Journal of Environmental Science and Engineering B 13 (2024) 199-209
doi:10.17265/2162-5263/2024.05.004

Antimicrobial Resistance of *Aeromonas* spp. and *Vibrio* spp. Isolated from Fresh Pangasius Fish in Cambodia

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Abstract: The study was conducted to identify *Aeromonas* spp. and *Vibrio* spp. from fresh Pangasius fish (n = 153) in Cambodia and test their antimicrobial susceptibility to antibiotics. The samples were collected from different wet markets of Phnom Penh city and Kampong Thom, and Siem Reap provinces. The bacteria were isolated by using selective medium and their AMR (Antimicrobial Resistance) profile was tested by API 20E technique, respectively. Susceptibility profile was determined for seven antibiotics commonly used. The *Vibrio* spp. (34.64%, n = 53) was found to be higher than *Aeromonas* spp. (24.83%, n = 38). Four *Vibrio* and four *Aeromonas* species were identified where *V. parahaemolyticus* (57%, n = 30) was the highest, followed by *V. cholerae* (38%, n = 20), *V. fluvialis* (3.8%, n = 2) and *V. aglinolyticus* (1.9%, n = 1), whereas *A. hydrophila* (47%, n = 18) was the highest, followed by *A. hydrophila/caviae* (45%, n = 17), *A. sobria* (5%, n = 2), *A. caviae* (2.6%, n = 1). All the species presented high multi-resistance to the tested antibiotics. The antibiotic susceptibility profile to ampicillin (74%-100%), ciprofloxacin (7%-100%), sulfamethoxazole/trimethoprim (14%-100%), florfenicol (14%-100%), oxytetracycline (7%-100%), erythromycin (10%-100%) and colistin sulphate (33%-100%) was revealed resistance level in *Aeromonas* spp. whereas few species of *Vibrio* spp. resistant to ampicillin (43%-100%), ciprofloxacin (14%-100%), sulfamethoxazole/trimethoprim (14%-100%), florfenicol (14%-100%), oxytetracycline (20%-100%), erythromycin (29%-100%), colistin sulphate (33%-100%) were also identified. The results revealed these *Aeromonas* spp. and *Vibrio* spp. are potentially reservoirs of antibiotic resistance genes. MDR (Multidrug Resistance) was widespread among the samples isolated. That is a high-risk source of contamination since those pathogens and antimicrobials are often used. Our findings highlight that the aquatic environment and fresh Pangasius fish act as reservoirs of AMR *Aeromonas* spp. and *Vibrio* spp. which underline the need for a judicious use of antimicrobials and timely surveillance of AMR in aquaculture. Overall, the findings of our study indicated the presence of *A. hydrophila*, *A. hydrophila/caviae*, *A. caviae*, *A. sobria*, *V. parahaemolyticus*, *V. cholerae*, *V. alginolyticus* and *V. fluvialis* and high MDR. This result will allow us to identify the potential risk over circulating isolates in animal health and public health and the spread through the food chain offering supports for appropriate sanitary actions.

Key words: *Aeromonas* spp., *Vibrio* spp., prevalence, MDR, AMR, and Cambodia's Pangasius.

1. Introduction

The total production in 2016 is 802,450 tones: 509,350 tons from freshwater fisheries, 120,600 from marine capture and 172,500 tones from aquaculture [1]. Cambodians are among the highest consumers of freshwater fish in the world, with annual per capita fish consumption estimated at 52.4 kg [2]. According to aquaculture in Cambodia, feed have been used and mixed with antibiotics. The farmers have decided to use antibiotics to prevent disease and increase yield without consultation and prescription toward AMR (Antimicrobial Resistance). AMR is a major global

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threat of increasing concern to animal, human and environmental health. ODC [3] and Van Boeckel et al. [4] said that antimicrobials are used in livestock production to maintain health and productivity. These practices contribute to the spread of antimicrobial resistant pathogens in both livestock and humans, posing a significant public health threat. We distribute the first global map (228 countries) of antibiotic consumption in livestock and conservatively estimate the total consumption in 2010 at 63,151 tons. We project that antimicrobial consumption will rise by 67% by 2030, and nearly double in Brazil, Russia, India, China, and South Africa. This rise is likely to be driven by the growth in consumer demand for livestock products in middle-income countries and a shift to large-scale farms where antimicrobials are used routinely. Our findings call for initiatives to preserve antibiotic effectiveness while simultaneously ensuring food security in low- and lower-middle-income countries. Nguyen and Dang [5] show that farmers have used antibiotics without any prescription for both prevention and treatment of fish disease, because they invested a lot of money, and they do not want to lose their products. In Cambodia, the surveyed fish disease control covered both extensive and intensive farms and reported any disease problems and oxytetracycline had been used for disease control on affected farms [6]. Om and McLaws (2016) interview with farmers, veterinarian and animal feed retailer and filed observation identified a broad range of antibiotics used on food animal farms including beta-lactam, fluoroquinolones, tetracycline, colistin and lincosamides. Farmers and veterinarians commonly spoke of antibiotics being useful for preservation and treatment of disease in their animal feed and believed that without antibiotics, their livestock would not thrive.

The aim of the present study was to identify *Aeromonas* spp. and *Vibrio* spp. from the fresh Pangasus fish in Cambodia and to evaluate their susceptibility to some commonly used antibiotics.

2. Research and Methodology

2.1 Location and Duration

This research study involved collection of fresh Pangasius fish samples from nine different local markets of Cambodia. There are three forms in Kampong Thom province including Phsar Kampong Thom (KP), Phsar Stoung (ST), and Phsar Romlong (RL), Siem Reap province including Phsar Sot Nikum (SN), Phsar Prasat Bakong (PB), and Phsar Leu Thom Tmey (TM), and Phnom Penh city including Phsar Prek Pnov (PK), Chamkar Doung (CD), and Phsar Deum Kor (DK) from Cambodia. The experiment was conducted at the Microbiology Laboratory of Faculty of Agro-Industry, Royal University of Agriculture. The experimental period lasts 90 days, starting from March 17th to May 16th 2022.

Bacteria were isolated from the collected fresh Pangasius fish and identified by morphological and biochemical characteristics of *Aeromonas* spp. and *Vibrio* spp. which were determined by PHE (Public Health England, 2015) and performed following the API 20E (API 20 system) (bioMérieux) [7]. Antibiotic susceptibility testing of selected bacteria was determined by the Kirby-Bauer disc diffusion method [8].

2.2 Sample Collection

A total of 153 fresh fish samples were collected form the target area. Ten grams (10 g) of the samples' intestine, gill and muscle were tested for isolation and identification on bacteria.

2.3 Isolation and Identification of Aeromonas spp.

A total of 153 samples obtained from Kampong Thom, Siem Reap and Phnom Penh were initially cultured. The samples should not have been damaged or changed during transport or storage. The samples' surfaces were disinfected using 70% alcohol. Ten grams (10 g) of fresh Pangasius (gill, intestine and muscle) mix well with Buffer Peptone Water (BPW) 90 mL. Blood agar incubated at 35-37 ℃ for 24-48 h. Colonies are distinctively circular, large, raised and are 1-3 mm in diameter. And MAC (MacConkey Agar) incubated at 35-37 ℃ for 24-48 h. Colonies are typically non-lactose fermenting although some lactose-fermenting *Aeromonas* spp. and then pure culture gram (- ve), rod shape and coccobacillary with rounded ends oxidase (+ ve) [9] and biochemical test API 20 NE biochemical characterization [7].

2.4 Isolation and Identification of Vibrio spp.

There are 153 of the fresh Pangasius fish. It should not have been damaged or changed during transport or storage. The samples surfaces were disinfected using 70% alcohol. Ten grams (10 g) of fresh Pangasius (gill, intestine and muscle) mix well with BPW 90 mL. Blood agar incubated at 35-37 ℃ for 18-24 h. Colonies are 2-3 mm in diameter; some colonies may be haemolytic. TCBS (Thiosulfate Citrate Bile Salts Sucrose Agar) incubated at 35-37 ℃ for 24-48 h. Yellow or green colonies are 2-3 mm in diameter. Following incubation, presumed colonies of *V. cholerae* (yellow on TCBS Agar, Difco agar) and *V. parahaemolyticus* (green on TCBS Agar, Difco agar). Pure culture gram (- ve), rods characteristically cured or comma-shape but can also be straight, oxidase (+ ve) all *Vibrio* spp. [10] and biochemical test using API (Analytical Profile Index 20) namely API 20E biochemical characterization [7].

2.5 Antibiotic Susceptibility Test of Selected Bacteria

Aeromonas spp. strains isolated in the present study were subjected to susceptibility testing against 28 antimicrobials commonly used. Susceptibility was determined by the disk-diffusion technique of Kirby-Bauer on Mueller-Hinton agar plates (Oxoid Basingstoke, UK) with inoculation adjusted to an optical density of 0.5 McFarland standard units.

Disks containing ampicillin (AMP 10 μg), carbenicillin (CAR 100 μg), amoxicillin (AML 10 μg), amoxicillin/clavulanic acid (AMC 30 μg), piperacillin (PRL 100 μg), piperacillin/ tazobactam (TZP 110 μg), ticarcillin (TIC 75 μg), ticarcillin/clavulanic acid (TIM 85 μg), cephalothin (KF 30 μg), cefoxitin (FOX 30 μg), cefotaxime (CTX 30 μg), cefoperazone (CFP 30 μg), ceftazidime (CAZ 30 μg), ceftriaxone (CRO 30 μg), cefepime (FEP 30 μg), aztreonam (ATM 30 μg), imipenem (IMP 10 μg), gentamicin (CN 10 μg), kanamycin (K 30 μg), tobramycin (TOB 10 μg), amikacin (AK 30 μg), netilmicin (NET 30 μg), tetracycline (TE 30 μg), ciprofloxacin (CIP 5 μg), norfloxacin (NOR 10 μg), erythromycin (E 15 μg), trimethoprim/sulfamethoxazole (SXT 25 μg) and chloramphenicol (C 30 μg) were used. All disks were obtained from Oxoid. After 24 h incubation at 30 ℃, organisms were classified as sensitive (S), intermediately (I) or resistant (R) based on the size of the zone of bacteria growth inhibition according to the guidelines of the CLSI.

The susceptibilities of the identified *Aeromonas* and *Vibrio* species to 7 antibiotics used include Ampicillin (10 μg), Florfenicol (30 μg), Colistin Sulphate (10 μg), Erythromycin (15 μg), Oxytetracycline (30 μg), Ciprofloxacin (5 μg), and Sulfamethoxazole/ trimethoprim (23.75/1.25 μg). Combination was used on Mueller Hinton agar (Merck KGaA, Germany) Kirby Bauer Disc Diffusion Method [8]. Antibiotic disks were purchased from OXOID (Thermo Fisher, UK). Isolates were identified as susceptible, intermediate or, resistant according to CLSI [11, 12] guidelines and M45/M49-S1.

2.6 Data Record and Analysis

All the data were recorded in Excel and analyzed by using descriptive.

3. Result

3.1 Isolation and Identification of Aeromonas and Vibrio spp

The obtained data in Fig. 1 distribution of *Aeromonas* spp. and *Vibrio* spp. could be isolated from fresh

Pangasius fish. The results show that an amount of 153 fresh Pangasius fish samples selected from retail markets level from Kampong Thom province, Siem Reap province and Phnom Penh city were positive of bacterial *Aeromonas* spp. within amount of 38 isolated (24.83%, *n* = 38) from 153 samples and *Vibrio* spp. in amount 53 isolated (34.64%, *n* = 53) from 153 samples as well. The distribution of *Aeromonas* spp. And *Vibrio* spp. isolated from fresh Pangasius fish in Kampong Thom, Siem Reap, and Phnom Penh has been shown in Figs. 1-6.

3.1.1 Prevalence Characterization of *Aeromonas* and *Vibrio* spp. Isolated from Kampong Thom Province

A total of 51 colonies were selected from different markets in Kampong Thom province, which were meet characteristic of *Aeromonas* spp. and *Vibrio* spp. In all, 20 colonies (39.21%, *n* = 20/51) including *Aeromonas* spp. (*n* = 9) and *Vibrio* spp. (*n* = 11) were presumptively screened as an *Aeromonas* and *Vibrio* spp. base on API 20E.

Figs. 1 and 2 showed that prevalence of *Aeromonas* spp. and *Vibrio* spp. was found to be highest in *A. hydrophila* (45%, *n* = 4), *A. hydrophila/caviae* (22%, *n* = 2), *A. sobria* (22%, *n* = 2), *A. caviae* (11%, *n* = 1), *V. cholerae* (55%, *n* = 6), *V. parahaemolyticus* (27%, *n* = 3), and *V. fluvialis* (18%, *n* = 2).

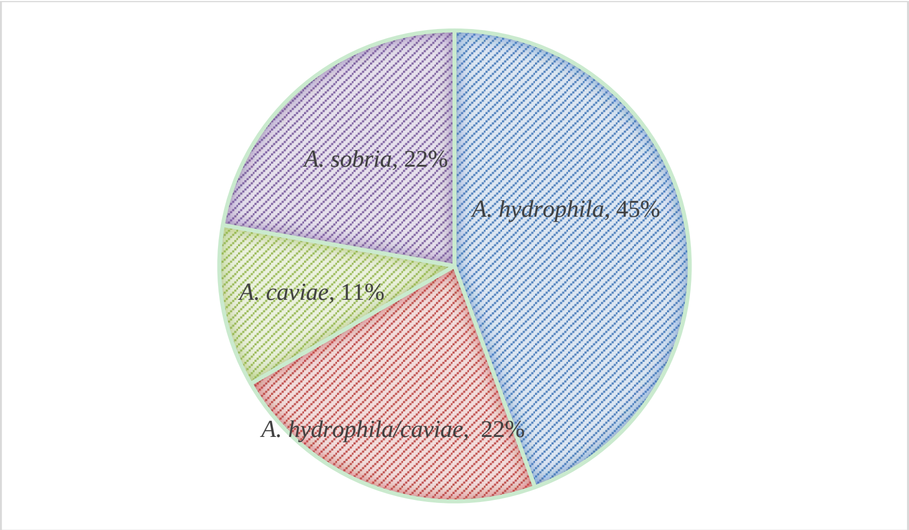

Fig. 1 Prevalence of *Aeromonas* spp. from Kampong Thom province.

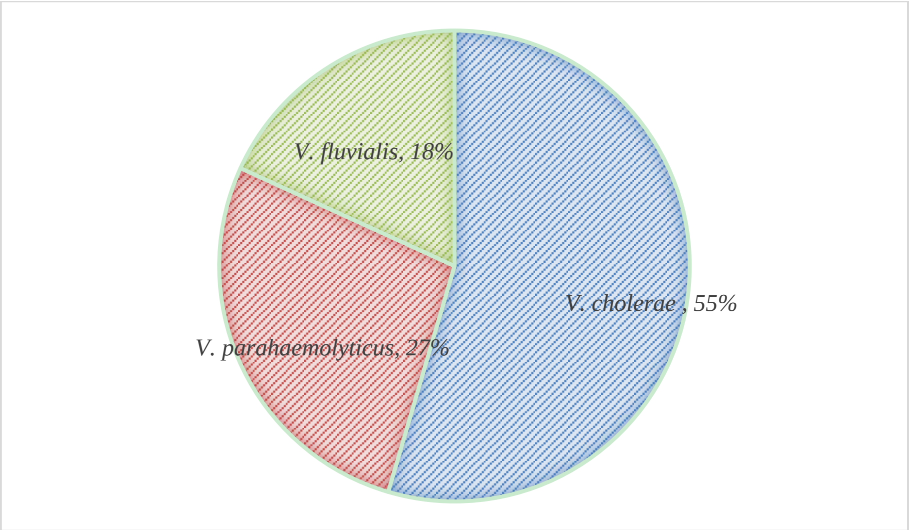

Fig. 2 Prevalence of *Vibrio* spp. from Kampong Thom province.

3.1.2 Prevalence of *Aeromonas* spp. and *Vibrio* spp. Isolated from Siem Reap Province

A total of 51 colonies were selected from different markets in Siem Reap province. In all, 45 colonies (88.23%, *n* = 45/51) including *Aeromonas* spp. (*n* = 15) and *Vibrio* spp. (*n* = 30) were presumptively screened as an *Aeromonas* and *Vibrio* spp. base on API 20E.

The results of the prevalence of *Vibrio* and *Aeromonas* species were reported in Figs. 3 and 4. The largest prevalence of *A. hydrophila* (93%, *n* = 14), *A. hydrophila/caviae* (7%, *n* = 1), *V. parahaemolyticus* (74%, n= *n* = 22), *V. cholerae* (23%, *n* = 7), and *V. aglinolyticus* (3%, *n* = 1) was identified.

3.1.3 Prevalence of *Aeromonas* spp. and *Vibrio* spp. Isolated from Phnom Penh

A total of 51 colonies were selected from different markets in Phnom Penh city. In all, 26 colonies (50.98%, *n* = 26/51) including *Aeromonas* spp. (*n* = 14) and *Vibrio* spp. (*n* = 12) were presumptively screened as an *Aeromonas* and *Vibrio* spp. base on API 20E.

The prevalence of *Aeromonas* and *Vibrio* species was found to be highest for *A. hydrophila/caviae* (100%, *n* = 14), *V. cholerae* (58%, *n* = 7), and *V. parahaemolyticus* (42%, *n* = 5), as shown in Figs. 5 and 6.

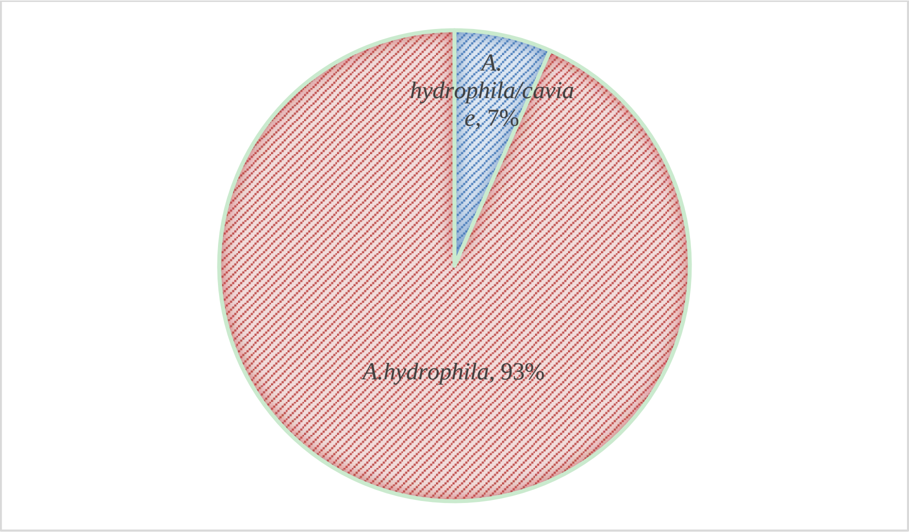

Fig. 3 Prevalence of *Aeromonas* spp. from Siem Reap province.

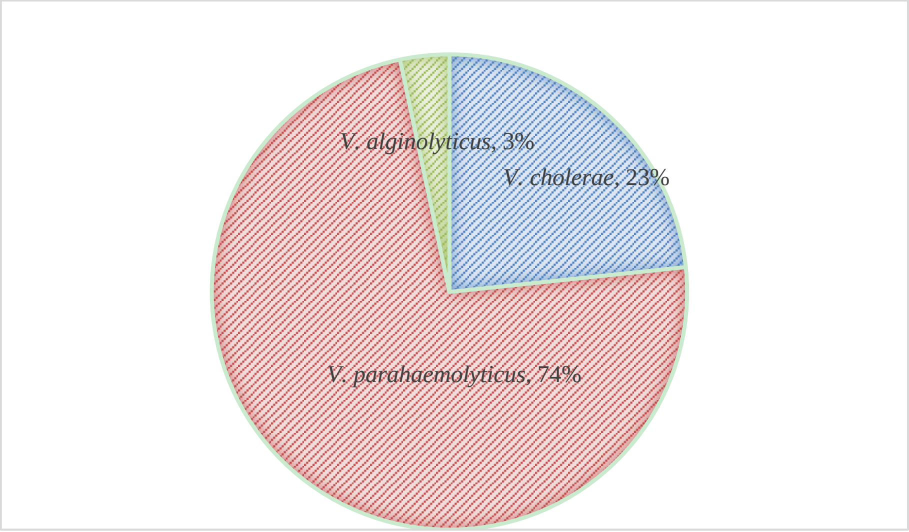

Fig. 4 Prevalence of *Vibrio* spp. from Siem Reap province.

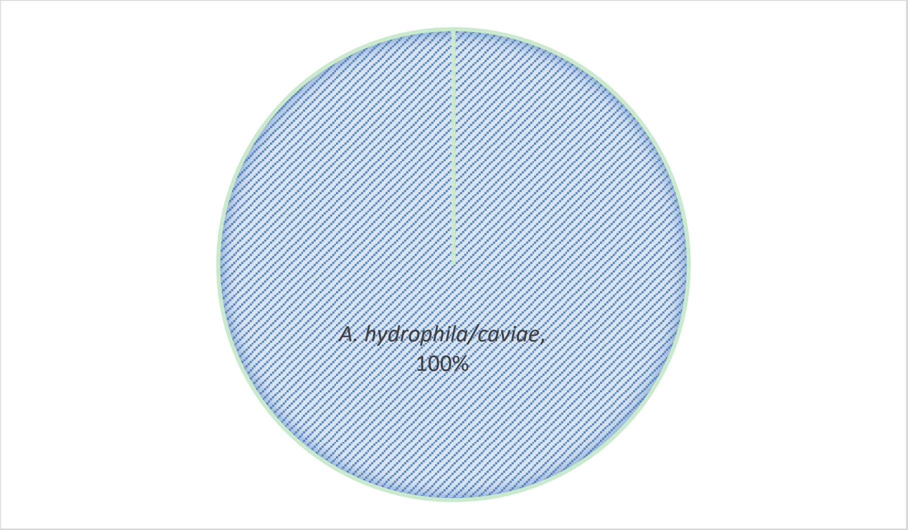

Fig. 5 Prevalence of *Aeromonas* spp. from Phnom Penh city.

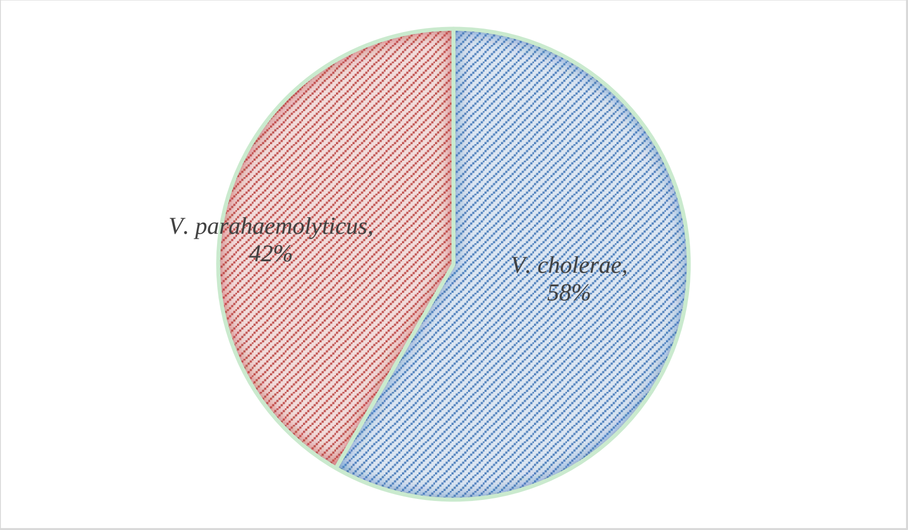

Fig. 6 Prevalence of *Vibrio* spp. from Phnom Penh city.

3.2 Total Isolation of Aeromonas spp. and Vibrio spp. in Cambodia

This study found the highest number of *Aeromonas* spp. is from Siem Reap (*n* = 15), Phnom Penh (*n* = 14) and Kampong Thom (*n* = 9) respectively and the highest of *Vibrio* spp. is from Siem Reap (*n* = 30), Phnom Penh (*n* = 12), and Kampong Thom (*n* = 11) is lowest.

The total 53 *Vibrio* spp. were isolated and 38 *Aeromonas* spp. isolated. The summary of the results of the prevalence from highest to lowest is: *V. parahaemolyticus* (57%, *n* = 30), *V. cholerae* (38%, *n* = 20), *V. fluvialis* (3.8%, *n* = 2), *V. aglinolyticus* (1.9%, *n* = 1); *A. hydrophila* (47%, *n* = 18), *A. hydrophila/caviae* (45%, *n* = 17), *A. sobria* (5%, *n* = 2), *A. caviae* (2.6%, *n* = 1) (Fig. 7).

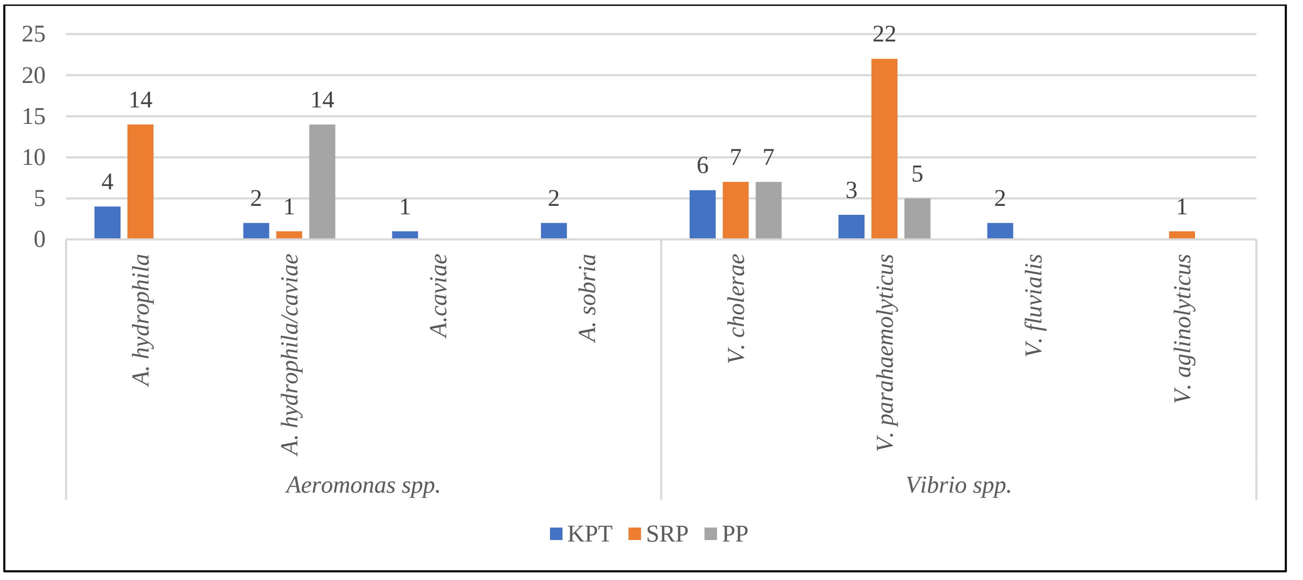

Fig. 7 The prevalence of *Aeromonas* spp. and *Vibrio* spp. isolated from difference markets in Cambodia.

3.3 The Prevalence of AMR of Aeromonas and Vibrio spp. Isolated from Fresh Pangasius Fish in Cambodia

3.3.1 The Prevalence of AMR of *Aeromonas* and *Vibrio* spp. Isolated from Fresh Pangasius Fish in Kampong Thom

Among seven antimicrobials of AMP, FFC, E, OT, CL, CIP and SXT were resistance to *V. cholerae* (100%, 0%, 67%, 17%, 50, 0%, 17%), *V. fluvialis* (50%, 0%, 0%, 0%, 50%, 0%, 0%), *V. parahaemolyticus* (100%, 0%, 0%, 0%, 50%, 0%, 0%), *A. hydrophila/caviae* (100%, 50%, 100%, 0%, 50%, 0%, 0%), *A. sobria* (100%, 0%, 0%, 0%, 50%, 0%, 0%), *A. caviae* (100%, 0%, 0%, 0%, 100%, 0%, 0%), and *A. hydrophila*(75%, 0%, 0%, 0%, 50%, 0%, 25%) respectively. CIP, FFC, OT, E and SXT were sensitive but there were three species of *V. cholerae*, *A. hydrophila* and *A. hydrophila/caviae* were MDR (Multidrug Resistance) (see Table 1).

3.3.2 The Prevalence of AMR of *Aeromonas* and *Vibrio* spp. Isolated from Fresh Pangasius Fish in Siem Reap province

Table 2 shows that seven antimicrobials of AMP, FFC, E, OT, CL, CIP and SXT were resistant to all pathogens: *V. cholerae* (57%, 57%, 86%, 43%, 86%, 43%, 29%), *V. parahaemolyticus* (73%, 41%, 73%, 55%, 95%, 41%, 9%), *V. aglinoluticus* (0%, 100%, 100%, 100%, 100%, 100%, 0%), *A. hydrophila/caviae* (100%, 0%, 100%, 100%, 100%, 0%, 0%), and *A. hydrophila* (100%, 14%, 21%, 7%, 57%, 7%, 29%) respectively. All the pathogens are MDR (see Table 2).

Table 1 The prevalence of AMR of *Aeromonas* and *Vibrio* spp. from Kampong Thom province.

Species	Antimicrobial							MDR
	AMP	FFC	E	OT	CL	CIP	SXT	
V. cholerae (*n* = 6)	100	-	67	17	50	-	17	5
V. fluvialis (*n* = 2)	50	-	-	-	50	-	-	-
V. parahaemolyticus (*n* = 3)	100	-	-	-	33	-	-	-
A. hydrophila/caviae (*n* = 2)	100	50	100	-	50	-	-	4
A. sobria (*n* = 2)	100	-	-	-	50	-	-	-
A. caviae (*n* = 1)	100	-	-	-	100	-	-	-
A. hydrophila (*n* = 4)	75	-	-	-	50	-	25	3

AMP: Ampicillin, FFC: Florfenicol, E: Erythromycin, OT: Oxytetracycline, CL: Colistin Sulphate, CIP: Ciprofloxacin, SXT: Sulfamethoxazole/trimethoprim.

Table 2 The prevalence of AMR in *Aeromonas* and *Vibrio* spp. from Siem Reap province.

Species	Antimicrobial							MDR
	AMP	FFC	E	OT	CL	CIP	SXT	
V. cholerae (*n* = 7)	57	57	86	43	86	43	29	7
V. parahaemolyticus (*n* = 22)	73	41	73	55	95	41	9	7
V. aglinoluticus (*n* = 1)	-	100	100	100	100	100	-	5
A. hydrophila/caviae (*n* = 1)	100	-	100	100	100	-	-	4
A. hydrophila (*n* = 14)	100	14	21	7	57	7	29	7

Table 3 The distribution of AMR in *Aeromonas* and *Vibrio* spp. from Phnom Penh city.

Species	Antimicrobial							MDR
	AMP	FFC	E	OT	CL	CIP	SXT	
V. cholerae (*n* = 7)	43	14	29	-	57	14	14	6
V. parahaemolyticus (*n* = 5)	100	40	40	40	40	40	100	7
A. hydrophila/caviae (*n* = 14)	79	57	36	43	50	14	36	7

3.3.3 The Prevalence of AMR of *Aeromonas* and *Vibrio* spp. Isolated from Fresh Pangasius Fish in Phnom Penh city

The seven antimicrobials of AMP, FFC, E, OT, CL, CIP and SXT were resistant to all pathogens: *V. cholerae* (43%, 14%, 29%, 0%, 57%, 14%, 14%), *V. parahaemolyticus* (100%, 40%, 40%, 40%, 40%, 40%, 100%) and *A. hydrophila/caviae* (79%, 57%, 36%, 43%, 50%, 14%, 36%) respectively (see Table 3).

4. Discussion

In this study, the results of distribution of *Aeromonas* spp. and *Vibrio* spp. isolated from fresh Pangasius fish shows that *Aeromonas* spp. (24.83%, *n* = 38) and *Vibrio* spp. (34.64%, *n* = 53): the species of *V. parahaemolyticus* (57%, *n* = 30), *V. cholerae* (38%, *n* = 20), *V. fluvialis* (3.8%, *n* = 2) and *V. aglinolyticus* (1.9%, *n* = 1), and also *A. hydrophila* (47%, *n* = 18), *A. hydrophila/caviae* (45%, *n* = 17), *A. sobria* (5%, *n* = 2), *A. caviae* (2,6%, *n* = 1).

The result reported by Abedin et al. [13] found *Aeromonas* spp. (39.33%), Ashraf and Abd-El-Malek [14] found (40%), Gupta et al. [15] found (39.58%) and Stratev et al. [16] found 37%-93%, higher than this result (24.83%). Other studies, which have also been reported Wamala et al. [17], Wu et al. [18] and Dhanapala et al. [19], identified *A. hydrophila* percentage (43.8%, 9.3% and 7.0%) respectively, lower than this research (47%). *A. sobria* (0.6%) reported by Dhanapala et al. [19] is lower than this study (5.0%) and *A. caviae* (2.63%) indicated nearly similar result (2.6%) but less than Wu et al. [18]; Abdelsalam et al. [20] and ODC [1] reported *A. caviae* (33.6%, 23.5% and 5.0%) respectively.

And also, other studies reported by Noorlis et al. [21] found *Vibrio* spp. (98.67%) has higher resistance than this result (34.64%). In comparison with the obtained results *V. cholerae* (22.22%) recorded by Wamala et al. [17] was less than this result (38%). *V. parahaemolyticus* with percentage 34.38% documented by Siddique et al. [22] and 25% recorded by Noorlis et al. [21] were less than result (57%). And also result was indicated by Siddique et al. [22] *V. parahaemolyticus* with percentage 81.25% and 79.16%, respectively higher than this result (57%). El-Sayed et al. [23] showed *V. alginolyticus* with percentage 35.7%-48.6% and (32.30%) indicated by Mohammad et al. [13] which were higher level than the result (1.9%). The tested positive *V. fluvialis* (32.63%) reported by Igbinosa [24] is higher than this result (3.8%).

Our research has indicated that *Aeromonas* spp. has resistance to AMP (74%-100%), CIP (7%-100%), SXT (14%-100%), FFC (14%-100%), OT (7%-100%), E (10%-100%), CL (33%-100%). And *Vibrio* spp. has resistance to AMP (43%-100%), CIP (14%-100%), SXT (14%-100%), FFC (14%-100%), OT (20%-100%), E (29%-100%), CL (33%-100%).

Other research has also reported that susceptibility of *Aeromonas* spp. was indicated by Fauzi in 2021 who shows that 100% resistance to AMP and 9.8%, 3.9% to CIP [5], 6.35% to SXT, 12.10% to FFC, 29.14% to OT [25] and 5.9% to SXT [26], 26.1% to E was highly resistance [19] and 90.90% to CL were resistance in *Aeromonas* spp. However, Long and Lua [5] have stated that *Vibrio* spp. has 18.70% resistance to AMP, 8.82% to FFC, 27.50% to OT and 50.68% to SXT, 10% to E [27], 72.20% to CL [28] and CIP [29]. MDR was widespread among the samples isolated. That is a high-risk source of contamination since pathogens and antimicrobials are often used. Our findings highlight that the aquatic environment and fresh Pangasius fish act as reservoirs of multidrug resistant *Aeromonas* spp. and *Vibrio* spp. which underline the need for a judicious use of antimicrobials and timely surveillance of AMR in aquaculture.

5. Conclusion

The study concluded that fresh Pangasius fish from different markets in Cambodia are reservoirs of multidrug-resistant Aeromonas spp. and Vibrio spp. The study discovered the presence of *A. hydrophila, A. hydrophila/caviae*, *A. caviae*, *A. sobria*, *V. parahaemolyticus*, *V. cholerae*, *V. alginolyticus* and *V. fluvialis* in the tested samples. The bacteria species showed high resistance to commonly used antibiotics like oxytetracycline, ampicillin, ciprofloxacin, sulfamethoxzole/trimethoprim, florfenicol, erythromycin, and colistin sulphate. As these fish and antimicrobials are commonly used, this can be a high-risk source of contamination, posing risks to both public health and animal health. Therefore, this calls for the need for judicious use of antimicrobials and regular surveillance of AMR in aquaculture. The findings can guide effective sanitary measures and strategic actions to manage and control antibiotic resistance in aquaculture and food chain.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and publication of this article.

Compliance with Ethical Standards

This article does not cover any studies with human contributors or animals performed by any of the author.

Acknowledgements

This was supported by grants from HEIP (Higher Education Improvement Project) Project and Royal University of Agriculture. We would like to thank Dr. Venn Vutey, Assoc. Prof. Dr. Sudsai Trevanich, Dr. Chea Rortana, Dr. Nguong Samnang, Dr. Chrun Rithy, Dr. Ségolène Calvez, Dr. Nora Navarro-Gonzalez and Dr. Kenny Oberle for excellent technical assistance.

References

[1] ODC (Open Development Cambodia). 2018. *Fishing, Fishery and Aquaculture*. Cambodia: ODC. https://opendevelopmentcambodia.net/topics/fishing-fisheries-and-aquaculture/.

[2] Joffre, O., Kura, Y., Pant, J., and Nam, S. 2010. *Aquaculture for the Poor in Cambodia—Lessons Learned*. Phnom Penh, Cambodia: The World Fish Center. http://pubs.iclarm.net/resource_centre/WF_2769.pdf.

[3] ODC (Open Development Cambodia). 2020. *Fisheries Production.* Cambodia: ODC. https://opendevelopment cambodia.net/topics/fisheries-production/.

[4] Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfella, B. T., Levina, S. A., Robinsoni, T. P., Teillant, A., and Laxminarayanb, R. 2015. "Global Trends in Antimicrobial Use in Food Animals." *Proc. Natl. Acad. Sci.* 112 (18): 5649-54. www.pnas.org/cgi/doi/10.1073/pnas.1503141112.

[5] Nguyen, V. L., and Dang, T. L. 2017. "Antimicrobial Usage and Antimicrobial Resistance in Vietnam." FAO: FMM/RAS/298: Strengthening Capacities, Policies and National Action Plans on Prudent and Responsible Use of Antimicrobials in Fisheries AS/298.

[6] Phillips, M. 2000. "The use of Chemicals in Carp and Shrimp Aquaculture in Bangladesh, Cambodia, Lao PDR, Nepal, Pakistan, Sri Lanka and Viet Nam." SEAFDEC/AQD Institutional Repository (SAIR). http://hdl.handle.net/10862/605.

[7] Carl Uhland, F. 2011. "Characterization of Antimicrobial Resistance in Aeromonas and Vibrio Isolated in Canada from Fish and Seafood." M.Sc. thesis, Univerisité de Montréal.

[8] Hudzicki, J. 2009. *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol.* Washington, DC: American Society for Microbiology.

[9] PHE (Public Health England). 2015. *Identification of Vibrio and Aeromonas Species*. London: Public Health England.

[10] ISO/TS, 21872.1. 2007. "Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Detection of Potentially Enteropathogenic *Vibrio* spp. Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*."

[11] CLSI. 2015. *Method for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*. *M45* (3rd ed.). Wayne: CISL.

[12] Abedin, M. Z., Islam, M. I., Yeasmin, F., Rahman, M. S., Islam, J., Arfat, M. E., Farnaz, N. E. K., Razu, M. H., Moniruzzaman, M., Karmaker, P., Khan, M., and Ahmed, A. A. 2021. "Microbiological Screening and Antibiotics Responsiveness of Pathogenic Bacteria Isolated from Pond Cultivated Pangas Fishes (*Pangasius hypothallus*) in Bangladesh." *Science Archives* 2 (3): 238-44.

[13] Ashraf, M., and Abd-El-Malek. 2017. "Incidence and Virulence Characteristics of *Aeromonas* spp. in Fish." *Veterinary World* 10 (1): 34-7.

[14] Gupta, B., Ghatak, S., and Gill, J. P. S. 2013. "Distribution and Characterization of Pathogenic Aeromonas spp. Isolated from Fish and Fish Products." *J. Vet. Public Health* 11 (1): 19-26. https://doi.org/a10.1016/j.micpath. 2021.105213.https://doi.org/10.3389/fmicb.2021.635539.

[15] Stratev, D., Vashin, I., and Rusev, V. 2012. "Distribution and Survival of Aeromonas spp.in Foods—A Review." *Revue Méd. Vét.* 163 (10): 486-94.

[16] Wamala, S. P., Mugimba, K. K., Mutoloki, S., Evensen, Ø., Mdegela, R., Byarugaba, D. K., and Sørum, H. 2018. "Occurrence and Antibiotic Susceptibility of Fish Bacteria Isolated from *Oreochromis niloticus* (Nile Tilapia) and *Clarias gariepinus* (African Catfish) in Uganda." *Fisheries and Aquatic Sciences* 21: 6.

[17] Wu, C. J., Ko, W. C., Lee, N. Y., Su, S. L., Li, C. W., Li, M. C., Chen, Y. W., Su, Y. C., Shu, C. Y., Lin, Y. T., and Chen, P. L. 2019. "*Aeromonas* Isolates from Fish and Patients in Tainan City, Taiwan." *Genotypic and Phenotypic Characteristics* 85 (21): e01360-19. doi: 10.1128/AEM.01360-19.

[18] Dhanapala, P. M., Kalupahana, R. S., Kalupahana, A. W., Wijesekera, D. P. H., Kottawatta, S. A., Jayasekera, N. K., Silva-Fletcher, A., and de S. Jagoda, S. S. S. 2021. "Characterization and Antimicrobial Resistance of Environmental and Clinical *Aeromonas* Species Isolated from Fresh Water Ornamental Fish and Associated Farming Environment in Sri Lanka." Microorganisms 9: 2106. https://www.mdpi.com/2076-2607/9/10/2106#:~:text=A%20total%20of%20161%20Aeromonas%20spp.%20were%20subjected%20to.

[19] Abdelsalam, M., Zaki Ewiss, M. A., Khalefa, H. S., Mahmoud, M. A., Elgendy, M. Y., Abdel-Moneam, D. A. 2021. "Coinfections of *Aeromonas* spp., *Enterococcus faecalis*, and *Vibrio alginolyticus* Isolated from Farmed Nile Tilapia and African Catfish in Egypt, with an Emphasis on Poor Water Quality." *Microb. Pathog.* 160: 105213.

[20] Noorlis, A., Ghazali, F. M., Cheah, Y. K., Tuan Zainazor, T. C., Ponniah, J., Tunung, R., Tang, J. Y. H., Nishibuchi, M., Nakaguchi, Y., and Son, R. 2011. "Distribution and Quantification of Vibrio Species and *Vibrio Parahaemolyticus* in Freshwater Fish at Hypermarket Level." *International Food Research Journal* 18: 689-95.

[21] Siddique, A. B., Moniruzzaman, M., Ali, S., Dewan, M. N., Islam, M. R., Islam, M. S., Amin, M. B., Mondal, D., Parvez, A. K., and Mahmud, Z. H. 2021. "Characterization of Pathogenic *Vibrio parahaemolyticus* Isolated from Fish Aquaculture of the Southwest Coastal Area of Bangladesh." *Front. Microbiol., Sec. Food Microbiology* 12: 635539.

[22] El-Sayed, M. E., Algammal, A. M., Abouel-Atta, M. E., Mabrok, M., and Emam, A. M. 2019. "Pathogenicity, Genetic Typing, and Antibiotic Sensitivity of *Vibrio alginolyticus* Isolated from *Oreochromis niloticus* and *Tilapia zillii*." *Revue Méd. Vét.* 170 (4-6): 80-6.

[23] Igbinosa, E. O. 2016. "Detection and Antimicrobial Resistance of Vibrio Isolates in Aquaculture Environments: Implications for Public Health." *Microbial Drug Resistance* 22 (3): 238-45. http://doi.org/10.1089/mdr. 2015.0169.

[24] Parven, M., Mahbub Alam, M. M., Khalil, S. M. I., Hamom, A., Goni, O., Rahman, M. M., and Abdullah-Al-Mamun, M. 2020. "Identification of Pathogenic Bacteria from Diseased Thai *Pangas Pangasius hypophthalmus* with Their Sensitivity to Antibiotics." *Microbiology Research Journal International* 30 (3): 7-21.

[25] Roges, E. M., Gonçalves, V. D., Cardoso, M. D., Festivo, M. L., Siciliano, S., Berto, L. H., de Almeida Pereira, V. L., dos Prazeres Rodrigues, D., and de Aquino, M. H. C. 2020. "Virulence-Associated Genes and Antimicrobial Resistance of *Aeromonas hydrophila* Isolates from Animal, Food, and Human Sources in Brazil." *BioMed Research International* 2020: 8. https://doi.org/10.1155/2020/ 1052607.

[26] Wan Norhana, M. N., Misol Jr., G., and Johari, R. 2020. "Aquaculture Component of National Action Plan on Antimicrobial Resistance in Malaysia. Fisheries and Aquaculture." FAO. https://www.fao.org/fishery/en/ openasfa/fda095f3-562f-46f2-b5d7-31d7d056dc7f.

[27] Lee, S., Najiah, M., Wendy, W., and Nadirah, M. 2010. "Antibiogram and Heavy Metal Resistance of Pathogenic Bacteria Isolated from Moribund Cage Cultured Silver

Catfish (*Pangasius sutchi*) and Red Hybrid Tilapia (*Tilapia* sp.).” *Front. Agric. China* 4 (1): 116-20. doi: 10.1007/s11703-009-0085-z.

[28] Akinbowale, O. L., Peng, H., and Barton, M. D. 2006. “Antimicrobial Resistance in Bacteria Isolated from Aquaculture Sources in Australia.” *Journal of Applied Microbiology* 100 (5): 1103-13. https://doi.org/10.1111/j.1365-2672.2006.02812.x.