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Research paper



Antimicrobial Resistance of Staphylococcus aureus and Escherichia Coli Isolated from Local Shrimp

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Abstract

Staphylococcus aureus and Escherichia coli are pathogens that can cause foodborne illnesses due to their ability to produce toxin. These bacteria are not naturally found in seafood, but recent studies have shown the prevalence of this microorganism in shrimp and its antibiotic resistance properties. In this study, shrimp from two species; Litopenaeus vannamei and Penaeus monodon were obtained to assess the prevalence of S. aureus and E. coli and to test their antibiotics susceptibility. The result obtained by isolation indicated that 87% and 58% of shrimp samples were contaminated with S. aureus and E. coli, respectively. S. aureus and E. coli occurrence in shrimps from retail for twelve weeks fluctuated in range between 2.00 to 4.94 $\log_{10}$ CFU/g and 2.00 to 6.39 $\log_{10}$ CFU/g, respectively. The identified bacteria were subjected to antibiotic susceptibility test. The result showed that most of S. aureus and E. coli isolates were resistant to penicillin G from β-lactam antibiotic group. Multidrug-resistant organism was identified in two isolates of S. aureus and seven isolates of E. coli. This indicated the need of improving handling of shrimp by food handlers and monitoring activities for S. aureus and E. coli contamination in shrimp as a preventive measure to ensure the safety of public health.

Keywords: *Staphylococcus aureus, Escherichia coli, shrimp, occurrence, antibiotic resistance*

1. Introduction

White leg shrimp, *Litopenaeus vannamei* and tiger shrimp, *Penaeus monodon* are the main species cultured in Malaysia [7]. White leg shrimp, *L. vannamei* or can be recognized as *Penaeus vannamei* is one of the most important shrimp species in farmed worldwide especially for developing country like Malaysia. It appears translucent in white colour whereas tiger shrimp can be identified by its greyish greenish body or dark greenish. According to Department of Statistics Malaysia (2015), shrimps are the second highest selected fisheries commodities in 2013 and 2014. Aquaculture industry in Malaysia has grown rapidly due to government role of the aquaculture sector in Third National Agriculture Policy (1998-2008) followed with National Agro-Food Policy (2011-2020) [18].

Seafood is high-risk commodities when it comes to pathogenic bacteria contamination [40]. Shrimps can be contaminated with water and sediments which naturally contain *Vibrio* spp., non-typhi *Salmonella*, and *Campylobacter* [14]. Furthermore, shrimps are also vulnerable to coliform and *Staphylococcus aureus* contamination which can be easily isolated from skin and mucosal surfaces of humans and animals. *S. aureus* may contaminate food when handling practices are not adequately practiced once this pathogen spread from the processing equipment or food handlers to the food system [2].

Due to growing production of shrimp's industry and its vulnerability to microbial contamination, veterinary drugs have been used in aquaculture to cater these problems. Antibiotic has been used in aquaculture as prophylactic, metaphylactic and therapeutic purpose. In spite of the fact that antibiotic is effective in controlling disease mainly from a microorganism, it has led to another problem which is antibiotic resistant bacteria. Aquaculture animals are the most affected by antibiotic resistance bacteria due to wide usage of antibiotic in farming which may be excreted in faeces or by the residual antibiotic that is not consumed by the aquatic animals.

Occurrence of antibiotic resistance *S. aureus* and *E. coli* are alarming as it can lead to several foods borne diseases such as haemorrhagic colitis, haemolytic uremic syndrome (HUS) [23], hospital-acquired infection (HAI) or nosocomial infections and community-acquired disease [22]. Antibiotic resistance *S. aureus* and *E. coli* might affect the efficiency of antimicrobial agents to human or animals and complicate the treatment to eliminate foodborne diseases. Several studies have found the prevalence of antibiotic resistance *S. aureus* and *E. coli* in shrimps [2]. Hence, this study would be focussed on the presence of *Staphylococcus aureus* and *Escherichia coli* in shrimps from retail for twelve weeks (sampling of every two weeks) and the occurrence of antibiotic resistance of *Staphylococcus aureus* and *Escherichia coli* in shrimps.

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2. Materials and method

2.1 Sample collection

A total of 24 samples which consists of 200 g of each white leg shrimp (*Litopenaeus vannamei*) and tiger shrimp (*Penaeus monodon*) were collected from retail located in Putrajaya for every two weeks for twelve weeks. All samples was brought to the lab with ice-box to keep the temperature below 4°C and immediately analysed. 200 g of shrimps have minced aseptically. Then, 25 g of the sample was transferred into a stomacher bag containing 225 ml buffered peptone water. The sample was stomached for one or two minutes to homogenize the mixture.

2.2 Isolation of bacteria

An aliquot of 1 ml from the sample was transferred and homogenized into 9 ml of sterile peptone water and serially diluted until 10^{-3} [36]. In order to determine the presence of bacteria, the selective media; Baird-Parker agar (BPA) and Eosin Methylene Blue (EMB) was used to isolate *S. aureus* and *E. coli*, respectively. Baird-Parker agar has been recognized and recommended by national and international bodies. 0.1 ml of each dilution (10^{-1} to 10^{-3}) was spread onto duplicate selective agar plates and incubated at 37°C for 24 hours. After a period of incubation, presumptive colonies of *S. aureus* and *E. coli* were observed. On Baird-Parker agar, presumptive colonies of *S. aureus* appeared black colonies surrounded with the clear zone [26] whereas presumptive *E. coli* on EMB agar appeared metallic green sheen colonies [16].

2.3 Identification of *S. aureus* and *E. coli*

A series of tests; Gram-staining, catalase test, and coagulase test were carried out to confirm colonies of the bacteria from the selective media. Five colonies from each sample were subjected to a series of tests.

2.3.1 Gram-staining

This test started with methanol fixation which to preserve the morphology of the host cells and proceeds with the application of crystal violet and iodine as mordant. Then, the decolourization by alcohol and followed by safranin. Gram-positive bacteria like *S. aureus* appeared purple and a cluster of cocci-shaped organism whereas Gram-negative bacteria like *E. coli* appeared red or pink, rod-shaped and appear in a large clump under 100x oil immersion observation by using a microscope [25].

2.3.2 Catalase test

Slide method was used to perform the catalase test. A drop of distilled water and the bacteria colonies were placed and emulsified onto the slide to form a smooth suspension. 3% hydrogen peroxide were dropped onto the suspensions, and the presence of bubbling or foaming was observed [39]. Positive test indicated by immediate bubbling or foaming which indicates *S. aureus* and *E. coli* [30].

2.3.3 Coagulase test: *S. aureus*

Slide coagulase test was carried out. A drop of distilled water was placed onto the slide and emulsified with a small amount of *S. aureus* to form a smooth suspension. The test suspension was treated with a drop of citrated plasma. The clumping of cocci can be observed within 5-10 seconds as a positive result of *S. aureus* [41].

2.4 Antibiotic Resistance Test

Antibiotic susceptibility analysis was carried out by using the Oxoid™ antibiotic disk. Total of nine antibiotics was tested against *S. aureus* and *E. coli* isolates (Table 1). It was chosen according to its activity which is; β-Lactams and non-β-Lactams. Antibiotic tested comes from five antibiotic classes to indicate the presence of multidrug-resistant bacteria.

Table 2.1: Antibiotics used to determine the susceptibility of *S. aureus* and *E. coli* isolated from *L. vannamei* and *P. monodon*.

Group	Antibiotic class	Antibiotic
β-Lactams	Penicillin	Penicillin G (P)
		Amoxicillin (AMC)
	Cephalosporin	Ceftazidime (CAZ)
		Cefotaxime (CTX)
Non β-Lactams	Aminoglycosides	Gentamicin (CN)
		Streptomycin (S)
	Quinolone Fluoroquinolone	Nalidixic acid (NA)
		Ciprofloxacin (CIP)
	Sulfonamides	Sulfisoxazole (SF)

Antibiotic resistance test was carried out according to CLSI standards by using the disk-diffusion method on Mueller-Hinton agar (MHA). This method was chosen due to its simplicity and the ability to analyze different kind of antimicrobial agents with huge number of microorganism. Antibiotics used to observe the prevalence of antibiotic resistance for *S. aureus* and E. *coli* were amoxicillin penicillin G, ceftazidime, cefotaxime, gentamicin, streptomycin, nalidixic acid ciprofloxacin and sulfisoxazole [12;5;17].
The test was started with inoculation of *S. aureus* and *E. coli* on the agar plates. Inoculum was adjusted to approximately 8 $\log_{10}$ CFU/g, a turbidity equivalent to a 0.5 McFarland standard. Then, antibiotic disks were placed onto the agar surface and incubated at 35°C for 16 to 18 hours. The antibiotic would diffuse into the agar and inhibit microbial growth which indicated by measuring the diameter of inhibition zones [4]. Zone of inhibition for each antibiotics was categorized as susceptible, resistant and intermediate based on CLSI standards for antimicrobial susceptibility testing and zone interpretation table (Sigma-Aldrich, US) (Table 2.2).

Table 2.2: CLSI standards for inhibition zone interpretation

Antibiotics	Resistant	Intermediate	Susceptible
	Zone diameter (mm)	Zone diameter (mm)	Zone diameter (mm)
Ciprofloxacin (5 µg)	≤12	13-14	≥15
Amoxycillin (10 µg)	≤28	-	≥29
Gentamicin (10 µg)	≤12	13-14	≥15
Penicillin G (10 µg)	≤28	-	≥29
Ceftriaxone (5 µg)	≤13	14-20	≥21
Sulphafurazole (300 µg)	≤12	13-16	≥17
Streptomycin (25 µg)	≤12	13-14	≥15
Ceftazidime (30 µg)	≤14	15-17	≥18
Cephalothin (30 µg)	≤14	15-17	≥18
Nalidixic acid (30 µg)	≤14	15-22	≥23
Cefotaxime (30 µg)	≤14	15-22	≥23

2.5 Statistical analysis

Statistical analysis was carried out to indicate a significant difference of bacterial occurrence between two species by using Minitab 17th version. All experiment was performed in triplicate, and the results are expressed in average. The data were analysed by using one-way ANOVA and Tukey test at 95% confidence interval.

3. Results and discussion

3.1 Identification of bacteria

3.1.1 Occurrence of *Staphylococcus aureus* in *Litopenaeus vannamei* and *Penaeus monodon*

Among 24 tested samples collected, 21 (87%) samples were found to be positive for the presence of *Staphylococcus aureus* in *Litopenaeus vannamei* and *Penaeus monodon*. Meanwhile, 11 (92%) and 10 (83%) samples of *L. vannamei* and *P. monodon*, respectively were contaminated with *S. aureus* (Table 3.1).

Table 3.1: Occurrence of *S. aureus* in *L. vannamei* and *P. monodon* in twelve weeks sampling from retail

Sample	Non-contaminated samples	Contaminated samples	Number of samples
L. vannamei	1 (8%)	11 (92%)	12 (100%)
P. monodon	2 (17%)	10 (83%)	12 (100%)
Total	3 (13%)	21 (87%)	24 (100%)

Microbial growth occurs due to environmental factors such as pH, temperature and water activity. Shrimp meat contains high nutritional values, high protein, vitamins and unsaturated fatty acids that could be an optimum condition for the microorganism to grow. Shrimp is one of the extremely perishable foods that need to be handled inadequate sanitary condition in every step of handling before it reaches the consumers. Shrimp can be contaminated by *S. aureus* from human contamination, and *E. coli* from both human and environmental contamination [2;5].

In this study, *S. aureus* was detected in 87% of retail shrimps sample from two species (*L. vannamei* and *P. monodon*). In Malaysia, data on the microbial safety of *S. aureus* in shrimps are limited. Prevalence of *S. aureus* from this study was higher than samples of raw shellfish (15%) obtained from retail market located in Bangi, Kajang and Serdang, Selangor [28]. The result from this study was also higher than samples of shrimp in Brazil (66.7%) [2] and Switzerland (9%) [5]. Differences in these results may be due to sampling size, different facilities, and maintenance of processing [3].

According to Arfatahery et al., [3] the authors were mentioned that contamination of *S. aureus* in shrimp could be influenced by the handling sanitary by food handlers, contact with contaminated surfaces and also environmental contamination before consumption. In addition, fresh samples contamination may be accumulated with microbial growth when food hygiene standards are not practiced by workers prolong of product transport and improper storage condition [3]. The contamination of *S. aureus* in seafood might be due to cross-contamination of food handlers. The main source of *S. aureus* contamination from food handlers into foods could be from their noses or hands which can be transmitted by manual contact or respiratory secretions [11]. According to Othman et al., [28], high protein foods such as seafood are usually associated with *S. aureus* as it favours the growth requirements of this bacterium.

In addition, the natural habitat of shrimp and the intestine of shrimp create a complex ecosystem that is optimum for bacterial communities whether good bacteria as well as toxin-producing bacteria such as *S. aureus* [43]. Occurrence of *S. aureus* in shrimp species obtained in 12 weeks were determined and there was no significant different ($p < 0.05$) between each sample of *L. vannamei* and *P. monodon* species except in week four to eight where occurrence of *S. aureus* in *L. vannamei* was significantly higher ($p < 0.05$) than occurrence in *P. monodon* (Figure 3.1). In week eight, there was no detectable contamination of *S. aureus* in *P.* monodon. The estimated number of colonies were less than 2.00 $\log_{10}$ CFU/g.

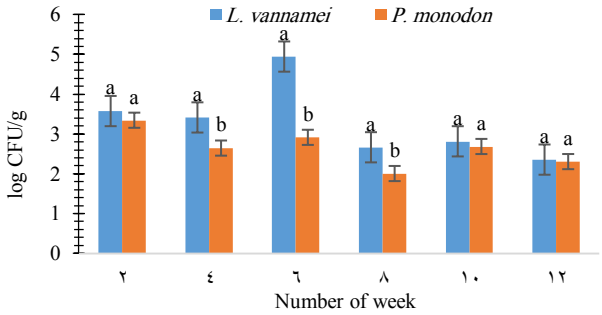

Fig 3.1: The occurrence of *S. aureus* in *L. vannamei* and *P. monodon* in twelve weeks sampling from retail.

The occurrence of *S. aureus* in *L. vannamei* and *P. monodon* over twelve weeks can be observed in Figure 3.1. The result showed the occurrence of *S. aureus* in *L. vannamei* and *P. monodon* fluctuated through twelve weeks in the range between 2.35 $\log_{10}$ CFU/g to 4.94 $\log_{10}$ CFU/g and 2.00 $\log_{10}$ CFU/g to 2.91 $\log_{10}$ CFU/g respectively. Presence of *S. aureus* in *L. vannamei* increased in week six and dropped steeply in the following weeks. Whereas, as for *P. monodon*, the highest peak of *S. aureus* occurrence happened in week two, and it reached its lowest point in week eight. Based on International Commission for the Microbiological Specification of Food (ICMSF) (1986), $\log_{10}$ CFU/g of *S. aureus* in *L. vannamei* in week two, four and six as well as *P. monodon* in week two and six had exceeded the maximum permissible human consumption for good shrimp quality which is 3.00 $\log_{10}$ CFU/g.

3.1.2 Occurrence of *Escherichia coli* in *Litopenaeus vannamei* and *Penaeus monodon*

Among 24 samples of *L. vannamei* and *P. monodon*, 14 (58%) samples were observed contaminated with *E. coli*. From 12 samples of each *L. vannamei* and *P. monodon*, the samples contaminated with *E. coli* were eight (67%) and six (50%), respectively (Table 3.2).

Table 3.2: Occurrence of *E. coli* in *L. vannamei* and *P. monodon* in twelve weeks sampling from retail

Sample	Non-contaminated samples	Contaminated samples	Number of samples
L. vannamei	4 (33%)	8 (67%)	12 (100%)
P. monodon	6 (50%)	6 (50%)	12 (100%)
Total	10 (42%)	14 (58%)	24 (100%)

The occurrence of *E. coli* in shrimp is expected since these organisms are present in the estuarine environment where contamination of faecal matter is commonly reported [13]. In this study, 58% samples of shrimp were contaminated with *E. coli* which is lower than *E. coli* load in shrimp imported to Switzerland (64%) [5], and shrimp from retail in Kolkata, India (85%) [13], but higher than shrimp from Tuticorin Coast, South-eastern India (10%) [19]. Study of *E. coli* in shrimp at Malaysia is minimal, however isolation of *E. coli* from other types of seafood had been carried out in these few years. *E. coli* contamination in Asian seabass ranged from 10^5 CFU/g to 10^7 CFU/g. Meanwhile, a study of *E. coli* contamination in shellfish showed that more than 50% samples were below the detection limit set by ICMSF and it indicates good quality seafood.

Presence of *E. coli* indicates poor hygiene and sanitary condition. Quality of seafood depends on the hygiene condition of the landing areas and the quality of water where the shrimp is farmed [13]. *E. coli* are not part of microflora in shrimp; therefore, the presence of *E. coli* might indicate a sign of faecal contamination from human or animal in the aquaculture environment and also cross-contamination that might occur during handling before it reaches the retail market and includes display time before it is bought by consumers [5].

Contamination of *E. coli* in *L. vannamei* and *P. monodon* that was obtained in 12 weeks showed a significant difference between samples species in week six only (Figure 3.2). In week two and four, there was no detectable count of *E. coli* contamination from both shrimp species which it was estimated to have less than 2.00 $\log_{10}$ CFU/g.

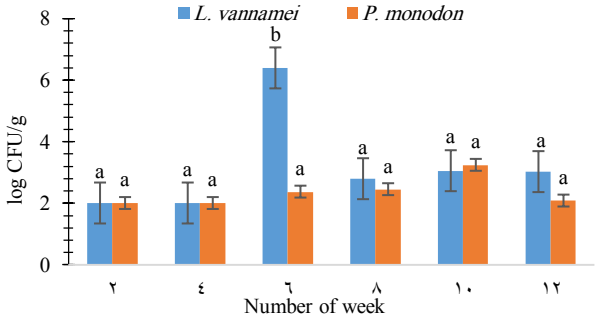

Fig 3.2: The occurrence of *E. coli* in *L. vannamei* and *P. monodon* in twelve weeks sampling from retail.

Figure 3.2 shows *E. coli* contamination in *L. vannamei* and *P. monodon* for twelve weeks in the range between 2.00 $\log_{10}$ CFU/g to 6.39 $\log_{10}$ CFU/g and 2.00 $\log_{10}$ CFU/g to 3.24 $\log_{10}$ CFU/g. The occurrence of *E. coli* in *L. vannamei* increased sharply and reached its highest occurrence point at week six. Meanwhile, *E. coli* occurrence in *P. monodon* reached its highest occurrence in week ten at the value of 3.24 $\log_{10}$ CFU/g.

According to ICMSF (1986), the acceptable limit of *E. coli* in shrimp is 2.70 $\log_{10}$ CFU/g. Several *E. coli* occurrences in this study which are week six to twelve for *L. vannamei* and week ten for *P. monodon* exceeded the acceptable limit of contamination and this might be harmful to human health and sufficient heat in cooking is extremely crucial to prevent food poisoning caused by *E. coli.*

S. aureus and *E. coli* occurrence showed fluctuation in 12 weeks of analysis (Figure 3.1; Figure 3.2). This could be explained by inconsistent practices and different ways of handling by food handlers which causes cross-contamination of human or surfaces that might happen from landing areas to the retail market. Besides that, contamination might occur due to temperature fluctuation to the point that it allows optimum temperature for microbial growth. The temperature during a display at the retail should be kept as near as possible to 0°C, and counter slab must have adequate drainage to ensure that there is no slime accumulated on the slab (Food and Agriculture Organization, 1998). In addition, during display, shrimp are exposed to the air which might result in food safety risk by exposure of flies.

3.1.3 Presumptive identification of bacteria

From 21 contaminated samples of *S. aureus* and 14 contaminated samples of *E. coli*, five colonies from each sample were selected for presumptive identification. A series of tests (Gram-staining, catalase test, coagulase test) were carried out to identify presumptive colonies of *S. aureus* and *E. coli.* Identification of presumptive *S. aureus* (*n*=105) and *E. coli* (*n*=70) yielded 62 isolates of interest: 34 isolates of *S. aureus* (*L. vannamei*, *n*=18; *P. monodon*, *n*=16) (Table 3.3) and 28 isolates of *E. coli* (*L. vannamei*, *n*=12; *P. monodon*, *n*=16) (Table 3.4).

Table 3.3: Identification of *Staphylococcus aureus* by Gram-staining, catalase test and coagulase test.

Biochemical test	Number of isolates (*n*)		Number of positive isolates (*n*)	
Gram-staining	105	LV: 55 PM: 50	80	LV: 43 PM: 37
Catalase test	80	LV: 43 PM: 37	41	LV: 20 PM: 21
Coagulase test	41	LV: 20 PM: 21	34	LV: 18 PM: 16

LV, *Litopenaeus vannamei*; PM, *Penaeus monodon.*

Table 3.4: Identification of *Escherichia coli* by Gram-staining, catalase test, and coagulase test.

Biochemical test	Number of isolates (n)		Number of positive isolates (n)	
Gram-staining	70	LV: 33 PM: 37	63	LV: 35 PM: 28
Catalase test	63	LV: 35 PM: 28	51	LV: 26 PM: 25
Coagulase test	51	LV: 26 PM: 25	28	LV: 16 PM: 12

LV, *Litopenaeus vannamei*; PM, *Penaeus monodon.*

3.2 Antimicrobial resistance among bacterial isolates

3.2.1 Inhibition zone diameter of *S. aureus* and *E. coli*

All isolates of *S. aureus* (*n*=34) and *E. coli* (*n*=28) which had been subjected to a series of presumptive identification tests were evaluated for antimicrobial susceptibility analysis to nine antibiotics from five different categories which are divided by group of β-Lactams (penicillin, cephalosporin) and non β-Lactams (aminoglycosides, quinolone fluoroquinolone, sulfonamides).

3.2.2 Resistance rate of *S. aureus* and *E. coli*

From Table 3.5, resistance rates of *S. aureus* were the highest for penicillin G (P) (85%) followed by nalidixic acid (NA) (39%), ceftazidime (CAZ) (21%), cefotaxime (CTX) (21%), amoxicillin (AMC) (18%) and sulfisoxazole (SF) (18%). There were no resistant isolates against gentamicin (CN), streptomycin (S) and ciprofloxacin (CIP). Meanwhile, highest resistance rate of *E. coli* was against P (86%) followed by SF (43%), NA (36%), CTX (25%), AMC (21%), CAZ (14%) and S (4%). All *E. coli* isolates were sensitive towards CN and CIP.

Table 3.5: Resistance rate of *S. aureus* and *E. coli* in the local shrimp.

	Percentage of resistance (%)								
	P	AMC	CAZ	CTX	CN	S	NA	CIP	SF
S. aureus	85	18	21	21	0	0	39	0	18
E. coli	86	21	14	25	0	4	36	0	43

P, Penicillin G; AMC, Amoxicillin; CAZ, Ceftazidime; CTX, Cefotaxime; CN, Gentamicin; S, Streptomycin; NA, Nalidixic acid; CIP, Ciprofloxacin; SF, Sulfisoxazole.

According to Arfatahery et al., [3], the authors were mentioned that antimicrobial resistance (AMR) genes spread from antibiotic residue in food products by transferring of a resistant foodborne pathogen or ingesting natural microflora of resistant bacterial strains. Resistant bacteria obtain resistance genes from mobile elements such as plasmids, transposons, and integrons from other resistant microorganism strains [23]. In farming activities, resistance genes might spread in shrimps due to the interaction between resistant bacteria to non-resistant bacteria and accumulation of antibiotic residues in the water whereas transmission to human might occur by direct consumption of contaminated shrimp.

In this study, *S. aureus* and *E. coli* were showed a resistance rate of 85% and 86%, respectively. Most of the identified bacteria were against penicillin G from the β-lactam group which was similar to *S. aureus* isolates from China (88%) [34], but higher than Iran (79%) [3] and imported shrimp in Switzerland (56%) [5]. The findings of antibiotic susceptibility patterns of *E. coli* isolated from shrimps showed that *E. coli* strains were most resistant to penicillin G (86%) which was similar to *E. coli* strains that were isolated from seafood in India [19].

Bacteria can become resistant against penicillin antibiotics (P and AMC) by hydrolysing β-lactam ring. Mechanism of inhibition by penicillin is done by inhibiting cell enzyme which is β-lactamase that contains a β-lactam ring. This delays the cell wall formation

which drops the osmotic pressure and destroys the bacterial cells. Bacteria become resistant towards penicillin due to the blaZ gene where it recognizes penicillinase enzyme and prevents it from binding to the β-lactam ring. *S. aureus* and *E. coli* were found to be resistant against penicillin since 1942 when it was found to be resistant to penicillin in hospitalized patients, and the resistant bacteria spread rapidly from hospital to public communities [24].

Cephalosporin antibiotic class (CAZ and CTX) has a similar mechanism with penicillin class in which it utilizes a β-lactam mechanism to kill bacteria. Cephalosporin is used in hospital as first-line therapy to treat infections from mild to severe ones. The emergence of resistance against this antibiotic class could be due to the abuse and misuse of antibiotics to human, veterinary drugs and agriculture [37]. Adesoji et al., [1] mentioned that bacteria become resistant to cephalosporin antibiotics due to two enzymes which are Extended Spectrum Beta-lactamases (ESBLs) and AmpC beta-lactamases.

Fluoroquinolones such as NA has been used widely in treating infections and used as prophylaxis in human medicine. Besides that, it is used in veterinary activities for medical purpose and act as a growth promoter to animals. Bacterial resistance against this class of antibiotic is expected due to high-level of usage which could be unnecessary, and it is used with poor activity in some developing countries [35]. Ruiz [35] stated that resistance might occur due to two mechanisms which are; alteration of the target in quinolones and impermeability of the membrane of efflux pump system.

Similar to cephalosporin class, sulfonamides antibiotic such as SF also recognized as antimicrobial agents that are commonly used in human medicine and veterinary practices. It is a synthetic veterinary antibiotic that is widely used by developing countries and European countries due to its low costs [42]. The drastic change in the microbial ecosystem due to the presence of antibiotics causes bacteria to adapt which resulted in the formation of resistance genes [38].

On the other hand, *S. aureus* and *E. coli* isolates in this study were sensitive towards aminoglycosides category (gentamicin, streptomycin) and ciprofloxacin. From previous studies, *S. aureus* isolated from imported shrimp in Switzerland [5] were susceptible to ciprofloxacin and gentamicin similar to *S. aureus* isolates in shrimp from Iran [3] where none of the isolates were resistant against ciprofloxacin. Susceptibility of *E. coli* towards ciprofloxacin similar with *E. coli* isolates in fish from Brazil [33] as well as streptomycin and gentamicin showed similar findings from fish samples [23] and seafood from Tuticorin Coast, South-eastern India [19]. According to Immaculate et al., [19], they suggested that ciprofloxacin is one of the best antibiotic to treat *E. coli* infection as most of the tested *E. coli* isolates were sensitive to this antibiotic.

According to Mohamed et al., [27], sulfonamides are the commonly used antibiotics in Malaysia's aquaculture. There are several strains of *S. aureus* and *E. coli* that were resistant to sulfisoxazole and amoxicillin whereas all isolates tested were sensitive towards ciprofloxacin. Antibiotic-resistant bacteria can be occurred due to several reasons. One of the reason is the usage of antibiotic in aquaculture which happens when antibiotic residue and resistant bacteria in the water are spread to the shrimp farms [15]. Even if the farms are no longer using antibiotic in their farming activities, antibiotic resistance bacteria may still occur due to accumulated antibiotic in the sediments of previous farming activities. Nevertheless, resistance towards other types of antibiotic is possible due to cross-resistance to another member of antibiotic of the same class [29].

Application of streptomycin in human medicine includes antimicrobial therapy for tuberculosis, and it was proven that it improved the condition of patients by assessment using lung radiography however it was found resistant to streptomycin by six months of the trial [21]. In addition, streptomycin is used in agriculture to eliminate microbial growth. The application of this antibiotic raised a concern that it may contribute to antibiotic resistance which led to bans in European countries and it was suggested that antibiotic formulations in animal feeds could be a potential vehicle for resistance genes [32].

3.2.3 Antibiotic susceptibility of *S. aureus* isolated from *Litopenaeus vannamei* and *Penaeus monodon*

Antibiotic susceptibility of *S. aureus* in *L. vannamei* (*n*=18) and *P. monodon* (*n*=16) can be discussed with the percentage of the resistance rate. The highest resistance rate of *S. aureus* in *L. vannamei* was against P (83%) followed with NA (39%), AMC (22%), SF (22%), CTX (17%) and CAZ (11%). On the other hand, resistant isolates of *S. aureus* from *P. monodon* were 14 (87%) to P, 6 (38%) to NA, 5 (31%) to CAZ, 4 (25%) to CTX and 2 (13%) to SF. *S. aureus* is known for its resistance towards antimicrobial agents, and it can be resistant in more than one way including mutations that occur either spontaneously or naturally [34].

Chambers and Deleo [6] mentioned that *S. aureus* is naturally susceptible to every antibiotic that has been developed. It acquires resistance by horizontal genes transfer from exterior sources, chromosomal mutation and selection of antibiotic. At the era of antibiotic discovery, *S. aureus* was sensitive towards penicillin but within ten years, it showed resistance which becomes a serious problem to the public. Human has the highest risk of infection and major source of transmission of *S. aureus* since human skin is the normal flora of this bacterium. Transmission of *S. aureus* can be due to direct contact, contaminated objects and surfaces [6].

3.2.4 Antibiotic susceptibility patterns of *E. coli* isolated from *Litopenaeus vannamei* and *Penaeus monodon*

Antibiotic susceptibility of *E. coli* in *L. vannamei* (*n*=16) and *P. monodon* (*n*=12) can be discussed in percentage. Total resistant isolates of *E. coli* from *L. vannamei* in descending order were 12 (75%) to P, 10 (63%) to SF, 6 (37%) to NA, 4 (25%) to AMC, 3 (19%) to CTX, 2 (12%) to CAZ and 1 (6%) to S. There was no *E. coli* isolates from *L. vannamei* were resistant to CN and CIP. Meanwhile, the highest resistant rate of *E. coli* from *P. monodon* was against P (100%) (Table 3.5) where all of the isolates were not susceptible to this antimicrobial agent. Lower resistance rates were found for CTX (33%), NA (33%), AMC (17%), CAZ (17%) and SF (17%). However none *E. coli* isolates from *P. monodon* were found resistant to aminoglycosides category (CN, S) and CIP from sulfonamides category.

Transmission of resistant *E. coli* to human could be via food and water consumption but there is no confirmation whether contamination happens due human or food animals [9]. *E. coli* resistance genes could be transmitted from food to human within gastrointestinal tract if the food contains resistant *E. coli* which subsequently is the source of resistance in feacal flora [31]. With insufficiently treated sewage, effluent from industry and improper waste dumping that are released in open areas might introduce resistant *E. coli* strains to the environment that could contribute to spreading of resistance genes [19] whereas resistant *S. aureus* might occur due to antibiotic discharged in wastewater [34].

3.3 Multidrug resistance among samples

Patterns of multidrug resistance (MDR) are shown in Table 3.6. Of the 24 shrimps' samples, nine isolates of *S. aureus* and *E. coli* displayed resistant to three or more category of antimicrobial agent were two (8%) harbored MDR *S. aureus* and seven (29%) had MDR *E. coli.* *L. vannamei* samples contained one MDR *S. aureus* and four MDR *E. coli* meanwhile *P. monodon* contained one MDR *S. aureus* and three MDR *E. coli.* It can be observed that the MDR organism was mainly from *E. coli* isolates. From Table 3.5, most MDR organism showed resistance to category I (Penicillin), category II (Cephalosporin), category IV (Quinolone Fluoroquinolone) and category V (Sulfonamides). However, there

was no MDR organism showed insusceptibility against CN and S from category III (Aminoglycosides).

Table 3.6 Multidrug resistance of *S. aureus* and *E. coli* isolated from *Litopenaeus vannamei* and *Penaeus monodon* from retail.

Resistant Strain	Isolates	Antimicrobial agent and susceptibility test								
		I	II				III		IV	V
		P	AMC	CAZ	CTX	CN	S	NA	CIP	SF
S. aureus	1(LV)	R	-	R	-	-	-	R	-	-
	1(PM)	R	-	-	-	-	-	R	-	R
E. coli	2(LV)	R	R	R	R	-	-	R	-	R
	2(LV)	R	-	-	-	-	-	R	-	R
	2(PM)	R	R	-	-	-	-	R	-	R
	1(PM)	R	-	-	R	-	-	R	-	-

*R indicate resistance; I, Penicillin; II, Cephalosporin; III, Aminoglycosides; IV, Quinolone Fluoroquinolone; V, Sulfonamides; LV, *L. vannamei*; PM, *P. monodon*; P, Penicillin G; AMC, Amoxicillin; CAZ, Ceftazidime; CTX, Cefotaxime; CN, Gentamicin; S, Streptomycin; NA, Nalidixic acid; CIP, Ciprofloxacin; SF, Sulfisoxazole.

Multidrug resistance bacteria might be happened due to the exploitation of antibiotic usage that causes drug-sensitive microorganism to be resistant against antimicrobial agents [19]. *E. coli* strains showed resistance to most of the antibiotic groups tested where seven out of 28 isolated were multidrug resistant compared to *S. aureus* where only two strains were multidrug resistant. This can be explained due to cell wall characteristics of a Gram-negative organism compared to Gram-positive organism and organism from Enterobacteriaceae family such as *E. coli* always seems resistant to various classes of antibiotics [19]. *E. coli* also recognized as a microorganism that capable of acquiring and transmitting resistance genes to another species of microorganism. Kumaran et al., [23] stated that previous studies have proven that *E. coli* isolates from animals to food possess resistance to many antibiotic classes which contributes to the reservoir for transmissible resistance genes.

4. Conclusion

The high prevalence of *Staphylococcus aureus* and *Escherichia coli* in two shrimp species*; Litopenaeus vannamei* and *Penaeus monodon* from retail in its antimicrobial resistant and multidrug-resistant were observed. Majority isolates of *S. aureus* and *E. coli* were resistant to penicillin G, and there was multidrug resistant (MDR) of both bacteria isolated from the shrimps. It is crucial to ensure that monitoring activities are carried out sufficiently for the prevalence of *S. aureus* and *E. coli* in shrimps. Improving handling procedure of shrimp from the farm to the retail could reduce the chance of cross-contamination and possible to eliminate the occurrence of *S. aureus*. Besides that, *E. coli* occurrence can be reduced by ensuring the hygienic sanitary condition and reducing direct human contact that might spread feacal contamination.

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