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Food Safety in Association with Bacteriological Quality of Some Marine Water Fishes

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Abstract

IN THE CURRENT study, one hundred and twenty random samples of marine water fishes represented by *Pagrus pagrus* (morgan), *Atherina hepsetus* (Basaria), *Mullus surmuletus* (barboni) and *Saurida undosquamis* (makarona) fishes (30 of each), which were collected from different retail fish markets in Qalubiya governorate, Egypt, were examined bacteriologically for their potential of fish-borne pathogenic bacteria of health hazard concerns. Results revealed *S. Aureus* as the most prevalent bacteria in the total of the examined samples, that was detected in the examined samples (45.0%), followed by *E. Coli* (30.8%), *V. Parahaemolyticus* (18.3%) and Salmonella spp. (5.0%). On the other hand, Basaria fish samples revealed higher incidence of *V. Parahaemolyticus, E. Coli* and *S. Aureus* than the other examined samples. Additionally, Salmonella spp. Was not detected in any of the examined Morgan samples. It is worth noted that *Ps. Aeruginosa* and *A. Hydrophila* were not detected in any of the examined samples. Furthermore, the isolated strains were subjected to *in vitro* antimicrobial susceptibility against amoxicillin/clavulanic acid, ampicillin/sulbactam, ceftazidime, ciprofloxacin, gentamicin, levofloxacin, Trimethoprim/Sulphamethoxazol and tetracycline; moreover, methicillin and vancomycin were especially applied against *S. Aureus* isolates; where it was proven that most of the isolates were sensitive to the used antibiotics in variable degrees, in which β-lactams and tetracycline recorded higher resistance levels than the other used antibiotics. Moreover, pathogenicity and antimicrobial profile of some of the detected isolates were estimated molecularly against some virulence factors and antibiotic resistance genes. After all, it can be concluded that marine water fishes may be considered as a possible source of foodborne pathogens, whereas it can be loaded with different other foodborne bacteria during storage and presentation.

Keywords: AMR, Hygienic quality, Marine water fish.

Introduction

Fish is an essential source of nutrients, particularly highly digestible proteins, and is nutrient-rich. Eating fish is linked to potential health advantages, including the development of the nervous system throughout pregnancy and infancy. Seafood can, however, also spread foodborne illnesses, which highlights the necessity of closely monitoring its bacteriological traits [1].

The application of bacterial analysis is used to assess the potential presence of microorganisms relevant to public health and to provide an indication of the hygienic quality of seafood. This covers improper handling and processing procedures and temperature abuse [2]. The bacterial pathogens linked to seafood can be classified into three groups: enteric bacteria resulting from fecal contamination, such as E. coli and Salmonella spp., environmental bacteria introduced during processing and handling, such as S. aureus, and normal inhabitant bacteria of the marine or estuarine environment, such as Vibrio spp., Pseudomonas spp., and Aeromonas spp. [3].

Seafood-related food poisoning is typically brought on by a variety of bacterial pathogens, including *V. parahemolyticus, E. coli, S. aureus,* Salmonella spp., A. hydrophila, and many others. These microbes can cause varying degrees of GIT disturbance, ranging from dysentery and abdominal pain to bloody diarrhea and even dehydration and deaths in advanced stages. These food poisoning episodes typically occur after consumption of raw or undercooked contaminated fish meat products and/or cross-contaminated ready-to-eat seafood [4].

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Aeromonas spp. are common marine inhabitants that are regarded as foodborne pathogens of rising significance due to their unique characteristics, which include their abundance in aquatic environments, multiplicity of virulence factors, and psychrotrophic nature. Although these bacteria are typically found in the microbiota, they can also be primary or secondary infections in fish. Furthermore, there are currently many species of the genus Vibrio that are found in aquatic habitats and are easily isolated from sediments, water, and seafood. In nations with warm coastal waters where fish and shellfish are eaten raw or very mildly cooked, some Vibrio species are harmful to humans and can cause alimentary sickness. Furthermore, Pseudomonas species are significant spoilage bacteria found in a variety of chilled food goods, particularly seafood, where they establish themselves as the predominant microflora either through chilling storage or by the use of tainted ice during market presentation [5].

Fish are known to have several Enterobacteriaceae food poisoning bacteria, which serve as a sign of fecal contamination. Being one of the most common bacteria that cause severe diarrhea in infants and food poisoning in adults, enteropathogenic *E. coli* is especially alarming. Additionally, because Salmonella spp. can contaminate fish and cause food poisoning, they pose a severe concern to the public's health [6]. However, *S. aureus* has been linked to significant heat-stable enterotoxins, which raise the risk of staphylococcal food poisoning when it comes into contact with food products. For this reason, it has been identified as a crucial environmental and personal hygiene concern [7].

Seafood contaminated by bacteria may undergo biochemical alterations brought on by microbial enzymatic activity, which can have a major effect on the fish's acceptability, freshness, and shelf life [8]. Therefore, the present study was planned to assess the food safety of some commercially marketed marine water fishes, in the Egyptian retails, through determination the presence of some pathogenic fish-borne bacteria.

Material and Methods

Collection of fish samples

A total of 120 random freshly caught, chilled samples of marine water fishes represented by *Pagrus pagrus* (morgan), *Atherina hepsetus* (Basaria), *Mullus surmuletus* (barboni) and *Saurida undosquamis* (makarona) fishes (30 of each) weighted about 100 to 300 g were collected from different fish markets located in Qalubiya governorate during the period between January to March 2024.

All samples were subjected to bacteriological examination following the next steps:

Bacteriological examinations

Detection of Aeromonas species

It was performed according to Palumbo *et al.* [9]. After incubation of 25g of fish sample on nutrient broth at 37^{O}C for 24h, loopful was taken and streaked on Aeromonas agar medium and incubated at 37^{O}C for 24h. The suspected colonies were purified and subjected for further biochemical identification.

Detection and identification of Vibrio parahaemolyticus

It was performed according to ISO 21872-1 [10]; in which 25g of fish sample were enriched in alkaline saline broth, and then loopfuls from each cultured sample were streaked onto Thiosulfate citrate bile and sucrose agar (TCBS) then incubated at 37°C for 24 hours. The suspected typical colonies were purified and furtherly identified biochemically.

Detection of Pseudomonas aeruginosa

It was performed according to ISO 22717 [11]. After incubation of 25g fish samples on nutrient broth at 37^{O}C for 24h, loopful was taken and streaked on Pseudomonas agar medium and incubated at 37^{O}C for 24h. The suspected colonies were purified and subjected for further biochemical identification.

Screening for Enteropathogenic Escherichia coli

It was performed according to ISO 16649-2 [12] included enrichment and plating steps on MacConkey broth and Tryptone Bile X-glucoronide agar (TBX agar) were seen as dark blue green round colonies.

Detection of Salmonella species was performed according to ISO 6579 [13]

Prepared sample was incubated in buffered peptone water broth at 37°C ± 1°C for 18 ± 2 hours, then transferred to Rappaport Vassilidis broth (RV broth) and incubated at 41.5°C\ 24hr. loopful of enriched sample was streaked on selective XLD agar and Brilliant Green agar, and incubated at 37°C\24h, plates were examined for suspected Salmonella colonies which then isolated for confirmation.

Detection of Staphylococcus aureus

It was performed according to ISO 6888- 1 [14]. After sample enrichment on nutrient broth at 37^{o}C for 24h, a loopful, of each sample, was streaked on Baird Parker agar supplemented with egg yolk tellurite, and then incubated at 35 ± 2^{O}C for 24-48h. *Staphylococcus aureus* colonies appear as black, shiny, circular, smooth, convex colony with narrow white margin and surrounded by a clear zone extending into opaque medium.

Estimation of in vitro antimicrobial susceptibility

It was conducted on each of the detected isolate using Mueller–Hinton broth and agar for bacterial enrichment and plating, and Oxoid standardized single antibiotic discs. Plates were incubated at 37^{o}C for 24h, followed by measuring inhibition zone and recording its antibiogram following the recommendations of CLSI [15].

All of the isolates were tested against amoxicillin/clavulanic acid (AMC, 30 µg), ampicillin/sulbactam (SAM, 10 µg), ceftazidime (CAZ, 10 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GN, 10 µg), levofloxacin (LEV, 5 µg), Trimethoprim/Sulphamethoxazol (SXT, 1.25/23.75 µg) and tetracycline (TE, 30 µg); moreover, methicillin (MET, 5 µg) and vancomycin (VA, 30 µg) were especially applied against *S. aureus* isolates.

Molecular examination of the strains

DNA Extraction

DNA preparation from bacterial culture was performed according to Shah *et al.* [16], following the instruction of QIAamp DNA mini kit instructions (Catalogue no.51304).

Oligonucleotide primers used in PCR

The examined target gene sequences of Salmonella, *V. parahaemolyticus*, *E. coli* and *S. aureus* were demonstrated in Table (1), respectively. Agarose gel electrophoreses was performed according to Sambrook et al. [25].

Statistical analysis

Simple descriptive analytics was used to analyze the data. Incidence of the isolated strains was calculated in relation to the total number of the examined samples.

Results.

Regarding with the incidence of some pathogenic fish-borne bacteria (Table, 2 and Fig, 1), S. aureus was the most prevalent bacteria in the total of the examined samples with the prevalence of 45.0%, followed *by E. coli* (30.8%), *V. parahaemolyticus* (18.3%) and Salmonella spp. (5.0%). On the other hand, Basaria fish samples revealed higher incidence of *V. parahaemolyticus, E. coli* and *S. aureus* than the other examined samples. Additionally, Salmonella spp. was not detected in any of the examined Morgan samples. It is worth noted that Ps. *aeruginosa* and *A. hydrophila* were not detected in any of the examined samples.

Regarding with the antimicrobial susceptibility profile of the detected Salmonella isolates (Fig., 2), Salmonella isolates were whole sensitive to ciprofloxacin and levofloxacin revealing that quinolone antibiotic group has higher inhibitory effect on salmonella spp. than the other examined antibiotics. In addition, salmonella isolates were moderately resistant toward β-lactams and tetracycline in the examined isolates.

Regarding with the antimicrobial susceptibility profile of the detected *V. parahaemolyticus* isolates (Fig. 3), the isolates showed higher resistance ratio for amoxicillin/clavulanic acid, ceftazidime and gentamicin (27.3, 22.3 and 27.3%, respectively), while the lowest resistance level was recorded against levofloxacin (4.5%).

Regarding with the antimicrobial susceptibility profile of the detected *E. coli* isolates (Fig., 4), the isolates showed higher resistance ratio for gentamicin, amoxicillin/clavulanic acid, ceftazidime and ampicillin/sulbactam (37.8, 29.7, 27.0 and 21.6%, respectively), while the lowest resistance level was recorded against Trimethoprim/Sulphamethoxazol (8.1%).

Regarding with the antimicrobial susceptibility profile of the detected *S. aureus* isolates (Fig., 5), the isolates showed higher resistance ratio for tetracycline, Trimethoprim/Sulphamethoxazol, ceftazidime, ampicillin/sulbactam and amoxicillin/clavulanic acid (46.3, 37, 33.3, 27.8 and 24.1%, respectively), while the lowest resistance level was recorded against gentamicin and levofloxacin (16.7%).

Referring to the molecular detection of some virulence factors and drug resistance genes of the present bacterial isolates, Fig. (6) revealed positive detection of *invA, aad*B and *bla*TEM genes in all of the examined Salmonella strains revealing them of highly invasive pathogenicity with gentamicin and B-lactams resistance. In addition, Fig. (7) positive detection of *recA, bla*TEM and *aad*B genes in *V. parahaemolyticus* (V1); while, V2 isolate revealed positive *rec*A and *bla*TEM genes only. Moreover, Fig. (8) showed that both *E. coli* isolates had *aad*B gene, while only E1 isolate had *sxt*1 gene, but *sxt*2 gene was absent in both samples. Furthermore, Fig. (9) proved that both of the examined *S. aureus* isolates were resistant to tetracycline (*tet*K) and methicillin (*mec*A), but only ST2 isolate was vancomycin resistant strain.

Discussion

Microbial food safety is dependably of a major health concern in our life. Food spoilage leads to reduction of shelf life, food poisoning, and economic losses.

Fish is a highly nutritious diet that contains a wide range of vitamins and minerals, as well as high-quality lipoproteins [7]. However, fish has a short shelf life due to the initial loss of freshness, which adversely affects the sensory characteristics of fish due to its high moisture content and easily utilized nutrient content. As a result, sensory evaluation is important in determining food acceptability, as human response determines the quality of any food consumed. According to Naeem and Selamoglu [26],

fish can be a significant source of foodborne infections, including many emerging diseases that pose a substantial threat to public health.

The source of the water used for processing, the distance between capture places, and polluted areas where anthropogenic activities (human and animal feces) are prevalent can all contribute to the contamination of seafood by foodborne pathogens [27].

Salmonella can infect fish and shellfish during processing and storage, or it can enter them from contaminated waters. Seafood has a great nutritional value and is a health food, but it also carries some hazards, especially when it comes to microbial contamination. Outbreaks associated to seafood in the United States [28], the European Union [29], and other nations have been linked to salmonella.

Escherichia coli is a commensal microbe that often lives in the colon's mucous layer in mammals. The main causes of *E. coli* food poisoning include contaminated water and/or unsanitary circumstances during food processing; in the tropics, where it is frequently found in large quantities, it is typically linked to contaminated seafood [30].

Worldwide, estuaries and marine ecosystems are naturally home to *Vibrio parahaemolyticus* [31]. This specific bacterium is particularly dangerous since, in many countries, it is the most prevalent pathogen that causes food-borne illnesses in humans who eat raw or undercooked seafood [32]. Furthermore, a great deal of Pseudomonas species are harmful to people, animals, and aquatic life. While some Pseudomonas species have been characterized as opportunistic pathogens, many species—including Pseudomonas aeruginosa—have also been identified as the main cause of a number of diseases in farmed fish [33].

Staphylococcus aureus is the most important pathogen found in sea foods. Food poisoning in human may happen due to the consumption of aqua products contaminated with this bacterium and its enterotoxin. The procedures carried out to maintain and preserve the quality of these products, from the time they are fished and transported to stores until they are consumed, can play a major role in the generation and growth of pathogenic bacteria and toxins [34].

Referring to the recorded results (Table, 1 and Fig., 1), *S. aureus* was the most prevalent bacteria in the total of the examined samples, that was detected in the examined samples (45.0%), followed by *E. coli* (30.8%), *V. parahaemolyticus* (18.3%) and Salmonella spp. (5.0%). On the other hand, Basaria fish samples revealed higher incidence of *V. parahaemolyticus, E. coli* and *S. aureus* than the other examined samples. Additionally, Salmonella spp. was not detected in any of the examined Morgan samples. It is worth noted that *Ps. aeruginosa* and *A. hydrophila* were not detected in any of the examined samples.The obtained results came in line with those recorded by Hassanien *et al.* [35] who recorded superiority of *S. aureus* prevalence over *E. coli* with prevalence of 32 and 24%, respectively. Ogur [36] who recorded that *S. aureus* was the most prevalent bacteria, followed by *E. coli* in the examined fish samples; on the other hand, Salmonella spp. was detected in higher prevalence (39.3%). While, came on the other side with those recorded by Moustafa *et al.* [37] who recorded positive detection of Pseudomonas spp. and Aeromonads spp. in their fish samples, where *S. aureus* was detected in only 3.67% of the examined samples, which was free of *V. parahaemolyticus*. Mok *et al.* [38] who detected *Vibrio parahaemolyticus* in higher prevalence (34.7%). Hussien [39] who detected *V. parahaemolyticus* in higher prevalence (44.74%), and isolated A. hydrophila and Ps. Aeruginosa in prevalence of 31.58 and 23.68%, respectively. Yacoub [40] who recorded detection of *V. parahaemolyticus, Aeromonas* spp., and Pseudomonas spp. in prevalence ranged from 83.34 to 90, 30 to 66.67, and 30 to 50 (%) according to the site of collection. El-Borolos [41] who detected *V. parahaemolyticus* in lower percentage (11%) in the examined fish samples, and Ahmedou *et al.* [42] who detected Salmonella spp. in higher prevalence than *E. coli* in the examined fish samples.

Foods of animal origin, including milk, meat, fish, and chicken, are associated with a majority of foodborne microbial infections. Due to the overuse of antibiotics in animal feed, the occurrence of multi-drug resistant (MDR) microorganisms in food is becoming a global public health concern. MDR infections can pose a serious threat to consumers and animals, making them an important entry point into the food chain [43].

The bacterial species, antimicrobial classes, and most importantly, the global location, all influence the occurrence of these infections. In the USA, antibiotic-resistant bacteria cause over 2 million infections annually, accounting for at least 23,000 deaths. The etiological agents responsible for these sharply rising infection rates were primarily identified as methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *E. faecium* (VRE), and fluoroquinolone-resistant *P. aeruginosa* [44].

The ability of microbes to adapt and endure antimicrobials is known as resistance. Antimicrobial resistance genes, which are found on mobile genetic components including integrons, transposons, and plasmids and which can spread by horizontal or vertical transfer, control this ability. Hospitalization, death, increased severity and duration of infections, and treatment failure are among the consequences for public health [45].

The complicated problem of antibiotic resistance has led to the emergence of resistant bacteria in a number of fields, including agriculture, animal husbandry, and human medicine. Products from

aquaculture as well as aquatic habitats have been reported to contain these bacteria. It is true that the food chain plays a part in the spread of resistant germs from animals to people, and there has occasionally been a connection made between this and the zoo technical use of antibiotics in farming. Antibiotics are mostly employed in animal husbandry, including aquaculture, as preventative and curative measures. Furthermore, the development of resistance has been aided by their use as growth promoters at subtherapeutic levels [46].

Regarding with the antimicrobial susceptibility profile of the detected Salmonella isolates of the current study**,** all of Salmonella isolates were sensitive to ciprofloxacin and levofloxacin revealing that quinolone antibiotic group has higher inhibitory effect on Salmonella spp. than the other examined antibiotics. In addition, they were moderately resistant toward β-lactams and tetracycline, which was confirmed by PCR that showed positive *inv*A, *bla*TEM and *aad*B genes revealing Salmonella isolates of high invasiveness and resistant to β-lactams and gentamicin; which came in line with Islam *et al*. [47] who reported similar result that the detected Salmonella isolates were highly resistant to Ceftazidime. Samantha *et al.* [48] also found out that 100% of their Salmonella isolates were resistant to Gentamicin. Barika *et al.* [49] who recorded that *Salmonella enterica* were resistant to amoxicillin\clavulinic acid (95%) and Cefotaxime (92.5%); while, sensitive to Ofloxacin (65%).

Regarding with the antimicrobial susceptibility profile of the detected *V. parahaemolyticus* isolates, the isolates showed resistance to amoxicillin/clavulanic acid, ceftazidime and gentamicin (27.3, 22.3 and 27.3%, respectively), while the lowest resistance level was recorded against levofloxacin (4.5%), which was confirmed by PCR that gave positive indication to *recA*, *blaTEM* and *aadB* genes revealing *V. parahaemolyticus* isolates of high pathogenicity and resistant to β-lactams and gentamicin. The current recorded results came in line with those recorded by Tan *et al.* [50] who found that *V. parahaemolyticus* isolates from the seafood samples, in Malaysia, were found to be susceptible to most antibiotics except ampicillin, cefazolin, and penicillin. In addition, authors confirmed their results molecularly revealing *V. parahaemolyticus* of high virulence and multi-drug resistant bacteria. Srinivasan and Ramasamy [51], Letchumanan *et al.* [52], Tan *et al.*, [53], Amalina *et al.*, [54], also, had recorded that *V. parahaemolyticus* strains isolated from different types of seafood were found to be highly resistant to beta-lactam class antibiotics, including penicillin and cephalosporins.

Regarding with the antimicrobial susceptibility profile of the detected *E. coli* isolates (Fig., 4), the isolates showed higher resistance ratio for gentamicin, amoxicillin/clavulanic acid, ceftazidime and ampicillin/sulbactam (37.8, 29.7, 27.0 and 21.6%, respectively), while the lowest resistance level was recorded against Trimethoprim/ Sulphamethoxazol (8.1%), which was confirmed by PCR that gave positive results for *stx1* and *aadB* genes revealing *E. coli* isolates of high toxigenicity and drug resistant.

The current obtained results not compatible with those recorded by Frances *et al.* [55] who recorded that gentamicin, amoxycillin/clavulanate were the antibiotics that *E. coli* was most sensitive to, while it agreed with that tetracycline, ciprofloxacin, levofloxacin was mostly effective against *E. coli* isolates. While, it came in line with the recorded results of Rubab and Oh [56] who found that STEC *E. coli* strains were phenotypically resistant to at least one antibiotic, with 98% resistance to gentamicin, and ampicillin (72%). Moreover, 70% of the examined isolates showed positive *aadA* gene.

Regarding with the antimicrobial susceptibility profile of the detected *S. aureus* isolates (Fig., 5), the isolates showed higher resistance ratio for tetracycline, Trimethoprim/Sulphamethoxazol, ceftazidime, ampicillin/sulbactam and amoxicillin/clavulanic acid (46.3, 37, 33.3, 27.8 and 24.1%, respectively), while the lowest resistance level was recorded against gentamicin and levofloxacin (16.7%). Moreover, isolates were examined molecularly for their methicillin and vancomycin susceptibility, where it appeared that the examined *S. aureus* isolates were resistant to tetracycline (*tetK*), methicillin (*mecA*) and vancomycin (*vanA*) revealing it of MDR strain.

The current obtained results came in line with Ed-Dra *et al.* [57] who recorded that 76.20% of isolated *S. aureus* were resistant to tetracycline and 42.86% to ampicillin. However, all isolated strains were sensitive to oxacillin, cefoxitin, gentamicin, and vancomycin. Naas *et al.* [58] who recorded detection of multi-drug resistant (MDR) *S. aureus* from their examined samples which was mainly resistant to cefotaxime, while MRSA strains were detected in lower ratio.

Such antimicrobial resistance profile of the detected bacteria should be considered as a significant threat on the human and animal health. Compatibility and incompatibility of the recorded results with other records may be attributed to the variation in the site of collection, animal species, rearing conditions and time of examination.

Foodborne bacterial pathogens are typically found in food by the use of basic biochemical identification techniques after the germs are cultured on agar plates. Traditional techniques are typically low-cost and straightforward, but they can take a lot of time since they rely on the microorganisms' capacity to proliferate in various culture media,

including selective plating media, pre-enrichment media, and enrichment media [59].

Referring to the molecular detection of some virulence factors and drug resistance genes of the present bacterial isolates, Fig. (6) revealed positive detection of *invA, aad*B and *bla*TEM genes in all of the examined Salmonella strains revealing them of highly invasive pathogenicity with gentamicin and B-lactams resistance. In addition, Fig. (7) positive detection of *recA, bla*TEM and *aad*B genes in *V. parahaemolyticus* (V1); while, V2 isolate revealed positive *rec*A and *bla*TEM genes only. Moreover, Fig. (8) showed that both *E. coli* isolates had *aad*B gene, while only E1 isolate had *sxt*1 gene, but *sxt*2 gene was absent in both samples. Furthermore, Fig. (9) proved that both of the examined *S. aureus* isolates were resistant to tetracycline (*tet*K) and methicillin (*mec*A), but only ST2 isolate was vancomycin resistant strain.

The obtained results came in line with those of El-Borolos [41] and Gao *et al.* [60] who recorded positive detection of fish-borne pathogens and drug resistant genes (DRGs) using PCR technique, revealing it as useful as application for rapid and confirmative detection method of drug-resistant, health-threaten fish-borne pathogens.

Conclusion

The examined marine water fishes appeared to be a possible source of some serious food-borne pathogens such as *E. coli*, *S. aureus*, Salmonella and *V. parahaemolyticus*. Superiority of *S. aureus* mostly indicates improper environmental and personal hygiene during storage and handling. Basaria samples had higher prevalence of bacterial contamination than the other examined samples. Isolated strains showed significant resistance β-lactams and tetracycline greater than resistance to the other used antibiotics giving red alarm for applications of such antibiotics in fish feeding and treatment. Molecular detection of some virulence and drug-resistant genes confirmed presence of virulence and MDR fish-borne bacteria, and proven to be a useful technique for their rapid and confirmative detection.

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This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Oligonucleotide primers sequences

Gene	Sequence	Amplified product	Reference
***Salmonella* spp.**			
invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284 bp	Oliveira *et al.*[17]
blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al.* [18]
aadB	GAGCGAAATCTGCCGCTCTGG CTGTTACAACGGACTGGCCGC	319 bp	Frana *et al.*,[19]
V. parahaemolyticus			
recA	TGARAARCARTTYGGTAAAGG TCRCCNTTRTAGCTRTACC	793 bp	Casandra *et al.*, [20]
blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al.*[18]
aadB	GAGCGAAATCTGCCGCTCTGG CTGTTACAACGGACTGGCCGC	319 bp	Frana *et al.*,[19]
E. coli			
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614 bp	Dipineto *et al.*, [21]
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp	
aadB	GAGCGAAATCTGCCGCTCTGG CTGTTACAACGGACTGGCCGC	319 bp	Frana *et al.*,[19]
S. aureus			
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure *et al.*, [22]
tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360 bp	Duran *et al.*, [23]
vanA	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC	885 bp	Patel *et al.*, [24]

TABLE 2. Incidence of the detected pathogens in the examined fish samples (n=30)

	Salmonella spp.		*V. parahaemolyticus*		*E. coli*		*S. aureus*	
	NO.	**%***	**NO.**	**%***	**NO.**	**%***	**NO.**	**%***
Barboni	3	10.0	5	16.7	10	33.3	15	50.0
Basaria	1	3.33	7	23.3	12	40.0	17	56.7
Makarona	2	6.7	6	20.0	9	30.0	12	40.0
Morgan	ND	--	4	13.3	6	20.0	10	33.3
Total**	6	5.0	22	18.3	37	30.8	54	45.0

*- Incidence in relation to the number of each examined fish species (30).
**- Incidence in relation to the total number of the examined samples (120).

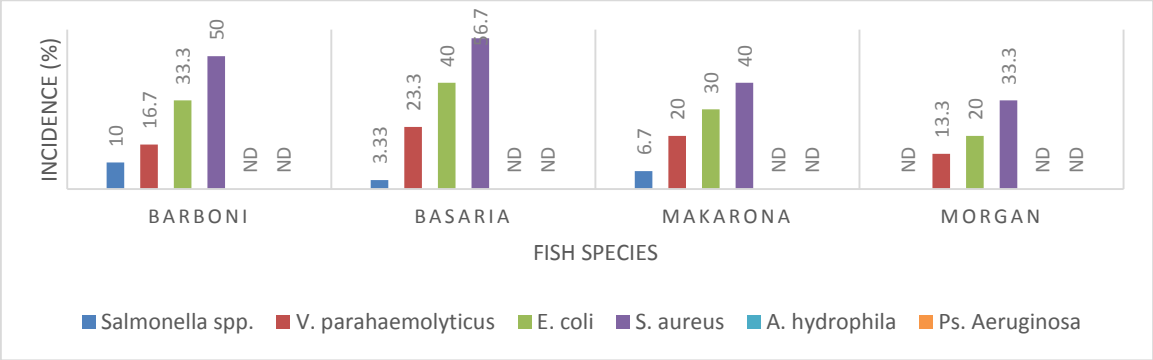

Fig. 1. Incidence of some pathogenic bacteria in the examined fish samples

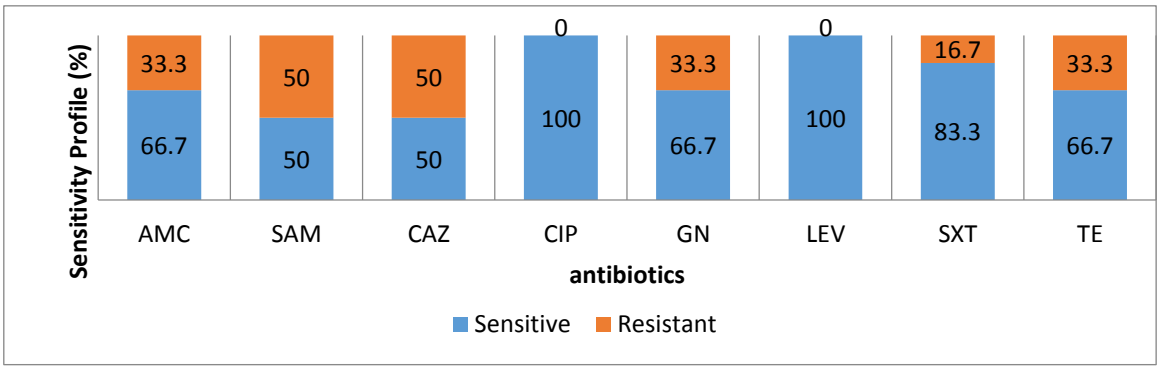

Fig. 2. Antibiotic susceptibility profile of Salmonella isolates (n=6)

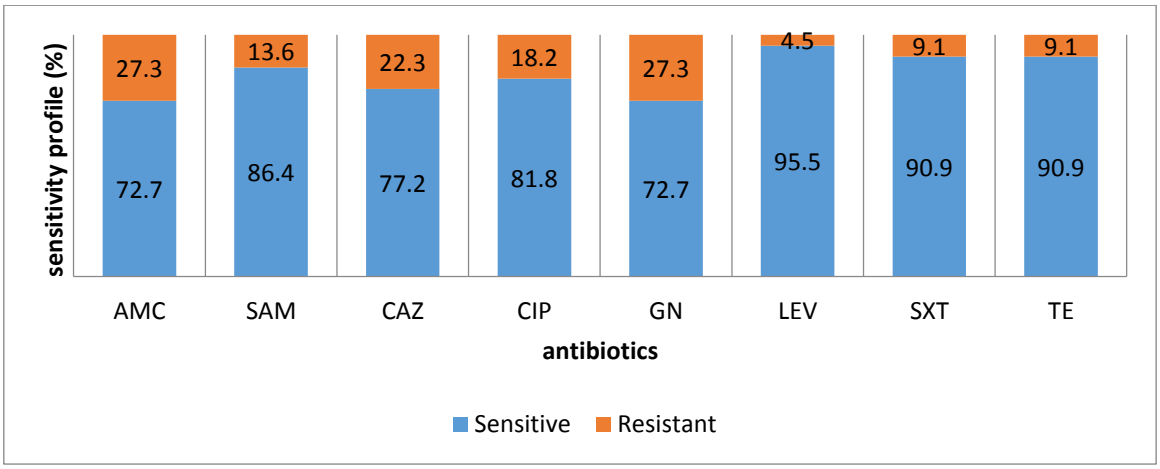

Fig. 3. Antibiotic susceptibility profile of *V. parahaemolyticus* isolates (n=22)

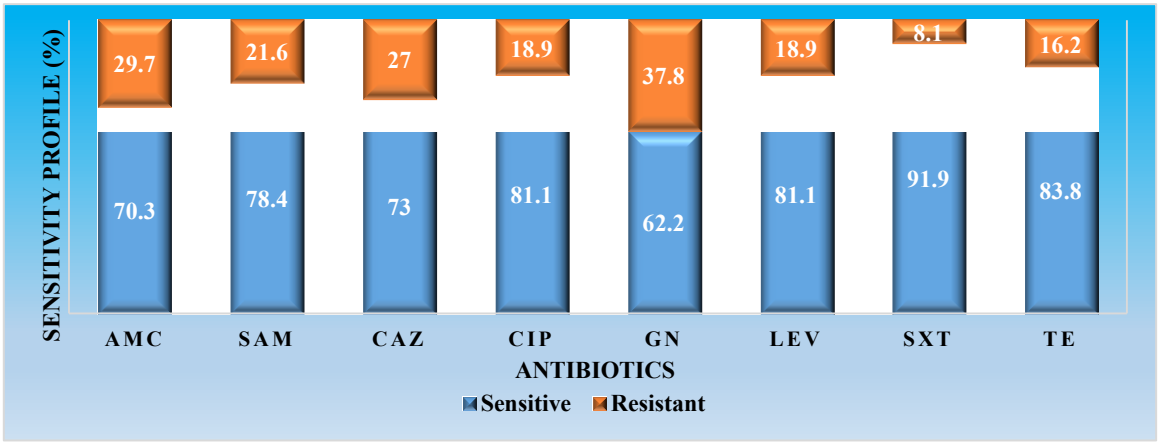

Fig. 4. Antibiotic susceptibility profile of *E. coli* isolates (n=37)

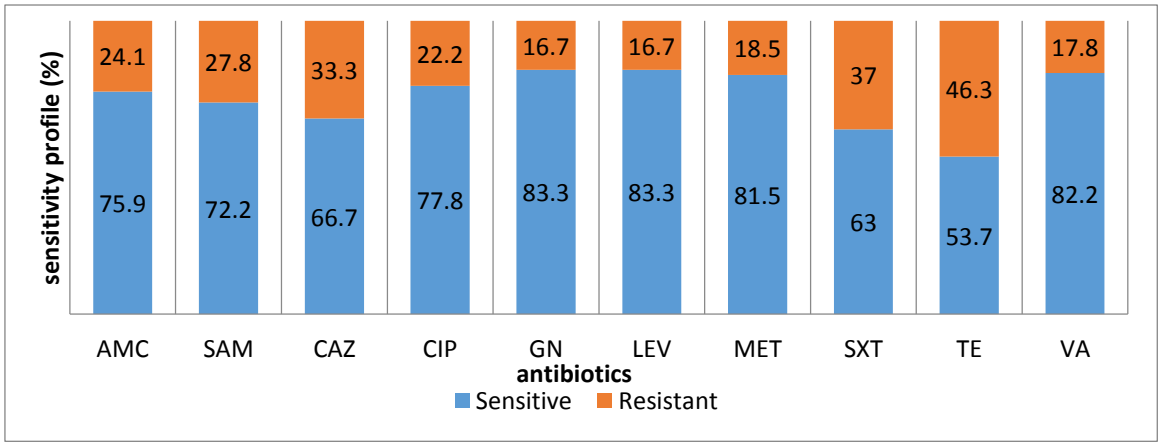

Fig. 5. Antibiotic susceptibility profile of *S. aureus* isolates (n=54)

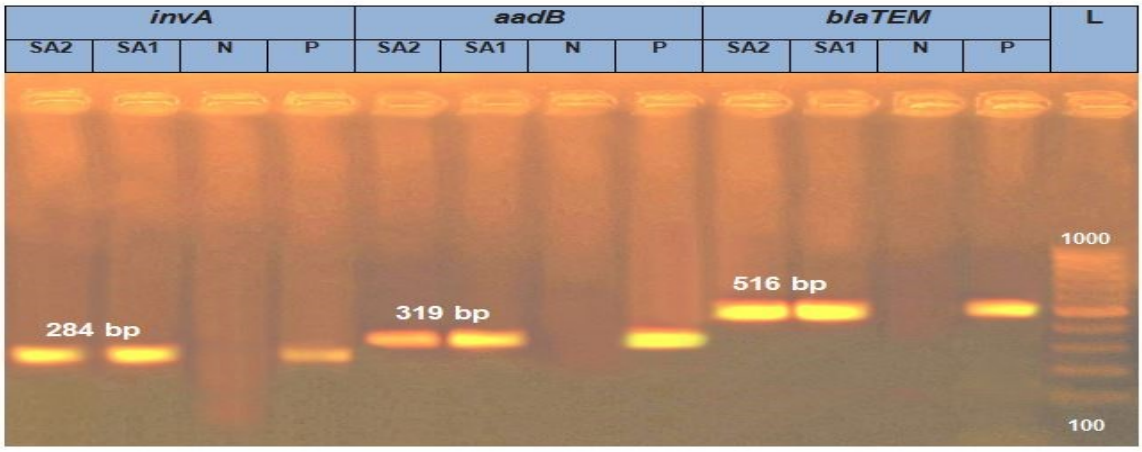

Fig. 6. Agarose gel electrophoresis of multiplex PCR of invA (284 bp), aadB (319bp) and blaTEM (516 bp) virulence genes of Salmonella isolates**. Lane L:** 100 bp ladder as molecular size DNA marker**, Lane N:** Control negative (E. coli), **Lane P:** Control positive Salmonella spp. for invA, aadB and blaTEM genes, **Lanes SA1 and SA2**: Positive *Salmonella* isolates for *invA, aadB* and *blaTEM* genes.

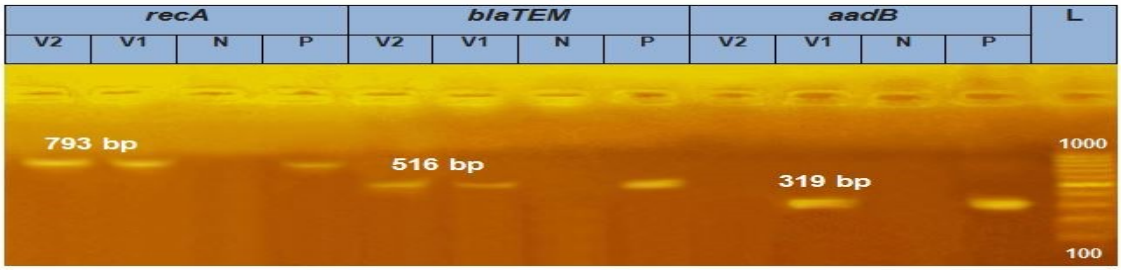

Fig. 7. Agarose gel electrophoresis of multiplex PCR of *rec*A (793 bp), aadB (319 bp) and *bla*TEM (516 bp) virulence genes of *V. parahaemolyticus* isolates. **Lane L:**100 bp ladder as molecular size DNA marker**, Lane N:** Control negative (*E. coli*), **Lane P:** Control positive *V. parahaemolyticus* for *recA, aadB* and *blaTEM* genes**, Lanes V1 and V2:** Positive *V. parahaemolyticus* isolates for *recA, aadB* and *blaTEM* genes, except V2 for aadB gene which gave negative results**.**

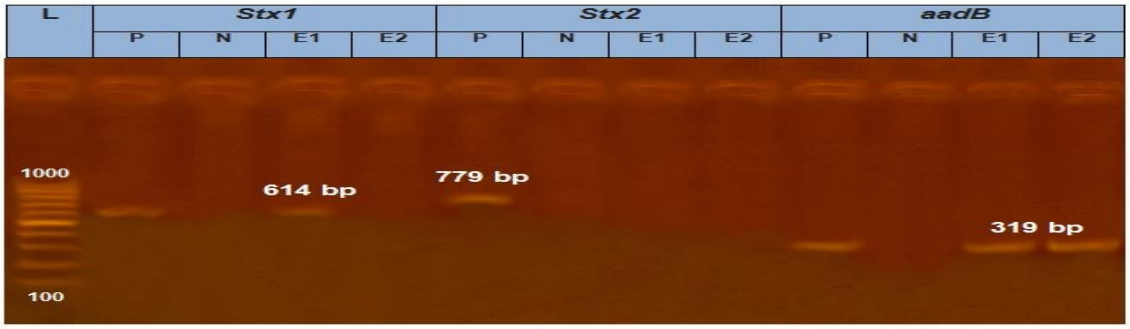

Fig. 8. Agarose gel electrophoresis of multiplex PCR of *stx*-1 (614 bp), *stx*-2 (779 bp) and *aadB* (319 bp) virulence genes of *E. coli* isolates, **Lane L**: 100 bp ladder as molecular size DNA marker, **Lane N:** Control negative (*S. aureus*). **Lane P:** Control positive *E. coli* for *stx-1, stx-2* and *aadB* genes. **Lane E1:** Positive *E. coli* isolate for *stx-1* and *aadB* genes, and negative for *stx-2* gene. **Lane E2**: Positive *E. coli* isolate for *aadB* gene, and negative for *stx-1* and *stx-2* genes.

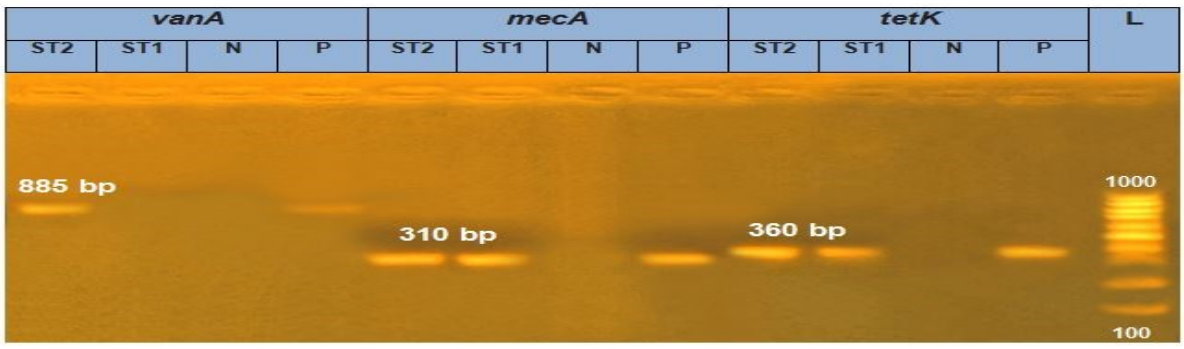

Fig. 9. Agarose gel electrophoresis of multiplex PCR of *vanA* (885 bp), *mecA* (310 bp) and *tetK* (360 bp) virulence genes of *S. aureus* isolates. **Lane L**: 100 bp ladder as molecular size DNA marker. **Lane N**: Control negative (*E. coli*). **Lane P**: Control positive *S. aureus* for *vanA, mecA* and *tetK* genes. **Lane ST1** : Positive *S. aureus* isolate for *mecA* and *tetK* genes, and negative for *vanA* gene. **Lane ST2**: Positive *S. aureus* isolate for *tetK, mecA* and *vanA* genes.

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سلامة الغذاء وعلاقتها بالجودة البكتريولوجية لبعض أسماك المياه البحرية

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الملخص

في الدراسة الحالية، تم فحص مائة وعشرين عينة عشوائية من أسماك المياه البحرية ممثلة بأسماك المرجان والبساريا والبربوني والمكرونة بواقع ثلاثين عينة من كل نوع، والتي تم جمعها من أسواق بيع الأسماك المختلفة في محافظة القليوبية بجمهورية مصر العربية، من الناحية البكتريولوجية لاحتمالية وجود بكتريا مسببة للأمراض منقولة بالأسماك تشكل خطرًا على الصحة العامة وصحة المستهلك. كشفت النتائج عن أن بكتريا الاستافيلوكوكس اوريس كانت هي البكتريا الأكثر انتشارًا في إجمالي العينات المفحوصة، والتي تم اكتشافها بنسبة 45%، تليها الايكولاي بنسبة 30.8%، والفبريو باراهيمولتكس بنسبة 18.3%، والسالمونيلا بنسبة 5%. من ناحية أخرى، كانت عينات أسماك البساريا الاعلى في معدلات التلوث ببكتريا الفبريوباراهيمولتكس و الايكولاي والاستافيلوكوكس اوريس من العينات الأخرى المفحوصة. بالإضافة إلى ذلك، لم يتم الكشف عن بكتريا السالمونيلا في أي من عينات اسماك المورجان المفحوصة. ومن الجدير بالذكر أنه لم يتم الكشف عن بكتريا السيدوموناس ايروجينوزا والالايروموناس هيدروفيلا في أي من العينات المفحوصة. علاوة على ذلك، تم إخضاع السلالات المعزولة لحساسية المضادات الحيوية في المختبر ضد أموكسيسيلين / حمض الكلافولانيك، أمبيسلين / سولباكتام، سيفتازيديم، سيبروفلوكساسين، جنتاميسين، ليفوفلوكساسين، تريميثوبريم / سلفاميثوكسازول وتتراسيكلين؛ كما تم اختبار حساسية معزولات الاستافيلوكوكس اوريس للميثيسيلين والفانكومايسين بشكل خاص؛ حيث ثبت أن معظم المعزولات كانت حساسة للمضادات الحيوية المستخدمة بدرجات متفاوتة، حيث سجلت مستويات مقاومة ضد البيتا لاكتام والتتراسيكلين أعلى من المضادات الحيوية الأخرى المستخدمة. كما تم اختبار بعض المعزولات لوجود بعض جينات الضراوة وجينات المقاومة للمضادات الحيوية جزيئيًا حيث ثبت إيجابية وجود تلك الجينات في المعوزولات التي تم فحصها مما يثبت امكانية ان تكون اسماك المياه المالحة قد تكون أحد مسببات الامراض البكتيرية المنقولة عن طريق الغذاء خاصة تلك المقاوم للمضادات الحيوية، في حين أنها قد تكون محملة ببكتيريا أخرى مختلفة منقولة بالغذاء أثناء التخزين والتداول.

الكلمات الدالة: مقاومة المضادات الحيوية، الجودة الصحية، اسماك المياه المالحة.