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PREVALENCE OF *ESCHERICHIA COLI* AND *SALMONELLA* IN FISH AND BLOOD CLAM (*ANADARA GRANOSA*) FROM WET MARKETS AND HYPERMARKETS IN KUALA PILAH

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Abstract

Food poisoning is one of Malaysia's top five infectious illnesses, with *Salmonella serovars* as the most known infectious agent. Pathogenic microorganisms, particularly *Salmonella* and *E. coli*, have been detected in various seafood, mostly fish and clamps. Thus, this study aims to assess the prevalence and antimicrobial resistance of *Salmonella* and *E. coli* isolated from wild-caught raw fishes and blood clam (*Anadara granosa*) from wet markets and hypermarkets in Kuala Pilah Negeri Sembilan. A total of 15 fish were sampled from three hypermarkets. Meanwhile, 18 blood clams were sampled from three wet markets in Kuala Pilah, Negeri Sembilan. The surface of fish (skin, gills, and guts) and blood clam (inner, outer, and meat) were swabbed to isolate *Salmonella* and *E. coli*. The isolates were then identified based on their morphological characteristics, and further confirmation was done using a biochemical test. The assessment of bacterial resistance was conducted using an antibiotic susceptibility test involving seven antibiotics: tetracycline (30 µg), streptomycin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), ampicillin (10 and 2 µg), chloramphenicol (30 µg), sulphamethoxazole/trimethoprim (25 µg) and Multiple antibiotic resistants (MAR). Findings showed that 6.7% (1/15) of isolates from fish samples tested positive for both bacteria. However, only 5.6% (1/18) of blood clam samples contained *Salmonella*. Most isolates were susceptible to antibiotics except for ampicillin, while MAR index results showed a value within 0.2 for both samples, indicating the samples had minimal exposure to antibiotics usage. In conclusion, the presence of *Salmonella* and *E. coli* in collected samples and their resistance to antibiotics may derive from contamination occurring in the natural aquatic environment, during processing, or due to unhygienic and improper handling. Therefore, effective control strategies should be implemented to prevent potential contamination, especially when handling and processing the fish and blood clam.

Keywords: *Anadara granosa*, Antimicrobial resistance bacteria, *Escherichia coli*; Fish, *Salmonella*

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Introduction

The presence of several bacterial species in fish, including human pathogenic bacteria, has been related to direct contact with a contaminated water environment and consumption of bacteria through sediments or contaminated feed (Beyari *et al*., 2021). Thakali and MacRae (2021) added that water pollutants such as heavy metals are dispersed throughout the aquatic systems and, therefore, may build in fish and other edible marine biotas, hence causing the fish to be unsafe to consume. Moreover, additional risks were also introduced during food preparation. Love *et al*. (2021) reported that contamination of seafood, including fish, during preparation was a contributing factor in 9% of seafood outbreaks, while food workers were responsible for 2%.

Salmonella contains many pathogenic species that cause different types of food poisoning. *S. enterica* subsp. enterica is responsible for 99% of salmonellosis in humans, causing symptoms such as diarrhoea, fever, and stomach pains (Jajere, 2019). Human salmonellosis infections are associated with consuming contaminated products such as meat, poultry, eggs, milk, shellfish, and fresh produce (World Health Organization, 2018). *Salmonella* naturally cannot be found in seafood. However, *Salmonella* can be introduced into fish by contact with contaminated water, poor handling hygiene, and other inappropriate fish breeding, processing, or storage procedures (Fernandes *et al*., 2018). In numerous recent articles, *Salmonella* has been found in fish and blood clams. A study by Amalia and Darmanto (2020) reported that 4 out of 9 fresh fish samples (44%) collected from three traditional markets in Semarang, Indonesia, were contaminated by *Salmonella* spp. *Salmonella* contamination in blood cockles has been studied by Atwill and Jeamsripong (2021), and they found that 78 per cent of the seafood sampled from Bangkok's retail markets contained the bacteria.

On the other hand, *Escherichia coli* is naturally found in the intestinal tracts of all warm-blooded animals, including humans. Most bacteria strains are non-pathogenic and perform essential roles in the intestine (Braz *et al*., 2020). *E. coli* affects the intestine and causes various symptoms such as abdominal pain, watery diarrhoea, bright red bloody faeces, nausea, fever, and exhaustion (Wyatt *et al*., 1979, as cited in Ava et al., 2020). However, *E. coli* in food or water is considered a sign of recent faecal contamination and the possibility of other pathogens (Feng *et al*., 2020). Assefa *et al*. (2019) reported that the total frequency of *E. coli* O157: H7 in fish collected from the landing site and retail market in Northern Ethiopia was 1.46 % (6/410). *E. coli* in blood cockles have the maximum limit to contamination in the product, which is <3.0MPN per gram of blood cockle (Khasanah *et al*., 2021).

Furthermore, it is a major global problem focusing on foodborne pathogens such as *Salmonella* and *E. coli*, particularly antibiotic-resistant bacteria, since affected people require specialized therapy to recover. Moreover, due to bacterial resistance to antimicrobial treatment during the past decade, today's society is in danger of returning to pre-antibiotic times. Hence, keeping track of antibiotic resistance among various hazardous bacteria is vital to sustaining and enhancing global public health. Therefore, this study focuses on detecting and determining *Salmonella* and *E. coli* contamination isolated from fish and blood clams and assessing the antibiotic's susceptibility against the bacteria isolated from the samples obtained.

Materials and methods

Sampling area and sample collection

A total of 15 fish were purchased randomly from four different local wet markets and two supermarkets located in Kuala Pilah, Malaysia, which consisted of 5 samples from hypermarket A, five samples from hypermarket B, and five samples from the wet market, meanwhile 18 blood clams were sampled from three wet markets (6 samples from each wet market). They were packed in a sterile plastic bag, immediately transferred to the laboratory, and analysed upon arrival. The surface of fish (skin, gills, and guts) and blood clam (inner, outer, and meat) were swabbed. The samples were then directly streaked on MacConkey and Eosin Methylene Blue agar plates (EMB) and incubated at 37°C for 24 hours. Selected colonies from both samples then proceeded to gram staining and biochemical tests, followed by an antibiotic susceptibility test.

Gram staining and Biochemical test

Both bacteria's presumptive colonies obtained in the previous steps were then taken for gram staining test, and confirmation of presumptive colony was done by performing biochemical testing which involves Indole test, Methyl Red test, Voges-Proskauer and Citrate test.

First, gram staining was conducted according to the methods previously described by Smith and Hussey (2005). Gram staining bacteria was applied to differentiate two large groups of bacteria based on their different characteristics. *E. coli* and *Salmonella* are classified as gram-negative bacteria because they stained negatively in the test and appeared pink to red under the microscope.

After that, a portion of the colony from the agar medium was injected into the tryptone broth tube and then incubated for 24 hours at 35°C. The presence of red or red-violet colour in the top layer of the solution within seconds after adding the reagent indicated a positive indole test; meanwhile, the reagent layer remaining yellow or somewhat cloudy indicated a negative indole test. Next, the methyl red test was conducted to determine the ability of bacteria to produce and maintain the final acid product from glucose or lactose fermentation. The appearance of red colour indicated a positive methyl red test, while no colour change indicated a negative methyl red test. The Voges-Proskauer test was then used to determine the ability of bacteria to produce methyl carbinol. An absence of change in colour after the addition indicated a negative test, while the formation of a red-brown colour after the addition indicated a positive test. The citrate test is the last biochemical test performed that determines the ability of bacteria to use citrate as the only source of carbon and ammonia salt as the only nitrogen source. Colour changes of slant from green to blue indicated a positive result; meanwhile, no colour changes indicated a negative result in this test.

Antibiotic susceptibility test and Multiple Antibiotic Resistant (MAR) tests

An antibiotic-resistant test was conducted by Kirby-Bauer disc diffusion assay against seven antibiotics: tetracycline (30 µg), streptomycin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), ampicillin (10 and 2 µg), chloramphenicol (30 µg), sulphamethoxazole/trimethoprim (25 µg). Briefly, one loopful of *Salmonella* and *E. coli* was cultured in nutrient broth overnight. Ten microliters of the broth were then pipetted and streaked on Mueller Hinton Agar (Merck) with a sterile cotton butt. Antibiotic discs were placed on the lawn and incubated at 37oC for 24 h. Clinical and Laboratory Standard Institute (CLSI) determined the resistance strain after measuring the clear zone. The MAR index for each isolate was also calculated to track the sources of antibiotic-resistant bacteria by dividing specific antibiotic resistance by the total number of antibiotics to which isolates have been exposed (Krumperman, 1983).

Results and discussion

According to the morphological characteristics on agar plates, there were four and two presumptive colonies of *Salmonella* and *E. coli*, respectively. Meanwhile, there were ten and seven presumptive *E. coli* and *Salmonella* for blood clam samples. Typical colonies of *Salmonella* appeared transparent and colourless, sometimes with a dark centre on MacConkey agar plates. On the other hand, *E. coli* colonies had a black or dark centre with a greenish metallic sheen grown on the EMB agar plates (Table 1).

Table 1. Description of colony morphology of isolated bacteria from fish and blood clam samples

Sample Code	Sample Description	Morphology on EMB agar *(E. coli)*	Morphology on MacConkey Agar (*Salmonella)*	Presumptive *Salmonella*	Presumptive *E. coli*
Wet market A (1)	Outer shell	Metallic green sheen, small colonies	Colourless, small colonies	Yes	Yes
Wet market A (2)	Inner shell	Metallic green sheen, small colonies	Colourless, small colonies	Yes	Yes
Wet market A (3)	Cockles meat	Pale pink, small colonies	Red, small colonies	No	No
Wet market A (4)	Outer shell	Metallic green sheen, large colonies	Red, small colonies	No	Yes
Wet market A (5)	Inner shell	Pink, large colonies	red, small colonies	No	No
Wet market A (6)	Cockles meat	Pale pink, large colonies	Colourless, large colonies	Yes	No
Wet market B (1)	Outer shell	Metallic green sheen, large colonies	Red, large colonies	No	Yes
Wet market B (2)	Inner shell	Pink, small colonies	Red, small colonies	No	No

Wet market B (3)	Cockles meat	Metallic green sheen, large colonies	Red, small colonies	No	Yes
Wet market B (4)	Outer shell	Pink, large colonies	Colourless, small colonies	Yes	No
Wet market B (5)	Inner shell	Metallic green sheen, small colonies	Red, large colonies	No	Yes
Wet market B(6)	Cockles meat	Metallic green sheen, small colonies	Red, small colonies	No	Yes
Wet market C (1)	Outer shell	Pale pink, large colonies	Colourless, small colonies	Yes	No
Wet market C (2)	Inner shell	Metallic green sheen, small colonies	Colourless, large colonies	Yes	Yes
Wet market C (3)	Cockles meat	Metallic green sheen, big colonies	Colourless, large colonies	Yes	Yes
Wer market C (4)	Outer shell	Pale pink, small colonies	Red, small colonies	No	No
Wet market C (5)	Inner shell	Pink, small colonies	Red, large colonies	No	No
Wet market C (6)	Cockles meat	Metallic green sheen, large colonies	Red, large colonies	No	Yes
Wet market A (1)	Fish gills	Dark colonies with a greenish metallic sheen	Colourless, mucoid colonies	No	Yes
Wet market A (2)	Fish guts	Dark colonies	Amber, mucoid colonies	No	Yes
Wet market A (3)	Fish outer skin	Pale pink, round colonies	Colourless, mucoid colonies	No	No
Wet market A (4)	Fish guts	Red, mucoid colonies	Colourless, round colonies	Yes	No
Wet market A (5)	Fish guts	Red, mucoid colonies	Colourless, mucoid colonies	No	No
Hypermarket A (1)	Fish gills	Pale pink, mucoid colonies	Colourless, round colonies	Yes	No
Hypermarket A (2)	Fish outer skin	Amber, mucoid colonies	Colourless, mucoid colonies	No	No
Hypermarket A (3)	Fish guts	Red, mucoid colonies	Colourless, round colonies	Yes	No
Hypermarket A (4)	Fish guts	Pale pink, mucoid colonies	Colourless, round colonies	Yes	No
Hypermarket A (5)	Fish gills	Red, round colonies	Amber, mucoid colonies	No	No
Hypermarket B (1)	Fish outer skin	Pink, mucoid colonies	Colourless, mucoid colonies	No	No
Hypermarket B (2)	Fish gills	Pink, mucoid colonies	Colourless, mucoid colonies	No	No
Hypermarket B (3)	Fish guts	Pink, mucoid colonies	Colourless, mucoid colonies	No	No
Hypermarket B (4)	Fish outer skin	Red, round colonies	Colourless, mucoid colonies	No	No
Hypermarket B (5)	Fish gills	Pink, mucoid colonies	Colourless, mucoid colonies	No	No

These presumptive colonies then proceeded with IMViC (Indol, Methyl red, Voges-Proskauer (VP), and Citrate) tests with gram staining. Out of 15 fish samples, only one sample (A1) resulted in positive methyl, positive indole, negative VP and negative citrate test that fulfilled the phenotypic characterization of *E.coli* meanwhile; one sample (A3) indicated positive methyl, negative indole, negative VP and positive citrate test fulfilled the characteristic of *Salmonella*. In blood clam samples, only one isolate (C1) was confirmed to be positive for *Salmonella*, and no samples tested positive for *E.coli*. The details of the data were described in Table 2.

Table 2: Observation of isolated colonies on gram staining, methyl red test, Voges Proskauer test and citrate test

Sample Code	**Sample Description**	**Gram Staining**	**Indole Test**	**Methyl Red Test**	**Voges Proskauer Test**	**Citrate Test**	**Presumptive *E. coli***	**Presumptive *Salmonella***
Wet market A (1) *E. coli*	Outer shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	Colour change from green to blue	No	-
Wet market A (2) *E.coli*	Inner shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	-	No	-
Wet market A (4) *E. coli*	Outer shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	-	No	-
Wet market B (1) *E. coli*	Outer shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	-	No	-
Wet market B (3) *E. coli*	Cockles meat	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	-	No	-
Wet market B (5) *E. coli*	Inner shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	-	No	-
Wet market B (6) *E. coli*	Cockles meat	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	-	No	-
Wet market C (2) *E. coli*	Inner shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	-	No	-
Wet market C (3) *E. coli*	Cockles meat	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	-	No	-

Wet market C(6) *E. coli*	Cockles meat	Gram-negative, rod-shaped	Formation of a thin yellow layer	-	-	-	No	-
Wet market A (1) *Salmonella*	Outer shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	Yellow coloration	-	-	-	No
Wet market A (2) *Salmonella*	Inner shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	Red coloration	The yellowish colouration on top of the layer	No colour changes	-	No
Wet market A (6) S*almonella*	Cockles meat	Gram-negative	Formation of the red reagent layer	-	-	-	-	No
Wet market B (4) *Salmonella*	Outer shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	Red coloration	The yellowish colouration on top of the layer	No colour changes	-	No
Wet market C (1) *Salmonella*	Cockles meat	Gram Negative, rod-shaped	Formation of a thin yellow layer	Red coloration	The yellowish colouration on top of the layer	Colour changes from green to blue	-	Yes
Wet market C (2) *Salmonella*	Inner shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	Yellow coloration	-	-	-	No
Wet market C (3) *Salmonella*	Outer shell	Gram-negative, rod-shaped	Formation of a thin yellow layer	Yellow coloration	-	-	-	No
Wet market A (1) *E. coli*	Fish gills	Gram-negative, rod-shaped	Formation of the red reagent layer	Red coloration	Yellowish colour on top of the culture	No colour changes	Yes	-
Wet market A (2) *E. coli*	Fish guts	Gram-negative, rod-shaped	Formation of the red reagent layer	Yellow colour produced	The red colouration on top of culture	Colour changes from green to blue	-	No
Wet market A (4) *Salmonella*	Fish guts	Gram-negative, rod-shaped	Formation of the red reagent layer	Yellow colour produced	The red colouration on top of culture	Colour changes from green to blue	No	-
Hyper market A (1)	Fish gills	Gram-negative,	Formation of the red	Yellow colour	The red colouration on top	Colour changes from	No	-

Salmonella		rod-shaped	reagent layer	produced	of culture	green to blue		
Hypermarket A (3) Salmonella	Fish guts	Gram-negative, rod-shaped	Formation of a thin yellow layer	Red colouration	The yellowish colouration on top of culture	Colour changes from green to blue	-	Yes-
Hypermarket A (4) *Salmonella*	Fish guts	Gram-negative, rod-shaped	Formation of a thin yellow layer	Yellow colour produced	The colouration on top of culture	Colour changes from green to blue	No	-

This study recorded the prevalence of *E. coli* and *Salmonella* as 6.7% (1/15) respectively of isolates from fish samples, whereas 5.6% (1/18) of blood clam samples contained *Salmonella* and no positive samples for *E. coli*. The prevalence of both pathogenic bacteria has been extensively documented in other studies all over the globe. A recent study by Dewi *et al*. (2022) involving 32 cultured fish farms from the Malaysian states of Selangor, Negeri Sembilan, Melaka, and Perak, revealed the prevalence of *E. coli* and *Salmonella* in tilapia fish of about 44.5% and 0.6%, respectively. Meanwhile, Pramono *et al*. (2019) discovered *Salmonella* infection in 93.1% of the fish and seafood items from Surabaya's traditional market, with eight of the *Salmonella* serotypes being antibiotic-resistant. Other than that, Dumen *et al*. (2020) have discovered *E. coli* in 67 raw fish samples, 21 raw mussels, 24 raw shrimp, and 19 raw squids collected from various sources in Istanbul, Turkey. Similar research has also been conducted in the Philippines (Tanyag *et al*., 2021), India (Prabhakar *et al*., 2020) and Italy (Ali *et al*., 2020). Nevertheless, the percentage in this study was lower due to many variables such as the small sample size used, different methodological approaches and sampling season. However, the presence of *E. coli* and *Salmonella* from fish and blood clams in this study depicted possible contamination of the food source, thus posing a risk of these pathogenic bacteria infection in humans if the fish is consumed raw or semi-cooked as well as handled with unsanitary practices.

The contamination of seafood with *Salmonella* and *E. coli* may derive from several factors, including transporting fish in dirty fishing boats, storing fish in dirty containers, and displaying seafood uncovered in open markets for purchasers (Sheng & Wang, 2020). The potential for *E. coli* infection in fish samples is most likely due to *E. coli* contamination in the water used (Wattimena *et al*., 2021; Jahan *et al*., 2019).

As for blood clam, although bivalve (e.g. clam, oysters, scallops, and mussels) is not a natural habitat for the growth of *Salmonella*, the incidence of *Salmonella* in bivalves still occurs a steady rise. For instance, Atwill *et al*. (2021) discovered the *Salmonella* and *E. coli* contamination in the retail market in Bangkok, Thailand, with 33% and 81% in blood cockles/ clam, respectively. Moreover, Miotto *et al*. (2018) reported all 40 samples of oysters and 60 samples of mussels (100%) collected from ten different locations in Brazil and 11/18 (61%) of oysters samples collected in Chesapeake Bay, Maryland, USA, were identified positive for *E. coli* with concentrations ranging from 20 to 18,000 MPN/100 g and <20 to 130 MPN/100, respectively. The presence of *Salmonella* in clams is because bivalves rapidly amass bacteria, viruses, and poisons in their body because they feed by taking in and filtering water. Therefore humans can be infected by *Salmonella* as they usually eat raw seafood, which causes the transmission of *Salmonella* into their body. According to Dr Laurence Knott (2019), he had identified the "4 Cs" as a way to improve food safety and avoid food poisoning, particularly *Salmonella* illness, which are cleanliness, cooking, chilling and cross-contamination. These factors combine to create complex epidemiology that must be investigated to determine the sources of pathogenic bacteria infection in humans. As a result, preventive and control measures must be implemented.

Apart from that, there are growing concerns that sublethal levels of antibiotic residues in aquaculture ponds or the environment contribute to developing resistance in both pathogenic and non-pathogenic bacteria (Pepi & Focardi, 2021). Stephen *et al*. (2021) also emphasized the prevalence of numerous antibiotic-resistant bacteria in Asian seafood and the unregulated use of antibiotics in aquaculture, both

of which must be addressed scientifically to develop methods to prevent the formation and spread of antibiotic-resistant bacteria in food fish.

Table 3 reveals that *Salmonella* and *E. coli* bacteria isolated from fish samples were susceptible to tetracycline, streptomycin, nalidixic acid, and ciprofloxacin. They were, however, ampicillin-resistant. On the other hand, *Salmonella* isolated from blood clams were exposed to chloramphenicol, ampicillin, tetracycline, ciprofloxacin, streptomycin, and sulphamethoxazole/ trimethoprim.

According to the results shown in Table 4, susceptibility to ciprofloxacin at a concentration of 5 µg and chloramphenicol at 30 µg for *Salmonella* isolated from blood clam samples provide the same inhibitory zone, 11 mm, which put them at rank 1 in inhibiting the growth of *Salmonella*. Meanwhile, despite its high concentration of 25 µg, sulphamethoxazole is the least susceptible to *Salmonella* as it recorded only 2 mm, ranking in last place. On the other hand, streptomycin is the second weakest antibiotic at rank 4 in inhibiting *Salmonella* growth since its inhibition zone, 3 mm, is the second smallest among the antibiotics tested. As for *E. coli* and *Salmonella* isolated from fish samples, ciprofloxacin is in the first rank of antibiotics that can inhibit both bacteria as it yielded the biggest inhibition zone, which was 24 mm and 26 mm, respectively. In contrast, tetracycline and nalidixic acid are least susceptible to *E. coli* and *Salmonella*, as they recorded the smallest size of inhibition zone, thus ranking in the lowest rank compared to other antibiotics.

Table 3. Multiple antibiotic resistance index of *Salmonella* and *E. coli* strain

Sample	**Strain**	**Name of antibiotic that strains resistant to**	**No. of antibiotics**		**MAR index (a/b)**
			Resistant (a)	**Tested (b)**	
Fish	*E. coli*	A	1	5	0.2
	Salmonella	A	1	5	
Blood clam	*Salmonella*	-	0	6	0.0

*Note: A (Ampicillin)

Table 4. Zone of inhibition of *Salmonella* isolated from blood clam fish samples

Sample	**Strain**	**Name of antibiotics (dose)**	**Inhibitory zone diameter to the nearest millimetre (mm)**	**Rank**
Blood clam	*Salmonella*	TET (30 µg)	9	2
		STM (10 µg)	3	4
		A (2 µg)	4	3
		CIP (5 µg)	11	1
		SMZ (25 µg)	2	5
		C (30 µg)	11	1
Fish	*E. coli*	TET (30 µg)	10	4
		STM (10 µg)	12	3
		NA (30µg)	20	2
		CIP (5µg)	24	1
		A (10µg)	0	5
	Salmonella	TET (30 µg)	20	2
		STM (10 µg)	14	3
		NA (30 µg)	12	4
		CIP (5µg)	26	1
		A (10µg)	0	5

*Note: rank represents the decreasing order of antibiotics from highest to lowest in having the potential to inhibit bacteria's growth; TET (Tetracycline); STM (Streptomycin); NA (Nalidixic acid); CIP (Ciprofloxacin); A (Ampicillin); SMZ (Sulphametoxazole); C (Chloramphenicol)

According to Krumperman (1983), a multiple antibiotic resistance (MAR) index value of less than or equal to 2.0 is considered to indicate that the bacterial isolates tested originated from an animal in which antibiotics are seldom or never used, but a MAR index greater than 2.0 indicates that the bacterial isolates originated from high-risk sources where antibiotics are widely used. A value higher than 0.2 indicated that bacterial isolates are likely to have come from a high-risk source, such as faecal contamination, where antibiotics are frequently used (Gufe *et al*., 2019). However, all three isolates from fish and blood clam had shown a MAR index not more than 0.2, and no Multi-Drug Resistance (MDR) patterns were identified (Table 3). Therefore, we conclude that fish and blood clams in this study are free from overused antibiotics since they're from the wild. However, a proper investigation with a large number of samples should be conducted to obtain valid and reliable results.

Additionally, early and exact genetic marker identification is required to monitor and limit the emergence of bacterial resistance. Therefore, it is necessary to use a wide range of genetic assays to confirm resistance gene determinants, support questionable phenotypic results, and provide a reliable scientific basis for global molecular surveillance of antimicrobial-resistant bacteria and resistance determinants (Galhano *et al*., 2021). In particular, multiplex PCR appears to be a viable method for simultaneously detecting numerous resistance determinants. A more practical technique to control pathogenic bacteria would be the application of biocontrol and rational use of antibiotics to make the industry more sustainable and preserve global public health.

Conclusion

This study demonstrated that the prevalence of *Salmonella* and *E. coli* isolated from raw fish and blood clams (*Anadara granosa*) sampled from wet markets and hypermarkets in Kuala Pilah, Negeri Sembilan, Malaysia, was categorized as mild since only one out of 15 fish samples (6.7%) were positive for *Salmonella* and *E. coli* respectively. Only one of 18 blood clams (5.6%) tested positive for *Salmonella*. However, there was also antibiotic resistance detected in *E. coli* and *Salmonella* isolated from samples; hence this issue needs to be addressed by the parties concerned to create the proper measures to prevent this condition from getting worse in the near future. The current findings suggest that those participating in fish and blood clams' production should adopt proper and good hygiene management, either at collection or cold storage centres, to maintain the quality and prevent the growth of pathogens such as *E. coli* and *Salmonella* that are harmful to humans.

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Author Contribution

FB Johari & NFF Zapri- data curation and writing; S Mohamed- study conception and design, supervision, writing manuscript, review and editing.

Conflict of Interest

Authors declare no conflict of interest.

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