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Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from *Pangasius* fish and fish processing handlers in the Mekong Delta, Viet Nam

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ABSTRACT

This study aimed to investigate the prevalence and antibiotic resistance of Staphylococcus aureus isolates from Pangasius fish (Pangasianodon hypophthalmus) and the hands of fish processors in fish processing plants. The results showed that 16 of 90 Pangasius fish (18%) and 6 of 54 fish processing handlers' samples (11%) harbored coagulase-positive for S. aureus. A high antibiotic resistance (68%) was observed for ampicillin, while 100% susceptibility for oxacillin, cefazolin, cefmetazole, flomoxef, imipenem, arbekacin, fosfomycin, and sulfamethoxazole-trimethoprim were recorded for both fish and hand samples. Remarkably, only one isolates from the fish was classified as methicillin-resistant S. aureus, whereas no isolates from the handlers were found. Among the total S. aureus isolates, the multidrug resistance rate was nearly 41%, posing a hazard to public health concerns. The obtained results may provide valuable information about the actual state of S. aureus contamination for efficient control in fish processing plants.

1. INTRODUCTION

The emergence of multidrug-resistant (MDR) bacteria is an increasing global concern because of the extensive use of antibiotics in livestock and aquaculture production. *Staphylococcus aureus* is an opportunistic common pathogen associated with the colonization of the skin and musical surfaces of humans (Hwa, 2003). *S. aureus* may grow in food by utilizing temperature and time, producing heat-stable intoxication and cooking-resistant enterotoxins (Hennekinne et al., 2012). The antibiotic resistance of *S. aureus* following the publication of methicillin-resistant *S. aureus* (MRSA) has been recognized as a public health problem throughout the world because of significant mortality and morbidity rates (Kong et al., 2016). Additionally, *S. aureus* is not a normal microbiota of fish; therefore, its presence in fish indicates cross-contamination originating from the harvest area or improper handling by fish handlers (Kumar et al., 2016). Several past studies have reported that the incidence of antibiotics resistance *S. aureus* in fish varied in a range from 30 to 60% (Obaidat et al., 2015; Kumar et al., 2016; Vaiyapuri et al., 2019).

Pangasius fish (*Pangasianodon hypophthalmus*) is a kind of freshwater fish classified as the leading aquaculture species in Viet Nam. Over the years, the high number of antimicrobial agents on *Pangasius* farms, and other agricultural sectors has been noticed because of the rapid development of the economy (Long & Lua, 2017). The blooming export of *Pangasius* fish to global markets led to more research focus on the antibiotic's resistance bacteria from the farm to the final product. However, information regarding the antibiotic resistance of *S. aureus* remains limited. Thus, this study aims to investigate the occurrence of *S. aureus* in *Pangasius* products and the hands of processing factory workers. The susceptibility of *S. aureus* to different antimicrobial agents was determined by using the broth microdilution method.

2. MATERIALS AND METHOD

2.1. Samplings

About 144 samples were processed for the isolation of *S. aureus*, including 90 samples from *Pangasius* fish at different steps of processing and fifty-four samples of hands/gloves of processing workers in two *Pangasius* fish processing plants in Mekong Delta, Viet Nam. Personnel hands were sampled by swab method with pre-wetted swabs placed in 5 mL maximum recovery diluent (Merck, Darmstadt, Germany).

2.2. Isolation and identification of *S. aureus*

Fish and swab samples were processed according to the International Organization for Standardization ISO 6888–1 (ISO, 2003) to isolate *S. aureus* on Baird Parker Agar (Merck, Darmstadt, Germany). *S. aureus* colonies with distinct characteristics (black or grey, shining, and convex with narrow surrounding a clear zone) were selected for coagulase tests (Bactident® Coagulase, Merck, Darmstadt, Germany).

2.3. Antibiotics resistance tests

The *S. aureus* isolates were performed antimicrobial susceptibility tests following the broth microdilution as described by the Clinical and Laboratory Standards Institute (CLSI, 2021). The DP 32 dry plates with 96-well (Eiken Chemical Co., Tokyo, Japan) including oxacillin, ampicillin, cefazolin, cefmetazole, flomoxef, imipenem, gentamycin, arbekacin, minocycline, cefoxitin, erythromycin, clindamycin, vancomycin, teicoplanin, linezolid, fosfomycin, sulfamethoxazole/trimethoprim, and levofloxacin at different concentrations were used in this test. The results were interpreted according to CLSI (2021).

3. RESULTS AND DISCUSSION

The microbiological contamination of fish and fishery products is associated with the aquatic environments, and the sanitary conditions from the farm to the table including cultivation, harvest, processing, storage, and transportation. Thus, ensuring food safety is the responsibility of the authorities and those who produce and market the processing product.

The incidence and *S. aureus* count in *Pangasius* fish and hands of fish processors are shown in Table 1. The results show that the occurrence of *S. aureus* in Pangasius fish was 18%, and count levels ranged from 1 to 1.6 log CFU/g. This incidence was lower than a past study in Spain where *S. aureus* was found a relatively high prevalence in 43% of fresh fish and 30% of frozen products but similar to a report in Turkey with 18% of raw fish, 17.5% of processed fish, and 10% of frozen fish contaminated with *S. aureus* (Vázquez-Sánchez et al., 2012; Sivaraman et al., 2022). Moreover, a similar value of < 2 log CFU/g has been reported by Herrera et al. (2006) but Stratev et al. (2021) stated a high count of 4.5 log CFU/g *S. aureus* in fish. Among the bacteria, *S. aureus* is a product of contamination of the aquatic environment where they come from and/or the poor production and handling conditions in the matter of hygiene (Hernández-Nava et al., 2020). The difference in *S. aureus* count and incidence rates found in this study and other studies may be a result of differences in the processing and hygienic practices of fish handlers.

Table 1. Prevalence and number of *S. aureus* count (log CFU/g) in the Vietnamese *Pangasius* fish and hands of fish processors.

Types of samples	No. of samples	No. of *S. aureus* positive samples (%)	Range of *S. aureus* count
Fish	90	(18)	1-1.6 log CFU/g
Hands/gloves of workers	54	6 (11)	1-1.8 log CFU/cm^2
Total	144	22 (15.3)	

As shown in Table 1, it has been observed that the relatively low 11% *S. aureus* incidence and count levels varied from 1 to 1.8 log CFU/cm^2 in the hands of fish processing handlers. The obtained results were not similar to previous research, which showed the high prevalence of *S. aureus* in 74% of food handlers.

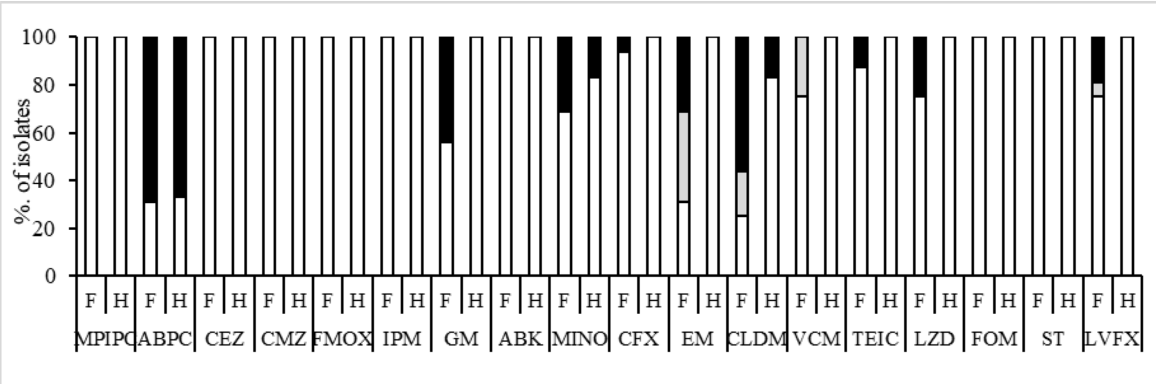

Figure 1. Antibiotic resistance profile including

susceptibility ☐ intermediate and resistance ■ of S.aureus isolates from Pangasius fish (F) and hands of workers (H) to antimicrobials. MPIPC, Oxacillin; ABPC, Ampicillin; CEZ, Cefazolin; CMZ, Cefmetazole; FMOX, Flomoxef; IPM, Imipenem; GM, Gentamycin; ABK, Arbekacin; MINO, Minocycline; CFX, Cefoxitin; EM, Erythromycin; CLDM, Clindamycin; VCM, Vancomycin; TEIC, Teicoplanin; LZD, Linezolid; FOM, Fosfomycin; ST, Sulfomethoxazole-Trimethoprim; LVFX, Levofloxacin

hands in Malaysia and 60 % of those in Brazil (Albuquerque et al., 2007; Tan et al., 2014). Additionally, Tan et al. (2013) illustrated a high prevalence of *S. aureus* in the hands because of improper hand-washing practices and not frequent use of face masks by food processing handlers. Moreover, skin lesions, sneezing, or coughing of food handlers may cause cross-contamination of *S. aureus* into the food (Bischoff et al., 2006). In this study, although the *S. aureus* counts from fish were under the maximum tolerable microbiological limit (<2 log CFU/g) according to the Vietnamese National Standards (TCVN, 2010), *S. aureus* on fish handlers exceeded the legal regulations, suggesting inadequate personal hygiene practice. This result shows that it is essential to make efforts to implement effective regulations and good hygiene practices in the fish industry to ensure food hygiene.

The results of antimicrobial susceptibility testing of all *S. aureus* isolates are shown in Figure 1 by using the broth microdilution method. A similar high resistance to ampicillin (68%) was exhibited by *S. aureus* isolates from both *Pangasius* fish and hands samples. Similarly, Obaidat et al. (2015) and Fri et al. (2020) reported a high percentage of 83% and 70% of *S. aureus* isolated from fish against ampicillin in Jordan and South Africa, respectively. In this study, 31% of the *S. aureus* isolates from fish and 17% from hands samples showed resistance to minocycline while 56% of the isolates from fish and 17% from hands were resistant to clindamycin. Only the *S. aureus* isolates derived from *Pangasius* fish was resistant to gentamycin (44%), erythromycin (32%), teicoplanin (12%), linezolid (25%), and levofloxacin (19%) whereas *S. aureus* isolates from hands samples exhibited the susceptible to these antibiotics. Moreover, no *S. aureus* isolate were resistant to oxacillin, cefazolin, cefmetazole, flomoxef, imipenem, arbekacin, fosfomycin, and sulfamethoxazole-trimethoprim. The differences among the source of food products and/or the sanitary of food handlers may contribute to the variations in the susceptibility profile of *S. aureus*.

Remarkably, only one *S. aureus* isolate derived from *Pangasius* fish revealed resistance to cefoxitin with MIC ≥ 16μg/ml which is classified as MRSA according to CLSI (2021) while no MRSA isolate was observed on the hand samples. The results were similar to previous studies that reported nearly 5% of MRSA from fish in Iran, sashimi fish in Japan, and aquatic products in China (Hammad et al., 2012; Noushin et al., 2016; Wu et al., 2019). Conversely, this finding is considerably low compared with 60% of MRSA from fish in Turkey, 50% from ready-to-eat fish in Poland, and 35% from farmed tilapia fish in Malaysia (Atyah et al., 2010; Chajecka-Wierzchowska et al., 2015; Siiriken et al., 2016). Several studies from different countries have showed that food handlers have a 30-60% of *MRSA* in Sudan, Turkey, Botswana, Brazil, and Portugal (Loeto et al., 2007; Sezer et al., 2015; Casto et al., 2016; Anderson et al., 2020; Ahmed, 2020). Obviously, food handlers' carriage of MRSA may contribute to the development of MRS and become an important source of transmission in humans. Although several studies reported a high prevalence of over 80% MRSA in the Vietnamese hospitals (Nguyen et al., 2019; Thai et al., 2019; Thai et al., 2020; Thuy et al., 2021), there is a lack of scientific information on MRSA in food and food handlers since no study focuses on these aspects in Viet Nam. Thus, further research to fill this gap should be done at the national level to gain insight into Viet Nam's circumstances.

Table 2. Antibiotics resistance pattern of *S. aureus* isolates derived from the Vietnamese Pangasius fish and hands of workers.

Resistance pattern[1]	Type of sample	Processing step	No.of isolates	%
ABPC,GM,MINO,CFX,EM,CLDM		Product	1	
ABPC,GM,MINO,EM,CLDM,LZD		Trimming	1	
ABPC,GM,MINO,CLDM,LVFX		Cooling	1	
ABPC,ABK,CLDM,MINO	Fish	Cooling	1	41[a]
ABPC,GM,MINO,LVFX		Product	1	
ABPC,GM,EM,CLDM		Filleting Filleting	2	
ABPC,MINO,CLDM	Hands	Filleting	1	
ABPC,TEIC,LZD		Product	1	
ABPC,CLDM		Product	1	
EM,CLDM		Filleting	1	
TEIC,LZD		Trimming	1	
LVFX		Cooling	1	
CLDM	Fish	Raw	1	
GM		Filleting	1	50[b]
ABPC	Hands	Filleting Filleting Product Filleting Product	5	

***Note**: [a] number of isolates resistant to three or more antibiotics over the total number of isolates; [b]number of isolates resistant to two or fewer antibiotics over the total number of isolates.[1] MPIPC, Oxacillin; ABPC, Ampicillin; CEZ, Cefazolin; CMZ, Cefmetazole; FMOX, Flomoxef; IPM, Imipenem; GM, Gentamycin; ABK, Arbekacin; MINO, Minocycline; CFX, Cefoxitin; EM, Erythromycin; CLDM, Clindamycin; VCM, Vancomycin; TEIC, Teicoplanin; LZD, Linezolid; FOM, Fosfomycin; ST, Sulfomethoxazole-Trimethoprim; LVFX, Levofloxacin; CIP, Ciprofloxacin; TET, Tetracycline; CHL, Chloramphenicol*

The antibiotic resistance pattern of *S. aureus* isolates is represented in **Table 2**. About 41% (9/22 isolates) were found to be MRD since they showed resistance to at least one antibiotic drug in three or more antibiotic classes. The results obtained in this study are lower than with high levels of up to 100 % of MRD – MRSA *S. aureus* in pig and retail foods in China (Wang et al., 2017), as well as in poultry and farmers workers in South Africa (Amoako et al., 2019), but comparable higher than 17% from the imported fish sample in Switzerland (Boss et al., 2016). Unregulated antimicrobials in the aquaculture sector for the production of farm-raised fish expose a risk to food safety and public health concerns (Okocha et al., 2018). The moderately high prevalence of MRD in this study enriches the ongoing database of MRD of *S. aureus* from fish processing plants in particular and in Viet Nam.

4. CONCLUSION

The findings of this study highlight the occurrence of antibiotic resistance of *S. aureus* in *Pangasius* fish and fish processing handlers in Viet Nam. It is essential for continuous food control, including effective audits, to minimize contamination with *S. aureus* from fish and personnel processing workers. Although the prevalence of MRSA and MRD in fish processing handlers was low, the potential dissemination of these isolates in the community cannot be ruled out. Thus, improving processing operations and implementing appropriate sanitation practices in fish processing handlers is recommended to ensure fishery product safety.

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