Prevalence of antibiotic resistance *Escherichia coli* isolated from *Pangasius* catfish (*P. hypophthalmus*) fillet during freezing process at two factories in Mekong Delta Vietnam

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**Abstract**

Total of 261 samples of fish and environmental samples (i.e. wash water, swabs of hand/gloves of workers, fish contact surfaces i.e. knives, cutting boards and working tables) were collected from two *Pangasius* processing factories (PPF1 and PPF2). A total of seventy-one (71) isolates of *Escherichia coli* were selected to study the prevalence of antibiotics resistance using disk agar diffusion method. Overall, it was determined that 61% (22/36) of PPF1 isolates were resistant except to colistin while 68.57% (24/35) of PPF2 isolates were resistant except kanamycin. High resistance was against ampicillin in both PPF1 and PPF2 isolates (47.22% and 42.86%), followed by cefotaxime (33.33% and 40%) respectively. Varying resistance response to all other tested antibiotics such as streptomycin, meropenem, tetracycline, sulfamethoxazole/trimethoprim and nalidixic acid was also observed among the *E. coli* isolates from both factories. About 50% of the multi-drug resistant (3-9 antibiotics) among PPF1 were observed whereas there were 45.83% multi-drug resistant (3-7 antibiotics) among PPF2 isolates. The result from this study reflected that there was a prevalence of multi-drug resistance of *E. coli* isolated during the processing of *Pangasius* at the studied factories. Therefore, there is a need for an effective risk management assessment models and management plans from stakeholders involved in the *Pangasius* value chain (i.e. farmers, processors and government) to ensure the food safety of production chain.

1. **Introduction**

*Pangasius hypophthalmus* is a key aquaculture species in Vietnam which accounts for more than 90% of what is sold on the international market (Little et al., 2012). This industry has become an important source of employment and wealth generation in the Mekong Delta as the product is almost totally exported to over 100 countries, as frozen fillets making it one of the key success stories of Asian aquaculture (Phan et al., 2009). To meet up with the booming exports of *Pangasius* fillets, there are currently almost 140 *Pangasius* catfish processing units operating with a cumulative capacity of nearly one million ton of processed *Pangasius* fillets (De Silva and Phuong, 2011). To ensure that safe and quality aquatic product is produced both for domestic and international markets, as well as the reduction in the number of food contamination, several quality regulations, standards and certifications, were put in place which all stakeholders in the industry must comply with. The processed *Pangasius* products had currently implemented Good Manufacturing and Hygienic Practices (GMP and GHP), Hazard Analysis Critical Control Point (HACCP), and/or other food quality or Food Safety Management Systems (FSMS) such as BRC, IFS, ISO 9001, ISO 14001, etc. (VASEP, 2020).

In aquaculture sectors, the intensive and semi-intensive practices employed to produce large stocks of fish result in the outbreak of diseases, and the use of antimicrobials has become a customary practice to control them (Santos and Ramos, 2018). So, over the years, antibiotics have also been used in animal husbandry and aquaculture for growth promotion, improvement of feed efficiency, prophylaxis, and treatment of infections in South Asian countries (Manage, 2018). It is highlighted that the lack of stringent control on the use of antimicrobial agents resulted in the emergence of antimicrobial resistant bacteria, representing a risk of dissemination of

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Antimicrobial Resistance (AMR) organisms and their genes to consumers (Manage, 2018). As such, antimicrobial resistance is now recognized as one of the most serious global threats to human health (Liu et al., 2016). High use of antibiotics has been noticed among Pangasius farms and Vietnamese aquaculture generally (Rico et al., 2013; Pham et al., 2015; Long and Lua, 2017; Hedberg et al., 2018). There was a rapid alert notification of ofloxacin categorized as an unauthorized substance in the exported Pangasius products to Switzerland recently (RASFF, 2019). On the other hand, antibiotics residue has also been reported in exported Pangasius products (Jansomboon et al., 2018).

E. coli has been reported to be a candidate vehicle for transfer of antibiotic resistance gene (Van et al., 2008). Multi-drug resistant bacteria was found on exported Pangasius fillets (Khan et al., 2006; Boss et al., 2016). It is evident that the prevalence of multi-drug resistance of E. coli on fresh Pangasius fish and pond water of cultured fish has also been reported (Sarter et al., 2007; Nguyen et al., 2016; Boss et al., 2016; Long and Lua, 2017). The report of multi-drug resistance in E. coli that is rampant in, food sources, environment and human beings in Mekong Delta region is alarming (Van et al., 2008; Dyar et al., 2012; Nhung et al., 2015).

With the booming export of Pangasius products from Vietnam, the prevalence of antibiotics resistant bacteria from farm levels or final fish products has been studied. Very little to no scientific information is available about the resistance prevalence in E. coli obtained from Pangasius processing factory (PPF) influenced by industrial processing. Therefore, the objective of the current research was to study the antibiotics resistance of E. coli isolated from Pangasius fish and environmental samples during processing from two processing factories located at Dong Thap and Vinh Long provinces of the Mekong Delta Region.

2. Materials and methods

2.1 Characteristics of Pangasius processing factories (PPF)

The processing factories evaluated in this study are located at Dong Thap (PPF1) and Vinh Long (PPF2) provinces of the Mekong Delta region, Vietnam. PPF1 has a production capacity of approximately 100 tons per day and between 800-900 people work in the factory. Food safety and/or quality management systems implemented include HACCP, BRC, GMP, SSOP, ISO 9001-2000, HALAL and SQF 2000. Pangasius products especially frozen fillets from this factory are being exported to South America including Brazil, Arab region, China, Hong Kong and Europe. PPF2, on the other hand, has a production capacity between 70-80 tons per day, with 500 employees working in the factory. Food safety and/or quality management systems implemented are HACCP and HALAL. The Pangasius frozen fillets from this factory are being exported to Spain, Poland, Germany, Malaysia, Indonesia and China.

2.2 Enumeration and isolation of E. coli

2.2.1 Sampling

Total of 261 samples (i.e. n = 144 from PPF1 and n = 117 from PPF2) of fish and environmental samples (i.e. wash water, swabs of hand/gloves of workers, fish contact surfaces i.e. knives, cutting boards and working tables) with 9 replicates per sample were collected at the different processing steps critical to the safety and quality of fillets such as raw material receiving, filleting, trimming, cooling and packaging as well as intervention steps of washing in water baths from two factories between January and August 2019 and subjected to microbiological analysis within 6 to 24 hrs of sampling.

2.2.2 Microbiological analysis

Approximately 25 g of fish samples were weighed and 225 mL of Maximum Recovery Diluent (MRD) was added to make first primary dilution, and a ten-fold serial dilution was done. For the water and swab samples, they were vortexed and a ten-fold serial dilution was done as well. Enumeration of E. coli was done by streaking 0.1 mL of inoculum of three consecutive dilutions taken from each sample on Coliform Agar ES (Enhanced Selectivity) (Merck Merck Darmstadt, Germany) and incubated for 18-24 hrs at 37°C. Colonies of blue color suspected as E. coli were picked and subsequently purified until pure E. coli isolates were obtained. Biochemical confirmation was done for the E. coli isolates using IMViC and KIA tests. E. coli isolates that tested positive were streaked on Tryptic Soy Agar (TSA, Merck Darmstadt, Germany) slant, incubated at 37°C for 18-24 hrs after which the purified E. coli were used for antimicrobial susceptibility tests.

2.3 Antimicrobial susceptibility testing

About seventy-one (PPF1, n = 36 and PPF2, n = 35) E. coli isolates were selected to be representative of the different samples and processing steps mentioned above. They were subjected to antimicrobial susceptibility test which was replicated twice using the disc agar diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2017). E. coli isolates were pre-cultured in Tryptic Soy Broth (TSB, Merck Darmstadt, Germany) and incubated at 37°C for 18-24 hrs. The turbidity of the isolates was then adjusted to 0.5 McFarland (approximately 10^8 CFU/mL). The isolates tested were spread on Mueller Hinton Agar (MHA,
3.1 Escherichia coli contamination during processing between PPF1 and PPF2

The trend of E. coli counts from fish and environmental samples along the processing steps in both PPF1 and PPF2 is shown in Figure 1 A-D. From PPF1, the highest count of E. coli was observed at the trimming (3.67 log CFU/g) and cooling steps (3.73 log CFU/g) for the fish samples compared to other steps (p<0.05). E. coli on packaged fish (2.07 log CFU/g) was not significantly different on the raw fish (1.28 log CFU/g) at PPF1 (p=0.05). From PPF2, E. coli counts on fish ranged from 1.0 to 2.0 log CFU/g among processing steps. For hands samples, the high E. coli counts on trimming, being 3.22 at PPF1 and 2.87 log CFU/100 cm² at PPF2. For food contact surfaces, there was a statistically significant difference (p<0.05) between E.coli at the skinning (3.58) and packaging (2.0 log CFU/100 cm²) steps at PPF1 whereas there was no significant difference between E.coli at the skinning (1.78) and packaging (1.67 log CFU/100 cm²) steps at PPF2 (p>0.05). For water samples, the highest E. coli was seen in bleeding (2.6 and 2.2 log CFU/ml) whereas the lowest E. coli was in glazing step (1.0 and 1.08 log CFU/ml) at PPF1 and PPF2, respectively (p<0.05). From this study, the fillets went through several handlers at the trimming step which increased the chances of contamination for the fillets. Also, during observation at the skinning step, several fillets passed through the machine surface where skinning is being done without intermittent cleaning of the surface during this operation, therefore, there is a greater possibility of contamination at this step as well. This shows that the contamination of Pangasius fillets with E. coli can occur at any point during production, and this might originate from the raw fish itself, food operators, environment and poor quality of wash water used for fish processing along the processing chain (Tong Thi et al., 2013; Divyashree et al., 2019). The increasing trend observed in the E. coli of sampled raw fish and fillets at the trimming step in this study is the same as observed by Tong Thi et al. (2014) with the counts from this study higher than those observed in their study. This might be because of the differences in sampled factories or some subtle differences in the processing steps of both factories. However, the count of E. coli conforms to the microbiological criteria for production of frozen Tra fish (Pangasius hypophthalmus) fillets (tolerance level for E. coli is 2 log CFU/g) according to (Ministry of Science and Technology of the Socialist Republic of Vietnam, 2010). The presence of E. coli on the packaged fillets is an indication that a contamination pathway exists between source of bacteria and the fillets across the processing chain. It is therefore recommended that improved hygiene practices among the food handlers as well as the cleaning efficiency of the contact surfaces of both factories should be implemented to reduce and/or prevent fillet contamination.

3.2 Antibiotic resistance

The comparison of the prevalence of resistance against 15 antibiotics is shown in Figure 2. Overall, it was determined that 61% (22/36) of PPF 1 isolates were resistant to at least one antibiotics except to colistin. A high level of resistance was against ampicillin (44.33%), followed by cefotaxime (33.33%) and streptomycin (22.22%). Furthermore, resistance response in the range of 11.01 to 16.67% was observed among the isolates to meropenem, tetracycline, sulfamethoxazole/trimethoprim and nalidixic acid while the rest of the tested antimicrobial agents was <10% except for colistin. A similar trend was also seen among PPF2 isolates, 68.57% (24/35) isolates were resistant to at least one antibiotics except for kanamycin. The highest resistance was to ampicillin (42.86%) and cefotaxime (40.00%).
Resistance to sulfamethoxazole/trimethoprim and nalidixic acid was 20.00% and 22.86%, respectively while resistance to streptomycin, tetracycline and chloramphenicol was in the range of 11.00% - 17.00%.

The differences observed in the resistance response might be as a result of different antibiotics commonly used in catfish ponds (Hong et al., 2018), sources of raw Pangasius fish produced (Tong Thi et al., 2013), origins of contamination with E. coli and seasonal periods of sampling (Le Nguyen et al., 2008) i.e. PPF1 sampling in the dry season (January 2019) versus PPF2 sampling in the rainy season (August 2019). This may be the first study of antibiotics resistance of E. coli isolated from processing factories during the processing chain of Pangasius product; however, high resistance of E. coli to ampicillin has been reported in previous studies from cultured catfish and pond water in Vietnam (Sarter et al., 2007; Divyashree et al., 2019; Shivakumaraswamy et al., 2019). Divyashree et al. (2019) reported maximum resistance of E. coli isolates obtained from effluents of two fish processing factories in Mangalore, India to ampicillin, tetracycline, and cefotaxime. According to Heuer et al. (2009), a wide range of antibiotic classes used extensively in aquaculture and human medicine lead to the incidence of resistant E. coli to higher generation antimicrobials. The present study clearly demonstrated that ampicillin and cefotaxime resistant - E. coli was present in Pangasius processing factories. These antibiotics (ampicillin, beta-lactam penicillin and cefotaxime as a 3rd generation cephalosporin) represent a group of antibiotics used in treating serious infections caused by E. coli and Salmonella (Nguyen et al., 2016). Resistance to meropenem was also observed among the E. coli isolates from both PPF1 (13.89%) and PPF2 (8.57%) isolates. Meropenem is a carbapenem which is one of the most important groups of antimicrobials considered as the last line of drugs for treatment of severe infection (Murugan et al., 2019). Likewise,
resistance to streptomycin, sulfamethoxazole/trimethoprim and nalidixic acid among *E. coli* isolates from this study is also of importance both in treating bacterial diseases in *Pangasius* aquaculture and also in human medicine. The implication of these results is that such antimicrobial resistant bacteria may be transmitted through the human gut thereby entering the food cycle when undercooked fish is consumed.

Table 1a and 1b shows the antibiotic resistance pattern of individual *E. coli* from PPF1 and PPF2. The resistant *E. coli* isolates from PPF1 possess 16 distinctive resistance patterns (Table 1a). 50.00% (11/22) of the isolates from PPF1 were resistant to two or fewer antibiotics; whereas the remaining 50.00% of the isolates were multi-drug resistant (resistance 3-9 antibiotics). These multi-drug resistant isolates represent fish (i.e. raw fish, filleting and packaging), water (i.e. bleeding, first and second washing steps) and hand/gloves (i.e. filleting and trimming steps) (Table 1a). For isolates from PPF2, there were 19 distinctive patterns (Table 1b). 54.17% of the isolates were resistant to two or fewer antibiotics whereas 45.83% are multi-drug resistant (3-7 antibiotics) (Table 1b). The results from both factories showed possible sources of contamination of *Pangasius* products with multi-drug resistant bacteria along the processing chain. Multiple antibiotics resistance in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes each encoding a single antibiotic resistance phenotype (Daini et al., 2008). For the raw fish, microbial flora present on fish is a representative of the aquaculture environment as well as the feed (Salgado-Miranda et al., 2010). Even though the fish were starved while awaiting processing, they may still be kept in the fishing boat with the catch nets holding fish possibly inside the Mekong River close to the processing factories, and multi-drug resistant bacteria may be abundant in water bodies (Holt et al., 2011). It is suggested that the processors should not only focus on reducing spoilage and pathogenic bacteria from the fillets but also put in high consideration the multi-drug resistant bacteria as they are present in almost every stage of processing and production chain. Some studies reported the prevalence of multi-drug resistant *E. coli* from cultured *Pangasius* catfish, other cultured fish species as well as wild fish species in Mekong Delta area.

Table 1a. Antibiotic resistance pattern of *E. coli* isolates from PPF1 (n = 36)

| No. | Resistance pattern | Number of antibiotics | Code | Processing step | Isolate source | % |
|-----|-------------------|-----------------------|------|----------------|----------------|---|
| 1   | NA                | 1                     | E33FT2 | Trimming | Fish | 50.00a |
| 2   | AMP               | 1                     | E22FP | Packaging | Fish | 50.00a |
| 3   | MEM               | 1                     | E12HF2 | Filleting | Hands | 50.00 |
| 4   | AMP-CTX           | 2                     | E22WB2 | Bleeding | Water | 50.00 |
| 5   | CAZ-CTX           | 2                     | E21FT4 | Trimming | Fish | 50.00 |
| 6   | AMP-CTX-SXT       | 3                     | E33FR1 | Raw fish | Fish | 50.00 |
| 7   | AMP-CTX-MEM       | 3                     | E33W13 | Washing | 1 | Water | 50.00 |
| 8   | AMP-CTX-CAZ       | 3                     | E12FP2 | Packaging | Fish | 50.00 |
| 9   | AMP-S-TE-SXT      | 4                     | E31HT2 | Filleting | Hand | 50.00 |
| 10  | AMP-S-CTX-FOX     | 4                     | E12FP1 | Filleting | Hand | 50.00 |
| 11  | AMP-S-CTX-SXT-GM  | 5                     | E33FF1 | Filleting | Fish | 50.00 |
| 12  | AMP-S-CTX-SXT-TF  | 5                     | E31HT2 | Filleting | Hand | 50.00 |
| 13  | AMP-S-CTX-GM-C-NA-SXT-CIP | 8 | E33FR4 | Raw fish | Fish | 50.00 |
| 14  | AMP-S-CTX-FOX-MEM-KM-TE-FOF | 8 | E12WB2 | Bleeding | Water | 50.00 |
| 15  | AMP-S-MEM-KM-C-NA-SXT-CIP | 9 | E32FR2 | Raw fish | Fish | 50.00 |
| 16  | AMP-S-MEM-KM-C-CTX-FOX-TE-FOF | 9 | E11W22 | Washing | 2 | Water | 50.00 |

*a*total number of resistant isolates (22/36), *a*number of isolates resistant to two or less antibiotics over total number of resistant isolates, *b*number of isolates resistant to three or more antibiotics over total number of resistant isolates. AMP: Ampicillin, CTX: Cefotaxime, CAZ: Ceftazidime, FOX: Cefoxitin, MEM: Meropenem, GM: Gentamicin, KM: Kanamycin, S: Streptomycin, TE: Tetracycline, C: Chloramphenicol, SXT: Sulfamethoxazole/trimethoprim, NA: Nalidixic acid, CIP: Ciprofloxacin, FOF: Fosfomycin, CL: Colistin

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of Vietnam (Sarter et al., 2007; Hon et al., 2016). These multi-drug resistant *E. coli* was not only limited to cultured fish but also prevalent in raw food items, food distribution systems and healthy human which also carried resistance and virulence genes (Van et al., 2008; Dyar et al., 2012; Rasheed et al., 2014; Done et al., 2015; Danielle Mroz, 2018). The emergence of *E. coli* with multiple antibiotics resistant phenotypes, including co-resistance to four or more unrelated families of antibiotics was therefore considered a serious health concern (Sarter et al., 2007). It is highly recommended that antibiotic resistant *E. coli* should be controlled as it was not only present in *Pangasius* catfish farms and environment but also prevalent in processing factories and among food operators. These findings are from two *Pangasius* processing factories and it can serve as background information for more studies to be done on several other *Pangasius* processing factories in the Mekong Delta region.

### 4. Conclusion

It can be concluded that even though both factories studied had implemented food safety and quality management systems such as HACCP, antibiotic resistant and multi-drug resistant *E. coli* were still found across the *Pangasius* processing chain. This showed that the current food safety management systems need to be reviewed to take into consideration multi-drug resistant bacteria. In addition, microbiological criteria required for *Pangasius* products destined for local and international markets should include multi-drug resistant bacteria to prevent the product from being a source of transfer of resistant genes both locally and internationally.

### Conflict of interest

The authors declare no conflict of interest.

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### Table 1b. Antibiotic resistance pattern of *E. coli* isolates from PPF2 (n = 35)

| No. | Resistance pattern | Number of antibiotics | Code | Processing step | Isolate source |
|-----|--------------------|-----------------------|------|----------------|----------------|
| 1   | CAZ                | 1                     | E11FT1 | Trimming     | Fish           |
| 2   | CAZ                | 1                     | E33FT1 | Trimming     | Fish           |
| 3   | CAZ                | 1                     | E33W11 | Washing I    | Water          |
| 4   | SXT                | 1                     | E12WB1 | Bleeding     | Water          |
| 5   | SXT                | 1                     | E22FR1 | Raw fish     | Fish           |
| 6   | AMP                | 1                     | E21FR1 | Raw fish     | Fish           |
| 7   | AMP                | 1                     | E22W11 | Washing I    | Water          | 54.17<sup>a</sup> |
| 8   | FOF                | 1                     | E13WB2 | Bleeding     | Water          |
| 9   | NA                 | 1                     | E33FF3 | Filleting    | Fish           |
| 10  | CTX-S              | 2                     | E33HT1 | Trimming     | Hand           |
| 11  | AMP-CAZ            | 2                     | E32FR2 | Raw fish     | Fish           |
| 12  | CAZ-FOX            | 2                     | E32WB1 | Bleeding     | Water          |
| 13  | GM-NA              | 2                     | E32WB2 | Bleeding     | Water          |
| 14  | AMP-S-SXT          | 3                     | E32FR1 | Raw fish     | Fish           |
| 15  | S-CAZ-CTX          | 3                     | E33FF2 | Filleting    | Fish           |
| 16  | AMP-CAZ-S-CL       | 4                     | E22FR4 | Raw fish     | Fish           |
| 17  | AMP-CAZ-S-TE       | 4                     | E22SS1 | Skinning     | Surface        |
| 18  | AMP-CAZ-MEM-C      | 4                     | E22WB1 | Bleeding     | Water          |
| 19  | AMP-TE-C-SXT       | 4                     | E31FR1 | Raw fish     | Fish           | 45.83<sup>b</sup> |
| 20  | AMP-CL-CAZ-TE-NA   | 5                     | E33HT2 | Trimming     | Hand           |
| 21  | AMP-CL-CAZ-TE-NA   | 5                     | E33SS2 | Skinning     | Surface        |
| 22  | AMP-CL-CAZ-CTX-MEM | 5                     | E31SS2 | Skinning     | Surface        |
| 23  | AMP-TE-SXT-CL-NA   | 7                     | E21W11 | Washing I    | Water          |
| 24  | AMP-TE-SXT-CL-NA   | 7                     | E33WB2 | Bleeding     | Water          |

<sup>a</sup>total number of resistant isolates (24/35), <sup>b</sup>number of isolates resistant to two or less antibiotics over total number of resistant isolates, <sup>c</sup>number of isolates resistant to three or more antibiotics over total number of resistant isolates. AMP: Ampicillin, CTX: Cefotaxime, CAZ: Ceftazidine, FOX: Cefoxitin, MEM: Meropenem, GM: Gentamicin, KM: Kanamycin, S: Streptomycin, TE: Tetracycline, C: Chloramphenicol, SXT: Sulfamethoxazole/trimethoprim, NA: Nalidixic acid, CIP: Ciprofloxacin, FOF: Fosfomycin, CL: Colistin.
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References

Boss, R., Overesch, G. and Baumgartner, A. (2016). Antimicrobial resistance of Escherichia coli, Enterococci, Pseudomonas aeruginosa, and Staphylococcus aureus from raw fish and seafood imported into Switzerland. Journal of Food Protection, 79(7), 1240-1246. https://doi.org/10.4315/0362-028X.JFP-15-463

CLSI. (2017). Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. https://doi.org/10.1039/C4DT01694G

Daini, O., Effiong, M. and Ogbulu, O. (2008). Quinolones Resistance and R-Plasmids of clinical isolates of Pseudomonas species. Sudan Journal of Medical Sciences, 3(2), 139-146. https://doi.org/10.4314/sjms.v3i2.38528

Danielle Mroz, M. (2018). Colistin-Resistant E. coli Found in Almost All Rural Vietnam Village. Contagion Infectious Diseases Today. Retrieved on April 18, 2019 from: https://www.contagionlive.com/news/colistin-resistant-e-coli-found-in-almost-all-residents-of-rural-vietnam-village.

Divyashree, M. Vijaya Kumar, D. Ballamoole, K.K. Shetty A, V. Chakraborty, A., and Karunasagar, I. (2019). Occurrence of antibiotic resistance among gram-negative bacteria isolated from effluents of fish processing plants in and around Mangalore. International Journal of Environmental Health Research, 2019(2), 45-73. https://doi.org/10.1080/09603123.2019.1618799

De Silva, S.S. and Phuong, N.T. (2011). Striped catfish farming in the Mekong Delta, Vietnam: a tumultuous path to a global success. Reviews inAquaculture, 3 (2), 45-73. https://doi.org/10.1111/j.1753-5131.2011.01046.x

Done, H.Y., Venkatesan, A.K. and Halden, R.U. (2015). Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture? The AAPS Journal, 17(3), 513-524. https://doi.org/10.1208/s12248-015-9722-z

Dyar, O.J., Hoa, N.Q., Trung, N.V., Phuc, H.D., Larsson, M., Chuc, N.T. and Lundborg, C.S. (2012). High prevalence of antibiotic resistance in commensal Escherichia coli among children in rural Vietnam.

BMC Infectious Diseases, 12(1), 92. https://doi.org/10.1186/1471-2334-12-92

Hedberg, N., Stenson, I., Pettersson, M.N., Warshon, D., Nguyen-Kim, H., Tedengren, M. and Kautsky, N. (2018). Antibiotic use in Vietnamese fish and lobster sea cage farms; implications for coral reefs and human health. Aquaculture, 495, 366-375. https://doi.org/10.1016/j.aquaculture.2018.06.005

Heuer, O.E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I. and Angulo, F.J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. Clinical Infectious Diseases, 49(8), 1248-1253. https://doi.org/10.1086/605667

Holt, K.E., Dolecek, C., Chau, T.T., Duy, P.T., La, T.T.P., Hoang, N.V.M., Nga, T.V.T., Campbell, J.L., Manh, B.H. and Chau, N.V.V. (2011). Temporal fluctuation of multidrug resistant Salmonella typhi haplotypes in the Mekong river delta region of Vietnam. PLoS Neglected Tropical Diseases, 5(1), e929. https://doi.org/10.1371/journal.pntd.0000929

Hon, N.T.N., Hoa, T.T.T., Thinh, N.Q. Hinenoya, A. Nakayama, T., Harada, K. Asayama, M., Warisaya, M. Hirata, K. and Phuong, N.T. (2016). Spread of antibiotic and antimicrobial susceptibility of ESBL-producing Escherichia coli isolated from wild and cultured fish in the Mekong Delta, Vietnam. Fish Pathology, 51(Special Issue), S75-S82. https://doi.org/10.3147/jsfp.51.S75

Hong, B., Ba, Y., Niu, L. Lou, F., Zhang, Z., Liu, H., Pan, Y. and Zhao, Y. (2018). A comprehensive research on antibiotic resistance genes in microbiota of aquatic animals. Frontiers in Microbiology, 2018, 01617. https://doi.org/10.3389/fmicb.2018.01617

Jansomboon, W., Boontanon, S., Boontanon, N., Polprasert, C. and Liamlaem, W. (2018). Food safety management of imported fishery products in Thailand: antibiotic standards and case study of enrofloxacin contamination in imported Pangasius catfish. International Food Research Journal, 25(5), 2081-2089.

Khan, A.A., Cheng, C.-M., Van, K.T., West, C.S., Nawaz, M. and Khan, S. (2006). Characterization of class 1 integron resistance gene cassettes in Salmonella enterica serovars Oslo and Bareilly from imported seafood. Journal of Antimicrobial Chemotherapy, 58(6), 1308-1310. https://doi.org/10.1093/jac/dkl416

Le Nguyen, D.D., Ngoc, H.H., Dijoux, D., Loiseau, G. and Montet, D. (2008). Determination of fish origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: An application on Pangasius fish from Viet Nam. Food Control, 19(5),

© 2020 The Authors. Published by Rynnye Lyen Resources
Little, D.C., Bush, S.R., Belton, B., Phuong, N.T., Young, J.A. and Murray, F.J. (2012). Whitefish wars: Pangasius, politics and consumer confusion in Europe. Marine Policy, 36(3), 738-745. https://doi.org/10.1016/j.marpol.2010.10.006

Liu, Y.-Y., Wang, Y., Walsh, T.R., Yi, L.-X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B. and Huang, X. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. The Lancet Infectious Diseases, 16(2), 161-168. https://doi.org/10.1016/S1473-3099(15)00424-7

Long, N.V. and Lua, D.T. (2017). Antimicrobial usage and Antimicrobial resistance in Vietnam. In Aquatic AMR Workshop 1, 10–11 April 2017. Mangalore, India.

Manage, P.M. (2018). Heavy Use of Antibiotics in Aquaculture; Emerging Human and Animal Health Problems—A review. Sri Lanka Journal of Aquatic Sciences, 23(1), 13–27. DOI: http://doi.org/10.4038/sljas.v23i1.7543

Ministry of Science and Technology of the Socialist Republic of Vietnam. (2010). Officially legal criteria for frozen tra fish Pangasius hypophthalmus fillet. TCVN 8338: 2010. Viet Nam.

Murugan, M.S., Sinha, D., Kumar, O.V., Yadav, A.K., Pruthivishree, B., Vadhana, P., Nirupama, K., Bhardwaj, M. and Singh, B. (2019). Epidemiology of carbapenem-resistant Escherichia coli and first report of blaVIM carbapenemases gene in calves from India. Epidemiology and Infection, 147, e159. https://doi.org/10.1017/S0950268819000463

Nguyen, D.T.A., Kanki, M., Do Nguyen, P., Le, H.T., Ngo, P.T., Tran, D.N.M., Le, N.H., Van Dang, C., Kawai, T. and Kawahara, R. (2016). Prevalence, antibiotic resistance, and extended-spectrum and AmpC β-lactamase productivity of Salmonella isolates from raw meat and seafood samples in Ho Chi Minh City, Vietnam. International Journal of Food Microbiology, 236, 115-236122. https://doi.org/10.1016/j.ijfoodmicro.2016.07.017

Nhung, N., Cuong, N., Campbell, J., Hoa, N., Bryant, J., Truc, V., Kiet, B., Jombart, T., Trung, N. and Hien, V. (2015). High levels of antimicrobial resistance among Escherichia coli isolates from livestock farms and synanthropic rats and shrews in the Mekong Delta of Vietnam. Applied and Environmental Microbiology, 81(3), 812-820. https://doi.org/10.1128/AEM.03366-14

Pham, D.K., Chu, J., Do, N.T., Brose, F., Degand, G., Delahaut, P., De Pauw, E., Dony, C., Van Nguyen, K. and Vu, T.D. (2015). Monitoring antibiotic use and residue in freshwater aquaculture for domestic use in Vietnam. EcoHealth, 12(3), 480-489. https://doi.org/10.1007/s10393-014-1006-z

Phan, L.T., Bui, T.M., Nguyen, T.T., Gooley, G.J., Ingram, B.A., Nguyen, H.V., Nguyen, P.T. and De Silva, S.S. (2009). Current status of farming practices of striped catfish, Pangasius hypophthalmus in the Mekong Delta, Vietnam. Aquaculture, 296(3-4), 227-236. https://doi.org/10.1016/j.aquaculture.2009.08.017

RASFF. (2019). Rapid alert notifications for food and feed. Retrieved on October 5, 2019 from RASFF website: https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationsList&StartRow=101

Rasheed, M.U., Thajuddin, N., Ahamed, P., Teklemariam, Z. and Jamil, K. (2014). Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources. Revista do Instituto de Medicina Tropical de São Paulo, 56(4), 341-346. https://doi.org/10.1590/S0036-46652014000400012

Rico, A., Phu, T.M., Satapornvanit, K., Min, J., Shahabuddin, A., Henriksen, P.J., Murray, F.J., Little, D.C., Dalsgaard, A. and Van den Brink, P.J. (2013). Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. Aquaculture, 412-413, 231-243. https://doi.org/10.1016/j.aquaculture.2013.07.028

Salgado-Miranda, C., Palomares, E., Jurado, M., Marin, A., Vega, F. and Soriano-Vargas, E. (2010). Isolation and distribution of bacterial flora in farmed rainbow trout from Mexico. Journal of Aquatic Animal Health, 22(4), 244-247. https://doi.org/10.1577/H09-004.1

Santos, L. and Ramos, F. (2018). Antimicrobial resistance in aquaculture: current knowledge and alternatives to tackle the problem. International Journal of Antimicrobial Agents, 52(2), 135-143. https://doi.org/10.1016/j.ijantimicag.2018.03.010

Sarter, S., Nguyen, H.N.K., Hung, L.T., Lazard, J. and Montet, D. (2007). Antibiotic resistance in Gram-negative bacteria isolated from farmed catfish. Food Control, 18(11), 1391-1396. https://doi.org/10.1016/j.foodcont.2006.10.003

Shivakumaraswamy, S.K., Deekshit, V.K., Vittal, R., Akhila, D.S., Mundanda, D.M., Raj, J.R.M., Chakraborty, A. and Karunasagar, I. (2019). Phenotypic and genotypic study of antimicrobial profile of bacteria isolates from environmental samples. The Indian Journal of Medical Research,
Tong Thi, A.N., Noseda, B., Samapundo, S., Nguyen, B.L., Broekaert, K., Rasschaert, G., Heyndrickx, M. and Devlieghere, F. (2013). Microbial ecology of Vietnamese Tra fish (*Pangasius hypophthalmus*) fillets during processing. *International Journal of Food Microbiology*, 167(2), 144-152. https://doi.org/10.1016/j.ijfoodmicro.2013.09.010

Van, T.T.H., Chin, J., Chapman, T., Tran, L.T. and Coloe, P.J. (2008). Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. *International Journal of Food Microbiology*, 124(3), 217-223. https://doi.org/10.1016/j.ijfoodmicro.2008.03.029

VASEP. (2020). Vietnam Association of Seafood Exporters and Producers. Retrieved on March 5, 2020 from VASEP website: http://www.pangasius-vietnam.com/378/Daily-Newsp/About-Pangasius.htm.