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INVESTIGATION OF THE ANTIBIOTIC RESISTANCE *ESCHERICHIA COLI* FROM THE WATER AND FISH SAMPLES FROM MOGAN LAKE: AN INSIGHT FOR THE POSSIBLE HUMAIN HEALTH RISKS

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MOUHYADIN ABDOURAHMMAN HARBI

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March 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

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Master of Science in Biology

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March 2023

The use of antibiotics, important for human and animal health, is constantly increasing. It is observed that antibiotic resistance increases in pathogenic or non-pathogenic bacteria. In this study, the presence of antibiotic-resistant *E. coli* in the intestines of Mogan Lake water and fish living here and offered for consumption was investigated. Thus, the risks in terms of human health were evaluated. 8 waters in total; A total of 16 fish samples, 3 from each of 2 invasive species (*A. boyeri* and *P. parva*) and *C. carpio* (carp), *Esox lucius* (pike) and *T. tinca* (velvet) fish, were taken. Intestinal contents and water samples from fish were cultured, DNA of *E. coli* isolates was isolated, and then RT-PCR was performed. According to the PCR results, the presence of some genes encoding amoxicillin-clavulanic acid (*OXA1*) quinolone (*qnrA*) and colistin (*mcr1-5*) resistance was also detected in most of the *E. coli* detected samples. These results suggest that the aquatic environment may play an important role in the development of resistant antibiotics and the spread of resistance genes among bacteria.

2023, 48 pages

Keywords: Antibiotic resistance, *E. coli*, Human health risk

ÖZET

MOGAN GÖLÜ SU VE BALIK ÖRNEKLERİNDE ANTİBİYOTİĞE DİRENÇLİ *ESCHERICHIA COLI* ARAŞTIRILMASI: OLASI İNSAN SAĞLIĞI RİSKLERİ

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İnsan ve hayvan sağlığı için önemli olan antibiyotiklerin kullanımı sürekli artmaktadır. Sonuç olarak patojenik veya patojenik olmayan bakterilerde antibiyotik direncin de arttığı görülmektedir. Bu çalışma ile Mogan Gölü suyu ile burada yaşayan ve tüketime sunulan balıkların bağırsaklarında antibiyotiğe dirençli *E. coli* varlığı araştırıldı. Böylece insan sağlığı açısından riskler değerlendirildi. Toplamda 8 su; 2 istilacı tür (*A. boyeri* ve *P. parva*) ile *C. carpio* (sazan), *E. lucius* (turna) ve *T. tinca* (kadife) türü balıklardan 3'er örnek olmak üzere 16 balık örneği alındı. Balıklardan alınan bağırsak içerikleri ile su örnekleri kültüre alındı, *E. coli* izolatlarının DNA'sı izole edildi ve ardından RT-PCR yapıldı. PCR sonucuna göre, *E. coli* tespit edilen örneklerin çoğunda ayrıca amoksisilin-klavulanik asit (*OXA1*) kinolon (*qnrA*) ve kolistin (*mcr1-5*) direncini kodlayan bazı genlerin varlığı tespit edildi. Bu sonuçlar, sucul ortamın antibiyotiklere direnç geliştirilmesinde ve direnç genlerinin bakteriler arasında yayılmasında önemli bir rol oynayabileceğini göstermektedir.

2023, 48 sayfa

Anahtar Kelimeler: Antibiyotik direnci, *E. coli*, İnsan sağlığı riski

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Mouhyadin ABDOURAHMAN HARBI

Çankırı-2023

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LIST OF SYMBOLS

mg	Milligram
L	Liter
kb	Kilobase
g	Gram
µL	Microliter
µg	Microgram
°C	Degree celsius

LIST OF ABBREVIATIONS

AMOX	Amoxicillin
ARG	Antibiotic resistance genes
CLV	Clavulanic acid
DDD	Daily defined dose
ICE	Integrative and conjugative elements
IME	Integrative and mobilizable elements
MIC	Minimum inhibitory concentration
WHO	World health organization
WTP	Water treatment plants

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1. INTRODUCTION

The discovery of antibiotics at the beginning of the 20th century was a real revolution for the treatment of infectious diseases of bacterial origin. They represent one of the greatest advances in medicine of the 20th century and their applications are now important for human medicine and aquaculture, veterinary medicine and animal husbandry. Their discovery and use have reduced the mortality rate from diseases of bacterial origin. In addition to their use Antibiotics are used in non-therapeutic contexts (prophylaxis or as a growth agent) (Brown *et al.* 2017).

However, their massive use since the 1950s has generated a significant release of antibiotic residues into the environment, and into the aquatic environment. This is due to several causes: first, antibiotics are now overused in many areas. Then, they are not fully assimilated by the recipient organizations. Between 30 and 90% of the product (or its metabolites) is excreted through urine or feces and often reaches the aquatic environment, either through direct wastewater discharges, or through soil leaching, or through treatment plants. treatment plants (STEP) which do not allow the complete reduction of antibiotic residues (Tang *et al.* 2015).

The main source of dispersion of antibiotics in the natural environment is the wastewater discharged by treatment plants. They are frequently detected at quantities of ng/L or g/L. Thus, their unique chemical structures influence their biodegradability and their persistence in the environment. The necessity and need for antibiotics is indisputable. But their presence in the environment raises more and more concern. Especially since water security is currently one of the greatest environmental threats (Välitalo *et al.* 2017).

When assessing water quality, *Escherichia coli* from feces has always been the primary sign of fecal contamination. *E. coli* is the source of various diseases in humans and animals (Wen *et al.* 2020). These infections are mainly caused by several pathogenic strains, such as *E. coli* O157:H7. *E. coli* has also been shown to be an important

reservoir of genes encoding antimicrobial drug resistance, making it a useful biomarker of antimicrobial resistance in bacterial communities (Fresia *et al.* 2019). Since the antimicrobial resistance profiles of *E. coli* can be used as an indicator of global antimicrobial resistance and that the results directly affect public health threats, the search for these patterns has implications on many levels (Klein *et al.* 2019).

In addition to the chemical pollution caused by the very presence of the antibiotic in the natural environment, the problem lies in the proliferation of bacteria resistant to antibiotics and genes coding for a resistance mechanism. In recent decades, more and more scientists support the fact that antibiotics enter the environment through several channels. They breed resistance bacteria or genes that can spread and cause harmful effects in humans or animals. In 2016, the United Nations General Assembly sounded the alarm on the use of antibiotics and recognized that these were, among other things, the cause of the increase in antibiotic resistance (Van Boeckel *et al.* 2017). Antibiotics are emerging pollutants, which means that they are not or poorly regulated (Sharma *et al.* 2016).

The aim of this study is to investigate the presence of antibiotic-resistant *E. coli* in the intestines of fish grown in the same waters as the Mogan Lake water and offered for human consumption, and to determine which antibiotics it is resistant. Thus, it is the assessment of potential risks to human health

2. LITERATURE REVIEW

2.1 Antibiotics and Their Actions

Antibiotics are “substances, of natural or synthetic origin, used against infections caused by bacteria” (Ramalingam 2015). These natural agents can be modified by chemical methods (semi-synthetic antibiotics), other substances are completely artificial (sulfonamides, trimethoprim and quinolones). Antibiotics are used in human and veterinary medicine. Various other uses are made of it (crop protection, markers for genetically modified organisms) (Gothwal *et al.* 2015).

The variety of known antibiotic molecules makes it necessary to attempt a classification of molecules that considers the chemical structure in relation to bacterial activity. This classification evolves more or less quickly depending on the arrival of new molecules and the interest aroused by their therapeutic use. The main families of antibiotics are currently the following: beta-lactams, aminoglycosides, macrolides, fluoroquinolones, peptides, cyclins. Other classes exist but are quantitatively less important (Edet *et al.* 2017).

Antibiotics interact with bacteria at specific targets. For example, some agents will act on the synthesis of the cell wall, others inhibit the synthesis of nucleic acids, the synthesis of proteins. The selective toxicity of antibiotics, based on the difference in structure or metabolism between bacterial and animal cells, allows the least possible harm to the host (Esmatabadi *et al.* 2017).

As illustrated in Figure 2.1, each family of antibiotics has its own mechanism of action. The main families of antibiotics are described below. This is a non-exhaustive list; other families of antibiotics exist but are much less used and/or studied.

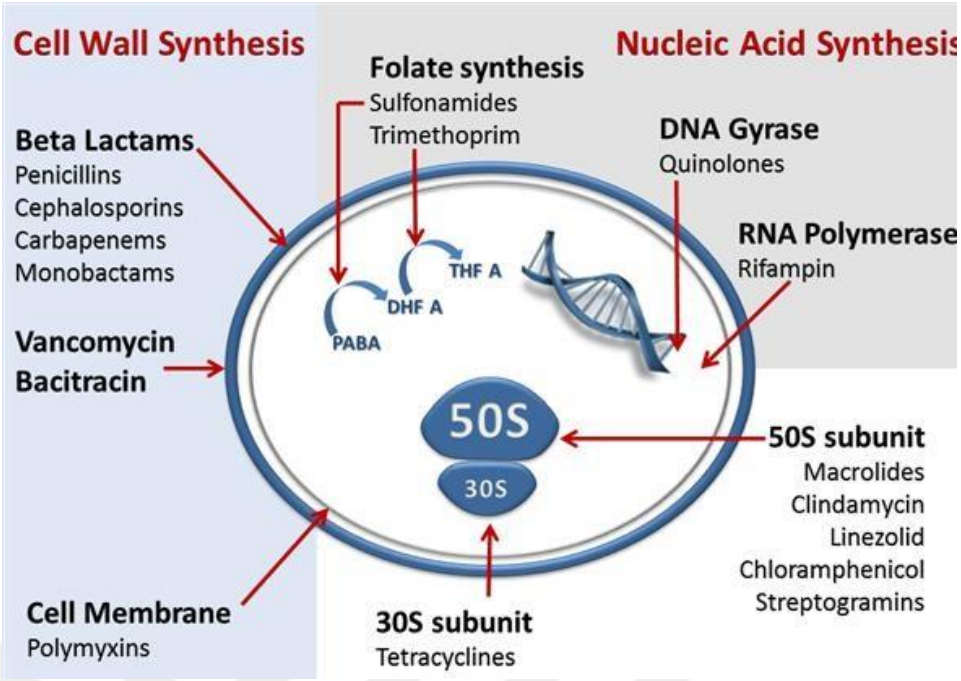


Figure 2.1 The action mechanisms of antibiotics (Garima Kapoor *et al.* 2017)

2.1.1 Sulfonamides

Sulfonamides are antibiotics that act as inhibitors of folic acid, a cofactor in the synthesis of purine and pyrimidine bases necessary for DNA synthesis. This leads to a decrease in DNA replication and therefore a decrease in bacterial multiplication. Sulfonamides are generally used in combination: the most common example is the combination of sulfamethoxazole with trimethoprim which forms co-trimoxazole. Trimethoprim comes from the diaminopyrimidine family, but it is used in combination with sulfamethoxazole (Grenni *et al.* 2018).

2.1.2 Quinolones/Fluoroquinolones

The quinolones (in particular oxolinic acid and flumequine) are said to be first generation and the fluoroquinolones (ciprofloxacin, norfloxacin, ofloxacin) second generation. Due to the development of antibiotic resistance to quinolones, these are practically no longer used. Fluoroquinolones are broad-spectrum antibiotics that act as nucleic acid (DNA) synthesis inhibitors by binding to major regulatory enzymes

(topoisomerase and DNA gyrase). Given their very broad treatment spectrum, the consumption of fluoroquinolones can lead to the emergence of resistance to all the bacteria that this family affects (Adriaenssens *et al.* 2021).

2.1.3 Tetracyclines

Tetracyclines are bacteriostatic antibiotics that act on ribosomes to inhibit protein synthesis. Without the possibility of protein synthesis, the multiplication of bacteria stops (Dmitriev *et al.* 2020).

2.1.4 Beta-lactams

Beta-lactams act as inhibitors of the synthesis of bacterial envelopes. They disrupt the synthesis of peptidoglycans, which leads to a weakening of the wall and an increased sensitization of the bacterium to external stresses. Beta-lactams are divided into two subgroups: penicillins and cephalosporins (Recacha *et al.* 2021).

2.1.5 Macrolides

Macrolides inhibit protein synthesis by binding to ribosomes. They are divided into two subgroups: clarithromycin, erythromycin, roxithromycin and clindamycin, lincomycin. The field of effectiveness of an antibiotic substance or antibiotic spectrum is either narrow (effectiveness of antibiotics on a limited variety of microorganisms) or broad (antibiotics attack many different types of pathogens) (Beckert *et al.* 2021).

2.2 Antibiotic Resistance

The definition of antibiotic resistance is still the subject of much discussion. Thus, antibiotic resistance is defined according to different points of view (Huijbers *et al.* 2019). According to the clinician, a strain is resistant to an antibiotic if the treatment is not effective. For the pharmacologist, a bacterial strain is resistant to an antibiotic if the

concentrations reached at the site of action are lower than the minimum inhibitory concentration (MIC). From the microbiologist's point of view, bacteria are said to be resistant if they have a resistance mechanism that increases the value of the MIC. Concerning the epidemiologist, a bacterial strain is resistant to an antibiotic if its MIC is significantly different from that of the normal population. Faced with this ambivalence in the definitions of resistance, the World Health Organization (WHO) has proposed two definitions of resistance. The first stipulates that a strain is said to be “resistant” when it supports a concentration of antibiotic notably higher than that which inhibits the development of most other strains of the same species. The second definition, based on pharmacological and clinical criteria, defines a resistant strain when the concentration of antibiotic that it can withstand is higher than the concentration that can be reached in vivo. There is natural resistance and acquired resistance (Balaban *et al.* 2019).

Natural antibiotic resistance exists in certain fungi or in certain bacteria that have learned to defend themselves against other bacteria. Resistance is transmitted from generation to generation through the bacterial chromosome. Natural resistance is a very stable phenomenon (Reygaert 2018).

Acquired resistance only concerns a few bacteria of the same species, it is less stable and spreads horizontally. It is a modification of the genetic capital of a particular bacterium, which allows it to be resistant to higher concentrations of a specific antibiotic. Acquired resistance has always existed since the use of antibiotics, but with the generalization and abusive and excessive use of these, the phenomenon has increased and strains resistant to multiple antibiotics are appearing. Acquired resistance is obtained in two ways: chromosomal and plasmid resistance (Landecker 2016).

2.3 Dissemination of Resistance Genes

Dissemination of antibiotic resistance genes is mainly due to bacterial conjugation and/or transposition. This last process will have a major role during horizontal transfers by transformation and transduction. The mutation may also be responsible for the emergence of antibiotic-resistant bacteria. Plasmid conjugation seems to be the main

mechanism involved in the dissemination of resistance in Gram-negative bacteria. This involves the unidirectional transfer of a copy of a so-called "conjugative" plasmid of close and specific cell contact between a recipient bacterium and a donor bacterium (Virolle *et al.* 2020). Although there are plasmids with a broad host spectrum, conjugative transfers between bacteria of the same species, or even of the same genus, have been more frequently observed. Many studies have determined frequencies of transfer of antibiotic resistance genes between bacteria in optimum laboratory conditions (from 10^{-1} to 10^{-3}) and in oligotrophic conditions (from 10^{-4} to 10^{-7}) close to those found in the environment. Transfers by conjugation seem to depend on the ratio between donor bacteria and recipient bacteria which, when it is high, increases the transfer probabilities, the presence of a carbon source and/or antibiotics which would favor the events of conjugation (Prensky *et al.* 2021).

The release of bacteria of faecal origin into the environment is accompanied by the dissemination of their genetic material during cell lysis. In the environment, DNA persistence times in water vary from a few hours to several months, depending on the methodology used (in situ studies, microcosms). In these environments, the concentration of extracellular bacterial DNA varies depending on enzymatic degradation, fragmentation and physical and chemical inactivation of DNA molecules. DNA from a strain of *E. coli* inoculated into soil was detected for up to 40 days, whereas after 15 days the strain was no longer cultivable (Abberton *et al.* 2016). The persistence of "naked" DNA in the environment indicates that it may be available for gene horizontals between allochthonous and autochthonous bacteria by transformation and thus participate in the dissemination of resistance genes. Transformation, a major mechanism in Gram-positive bacteria, allows the acquisition of a "naked" extracellular DNA molecule by a competent bacterium. During this state of competence demonstrated in *Neisseria gonorrhoeae* (Gram negative) and *Bacillus subtilis* (Gram positive), the bacteria develop permeability to DNA macromolecules using a type IV secretion system. To maintain itself, the single-stranded DNA that has entered the bacterium must be integrated into a replicon (plasmid, chromosome). Otherwise, this naked DNA fragment will be degraded by nucleases (Kittredge *et al.* 2022). Dissemination mechanism is given in Figure 2.2.

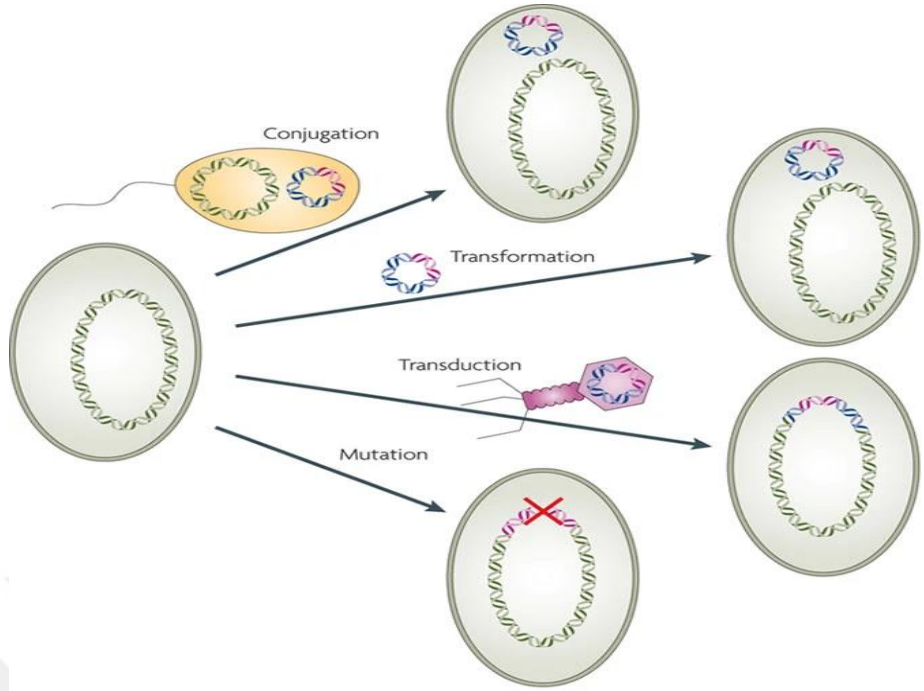


Figure 2.2 Mechanisms of dissemination of antibiotic resistance genes in bacteria (Andersson and Hughes 2010)

Transduction will allow the transfer of bacterial DNA via a bacteriophage from an infected bacterium to a recipient bacterium. This phenomenon requires specificity of the bacteriophage for the host bacterium. It can infect a bacterium and carrying out either a lytic cycle there which will lead either to cell lysis or to lysogeny with the insertion of the bacteriophage genome into the bacterial genome while waiting for optimal environmental conditions to complete its lytic cycle. The transfer of genes by this mechanism is the result of an encapsidation error in the head of the bacteriophage during the lytic cycle: generalized transduction. Few studies have been performed on this transfer mechanism. However, in the environment, frequencies of transfer of antibiotic resistance genes by transduction and transposition have been estimated between 10^{-9} and 10^{-7} (Torres-Barceló *et al.* 2018). This phenomenon, which is rarely described as being involved in the dissemination of antibiotic resistance genes, has however recently been demonstrated in the emergence of pathogenic strains producing a shiga toxin and responsible for hemolytic uremic syndrome (Smith *et al.* 2012).

The frequencies of transfer of antibiotic resistance genes by horizontal transfer and/or transposition remain low and require high cell densities to observe dissemination of antibiotic resistance genes. However, these densities could be reached in areas of accumulation of faecal bacteria which are added to indigenous communities, mudflats and periphytons (hot spots). Quantitative and metagenomic PCR approaches have observed an enrichment in antibiotic resistance genes in sediments from highly anthropized rivers (Kristiansson *et al.* 2011).

2.4 The Mobile Genetic Elements Involved in Antibiotic Resistance

All resistance mechanisms originating from a resistance gene can be transmitted vertically from one generation to another, but also horizontally thanks to external DNA integration mechanisms. These mechanisms are transformation mentioned above, during which bacteria integrate free DNA, transduction, which designates the entry of DNA into the cell thanks to a vector (a bacteriophage virus for example), and conjugation, during which the transfer takes place by contact between the bacteria. The genes concerned are transferred thanks to mobile genetic elements, among which may be cited transposons, plasmids and genomic islands. Phages can also serve as vectors for the transfer of antibiotic resistance genes. These elements can contain several resistance genes (ARG) and thus confer several resistances on the bacterium which expresses them. In the study of antibiotic resistance in bacterial communities of water systems, the transfer of resistance genes or resistant strains from one environment to another is a key element. The hospital environment and the water environment are two sources of contamination by which the resistance of strains can be enriched, either by the transfer of communities carrying resistances or by the transfer of genes from one community to the 'other. In this context, the resistances carried by plasmid and chromosomal genes must be taken into consideration (Durão *et al.* 2018).

2.4.1 Plasmids

Plasmids are non-chromosomal DNA fragments, the size of which can vary from a thousand to several million base pairs. They can carry several resistance genes and are

transmitted from one bacterium to another by a process of conjugation, that ‘is a horizontal transfer of genetic material by direct contact between bacterial cells. Conjugative plasmids have in their sequence the genetic elements necessary for their transfer, while mobilizable plasmids rely on the mechanisms of the host cell to carry out this transfer. The dissemination of plasmids containing antibiotic resistance genes is favored by the pressure exerted using antibiotics in human and animal health (Bennett 2008). The capacity of a plasmid to be transferred to a bacterium depends on its host spectrum, defined experimentally. In addition, the persistence potential of a plasmid in a bacterium depends on its incompatibility group, which defines the ability of different plasmids to cohabit in the same host cell. Thus, two plasmids from the same incompatibility group cannot coexist. Some plasmids are transferable to many hosts, such as the RK2 plasmid which seems to be compatible with most Gram-negative bacteria (Gruber *et al.* 2015).

2.4.2 Transposon

Transposable elements or transposons were discovered by Barbara McClintock during work on the chromosomes of maize cells, parts of which seemed to change position during cell division (McClintock 1953). These elements are DNA sequences capable of moving in the genome from one cell or from one cell to another, categorized in prokaryotic organisms into composite or non-composite transposon. Composite transposons consist of a gene coding for a transposase, framed by two inverted repeated sequences, and this trinomial called the insertion sequence itself frames the gene or genes that will be mobilized. This set constitutes a transposon. The transposase encoded by the genes of the insertion sequences is the enzyme which will catalyze the displacement of the transposon by separating it from the rest of the genome and integrating it at another location (Siguier *et al.* 2014) (Figure 2.3).

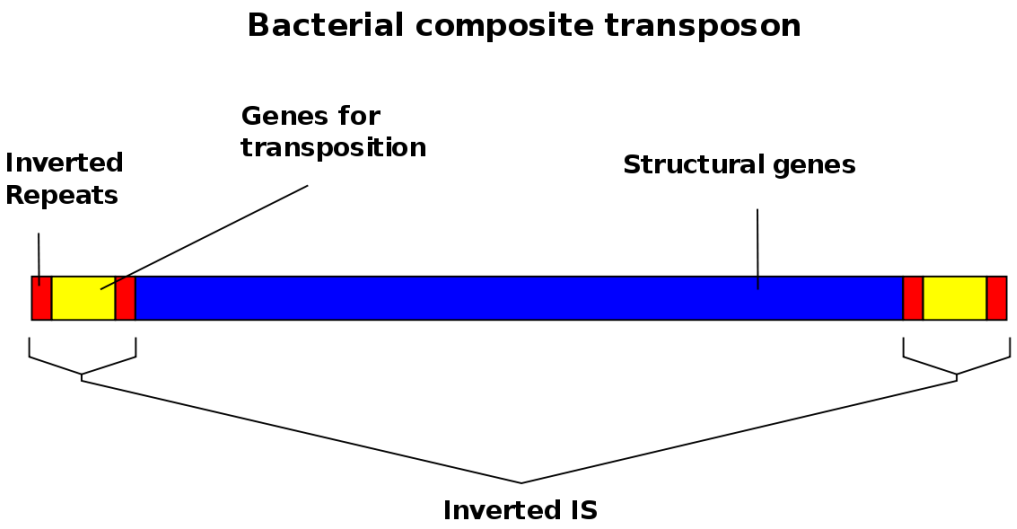


Figure 2.3 A schematic structure of the transposon (Anonymous 2022)

Non-composite transposons do not have insertion sequences. They are flanked by inverted repeat sequences, including the terminal sequence, which is recognized by transposase, which allows transposition. These transposons also contain a gene encoding a resolvase, which represses the transposase and allows replication of the transposon (Lima-Mendez *et al.* 2020).

In the transmission of antibiotic resistance genes, transposons play a determining role. They allow the movement of a gene between different DNAs, for example between a plasmid and the chromosome of a bacterium, or between several mobile genetic elements. Thus, transposons can mobilize chromosomal resistance genes to a plasmid or conversely integrate plasmid resistance genes into a chromosome, which will allow their persistence in subsequent bacterial generations. The non-composite transposon Tn1696 confers, for example, resistance to gentamicin, streptomycin, chloramphenicol and sulfamethoxazole in Pseudomonas aeruginosa (Smith and Kronvall 2015).

2.4.3 Genomic Islands: ICE and IME

Genomic islands are elements that can be excised and integrated into the chromosomes or plasmids of bacteria. They can be separated into two categories: conjugative genetic islands, which is called integrative and conjugative elements (ICE) and mobilizable genetic islands, which is called integrative and mobilisable elements (IME) (Bellanger *et al.* 2014). ICEs can conjugate thanks to the presence in their structure of a conjugation module allowing their mobility, whereas IMEs require an external mechanism to be mobilized. The genomic islands carry genes allowing the adaptation of the host cell to the environment, including genes for resistance to antibiotics. ICE Tn5397, for example, has been described in *Clostridioides difficile* and notably contains the tetracycline resistance gene *tetM*. Mention may also be made of the IME *SGI1* described in *Salmonella*, which contains several genes conferring resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline (Hawkey *et al.* 2019).

2.4.4 Integron

Integrons are not mobile genetic elements, but they allow the dissemination and capture of genes involved in the genetic adaptation of bacteria to environmental conditions. These are molecular structures allowing rapid evolution of the bacterial genome. They consist of an *intI* gene, an adjacent *attI* insertion site, a variable gene cassette and a transcriptional promoter (Pc) (Cury *et al.* 2016).

Gene cassettes are non-replicating genetic elements. They contain an *attC* recombination site, allowing their integration or excision of the integron, as well as one or more genes which will be integrated with the Pc promoter into the plasmid or into the chromosome by the integrase encoded by the *intI* gene. This insertion requires the integrase to recognize the attI insertion site to excise the cassette and insert it into the integron. Once the insertion has been carried out, the promoter will allow the transcription of the genes of the cassette (Kovalevskaya 2002). Integron structure and function is given in Figure 2.4.

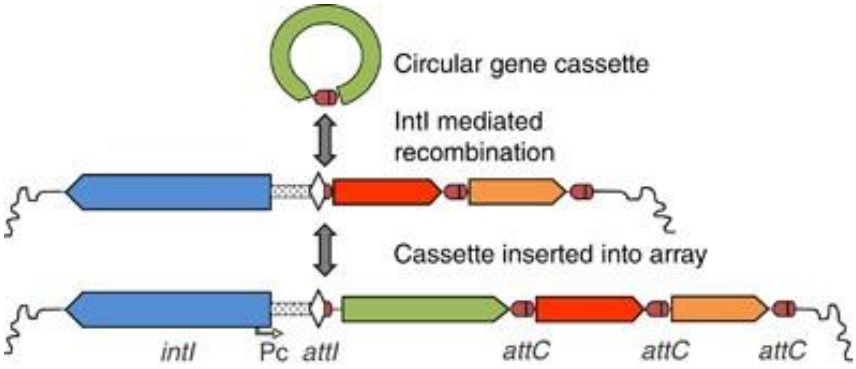


Figure 2.4 Structure and functioning of an integron (Gillings *et al.* 2015)

The cassettes are therefore the elements carrying the resistance genes which can be disseminated by horizontal transfer. More than 130 gene cassettes conferring resistance to antibiotics have been described, the presence of which is often linked to multi-resistance profiles (Partridge *et al.* 2009).

Antibiotic selection pressure exerted on a bacterial population can select strains possessing resistance genes present on plasmids or transposons, which themselves can possess integrons. The transfer of these elements leads to the transfer of integrons possessing cassettes carrying resistance genes and promotes the appearance of multi-resistance profiles (Partridge *et al.* 2018).

2.5 Antibiotic Resistance and The Fish Farming Industry Around the World

World aquaculture production represented 82 million tonnes in 2018 of which 88.7% is produced in Asia. The main producing countries are China (57.9% of world volume), India, Indonesia, Vietnam and Chile (Westerling *et al.* 2020). As mentioned above, products from the fish farming sector are likely to contain bacteria resistant to antibiotics. Farming practices are likely to have a strong impact on levels of antibiotic resistance in the environment. In a review published in 2019, studied the uses of antibiotics in aquaculture in the 15 main countries producing aquaculture products. Of 67 antibiotics mainly used in these countries, Vietnam used 39 and China 33 (Lulijwa *et al.* 2019). This high number of antibiotics used in aquaculture, combined with the use of

antibiotics in other sectors, may explain the high rates of resistance to antibiotics in aquaculture products and river water in these countries. For example, high prevalences of *E. coli* bacteria in farmed and wild fish have been reported in Vietnam. In these studies, 38.5% of the fish reared in tanks carried a strain with the *blaCTX-M-1* gene and 48.7% of them a strain with the *sul2* gene, conferring resistance to sulfonamide antibiotics. Similarly, 65% of caged fish carried a strain with the *blaCTX-M-1* and *blaTEM* genes conferring resistance to β-lactams, and 20.5% of wild fish carried the *sul1* and *sul2* genes (Hoa *et al.* 2020). It is described that,treatments applied to land-based farms can also impact the antibiotic resistance of bacteria associated with fish farming ponds (Dang *et al.* 2011). In an integrated pig-fish farming system, antibiotics administered to pigs through feed increased the resistance of *E. coli* and *Enterococcus spp*. which impacted the resistance levels of the bacteria present in the pool water. Regarding other Asian countries, phenotypic resistance to one or more antibiotics among chloramphenicol, clindamycin, rifampicin, spectinomycin and tetracycline has been observed in strains of *Salmonella spp*. isolated from tilapia and catfish from markets in Malaysia (Budiati *et al.* 2013). The *blaCTX-M-1*, *qnrS*, *qnrB* (conferring quinolone resistance), *sul1* and *sul2* genes have also been detected 29 in strains of *E. coli* isolated from river sediments in India (Diwan *et al.* 2018).

In Europe, resistance profiles to penicillin, ampicillin, meropenem, erythromycin and the trimethoprim-sulfamethoxazole combination have been characterized in strains of *Listeria monocytogenes* isolated by swabbing different surfaces in a fish processing workshop in Poland (Skowron *et al.* 2018). Two strains were resistant to 11 antibiotics, and one was resistant to 12 antibiotics tested. The application of high-throughput molecular technologies to the study of the resistome of farmed fish has made it possible to describe the presence of ARGs and mobile genetic elements, mainly from samples of intestinal contents, and ARGs have also been described in some skin and gill samples (Muziasari *et al.* 2017). A study was conducted on the levels of antibiotic resistance of strains of *E. coli* and *S. aureus* isolated from fish. The authors observed that 41.4% of *E. coli* and 87% of *S. aureus* were resistant to ciprofloxacin and 33.3% of *E. coli* and 61.3% of *S. aureus* were resistant to gentamicin (Okyere *et al.* 2018). Multidrug resistance patterns have been detected in Gram-negative bacteria isolated from farmed

salmon in Chile. It was thus possible to identify the *floR* gene, conferring resistance to chloramphenicol and florfenicol, in Gram-negative bacteria isolated from salmon farmed in Chile (Fernández-Alarcón 2010).

2.6 Increase in Antibiotic use and Antibiotic Resistance in Turkey.

Turkey is one of the countries with the highest antibiotic consumption and antibiotic resistance. Antibiotic use, which was 6722 defined daily doses (DDD), per 1000 people in 2000, has increased over the years and reached 18095 DDD per 1000 people in 2015 (Anonymous 2021).

According to the National Bacterial Drug Consumption Monitoring Report, the total consumption of systemically consumed antimicrobial drugs; The DID (Defined Inhabitant Dose), per 1000 people in one day, was 46.32 DID in 2012 and 44.77 DID in 2021 (Anonymous 2021). According to the National Bacterial Drug Consumption Monitoring Report of 2013, the group of antibacterial drugs used systemically constitutes the highest consumption among antimicrobials with 40.80 DID (Anonymous 2021). This value was 42.28 in 2011 and 42.21 in 2012. According to the 2013 report, beta-lactam-penicillin antibacterials are the most widely used among antibacterials (18.15 DID) (Anonymous 2021).

According to World Health Organization Antibiotic Consumption Report 2016-2021, the defined daily dose for 1000 people in Turkey in 2021 was declared as 38.18 and it was determined that this is the one of the countries with the highest antibiotic use (Anonymous 2021). The use of antibiotic use in Western Europe is given in Figure 2.5.

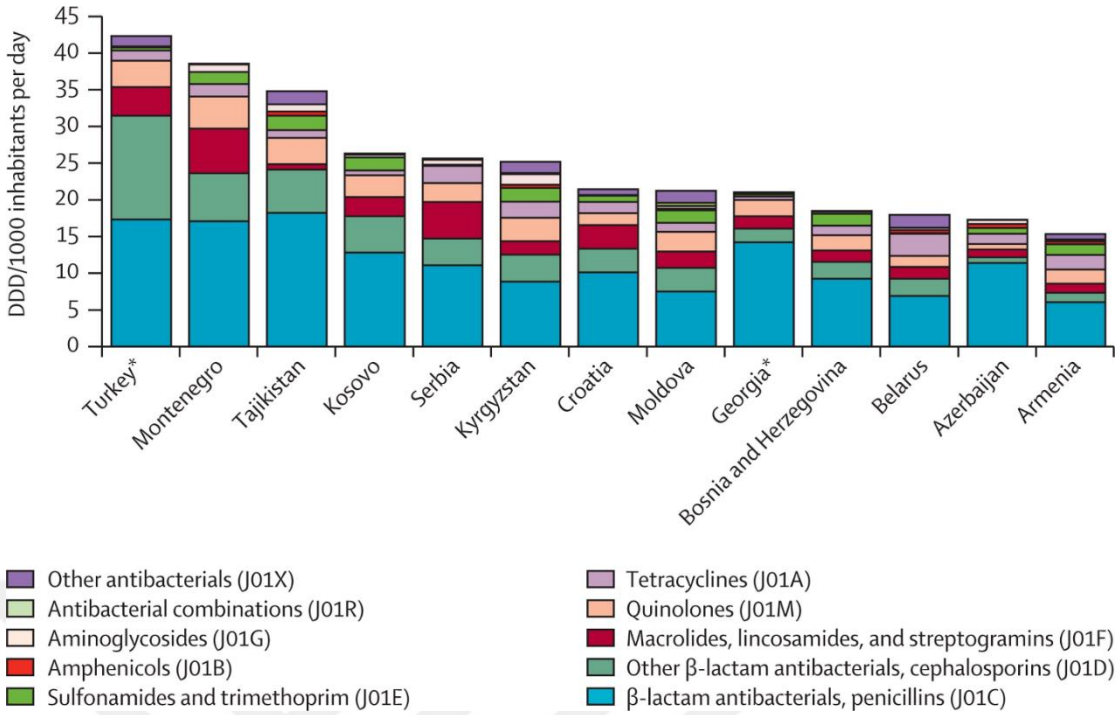


Figure 2.5 Antibiotic use in Eastern Europe (Robertson *et al.* 2019)

Considering the high consumption of antibiotics and the direct relationship between the frequency of consumption and the development of resistance, antibiotic resistance is high in Turkey and is expected to increase in the future (Anonymous 2019). According to WHO data from 2015, when antimicrobial resistance is examined in Turkey, the frequency of *E. coli* resistant to 3rd generation cephalosporins is >50%, the frequency of *E. coli* resistant to carbapenems is 1-5%, the frequency of multi drug-resistant-*Klebsiella pneumoniae* is 25-50%, the frequency of carbapenem-resistant *K. pneumoniae* its incidence is 25-50%, *Acinetobacter spp*. prevalence is >50%, methicillin resistant *S. aureus* (MRSA) prevalence is 25-50%, and Turkey ranks highest in resistance levels (Anonymous 2019).

It is predicted that Turkey is at risk of incurring an economic loss of between $220 billion and $1.4 trillion due to high antibiotic resistance until 2050. If the current level of antibiotic resistance continues, the total loss that will affect the economy from 2010 is estimated at 220 billion dollars; it is estimated that this value will rise to $1.4 trillion if action is not taken and resistance rates increase (Anonymous 2020).

Considering this situation of antibiotic use and resistance, national policies and programs have been determined to prevent antibiotic resistance, which has become an important program for Turkey. Studies on the rational use of drugs began in Turkey in the 1990s and the Turkish Pharmaceutical Guide was published in 1999. In the 2000s, books on diagnosis and treatment, prescription guide and education of the public on the rational use of medicines have been published. The Directorate General for the Rational Use of Medicines was created in 2010 and the provincial coordinations were established in 2016. The National Action Plan for the Rational Use of Medicines 2014-2017 was implemented in order to improve the knowledge, attitudes and behavior towards doctors, pharmacists, paramedical staff, the public and the pharmaceutical industry. Within this framework, 99 activities have been determined in 20 strategic targets (Anonymous 2018a).

The National Antimicrobial Resistance Surveillance System was established in 2011 under the coordination of the Public Health Institution of Turkey, and data has been collected and the results of antibiotic susceptibility testing of these isolates are monitored. With these data, the status of antimicrobial resistance is determined, measures to reduce resistance are determined, and the effectiveness of the measures taken is evaluated (Anonymous 2011).

In addition, monitoring for invasive device-associated infections, catheter-associated central bacteraemia (CBSI-CIS), ventilator-associated pneumonia (VAP) or ventilator-associated event (VIO), infection of catheter associated urinary tract (CI-UTI) in second and third level intensive care units); Surveillance of infections associated with invasive devices (SKI-BSI, VAP) categorized according to birth weight is carried out in neonatal intensive care units. Surgical site infection (SSI) surveillance specific to the type of surgery is carried out in offices determined from the relevant list according to the number of beds. Surveillance data was assessed across Turkey and based on four types of institutions (Ministry of Health public hospitals, Ministry of Health training and research hospitals, university hospitals, private hospitals) (Anonymous 2021).

2.7 Impact of Antibiotic Resistance on Human Health

Impact assessment is possible using three criteria: persistence, bioaccumulation and toxicity (PBT). These criteria are used at European level for reference documents concerning the evaluation and authorization of certain chemical substances. But you have to keep in mind that if the evaluation of a compound by these three criteria indicates that it is safe, it is not necessarily the case. For example, if a weakly persistent antibiotic is continuously released in small quantities, this can lead to very significant impacts (Carre *et al.* 2021).

Persistence is by their nature, antibiotics must be stable enough to achieve their purpose, but must be eliminated to avoid long-term effects. Studies on the persistence of antibiotics have mainly focused on their persistence in sediments. The results often indicate strong persistence from antibiotics of the fluoroquinolone and cyclin families, which are therefore poorly biodegradable (Martins *et al*. 2019).

Bioaccumulation corresponds to the accumulation in the body of a substance from the surrounding physical environment (bioconcentration) and from food. Bioaccumulation is influenced by the persistence and lipophilic potential of the compound (Li *et al*. 2020).

Ecotoxicity studies are toxicity of pharmaceutical products on aquatic organisms are carried out using indicator organisms. In general, control microorganisms are used to assess toxicity. In the aquatic environment, these are often cyanobacteria, proteobacteria, micro-algae or green algae. But if some molecules show a low toxicity threshold, this does not mean that they are harmless. Indeed, in the case where exposure to pollutants is chronic and long-term or if there is simultaneous exposure to several substances, the impacts can multiply. Not all antibiotics are equally harmful. Ecotoxicity depends on several 66 criteria: the rate of consumption of the product, the frequency of detection, the persistence in the environment, the exposure time and the toxic effects detected (Välitalo *et al*. 2017).

The effect of antibiotics on the activity of microbial communities in the environment raises questions about the possible selection of antibiotic-resistant strains in water. Except for discharges from pharmaceutical production plants in Asia, the concentrations of antibiotics observed in the waters are below the MIC. It was recently demonstrated that concentrations 100 times lower than these MICs would promote the maintenance and emergence of strains of *E. coli* and antibiotic-resistant *Salmonella* (Gullberg *et al*. 2011). Kohanski *et al*. (2010) highlighted the effect of these "sub-inhibitory" concentrations (norfloxacin, 50 ng/mL) on the production of free radicals responsible for the emergence of multi-resistant bacteria to antibiotics following mutations linked to regulation by the SOS system. These genotoxic effects are observed for ciprofloxacin concentrations of the order of 10 µg/L.

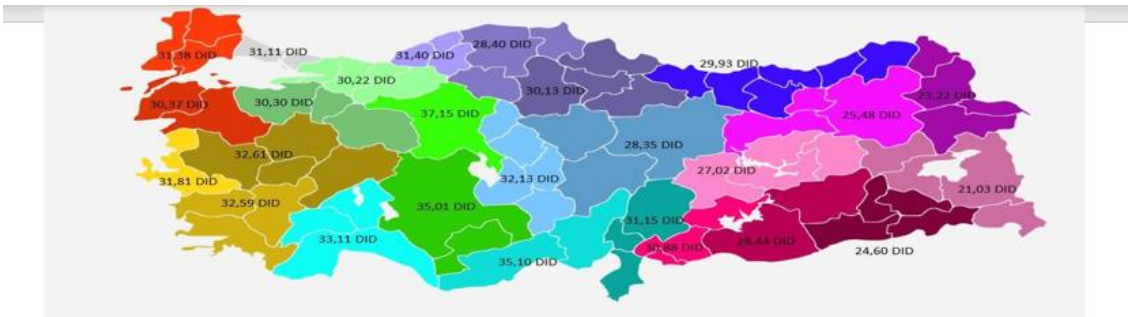
Human beings can be subjected to the presence of antibiotic residues through two means: drinking water or fish products containing traces of antibiotics. But the treatment of raw water in a drinking water treatment plant is generally very effective and achieves an almost complete elimination of antibiotics. Regarding aquatic organisms, they may indeed contain traces of antibiotics, but at very low concentrations (Okeke *et al*. 2022).

2.8 Context and Choice of Sampling Location

The discovery of antibacterials, which is a group of drugs used in the treatment of infectious diseases caused by bacteria and which is of great clinical importance, was an important turning point in terms of human health. The introduction of these drugs has been reflected in the mortality and morbidity rates from infections, and these rates have dropped dramatically. With the use of antibacterials, the resistance of bacteria to these drugs began to be observed almost simultaneously. Problems caused by resistant bacteria pose a serious threat, especially in intensive care patients with weakened immune systems. Considering the importance of antibacterials, it becomes clearer how antibacterial resistance poses a threat to humanity, which can negate the effect of antibacterials. For this reason, it is obvious that it is very important to fight against the problem which has become a global threat. Countries are taking various initiatives to

ensure rational use of antibacterial drugs under the guidance of WHO. In recent years, serious measures have been taken in Turkey within the framework determined by the WHO in this regard. Within the framework of the "National Action Plan for the Rational Use of Medicines 2014-2017", which is one of these stages, a series of activities are planned with the aim of popularizing the rational use of antibacterial medicines. One of the monitoring and evaluation activities included in the action plan is to determine the consumption of antibacterial in Turkey (Anonymous 2018a).

In the reportprepared by Ministry of Health of Republic of Turkey, the use of antibacterial drugs by outpatients across Turkey, Anatomical Therapeutic Chemical (ATC), and Chemical Classification System DDD, which is a method recommended by WHO, where comparisons can be made between data in national and international platforms. DDD and a detailed evaluation of these drugs according to variables such as “subgroups, province, region” was made. According to the calculation made on a provincial basis for 2018, the highest consumption of antibacterial drugs was found in Ankara with 37.15 DID (Anonymous 2018a). Distributions of antibacterial drug consumption is given in Figure 2.6.



Ankara Alt Bölgesi	37.15 DID	Izmir Alt Bölgesi	31.81 DID	Bursa Alt Bölgesi	30.30 DID	Kayseri Alt Bölgesi	28.35 DID
Adana Alt Bölgesi	35.10 DID	Zonguldak Alt Bölgesi	32.40 DID	Kocaeli Alt Bölgesi	30.22 DID	Malatya Alt Bölgesi	27.02 DID
Konya Alt Bölgesi	35.02 DID	Tekirdağ Alt Bölgesi	32.38 DID	Samsun Alt Bölgesi	30.13 DID	Erzurum Alt Bölgesi	25.48 DID
Antalya Alt Bölgesi	33.11 DID	Hatay Alt Bölgesi	31.15 DID	Trabzon Alt Bölgesi	29.93 DID	Mardin Alt Bölgesi	24.60 DID
Manisa Alt Bölgesi	32.61 DID	Istanbul Alt Bölgesi	31.11 DID	Şanlıurfa Alt Bölgesi	28.44 DID	Ağı Alt Bölgesi	23.22 DID
Aydin Alt Bölgesi	32.59 DID	Gaziantep Alt Bölgesi	30.88 DID	Kastamonu Alt Bölgesi	28.40 DID	Van Alt Bölgesi	21.08 DID
Kırıkkale Alt Bölgesi	32.13 DID	Balıkesir Alt Bölgesi	30.37 DID				

Figure 2.6 DID distributions of antibacterial drug consumption for systemic use on a provincial basis in Turkey (Anonymous 2018a)

In this study, we chose to assess Lake Mogan in Gölbaşı province because rainfed agriculture is generally practiced in the basin. The cropping pattern is mainly cereals and, in irrigated lands (particularly in the Sukesen Stream Valley), vegetables to a limited extent. There are pastures in part of the basin. The intensive use of pesticides and fertilizers in these areas has an inevitable effect on the lake, particularly in terms of nitrogen-phosphorus pollution. In fact, intense reed development is observed in the parts where the Çölova stream reaches the lake to the south, indicating overfeeding. In the 2001 report prepared by the IEEI; "The Çökek wetland south of Lake Mogan, which constitutes 83% of the area where Lake Mogan is fed, provides many positive contributions to the lake ecosystem such as flooding, sediment load and water pollution control. Major industrial facilities in the Lake Mogan Basin are mentioned below. Its proximity to the center of Ankara and efficient road transport increases the industry suitability of this region. The main facilities in the region are: Mekon Aluminum Cladding Factory, Pi Makina, Furniture Factory, Ensa Tube Factory, Guris Makina Factory, Ankara Plaster Industry (Alçısan), AD Atilla Assembly Factory Doğan, Prekons Construction Industry, ABS Plaster Factory, Tipo Poultry, Meat and Fish Institute Nursing Home, BM Holding Warehouses, FMC Nurol Tank Factory, Mohair Birlik Wool Storage, Machinery Factory Gülermak, Gazi Application and Research University Hospital, Özmak Toprak Industry, Tile Factory and Özmak Machinery Factory. In addition, there are Petrol Ofisi facilities on the east side of Lake Mogan, machinery factories which do not contain hazardous waste on the east and west, and iron processing facilities on the east side. Although most of these facilities are connected to the sewer network, unexpected and uncontrolled spills into the lake can occur from time to time (Cengiz 2010).

According to State Hydraulic Works (DSI) report in 1994; Mogan Lake, within the borders of Gölbaşı district, has been used for many years for hunting, swimming, sports activities and recreation area. After 1987, it was observed that there were significant changes in the ecological balance of the lake, bottom plants increased, algae and odors

appeared and a decrease in fishery and aquaculture products. In the same report, “The lake is still in a state where illegal fishing is widespread, far from any type of control. The lake and its surroundings are still used as open dumps. When the level drops on the north side of the lake, construction debris, broken bottles, nylons, etc. all kinds of trash. Also, the garbage dumped in the Sukesen class reaches the lake. There are always fishing nets in the lake, the fish caught in the nets are caught by boats, but the nets are not picked up. The steel covers made by DSI to protect the level of the lake are broken by fishermen and local people by breaking the control devices and water is taken from the lake. Wool and carpets are washed in the lake outlet channel. Unless the public is aware, it seems impossible to establish a healthy operation on the lake. Materials entering the lake through erosion, snowmelt and drainage have caused the formation of bottom sediments and a decrease in the depth and therefore the volume of the lake. Sediment accumulation, sewage discharge, and nutrients entering the lake with rainwater from the field, which has been going on for many years, have accelerated biological developments in the lake. In the lake, flooding began in places, an excessive increase and spread of tall aquatic plants began, and the shallowness of the lake accelerated. With the increase in sediments and nutrients in the lake, waterlogging and eutrophication have become dominant (Yilmaz 2022).

3. MATERIAL AND METHOD

3.1 Study of The Sampling Site

Gölbaşı is about 20 km inside the borders of Ankara. It is a district center with a population of around 40000, located on the highway from Konya to the south. The basin, which includes Lake Mogan and Lake Eymir and the rivers that feed them, was declared a special area of environmental protection by decision of the Council of Ministers of 22.10.1990 and numbered 90/1117 (Anonymous 1990). The accepted special environmental protection zone includes 245 km^2 of the total lake precipitation area. Lake Mogan is fed by small streams which usually dry up in the summer, and water from the lake flows into Lake Eymir under the control of the regulator to the northeast. About 750 hectares of swamps and wet meadows south of the lake provide habitat for many different animals, especially bird species. Mogan and Eymir lakes are the largest and most important of the limited number of wetlands located near Ankara. There are wide reeds and restaurants around Lake Mogan. Lake Eymir is located within METU borders. There are 10 villages in the special environmental protection zone of Gölbaşı (Anonymous 2003). The study field maps are given in Figure 3.1.

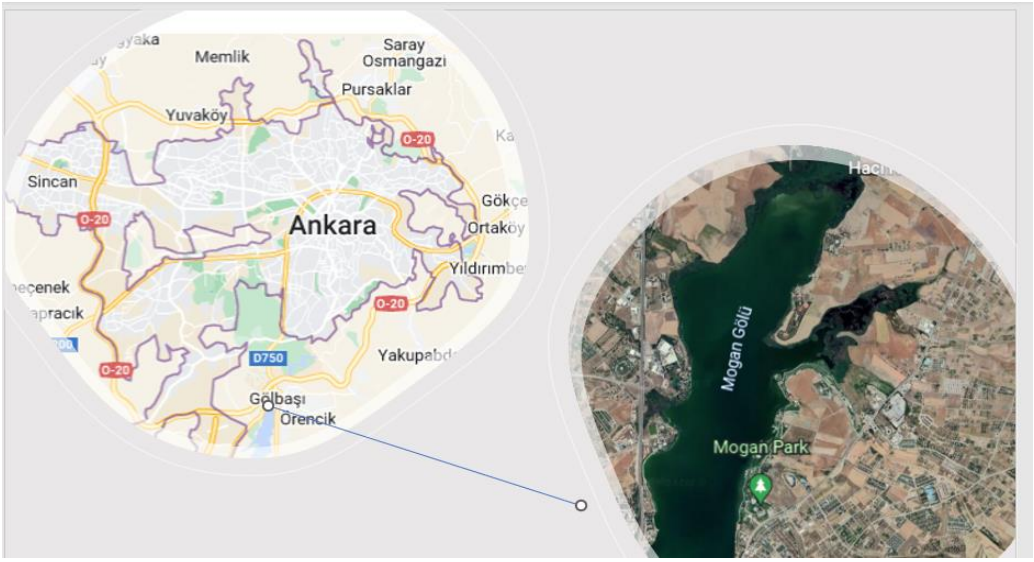


Figure 3.1 The study field: Mogan Lake (Turkey) (Google maps)

3.2 Sampling

3.2.1 Fish sampling

Five species of fish were selected for this study. These are *Atherina boyeri* which belongs to the Atherinidae family, *Pseudorasbora pavra*, *Tinca tinca*, *Cyprinus carpio* (they are all 3 members of the Cyprinidae family) and the species *Esox lucius* (It belongs to the family *Esocidae*). These five species of fish were caught by a specialized fisherman who carries out these kinds of missions for the university. A total of 16 fish were collected. Then the samples were brought to the laboratory of the Department of Pharmacology and Toxicology of the Faculty of Veterinary Medicine of the University of Ankara for study. Images of the fish species are given in Figure 3.2 and biometric datas are given in Table 3.1.

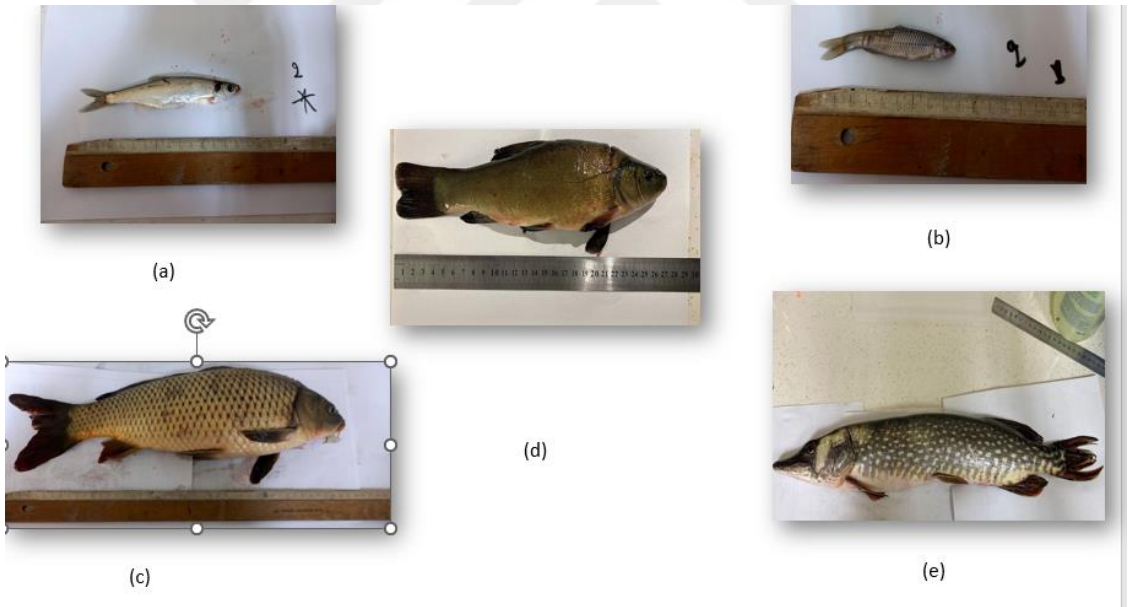


Figure 3.2 The fish species (a) *A. boyeri*, (b) *P. pavra*, (c) *C. carpio*, (d) *T. tinca* (e) *E. lucius*

Table 3.1 Table showing the biometric data (weight and size) of the fish species

Scientific Name	Local Name	Total Numbers	Weight	Length
C. carpio	Carp	3	+400 g	±35.5 cm
P. parva	Pebble fish	3	2.72 g	±10 cm
A. boyeri	Small Silverfish	3	5 g	±15 cm
E. lucius	Pike	2	+400 g	±55 cm
T. tinca	Tench Fish	5	±245 g	±26 cm

3.2.2 Water sampling

The water samples were taken around the lake at 4 different sampling points. We chose these points because they were places where there was a water inlet to the lake. These waters supplying the lake, agricultural lands, dwellings, recreation areas, picnic areas. The GPS positions of the samples are shown in the Figure 3.3. On each sampling point, the samples were taken in duplicate to avoid errors of contamination, loss or insufficiency of the sample for a volume of 150 ml.

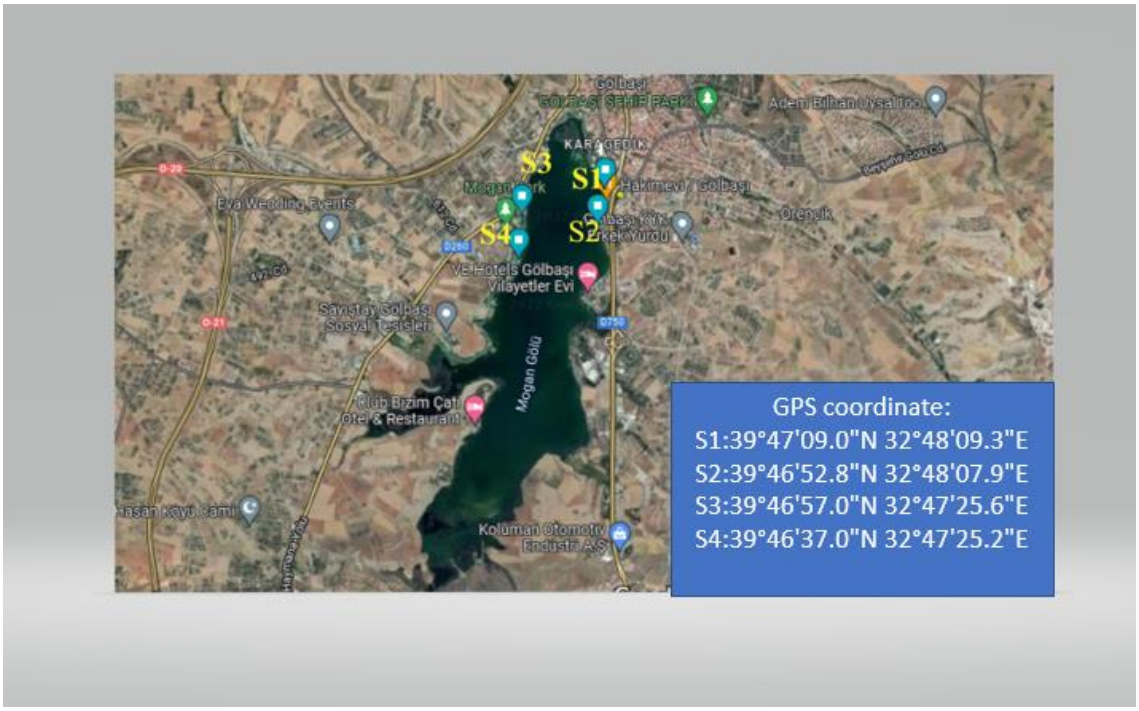


Figure 3.3 Water sampling stations on Mogan Lake (Google maps)

3.3 Sample Preparations for Detection

After the samples arrived at the laboratory, the biometric measurements (weight and size) of each fish were taken. Then we moved on to dissecting them. It should be noted that the fish were slaughtered by cranial percussion in accordance with animal welfare regulations (Anonymous 2009).

The incision method is given in Figure 3.4. A first vertical incision from the backbone to the belly about 2 cm from the gill (1) followed by a horizontal incision along the backbone (2), a third vertical incision about 2 cm from the base of the caudal fin (3) and a final horizontal incision along the belly to join the first incision (4). The tenderloin was then removed by lifting the flesh along the rib cage without piercing the digestive tract (5). All the dissection of this study was carried out by the same operator, under sterile conditions (under PSM) in order to put themselves in optimal conditions for the detection-recovery of the microbiota.

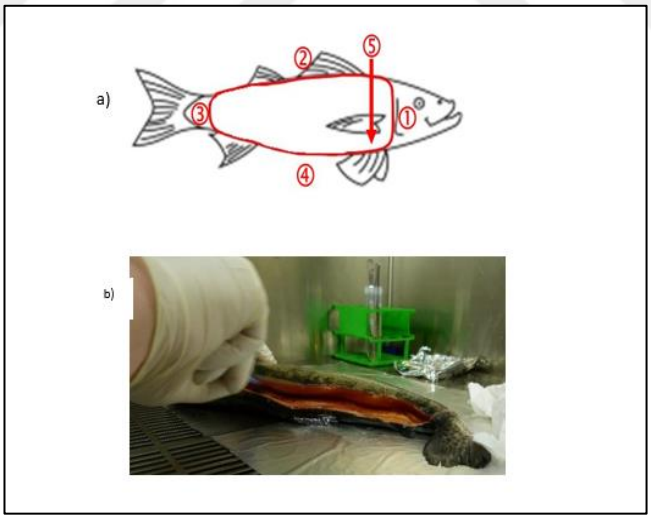


Figure 3.4 Tissue sampling a) Diagram of the cuts made, numbered from 1 to 5 in the order in which they were made and b) Photograph taken during the dissection in the laboratory

3.4 Microbial DNA Isolation

DNA purification relies on silica-based column technology that eliminates the need for unhealthy phenol/chloroform, time-consuming alcohol precipitation, or compensated resins.

DiaRex columns bind DNA under highly optimized salt concentrations and release bound DNA under low salt and mildly alkaline conditions. The master protocol takes less than 45 minutes after cell lysis and produces conformal DNA larger than 30 kb.

Extraction of various tissue and fluid samples was performed with the Diagen brand DiaRex® DNA Extraction Kit from Bacteria (Catalogue No.: BD-0865, Ankara). First, 300 µL of lysis solution was added to 50 mg of tissue or 200 µL of liquid sample. 15 mg of glass and 10 pieces of zirconia beads were added thereto and the application was made in the homogenizer device at 4000 rpm for 2 x 20 seconds. Then 25 µL proteinase K was added and after a short vortex they were incubated at 56 °C for 60 minutes. After incubation, centrifugation was performed at 5000 g for 1 minute and the supernatant was transferred to a new tube. 300 µL of binding solution was added thereto. After a short pipetting, the entire contents were transferred to the spin column. The spin column was spun at 8000g for 1 minute. The column was then transferred to a new tube and the washes were performed according to the manufacturer's instructions. After the washes were completed, the column was treated with 50 µL of elution solution and the genomic DNA was recovered. These steps are given in Figure 3.5.

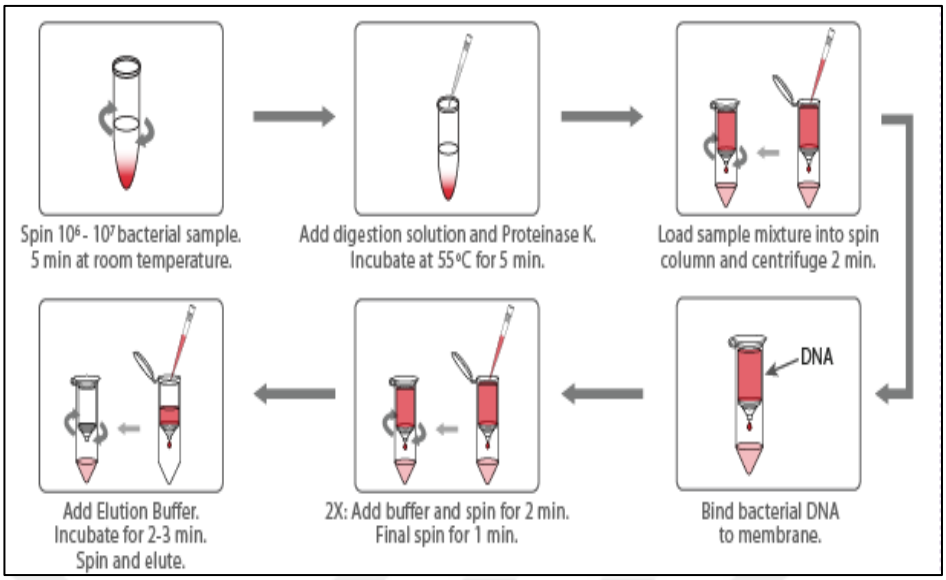


Figure 3.5 DNA isolation steps

3.5 Detection of genes by real-time PCR

With the development of thermal cyclers integrating fluorescent detection, PCR finds a new innovative application. In routine PCR, the critical result is the final amount of amplicon generated after the process. Real-time or quantitative PCR and RT-PCR use the linearity of DNA amplification to determine the absolute or relative amounts of a known sequence in a sample. By using a fluorescent reporter in the reaction, it is possible to measure DNA generation. The Schematic representation of the real-time PCR amplification process is given in Figure 3.6.

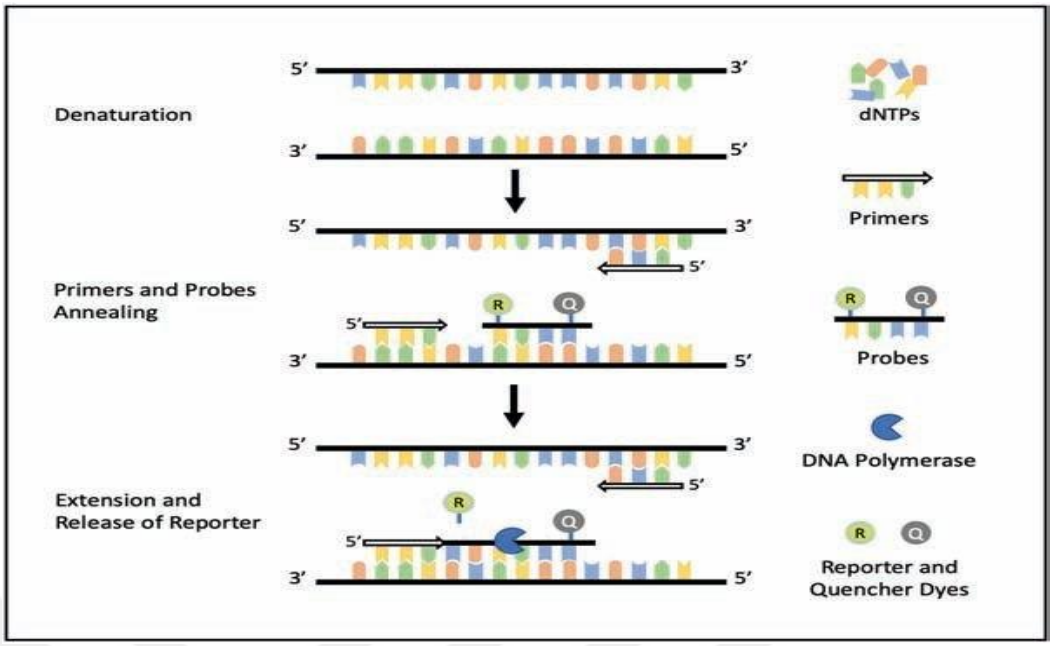


Figure 3.6 Schematic representation of the real-time PCR amplification processes

Steps in a single RT-PCR thermal cycle to detect the presence of RNA, samples are combined with primer/probe sets, reverse transcriptase (RT), DNA polymerase, buffer, and deoxynucleoside triphosphates (dNTPs). This mixture then undergoes an initial phase of annealing where the first strand of complementary DNA (cDNA) is formed at the target sequence. Thermal cycling begins with denaturation to separate the strands, followed by annealing of strand-specific primers and probes. Probes containing reporter (R) and quencher (Q) dyes are designed to anneal to portions of the target genetic sequence between primers. In the next step, the polymerase extends the primer (extension phase) towards the probe and releases R generating visible fluorescence. This thermal cycle repeats itself to amplify the genetic material with each cycle. A cycle threshold, defined as the number of cycles to reach a certain fluorescence, is often used as a determination of positivity. It is important that primers and probes are designed to be both specific and sensitive in identifying highly conserved regions unique to the gene of interest.

For real-time PCR studies, the primers in Table 3.2 and 3.3, which were designed specifically for bacteria and antibiotic genes, were first synthesized (Biomers, Germany). Then, the freeze-dried primers were dissolved with 100 Mm nuclease-free

dH_2O. Diagen 2X Sybrgreen master mix (Diagen, Turkey) was used for qPCR. The total volume was brought to 20 µl with a 1X (2X) master mix, 0.5 mM (10 mM) forward and reverse primers and 5 µl of gDNA. The reaction was performed in a real-time PCR machine (Roche 480 II, USA) using 8 strip tubes, initial denaturation at 95 °C 5 min, 40 repeats at 95 °C 10 sec, 60 °C 30 sec (read), 72 °C 30 sec and 95 °C 5 sec, 65 Fragment augmentation was performed with HRM analysis at 1 min at oC and up to 97 °C. The Ct values obtained as a result of the real-time PCR process were verified.

Table 3.2 Bacterial primer information

E. coli-malK-F	TAAGACTGAAACACCATACC
E. coli-malK-R	CAAAATGTAACGAAAGCCTG

Table 3.3 Antibiotic PCR primer information

Antibiotic	**Gene**	**Sequence**
Amoxicillin+clavulanic acid	*OXA1*-F	TTTTCTGTTGTTTGGGTTTC
	OXA1-R	CTATGGTGTTTTCTATGGCT
	TEM-F	GAAGCCATACCAAACGAC
	TEM-R	CAGCAATAAACCAGCCAG
Cefepime	*ampC-F*	TTCTTGTCTACTTTTATCCCC
	ampC-R	ACTGCTATTTACGGCTTTTT
Ceftriaxone	Ceftriaxone-F	CGCAACCGTGCCGTT
	Ceftriaxone-R	GGGTATTGAATGTGTCTGTTGGA
Ciprofloxacin-Escherichia	*gyrA-F*	GGCAATGACTGGAACAAA
	gyrA-R	GATAGAACCGAAGTTACCC
Colistin	*mcr-1-F*	GTCCGTTTGTTCTTGTGG
	mcr-1-R	GTCTGTAGGGCATTTTGG
	mcr-2-F	GTATTCTGTGCCGTGTATG
	mcr-2-R	GTATTGTTGGTTGCTGATTT
	mcr-3-F	GCCTCATTTTGATTGGTTTC
	mcr-3-R	TAAGTTTGGTTTCGCCATTT
	mcr-4-F	CCCGAACACTAAACCTAAC
	mcr-4-R	AAACATACAGGGTAGAGACA
	mcr-5-F	ACTGATTCTGCTTGCTGT
	mcr-5-R	TCATTACCGCTTGTTTCC
Imipenem	Imipenem-F	GAGTGGCTTAATTCTCRATC
	Imipenem-R	AACTAYCCAATAYRTAAC
Methicillin	*MecA-F*	GCAGTTATTGGTAAAAAGGGA
	MecA-R	GTTTGAGGGTGGATAGCA
Quinolone	*qnrA-F*	TTTGGCATAGAGTTCAGG
	qnrA-R	CATTGCTCCAGTTGTTTT
Vancomycin	*vanA-R*	GATACAGGAAACGGCAAAAA
	vanA-F	CATCATACGGGGATAACGA
	vanB-F	GCTTACCTACCCTGTCTTT
	vanB-F	AATCAAATCATCCTCGTTCC

4 RESULTS AND DISCUSSION

It must first known a fundamental concept at the base of quantitative PCR which is the CT or "cycle threshold", also known by the terminology of CP "crossing point" or TOP "take-offpoint". The CP or CT corresponds to the number of cycles from which the fluorescent signal of the targeted gene is greater than that of the background noise (Rasmussen 2001). In other words, the fluorescence emission is statistically and significantly higher than the baseline or threshold. The baseline is made up of the line parallel to the abscissa axis and it intersects all the kinetics at a point in their exponential phase. That is to say, the smaller the value of the CT, the more the target genes are present.

After recovering the genomic DNA of the *E. coli* bacterial strains obtained in the fish isolates. We amplified this DNA by RT-PCR and the result obtained (CT value) at the end of the process were reported in Table 4.1.

Table 4.1 Antibiotic resistant E. coli in fish samples reared in Lake Mogan water

Sample names	***E. coli***	***TEM***	***OXA1***	***qnrA***	***gyrA***	***mcr2***	***mcr3***	***mcr4***	***mcr5***
P. pavra 1	36	-	-	25	-	36	-	-	-
P. pavra 2	32	-	-		-	-	-	-	-
T. tinca 2	19	17	16	12	-	-	-	-	-
T. tinca 3	19	14	14	12	12	-	14	13	-
T. tinca 5	14	12	12		-	-	-	11	12
Esox Lucius 1	36	32	37	-	-	-	-	-	-
C. carpio 2	34	31	-	23	-	-	-	-	-

4.1 Presence of Antibiotic Resistant *E. coli* in Fish Samples

According to the reading of the PCR results obtained (present in the table) we note 6 samples of fish sensing *E. coli* resistant to the antibiotic panels tested on these samples:

Pseudorasbora pavra (1), which shows resistance against the gene coding for quinolones (*qnrA*) and colistin (*Mcr2*) with ct values of 25 and 36 respectively. *Tinca tinca* (5), which shows resistance to the combination amoxicillin-clavulanic acid (*TEM*

and *OXA1*) and colistin (*Mcr4* and *Mcr5*) with ct values of 12,12,11 and 12 respectively. Graphs showing RT-PCR result of amoxicillin-clavulanic acid association genes ,quinolones and colistin resistance that most samples present are presented in APPENDIX 1 and 2. As these are low CT values, this means that the resistance genes to these antibiotics are strongly detected.

Tinca tinca (3), which is the sample that shows resistance against several antibiotics. The amoxicillin (*TEM* and *OXA1*), quinolones (*qnrA*), ciprofloxacin (*gyrA*) and colistin (*Mcr3* and *Mcr4*) with CT values of 14,14,12,12,14 and 13 respectively. *Tinca tinca* (2), shows resistance to the combination amoxicillin-clavulanic acid (*TEM* and OXA1) and Quinolones (qnrA) with CT values of 17, 16 and 12 respectively. *Esox lucius* (1), shows resistance to only the amoxicillin (*TEM and* OXA1) with CT values of 32 and 37 respectively. *Cyprinus carpio* (2), shows resistance to the combination amoxicillin-clavulanic acid (*TEM*) and quinolones (*qnrA*) with CT value 32 and 23 respectively. We also note that the species *Tinca tinca* is the species that has shown the presence of the highest number (6 genes) of antibiotic resistance genes. Presence of 6 genes out of 15 genes coding for antibiotic resistance. On the other hand, the species *A. pavra* showed no presence of resistant genes.

4.2. Presence of Antibiotic Resistant *E. coli* in Water Samples

In parallel with the fish sampling, we took water samples from Lake Mogan. And according to the table below which shows the results of RT-PCR (CT value).

There is only resistance or presence of the Ciprofloxacin gene with a ct value of 33, which is a very high value, and which could mean that the genes of interest present here, that of the ciprofloxacin antibiotics is not strongly detected.

Antibiotics are commonly used around the world to treat bacterial infections in humans and other animals, including fish. Antibiotics used inappropriately or unintentionally can accelerate the acquisition of ARGs in fish pathogenic bacteria and the transfer of

antibiotic resistance from bacteria in the environment around fish farms or in food chains. Genetic mechanisms involved in the horizontal transfer of ARGs among environmental bacteria include conjugative transfer by mobile elements including plasmids, transposons, and integrons on plasmids or transposons (Bie *et al.* 2017).

In the present study, ARGs were observed in isolates of *E. coli* isolated in water and in fish from Lake Mogan (Turkey). Bacteria present in the surrounding environment can acquire resistance to antibiotics because they can be exposed to antibiotics eliminated in the environment by houses and/or hospitals, and agricultural culture located near the lake.

In our studies, the majority of the samples present ARGs coding for resistance to the amoxicillin-clavulanic acid association 90% (5 enchantions/6 sample) and to Quinolones 66.7% (4 samples/6 samples) and to colistins 50% (3 samples/6 samples). This can be explained by the irrational use of antibiotics in the human health sector, in particular self-medication, therapeutic non-compliance and the daily intake of antibiotics. So far, few studies have investigated the presence of *E. coli* resistant to quinolones, beta lactams and colistins in river water. Rivers and lakes are known to be a relevant putative reservoir of multidrug-resistant bacteria because they collect surface water containing materials of different origins, for example, sewage treatment plants, urban or industrial effluent water, agricultural activities, and they harbor a huge antibiotic resistome, distributed to pathogenic and non-pathogenic bacteria (Balasa *et al.* 2021).

A study in Tanzania (Africa) to determine the presence and genomic characteristics of ESBL-producing *E. coli* isolated from offshore fishing waters and Nile perch (Lates niloticus) from Lake Victoria has was conducted in February to July 2017. A total of 180 Nile perch samples and 60 water samples were taken. Of the 180 Nile perch samples analyzed, 8 ESBL-producing *E. coli* were detected and only one water sample was positive (1.7%) out of the 60 samples. Isolates were resistant to ampicillin/cloxacillin (100%), tetracycline (90%), sulfonamide (83.3%) and 72.7% of quinolones (Baniga *et al.* 2020).

In another study conducted out in lake taihu in china, researchers aimed to study the prevalence and the potential for dissemination of transferable resistance to antibiotics in the aquatic environment. The antimicrobial resistances observed were: resistance to ampicillin (58.3%) and tetracycline (57.1%) which were higher than that of streptomycin (20.1%) and gentamicin (15.4%) (Yin *et al.* 2013).

A cross-sectional study and standard microbiological methods were used to determine the prevalence and antimicrobial sensitivities of *E. coli* isolated from water and two species of fish (Rastrineobola argenta and Oreochromis niloticus) collected from the Lake Victoria Basin a western Kenya. The isolates of *E. coli* in fish were resistant to ampicillin (73.7%), cotrimoxazole (63.2%), tetracycline (73.7%), chloramphenic (26.3%) and gentamicin (21.1%). And in water, E. coli resistant to ampicillin (64%), chloraphenic (28%), tetracycline (76%), cotrimoxazole (80%) and gentamicin (32%) have been observed (Figure 4.1) (Onyuka *et al.* 2011).

Fish because of their mobility, they can be considered as powerful disseminators of resistant bacteria. Knowing that *E. coli* is not a permanent resident of the intestinal tract of fish, but reflects the character of the aquatic habitat and the bacterial load in the water (Hansen Dl *et al.* 2008). Due to fish exposure to a plethora of environmental bacteria on their outer mucus layer. Fish therefore provide an excellent condition for the horizontal transfer of antibiotic resistance genes within the aquatic bacterial community (Abgottspon *et al.* 2014).

The occurrence and distribution of antimicrobial-resistant *E. coli* in surface waters of seven rivers in southeast Queensland, Australia was studied in 2017. Resistance to four antimicrobials in water was determined: ampicillin (15%), tetracycline (7%), sulfamethoxazole (4%) and trace ciprofloxacin (weak) (Figure 4.2) (Watkinson *et al.* 2017).

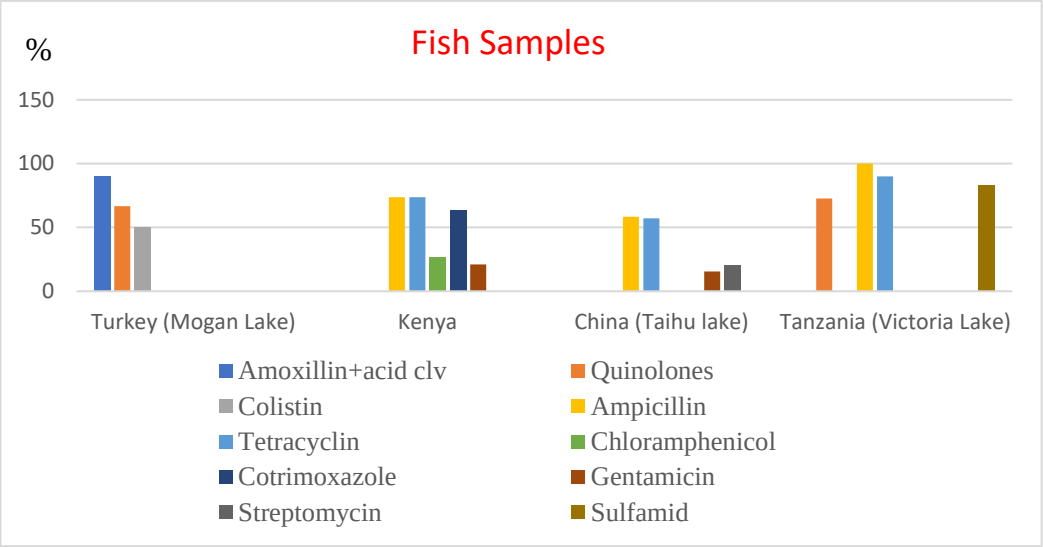


Figure 4.1 The rates of antimicrobial resistance in fish (Onyuka *et al.* 2011, Yin *et al.* 2013, Baniga *et al.* 2020)

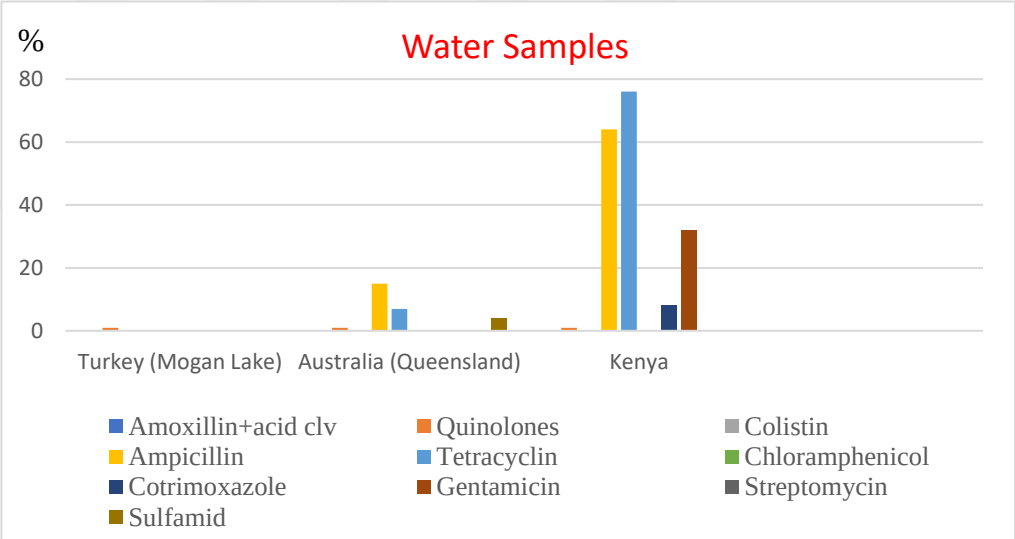


Figure 4.2 The rates of antimicrobial resistance in the Lakes water (Onyuka *et al.* 2011, Waltkinson *et al.* 2017)

5. CONCLUSION AND RECOMMENDATION

The World Health Organization has described antimicrobial resistance as one of the major global health problems and a profound threat to human health (Anonymous 2015). Due to the overuse and mismanagement of antimicrobials, we now find ourselves in a precarious situation where careful management of antimicrobials is essential to combat the widespread emergence of resistant bacteria.

Even if the interpretation of the results obtained during the field study is not sufficiently advanced to conclude on the precise origin of the acquired resistance revealed, the identification of multidrug resistance to antibiotics in *E. coli* isolated from the lake used for water intake is of particular concern. The bacterial genera *E. coli* chosen made it possible on the one hand to link the antibiotic resistance profiles of the sampling site that it is subjected to faecal contamination most likely of animal origin (human) and on the other hand to approach the public health risks that would result from the transmission of this resistance to human pathogens. By analyzing the existing antimicrobial resistance surveillance networks, we realize that the environmental compartment is not considered.

In addition, scientific data on the resistance of environmental bacteria resulting from human activities are scarce. However, the environment and more particularly water is a link in the network of transfer of antibiotic resistance. The presence of saprophytic or pathogenic bacteria that can transfer their antibiotic resistance genes to pathogenic microorganisms for humans is a threat to public health.

Scientists have thought about the implementation of new microbiological criteria for water quality integrating the antibiotic resistance profiles of multi-resistant bacteria (*E. coli*) isolated from water environments subjected to discharges from farms or human wastewater. These criteria essentially make it possible to identify the sources of faecal contamination, but they are not fully developed (Mutuku *et al.* 2022).

The advantage of setting up an environmental monitoring network lies mainly in monitoring the resistance phenotypes of aquatic bacteria such as *E. coli* present in recreational and natural waters and which can be found in treated water and in public water distribution networks. Their presence in the water environment could pose public health problems if their resistance genes were transferred to a bacterium pathogenic for humans.

However, certain limitations exist (mainly due to the technique of the antibiogram): (1) limits to the interpretation of the results (the clinical interpretation criteria are not necessarily adapted to the bacteria in the environment; only viable bacteria are examined) and (2) the risks of transfer of resistance genes from the natural environment to the consumer's tap are currently unquantifiable given the path traveled by the water (treatment, distribution network) and the complexity of the exposure pathways of man according to the use that is made of the water.

It seems more urgent to monitor the consumption of antibiotics in human and animal medicine as a priority, to define rational strategies for the use of antibiotics and to put in place measures to prevent the transmission of antibiotic resistance in order to control the emergence and spread of resistant bacteria. The data provided by existing or future monitoring networks will make it possible to identify the link between antibiotic consumption and the development of bacterial resistance and to initiate actions to improve the choice of prescriptions and make the best use of existing antibiotics.

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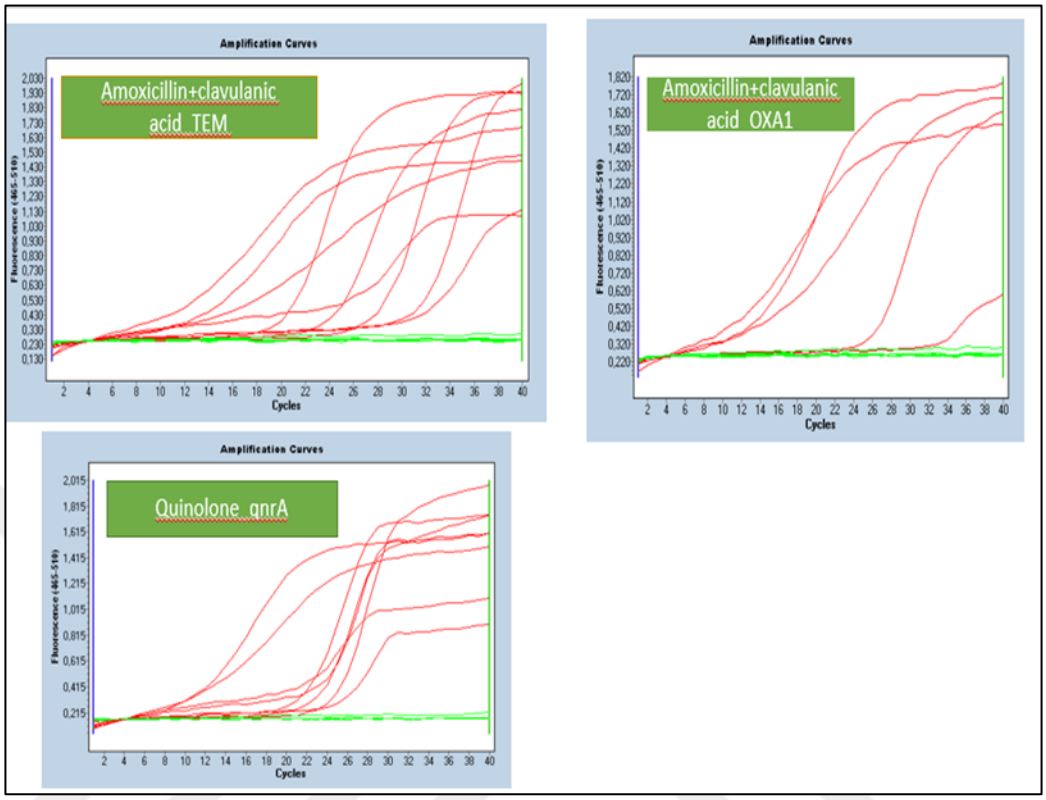
APPENDICES

APPENDIX 1. Graph showing RT-PCR result of amoxicillin-clavulanic acid association genes and quinolones that most samples present

APPENDIX 2. Graph showing RT-PCR results of colistin resistance genes

APPENDIX 1. Graph showing RT-PCR result of amoxicillin-clavulanic acid association genes and quinolones that most samples present



APPENDIX 2. Graph showing RT-PCR results of colistin resistance genes