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Antibiotic Resistance Profiles of the Bacteria Isolated from some Finfish Species in Iskenderun Bay, (Northeastern Mediterranean Sea), Turkey

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ABSTRACT

This study was conducted on the microbial flora isolated from gills and intestine tissues of commercially important finfish species: saddled seabream (*Oblada melanura*), gilthead sea bream (*Sparus aurata*), Bogue (*Boops boops*), horse mackerel (*Trachurus mediterraneus)*, red mullet (*Mullus barbatus*), brushtooth lizardfish (*Saurida undosquamis*), grey mullet (*Mugil cephalus*), threadfin bream, *(Nemipterus randalli*), which were caught from the Iskenderun Bay at 6 different locations (local fishermen's fish market) were evaluated. The *Serratia* spp., *Enterobacter aerogenes, Klebsiella pneumoniae,* and *Enterobacter cloacae* were isolated from the gill samples, while *E. aerogenes, E. cloacae, Proteus vulgaris, K. pneumoniae* and *Pseudomonas fluorescens* were isolated from the fish intestine samples. *E. aerogenes* (68.4%), *Serratia* spp. (53.3%), *E. cloacae* (50%) and *K. pneumoniae* (44.4%) showed high level of resistance against ampicillin. *K. pneumoniae* also showed high resistance against Cefepime (52.9%) and Ciprofloxacin (47.1%). *Pseudomonas* species exhibited high level of resistance against Cephalosporin.

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Authors' Contribution
YBY designed the study, supervised the work and wrote the article. YBY and HTV collected samples and performed the laboratory experiments.

Key words
Antibiotic resistance, Bacteria, Gill, Intestine, Marine fish, Bay.

INTRODUCTION

The community of the microorganisms on the fish skins, gills, and intestinal microflora varies according to fish species, and to the pollution levels of the fishing area (Cooke, 1976; Austin and Austin, 1987; Diler and Diler, 1995; Robertson *et al*., 2000; Olafsen, 2001; Terzi, 2006; Kaynar and Beyatli, 2006; Korun and Toprak, 2010).

The use of antimicrobials as growth promoters in animal husbandry has been linked to certain antimicrobial resistance patterns among human bacterial pathogens, suggesting that there is a possible flow of antimicrobial resistance genes between animal and human pathogens. Potential transfer of resistant bacteria and resistance genes from environments to humans may occur through direct consumption of antimicrobial-resistant bacteria present in fish and associated products (Bager *et al*., 1997; Wegener *et al*., 1999; Schmidt *et al*., 2000; Petersen *et al*., 2002; Vivekanandhan *et al*., 2002; Gil *et al*., 2004; Akkan, 2009).

Iskenderun Bay is located in the northeastern part of the Mediterranean Sea. There are some industrial pollution sources such as iron, steel, fertilizer, refineries, and the thermal power station in the Iskenderun Bay (Matyar *et al*., 2009). It is known that the bay commercially important trade harbor and fisheries area. For this reason, there are many studies on pollution in this region. The Iskenderun Bay is polluted by iron and steel processing and production, a fertilizer factory, a refinery, a coal-fired power plant, and the wreck of the M/V Ulla carrying coal-fired power plant ash, all of which discharge a lot of amount of processed or unprocessed wastes into the Bay. In addition, domestic wastes, including hospital wastes are released into the Bay (Yılmaz *et al*., 1992; Doygun and Alphan, 2006; Türkmen *et al*., 2008; Dural *et al*., 2010).

In this study, the microbial flora isolated from gills and intestine tissues of some finfish species; saddled seabream (*Oblada melanura)*, gilthead sea bream (*Sparus aurata)*, bogue (*Boops boops*), horse mackerel (*Trachurus mediterraneus)*, red mullet (*Mullus barbatus)*, brushtooth lizardfish (*Saurida undosquamis)*, grey mullet (*Mugil cephalus*), threadfin bream (*Nemipterus randalli*) which caught from Iskenderun Bay from 6 different locations/ local fish market were evaluated. The resistance patterns of the isolates were determined using disk diffusion method by taking the Clinical and Laboratory Standards Institute criteria as the basis (CLSI, 2005). For Gram-negative bacteria, we used erythromycin, ampicillin, gentamycin, ciprofloxacin, tetracycline, streptomycin, cefoperazone, and cefuroxime.

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MATERIALS AND METHODS

Study area and sample collection

The fish samples were obtained from 6 stations (local fishermen's fish market), Arsuz, Karaağaç, Iskenderun Port, Isdemir, Dörtyol, and Yumurtalık to represent the Iskenderun Bay (Fig. 1).

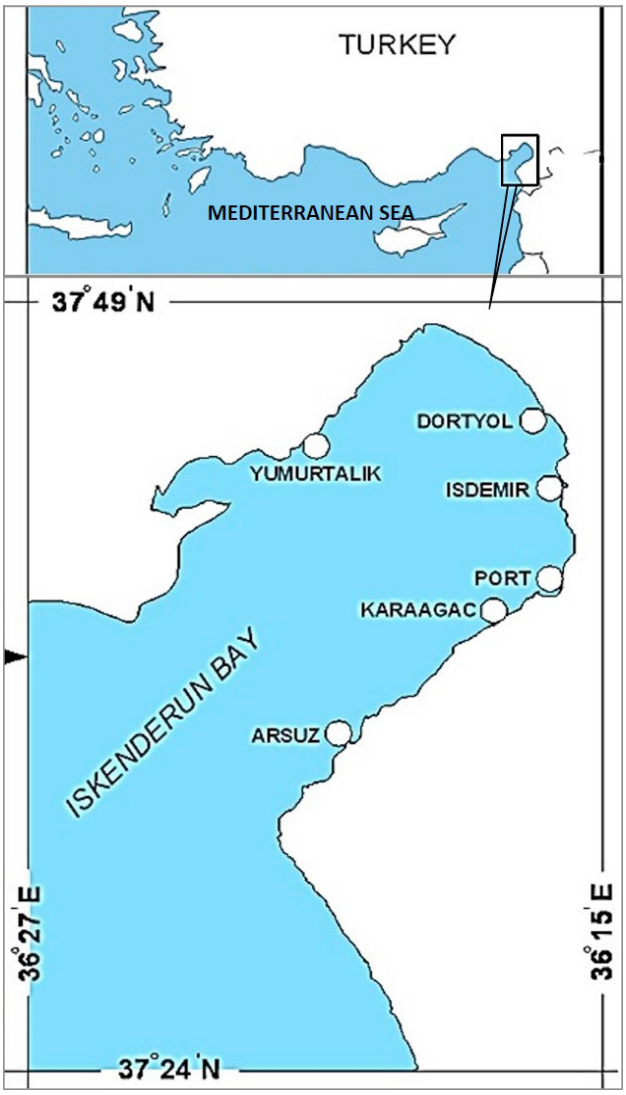

Fig. 1. Sampling area and local fish markets in Iskenderun Bay.

The captured and newly landed (fresh fish) 8 different finfish species were sampled. Sampled fish (*Oblada melanura, Sparus aurata, Boops boops, Trachurus mediterraneus, Mullus barbatus, Saurida undosquamis, Mugil cephalus,* and *Nemipterus randalli*) were collected individually in sterilized polyethylene bags, and they were transported to the laboratory in an icebox. All fish were examined for external and internal signs for health assessment. Processing and inoculation of samples for bacteriological analysis were completed within 2–4 h. Aseptic procedures were strictly followed during collection, transportation, and analysis (AOAC, 1975).

Isolation and identification

Gill epithelium of fish was aseptically swabbed using sterile cotton buds, inoculated into 5 ml Mueller-Hinton Broth medium. After 72 h at 37°C, samples from the medium were streaked on sheep blood agar, Mueller-Hinton agar, Eosin Methylene Blue (EMB) agar, and Sabouraud dextrose (SDA) agar. Preliminary identification of strains obtained in pure culture was based on Gram staining and the typology process was performed by assessing the samples with catalase, coagulase, sucrose use (glucose or saccharose), indole test, methyl red test, Voges-Proskauver test, and using the citrate as carbon source, urease activity regarding biochemical and physical properties.

To assess the intestine samples taken from the fish, the contents of the intestines were taken into sterile Petri dishes, and 0.1 g sample was weighed, and serially diluted. 100 µl was taken from each dilution and plated on sheep blood agar, EMB, and SDA broth medium. The plates obtained from each dilution were left for incubation both in aerobe and anaerobe media. The plates were incubated at 37°C for 72 h. After the incubation Gram staining from colonies, and bacterial identification processes were performed for Gram-negative bacteria with oxidase and IMVIC tests. "I" is for indole; "M" is for methyl red; "V" is for Voges-Proskauer, and "C" is for citrate, lowercase "i" is added for ease of pronunciation. IMVIC is an acronym that stands for four different tests; Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test to obtain the results of these four tests, three test tubes are inoculated: tryptone broth (indole test), methyl red/Voges-Proskauer broth (MR-VP broth), and citrate. Vitek - 2 Automatized Culture Systems (Biomerieux, France) were used for bacterial identification when needed. *C. albicans*, non-albicans distinction was made after the germination test and identification of the yeasts.

Susceptibility testing

The resistance patterns of the Gram-negative isolates for erythromycin, ampicillin, gentamycin, ciprofloxacin, tetracycline, streptomycin, cefoperazone, cefuroxime were determined with disk diffusion method by taking the Clinical and Laboratory Standards Institute (CLSI) criteria as the basis. The minimum inhibition concentration was determined by the disk diffusion method in the Mueller-Hinton medium. This method allows for the determination

of total levels of antibiotic resistance from typical colonies, thereby detecting the most resist phenotypes present and the results were separately interpreted by using the breakpoints from the CLSI guidelines (CLSI, 2005).

RESULTS AND DISCUSSION

Distribution of the Gram-negative bacteria isolated from the gill samples was examined. It was observed that 49.7% of the samples had *E. aerogenes*, 43.6% had *K. pneumoniae*, and 41% had *Serratia* spp. The microorganisms isolated the most rarely from the samples of the gills were *Y. ruckeri* at a rate of 7.7%, and *V. alginolyticus* at a rate of 5.1%. The percentage of Gram-negative bacteria is given in Table I. Gram-negative bacteria were isolated from intestine samples as *E. aerogenes* (59%), *E. cloacae* (48.7%), *P. vulgaris* (46.2%), *K. pneumoniae* (38.5%), *E. tarda* (7.7%) and *Y. ruckeri* (5.1%) (Table I).

The most frequently isolated *Serratia* type microorganisms from gill samples were *E. aerogenes, K. pneumoniae, E. cloacae,* and their antibiotic resistance patterns were examined. When the antibiotic resistance patterns were examined, no imipenem and meropenem resistance were observed in these microorganisms isolated from gill samples; and the amikacin and gentamicin resistance in these bacteria have been determined to be extremely low. High ampicillin resistance was observed especially in *E. aerogenes* types (68.4%), in *Serratia* spp. (53.3%), *E. cloacae* (50%) and *K. pneumoniae* (44.4%).

Table I.- The percentage distribution of the number of the Gram-negative bacteria from gills and intestine.

Bacteria isolated from gill samples	n=39	%	Bacteria isolated from intestine samples	n=39	%
Enterobacter aerogenes	19	49.7	*Enterobacter aerogenes*	23	59.0
Klebsiella pneumoniae	17	43.6	*Proteus vulgaris*	19	48.7
Serratia spp.	16	41.0	*Klebsiella pneumoniae*	18	46.2
Enterobacter cloacae	14	35.9	*Vibrio alginolyticus*	15	38.5
Serratia marcescens	14	35.9	*Pseudomonas fluorescens*	12	30.8
Escherichia coli	11	28.2	*Klebsiella oxytoca*	12	30.8
Shigella sonnei	11	28.2	*Pseudomonas* spp.	10	25.6
Citrobacter freundii	10	25.6	*Aeromonas salmonicida*	9	23.1
Klebsiella oxytoca	9	23.1	*Citrobacter freundii*	9	23.1
Edwardsiella tarda	9	23.1	*Serratia marcescens*	9	23.1
Proteus mirabilis	8	20.5	*Esherichia coli*	8	20.5
Pseudomonas spp.	6	15.4	*Shigella* spp.	7	17.9
Proteus vulgaris	5	12.8	*Serratia* spp.	6	15.4
Shigella spp.	4	10.3	*Edwardsiella tarda*	4	10.3
Yersinia ruckeri	3	7.7	*Yersinia ruckeri*	3	7.7
Vibrio anguillarium	2	5.1	*Vibrio anguillarum*	2	5.1

Table II.- Antibiotic resistance patterns of Gram-negative bacteria from intestine samples*.

Antibiotics	*E. aerogenes* (n=23)		*E. cloacae* (n=19)		*P. vulgaris* (n=18)		*K. pneumoniae* (n=15)		*P. fluorescens* (n=12)	
	n	%	n	%	n	%	n	%	n	%
Imipenem	0	0	0	0	0	0	0	0	0	0
Meropenem	1	4.3	0	0	0	0	1	6.7	2	16.7
Amikacin	1	4.3	0	0	1	5.6	2	13.3	1	8.3
Ciprofloxacin	7	30.4	3	15.8	11	61.1	6	40	7	58.3
Ampicilin	14	60.9	14	73.7	11	61.1	9	60	8	66.7
Piperacillin	11	47.8	7	36.8	6	33.3	5	33.3	9	75
Gentamicin	4	17.4	3	15.8	4	22.2	6	40	1	8.3
Cefepime	7	30.4	6	31.6	3	16.7	5	33.3	5	58.3
Ceftazidime	8	34.8	6	31.6	2	11.1	5	33.3	7	41.7

E, *Enterobacter*; P, *Proteus*; K, *Klebsiella*; P, *Pseudomonas*.

Similarly, high piperacillin resistance was also observed in these microorganisms. Aside from these, high resistance was observed in *K. pneumoniae* types to cefepime (52.9%) and ciprofloxacin (47.1%). Except for the microorganisms isolated at the most frequent level from gill samples, the antibiotic resistance patterns were examined for *E. aerogenes, E. cloacae, P. vulgaris, K. pneumoniae* and *P. fluorescens* isolated at a high rate from the intestine samples (Table II).

The imipenem resistance was not observed like in the Gram-negative bacteria in the gill samples. However, meropenem-resistant origins were detected in one of the *E. aerogenes* isolated from intestinal bacteria (4.3%) in one of the *K. pneumoniae* samples (6.7%), and in two of the *P. fluorescens* samples (16.7%). The imipenem and meropenem resistance of the bacteria was found as the lowest levels compared to the amikacin resistance microorganisms.

The high ampicillin resistance was observed among these bacteria that were isolated from the gills especially in *E. cloacae* (73.7%), *P. fluorescens* (66.7%), and *P. vulgaris* (61.1%) (Table III). Aside from these, ciprofloxacin resistance was determined in *Proteus vulgaris* at a rate of 61.1%, and cefepime resistance in *P. fluorescens* origins with a rate of 58.3% and ceftazidime resistance at a rate of 41.7%.

In our study, the ampicillin resistance was 73.7% in *E. cloacae*. However, Goñi-Urriza *et al.* (2000) reported that common resistance to tetracycline (24%) and β-lactam (20.5%) in Enterobacteriaceae and 27.5% tetracycline resistance was determined in *Aeromonas* which were isolated from the water samples in Arga River (Spain).

Akşit and Kum (2007) determined the bacterial disease agent in rainbow trout and investigated the sensitivity levels of the fish for various antibacterial medications. They found that all of the species isolated have sensitivity for enrofloxacin, fluorphenikol and ciprofloxacin; and also for amoxicillin, ampicillin, bacitracin, erythromycin, fucidic acid, gentamicin, chloramphenicol, lincomycin, nalidixic acid, neomycin, novobiocin, oxytetracycline, cefoxitin and sulfamethoxazole-trimethoprim at varying levels. In our study, the antibiotic resistances among Gram-negative bacteria isolated from gill samples at high resistance rates were detected especially for the ampicillin, piperacillin, and cephalosporin-group antibiotics and the meropenem, cefepime, and ceftazidime.

Savaşan *et al.* (2008) isolated 26 *E. faecalis* strains from marine fish in Agean Sea. They examined the sensitivity of *E. faecalis* strains to ciprofloxacin, erythromycin, tetracycline, streptomycin, ampicillin, gentamycin, penicillin, and vancomycin. They reported that resistant strains were commonly found in fish for streptomycin, gentamicin, and ciprofloxacin and also low level for vancomycin. In the present study, the imipenem and meropenem resistance being not detected among the Gram-negative bacteria isolated from the gill samples, and the amikacin resistance and gentamycin resistance being found at low levels.

E. aerogenes (%59) and *E. cloacae* (48.7%) were the most frequently isolated microorganisms in the present study. Matyar *et al.* (2009) determined the microbial diversity, antibiotic resistance pattern levels, and the heavy metal resistance distribution of the bacterial isolates in Eastern Mediterranean coastline and reported that the most frequent strains isolated from all samples were *Citrobacter koseri* (9.0 %), *Escherichia coli* (8.2 %) and *Pantoea agglomerans* (8.2%). In our examination, the detection of antibiotic resistance in Gram-negative bacteria isolated from 8 different finfish samples. The water/marine pollution may be defined as damage to the ecosystem it disrupts directly to the aquatic life/animal health and indirectly to the terrestrial animal/human health. Environmental health is one of the branches of public health that is concerned with all aspects of the natural and built environment that may affect animal/human health.

Table III.- Antibiotic resistance patterns of Gram-negative bacteria from gill samples*.

Antibiotics	*Serratia marcescens* + *Serratia* spp. (n=30)		*E. aerogenes* (n=19)		*K. pneumoniae* (n=17)		*E. cloacae* (n=14)	
	n	%	n	%	n	%	n	%
Imipenem	0	0	0	0	0	0	0	0
Meropenem	0	0	0	0	0	0	0	0
Amikacin	2	6.7	1	5.3	1	5.9	2	14.3
Gentamicin	3	10	4	21.1	2	11.8	2	14.3
Ciprofloxacin	6	20	5	26.3	8	47.1	6	42.9
Ampicilin	16	53.3	13	68.4	8	44.4	7	50
Piperacillin	13	43.3	11	57.9	9	52.9	8	57.1
Cefepime	11	36.7	7	36.8	9	52.9	6	42.9
Ceftazidime	7	23.3	6	31.6	5	29.4	4	28.6

*E, *Enterobacter*; K, *Klebsiella*.

Many of the available treatment options for common bacterial infections are becoming more and more ineffective. As a consequence, there are situations where infected human and animal cannot be treated adequately by any of the available antibiotics. This resistance may delay and hinder treatment, resulting in complications or even death. A recent WHO report made a clear case that resistance of common bacteria to antibiotics has reached alarming levels in many parts of the world. In Europe, for example, there is an increase of the resistance to major antibiotics of common bacteria such as *E. coli* which causes, among others, urinary tract infections, and also *K. pneumoniae* and *Pseudomonas aeruginosa*. For WHO, the consequence is that progress in modern medicine, which relies on the availability of effective antibacterial drugs, is now at risk (WHO, 2001, 2014; CDC, 2013; Davies and Davies, 2010).

In this study the bacteria isolated from gill samples *E. aerogenes*, *K. pneumoniae* and *Serratia* spp. were found at high prevalence levels as 49.7% 43.6% and 41.0%, respectively, and also *E. aerogenes*, *P. vulgaris* and *K. pneumoniae* (59.0%, 48.7%, and 46.2%) were detected as a higher percentage of the intestine samples. Therefore, the antibiotic resistance microorganisms should be considered to environmental health and food security/safety. Similar studies must become routine and conducted at certain intervals, and the bacteria in the fish must be determined, and the antibiotic resistance profiles must be revealed and discussed and shared with the public.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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