. The high level of contamination of the different bacteria in cage waters from these locations could be linked with the nature and type of fish raised in those cages. While some of the bacteria may constitute part of the water flora, most of the bacteria by the cage waters are likely to be introduced by the fish as well as human activities [14], [15]. In this study, the highest bacterial contamination was also found to be V. cholorae in ketam and this finding is in agreement with the findings of a previous study [16], who reported higher level of V. cholorae in water bodies compared to other bacteria. the level of bacterial water contamination and microbial diversity[6],[17].

**Table 1: Bacterial contamination levels of water and sediments from different locations**

| | TCBS | | | Mrs |
|---|---|---|---|---|
| **Sampling sites** | *V. cholera* | *V. parahaemolyticus* | *V. vulnificus* | *Aeromonas*spp. |
| **Ketam/water from cage** | $3.6x10^4$ | $1.3x10^4$ | $2.2x10^4$ | $1.43x10^4$ |
| **Ketam/ sea water** | $1.8x10^3$ | $2.9x10^3$ | $4.3x10^3$ | $0.33x10^3$ |
| **Ketam/sediment** | $2x10^3$ | $0.4x10^3$ | $3.7x10^3$ | $0.12x10^2$ |
| **Kukup/water from cage** | $0.4x10^3$ | $0.5\ x10^2$ | 0 | $0.3\ x10^2$ |
| **Kukup/sea water** | $0.2\ x10^3$ | $0.3\ x10^2$ | $0.1\ x10^2$ | $1.4\ x10^2$ |
| **Kukup/sediment** | $0.1x10^3$ | $0.3x10^2$ | 0 | $0.5x10^2$ |
| **Kualalingii/water from cage** | $1.80x10^4$ | $0.88x10^4$ | $4.9x10^3$ | $3.7x10^3$ |
| **Kualalingii/sea water** | $1.22\ x10^4$ | $0.10\ x10^4$ | TNTC | TNTC |
| **Kualalingii/sediment** | $1.34x10^4$ | $1.61x10^4$ | $3.8x10^2$ | $2x10^2$ |

### 3.2:Tissue bacterial load

The mean bacterial load of different organs collected from the fish in the different locations in this study is depicted in table 2. The TPC tissue bacterial load in Ketam were 5.1 x $10^5$, 6.3 x $10^5$, 4.4 x $10^5$ and 8.8 x $10^5$ in the kidney, intestine, gills and skin respectively. The highest TPC tissue burden were from the skin while the lowest TPC bacterial load were from the gills. In Kukup, TPC bacterial load in the kidney, intestine, gills and skin were 2.2 x $10^5$, 2.7 x $10^5$, 1.4 x $10^5$ and 3.1 x $10^5$ respectively. The highest TPC bacterial burden in kukup was recorded in the skin while the lowest was from the gills. In kualaLingii, TPC tissue bacterial load were 17.4 x $10^5$, 16.9 x $10^5$, 12.6 x $10^5$ and 17.7 x $10^5$ for the kidney, intestine, gills and skin respectively. In Seri Serdang, TPC tissue bacterial loads were 21.5 x $10^5$, 18.4 x $10^5$, 12.8 x $10^5$ and 12.8 x $10^5$ respectively. Tissue TPC bacterial loads in the fish were found to be higher in KualaLingii and Seri Serdang compared to Ketam and kukup.

For coliforms in ketam, the tissue bacterial burden were 6.8 x $10^5$, 7.6 x $10^5$, 3.8 x $10^5$ and 7.2 x $10^5$ for the kidney, intestine, gills and the skin respectively. In kukup, coliform tissue loads were 1.5 x $10^5$, 2.2 x $10^5$, 1.0 x $10^5$ and 1.4 x $10^5$ for the kidney, intestine, gills and skin respectively. In KualaLingii, the tissue coliform ranges from 4.7 x $10^5$ to 9.9 x $10^5$, with the highest load in the intestine and the lowest load in the gills. Seri Serdang appeared to have the highest tissue coliform load ranging from 10.2 x $10^5$ in the kidney to 11.1 in the intestine. For coliform tissue loads, the intestine was consistently found to have the highest bacteria burden for all the locations.

*Pseudomonas aeruginosa* tissue bacterial load vary between locations. In ketam, *P. aeruginosa* tissue bacterial load were 4.9 x $10^5$, 11.2 x $10^5$, 3.2 x $10^5$ and 13.3 x $10^5$ for the kidney, intestine, gill and the skin respectively while in Kukep, *P. aeruginosa* tissue bacterial load were 5.4 x $10^5$, 3.7 x $10^5$, 5.1 x $10^5$ and 4.4 x $10^5$ for the kidney, intestine, gills and skin respectively. In KualaLingii, the bacterial load in the tissues ranged from 8.3 x $10^5$ in the kidney to 7.5 x $10^5$ in the intestine while in Seri Serdang, the bacterial load were relatively higher, ranging from 18.8 x $10^5$ in the intestine to 12.4 x $10^5$ in the kidney. The highest *P. aeruginosa* bacterial loads were found in the skin and the intestine respectively and Seri Serdang appeared to have the highest tissue bacterial loads amongst the different locations.

There was variations in *V. cholerae* tissue bacterial load between the different locations and generally, tissue bacterial load were relatively low compared to other bacterial isolates. In Ketam, the tissue bacterial of *V. cholerae* ranged from nill in the gills to 4.3 x $10^5$ in the intestine While in Kukup, the tissue bacterial load of *V. cholerae* ranged from nil in both kidney and gills to 1.3 x $10^5$ in the skin. In Kualalingii however, the tissue bacterial load were relatively higher, ranging from 7.9 x $10^5$ in the intestine to 3.3 x $10^5$ in the gills and in Seri Serdang, *V. cholerae* bacterial load ranged from 4.2 x $10^5$ in skin to 4.8 x $10^5$ in the intestine. The *V. cholerae* bacterial load were found to be relatively higher in the intestine and least in the gills.

Tissue bacterial load for *V. vulnificus* equally vary between locations and organs. In Ketam, the bacterial load ranged from 6.2 x $10^5$ in the skin to 2.7 x $10^5$ in the gills while in Kukup, bacterial load ranged from nil in the kidney and intestine to 2.6 x $10^5$ in the skin. Tissue bacterial load in Kualalingii ranged from 8.5 x $10^5$ in the skin to 5.8 x $10^5$ in the gills while in Seri Serdang, bacterial load in the tissue ranged from 8.9 x $10^5$ in the gills to 10.5 x $10^5$ in the kidney. Generally, the gills appeared to have the least bacterial load while the skin was found to have the highest *V. vulnificus* bacterial load.

*V. parahaemolyticus* tissue bacterial load vary between locations and between organs. In ketam, tissue bacterial loads were found to range from 8.4 x $10^5$ in the skin to 4.9 x $10^5$ in the gills while in Kukup, bacterial loads from the tissue were found to range from 4.2 x $10^5$ in the skin to 2.8 x $10^5$ in the intestine. In Kualalingii, tissue bacterial loads for *V. parahaemolyticus* ranged from 10.6 x $10^5$ in the skin to 7.9 x $10^5$ in the kidney and in Seri Serdang, tissue bacterial loads ranged from 10.3 x $10^5$ in the kidney to 11.7 x $10^5$in the gills. While the gills appeared to consistently have the least bacterial loads, the skin on the other hand was found to consistently have the highest *V. parahaemolyticus* bacterial load.

*Aeromonashydrophilia* tissue bacterial loads were found to vary between location and between organs. In Ketam, *A. hydrophilia* tissue bacterial loads were found to range from 8.2 x $10^5$ in the kidney to 5.9 x $10^5$ in the intestine while in Kukup, bacterial loads ranged from 6.5 x $10^5$ in the skin to 2.8 x $10^5$ in the intestine. *A. hydrophilia* tissue bacterial loads in Kualalingi were found to range from 10.9 x $10^5$ in both kidney and intestine to 14.8 x $10^5$ in the gills and skin while in Seri Serdang, tissue bacterial loads were found to range from 16.3 x $10^5$ in the kidney to 13.2 x $10^5$ in the intestine. *Aeromonas hydrophilia* tissue bacterial loads were found to be higher in

kidney and lowest in the intestine. The conduct of periodic surveillance on the presence, both in different water bodies as well as different organs of fish and the various environmental factors associated with potential fish pathogens and the consequent link to disease outbreak in the fish industry in Malaysia is imperative[6].Unlike higher vertebrates, fishes are less immuno-competent and they may be predisposed to numerous disease outbreaks [18]. Outbreak of bacterial diseases in fish remains one of the most vital limiting factors affecting the global fish culture industry[19]. This investigation describes the isolation and identification of potential fish pathogens in water, sediments and tissues of sea bass, snapper, grouper and tilapia from four different water bodies in Malaysia In this study, all the bacteria investigated were isolated in all the locations and from different organs as previous reported in other related studies [20],[21]. However, the variation in the tissue bacterial load as seen between locations and between organs could be associated with level of bacterial water contamination, microbial diversity and tissue tropism for those bacterial isolates [17], [6]. Fish from Seri Serdang were found to have the highest tissue bacterial load. This could be linked to the fact that almost all water bodies in Seri Serdang are either directly or indirectly connected with waste waters which constitute heavy sources of contamination[21].

Since the level of bacterial contamination is associated with tissue bacterial load in fish living in such waters[22], [17], [21]. this perhaps explains the higher tissue bacterial loads found in the tissue of fish in this are a.Amongst the different organs, coliforms were found to be consistently higher in the intestine compared to the other organs in all the locations. This finding is in accord with an earlier study [21] where fish from polluted water and fresh water were collected and tissue coliform bacterial load were determined and reported to be higher in the intestine compared to other organs. Tissue bacterial loads for E. coli were observed to follow similar pattern with those of the coliforms in this study and this findings agree with the report in an earlier study [21] who reported similar pattern in tissue bacterial load between coliforms and E. coli. Tissue bacterial load for P. aeruginosa were found to be higher in the intestine and the skin in almost all the locations. Pseudomonas aeruginosa has been demonstrated to be an opportunistic aquatic pathogen [23] and it often lead to infection of fish under unfavourable environmental condition and the bacteria has been similarly isolated from organs of fish in other related studies [24],[25]. All Vibrio spp isolated in this study were found to be consistently higher on the skin compared to other organs in all the locations. However, the tissue bacterial

load were found to be consistently higher in the fish form Seri Serdang. This findings agree with the findings of other related studies [21], [6] where tissue bacterial load were associated to the level of bacterial contamination of the water, owing to human activities. Higher bacterial loads of A. hydrophilia were similarly found in tissues of fish from Seri Serdang compared to other locations. Tissue bacterial load of A. hydrophilia in fish has been associated with the level of bacterial contamination or rather pollution of the water and the high bacterial load obtained from the intestines of fish from this area is in accord with the reports of other studies [26], [6]. Pathogen contamination of water is a challenging issue for most types of water bodies, which is increasingly attracting more interests. Monitoring the levels of indicator organisms such as faecal coliforms and E. coli is one of the common approaches used in quantifying the potential pathogen loads in different water bodies [27].

**Table 2: Tissue bacterial load of different organs of fish from different locations**

| | Parts | TPC | coliforms | *P.aureuginosa* | *V.cholerae* | *V. vulnificus* | *V. parahaemolyticus* | *A. hydrophila* |
|---|---|---|---|---|---|---|---|---|
| **Location** | CFU/g | Cfu/g | Cfu/g | Cfu/g | Cfu/g | Cfu/g | Cfu/g | Cfu/g |
| | $10^3$ | $10^5$ | $10^5$ | $10^5$ | $10^5$ | $10^5$ | $10^5$ | $10^5$ |
| **Ketam** | KIDNEY | 5.1 | 6.8 | 4.9 | 3.4 | 4.2 | 5.6 | 8.2 |
| | INTESTINS | 6.3 | 7.6 | 11.2 | 4.3 | 3.9 | 7.5 | 5.9 |
| | GILL | 4.4 | 3.8 | 3.2 | - | 2.7 | 4.9 | 6.5 |
| | SKIN | 8.8 | 7.2 | 13.3 | 2.7 | 6.2 | 8.4 | 7.3 |
| **Kukup** | KIDNEY | 2.2 | 1.5 | 5.4 | - | - | 3.3 | 4.2 |
| | INTESTINS | 2.7 | 2.2 | 3.7 | 1.0 | - | 2.8 | 2.8 |
| | GILL | 1.4 | 1.0 | 5.1 | - | 1.6 | 3.5 | 4.5 |
| | SKIN | 3.1 | 1.4 | 4.4 | 1.3 | 2.4 | 4.2 | 6.5 |
| **Kualalingii** | KIDNEY | 17.4 | 9.8 | 18.3 | 5.4 | 7.9 | 7.9 | 10.9 |
| | INTESTINS | 16.9 | 9.9 | 7.5 | 7.9 | 6.6 | 10.2 | 10.9 |
| | GILL | 12.6 | 4.7 | 8.8 | 3.3 | 5.8 | 10.1 | 14.8 |
| | SKIN | 17.7 | 6.4 | 10.5 | 6.5 | 8.5 | 10.6 | 14.8 |
| **Sri Serdang** | KIDNEY | 21.5 | 10.2 | 12.4 | 4.7 | 10.5 | 16.3 | 16.3 |
| | INTESTINS | 18.4 | 11.1 | 14.4 | 4.8 | 9.5 | 12.1 | 13.2 |
| | GILL | 12.8 | 11.0 | 18.8 | 4.6 | 8.9 | 11.7 | 14.5 |
| | SKIN | 12.8 | 10.5 | 17.5 | 4.2 | 9.4 | 12.8 | 13.6 |

### 3-3:Percentage of individual bacterial specie contamination for the different locations

The mean percentage of bacterial contamination for the different locations and the different bacteria investigated in this study is presented in Table 2. It was observed that perc4-3:entage of bacteria contaminations vary between location and species. The highest level of E. coli contamination (35%) was obtained in Kualalingii and this was followed by Seriserdang which recorded 28% E. coli contamination. The level of E. coli contamination in Kuala Selangor, Ketam and Kukup were 23%, 9% and 5% respectively. For Pseudomonas, the highest level of contamination (35%) was found in Kualalingii, followed by Seriserdang which had 33% bacterial contamination. Locations such as Kulaselangor, Ketam and Kukup recorded 22%, 6% and 4% respectively. The highest bacterial contamination for Vibrio species (45%) was similarly obtained from kualalingi followed by 26% in Seriserdang, 16% in Kualaselangor, 8% in Ketam and 5% in Kukup. For Aeromonas species, highest percentage (41%) was obtained from Kualalingii while 25% was obtained in Seriserdang. Others were 20% in Kualaselangor, 10% in Ketam and 4% in Kukup. From the generality of the results presented in this table, Kualalingii appeared to consistently have the highest level of bacterial contamination for the the 4 bacterial species investigated while Kukup recorded the least bacterial contamination for these bacteria. Based on the level of bacterial contamination as determined in this study, Kukup water bodies have the lowest level of bacterial contamination with Pseudomonasspp and Aeromonassppbeing the least in this water body. This findings correlated with tissue bacterial loads of fish from kukep, thereby affirming the assertion that bacterial load of fish depends on the level of contamination of the water [6] ,[21]. On the other hand, the highest level of bacterial contamination were found in Kualalingii and Seri Serdang water bodies which similarly correlated with tissue bacterial loads of fish from those water bodies[28], [29]. In this study, E. coli, Pseudomonas, Vibrio and Aeromonas species were found at varying percentages for the different water bodies sampled and there was no consistency in the percentages of the different bacteria detected in the different water bodies. The variation in percentages could be associated with differences in contamination levels by different sources in each of the water bodies which probably have different microbial diversity[13], [22].

**Table 2: Total percentage of bacterial contamination for the different bacteria spp.**

| **Bacteria species** | Ketam | Kualaselangor | Seriserdang | Kukuep | Kualalingii |
|---|---|---|---|---|---|
| ***Escherichia Coli*** | 9% | 23% | 28% | 5% | 35% |
| ***Pseudomonas*** | 6% | 22% | 33% | 4% | 35% |
| ***Vibrio*** | 8% | 16% | 26% | 5% | 45% |
| ***Aeromonas*** | 10% | 20% | 25% | 4% | 41% |

### 3.4:Rates of isolation from the four species of fish:

The mean rate of isolation of the four bacteria isolates in this study are shown in table 3. In sea bass, the rate of isolation of*V. cholera, V. parahaemolyticus, V. vulnificus, V fluvialis, V alginolyticus, A. hydrophilia, A. caviae* and *A. sobria* were 15, 44, 14, 9, 9, 43, 31 and 15 respectively. From these values, it was observed that V. parahaemolyticus and *A. hydrophila*recorded the highest rate of isolation which were 44 and 43 respectively. For Snapper, the rate of isolation of *V. cholera, V. parahaemolyticus, V. vulnificus, V fluvialis, V alginolyticus, A. hydrophilia, A. caviae* and *A. sobria* were 18, 38, 17, 12, 12, 33, 16 and 10 respectively. Rate of isolation of*V. cholerae, V. parahaemolyticus, V. vulnificus, V fluvialis, V alginolyticus, A. hydrophilia, A. caviae* and *A. sobria* in grouper were 18, 31, 22, 6, 6, 28, 8 and 11 respectively. The rate of isolation of *V. cholera, V. parahaemolyticus, V. vulnificus, V fluvialis, V alginolyticus, A. hydrophilia, A. caviae* and *A. sobria* in tilapia were 28, 61, 41, 20, 20, 51, 33 and 21 respectively. In tilapia, the highest rate of isolation (61) were those of *V. parahaemolyticus*followed by*A. hydrophila*whose rate of isolation were 51 and the least bacteria isolated from tilapia were *V. fluvialis* and *V, alginolyticus* which had 20 rate of isolation each. Generally, *V. parahaemolyticus*and *A. hydrophila*appeared to be consistently isolated at a higher rates compared to the other species.

The rate of isolation of the 5 vibrio spp. and A. hydrophilia in this study showed variation between the types of fish and the spp of bacteria involved. The highest rate of isolation were V. parahaemolyticus and A. hydrophilia in the four types of fish used in this study and the highest rate of bacterial isolation was from tilapia followed by sea bass. The high rate of isolation of V. parahaemolyticus recorded in this study is in accord with earlier study [17] conducted in the West coast of Sabah, Malaysia, where V. parahaemolyticus was reported as one of the three species with highest rate of isolation. However, A. hydrophilia was not isolated in the said study [17] unlike in this study where A. hydrophilia was also isolated in a high rate. Interestingly, in another related study in the Peninsula Malaysia [6] A hydrophila was reported to be isolated at a high rate in tilapia.

**Table 3:** Rate of isolation from the four species of fish

| | *V. cholera* | *V. parahaemolyticus* | *V. vulnificus* | *V. fluvialis* | *V. alginolyticus* | *A. hydrophila* | *A. caviae* | *A. sobria* |
|---|---|---|---|---|---|---|---|---|
| **Sea bass** | 15 | 44 | 14 | 9 | 9 | 43 | 31 | 15 |
| **Snapper** | 18 | 38 | 17 | 12 | 12 | 33 | 16 | 10 |
| **Grouper** | 18 | 31 | 22 | 6 | 6 | 28 | 8 | 11 |
| **Tilapia** | 28 | 61 | 41 | 20 | 20 | 51 | 33 | 21 |

### 3.5:Biochemical tests on the isolates

All isolates were subjected to biochemical tests and the results are presented in table 4. For colour of growth in TCBS, *V. cholorae, V. fluvialis, V. alginolyticus* and *A hydrophilia* were yellow while *V.parahaemolyticus* and *V. vulnificus* were green and *A. sorbia* and *A. caviae* were colourless in TCBS. For colony morphology, All Vibrio species except *V. parahaemolyticus* which showed smooth colonies, were mixtures of both smooth and rough colonies. *Aeromonasspp, A. sobia* and *Acaviae* had smooth colonies. The sizes of the colonies ranges from 2.4 to 3.2 across all the isolates. On gram staining, all the isolates in this study were gram negative. Only *V.cholerae, A. hydrophilia, A. sorbia* and *A. caviae* showed growth in 0% NaCl and did not grow in 6% NaCl while *V. paraphaemolyticus, Vvulnificus, V fluvialis*and *V. alginolyticus* did not grow in 0% NaCl but grew in 6% NaCl.

All isolates were positive on oxidase test while on ONPG, only V. parahaemolyticus and V alginolyticus were negative. For Voges-Proskauer, only *V. alginolyticus* and *A. hydrophilia* were negative, the rest of the isolates were positive. For lysine decarboxylase, only *V. vulnificus* and *V. fluvialis* were negative while all other isolates were positive. All islates were positive for catalase and negative for Ornithine decarboxylaseThe isolates were subjected to biochemical tests and the results obtained were consistent with previously reported biochemical characteristics of the species identified[6], [17]. Gram staining also revealed that all isolates were gram negative which were consistent with the bacterial species identified

**.Table 4: Biochemical tests**

| | *V.cholerae* | *V.parahaemolyti* | *V.vulnificus* | *V.fluvialis* | *V.alginolyticuss* | *A.hydrophila* | *A.caviae* | *A.sorbia* |
|---|---|---|---|---|---|---|---|---|
| **Growth in TCBS Colour** | Y | G | G | Y | Y | Y | colourless | colourless |
| **Morphology** | R.S | S | R.S | R.S | R.S | s | S | s |
| **Staining** | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| **Size of colony( µm)** | 2.4 | 3 | 3.2 | 3.2 | 2.8 | 3.2 | 2.7 | 2.7 |
| **Growth in[(1)] 0 %NaCl** | + | - | - | - | - | + | + | + |
| **6 %NaCl** | - | + | + | + | + | - | - | - |
| **Oxidase** | + | + | + | + | + | + | + | + |
| **ONPG** | - | + | + | + | - | + | + | + |
| **Voges-Proskaue** | - | V | - | - | + | + | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **r** | | | | | | | | |
| **Lysine Decarboxylase** | + | + | - | - | + | + | | + |
| **Catalase** | + | + | + | + | + | + | + | + |
| **Ornithine decarboxylase** | - | - | - | - | - | - | - | - |

### 3.6:Antibiotic resistance

All the isolates were subjected to antibiotic susceptibility tests and the results are presented in Table 5. From the test results, only *V. cholorae* and *V. parahaemolyticus* isolates were resistant to Chloramphenicol at 30 µg while the remaining isolates were susceptible to this antibiotic.For tetracycline at 30 µg, only *V. parahaemolyticus , V. vulnificus* and *V. fluvialis* were resistant to the antibiotic while the rest were susceptible. Interestingly, all isolates were susceptible to Streptomycin at 20 µg with varying degrees on zone of inhibition. On the other hand, only *A. hydrophilia, Asorbia*and *A. caviae* were resistant to Bacitracin at 10 µg while all vibrio isolates were susceptible. All isolates were found to also be susceptible to Gentamycin at 10 µg concentration. All isolates were resistant to Polymyxin B at 10 µg except *V. vulnificus, V. fluvialis* and *V. alginolyticus* which were susceptible to the antibiotic. In a similar manner, all isolates were found to be resistant to Penecillin G at 10 µg except *V. vulnificus, V. fluvialis, V. alginolyticus* and *A. sorbia*. All isolates were also susceptible to Methicillin at 5 µg with varying degrees in the zone of inhibition. For Ampicillin at 10 µg, all isolates were resistant except *V. vulnificus, V. fluvialis* and *V. alginolyticus* while all isolates were susceptible to Nitrofurantoin at 200 µg and Kanamycin at 30 µg. For Novobiocin at 5 µg, only *A. hydrophilia* was resistant while all other isolates were susceptible. All isolates were susceptible to Vancomycin at 30 µg except *V. vulnificus* and *V. fluvialis*. All isolates were found to be susceptible to Streptomycin at 20 µg, gentamycin at 10 µg, methicillin at 5 µg, nitrofurantoin at 200 µg and kanamycin at 30 µg while most of the isolates were resistant to Polymixin B, Penecillin G, Ampicillin and Vancomycin. The resistance to ampicillin, penicillin and vancomycin as observed in this study

was consistent with the findings of a previous related study[30], [17] who reported that Vibrio isolates were resistant to these antibiotics. The highest rate of resistance was found with Vancomycin in which only 2 of the 8 isolates were susceptible. This level of resistance has been similarly reported in an earlier related study[17]. The presence of both penicillin G and vancomycin-resistant Vibrio strains in these water bodies and fish is an evidence of environmental contamination from from human sources as previously opined [31] The failed effect of these antibiotics is worrisome since they are important drugs for the treatment of gastroenteritis induced by pathogenic strains of these bacteria [32].

**Table 5: Antibiotic resistance (Zone of inhibition size)**

| | *V.cholerae* | *V.parahaemolyti* | *V.vulnificus* | *V.fluvialis* | *V.alginolyticuss* | *A.hydrophilia* | *A.s* | *A.cavi* |
|---|---|---|---|---|---|---|---|---|
| **Chloramphenicol (c)-30µg** | R | R | 23 | 12 | 12 | 17 | 21 | 23 |
| **Tetracycline (TE)-30µg** | 28 | R | R | R | 12 | 19 | 12 | 16 |
| **Streptomycin(S)-20µg** | 27 | 25 | 22 | 28 | 25 | 17 | 12 | 15 |
| **Bacitracin(B)-10µg** | 10 | 8 | 14 | 12 | 12 | R | R | R |
| **Gentamicin(CN)-10µg** | 26 | 22 | 18 | 14 | 28 | 20 | 21 | 20 |
| **Polymyxin B(PB)-10 µg** | R | R | 13 | 16 | 12 | R | R | R |
| **Penicillin G(P)-10µg** | R | R | 18 | 21 | 14 | R | 8 | R |
| **Methicillin (MT)-5µg** | 11 | 16 | 14 | 14 | 18 | 12 | 12 | 11 |
| **Ampicillin(AMP)-10µg** | R | R | 12 | 8 | 12 | R | R | R |
| **Nitrofurantoin(F)-200µg** | 12 | 18 | 15 | 12 | 17 | 18 | 20 | 21 |
| **Kanamycin(K)-30µg** | 29 | 14 | 21 | 17 | 14 | 12 | 18 | 14 |
| **Novobiocin(NV)-5µg** | 21 | 22 | 18 | 18 | 22 | R | 18 | 17 |
| **Vancomycin(VA)-30µg** | R | R | 24 | 18 | R | R | R | R |

## 4. CONCLUSION,

The isolation of several pathogenic species of bacteria from the water, sediments and fish in this study may be associated with the nature of feed, sediments and poor water quality due to human activity. In view of the dangers posed by the pathogenic bacteria isolates identified in this study, and the multi-drug resistant nature of some of the bacteria, passive surveillance of this nature is crucial for the mitigation of possible disease outbreaks associated with the pathogenic bacteria observed especially the multi-drug resistant bacteria species.

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