Research Article

Prevalence of Multidrug Resistant *Aeromonas hydrophila* in Crustaceans

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Abstract | Crustaceans such as crab, lobster, and shrimps, are considered as rich source of omega-3 polyunsaturated fatty acids, high biological value protein, minerals, and vitamins. However, crustaceans are regarded as potential sources for the transmission of foodborne pathogens. *Aeromonas* spp. is one of the opportunistic microorganisms, that normally inhabits aquatic environments such as fresh, and marine water bodies. *Aeromonas* spp. might cause food-borne gastroenteritis that might be complicated to cause septicemia, meningitis, endocarditis, and osteomyelitis with high mortalities in immune-compromised persons. This international collaborative research was conducted to investigate the prevalence of Aeromonas *spp*., particularly *A. hydrophila* in three crustaceans (crab, lobster, and shrimp) retailed in Zagazig, Egypt, and Al-Ahsa, Saudi Arabia. Detection of virulence associated genes in the recovered *A. hydrophila* isolates was additionally screened using PCR. Furthermore, antimicrobial resistance profiling was examined among the recovered *A. hydrophila* isolates. The obtained results of the present study revealed overall isolation rates of *Aeromonas spp.* at 48.33%, and 21.66% in the examined crustacean samples collected from Egypt and Saudi Arabia, respectively. Crab samples had the highest prevalence rates, followed by lobster, and shrimp in samples collected from Egypt and Saudi Arabia, respectively. Four *Aeromonas spp.* were identified, namely *A. hydrophila*, *A. sobria, A. caviae* and *A.veronii*. The recovered isolates of *A. hydrophila* harbored coding genes for hemolytic toxins such as *aerolysin (aerA)* and *haemolysin (ahhl)* with multidrug resistance profiling.

Keywords | Crustaceans, *Aeromonas hydrophila*, Drug resistance, Virulence genes

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INTRODUCTION

Crustaceans such as crab, lobster, and shrimps, are considered as emerging sources of high biological value protein (16.0-19.2%), rich source of omega-3 polyunsaturated fatty acids, moisture (75.3-79.5%), carbohydrate (1.3-3.6%), minerals such as calcium, and phosphorus, and vitamins, particularly vitamin *D. Crustaceans* are rich in amino acids (% of total protein) such as alanine (3.9-8.1%), arginine (4.7-7.1%), aspartic acid (7.7-10.7%), glutamic acid (10.7-16.2%), glycine (4.5-8.7%), and lysine (5.5-9.4%) (Sidwell, 1981). However, crustaceans are regarded potential sources for the transmission of foodborne pathogens such as *Staphylococcus aureus* (Rajkovic, 2016), *Salmonella* spp., (Hatha and Lakshmanaperumalsamy, 1997), *Vibrio* spp., (Traore et al.,

2012), and *Aeromonas* spp., (Colakoglu et al., 2006).

Aeromonas spp., is one of the emerging foodborne pathogens with opportunistic characters. It normally inhabits aquatic environments such as fresh, and marine water bodies (Figueras and Beaz-Hidalgo, 2014). *Aeromonas* spp., is a major pathogen of fish and shellfish and can be isolated from healthy or diseased fish. This organism is responsible for massive economic losses in fish industry (Karunasagar et al., 2003). Several *Aeromonas* spp., have been identified in fish and shellfish worldwide including *A. hydrophila*, *A. allosaccharophila, A. salmonicida,* and *A. veronii* biovar *sobria* (Beaz-Hidalgo and Figueras, 2013). In humans, *Aeromonas* spp., might cause foodborne gastroenteritis that might be complicated to cause septicemia, meningitis, endocarditis, and osteomyelitis with high mortalities in immune-compromised persons (Koca and Sarimehmetoglu, 2009). Reports related to the pathogenicity of *Aeromonas* spp., are recently increasing (Carvalho et al., 2012; Pessoa et al., 2019). The pathogenicity of *Aeromonas* spp., depends mainly on the release of several virulent-associated attributes encoded for the production of enterotoxins, hemolysins, and adhesion-related factors (Puah et al., 2013; Wu et al., 2012).

Microbial drug resistance is an emerging issue with significant public health concern. Antimicrobials are frequently used in aquacultures for the purposes of prevention and control of bacterial diseases. However, the abuse of such antimicrobials, the direct release of animal dead bodies, and the hospitals drainage to water bodies in developing countries might lead to the development of drug resistant pathogens (Alsayeqh et al., 2021; Darwish et al., 2013).

Egypt and Saudi Arabia are big countries in the Middle East that share many eating habits and culture. Both countries share Red sea shores and regarded as rich countries for sea food. However, there are few reports studying the prevalence of *Aeromonas* spp. in crustaceans retailed in the fish markets of both countries. Therefore, international collaborative research was done to investigate the prevalence of *Aeromonas* spp., particularly *A. hydrophila* in three different crustaceans, namely crab, lobster, and shrimp retailed in fish markets in Zagazig, Egypt, and Al-Ahsa, Saudi Arabia. Detection of virulence associated genes in the recovered *A. hydrophila* isolates was additionally screened using PCR. Furthermore, antimicrobial resistance profiling was examined among the recovered *A. hydrophila* isolates.

MATERIALS AND METHODS

Collection of crustaceans samples

Hundred and twenty crustaceans' samples of crab (*Portunus pelagicus*), lobster (*Panulirus penicillatus*), and shrimp (*Penaeus monodon*) (40 of each) were collected randomly from different fish markets at Zagazig city, Egypt (60 samples, 20 from each species), and Al-Ahsa, Saudi Arabia (60 samples, 20 from each species). The collected samples were identified and packaged separately in a sterile plastic bag. Samples collected from Egypt were transported cooled with undue delay to the Laboratory of Meat Hygiene, Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt for bacterial isolation and identification. While in parallel, samples collected from Saudi Arabia were similarly transported cooled without delay to the Department of Public Health, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia for bacterial isolation and identification.

Preparation of crustaceans samples for bacteriological examination

After removal of the shell using sterile scissors and forceps, a piece of the muscle (about 10 g) is obtained. The muscle was cut into small pieces and then aseptically homogenized in 90 ml sterile 0.1% peptone water for 3 min by using a stomacher, and then to stand for 5 min (Abd-El-Aziz and Moharam, 2016).

Bacterial isolation

One mL from each prepared homogenate was enriched in 0.1% alkaline peptone water for 24 h at 37°C. Then, a loopful of the enriched cultured was streaked on the surface of *Aeromonas* agar medium (Himedia, Mumbai, India) and incubated for 24 h at 37°C (Palumbo et al., 1985). Typical colonies appeared as pale green with dark centers, 0.5 to 1.5-mm in diameter. Typical colonies were purified on Nutrient agar plates and then stored on Nutrient slope agar (Himedia, Mumbai, India), and kept for further identification.

Primary characterization and identification of the isolates

Pure Aeromonas isolates were identified based on their morphological, biochemical, and serological characteristics following the criteria described in the Bergey's Manual of Determinative Bacteriology (Garrity, 2001).

Molecular confirmation and detection of virulence-coding genes of *A. hydrophila* by PCR

One or two colonies of an overnight culture were suspended in 5 ml of sterile distilled water, and heated at 100°C for 20 min (Hafez et al., 2018). After cooling, 200 µl of the suspension were placed in Eppendorf tube for bacterial DNA extraction using QIA amp kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Molecular confirmation of *A. hydrophila* was done via detection of the species-specific *16SrRNA* gene using

PCR. Similarly, two virulence-associated genes, namely *aerolysin (aerA),* and *haemolysin (ahh1)* were screened in the recovered *A. hydrophila* isolates using PCR. Primer sequences of the targeted genes were displayed in Table 1, and primers were purchased from Pharmacia Biotech, Sweden. PCR amplification reaction of *A. hydrophila* DNA was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). The reaction started with an initial denaturation at 94˚C for 4 min, then proceeded with 35 cycles consisting of 94˚C for 30 sec, 59 ˚C for 30 sec, and 72 ˚C for 30 sec, followed by a final extension at 72˚C for 7 min. Amplified DNA fragments were analyzed on 1.5% agarose gel electrophoresis (Applichem, GmbH, Germany) and visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes (Stratev et al., 2016). Nucleic acid of the reference strain *A. hydrophila* ATCC 7966 was used as a positive control, while DDW was loaded instead of the PCR product as a negative control.

Antibiotic resistance of the isolated *A. hydrophila*

Antimicrobial susceptibility of the recovered *A. hydrophila* isolates was examined using the disk diffusion method. The used antimicrobial discs and Nutrient agar were purchased from Oxoid, Hampshire, UK. We applied the experimental guidelines of the Clinical and Laboratory Standards Institute (CLSI) (Wayne, 2013). In addition, the Multiple Antibiotic Resistance (MAR) index for each tested *A. hydrophila* isolate was determined according to the formula stipulated by Singh et al. (2010) as follow:

MAR index= No. of resistance / Total
No. of tested antibiotics

Isolates classified as intermediate were considered sensitive for MAR index

The used antimicrobial discs (Oxoid Limited, Hampshire, UK) were ampicillin (10 µg) (AM), cephalothin (30 µg) (CET), chloramphenicol (30 µg) (C), ciprofloxacin (5 µg) (CIP), enrofloxacin (5 µg) (ENR), erythromycin (15 µg) (E), gentamicin (10 µg) (GEN), kanamycin (30 µg) (K), nalidixic acid (30 µg) (NA), neomycin (30 µg) (N), oxacillin (1 µg) (OX), oxytetracycline (30 µg) (OXY), penicillin (10 IU) (P), and trimethoprim/sulfamethoxazole (25 µg) (SXT).

RESULTS AND DISCUSSION

The obtained results of the present study revealed overall isolation rates of *Aeromonas* spp. at 48.33% (29 out of 60 samples), and 21.66% (13 out of 60 samples) in the examined crustacean samples collected from Egypt and Saudi Arabia, respectively. Crab samples had the highest prevalence rates 60%, and 30%, followed by lobster at 50%, and 20%, and shrimp at 35%, and 15% in samples collected from Egypt and Saudi Arabia, respectively (Figure 1). In agreement with the obtained results of the present study *Aeromonas* spp. was isolated from fish and seafoods collected from the Eastern province in Saudi Arabia at 10.4% (Ibrahim et al., 2016). In Egypt, *Aeromonas* spp. was isolated from retailed frozen fish at 24% in fillet, 32% in Mackerel, and 80% in herrings (Hafez et al., 2018). Further identification of the recovered Aeromonas isolates revealed that *A. hydrophila* had the highest prevalence rates at 20% (crab), 15% (lobster), and 10% (shrimp) in Egyptian samples with an overall isolation rate of 15% (9 out of 60 samples), followed by *A. veronii* which was isolated at 15% (crab), 15% (lobster), and 10% (shrimp) with an overall isolation rate of 13.33% (8 out of 60 samples), followed by *A. sobria* which was isolated at 10% (crab), 15% (lobster),

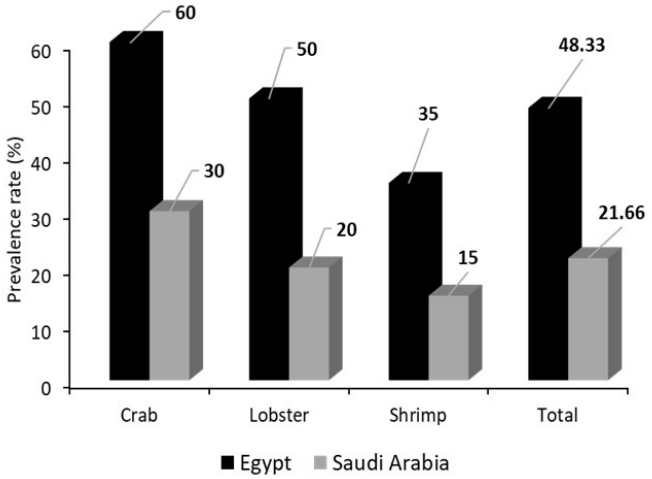

Figure 1: Prevalence rate (%) of *Aeromonas* spp. in the examined crustaceans collected from Egypt and Saudi Arabia.

Table 1: Primer sequences of virulence-coding genes in *A. hydrophila.*

Target	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
16SrRNA (F)	5′GGGAGTGCCTTCGGGAATCAGA′3	356	Stratev *et al.* (2016)
16SrRNA (R)	5′CAAGAACAAGTTCAAGTGGCCA′3		
aerA (F)	5′CAAGAACAAGTTCAAGTGGCCA′3	309	
aerA (R)	5′ACGAAGGTGTGGTTCCAGT′3		
ahh1 (F)	5′GCCGAGCGCCCAGAAGGTGAGTT′3	130	
ahh1 (R)	5′GAGCGGCTGGATGCGGTTGT′3		

and 5% (shrimp) with an overall isolation rate of 10% (6 out of 60 samples), and *A. caviae* with isolation rates of 15%, 5%, and 10% in crab, lobster, and shrimp respectively, with an overall isolation rate of 10% (6 out of 60 samples) (Table 2). In samples collected from Saudi Arabia, *A. veronii* had the highest isolation rates at 15% (crab), 10% (lobster), and 5% (shrimp) with an overall isolation rate of 10% (6 out of 60 samples), followed by *A. hydrophila* with isolation rates at 10% (crab), 10% (lobster), and 5% (shrimp) with an overall isolation rate of 8.33% (5 out of 60 samples), while both of *A. caviae,* and *A. sobria* were isolated at 1.66% (Table 2). In correspondence with the obtained results of the present study *A. hydrophila* was isolated from shrimp collected from the Eastern province in Saudi Arabia at 6.12% (Ibrahim et al., 2016). In Egypt, *A. hydrophila* was isolated from Nile tilapia at 19.2% (48 out of 250 samples) (Zaher et al., 2021). Globally, *Aeromonas spp.* was isolated from imported shrimps in USA. The identified Aeromonas spp. were *A. enteropelogenes*, *A. caviae*, and *A. sobria* (Shakir et al., 2012). Similarly, *Aeromonas spp.* was also isolated from marine shrimps cultured in Thailand, and the identified Aeromonas species were *A. veronii* (70%), *A. aquariorum* (18%), *A. caviae* (7%), *A. jandaei* (2%), and *A. schubertii* (2%) (Yano et al., 2015). Microbial contamination of fish and shellfish might be attributed to a variety of sources such as soil, water, fish handlers, improper handling of fish during transportation, processing, and preservation. Such factors facilitate bacterial cross contamination, and bacterial migration from gut to the fleshy parts (Hafez et al., 2018).

The pathogenicity of *Aeromonas* spp. is attributed to various virulence associated genes (Chacon et al., 2003). The mechanisms of pathogenesis are complex and multifactorial. Aeromonas spp. have the ability to produce four different kinds of enterotoxins (Gonzalez-Serrano et al., 2002), and many of these virulence attributes exhibit hemolytic, cytotoxic, and enterotoxic activities (Chopra and Houstan, 1999). The obtained results of the present study revealed detection of hemolytic toxins such as *aerolysin (aerA)* and *haemolysin (ahhl)*. As *aerA* was detected in all recovered *A. hydrophila* isolates from Egypt and Saudi Arabia. While *ahh1* gene was detected in 8 out of 9 *A. hydrophila* isolates from Egypt, and 2 out of 5 *A. hydrophila* isolates from Saudi Arabia (Tables 3, 4). Hemolysin is a group of enzymes with multifunction, which contribute significantly to the pathogenicity of *A. hydrophila*. Hemolysins include *aerA, ahh1, ahyA*, and *asa1*; *ahh1* is the most widely distributed extracellular heat-labile hemolysin, the synergistic combination of *aerA* and *ahh1*is the most cytotoxic genotype (Wang et al., 2003). In agreement with the obtained results of the present study, *aerA* and *ahh1* were detected in *A. hydrophila* and other Aeromonads isolated from imported shrimps in USA (Shakir et al., 2012), cultured marine shrimp in Thailand (Yano et al., 2015), seafoods in Saudi Arabia (Ibrahim et al., 2016), frozen fish (Hafez et al., 2018), and Nile tilapia retailed in Egypt (Zaher et al., 2021). *Aeromonas* induced foodborne disease occur in two forms; an acute self-limiting gastroenteritis which is more apparent in children and elders; and a fatal extra intestinal infection especially among the immunocompromised patients (Rasmussen-Ivey et al., 2016).

The abuse of antimicrobials in aquacultures without a proper veterinary supervision led to development of multidrug resistance among foodborne pathogens (Alsayeqh et al., 2021). In this regard, the recovered *A. hydrophila* isolates were subjected to antimicrobial sensitivity testing. Interestingly, all collected isolates from Egypt, and Saudi Arabia (100%) exhibited multidrug resistance profiling with at least resistance to three tested antimicrobial classes The calculated MAR index for the recovered *A. hydrophila* isolates from Egypt ranged between 0.214 to 1.0 with an average of 0.587 (Table 3). *A. hydrophila* isolates collected from Saudi Arabia had relatively similar MAR index values as MAR index ranged between 0.286 and 0.786 with an average of 0.557 (Table 4). The recovered *A. hydrophila* isolates from Egyptian samples showed resistance to the tested antimicrobials in the following order: 88.88% to erythromycin > 77.77% to gentamicin, kanamycin, nalidixic acid, neomycin, and oxacillin > 66.66% to cephalothin, and oxytetracycline > 44.44% to ampicillin, enrofloxacin, and trimethoprim/sulfamethoxazole > 33.33% to ciprofloxacin,

Table 2: Prevalence of *Aeromonas* spp. in the examined crustaceans' samples collected from Egypt and Saudi Arabia.

Sample origin	Crustacean samples	Positive samples	*A. hydrophila*	*A. sobria*	*A. caviae*	*A. veronii*
Egypt	Crab	12/20 (60%)	4 (20%)	2 (10%)	3 (15%)	3 (15%)
	Lobster	10/20 (50%)	3 (15%)	3 (15%)	1 (5%)	3 (15%)
	Shrimp	7/20 (35%)	2 (10%)	1 (5%)	2 (10%)	2 (10%)
	Total	29/60 (48.33%)	9 (15%)	6 (10%)	6 (10%)	8 (13.33%)
Saudi Arabia	Crab	6/20 (30%)	2 (10%)	0 (0%)	1 (5%)	3 (15%)
	Lobster	4/20 (20%)	2 (10%)	0 (0%)	0 (0%)	2 (10%)
	Shrimp	3/ 20 (15%)	1 (5%)	1 (5%)	0 (0%)	1 (5%)
	Total	13/60 (21.66%)	5 (8.33%)	1 (1.66%)	1 (1.66%)	6 (10%)

Table 3: Molecular confirmation, virulence attributes, and antimicrobial resistance profiles of A. hydrophila isolates recovered from crustaceans collected in Egypt.

Isolate ID	Origin	16S rRNA	Virulence factor	Resistance profile	MAR value
A. hydrophila 1	Crab	+	aerA, ahh1	AM, CET, C, CIP, ENR, E, GEN, K, NA, N, OX, OXY, P, SXT	1
A. hydrophila 2	Crab	+	aerA, ahh1	AM, CET, CIP, ENR, E, GEN, K, NA, N, OX, OXY, P, SXT	0.929
A. hydrophila 3	Crab	+	aerA, ahh1	CET, CIP, ENR, E, GEN, K, NA, N, OX, OXY	0.714
A. hydrophila 4	Crab	+	aerA	CET, GEN, K, NA, N, OX, OXY, P, SXT	0.643
A. hydrophila 5	Lobster	+	aerA, ahh1	ENR, E, GEN, K, NA, N, OX, OXY	0.571
A. hydrophila 6	Lobster	+	aerA, ahh1	E, GEN, K, NA, N, OX, OXY	0.500
A. hydrophila 7	Lobster	+	aerA, ahh1	E, GEN, K, NA, N, OX	0.429
A. hydrophila 8	Shrimp	+	aerA, ahh1	AM, CET, E, SXT	0.286
A. hydrophila 9	Shrimp	+	aerA, ahh1	AM, CET, E	0.214
				Average	0.587

Table 4: Molecular confirmation, virulence attributes, and antimicrobial resistance profiles of A. hydrophila isolates recovered from crustaceans collected in Saudi Arabia.

Isolate ID	Origin	16S rRNA	Virulence factor	Resistance profile	MAR value
A. hydrophila 1	Crab	+	aerA	AM, CET, C, CIP, E, GEN, K, NA, N, OX, OXY	0.786
A. hydrophila 2	Crab	+	aerA, ahh1	AM, CET, CIP, ENR, E, GEN, K, NA, N	0.643
A. hydrophila 3	Lobster	+	aerA	CET, GEN, K, NA, N, OX, OXY, P	0.571
A. hydrophila 4	Lobster	+	aerA	E, GEN, K, NA, N, OX, OXY	0.500
A. hydrophila 5	Shrimp	+	aerA, ahh1	AM, CET, P, SXT	0.286
				Average	0.557

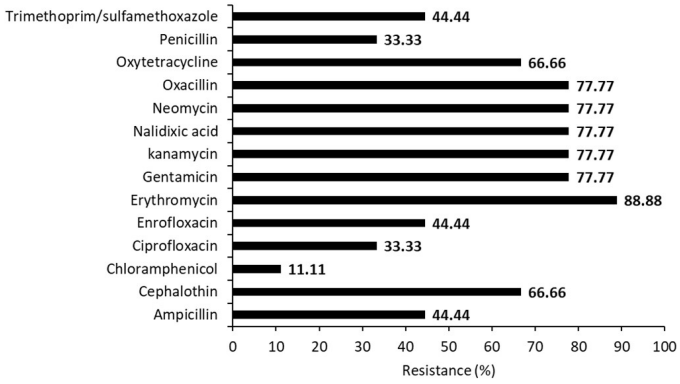

Figure 2: Antimicrobial resistance rates (%) of the recovered *A. hydrophila* isolates recovered from raw crustaceans in Egypt.

and penicillin > 11.11% to chloramphenicol, respectively (Figure 2). While the recovered *A. hydrophila* isolates from Saudi samples had antimicrobial resistance profiling in the following descending order: 80% to cephalothin, gentamicin, kanamycin, nalidixic acid, and neomycin, > 60% to ampicillin, erythromycin, oxacillin, and oxytetracycline > 44.44% to ampicillin, enrofloxacin, and trimethoprim/sulfamethoxazole > 40% to ciprofloxacin, and penicillin > 20% to chloramphenicol, and trimethoprim/sulfamethoxazole, respectively (Figure 3). In agreement with the obtained results of the present study, *A. hydrophila* isolated from seafoods in Saudi Arabia (Ibrahim et al., 2016), and from frozen fish in Egypt (Hafez et al., 2018) had multidrug resistance profiling. Therefore, it would be of interest to minimize the use of antimicrobials in aquaculture and avoid direct disposal of animal dead bodies and hospital wastes to the main water streams in Egypt, and Saudi Arabia.

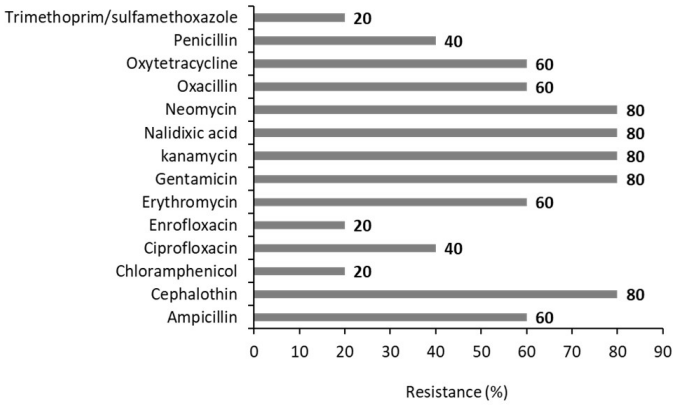

Figure 3: Antimicrobial resistance rates (%) of the recovered *A. hydrophila* isolates recovered from raw crustaceans in Saudi Arabia.

CONCLUSIONS AND RECOMMENDATIONS

The obtained results of the current study revealed contamination of crustaceans like crab, lobster, and shrimp retailed in Egypt and Saudi Arabia with *Aeromonas*

spp. The isolates of *A. hydrophila* harbored virulence-associated genes in addition to their resistance to different antimicrobials. Therefore, hygienic measures should be followed to minimize the microbial contamination of crustaceans either in the aquatic environment or in fish markets. In addition, efficient cooking of crustaceans before serving to humans is highly recommended. To the best of our knowledge, this is the first report to investigate the prevalence, antibiogram and virulence attributes of *A. hydrophila* in crustaceans retailed in Egypt, and Saudi Arabia.

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NOVELTY STATEMENT

This is the first report to investigate the prevalence, antibiogram and virulence attributes of *A. hydrophila* in crustaceans retailed in Egypt, and Saudi Arabia.

AUTHOR'S CONTRIBUTION

Waleed Rizk El-Ghareeb and Wageh Sobhy Darwish designed the study and supervised the work and wrote the first draft of the manuscript. Mohammad Belal Shaker, Waleed Rizk El-Ghareeb and Bassam Abdulla Alhawas collected the samples in Saudi Arabia and did the microbiological examination and collected the data. Marwa Magdy Seliem, Wageh Sobhy Darwish Ahmed E. Tharwat collected the samples in Egypt and did the microbiological examination, and the molecular confirmation, and collected the data. All authors revised and approved the final version of the manuscript and approved the order of the authors.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abd-El-Aziz NA, Moharram YG (2016). Microbiological quality of imported frozen shrimp in Egypt. Annals Agric. Sci., 61(1): 35-40. https://doi.org/10.1016/j.aoas.2016.04.002

Alsayeqh AF, Baz AHA, Darwish WS (2021). Antimicrobial-resistant foodborne pathogens in the Middle East: A systematic review. Environ. Sci. Pollut. Res. Int., 28(48): 68111-68133. https://doi.org/10.1007/s11356-021-17070-9

Beaz-Hidalgo R, Figueras MJ (2013). *Aeromonas* spp. whole genomes and virulence factors implicated in fish disease. J. Fish Dis., 36: 371-388. https://doi.org/10.1111/jfd.12025

Carvalho MJ, Martínez-Murcia A, Esteves AC, Correia A, Saavedra MJ (2012). Phylogenetic diversity, antibiotic resistance, and virulence traits of *Aeromonas* spp. from untreated waters for human consumption, Int. J. Food Microbiol., 159(3): 230-239. https://doi.org/10.1016/j.ijfoodmicro.2012.09.008

Chacon MR, Figueras MJ, Castro-Escarpulli G, Soler L, Guarro J (2003). Distribution of virulence genes in clinical and virulence genes of *Aeromonas* spp. Antonie Van Leeuwenhoek, 84: 269-278. https://doi.org/10.1023/A:1026042125243

Chopra AK, Houston CW (1999). Enterotoxins in Aeromonas-associated gastroenteritis. Microb. Infect., 1: 1129-1137. https://doi.org/10.1016/S1286-4579(99)00202-6

Colakoglu FA, Sarmasik A, Koseoglu B (2006). Occurrence of *Vibrio* spp. and *Aeromonas* spp. in shellfish harvested off Dardanelles cost of Turkey. Food Contr., 17(8): 648-652. https://doi.org/10.1016/j.foodcont.2005.04.014

Darwish WS, Eldaly EA, El-Abbasy MT, Ikenaka Y, Nakayama S, Ishizuka M (2013). Antibiotic residues in food: The African scenario. Jpn. J. Vet. Res., 61(Supplement): S13-S22.

Figueras MJ, Beaz-Hidalgo R (2014). Aeromonas introduction. Encycl. Food Microbiol., 1: 24-30. https://doi.org/10.1016/B978-0-12-384730-0.00004-5

Garrity GM (2001). Bergey's manual of systematic bacteriology. Springer Verlag, New York, USA.

Gonzalez-Serrano CJ, Santos JA, Garcia-Lopez ML, Otera A (2002). Virulence markers in *Aeromonas hydrophila* and *Aeromonas veronii* biovar sobria isolates from freshwater fish and from a diarrhoea case. J. Appl. Microbiol., 93: 414-419. https://doi.org/10.1046/j.1365-2672.2002.01705.x

Hafez AE, Darwish WS, Elbayomi RM, Hussein MA, El Nahal SM (2018). Prevalence, antibiogram and molecular characterization of *Aeromonas hydrophila* isolated from frozen fish marketed in Egypt. Slov. Vet. Res., 55: 445-54.

Hatha AM, Lakshmanaperumalsamy P (1997). Prevalence of Salmonella in fish and crustaceans from markets in Coimbatore, South India. Food Microbiol., 14(2): 111-116. https://doi.org/10.1006/fmic.1996.0070

Ibrahim MM, Al Shabeeb SS, Noureldin EA, Al Ramadhan GH (2016). Occurrence of potentially pathogenic vibrio and related species in seafoods obtained from the eastern Province of Saudi Arabia. Int. J. Adv. Res. Biol. Sci., 3(12): 71-80. https://doi.org/10.22192/ijarbs.2016.03.12.009

Karunasagar I, Karunasagar I, Otta, SK (2003). Disease problems affecting fish in tropical environments. J. Appl. Aqua., 13: 231-249. https://doi.org/10.1300/J028v13n03_03

Koca C, Sarimehmetoglu B (2009). Isolation, and identification of motile *Aeromonas* spp. in turkey meat. Ankara Univ. Vet. Fak. Derg., 56: 95-98. https://doi.org/10.1501/Vetfak_0000002170

Palumbo SA, Maxino F, Williams AC, Buchanan RL, Thayer DW (1985). Starch ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. Appl. Environ. Bacteriol., 50: 1027-1030. https://doi.org/10.1128/aem.50.4.1027-1030.1985

Pessoa RB, de Oliveira WF, Marques DS, dos Santos Correia MT, de Carvalho EV, Coelho LC (2019). The genus Aeromonas: A general approach. Microbial Pathog., 130: 81-94. https://doi.org/10.1016/j.micpath.2019.02.036

Puah SM, Puthucheary SD, Liew FY, Chua KH (2013). *Aeromonas aquariorum* clinical isolates: antimicrobial profiles, plasmids and genetic determinants. Int. J. Antimicrob. Agents, 41(3): 281-284. https://doi.org/10.1016/j.ijantimicag.2012.11.012

Rajkovic A (2016). Staphylococcus food poisoning. Encyclopedia of Food and Health; Caballero, B., Finglas, PM, Toldrá, F., Eds, pp. 133-139. https://doi.org/10.1016/B978-0-12-384947-2.00655-3

Rasmussen-Ivey CR, Figueras MJ, McGarey D, Liles, MR (2016). Virulence factors of *Aeromonas hydrophila* in the wake of reclassification. Front. Microbiol., 7: 1337. https://doi.org/10.3389/fmicb.2016.01337

Shakir Z, Khan S, Sung K, Khare S, Khan A, Steele R, Nawaz M (2012). Molecular characterization of fluoroquinolone-resistant *Aeromonas spp.* isolated from imported shrimp. Appl. Environ. Microbiol., 78(22): 8137-8141. https://doi.org/10.1128/AEM.02081-12

Sidwell VD (1981). Chemical and nutritional composition of finfishes, whales, crustaceans, mollusks, and their products (Vol. 55). US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Singh A, Yadav S, Singh S, Bharti P (2010). Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Res. Int., 43: 2027- 2030. https://doi.org/10.1016/j.foodres.2010.06.001

Stratev D, Gurova E, Vashin I, Daskalov H (2016). Multiplex PCR detection of heamolysin genes in β-heamolytic *Aeromonas hydrophila* strains isolated from fish and fish products. Bulgarian J. Agri. Sci., 22: 308-314.

Sudhakar M, Manivannan K, Soundrapandian P (2009). Nutritive value of hard and soft shell crabs of *Portunus sanguinolentus* (Herbst). Int. J. Anim. Vet. Adv., 1(2): 44-48.

Traore SG, Bonfoh B, Krabi R, Odermatt P, Utzinger J, Rose KN, Tanner M, Frey J, Quilici ML, Koussémon M (2012). Risk of Vibrio transmission linked to the consumption of crustaceans in coastal towns of Côte d'Ivoire. J. Food Prot., 75(6): 1004-1011. https://doi.org/10.4315/0362-028X.JFP-11-472

Wang G, Clark C, Liu C, Pucknell K, Munro C, Kruk T, Caldeira R, Woodward D, Rodgers F (2003). Detection and characterization of the hemolysingenes in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR. J. Clin. Microbiol., 41: 1048-1054. https://doi.org/10.1128/JCM.41.3.1048-1054.2003

Wayne P (2013). Performance standards for antimicrobial susceptibility testing. CLSI approved standard M100–S23. Clin. Lab. Stand. Inst. 33: 118-156.

Wu CJ, Tsai PJ, Chen PL (2012). *Aeromonas aquariorum* septicemia and enterocolitis in a cirrhotic patient. Diagn. Microbiol. Infect. Dis., 74(4): 406-408. https://doi.org/10.1016/j.diagmicrobio.2012.08.005

Yano Y, Hamano K, Tsutsui I, Aue-Umneoy D, Ban M, Satomi M (2015). Occurrence, molecular characterization, and antimicrobial susceptibility of *Aeromonas* spp. in marine species of shrimps cultured at inland low salinity ponds. Food Microbiol., 47: 21-27. https://doi.org/10.1016/j.fm.2014.11.003

Zaher HA, Nofal MI, Hendam BM, Elshaer MM, Alothaim AS, Eraqi MM (2021). Prevalence and antibiogram of *Vibrio parahaemolyticus* and *Aeromonas hydrophila* in the flesh of Nile Tilapia, with special reference to their virulence genes detected using Multiplex PCR technique. Antibiotics, 10(6): 654. https://doi.org/10.3390/antibiotics10060654