ISOLATION, IDENTIFICATION AND ANTIBACTERIAL AGENTS RESISTANCE AMONG ENTEROBACTERIACEAE SPP. IN FISH OF THE EASTERN MEDITERRANEAN

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Abstract

The aim of the present study was to determine the level of antibiotic resistance patterns of bacterial isolates from Eastern Mediterranean fish in Turkey. The resistance of 93 Enterobacteriaceae isolates from gills to 10 different antibiotics belonging 7 classes (which most using in Turkey), was investigated by agar diffusion method. A total 13 species of bacteria were isolated: the most common strains were Enterobacter cloacae (49.5%) following by Klebsiella oxytoca (15.1%). Fish may contaminate with discharge of urban or hospital sewages. There was a high incidence of resistance to ampicillin (66.7%), and cefazolin (47.3%), and a low incidence of resistance to kanamycin (1.1%) and cefepime (1.1%). Results show that Eastern Mediterranean fish have significant proportion of antibiotic resistant Gram-negative bacteria, and these bacteria constitute a potential risk for fish population and public health. At the same time, antibiotic resistant pathogens in the aquatic environments may play role as a reservoir for resistance genes.

Keywords: Mediterranean, fish, antibiotic resistance, public health.

Introduction

Bacteria which are procaryotic, existed in single cells or chains of cells found in almost every environment. The *Enterobacteriaceae* are a large family of Gram-negative bacteria that includes along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella* and *Shigella*. Other disease-causing bacteria in this family include *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter*. Members of *Enterobacteriaceae* are usually found in the gastrointestinal tract of humans and animals, and certain strains are primary or opportunistic pathogens (Linton & Hinton, 1988). Antibiotic resistance in pathogen bacteria is a global problem for public health all over the world. Widespread of antibiotic usage for treatment of infectious diseases prompting an extensive spread of multidrug-resistant bacteria in various environments including the aquatic environment (Young, 1993). In developing countries, antibiotics are extensively applied in aquaculture. The use of antibiotics is the most important factor amplifying the incidence of resistance in pathogen bacteria. In recent years environmental pollution effects to human health direct or indirectly. One of the changes in the microbial flora of the fish, effect the human that use it nutrition source. Contamination of the sea rapidly and irreversibly affect the fish population.

The present study was performed to isolation and identification bacteria from gills of East Mediterranean fish and to determine antibiotic resistance patterns of bacterial isolates to antibacterials most used in Turkey.

Materials and Methods

Five different fish species (Sea bream, *Sparus aurata*; Red mullet, *Mullus barbatus*; Smelt, *Atherina boyeri*; Sardines, *Sardina pilchardu*; Sand steenbras, *Lithognathus mormyrus*) was catch from Iskenderun Bay (East Mediterranean). Samples were packed in sterile bags, placed on ice, immediately transported to the laboratory and processed within 4 hours after collection. Fish were externally washed with sterilized water to reduce potential contamination with skin bacteria. Gills were aseptically removed and placed on sterile petri dishes, and grinded by hand using sterile mortar. For the recovery *Enterobacteriaceae*, were made by the spread plate method using MacConkey agar (Merck). Bacterial identificaion was performed using the Becton and Dickinson Crystal E/NF ID system (BBL, MD, USA). These strains were identified using the E/NF identification software (BBL, MD, USA). Antibiotic resistance was determined by an agar diffusion test (NCCLS (1997) using Mueller-Hinton agar (Difco). Ten different antibiotics (representing seven classes) were used.

The antibiotics tested and their sensidisk concentrations were ampicillin (AM; 10 μg) kanamycin (K; 30 μg), imipenem (IPM; 10 μg), cefazolin (CZ; 30 μg), ceftizoxime (ZOX; 30 μg), cefuroxime (CXM; 30 μg), cefepime (FEP; 30 μg), chloramphenicol (C; 30 μg), tetracycline (TE; 30 μg) and trimethoprim–sulphamethoxazole (SXT; 1.25 and 23.75 μg).

Reference strains of *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, as recommended by NCCLS (1997), were used as control organisms for verification of the anti-bacterial effect of the discs on Mueller–Hinton agar plates.

Results and Discussion

A total of 93 bacterial isolates were obtained, representing 11 genus of *Enterobacteriaceae* strains from 5 different species of fish gills (Table 1). In this study, *Enterobacter cloacae* (49.5%) was the most frequently detected microorganism. Like most members of the family *Enterobacteriaceae*, *Enterobacter cloacae* is capable of causing opportunistic infections in hospitalized or debilitated patients (Flynn et al., 1987). The second most prevalent microorganism was *Klebsiella oxytoca* (15.1%), a ubiquitous inhabitant of hospital sewage environments. *Klebsiella oxytoca* is important as an infectious agent. Most infections of *K. oxytoca* are nosocomial, spreading via the hands of medical staff. Outbreaks occur in patients with immunodeficient diseases and patients whom are being treated with antibiotics. Outbreaks have also been reported occurring in both prenatal and neonatal infants (Berkowitz & Umeh, 2011*).* The third most prevalent species was *Proteus vulgaris* (11.8%) has been considered to be an important cause of nosocomial infections. *Vibrio metscchnikovii* was fourth most isolated bacterium in this study (3.2%). Vibrios are indigenous bacteria of the sea water. *Vibrio metschnikovii* is widely found in aquatic environments. Among the vibrios *V. parahaemolyticus* is a pathogen causing acute gastroenterisis. 50-70% of all foodborne gastroenteritis due to consumption of raw or cooked seafood in Japan is produced by this pathogen (Farmer et al., 2003). Marine vibrios have long been recognized as important reservoirs and vehicles of antibiotic resistance because of their importance as potential human and/or marine animal pathogens (Thompson et al., 2004), their abundance and diversity in coastal waters (Thompson et al., 2005), their ability to readily develop and acquire antibiotic resistance in response to selective pressure, and their ability to spread resistance by horizontal genetic material exchange (Aoki, 2000).

Table 1. Bacterial species isolated from fish gills

	Gills	
Microorganisms	No	%
Citrobacter freundii	2	2.2
Enterobacter cloacae	46	49,5
Klebsiella oxytoca	14	15.1
Klebsiella pneumoniae ssp. pneumoniae	3	3.2
Kluyvera cryocrescens	3	3.2
Morgenalla morganii	2	2.2
Pantoea agglomerans	3	3.2
Proteus vulgaris	11	11.8
Providencia rustigianii	3	3.2
Serratia odorifera	1	1.1
Shigella dysenteria	1	1.1
Shigella sp.	1	1.1
Vibrio metschnikovii	3	3.2
Total	93	100

Among the isolates, a high percentage of bacteria were resistant to ampicillin (66.7%), and cefazolin (47.3%), whereas a low percentage of bacteria were resistant to kanamycin (1.1%), and cefepime (1.1%). It was found no resistance to imipenem, and ceftizoxime (Table 2). The high degree of resistance to ampicillin in the present study was similar to the findings of Vaseeharan et al., (2005). Extensive use and misuse of several antibiotics in medication, veterinary, agriculture, and aquaculture have caused antibiotic-resistant bacteria to be widespread in the environment, including aquatic systems (Kummere, 2004). Environmental bacteria may play an important role as reservoirs of antibiotic resistance, and resistance genes are exchanged by bacteria from different ecosystems (Roberts, 1996). Epidemic diseases caused by antibiotic-resistant bacteria have been reported all over the world. More than 10,000 deaths caused by cholera were reported to the World Health Organization in 1998, and antibiotic-resistant Vibrio cholera strains have been reported frequently (Dalsgaard et al., 1999).

Multiple antibiotic-resistant pathogen bacteria may pose a particular threat in coastal areas.. In this research significant amounth of bacteria, that important for human health isolated from the fish. The presence, antibiotic resistance of *Enterobacteriaceae* in North-east of Mediterranean fish were investigated. These results suggests that North-east of Mediterranean fish are reservoir for antibiotic resistant *Enterobacteriaceae* strains.

Table 2. Antibacterial resistance among *Enterobacteriaceae* isolated from northeast-Mediterranean fish

Classes of antibiotics	Antibiotics	Source
		Gills n:93 (%)
Penicillins	Ampicillin (AM,10µg)	66.7
Aminoglycosides	Kanamycin (K, 30µg)	1.1
Carbapenems	Imipenem (IPM, 10µg)	-
Cephalosporins	Cefazolin (CZ, 30µg)	47.3
	Ceftizoxime (ZOX, 30µg)	-
	Cefuroxime (CXM, 30µg)	6.5
	Cefepime (FEP, 30µg)	1.1
Chloramphenicol	Chloramphenicol (C, 30µg)	3.2
Tetracyclines	Tetracycline (TE, 30µg)	12.9
Trimethoprim-sulphamethoxazole	Trimethoprim-sulphamethoxazole (SXT, 1.25 and 23.75µg)	3.2

McIntosh et al., (2008) have found an important incidence of R-plasmids in multiple antibiotic and mercury resistant *Aeromonas* isolates from Atlantic salmon. The study of Rhodes et al., (2000) provided evidence that related tetracycline-resistance encoding plasmids have been transferred between different *Aeromonas* species and *E. coli* and between the hospital and aquaculture environments in distinct geographical locations.

Fish are usually consumed after being cooked in Turkey, and therefore, fish may be a low risk food, even if contaminated with enteric bacteria species. However in recent years, the trend of consuming ready to eat raw seafood for example mussels, shrimps and fish in public places is getting popular and thus, there is always the possibility of cross-contamination at processing food preparation and service steps. Occurrence of foodborne infections due to antibiotic resistant enteric bacteria has been reported in a study in Turkey (Temelli, 2002). The presence of high numbers of antibiotic-resistant bacteria in gills of fish may have ecological and public health implications.

Further works are needed to understand better antibiotic resistant *Enterobacteriaceae* strains. Along with other parts of the other country coasts on the Mediterranean and Aegean Seas.

Biography

Mr. Fatih Matyar

He completed the bachelor in Biology at Cukurova University. He started his academic career as a research assistant at 1996 at the faculty of Science and Arts at Cukurova University. He completed his PhD in microbiology at 2002 at Cukurova University. He is currently working as an associated professor at the Education Faculty, Cukurova University. Dr Matyar has extensive research experience having successfully led and completed several research projects on a wide range of subject.

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References

Aoki, T. 2000. Transferable drug resistance plasmids in fish pathogenic bacteria. In: Arthur JR, Lavilla-Pitogo CR, Subasinghe RP (eds) *Use of chemicals in aquaculture in Asia. SEAFDEC Aquaculture Department* Tigbauan, Iloilo, Philippines, pp 31–33.

Berkowitz, L.B. & Umeh, O. 2011. Klebsiella Infections. Medicine Specialties. *Infectious diseases. Medical Topics.*

Dalsgaard, A., Forslund, A., Sandvang, D., Arntzen, L. & Keddy, K. 2001. *Vibrio cholerae* O1 outbreak isolates in Mozambique and South Africa in 1998 are multiple-drug resistant, contain the SXT element and the aadA2 gene located on class 1 integrons. *Journal of Antimicrobial and Chemotheraphy*, 48, pp. 827–838.

Farmer, J. J., Janda, M. & Birkhead, K. 2003. Vibrio, In: Murray, PR, Baron, EJ, Jorgensen JH, Pfaller MA, Yolken, R.H. (Eds.), *Manual of Clinical Microbiology, 8th edition. ASM Press*, Washington, D C., pp. 706–718.

Flynn, D. M., Weinstein, R. A., Nathan, C., Gaston, M. A. & Kabins, S.A. 1987. Patients' endogenous flora as a source of "nosocomial" *Enterobacter* in cardiac surgery. *Journal of Infectious Diseases*, 156, pp. 363–368.

Kummere. K. (2004). Resistance in the environment *Journal of Antimicrobial and Chemotheraphy*, 54, pp. 311–320.

Linton, A.H. & Hinton, M.H. 1988. *Enterobacteriaceae* associated with animals in health and disease. *Journal of Applied Bacteriology Symposium Supplement*, 17, pp. 71–85

McIntosh, D., Cunningham, M., Ji, B., Fekete, F. A., Parry, E. M., Clark, S. E., Zalinger, Z. B., Johnson, K. A., Beattie, M. &Ritchie, R. 2008. Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of *Aeromonas salmonicida* subsp. *salmonicida* is associated with carriage of an IncA/C plasmid similar to the *Salmonella enterica* plasmid pSN254. *Journal of Antimicrobial Chemotherapy, 61*, pp. 1221–1228.

NCCLS - National Committee for Clinical Laboratory Standards. ApprovedStandards M2-A6., (1997). *Performance Standards for Antimicrobial Disk Susceptibility Tests, 6th edn., NCCLS., Wayne, Pennsylvania*, USA.

Rhodes, G., Huys, G., Swings, J., McGann, P., Hiney, M., Smith, P. & Pickup, R.W., 2000,. Distribution of oxytetracyline resistance plasmids between aeromonads in hospital and aquaculture environments: Implication of Tn1721 in dissemination of the tetracycline resistance determinant TetA. *Applied and Environmental Microbiology,66*, pp. 3883–3890.

Temelli, S., 2002. Food poisoning agent *Escherichia coli* O157:H7 and its importance. *Journal of the Faculty of Veterinary. Medicine*, 21, pp. 133-138.

Roberts, M. C., 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiology Reviews*, 19, pp. 1–24.

Thompson, F. L., Iida, T. & Swings, J., 2004. Biodiversity of vibrios. *Microbiology and Molecular Biology Reviews*, 68, pp. 3403–3431.

Thompson, J. R., Pacocha, S., Pharin,o C., Klepac-Ceraj, V., Hunt, D. E., Benoit, J., Sarma-Rupavtarm, R., Distel, D. L. & Polz, M .F., 2005. Genotypic diversity within a natural coastal bacterioplankton population. *Science*, 307, pp. 1311–1313.

Vaseeharan, B., Ramasamy, P., Murugan, T. & Chen, J. C., 2005. In vitro susceptibility of antibiotics against Vibrio spp. and Aeromonas spp. isolated from Penaeus monodon hatcheries and ponds. *International Journal of Antimicrobial Agents*, 26, pp. 285–91.

Young, H. K., 1993. Antimicrobial resistance spread in aquatic environments. *Journal of Antimicrobial Chemotherapy*, 31, pp. 627-635.