Antimicrobial-resistant Salmonella spp. isolated from retail farmed shrimp in Kuala Lumpur

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Abstract

Antimicrobial resistant Salmonella is recognized as a potential food safety threat and its prevalence in farmed shrimps is very concerning. However, information about antimicrobial resistant Salmonella in retail farmed shrimps in Malaysia is very limited. Therefore, this study was aimed at determining the level of Salmonella contamination of farmed shrimps at selected retail in Kuala Lumpur and antibiotic resistance pattern of Salmonella isolated from retail farmed shrimps. Farmed shrimp samples were collected from selected hypermarkets and indoor markets. Salmonella was detected by conventional methods. All presumptive colonies of Salmonella were subjected to antimicrobial susceptibility test. A high rate of Salmonella contamination was detected (n = 9, 60%) with an estimated Salmonella load ranging between 0 and >800 x 10⁸ cfu/ml. All isolates (100%) were resistant to at least one antimicrobial agent, and 17 isolates (94.4%) were multidrug-resistant. 2 isolates (11.1%) were resistant to all eight types of antimicrobial agents. High rates of resistance were observed for erythromycin (100%), doxycycline (77.8%), tetracycline (72.2%), nalidixic acid (61.1%), ampicillin (55.6%) and chloramphenicol (44.4%). Findings from the present study provide insight on antibiotic resistant Salmonella contamination in retail farmed shrimps and suggest its potential food safety risk to the public. These data are valuable to warrant further investigation of risk management and public health strategies.

Keywords: Antimicrobial resistant, Salmonella, Farmed shrimp, Retail

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1. Introduction

Shrimp is appreciated given its uniqueness in taste and texture. Thus, it is unsurprising that shrimp is one of the most popular seafood throughout the world. The popularity has led to high demand from both local and international markets and driven rapid growth in shrimp agriculture industry. In order to fulfill the great capacity, many shrimp farmers shifted to more intensive farming system. While the new system has brought about significant yield improvement, disease outbreaks have frequently arose causing substantial economic losses to the farmers. Antibiotics have often become the quick solution when dealing with shrimp disease issue. Nonetheless, the uncontrolled use of antibiotics including the banned ones in shrimp farming will give rise to the development of antibiotic resistance pathogens.

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Salmonella has been identified as one of the major microbial contributors to shrimp import detention in the United States (Wan Norhana et al., 2010). Whereas in the European region, Salmonella was recorded as the second main cause of shrimp import rejection under microbial contamination category between 1999 and 2002 (Huss et al., 2004). Salmonella has been notorious for causing foodborne illness. People infected with Salmonella will likely develop food poisoning symptoms, however, to a vulnerable population, Salmonella infection can be lethal (CDC, 2016). In addition to the emergence of antimicrobial resistant Salmonella, it will certainly become a serious food safety threat to the public (CDC, 2015).

Realizing the urgency of the issue, several researches (Carvalho et al., 2013; Banerjee et al., 2012; Jeyasekaran and Ayyappan, 2002; Wan Norhana et al., 2001; and Bhaskar et al., 1995) have intensively investigated the prevalence of antibiotic resistant Salmonella in shrimp at farming sites and majority of the findings indicated Salmonella was widespread. However, there was limited information regarding the antimicrobial resistance patterns of Salmonella in farmed shrimps at retail level, especially in Malaysia. Thus, the objectives of this study are to determine the level of Salmonella contamination in retail farmed shrimp at selected retails in Kuala Lumpur and to study the antimicrobial resistance pattern of Salmonella isolated from retail farmed shrimps.

2. Materials and methods

2.1. Sample collection

According to data provided by Roslan et al. (2016), there are nine hypermarkets and 28 indoor markets situated in Kuala Lumpur (Figure 1). A 10% of retailers were selected based on the above-mentioned data. Samples were collected from three indoor markets (WMC, WMT and WMS) and two hypermarkets (HMA and HMB) with three replicates for each market. Shrimp were sampled and contained in sterile poly-bags, labeled and stored in the ice box. Samples were collected and transferred to the laboratory for microbiological analysis on the same day.

2.2. Detection and enumeration

Each sample (25 g) was homogenized with 225 ml of buffered peptone water (OXOID, England) in a stomacher bag for one minute and incubated for 18-20 h at 35 °C as pre enrichment. After incubation, 1 ml of the pre enriched broth was added into 10 ml of Rappaport-Vassiliadis (RV) broth (OXOID, England) followed by serial dilution up to $10^{-8}$ and mixed well, then incubated at 35 °C for 24 h. 0.1 ml of RV broth cultures were spread onto Hektoen Enteric Agar (HEA) (OXOID, England) and incubated at 35 °C for 24 h. Incubated agar plates were placed on colony counter to record the number of presumptive Salmonella colonies exhibited as blue-green to blue and black colonies on the selective agar. Presumptive colonies were then streaked onto nutrient agar plates (OXOID, England) and incubated for 24 h at 35 °C to obtain pure colonies prior to antimicrobial susceptibility test.

2.3. Antimicrobial susceptibility test

Antimicrobial susceptibility of Salmonella was tested using Kirby-Bauer disk diffusion method using Mueller-Hinton agar (OXOID, England) based on National Committee for Clinical Laboratory Standards (CLSI, 2017) guidelines. Pure colonies of Salmonella cultured on nutrient agar were suspended in normal saline (0.85%) until turbidity was comparable to 0.5 McFarland turbidity standards. Next, the suspension was swabbed onto Mueller-Hinton agar then antimicrobial discs were fixed onto the Salmonella seeded agar surface. Antimicrobial agents used in this test and their corresponding concentrations are as follow: Doxycycline 30 µg, Gentamicin 10 µg, Tetracycline 30 µg, Ampicillin 10 µg, Chloramphenicol 30 µg, Nalidixic acid 30 µg and (Oxoid, England). After incubation for 24 h at 37 °C, the diameter of the zone of inhibition of each antimicrobial disc on agar was measured and the results were interpreted according to interpretive criteria provided by CLSI (2017).

2.4. Statistical analysis

All data obtained from the analyses were entered into Microsoft Excel to generate descriptive information including charts and graphs. Minitab 16 was used for further statistical analysis with differences accepted as significant at values of $p \leq 0.05$.

3. Results and discussion

3.1. Prevalence and enumeration of Salmonella spp. in retail farmed shrimps

Out of 15 shrimp samples, 9 (prevalence rate of 60%) were positive for Salmonella spp. Salmonella spp. was
recovered from all five different sampling locations. Table 1 presents the screening and enumeration of Salmonella spp. in retail farmed shrimps in the current study. This is in accord with the study conducted by Nguyen et al. (2016) where the team detected a high rate of Salmonella contamination in raw shrimps (49.1%) sold in fresh markets of Ho Chi Minh City, Vietnam. Contrary, in China, Yang et al. (2015) reported lower (13%) prevalence of Salmonella in retail raw shrimp samples. On the other hand, 5.71% of raw shrimps from seven randomly selected markets in Dhaka city tested by Hossain et al. (2012) were positive for Salmonella. In Iran, Rahimi et al. (2011) also reported low percentage (1.8%) of Salmonella in fresh shrimps. Comparing to data obtained in 1995, Arumugaswamy and colleagues discovered a prevalence rate of 25% in raw shrimp samples collected from various retail markets and shops surrounding Kajang, Serdang and Kuala Lumpur. Although Arumugasamy et al. (1995) did not mention the specific types of shrimp that was being studied, it is worthy to suggest the possibility of growing trend in the prevalence rate of Salmonella spp. in raw shrimps within two decades in Malaysia.

Despite the absence of international agreement on microbiological specification for Salmonella in food including shrimp and its related products, several countries such as the United States (US), the European Union (EU), Australia, New Zealand and China (including Hong Kong) have taken initiative to impose regulatory requirement on raw and ready-to-eat shrimps in order to protect public from major Salmonellosis outbreak (Wan Norhana et al., 2010). Generally, zero tolerance policy is applied. For instance, Australia, New Zealand, and China require the absence of Salmonella in 25 g of raw shrimp sample. On average, enumeration data in this investigation showed a heavy load of Salmonella spp. in both indoor markets and hypermarkets, which far exceeded beyond the well-recognized zero tolerance limits. Therefore, this finding could indicate a substantial food safety risk to the public.

The root cause to Salmonella spp. contamination in raw shrimps can be vague. While Amagliani et al. (2012), Lunestad and Barlaug (2009), and Dalsgaard et al. (1995) emphasized that Salmonella present in either aquatic environment or its associated food products as a result of fecal contamination during farming stage, some researchers (Wan Norhana et al., 2001; Bhaskar et al., 1998, 1995; Reilly et al., 1992; and Iyer and Varma, 1990) argued that Salmonella is a part of the natural aquatic microflora in shrimp farming environment, thus rationalizing the prevalence of the pathogen in raw farmed shrimps at later stages of supply chain. The latter

| Sampling location | First sampling |          | Second sampling |          | Third sampling |          |
|-------------------|----------------|----------|-----------------|----------|----------------|----------|
|                   | Rep. 1         | Rep. 2   | Avg.            | Rep. 1   | Rep. 2         | Avg.     |
| Hypermarket (HMA)| >8 x 10^4      | >8 x 10^4| >8 x 10^4       | <1 x 10^2| <1 x 10^2      | <1 x 10^2|
| Hypermarket (HMB)| <1 x 10^2      | <1 x 10^2| <1 x 10^2       | >8 x 10^2| >8 x 10^2      | >8 x 10^2|
| Wet market (WMA) | <1 x 10^2      | <1 x 10^2| <1 x 10^2       | <1 x 10^2| <1 x 10^2      | <1 x 10^2|
| Wet market (WMB) | <1 x 10^2      | <1 x 10^2| <1 x 10^2       | 1 x 10^9 | 5 x 10^9       | 3 x 10^10|
| Wet market (WMC) | <1 x 10^2      | <1 x 10^2| <1 x 10^2       | 7 x 10^7 | 9 x 10^10      | 5 x 10^10|

Table 1: Microbial loads of Salmonella spp. in farmed shrimps at selected retails in Kuala Lumpur
conclusion is supported by the WHO who declared Salmonella as “geonotic” disease and removal of foodborne zoonoses from the food chain can be very challenging (Wan Norhana et al., 2010). Besides upstream factors, downstream controls are also a critical determinant in Salmonella load in retail farmed shrimps. Sloppy distribution, unhygienic retail environment, mishandling, cross contamination during transportation or storage and temperature abuse are among the typical contributors (Dib et al., 2014; Carrasco et al., 2012; Wan Norhana et al., 2010; and Panisello et al., 2000).

By studying the findings from previous similar studies (Begum et al., 2010; and Minami et al., 2010), fresh market is expected to have higher load of Salmonella in raw shrimp compared to supermarket because the latter type of retailer tend to have better resources, facilities and environment in lowering risk of contamination. Surprisingly, the results from the current study are contradicting against previous findings (Begum et al., 2010; and Minami et al., 2010). Average Salmonella spp. count in samples obtained from hypermarket is higher than the indoor market ($p > 0.05$). Even though hypothesis testing did not yield statistical significance, it could be attributed to the relatively small sampling size in this study.

Besides, it can be observed from the data that load of Salmonella spp. in the samples did not exhibit a steady trend. In one sampling location, Salmonella spp. was found absent and highly prevalent at different sampling dates. High load of Salmonella spp. from one particular date gave drastic rise to total contamination load. Hence, it is inconclusive to reflect Salmonella spp. contamination in raw farmed shrimps in hypermarket was more severe than indoor market. Minami et al. (2010) encountered similar Salmonella prevalence trend in their study, in which Salmonella was detected in the first batch of sampling but not in the second batch. The team was supposing that these retailers are engaging to the same suppliers or transportation service, then the inconsistency in the trend might suggest cross-contamination during these stages. Nonetheless, the findings from the current study provide preliminary insight into current Salmonella spp. contamination status in farmed shrimps at the retail stage that warrants further investigation. The uncertainties in current study should be overcame by increasing sampling size to develop a more established contamination trend and to yield more meaningful statistical hypothesis testing outcome.

3.2. Antimicrobial resistance pattern of Salmonella isolated from retail farmed shrimps

Table 2 and Figure 2 summarize the resistance pattern of 185 Salmonella spp. isolates against eight antimicrobial drugs tested in the present study. The prevalence rate of antimicrobial-resistant Salmonella spp. was high
Table 2: Antimicrobial resistance of *Salmonella* spp. isolates from farmed shrimp in each sampling location

| Sampling location     | CN | TE | NA | CIP  | E  | DO | AMP | C  |
|-----------------------|----|----|----|------|----|----|-----|----|
| Hypermarket (HMA) (n = 3) | 0  | 2  | 1  | (2)* | 3  | 2  | (1) | 1  |
| Hypermarket (HMB) (n = 4) | (1) | 2  | 3  | 1(3) | 4  | 2(1)| 2(1)| 1(1)|
| Wet market (WMC) (n = 1) | 0  | 1  | 0  | 0    | 1  | 1  | 0   | (1)|
| Wet market (WMT) (n = 5) | 2(1)| 4  | 3(1)| 1(4) | 5  | 4  | 5   | 3  |
| Wet market (WMS) (n = 5) | 1  | 4  | 4  | 1(2) | 5  | 5  | 3   | 3(1)|

Note: * The number with parentheses indicate intermediate resistance; * CN: gentamycin, TE: tetracycline, NA: nalidixic acid, CIP: ciprofloxacin, E: erythromycin, DO: doxycycline, AMP: ampicillin, and C: chloramphenicol.

Figure 2: Percentage of resistant, *Salmonella* spp. isolates from retail farmed shrimp to commercial antibiotics

Figure 3: Percentage of resistant, intermediate and susceptible *Salmonella* spp. isolates from retail farmed shrimp to commercial antibiotics
(100%); all of the Salmonella spp. isolates were resistant to at least one antimicrobial drug. High levels of resistance were found to erythromycin (100%), doxycycline (77.8%), tetracycline (72.2%), nalixidic acid (61.1%), ampicillin (55.6%), and chloramphenicol (44.4%). There were 16.7% of isolates resistant towards gentamycin and ciprofloxacin. It is crucial to highlight the high prevalence rate of antimicrobial resistance found in this study upon comparison against data from other countries. Even though most Salmonella isolates recovered by Hossain et al. (2012) and Wan Norhana et al. (2001) were also resistant to erythromycin, however; in contrast, Hossain and the team’s (2012) isolates were all (100%) susceptible to chloramphenicol, ciprofloxacin and doxycycline. Also, Nguyen et al. (2016) and Woodring et al. (2012) reported overall lower occurrence rate of antimicrobial resistance in Salmonella isolated from shrimp retailers in Vietnam and Thailand respectively compared to the present study. In addition, Rahimi et al. (2013) even reported zero incidence of antimicrobial resistance in Salmonella isolates obtained from retail raw shrimp samples in Iran.

The susceptibility of a bacterium to a given antibiotic is categorized as intermediate when its growth is inhibited in vitro by a concentration of the drug that is associated with an uncertain therapeutic effect (CLSI, 2017). Notably, in the present study, high prevalence rate of intermediate resistance was observed in ciprofloxacin.

The majority of the researchers (Yang et al., 2015; Zhang et al., 2015; and Hossain et al., 2012; and Cabello, 2006) linked prevalence of antimicrobial resistance in Salmonella to abuse of antimicrobial in aquaculture and agriculture either to promote growth or therapeutic purpose. Hossain and the team (2012) further suggest that the use of probiotics in shrimp farming can contribute to the development of antimicrobial resistance. It is also worth to note that ampicillin is rarely used in shrimp farming (Hossain et al., 2012). Therefore, ampicillin resistance in Salmonella spp. recovered in this study could be acquired from other sources (Hossain et al., 2012). As highlighted by Zhang et al. (2015), ciprofloxacin is commonly used as the front-line drug that is prescribed to treat Salmonellosis in adults. Therefore, the emergence of ciprofloxacin-resistant Salmonella (16.7%) could lead to serious medical complication.

Multidrug resistance is defined as the resistance to more than one type of antimicrobial drugs (Zhang et al., 2015). 94.4% (n = 17) Salmonella spp. isolates from the present study were multidrug-resistant (Figure 3). Notably, two isolates were resistant to all eight antimicrobial agents. These particular two isolates were recovered from wet market, WMT and WMS. The high prevalence rate of multidrug-resistant Salmonella spp. is another critical highlight in this study as this phenomenon will limit therapeutic options for clinical cases that require the prescription of antimicrobial agents. This is especially crucial to the vulnerable population such as the elders, infants and immunocompromised individuals since the delay in receiving effective treatment will significantly increase their risk of mortality.

4. Conclusion

This study leads to a preliminary insight of current antimicrobial-resistant Salmonella spp. contamination status in raw farmed shrimp at retail level. In conclusion, the prevalence rate of Salmonella spp. was found to be very high at retail level. Therefore, raw consumption should be avoided and farmed shrimps should be thoroughly cooked before serving to minimize health risk associated with Salmonella contamination. Hypermarkets which were perceived to be more hygienic were just as likely to be contaminated as those bought from the more austere indoor market. On the other hand, based on the antibiotic resistance pattern obtained from the present study, the prevalence rate of multidrug resistant Salmonella spp. in retail farmed shrimps is very high. Further investigation is warranted to confirm and expand upon the food safety risks reflected in the findings from the present study. Besides, surveillance programs need to be implemented to closely monitor on antibiotic use in aquaculture and agriculture as well as the microbiological status of farmed shrimps. Information from these investigations and monitoring programs are valuable to establish science-based public health policy and to develop effective interventions such as Hazard Analysis Critical Control Point (HACCP) programs in farmed shrimp supply chain.

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