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RESEARCH ARTICLE

Prevalence, Antimicrobial Susceptibility, and Virulence Gene Profile of *Enterococcus* Species isolated from Some Farmed Fish Retailed in Zagazig City, Egypt

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Abstract

The current study aimed to assess the prevalence of Enterococcus species isolated from some farmed fish species, including tilapia (Oreochromis niloticus), brush tooth lizard (Saurida undosquamis), horse mackerel (Trachurus trachurus), and red porgy (Pagrus pagrus) sold in Zagazig city, Sharkia Governorate, Egypt. Additionally, antimicrobial susceptibility testing was performed for Enterococcus species using the disc diffusion method. Furthermore, multiplex PCR was performed to identify virulence-associated genes of Enterococcus faecalis isolates. The results indicated contamination of the examined fish with various Enterococcus species with an overall total prevalence of 52.5%. The identified Enterococcus species were E. faecalis (26.25%), E. faecium (15%), and E. hirae, E. raffinosus, and E. durans (3.75%, each). The antibiotic sensitivity test revealed variable resistance patterns of the retrieved isolates to various antimicrobial agents, such as kanamycin (100%), clindamycin (76.9%), sulfamethoxazole (69.2%), ampicillin (69.2%), and colistin (61.5%). PCR screening of virulence genes revealed that E. faecalis harbored sodA (100%), gelE (83.3%), and ace (50%) genes. Consequently, urgent measures are needed to implement hygienic practices to control microbial contamination in both the aquatic environment and fish markets.

Keywords: Fish, E. faecalis, E. faecium, Antimicrobial susceptibility, Virulence genes.

Introduction

Fish is a significant component of the human diet, valued for its high-quality protein, essential omega-3 fatty acids, and various micronutrients. The consumption of fish and its meat is associated with numerous health benefits, including cardiovascular health and brain development. In addition to its nutritional value, fish holds cultural and economic importance worldwide, contributing to the livelihoods of millions of people [1]. However, the safety of fish consumption is a concern due to potential contamination with microorganisms such as *Enterococcus* species. In Egyptian aquaculture, the presence and emergence of *Enterococcus* species within certain fish species have become a subject of scientific inquiry due to its potential implications for both aquatic ecosystems and public health [2]. *Enterococcus* spp. are Gram-positive diplococcal bacteria that inhabit the intestines of humans and animals. While they play a role in maintaining the balance of the gut microbiota, certain strains of *Enterococcus*, particularly *Enterococcus faecalis* and *Enterococcus faecium* are opportunistic pathogens capable of

causing infections in humans like urinary tract infections, endocarditis, and nosocomial infections [3]. *Enterococcus* can enter aquatic ecosystems through various sources, including sewage discharge, agricultural runoff, and industrial discharges. Once introduced into water bodies, these bacteria can persist, multiply, and potentially contaminate fish and seafood [4]. The presence of *Enterococcus* in fish is a concern not only due to its potential pathogenicity but also because it serves as an indicator of fecal contamination. High levels of *Enterococcus* in fish can be indicative of poor water quality and unsanitary conditions during fish handling and processing [5]. The escalation of multidrug resistance on a global scale represents a substantial challenge to public health. Recent research has underscored the emergence of multidrug-resistant bacterial pathogens from diverse origins, emphasizing the critical need for prudent antibiotic administration. Moreover, the routine application of antimicrobial susceptibility testing is indispensable for pinpointing appropriate antibiotics and detecting the emergence of multidrug-resistant strains [6, 7]. *Enterococcus* spp. exhibit natural resistance to a broad spectrum of pharmaceutical antibiotics. Additionally, they have the ability to acquire drug resistance through different methods such as plasmid transfer or transferring genetic sequences that confer resistance in other bacteria [8]. Enterococci commonly develop resistance against a wide range of antibiotic classes, including β-lactams such as cephalosporins, aminoglycosides, lincosamides, and streptogramins. Additionally, acquired resistance is observed in glycopeptides (vancomycin), macrolides, tetracyclines, and phenicols [9]. Numerous factors have been proposed as contributing to the virulence of *Enterococcus* species, specifically to infections linked to *E. faecalis*. Bacterial adhesion to heart endocardial cells and renal tubular cells has been linked to the collagen-binding protein gene *ace* [10]. The chromosomal *gelE* -encoded extracellular gelatinase mediates virulence through tissue degradation and host immune response modulation [11]. The superoxide dismutase (*soda*) gene contributes to oxidative stress resistance [12].

Therefore, this study aimed to determine the frequency and antimicrobial sensitivity patterns of Enterococci in some farmed fish species, including tilapia (*Oreochromis niloticus*), brush tooth lizard (*Saurida undosquamis*), horse mackerel (*Trachurus trachurus*), and red porgy (*Pagrus pagrus*) collected from fish markets in Sharkia Governorate, Egypt. In addition, the virulence screening of *E. faecalis* was also evaluated.

Materials and Methods

Samples collection

A total of apparently healthy 80 farmed fish samples, including tilapia (*Oreochromis niloticus*), brush tooth lizard (*Saurida undosquamis*), horse mackerel (*Trachurus trachurus*), and red porgy (*Pagrus pagrus*) (20 for each) were randomly collected from different fish markets at Zagazig city, Sharkia Governorate, Egypt. The collected samples were aseptically handled and immediately transferred in an icebox to Meat Hygiene laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt for further bacteriological examination, antibiotic sensitivity testing, and PCR screening of some encoded virulence genes.

Isolation and identification of Enterococcus spp.

Enterococcus spp. isolation was carried out in an accordance with ISO 6887-2 [13]. Twenty-five gm of each fish flesh samples were aseptically homogenized with 225 mL of 0.1 % Buffered Peptone Water (BPW, HIMEDIA, M614-500G) in a stomacher (Colworth, 400) for 2.5 min at room temperature (25ºC) and then allowed to stand for 5 min to provide a homogenate which represents the dilution of 10^{-1} (as an initial dilution). One mL of the homogenate was transferred into a sterile test tube containing 9 ml of 0.1% BPW, then tenfold serial dilutions were prepared up to the required dilution (10^{-4}). Isolation of Enterococci was carried out on Bile Esculin Azide ager (BEA, HIMEDIA, M340). The BEA agar was inoculated by spreading 0.1 mL of the ready prepared serial dilutions onto the surface. The agar plates were incubated for 24 h at 37± 0.5ºC aerobically. Typical pinpoint colonies, greyish white, surrounded by black or brown zone due to esculin hydrolysis, with 1 mm diameter were identified as Enterococci. The suspected colonies were then purified on Brain Heart Infusion broth (BHI, OXOID, CM1135) and incubated at 37ºC for 24 h for further morphological, biochemical, and serological identification according to ISO [14], MacFaddin [15], and Lancefield [16], respectively.

Sensitivity to antibiotics

The Kirby-Bauer disc diffusion method was used to assess the susceptibility of *Enterococcus* isolates (n=26) to 16 different antibiotics according to Ferede *et al.* [17]. The antimicrobial agents are; tetracycline (TE, 30 µg), ampicillin (AMP, 10 µg), ciprofloxacin (CIP, 5 µg), clindamycin (DA, 10 µg), vancomycin (VA, 5 µg), tobramycin (TOB, 10 µg), amikacin (AK, 30 µg), linezolid (LZD, 30 µg), erythromycin (E, 15 µg), meropenem (MEM, 10 µg), gentamicin (CN, 10 µg), levofloxacin (LEV, 5 µg), kanamycin (K, 10 µg), colistin (CT, 25 µg), cefepime (FEP, 30 µg), and sulfamethoxazole (SXT, 25 µg) (Oxoid Limited, Basingstoke, Hampshire, UK). According to National Committee for Clinical Laboratory Standards (NCCLS) [18], zones of inhibition were identified. Multiple antibiotic resistance (MAR) indices were determined. This formula is used to calculate the MAR index: MAR index is equal to a/b, where a and b stand for the number of antibiotics to which the isolates are resistant and the total number of antibiotics tested, respectively. The selection of antimicrobials was based on their common usage in clinical practice for treating bacterial infections in both humans and animals. This choice took into account the potential for cross-species transmission of antimicrobial resistance.

Molecular characterization of E. faecalis virulence genes

The colonies, which were serologically identified as *E. faecalis* (n=6), were subsequently subjected to virulence genes detection using primers obtained from Metabion, Germany. The genomic DNA of all tested isolates were extracted using a QIAamp DNA Mini kit (QIAGEN GmbH, Hilden, Germany, Catalogue no. 51304) according to the manufacturer's instructions for the subsequent molecular analysis. The molecular characterization of virulence factors involving superoxide dismutase (*sodA*), extracellular gelatinase (*gelE*), and collagen-binding protein (*ace*) as virulence factors of *E. faecalis* isolates is illustrated in Table 1. The optimized

multiplex PCR reaction was conducted with 2 µL template DNA, 0.25 µm of each primer, 0.2 mM deoxyribonucleoside triphosphates, 1×reaction buffer, 2 mM MgCl2, and 0.5 U Prime Taq DNA polymerase (Genet Bio) in a total volume of 25 µL. DNA amplification followed the protocol of initial denaturation (94°C for 5 min), followed by 30 cycles of denaturation (94°C for 45 seconds), annealing (68°C for 1 minute), and extension (72°C for 1 minute), with a single final extension of 7 min at 72°C. Amplified DNA fragments were resolved by gel electrophoresis using 1% agarose gel stained with ethidium bromide solution (0.5µg/mL), visualized under an ultraviolet transilluminator, and photographed. A 100 bp DNA ladder was utilized to determine the fragment size.

Table 1. Molecular characterization of virulence factors for *E. faecalis*

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
sodA	F: ACTTATGTGACTAACTTAACC ′3	360	[19]
	R: TAATGGTGAATCTTGGTTTGG ′3		
gelE	F: ACC CCG TAT CAT TGG TTT ′3	419	[20]
	R: ACG CAT TGC TTT TCC ATC ′3		
ace	F: 5′GGAATGACCGAGAACGATGGC′3	616	[21]
	R: 5′GCTTGATGTTGGCCTGCTTCCG′3		

RESULTS

Bacteriological assay

Following a bacterial analysis of fish samples in the current study, it was found that 42 (52.5%) of the samples were contaminated with *Enterococcus* species. The prevalence was 12 (60%) and 15 (75%) for tilapia and brush tooth lizard fish, respectively. Furthermore, the prevalence was 3 (15%) and 12 (60%) for horse mackerel and red porgy, respectively. The highest prevalence of *Enterococcus* species was for *E. faecalis* (26.25%) and *E. faecium* (15%), whereas *E. hirae*, *E. raffinosus*, and *E. durans* had the lowest percentage of 3.75% each (Table 2).

Table 2. Prevalence of *Enterococcus* species among the examined fish species (n= 20, each).

***Enterococcus* species**	***Examined fish species***				**[a] Total no. (%) of isolated spp**
	Tilapia	**Brush tooth lizard fish**	**Horse mackerel**	**Red porgy**	
E. Faecium	3 (15%)	3 (15%)	-	6 (30%)	12 (15%)
E. Faecalis	6 (30%)	9 (45%)	3 (15%)	3 (15%)	21 (26.25%)
E. raffinosus	3 (15%)	-	-	-	

E. durans	-	3 (15%)	-	-	3 (3.75%)
E. Hirae	-	-	-	3 (15%)	3 (3.75%)
Total positive	12 (60%)	15 (75%)	3 (15%)	12 (60%)	42 (52.5%)

[a] The percentage of *Enterococcus* spp. was calculated from the number of total examined fish species (n=80).

Antibiotic susceptibility testing

Enterococcus strains were resistant to kanamycin (100%), clindamycin (76.9%), sulphamethoxazol (69.2%), ampicillin (69.2%), and colistin (61.5%). On the other side, the isolates were sensitive to linezolid (92.3%), vancomycin (84.6%), levofloxacin (69.2%), amikacin (69.2%), and gentamicin (61.5%) (Table 3).

Table 3. Antimicrobial susceptibility of *Enterococcus* species (n=26).

Antimicrobial agent	S		I		R	
	NO	%	NO	%	NO	%
Kanamycin (K)	-	-	-	-	26	100
Clindamycin (CL)	4	15.4	2	7.7	20	76.9
Sulphamethoxazol (SXT)	2	7.7	6	23.1	18	69.2
Ampicillin (AM)	8	30.8	-	-	18	69.2
Colistin (C)	6	23.1	4	15.4	16	61.5
Erythromycin (E)	8	30.8	4	15.4	14	53.8
Tobramycin (TO)	10	38.5	2	7.7	14	53.8
Cefepime (FEP)	8	30.8	6	23.1	12	46.2
Tetracycline (T)	12	46.2	4	15.4	10	38.5
Ciprofloxacin (CP)	12	46.2	6	23.1	8	30.8
Meropenem (M)	14	53.8	4	15.4	8	30.8
Gentamicin (G)	16	61.5	2	7.7	8	30.8
Amikacin (AK)	18	69.2	2	7.7	6	23.1
Levofloxacin (L)	18	69.2	4	15.4	4	15.4
Vancomycin (V)	22	84.6	2	7.7	2	7.7
Linezolid (LZ)	24	92.3	-	-	2	7.7

S: Sensitive **I: Intermediate** **R: Resistant**

Most of tested isolates were categorized as multi-drug resistant (MDR) *Enterococcus*, and their multiple antibiotic resistance (MAR) index ranged from 0.062 to 1.00, with an average of 0.447 (Table 4).

Table 4. Antimicrobial resistance profile of *Enterococcus* species (n=26).

Pattern	*Enterococcus* Spp.	Antimicrobial resistance profile	No. of isolates	No. of antibiotic	MAR index
I	***E. faecalis***	K, CL, SXT, AM, C, E, TO, FEP, T, CP, M, G, AK, L, V, LZ	4	16	1
II		K, CL, SXT, AM, C, E, TO, FEP, T, CP, M, G	2	12	0.750
III		K, CL, SXT, AM, C, E, TO, FEP	2	8	0.500
IV		K, CL, SXT, AM, C, E, TO	1	7	0.438
V		K, CL, SXT, AM	2	4	0.250
V I		K, CL	1	2	0.125
I	***E. faecium***	K, CL, SXT, AM, C, E, TO, FEP, T, CP, M, G, AK, L	3	14	0.875
II		K, CL, SXT, AM, C, E, TO, FEP, T	2	9	0.563
III		K, CL, SXT, AM, C	2	5	0.312
IV		K	1	1	0.062
I	***E. durans***	K, CL, SXT, AM, C, E, TO, FEP, T, CP, M, G, AK	2	13	0.813
I	***E. raffinosus***	K	2	1	0.062
I	***E. hirae***	K	2	1	0.062
		Average	**0.447**		

Molecular characterization of virulence genes of E. faecalis

PCR results revealed that the tested isolates harbored *SodA* (100%), *gelE* (83.3%), and *ace* (50%) virulence genes (Table 5) (Figure 1).

Table 5. Distribution of virulence genes among the tested *Enterococcus faecalis* strains (n=6).

Target genes	No. of examined isolates	Positive strains	
		NO	%
SodA	6	6	100
GelE	6	5	83.3
Ace	6	3	50

SodA**:** superoxide dismutase gene, ***GelE*****:** gelatinase gene, ***Ace*****:** collagen-binding protein gene

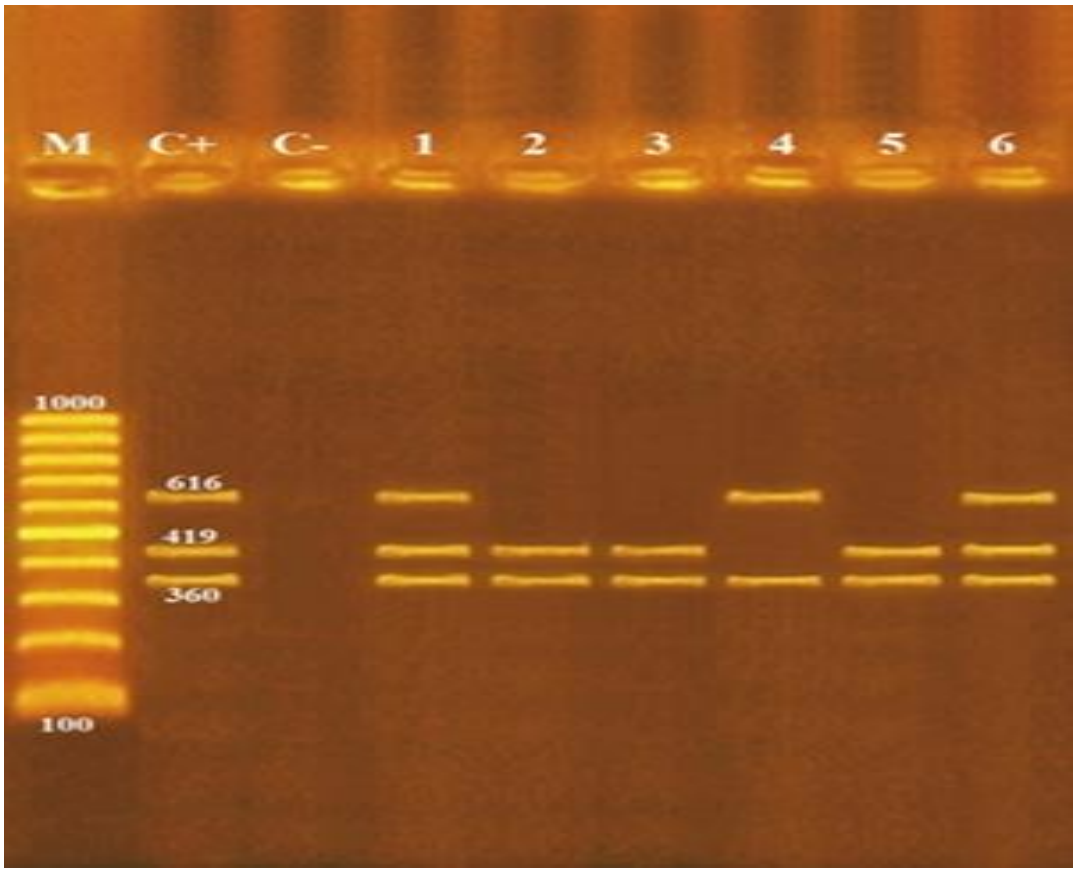

Figure (1): A multiplex PCR of *sodA* (360 bp), *gelE* (419 bp), and *ace* (616 bp) genes for characterization of *Enterococcus faecalis*, M: 100 bp ladder; C+: Control positive *E. faecalis* for *sodA*, *gelE* and *ace* genes; C-: Control negative; 1 & 6: Positive *E. faecalis* for *sodA*, *gelE* and *ace* genes; 2, 3 & 5: Positive *E. faecalis* for *sodA* and *gelE* genes; 4: Positive *E. faecalis* for *sodA* and *ace* gene. (Positive control: ATCC 33186, Negative control: PCR mixture without DNA template)

DISSCUSSION

In recent years, catastrophic outbreaks in aquaculture operations have been linked to opportunistic bacterial infections in fish. One of the most serious infections, *Enterococcus* species, has gained global recognition and is significantly impacting aquaculture practices [22]. In this study, bacteriological examination of 80 fish samples revealed the occurrence of *Enterococcus* species in 42 (52.5%) of the examined fish samples, where *E. faecalis* and *E. faecium* were the most identified species. A higher result was obtained by Mendoza *et al.* [23] who recorded 100% prevalence rate of *Enterococcus* spp. in *O. niloticus* fish from the BUDAMASA areas of Minalin, Pampanga, Philippines, and attributed this result to the interconnection of tilapia farms as these farms are dependent on Maniango River for water. Furthermore, Enany *et al.* [5] detected a prevalence of *Enterococcus* (58.5% and 62%) in *O. niloticus* and *Ictalurus punctatus*, respectively from fresh water farms, Ismailia Governorate, Egypt, and only two species of Enterococci, *E. faecalis* (75%) and *E. faecium* (25%) were identified. On contrary, Hassan *et al.* [2] investigated a

lower percentage of *Enterococcus* (50.5%) amongst cultured *O. niloticus* from fresh water farm along the Suez Canal area, Ismailia, Egypt. Moreover, Khafagy *et al.* [24] isolated *Enterococcus* from 23.76% of *O. niloticus* in Lake Temsah in Ismailia Governorate, Egypt, which is lower than the result of the present study. Additionally, a lower result of 22% *Enterococcus* in fresh water fish (*Salmo salar* and *Dicentrararchus labrax*) from different fish markets in Ankara, Turkey was obtained by Külahci and Gündoğan [25]. As well, much lower percentages of 4% and 2.8% were recorded by Adamu *et al.* [26] and El-Kader Mousa-Balabel [27] from fresh water fish in Nigeria (*Clarias gariepinus*, *Labeo senegalensis*, and *Clarias angularis*) and Egypt (*O. niloticus*), respectively. The difference in results may be attributed to various factors, including differences in geographic location and season, as well as differences in the fish species examined. According to Byappanahalli *et al.* [28] *Enterococcus* species typically do not thrive in fresh water habitats under normal conditions; their detection suggests the influence of point (such as a specific discharge from a sewage pipe) or non-point (like runoff from urban areas or agricultural fields) source pollution, or the possibility of re-suspension from other reservoirs (water currents, wind action, disturbances to the sediment bed, or even human activities like dredging or construction). Furthermore, Ullah *et al.* [29] identified *Enterococcus* in marine water fish (*Pampus chinensis*, *Euthynnus affinis*, and *Harpadon nehereus*) in Bangladesh with a percentage of 34.7%. Additionally Külahci and Gündoğan [25] documented the occurrence of enterococci in marine water fish (*Salmo trutta* and *Sarda sarda*) in Ankara, Turkey with a percentage of 21%. High *Enterococcus* prevalence (72.1%) from marine water fish (*Dicentrararchus labrax*, *Chelon labrosus*, and *Sardina pilchardus*) in Tunisia was recognized by Ben Said *et al.* [30], where *E. faecalis* was the most predominant isolated species followed by *E. faecium*. In addition, Barros *et al.* [31] and Hammad *et al.* [32] explored a high rate of *Enterococcus* (61.9% and 45%) in marine water fish in Portugal (*Sparus aurata*) and Japan (*Scomberomorus maculatus*, *Salmo salar*, *Paralichthys dentatus*, and *Thunnus obesus*), respectively. Also, Boss *et al.* [33] cited high *Enterococcus* prevalence (59%) from marine water fish (*Salmo salar* and *Pangasius hypophthalmus*) in Switzerland. These variations in *Enterococcus* prevalence could be explained by the environmental conditions and the microbial quality of fish farms. Finding *E. faecium* in marine water samples is a common occurrence, as numerous studies have suggested that marine fish can naturally acquire contamination from their surrounding environments during the collection process [3, 34, 35]. The results of the present study indicate that marine water samples may be susceptible to contamination during fish evisceration and from environmental sources during processing and handling. Additionally, the detection of *E. faecium* in seafood serves as an indicator of potential fecal contamination originating from diverse sources, including feces of domesticated mammals and birds, environmental pollution from human sources such as sewage and its by-products (e.g., biosolids), as well as fecal shedding from recreational water users. Additionally, agricultural contributions represent another significant source, highlighting a potential risk to human health associated

with the presence of *E. faecium* in seafood [36].

Unregulated usage of antibiotics, particularly in aquatic environments, is contributing to the widespread emergence of antibiotic resistance. The main cause of antibiotic resistance in *Enterococcus* is the incorporation of resistance genes through horizontal gene transfer [37]. The results of the present study were in harmony with those obtained in Iran by Norooz *et al.* [38] who documented *Enterococcus* strains from marine water fish, demonstrating a resistance to erythromycin, sulfamethoxazole, and clindamycin. Additionally, these strains exhibited sensitivity to linezolid and vancomycin. Furthermore, the obtained results were in agreement with Enany *et al.* [5] who reported the resistance of *Enterococcus* from fresh water fish in Egypt to erythromycin, tetracycline, ampicillin, and meropenem, in addition to its high sensitivity to linezolid. It is noteworthy that fish samples in this study were exposed to these antimicrobials during their lifetime as growth promoters. The high susceptibility to linezolid may be attributed to the deficient administration of this antibiotic, rendering bacterial isolates responsive to its effects, and this is detected by Chen *et al.* [39] who confirmed that linezolid is employed in the treatment of severe invasive infections caused by multidrug-resistant *Enterococcus*. As well , the obtained results were in agreement with those displayed in Bangladesh by Rahman *et al.* [40] who detected *Enterococcus* isolates from fresh water fish, exhibiting resistance to ampicillin and erythromycin, however, these isolates revealed variable levels of susceptibility to gentamycin and vancomycin. Moreover, in corroboration with the present study, Sergelidis *et al.* [41] stated that *Enterococcus* isolates from fresh water fish in northern Greece possessed the high resistance rate against cephalosporins, penicillins, and erythromycin antimicrobials. On the other hand, AL-Ghanayem *et al.* [42] documented the high resistance of *Enterococcus* isolated from fresh water fish in Kingdom of Saudi Arabia to ciprofloxacin and erythromycin. Likewise, Boss *et al.* [33] demonstrated low resistance of *Enterococcus* isolates obtained from different fish samples in Switzerland to tetracycline, and no resistance to ampicillin, ciprofloxacin, and vancomycin was noticed. Similarly, lower *Enterococcus* resistance to erythromycin, tetracycline, and ciprofloxacin was established by Araujo *et al.* [43] from fresh water fish in Spain. Correspondingly, none of the *Enterococcus* isolates demonstrated resistance to ampicillin, tetracycline, vancomycin, and gentamicin in the study of Sarra *et al.* [44], which was done on marine water fish in Tunisia.

Enterococcus species harbor virulence factors can colonize and invade host tissues, displace through epithelial cells, and evade the host's immune response [45]. Our choice of *Enterococcus faecalis* for the detection of virulent genes is driven by its clinical significance in humans, prevalence, genetic diversity, and antibiotic resistance concerns. In comparison with the obtained results, higher percentages of *ace* (92.7%) and *gelE* (63.4%) virulence genes were exhibited by Ullah et al. [29] from marine water fish in Bangladesh. Besides, *gelE* and *ace* virulence genes with percentages of 30.5% and 79.7%, respectively were presented by Igbinosa and Beshiru [46] from marine water fish in Nigeria. In addition, *gelE* (85.7%) and *ace* (74.3%) virulence genes were displayed with Chajęcka-Wierzchowska *et al.* [47] from

marine water fish in Poland. In contrast to the obtained results, no virulence factors were detected from 13 *Enterococcus* isolated from fresh water fish in China by Xiao *et al.* [48].

Conflict of Interest

The authors have no conflict to declare.

Conclusion

The current investigation proved that several fish species in Egyptian markets have potentially harmful *Enterococcus* species. High percentage of isolated *E. faecalis* strains harbored virulent genes, posing a potential risk to human health. The findings underscore the urgency of implementing stringent hygienic measures to control microbial contamination in both the aquatic environment and fish markets. Addressing these contamination issues is crucial for safeguarding public health and ensuring the safety of consumers. The study emphasizes the importance of ongoing monitoring, regulatory measures, and responsible antibiotic use in aquaculture to mitigate the prevalence of antibiotic-resistant strains and protect both the environment and human health.

Author contribution

All authors contributed equally.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Morshdy, A.E.M.; Ahmed, G.E.; El-tahlawy, A.S.; El Bayomi, R.M. and Mahmoud, A.F.A. (2023): Virulence and Antimicrobial Resistance Profiling of Aeromonas hydrophila Recovered from Retail fish in Sharkia Province, Egypt. J. Adv. Vet. Res, 13: 1320-1324.

[2] Hassan, M.A.; Abdel-Naeim, N.S.; Mabrok, M.; Dessouki, A.A. and Hassan, A.M. (2022): Isolation and identification of Enterococcus faecalis from cultured Oreochromis niloticus and Mugil cephalus with a special emphasis on a possible integrated control strategy. Aquac. Res, 53 (16): 5521-35.

[3] Valenzuela, A.S.; Benomar, N.; Abriouel, H.; Cañamero, M.M. and Gálvez, A. (2010): Isolation and identification of Enterococcus faecium from seafoods: antimicrobial resistance and production of bacteriocin-like substances. Food Microbiol, 27: 955-961.

[4] Akter, T.; Haque, M.N.; Ehsan, R.; Paul, S.I.; Foysal, M.J.; Tay, A.C.Y.; Islam, M.T. and Rahman, M.M. (2023): Virulence and antibiotic-resistance genes in Enterococcus faecalis associated with streptococcosis disease in fish. Sci. Rep, 13: 1551.

[5] Enany, M.; Tartor, Y.H.; Kishk, R.M. and Ali, E.M. (2022): Occurrence of Multidrug-Resistant Enterococci in Fresh Water Fishes. Suez Canal Vet. Med. J, 27: 91-100.

[6] Raharjo, H.M.; Budiyansah, H.; Mursalim, M.F.; Chokmangmeepisarn, P.; Sakulworakan, R.; Debnath, P.P.; Sivaramasamy, E.; Intan, S.T.; Chuanchuen, R. and Dong, H.T. (2023): The first evidence of blaCTX-M-55, QnrVC5, and novel insight into the genome of MDR Vibrio vulnificus isolated from Asian sea bass (Lates calcarifer) identified by resistome analysis. Aquaculture, 571: 739500.

[7] Algammal, A.M.; Alfifi, K.J.; Mabrok, M.; Alatawy, M.; Abdel-Moneam, D.A.; Alghamdi, S.; Azab, M.M.; Ibrahim, R.A.; Hetta, H.F. and El-Tarabili, R.M. (2022): Newly Emerging MDR B. cereus in Mugil seheli as the First Report Commonly Harbor nhe, hbl, cyt K, and

pc-plc Virulence Genes and bla 1, bla 2, tet A, and erm A Resistance Genes. Infect. Drug Resist, 15: 2167-2185.

[8] Hammerum, A.M.; Lester, C.H. and Heuer, O.E. (2010): Antimicrobial-resistant enterococci in animals and meat: a human health hazard? Foodborne Pathog. Dis, 7: 1137-1146.

[9] Hollenbeck, B.L. and Rice, L.B. (2012): Intrinsic and acquired resistance mechanisms in Enterococcus. Virulence, 3: 421-569.

[10] Vankerckhoven, V.; Van Autgaerden, T.; Vael, C.; Lammens, C.; Chapelle, S.; Rossi, R.; Jabes, D. and Goossens, H. (2004): Development of a multiplex PCR for the detection of asa1, gelE, cylA, esp, and hyl genes in enterococci and survey for virulence determinants among European hospital isolates of Enterococcus faecium . J. Clin. Microbiol, 42: 4473-4479.

[11] Park, S.Y.; Shin, Y.P.; Kim, C.H.; Park, H.J.; Seong, Y.S.; Kim, B.S.; Seo, S.J. and Lee, I.H. (2008): Immune evasion of Enterococcus faecalis by an extracellular gelatinase that cleaves C3 and iC3b. J. Immunol, 181: 6328-6336.

[12] Riboulet, E.; Verneuil, N.; La Carbona, S.; Sauvageot, N.; Auffray, Y.; Hartke, A. and Giard, J.-C. (2007): Relationships between oxidative stress response and virulence in Enterococcus faecalis. Microb. Physiol, 13: 140-146.

[13] ISO 6887-2 (2003): Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1-3: Specific rules for the preparation of meat and meat products.

[14] ISO (2013): Microbiology of food and animal feeding stuffs: Preparation of test sample, initial suspension and decimal dilutions for microbiological examination, International Standards Organization, Geneva.

[15] MacFaddin, J.F. (2000): Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.

[16] Lancefield, R. C. (1933). A serological differentiation of human and other groups of hemolytic streptococci. J. Exp. Med, 57, 571-595.

[17] Ferede, Z.T.; Tullu, K.D.; Derese, S.G. and Yeshanew, A.G. (2018): Prevalence and antimicrobial susceptibility pattern of Enterococcus species isolated from different clinical samples at Black Lion Specialized Teaching Hospital, Addis Ababa, Ethiopia. BMC Res. Notes, 11: 1-6.

[18] NCCLS (2001): Performance standards for antimicrobial susceptibility testing. Supplement M100-S11. Villanova, PA, USA.

[19] Jackson, C.R.; Fedorka-Cray, P.J. and Barrett, J.B. (2004): Use of a genus-and species-specific multiplex PCR for identification of enterococci. J. clin. microbiol, 42: 3558-3565.

[20] Eaton, T.J. and Gasson, M.J. (2001): Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. Appl. Environ. Microbio, 67: 1628-1635.

[21] Creti, R.; Imperi, M.; Bertuccini, L.; Fabretti, F.; Orefici, G.; Di Rosa, R. and Baldassarri, L. (2004): Survey for virulence determinants among Enterococcus faecalis isolated from different sources. J. Med. Microbiolo, 53: 13-20.

[22] Mukherjee, M.; Cuthbert, R.E.; Aitken, E.; Gay, K.A.; McKinney, K. and Brooks, J.P. (2023): Revealing an Abundance of Microbial Fecal Contamination and Multidrug Resistant

Bacteria in the Mississippi Gulf Coast. Water, 15: 2339.

[23] Mendoza, D.; Aquino, M.G. and Reyes, A. (2023): Assessment of Enterococcus in fish and its environment: a study in tilapia farms operating in the BUDAMASA areas of Minalin, Pampanga, Philippines. AACL Bioflux, 16: 1007-1024.

[24] Khafagy, A.; Eid, H.; Abou El-Atta, M. and Abd El-Fattah, L. (2009): Isolation of Enterococcus faecalis from tilapia in Lake Temsah in Ismailia governorate. Suez Canal Vet. Med. J, 14: 45-54.

[25] KÜLAHCI, M.B. and Gündoğan, N. (2021): Occurrence and characteristics of staphylococci and enterococci in retail fish used for human consumption in Turkey. Europ. J. Sci. Technol, 28: 911-916.

[26] Adamu, K.; Muhammad, H.; Ahmad, S.; Ahmad, M. and Yakubu, A. (2020): Diversity of Bacteria and Fungi associated with freshwater fishes from Mijawal River, Nasarawa, Nigeria. J. Appl. Sci. Environ. Manage, 24: 1085-2020.

[27] El-Kader, M. and Mousa-Balabel, T.M. (2017): Isolation and molecular characterization of some bacteria implicated in the seasonal summer mortalities of farm-raised Oreochromis niloticus at Kafr El-Sheikh and Dakahlia Governorates. Alex. J. Vet. Sci, 53: 107-113.

[28] Byappanahalli, M.N.; Nevers, M.B.; Korajkic, A.; Staley, Z.R. and Harwood, V.J. (2012): Enterococci in the environment. Microbiol. Mol. Biol. Rev. 76(4): 685-706.

[29] Ullah, M.A.; Islam, M.S.; Rana, M.L.; Ferdous, F.B.; Neloy, F.H.; Firdous, Z.; Hassan, J. and Rahman, M.T. (2023): Resistance Profiles and Virulence Determinants in Biofilm-Forming Enterococcus faecium Isolated from Raw Seafood in Bangladesh. Pathogens, 12: 1101.

[30] Ben Said, L.; Hamdaoui, M.; Klibi, A.; Ben Slama, K.; Torres, C. and Klibi, N. (2017): Diversity of species and antibiotic resistance in enterococci isolated from seafood in Tunisia. Ann. Microbiol, 67: 135-141.

[31] Barros, J.; Igrejas, G.; Andrade, M.; Radhouani, H.; López, M.; Torres, C. and Poeta, P. (2011): Gilthead seabream (Sparus aurata) carrying antibiotic resistant enterococci. A potential bioindicator of marine contamination? Mar. Pollut. Bull, 62: 1245-1248.

[32] Hammad, A.M.; Shimamoto, T. and Shimamoto, T. (2014): Genetic characterization of antibiotic resistance and virulence factors in Enterococcus spp. from Japanese retail ready-to-eat raw fish. Food microbiol, 38: 62-66.

[33] 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. J. Food Prot, 79: 1240-1246.

[34] Çardak, M.; Özmen Toğay, S.; Ay, M.; Karaalioğlu, O.; Erol, Ö. and Bağcı, U. (2022): Antibiotic resistance and virulence genes in Enterococcus species isolated from raw and processed seafood. J. Food Sci. Technol, 59: 2884–2893.

[35] Zhou, X.; Willems, R.J.; Friedrich, A.W.; Rossen, J.W. and Bathoorn, E. (2020): Enterococcus faecium : from microbiological insights to practical recommendations for infection control and diagnostics. Antimicrob. Resist. Infect. Control, 9: 1-13.

[36] Jennings, W.C.; Chern, E.C.; O'Donohue, D.; Kellogg, M.G. and Boehm, A.B. (2018): Frequent detection of a human fecal indicator in the urban ocean: environmental drivers and

covariation with enterococci. Environ. Sci.: Processes Impacts, 20: 480-492.

[37] Werner, G.; Coque, T.M.; Franz, C.M.; Grohmann, E.; Hegstad, K.; Jensen, L.; van Schaik, W. and Weaver, K. (2013): Antibiotic resistant enterococci—Tales of a drug resistance gene trafficker. Int. J. Med. Microbiol, 303: 360-379.

[38] Noroozi, N.; Momtaz, H. and Tajbakhsh, E. (2022): Molecular characterization and antimicrobial resistance of Enterococcus faecalis isolated from seafood samples. Vet. Med. Sci., 8: 1104-1112.

[39] Chen, C.; Sun, J.; Guo, Y.; Lin, D.; Guo, Q.; Hu, F.; Zhu, D.; Xu, X. and Wang, M. (2015): High prevalence of vanM in vancomycin-resistant Enterococcus faecium isolates from Shanghai, China. Antimicrob. Agents Chemother, 59: 7795-7798.

[40] Rahman, M.; Rahman, M.M.; Deb, S.C.; Alam, M.S.; Alam, M.J. and Islam, M.T. (2017): Molecular identification of multiple antibiotic resistant fish pathogenic Enterococcus faecalis and their control by medicinal herbs. Sci. rep, 7: 3747.

[41] Sergelidis, D.; Abrahim, A.; Papadopoulos, T.; Kirkoudis, J.; Anagnostou, V.; Papavergou, A. and Papa, A. (2013): Antimicrobial susceptibility of Enterococcus spp. isolated from freshwater fish and personnel and equipment of fish markets in northern Greece. J. Hellenic Vet. Med. Soc, 64: 239-249.

[42] Al-Ghanayem, A.; Joseph, B.; Bin Mahdi, M.; Scaria, B. and Saadabi, A.M. (2020): Multidrug Resistance Pattern of Bacteria Isolated from Fish Samples Sold in Retail Market. J. Clin. Diagn. Res, 14: 13-16.

[43] Araujo, C.; Munoz-Atienza, E.; Hernandez, P.E.; Herranz, C.; Cintas, L.M.; Igrejas, G. and Poeta, P. (2015): Evaluation of Enterococcus spp. from rainbow trout (Oncorhynchus mykiss, Walbaum), feed, and rearing environment against fish pathogens. Foodborne Pathog. Dis, 12: 311-322.

[44] Sarra, M., Taoufik, G., Patrick, L., Benjamin, B., Yannick, F. and Khaled, H. (2013): Isolation and Characterization of Enterococci Bacteriocinic Strains from Tunisian Fish Viscera. Food nutrition sci. 4: 701-708.

[45] Jaafar, S.S. (2022): Enterococcus faecalis: A Mini-Review. JUBPAS, 30: 191-200.

[46] Igbinosa, E.O. and Beshiru, A. (2019): Antimicrobial resistance, virulence determinants, and biofilm formation of Enterococcus species from ready-to-eat seafood. Front. Microbiol, 10: 728.

[47] Chajęcka-Wierzchowska, W.; Zadernowska, A. and Łaniewska-Trokenheim, Ł. (2016): Virulence factors, antimicrobial resistance and biofilm formation in Enterococcus spp. isolated from retail shrimps. LWT-Food Sci. Technol, 69: 117-122.

[48] Xiao, H.; Zhang, Y.; Wang, Y.R. and NI, Y.Q. (2018): Antibiotic Resistance and Resistance Mechanism in Enterococcus faecium from Cold-water Fish in Aletai Prefecture of Xinjiang. Biotechnol. bull, 34: 143-149.

الملخص العربي

مدي إنتشار، قابلية المضادات الحيوية ، ونمط جينات الضراوة لأنواع المكورات المعوية المعزولة من بعض الأسماك المُستزرعة المعروضة للبيع في مدينة الزقازيق، مصر

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أُجريت هذه الدراسة لتقييم تواجد المكورات المعوية في عينات مختلفة من الأسماك المُستزرعة (البلطي النيلي، المكرونة، الباغة، والمرجان) المعروضة للبيع في مدينة الزقازيق، محافظة الشرقية، مصر. بالإضافة إلى ذلك، تم إجراء اختبار الحساسية للمضادات الحيوية على عزلات المكورات المعوية بإستخدام المضادات الحيوية المستخدمة على نطاق واسع في مصر من خلال طريقة الإنتشار القرصي. علاوة على ذلك، تم إستخدام تفاعل البوليميريز المتسلسل للكشف عن جينات الضراوة المتواجدة في ميكروب إنتيروكوكس فيكاليس. أظهرت النتائج تلوث العينات المفحوصة بأنواع مختلفة من المكورات المعوية بنسبة إجمالية تبلغ 52.5٪، مُمثلة في إنتيروكوكس فيكاليس (26.25%)، إنتيروكوكس فاشيوم (15%)، وإنتيروكوكس هيري، إنتيروكوكس رافينوسس، و إنتيروكوكس ديورانس بنسبة 3.75% لكل منها. أظهر اختبار الحساسية للمضادات الحيوية مقاومة العزلات للكاناميسين (100%)، الكليندامايسين (76.9%)، سلفاميثوكسازول (69.2%)، الأمبيسيلين (69.2%)، والكوليستين (61.5%). كشف تفاعل البوليمراز المتسلسل عن وجود ثلاثة أنواع من جينات الضراوة في عزلات إنتيروكوكس فيكاليس، وهي جين sodA (100%)، جين gelE (83.3%)، و جين ace (50%). خَلُصت هذه الدراسة إلي أنه من الضروري تنفيذ تدابير صحية عاجلة للتحكم في التلوث الميكروبي سواء في البيئة المائية أو في أسواق الأسماك.