

UNIVERSIDADE FEDERAL DA FRONTEIRA SUL
CAMPUS ERECHIM
CURSO DE ENGENHARIA AMBIENTAL E SANITÁRIA

FELIPE OLIVEIRA DA SILVA

**OCORRÊNCIA DE COLIFORMES TERMOTOLERANTES EM ÁGUA
DESTINADA AO CONSUMO HUMANO E SUA RESISTÊNCIA A
ANTIBIÓTICOS**

ERECHIM

2023

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Trabalho de Conclusão de Curso
apresentado ao Curso de Engenharia
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Erechim, como requisito para obtenção
do título de Bacharel em Engenharia
Ambiental e Sanitária.

Orientadora: Prof^ª. Dra. Helen Treichel
Coorientadora: Ma. Aline Frumi
Camargo (Doutoranda em Biotecnologia
e Biociências – UFSC)

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Este trabalho foi defendido e aprovado pela banca em __/__/____.

BANCA EXAMINADORA

Prof.^a Dr.^a Helen Treichel

Orientadora

M.^a Aline Frumi Camargo

Coorientadora

Prof.^a Dr.^a Cristiane Funghetto Fuzinato

UFFS – Erechim

M.^a Suzana Bazoti

UFSC

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Introdução Geral

Com o intuito de publicar uma revisão sobre a ocorrência de coliformes termotolerantes em águas destinadas ao consumo humano e sua resistência a antibióticos, foi realizada extensa pesquisa para compreender o mecanismo de resistência de coliformes termotolerantes a antibióticos, os tipos de coliformes termotolerantes mais encontrados nessas pesquisas, bem como as doenças causadas pelos mesmos, além dos antibióticos mais comumente encontrados em corpos hídricos e os métodos legislativos e de mitigação dessa problemática ambiental. A metodologia utilizada para a construção deste estudo em forma de revisão literária foi a pesquisa, leitura e compilação de dados e informações encontrados em artigos, publicações e revistas acadêmicas disponibilizadas nas bases de dados científicos: SCIELO e CAPES nas línguas portuguesa, inglesa e espanhola. O cronograma para a realização da pesquisa se estendeu desde o momento de decisão do tema em dezembro de 2021, onde foram buscadas as primeiras referências bibliográficas para o desenvolvimento, até a finalização de sua parte escrita em setembro de 2023.

Através desta pesquisa foram elaborados dois artigos intitulados “Thermotolerant Coliform Bacteria Present in Water Bodies” e “Antibiotics Found in Water Bodies and Treatment Methods”, ambos escritos e submetidos a revistas em língua inglesa, são apresentados no presente trabalho na forma de capítulos.

Chapter 1

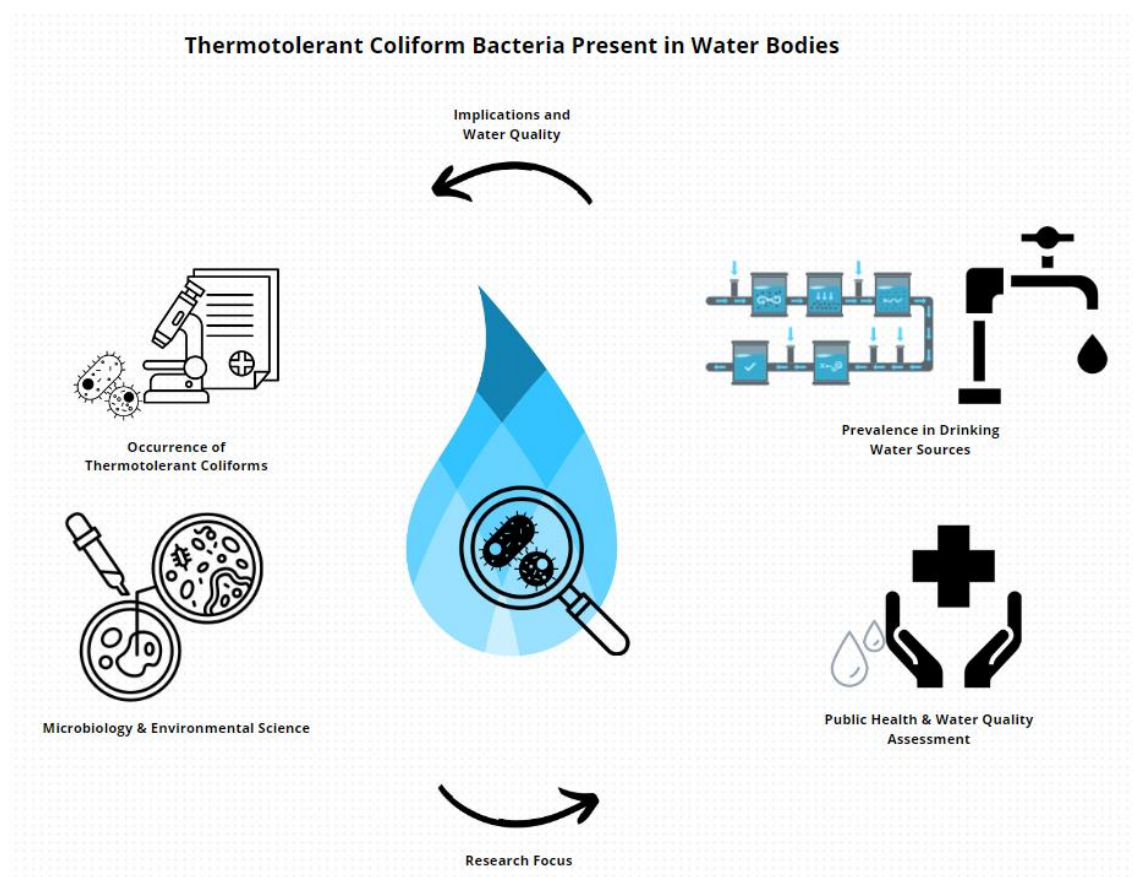
Thermotolerant Coliform Bacteria Present in Water Bodies

Felipe Oliveira da Silva¹, Aline Frumi Camargo^{1,2}, Helen Treichel^{1,2}

¹Laboratory of Microbiology and Bioprocesses, Federal University of Fronteira Sul, Erechim, Brazil.

²Graduate Program in Biotechnology and Biosciences, Federal University of Santa Catarina, Florianópolis, Brazil.

Graphical Abstract



Abstract

This work comprehensively reviews the Occurrence of Thermotolerant Coliforms in Waters Intended for Human Consumption. The research explores a multifaceted question at the intersection of public health, microbiology, and environmental science, shedding light on the prevalence of thermotolerant coliforms in drinking water sources. This review investigates the various types of thermotolerant coliforms commonly found in water sources intended for human consumption. An extensive literature examination establishes the importance of recognizing these organisms and their potential for water contamination, emphasizing the urgency of practical water quality assessment and monitoring.

Keywords: Human Health, Microbiology, Environmental Science, Water Quality.

Introduction

Water is essential for human life, and its potability is a concern. However, this was not always the case in ancient Greek and Sanskrit, dating back to 2000 BC, describing knowledge in initial water treatment methods such as heating, boiling, sand, and gravel filtration. The reasons for the treatment at the time differed from the current ones since the water went through these processes as a matter of taste, as there was still no knowledge of microorganisms or their possible pathogenic effects. After 1500 BC, the Egyptians discovered the principle of coagulation by applying chemical alum to precipitate suspended particles. Since then, humanity has advanced in the processes and discoveries about water treatment, developing filters, building aqueducts desalinators, and discovering and identifying microorganisms.

In Brazil, the first record of sanitation dates back to 1560, during the founding of Rio de Janeiro. Many factors hindered sanitation progress, such as lack of adequate planning, little investment, poor management by sanitation companies, and low technical quality in projects. After the 1940s, sanitation services began to be commercialized, with the emergence of municipalities and financing mechanisms for water supply. There was also the emergence of more specific legislation on the subject, such as the National Sanitation Plan (1971). Currently, the sanitation sector's public policies, goals, and strategies are led by the National Basic Sanitation Plan, where laws and guidelines are monitored by bodies such as the National Water Agency and the National Sanitation

Information System.

According to data from the United Nations, 35% of the world's population does not have access to clean water, and 43% does not have adequate sanitation services. Basic sanitation proves the social inequality between developed countries and developing countries development (BARROS, 2017). In Brazil, around 35 million people do not have access to treated water, and almost half of the population does not have access to sewage collection and treatment. Water is essential for life, so its safety and accessibility should be available. What seems to be a norm in European countries and North America is not a reality in the rest of the world, favoring infections and the transmission of diseases through water. According to data from the World Health Organization, mortality associated with water exceeds 5 million people per year, of which more than half are microbial intestinal infections, such as those caused by thermotolerant coliforms (CABRAL, 2010). Generally, the main microbial risks are associated with ingesting water contaminated by human or animal feces, directly or indirectly discharged into freshwater bodies or coastal marine waters. Those most affected by diarrheal diseases are those with less financial resources and precarious hygienic conditions, especially children under five. Coliforms in water, such as *Escherichia coli*, make it unfeasible for human consumption without prior treatment but allow it to be used for irrigation, recreation, and fishing (FENWICK, 2006).

After ingesting contaminated water, coliforms can colonize the intestine or share resistance genes with bacteria from the intestinal microbiota through conjugation (MISHRA et al., 2018). In this way, populations of antibiotic-resistant bacteria in the animal or human body are disseminated in the environment by defecation (YANG et al., 2016; ZHANG et al., 2021). Coliforms can move from feces to the soil and be transported to plants or water bodies due to surface runoff (VANDERZAAG et al., 2010).

The increasing frequency of microorganisms resistant to drugs, such as antibiotics, in the environment, poses a significant risk to human health (STREICHER, 2021). Antibiotic treatment can become challenging, expensive, or even impossible if an infection develops, as they are mostly excreted unchanged from the warm-blooded animals in which they colonize. It is estimated that around 700,000 people die annually from diseases caused by antibiotic-resistant 10ermente worldwide. The World Health Organization believes that this will be the leading cause of death in the world by the year 2050 (BONECA, 2021).

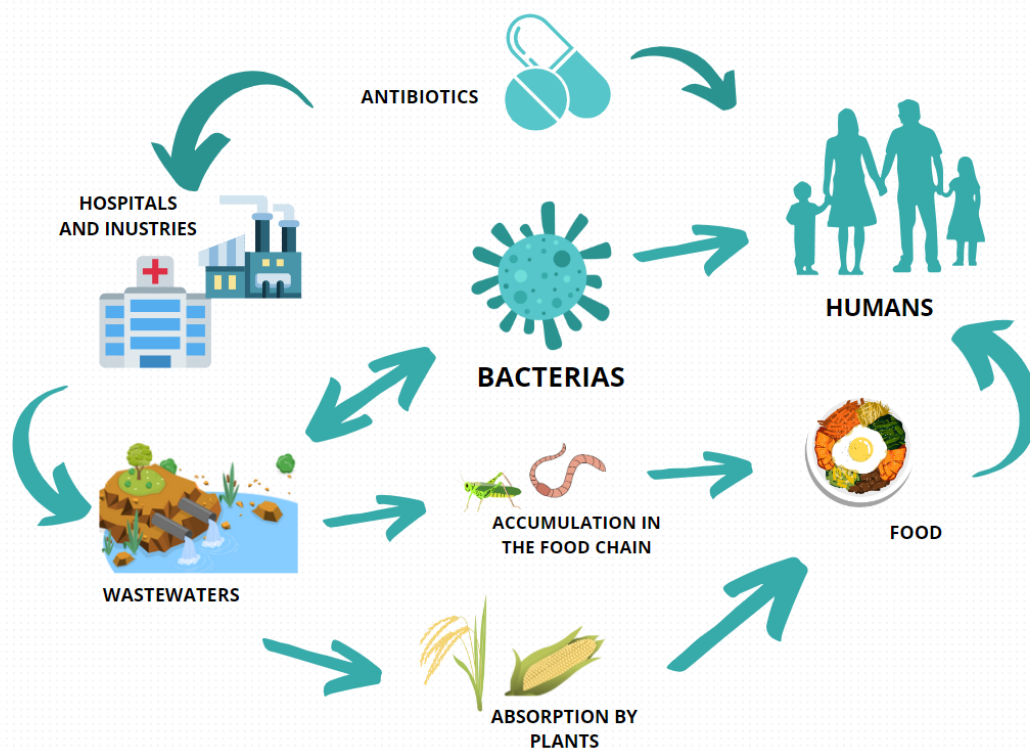
The United Nations (BEM) estimates that 2 million tons of waste reach, directly

or indirectly, water bodies across the planet (UNWAP, 2003; Ross, 2010). Approximately 1.2 billion people defecate along rivers and their banks in underdeveloped and developing countries (WHO and UNICEF, 2008). It is estimated that 70% of industrial waste is discarded without treatment in water bodies (BEM-WATER, 2009). However, treated sewage that is allowed to be discharged into rivers must not exceed the Biochemical Oxygen Demand (BOD) 11ermente 30 mg/L and Total Suspended Solids (TSS) 11ermente 50 mg/L (TYAGI et al., 2013).

To monitor the quality of water and its potability, investigations have been carried out on several parameters, of which the microbiological ones largely influence the usability of the water, as several scientific studies prove (OMS, 2006; MISHRA et al., 2012, 2013; HARNISZ et al., 2015; PARVEZ et al., 2016; GOMES FREITAS et al., 2017).

Thus, this study aims to research, discuss, and clarify, in the 11erment a review, the occurrence of thermotolerant coliforms 11ermente in water bodies intended for human consumption and their resistance to antibiotics. Residual antibiotics from pharmaceutical or industrial uses enter rivers and lakes through wastewater and accumulate in the soil, 11erme plants and animals absorb them. Figure 1 illustrates how the circulation of antibiotics occurs in 11ermente11 environments.

Figure 1. Scheme of antibiotic circulation.



Source: The Author

Potable Water as a Contamination Vehicle

Due to life's need for water, a sufficient, safe, and accessible supply is essential. Water is a common good, available for all types of use, being indispensable to all human activities, constituting a premise for the development of human actions, and a shaping element of civilizations and cultures, being linked to the history of humanity (LIMA, 1986).

Water quality is directly related to the use and occupation of a premise in a watershed. In densely occupied premises, contamination may occur due to irregular deposits of solid waste and sewage, influencing the water quality available to the premise (LIMA et al., 2018).

The World Health Organization (WHO, 2019) estimates that 25 million people die yearly from waterborne diseases such as cholera and diarrhea. The WHO indicates that 70% of the rural and 25% of the urban populations in developing countries lack adequate drinking water (WHO). It is estimated that 80% of hospital beds in developing countries are occupied by people with waterborne diseases (AZEVEDO NETO & BOTELHO, 1991).

Coliforms

The group of Coliform premise comprises more than twenty bacterial species, divided into two subgroups: total coliforms and thermotolerant coliforms.

The subgroup of total coliforms is composed of premise that are capable of fermenting lactose, with the production of gases at 35°C, a capacity that, with the formation of gas in culture media, serves as the basis for traditional methods of detection of total coliforms. They are common in food manufacturing environments and can become the resistant microbiota.

Thermotolerant coliforms, commonly called fecal coliforms, are restricted to premise capable of fermenting lactose at 44.5 – 45.5°C with the production of gases. Some originate from the gastrointestinal tract of warm-blooded animals, such as *Escherichia*, *Klebsiella*, and *Enterobacter* (DUFOR, 1977).

Detecting total and thermotolerant coliforms in food is a vital guideline instrument for food handlers and applying good practices in food manufacturing and production. As indicators of water quality, coliform premise have been used to monitor potability since 1920 due to their economic viability and ease of detection (ASHBOLT et al., 2001).

Detection Methods – Past and Present

Water bacteria were counted by Percy and Grace in a Robert Koch gelatin solid medium (HUTCHINSON & RIDGWAY, 1977). Theodor Escherich discovered *Bacillus coli* (ESCHERICH, 1885), later renamed *Escherichia coli* by Castellani and Chambers (1919). 1893, using the Wurtz method, sanitary bacteriologists enumerated *B. coli* by plating water samples on litmus lactose agar, forming acid from lactose fermentation (ASHBOLT et al., 2001). I

n 1893, Durham tubes were used to detect the formation of gases from fermentations (Durham, 1898). As early as 1905, MacConkey broth was used to diagnose bile-tolerant lactose-fermenting bacteria (MacConkey, 1905).

The preferred and most cost-effective method for detecting coliforms is the Most Probable Number (MPN). This technique allows statistically evaluating the number of microorganisms present in a sample and measuring the metabolically active viable proportion, which can be used to estimate the total population or a specific group of microorganisms.

A set of double and single robust lactose broth in inverted Durham tubes are inoculated and tested in measured amounts of water. The coliforms ferment the lactose present in the broth and produce acids and gases. The MPN of coliforms present in 100ml of water is estimated by the number of positive tubes concerning the MPN table. Samples from positive tubes are stained on EMB agar plates; black nucleated colonies with bright metallic green are *E. coli*. Single colonies are reinoculated in the bright green lactic broth, while the bile inhibits the growth of gram-positive microbes; bright green blocks the propagation of certain gram-negative bacilli, which allows the development of lactosefermenting coliforms (MISHRA et al., 2012, 2013). The WHO proposed the modified Eijkman test for thermotolerant coliforms based on the principle that only this type can ferment lactose at temperatures between 44.5 and 45.5°C, therefore using inverted Durham tubes to inoculate with several samples of water and incubate at 44.5°C, causing acid and gas (WHO, 2011). Other existing detection methods are the membrane filter and MMO-MUG (Colilert). The different methods, along with the categorization of coliforms, their health risks, and resistance mechanisms, are illustrated in the figure. Next, some detection methods found in the literature are listed in Board 1. Board 1. Coliform detection methods found in the literature.

Detection Method	Principles	References
Durham tubes	Coliforms are identified based on acids and gases produced by lactose fermentation.	DURHAM, 1898
MacConkey Broth	Coliforms were identified based on bile-tolerant lactose fermenters.	MacCONKEY, 1905
Robert Koch's Solid	Bacteria are counted based on the	HUTCHINSON &

Gelatin Medium	unit of colonies formed.	RIDGWAY, 1977
Most likely number	Based on the ability to ferment lactose by coliforms that form acids and gases; Ability of <i>E. coli</i> to reduce methylene blue to exhibit green metallic luster on 'Eosin methylene blue' agar; Ability of coliforms to ferment lactose-producing gases in the presence of bile.	CLESCERI et al., 1996 MISHRA et al., 2012; MISHRA et al., 2013
MMO-MUG (Colilert, Coliscan and Colitag)	It consists of enzyme-based detection of <i>E. coli</i> .	United States Environmental Protection Agency [USEPA], 2002
Modified Eijkman test	It consists of the ability to ferment lactose by thermotolerant coliforms that form acids and gases.	MISHRA et al., 2012, 2013
Biosensors	It is based on the interaction between substrate and chromogenic enzymes.	THAKUR & RAGAVAN, 2013; KIM & MYUNG, 2015; HESARI et al., 2016

Source: The author

Escherichia coli

Escherichia coli (better known by the abbreviation *E. coli*), is a Gram-negative bacillary bacterium that is normally found in the lower gastrointestinal tract of warm-blooded organisms (endotherms). Most strains of *E. coli* are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for food recalls due to contamination (VOGT & DIPPOLD, 2005).

E. coli takes the form of a bacillus and belongs to the Enterobacteriaceae family. They are aerobic and facultative anaerobic. Its natural habitat is the intestinal lumen of humans and other warm-blooded animals. It has multiple flagella arranged around the cell. Some species are so similar to *Shigella* and so different from each other that some biologists recommend that both be reclassified (LAN & REEVES, 2002).

E. coli strains isolated from intestinal diseases have been grouped into at least six significant groups based on epidemiological evidence, phenotypic characteristics, clinical disease factors, and specific virulence factors. Of these, the enterotoxigenic (ETEC),

enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC), and diffuse adhesion (DAEC) serotypes can be highlighted, which can be transmitted through contaminated water (BETTELHEIM,2003; SCHEUTZ and STROCKBINE,2005).

Enterotoxigenic E. coli

Enterotoxigenic *E. coli* (ETEC) serotypes can cause infantile gastroenteritis, especially in children living in developing countries without adequate drinking water and poor sanitation. In these countries, strains of this enteropathogen are commonly isolated in children under five years of age, causing hundreds of millions of cases of diarrhea and tens of thousands of deaths per year. (Bettelheim, 2003; WHO, 2010). The disease is caused by ingesting contaminated food or water and is characterized by profuse, watery diarrhea lasting several days, beginning about 14 to 50 hours after ingestion of the bacteria. Previous studies have shown that ETEC can survive in feces for over half a year, and it usually occurs in water in the form of biofilms, which gives it more significant survival potential. Diarrhea can be mild or prolonged, lasting for weeks and leading to dehydration. Other symptoms are abdominal cramps, low fever, nausea, headache, and muscle aches. (BETTELHEIM, 2003; WHO, 2010). This strain is the most common cause of so-called travelers' diarrhea, affecting individuals from developed countries who travel to developing regions (BETTELHEIM, 2003).

ETEC can infect its host using several virulence factors, including adhesion proteins (fimbrial adhesins) that mediate the adhesion of bacteria to the surface of host epithelial cells and allow bacterial colonization and toxin production (ZHANG et al., 2022). The adensin-producing ETEC strains in pigs are the most common currently associated with diarrheal diseases. These fimbriae bind to glycoconjugates in enterocytes (intestinal cells), and the absence of the respective glycoconjugate makes the animal resistant to bacterial colonization and consequent diarrheal diseases.

Until now, antibiotics and oral rehydration are the most popular treatments, while antibiotics create large numbers of resistant strains and eliminate beneficial bacteria in the gut, making it imperative to find alternative therapies. Treatment depends on the individual, as there may be recovery from the infection only with the replacement of hydrolytes, without antibiotics or other medicines. More severe cases require adequate medical treatment and the use of antibiotics and other medications (ZHANG et al., 2022).

Enterohemorrhagic E. coli

The reported cases are primarily associated with consuming contaminated food, such as raw or undercooked meat and raw milk, as it has been discovered that the primary reservoir of this bacterium is healthy cattle (BETTELHEIM, 2003).

The serotype of this bacteria, also known as O157, produces Shiga-like toxins. Its incubation period is 3 to 4 days, and symptoms occur from 7 to 10 days. It is estimated that somewhere between 2 and 7% of O157 infections result in cases of acute renal failure. Its main symptoms are abdominal pain, bloody diarrhea, and hemolytic uremic syndrome (WHO, 2011).

Although O157 is generally not a concern in treated drinking water, outbreaks involving drinking water contaminated with human sewage or livestock feces have been documented. The number of episodes associated with consuming fruits and vegetables contaminated by animal feces is increasing. As well as the cases of isolation of the serotype in bodies of water, mainly wells and drinking fountains (HEALTH CANADA, 2006; WHO, 2011).

To cause damage to its host, this pathogen has been characterized by producing virulence factors such as periplasmic catalase and Shiga-type toxins. Periplasmic catalase is encoded in plasmid pO157, providing oxidative protection during infection (KAISTHA and SINHA, 2009). Remember, the oxidative burst is central to the macrophage's arsenal against bacteria and parasites. If the oxidative burst is ineffective, the host must find an alternative route to kill the pathogen.

Treatment consists of fluid replacement and supportive therapy. Ciprofloxacin is indicated as first-line empiric therapy in patients with suspected invasive diarrhea and malaise. Azithromycin is indicated as second-line therapy. Ciprofloxacin is the first choice antibiotic for patients with EHEC (GONZALES et al., 2011).

Enteroinvasive E. coli

Enteroinvasive *E. coli* (EIEC) behaves similarly in many ways to *shigellae*. Both can invade and multiply in the epithelial cells of the human distal large intestine. The mechanism of pathogenicity of EIEC is the invasion of the colon epithelium; for this, the first step is the adherence of the bacteria to the mucosal villi, requiring mucinous and adhesins to subsequently enter the cell by endocytosis and subsequent multiplication of the EIEC within the cell and spread to adjacent healthy cells (NATARO and KAPER, 1998; RICO-MARTINEZ, 1995).

EIEC does not produce toxins but damages the intestinal wall through the mechanical destruction of cells. The bacterium has a highly virulent plasmid called pINV, also found in *Shigella*. This plasmid contributes to the disease phenotype similar to that of EIEC and *Shigella*. EIEC strains also lost the ability to synthesize cadaverine, produce the OmpT protein, and form curls, traits shared with *Shigella*.

The genetic information for this mechanism is in the chromosomal and plasmid sites, in addition to having the ability to produce one or more enterotoxins that may be important in the production of diarrhea. The genes necessary for infection are found on a 140 MDa plasmid called pInv, which encodes proteins such as Ipa and others that are involved in the pathogenesis process (ESLAVA and MATEO, 1994; HALE et al., 1983; SETHABUTR et al., 2000).

Where the disease is characterized by abdominal pain, diarrhea, vomiting, fever, general malaise, and the appearance of blood and mucus in the feces of the infected individual (BETTELHEIM, 2003) (SCHEUTZ and STROCKBINE, 2005), EIEC strains are more associated with outbreaks than isolated cases, in which transmission can be from person to person through ingesting contaminated food and water, becoming an important pathogen in children older than six months.

Treating enteroinvasive *Escherichia coli* (EIEC) infections focuses primarily on supportive care to control symptoms and prevent dehydration. In cases where antibiotic treatment is warranted, certain antibiotics can target non-Shiga toxin-producing (non-STEC) strains of *E. coli*, including EIEC.

Patients with profuse diarrhea or vomiting associated with EIEC infection should receive rehydration therapy. This involves fluid and electrolyte replacement to prevent or correct dehydration. Oral rehydration solutions containing a balanced amount of salts and sugars can be administered to replace lost fluids. Symptomatic relief measures can be employed to control abdominal cramps, fever, and other discomforts associated with EIEC infection. This may include over-the-counter antipyretics (fever reducers) and analgesics (pain relievers) (MURRAY et al., 2021).

Antibiotic therapy may sometimes be necessary, especially for severe or prolonged EIEC infections. The choice of antibiotics depends on factors such as the severity of symptoms, the susceptibility of the bacteria, and the patient's circumstances. Commonly used antibiotics for treating non-STEC diarrheal *E. coli*, including EIEC, may include Fluoroquinolones and Macrolides (MURRAY et al., 2021).

Enteropathogenic E. coli

The enteropathogenic *E. coli* (EPEC) was the first group to be serologically identified as associated with diarrhea cases in infants, with adherence as its most significant pathogenicity factor. The adhesion process between bacteria and the intestinal epithelial cell membrane, followed by the destruction of microvilli, with actin polymerization, which causes alteration of the cytoskeleton at the site where the bacteria attach due to higher levels of intracellular calcium and protein kinase C, being called adhesion and desquamation (A/D) (ESLAVA and MATEO, 1994) (KNUTTON et al., 1990).

Any food contaminated with human feces from a sick individual, either directly or through contaminated water, can cause illness in others. There are outbreaks associated with meat and unpasteurized milk.

EPEC is known to cause watery and bloody diarrhea (dysentery), the leading cause of diarrhea in children in developing countries (mainly under two years of age); infection usually occurs through improperly treated drinking water and sometimes meat products. Transmission of EPEC has also been reported through contact with domestic animals (DEAN and KENNY, 2009). People who are sick with EPEC may need to be given fluids so they do not lose too much water. Most people recover with supportive care alone and do not need other treatment, in severe cases, the use of ciprofloxacin or azithromycin is recommended (DENHAM et al., 2018).

The most commonly used diagnostic techniques for EPEC include monoclonal antibodies against Bfp, the EPEC-expressed pili used by the pathogen for adhesion purposes). Remarkably, the EPEC Bfp genes are 100% specific for this pathogen and cannot be amplified by PCR in any other known bacterial enteropathogen. More trivial symptoms of the disease (diarrhea, usually lasting up to two weeks, typically bloody) can be used to identify EPEC as a possible cause (DEAN and KENNY, 2009).

Enteraggative E. coli

Strains isolated from patients were found by Scaletsky and Nataro, which serologically did not correspond to the EPEC group. Still, they had a characteristic adherence pattern different from EPEC and were negative on the EAF test (ESLAVA and MATEO, 1994). Later studies found an aggregative adherence phenotype, which is characterized by self-agglutination of bacteria among themselves and also for being nonspecific since bacteria adhere to the surface of Hep-2 cells and the surface of the

coverslip-free Hep-2 cells (NATARO and KAPER, 1998).

The bacterium and several of the molecules it produces are involved in an EAEC pathogenicity system, and its strains can increase the production and secretion of mucus in the mucosa that traps the bacteria that self-agglutinate in a thin film on the intestinal epithelium; this increased production is related to the ability of EAEC to colonize the intestine, causing diarrhea persistently.

EAEC is increasingly recognized as an emerging enteric pathogen and a cause of persistent diarrhea and malnutrition in children and HIV-infected people living in developed countries. It is the second most common cause of traveler's diarrhea. It is a common cause of acute diarrheal illness in children and adults presenting to emergency departments and inpatient units in the US (NATARO et al., 2006). The US National Institutes of Health has classified EAEC as a potential category B bioterrorism agent (HUANG and DUPONT, 2004).

The leading site of action of EAEC is the mucosa of the large and small intestine, with an incubation period of less than 8 hours, and can persist for up to 20 days. This bacteria can cause outbreaks or isolated cases of persistent diarrhea. In children, it can manifest as watery diarrhea, green in color, with mucus, without blood, and with the capacity to worsen, leading to intravenous hydration. It may also cause vomiting and low fever (NATARO and KAPER, 1998; COBELJIC et al., 1996; CRAVIOTO et al., 1991).

Diffuse Adherence E. coli - DAEC

Diffuse Adherence *E. coli* (DAEC) strains do not form microcolonies when adhering to Hep-2 cells. Not much is known about its mechanism of pathogenicity, but a surface fimbria, known as F1845 and involved in the diffuse adherence phenomenon, has been characterized (ESLAVA and MATEO, 1994). Fimbriae coding genes can be found on the chromosome or a plasmid (BILGE et al., 1989).

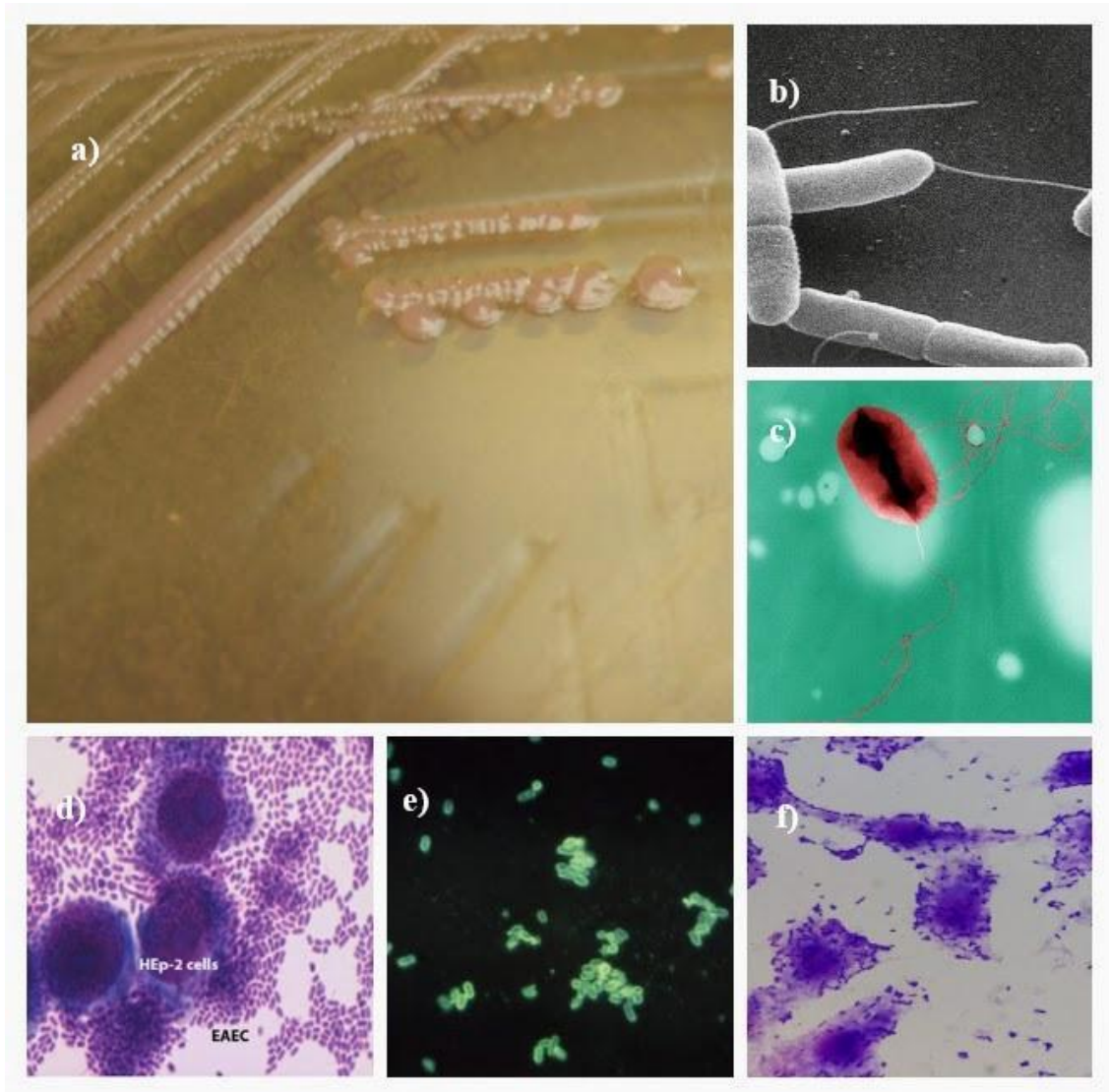
Diffuse adherence has also been associated with an outer membrane protein of 100 kDa in a strain of serotype O126:H27, where its genes have been sequenced. Still, they have only been found in a minority of isolates (BENZ and SCHMIDT, 1992).

When performing in vitro tests on CaCo and Hep-2 cells, DAEC strains can induce the formation of structures in the form of protruding fingers, which confer protection to bacteria. Still, the presence of such systems has not been demonstrated in vitro (POITRINEAU et al., 1995).

Although recent epidemiological studies indicate a high prevalence of DAEC

strains isolated from diarrheal stools, their pathogenicity has not been identified in adults (JALLAT et al., 1993). The relative risk of diarrhea associated with DAEC has been found to increase with a child aged 18 months to 5 years (SERVIN, 2005). The reason for such an age-related phenomenon and the mode of acquisition of the DAEC has yet to be determined (NATARO and KAPER, 1998). As a method of understanding and visualization, a montage with figures of each of the bacteria studied in this article is below.

Figure 2. Types of *Escherichia coli*, where: a) Enteroinvasive *E. coli* colonies on MacConkey sorbitol agar. b) Scanning electron micrograph of the morphological details exhibited by two Gram-negative enterotoxigenic bacteria, *E. coli*. c) Colored transmission electron micrograph of enterohemorrhagic *E. coli* showing its flagella. d) The enteroaggregative *E. coli* (EAEC) adhesion pattern to HEp-2 cells. EAEC adheres to HEp-2 cells in a pattern known as self-aggregation, in which bacteria adhere to each other in a 'stacked brick' configuration. e) Magnified at 600X, this photomicrograph stained with fluorescent antibodies revealed the presence of the enteropathogenic *E. coli* bacteria found in a fecal smear from a child with diarrhea. f) Microscopic appearance of adherence patterns on HEp 2 cells of diffusely adherent *E. coli* (DAEC) isolates from children with diarrhea.



Source: a) American Society of Microbiology, 2019; b) Pathogen Profile Dictionary; c) Pathogen Profile Dictionary; d) Navarro-Garcia et al., 2010; e) Pathogen Profile Dictionary; f) Javadi et al, 2020.

Enterobacter

Enterobacter are members of the Enterobacteriaceae, characterized by being straight, mobile rods. Your cells are positive in the Voges-Proskauer test and Simmons citrate agar. They do not decarboxylate lysine but are positive for ornithine. Malonate is used typically, and the gelatin is slowly liquefied. Cells do not produce H₂S, lipase, and deoxyribonuclease (HOLT et al., 1994).

A study by Gordon and FitzGibbon on enteric bacteria present in the feces of Australian mammals reported the isolation of *Enterobacter cloacae* (*E. cloacae*), *E. cancerogenus* (*E. taylorae*), and *E. aerogenes* (*Klebsiella mobilis*) (GORDON and FITZGIBBON, 1999).

Before the rise in popularity and use of antibiotics, Enterobacter species were

rarely encountered as pathogens. Still, lately, they have been found to cause nosocomial infections such as urinary tract infections and bacteremia, as well as potentially causing community-acquired infections. (GRIMONT and GRIMONT, 2003, 2005).

In the US, the Surveillance and Control of Pathogens of Epidemiological Importance project analyzed 24,179 nosocomial bloodstream infections from 1995 to 2002. Enterobacter species were the second most common gram-negative organism after *Pseudomonas aeruginosa*. Both bacteria have been reported, each accounting for 4.7% of bloodstream infections in intensive care units. Enterobacter species accounted for 3.1% of bloodstream infections in non-intensive care units. Of the approximately 75,000 gram-negative organisms collected from intensive care unit patients in the US between 1993 and 2004, Enterobacter species comprised 13.5% of the isolates. Multidrug resistance will increase, especially in infections caused by *E. cloacae subsp. cloacae* (LOCKHART et al., 2007).

Also, the National Health Safety Network reported a study on healthcare-associated infections between 2006 and 2007 in the US. It found that Enterobacter species are the eighth most common cause of healthcare-associated diseases (5% of all conditions) and the fourth most common gram-negative cause of these infections (HIDRON et al., 2008).

Enterobacter cloacae subsp. cloacae (*E. cloacae*) occurs in the intestinal tract of humans and animals, in hospital environments, on the skin, in water, sewage, soil, and meat. Nitrogen-fixing strains were isolated from the roots of rice plants. *E. amnigenus* has been isolated from water, but some strains have been isolated from clinical specimens from the respiratory tract, wounds, and feces. *E. asburiae* strains have been isolated from clinical specimens, mainly urine, respiratory tract, feces, wounds, and blood (GRIMONT and GRIMONT, 2003, 2005).

Enterobacter is a genus of a common gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacterium belonging to the Enterobacteriaceae family. Two of its well-known species, *Enterobacter aerogenes* and *E. cloacae* have assumed clinical significance as opportunistic bacteria and have emerged as nosocomial pathogens of pathogenic intensive care patients, especially those on mechanical ventilation (MEZZATESTA et al., 2012).

Enterobacter cloacae is ubiquitous in terrestrial and aquatic environments (water, sewage, soil, and food). The species occurs as a commensal microflora in the intestinal tract of humans and animals and is also a pathogen in plants and insects. This habitat

diversity is mirrored by the genetic variety of *E. cloacae* (MEZZATESTA et al., 2012). Recently, data from the MLST and PFGE epidemiological methods revealed the worldwide circulation of several epidemic clonal complexes (IZDEBSKI et al., 2014).

It is also a well-known nosocomial pathogen that contributes to bacteremia, endocarditis, septic arthritis, osteomyelitis, skin/soft tissue infections, and lower respiratory tract, urinary tract, and intra-abdominal infections (FATA et al., 1996). *E. cloacae* tends to contaminate various medical devices, intravenous and other hospital devices (DUGLEUX et al., 1991).

Enterobacter cloacae have intrinsic resistance to ampicillin, amoxicillin, first-generation cephalosporins, and cefoxitin due to constitutive AmpC β -lactamase production. It has a high frequency of enzymatic resistance to broad-spectrum cephalosporins (DAVIN-REGLI and PAGÈS, 2015).

Enterobacter aerogenes, currently known as *Klebsiella aerogenes*, is a gram-negative bacterium isolated as a human clinical specimen from the respiratory, urinary, blood, or gastrointestinal tract (LANGLEY et al., 2001; TINDALL et al., 2017).

The prevalence of *Enterobacter* species infections in clinical wards has also increased due to the introduction of cephalosporins and extended-spectrum carbapenems in antibiotic therapy (ARPIN et al., 1996; ANASTAY et al., 2013).

Some infections caused by *K. aerogenes* result from treatments with specific antibiotics, insertions of venous catheters, and surgical procedures. It can be found in the human gastrointestinal tract, where it usually does not cause disease in healthy individuals but in various types of waste, hygiene chemicals, and soil and water.

The consequence of this antibiotic therapy is the emergence of “pan-drug isolates of *E. aerogenes*” resistant to last-line antibiotics, such as carbapenems and colistin, for which there was no therapeutic option available (CHEVALIER et al., 1999; THIOLAS et al., 2005; DIENE et al., 2013).

Klebsiella

The species of the genus *Klebsiella* belong to the Enterobacteriaceae family, being gram-negative bacilli, facultative anaerobes, oxidase-negative, and devoid of flagellum. Widespread in nature, these bacteria are ubiquitous and can be found in water, sewage, soil, and plants, in addition to colonizing the mucosa of mammals such as humans, horses, and pigs. These microorganisms have a thick layer of polysaccharide capsule responsible for hypermucoviscosity that allows these bacteria to evade some host defense

mechanisms, such as phagocytosis (PODSCHUN and ULLMANN, 1998).

The genus is composed of the species *Klebsiella oxytoca*, *Klebsiella variicola*, *Klebsiella granulomatis*, *Klebsiella singaporensis*, *Klebsiella michiganensis*, *Klebsiella pneumoniae*, and the latter has three subspecies - *Klebsiella pneumoniae* subsp. *pneumoniae*, *Klebsiella pneumoniae* subsp. *ozaenae*, *Klebsiella pneumoniae* subsp. *rhinoscleromatis* (ROSENBLUETH et al., 2004). Despite the variety of known species, new species continue to be discovered. Two new species were recently described: *Klebsiella quasipneumoniae* (BRISSE et al., 2014) and the most recently told *Klebsiella grimontii* (PASSET and BRISSE, 2018).

Klebsiella species have become an important human pathogen and are directly implicated in the increased mortality of patients. The main factors that increase the risks of infections and drug resistance are multiple comorbidities, compromised immune status, and exposure to various antibiotics. Colonization of the respiratory and gastrointestinal tracts is common in hospitalized patients, being a frequent cause of bronchopneumonia, urinary tract infection, and septicemia.

Klebsiella oxytoca (*K. oxytoca*) is a gram-negative, immobile rod-shaped bacterium with a prominent polysaccharide capsule, which provides resistance against host defense mechanisms. *Klebsiella* species are commonly associated with hospital-acquired infections, particularly in immunocompromised patients.

K. oxytoca has evolved extended-spectrum β -lactamases (ESBL) and carbapenemases enzymes that lead to bacterial resistance to β -lactam antibiotics. Therefore, *K. oxytoca* is often resistant to cefotaxime, ceftazidime, and aztreonam. Recently, biofield treatment has been reported to change the sensitivity of antimicrobials against the tested microorganism (Amesh et al., 2022).

K. oxytoca is also an opportunistic pathogen capable of causing a variety of infections, in particular, antibiotic-associated hemorrhagic colitis (AAHC) following the administration of antimicrobial agents and other healthcare-associated disorders in patients with underlying diseases or immunocompromised conditions. (YANG et al., 2022).

Despite its importance, *K. oxytoca* is relatively under the radar and is primarily masked by its notorious relative, *Klebsiella pneumoniae*. However, *K. oxytoca* differs from *K. pneumoniae* in many aspects, such as antimicrobial resistance, virulence, and disease spectrum. Recently, discoveries have significantly advanced our knowledge of this important pathogen. For example, genome-based taxonomic studies have shown that

K. oxytoca is not a single species but a complex comprising at least six species, namely *Klebsiella grimontii*, *Klebsiella huaxiensis*, *Klebsiella michiganensis*, *K. oxytoca*, *Klebsiella pasteurii* and *Klebsiella spallanzanii* (Yang et al., 2022).

Klebsiella variicola is a gram-negative, facultatively anaerobic, immobile bacillus belonging to the Enterobacterales family. This species is mainly associated with opportunistic infections, such as bloodstream infections (BSI) (Maatallah et al., 2014), urinary tract (Potter et al., 2018), respiratory tract (Rodríguez-Medina et al., 2019), and neonatal outbreaks (Piepenbrock et al., 2020). *K. variicola* is often misidentified as *K. pneumoniae* by conventional biochemical tests and automated microbiology systems used worldwide (Rodríguez-Medina et al., 2019). However, surveys of genomic information have recently demonstrated that this species is also associated with severe human infections (Srinivasan and Rajamohan, 2020). Although it is not widely studied, like *K. pneumoniae*, reports indicate that *K. variicola* has apparent differences related to the infection, highlighting the importance of its early diagnosis (Rodríguez-Medina et al., 2019).

The *Klebsiella pneumoniae* bacterium was first described by Carl Friedlander at the end of the 19th century. This bacterium was isolated from the lungs of patients who died of pneumonia and was initially named Friedlander's bacterium (HOLT et al., 2015). It is considered an opportunistic pathogen due to the various human and animal infections it causes, such as bloodstream infections, urinary tract infections, pneumonia, postoperative infections, pyogenic liver abscesses, and meningitis.

Despite the similarity concerning biochemical patterns, virulence, and pathogenicity between environmental and clinical isolates of *K. pneumoniae* (HOLT et al., 2015; PODSCHUN and ULLMANN, 1996), antimicrobial susceptibility is higher in environmental isolates (PODSCHUN and ULLMANN, 1998), probably due to the selective pressure of the hospital environment (HOLT et al., 2015).

This bacterium is gaining interest due to the increase in the number of infections and the growing variety of resistance against antibiotics. Among the types of resistance to antibiotics, two mechanisms have been frequently observed in *K. pneumoniae*: (1) resistance to broad-spectrum beta-lactam antibiotics, mainly carried in plasmids, making bacterial cells resistant to last-generation beta-lactam antibiotics such as cephalosporins, for example; and (2) the expression of carbapenemases, bacteria that have this type of BLSE enzyme (extended-spectrum beta-lactamase) are resistant to almost all existing antibiotics, even carbapenems. (IREDELL; BROWN; TAGG, 2016).

Citrobacter

The genus *Citrobacter* belongs to the Enterobacteriaceae family. It comprises 11 different species of Gram-negative, facultatively anaerobic, motile bacilli that are oxidase-negative and typically use citrate as the sole carbon source.

In the environment, citrobacteria are commonly found in water, soil, and food and as occasional colonizers of the gastrointestinal tract of animals and humans. Although the *Citrobacter* strains that colonize the human gastrointestinal tract are traditionally considered to be of low virulence, they can be the source of many types of infections, such as urinary tract, respiratory, intra-abdominal, wound, bone, bloodstream, and intestinal disorders. *Citrobacter* infections usually occur in debilitated, hospitalized patients with multiple comorbidities, who are at greater risk of acquiring a strain of *Citrobacter* in the hospital environment (SAMONIS et al., 2009).

Although the genus *Citrobacter* comprises 13 species (*Citrobacter amalonaticus*, *Citrobacter braakii*, *Citrobacter diversus*, *Citrobacter farmeri*, *Citrobacter freundii*, *Citrobacter gillenii*, *Citrobacter koseri*, *Citrobacter murlinae*, *Citrobacter pasteurii*, *Citrobacter rodentium*, *Citrobacter sedlakii*, *Citrobacter werkmanii*, and *Citrobacter youngae*), *C. freundii* and *C. koseri* mainly cause infections in humans.

Citrobacter koseri, formerly *Citrobacter diversus*, is a facultative, motile, anaerobic, gram-negative bacillus of the Enterobacteriaceae family. *Citrobacter* species have been implicated in various pathological processes, including urinary tract infections, pneumonia, and bacteremia. Most commonly infecting individuals with severe comorbidities or immunocompromised status, *C. koseri* has rarely been described as causing infections in healthy immunocompetent adults.

Newborns infected with *C. koseri* usually present with sepsis, meningitis, seizures, apnea, and bulging fontanelles. No evidence of torticollis or high fever is current. It occasionally causes meningitis, but it can cause sepsis and ventriculitis. Arterial and venous infarcts are possible due to bacterial infiltration along the main vessel; exudates within the ventricles and ventriculitis can obstruct the ventricular foramina and result in multicystic hydrocephalus with consequent long-lasting shunt difficulties and necrotizing meningoencephalitis with pneumocephalus has been reported.

As with any organisms that cause serious illness, antibiotic susceptibility should guide the final choice of antibiotics. *C. diversus* and *C. freundii* are virtually always resistant to ampicillin, although they occasionally have intermediate susceptibility. Susceptibility to aminoglycosides is variable. *C. koseri* is typically susceptible to

gentamicin and other aminoglycosides. Third-generation cephalosporins are most active against the cause of neonatal disease. *C. koseri* than the first or second-generation cephalosporins (DORAN, 1999).

Citrobacter freundii (*C. freundii*) refers to a facultative anaerobic Gram-negative bacillus that has been reported to exist in soil, water, food, and the natural environment (e.g., hospitals). It belongs to the Enterobacteriaceae family and is found in the normal flora of the intestines of fish and humans. It is a conditionally pathogenic bacterium capable of infecting people with low immunity, causing various diseases (e.g., pneumonia, meningitis, sepsis, bacteremia, and urinary tract infections).

C. freundii has been reported to be resistant to several commonly used antibiotics since its discovery. In recent years, impacted by the indiscriminate use of antibiotics and poor supervision of drug-resistant bacteria, an increasing number of multidrug-resistant *C. freundii* has been clinically detected (AHAD et al., 2017).

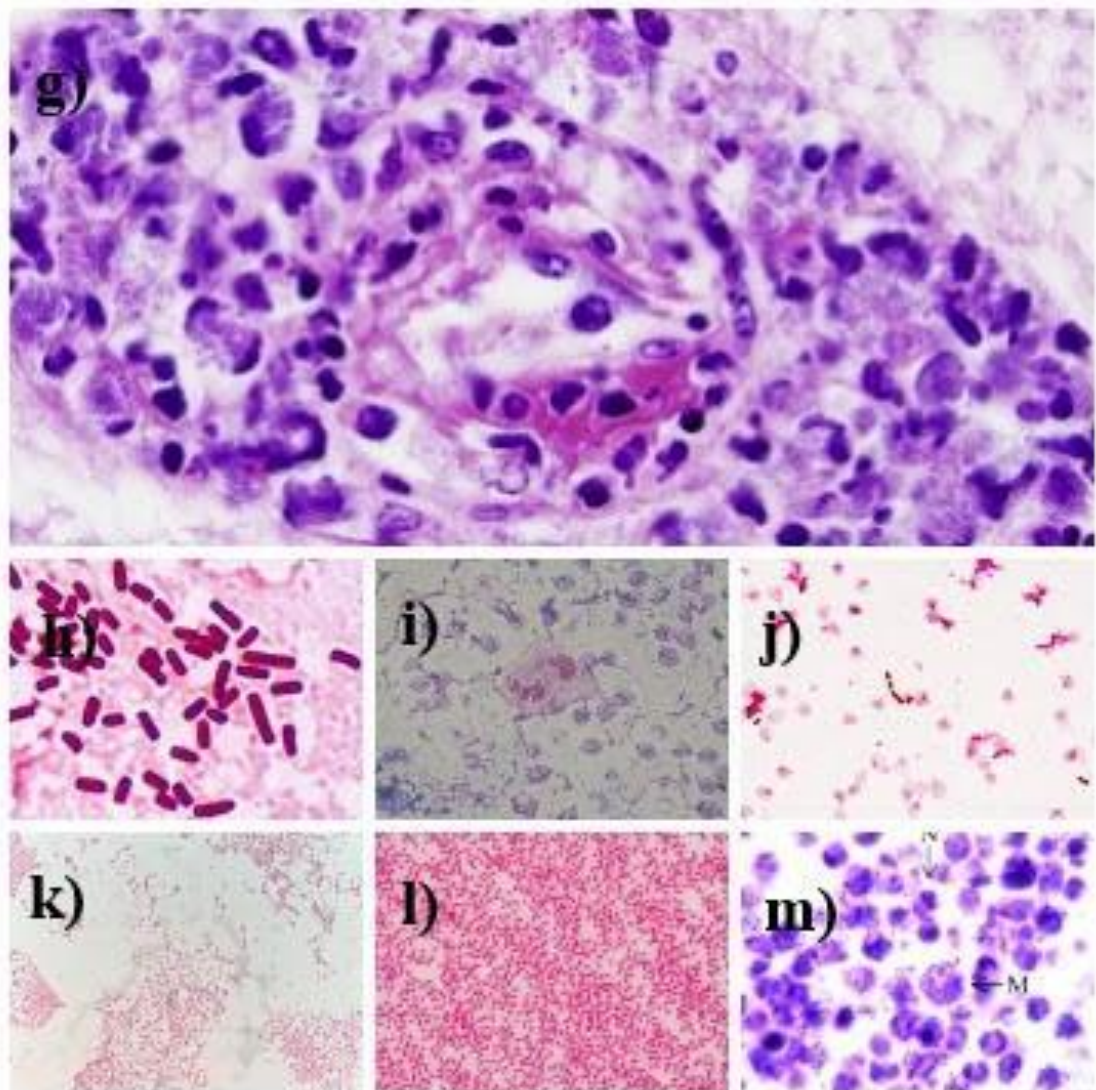
C. freundii may also positively impact the treatment of some types of cancer; specifically, it helps kill and treat cervical carcinoma. Certain strains isolated from *C. freundii* have toxicity against HeLa cells, an immortal human cell line originating from cervical cancer cells (CHOUDHURI et al., 2021).

Biofilm formation plays a vital role in *C. freundii* infection rates, demonstrating different modes of infection that include adhesion and biofilm formation at room temperature. Strong biofilm adhesion can also lead to a reservoir of resistance in antimicrobial genes, meaning that even if bonding is not directly causing infection in these cases, adhesion creates resistance. Large biofilms are formed at 25°C by non-cytotoxic multidrug-resistant strains of *C. freundii* that form due to adhesion in an aggregative pattern to colonic epithelial cells. They have no visible fimbriae on the surface. The expression of fimbriae to form biofilms can be regulated by temperature, similar to those in some enterobacterial bacteria. Rates of biofilm formation are relatively low, but upon growth at room temperature, more strains of *C. freundii* have been shown to create robust biofilms that aid in the persistence of this strain in hospital settings. This suggests that these strains use a different strategy to achieve success and continuation of the disease in the host. This demonstrates that different temperatures can strongly influence biofilm formation (RAMOS-VIVAS et al., 2020).

As a method of understanding and visualization, a montage with figures of each of the bacteria studied in this article is below.

Figure 2. Types of Enterobacters, Klebsiellas e Citrobacters, where: g) Microscopic

image of septicemia caused by *C. koseri* in a calf. h) Microscopic view of *E. cloacae* colony. i) Enterobacter aerogenes infecting the bloodstream and causing septic shock. j) Microscopic (histological) image of *K. oxytoca* Gram stain contributed by Joshua A. Lieberman. k) Gram stain of *K. variicola*, a positive culture, demonstrates Gram-negative bacilli on microscopy (x100). l) Culture of *K. pneumoniae* under an optical microscope. m) Tissue section from a brain abscess that was Gram stained using the MacCullum-Goodpasture technique, showing extracellular *C. koseri* and intracellular *C. koseri* within macrophages and neutrophils.



g) KOMINE et al., 2014; h) Microbe Canvas; i) Quip Labs, 2023; j) LIEBERMAN, 2023; k) MONDO et al., 2021; l) FERREIRA and PIMENTEL, 2022; m) TOWNSEND et al., 2003.

Conclusion

Therefore, the presence of thermotolerant coliforms in water is an issue of great concern for public health and environmental safety. When exploring this topic, the importance of monitoring and managing water quality to mitigate the risks associated with these bacteria is highlighted. Thermotolerant coliforms are widely recognized as indicators of fecal contamination, which can transmit a range of waterborne diseases. Its detection in water sources, especially those intended for human consumption or recreational activities, should motivate immediate actions to ensure human safety.

The importance of advanced testing methods and monitoring protocols cannot be overstated as they enable timely identification and response to contamination events. However, it is equally important to remember that managing thermotolerant coliforms in water is a multifaceted challenge that requires a holistic approach that involves not only improved testing, but also public education, regulatory enforcement and infrastructure development.

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Chapter 2

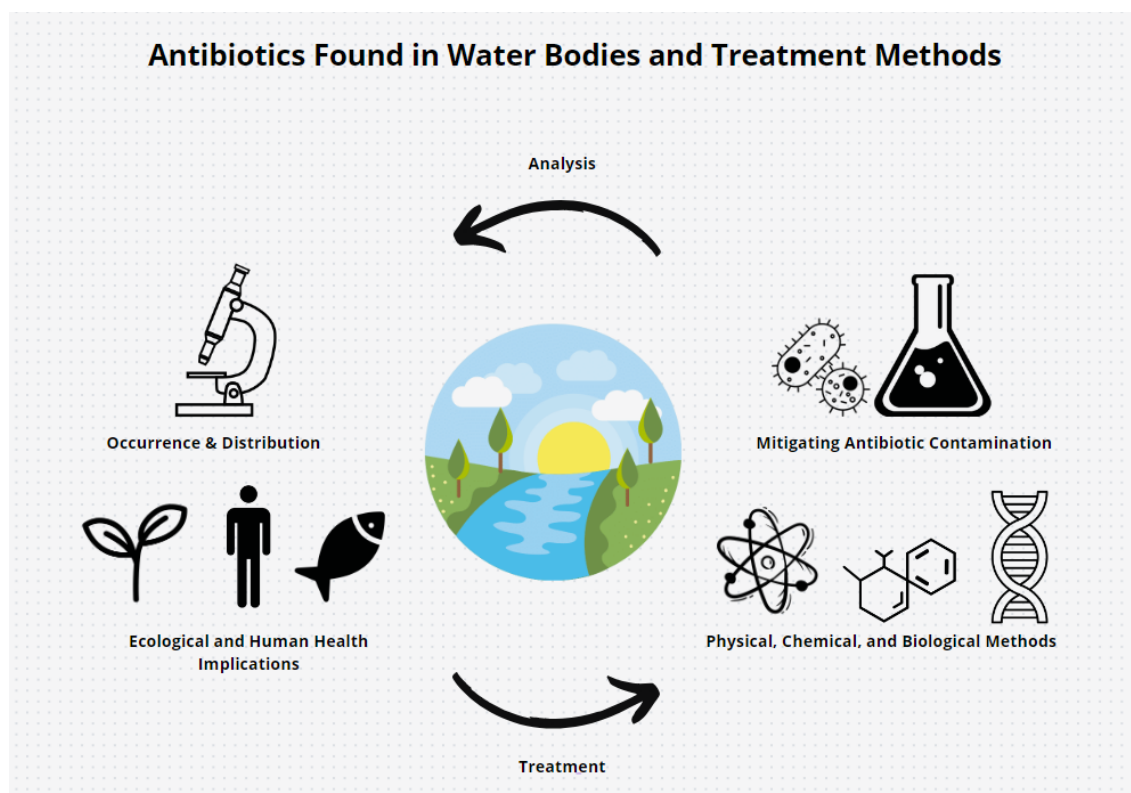
Antibiotics Found in Water Bodies and Treatment Methods

Felipe Oliveira da Silva¹, Aline Frumi Camargo^{1,2}, Helen Treichel^{1,2}

¹Laboratory of Microbiology and Bioprocesses, Federal University of Fronteira Sul, Erechim, Brazil.

²Graduate Program in Biotechnology and Biosciences, Federal University of Santa Catarina, Florianópolis, Brazil.

Graphical Abstract



Abstract

This comprehensive review article provides an in-depth analysis of the occurrence and distribution of antibiotics in aquatic ecosystems, investigating their resistance capability. It also explores the potential ecological and human health implications associated with antibiotic residues in water bodies. Recognizing the significance of mitigating antibiotic contamination in water bodies, this review dedicates substantial attention to the various treatment methods available. These methods are diverse, encompassing physical, chemical, and biological strategies.

Keywords: Drug resistance, Antibiotics, Remediation Techniques, Human and Ecological Health.

Introduction

In a world where the abuse and misuse of antibiotics pose a growing threat to global health, a worrying issue has emerged from an unexpected source: our water supply. Antibiotics, once considered miracle drugs that saved countless lives, are now becoming silent threats as they enter rivers, lakes, and drinking water sources. This contamination of water with antibiotics and antibiotic-resistant bacteria has raised significant concerns about potential impacts on human health and the environment.

With the revolution in medicine, caused by the discovery of antibiotics, came large-scale consumption that had widespread effects on the microbial biosphere. Analyzed by Van Boeckel et al., they showed that humans' worldwide consumption of antibiotics reached 54 billion units – whether pills, capsules, or ampoules – in 2000 and increased by 36% in 2010 (VAN BOECKEL et al., 2014). These authors also stated that this type of medicine in animals for food, supposedly higher than in humans, was more excellent than 63 million kg in 2010. (VAN BOECKEL et al., 2014). Such significant and still increasing amounts of antibiotics overwhelm natural production, increasing selection pressure on bacterial populations in exposed environments (VON WINTERSDORFF et al., 2016). The use and misuse of antibiotics in medicine, agriculture, and aquaculture are associated with the emergence of resistant bacteria in these environments (CABELLO, 2006; PENDERS and STOBBERINGH, 2008; ECONOMOU and GOUSIA, 2015). Most antibiotics are expelled unchanged, being

introduced into the territory directly or through waste and water streams (SARMAH et al., 2006).

As the development of this work delves deeper into the panorama of antibiotic pollution in waterways, it is intended to provide a critical assessment of the methods available for removal, weighing their advantages and limitations. By understanding the implications of these methods, we hope to contribute to the ongoing discourse on preserving water resources and protecting the health of ecosystems and communities.

Antibiotics

Despite the miraculous label, antibiotics are increasingly being misused. Their unsystematic use and disposal have led to resistance to various drugs among the microbiota. In case of reinfection, these antibiotics become useless for therapy. Their increasing use in the clinical, agricultural, and veterinary sectors is analogous to the growing resistance of bacteria to these frequently used antibiotics (DHANORKAR and TAMBEKAR, 2004; AHMED et al., 2010).

Antibiotic resistance has been established to be transmitted in the gut of warm-blooded animals (CHOPRA and ROBERTS, 2001; LAUNAY et al., 2004; FUKAO and YAJIMA, 2012). Studies by Larsson and Fick (2009) and Laxminarayan and Heymann (2012) described the daily infiltration of around 45 kg of Ciprofloxacin from factories into nearby bodies of water. Sjolund et al. report that the spread of environmental pollution led to the discovery of antibiotic-resistant bacteria in uninhabited lands such as Antarctica (SJÖLUND et al., 2008; HERNÁNDEZ et al., 2012).

Prolongation of illness due to a shortage of antibiotics or excessive use of antibiotics in circumstances where their use is not mandatory has become common. The use of antibiotics has skyrocketed in recent years. Sales of cephalosporins increased by 60% from 2005-2009, and sales of other last-line antibiotics increased sixfold from 2005 to 2010 (GANGULY et al., 2011; WESTLY, 2012).

Drug-resistant coliforms are the most commonly infecting bacteria, and the infection is contracted by exposure to unhygienic food, water, bacteria, or their carriers and reckless use of antibiotics (MISHRA et al., 2018). Hanna et al. (2005) investigated the association of drug-resistant *E. coli* infections with epidemiological factors: demographic variables, diet, health, and use of antibiotics and found a positive correlation between *E. coli* infection and contact with infected people, contaminated food, outpatient

visit, misuse of antimicrobials, prolonged medication and frequent consumption of meat with self-prescribed medication. Overconsumption of antibiotics kills the beneficial bacteria in our system, increasing the growth of pathogens that may be insensitive to the antibiotics used, becoming resistant (HANNA et al., 2005). The antimicrobial susceptibility with the production of Extended Spectrum Beta-Lactamases (ESBL) among coliform isolates is cause for concern, as it renders therapy with beta-lactam antibiotics ineffective (PITOUT and LAUPLAND, 2008; PEIRANO and PITOUT, 2010; ROGERS et al., 2011; SHIBL et al., 2012).

Mishra et al. (2013) worked on the prevalence of antibiotic-resistant *E. coli* in the Mahanadi River. The water there involuntarily experiences the influence of varying composition. NMP, modified Eijkman test, biochemical fingerprinting, antimicrobial susceptibility testing, and 16S rDNA ribotyping methods were employed to isolate, enumerate, and identify coliform isolates such as *E. coli* with established resistance to beta-lactam antibiotics, carboxypenicillin coupled with β -lactamase, glycopeptides, carbapenems, macrolides, up to fourth generation fluoroquinolones, cephalosporins. Also reported were resistance rates of isolates against 42 antibiotics used with MAR indices ranging from 0.51 to 0.90, which indicate severe pollution and risk to public health.

El-Zanfaly (2015) reported that antibiotic resistance could increase during sewage treatment processes; his study applied enumeration, antimicrobial susceptibility, granular activated carbon test, and transfer of antibiotic-resistant character tests to characterize ampicillin, sulfaguanidine penicillin, 2-sulfanilamide pyrimidine-resistant coliforms, tetracycline, chloramphenicol, neomycin, and streptomycin. The application of activated charcoal in a pilot experiment at a water treatment plant showed its prevalence with easy resistance transfer. The work insists on including antibiotic resistance in coliforms as a decisive criterion for judging water quality standards (EL-ZANFALY, 2015).

Characterization of the types of antibiotics found in water bodies

According to their chemical structures, antibiotics are classified into several groups, including macrolides, beta-lactams, tetracyclines, quinolones, fluoroquinolones, sulfonamides, phenols, and penicillins (MUHAMMAD et al., 2020).

The tendency of antibiotics to be present in different environments - such as water, soil, and atmosphere - depends on their physicochemical properties, octanol/water split coefficient (K_{ow}), distribution coefficient (K_d), separation constants (pK_a), vapor pressure, and Henry's law constant (K_H) (LEONARD et al., 2022).

The groups and types of antibiotics that are found in water bodies around the world, according to the literature, are expressed in the following board:

Board 2. Classes of antibiotics are found in water bodies around the world.

Groups	Antibiotic Names	Symbol	Excretion	References
Macrolides	Erythromycin	ERY	Excreted in the bile, less than 5% of an orally administered erythromycin dose is excreted in the urine.	KAVI et al., 1988
	Azithromycin	AZI	Biliary excretion of azithromycin, mainly as an unchanged drug, is the primary route of elimination.	ZUCKERMAN, 2004
	Clarithromycin	CLA	Approximately 20% of the dose is excreted in the urine.	MALHOTRA-KUMAR et al., 2007
	Roxithromycin	ROX	More than half of the parent compound excreted unchanged.	ZHONG et al., 2000
Tetracyclines	Tetracycline	TET	Excreted in the urine and feces in high concentrations in a biologically active form.	FDA, 2019
	Doxycycline	DXC	Excreted in the urine and feces in high concentrations in a biologically active form.	FDA, 2019
	Oxytetracycline	OTC	Excretion not described.	-
	Chlortetracycline	CTC	Is mainly eliminated in the feces.	AGWUH and MACGOWAN, 2006
Fluoroquinolones	Ofloxacin	OFL	Between 65% and 80% of an orally administered dose of ofloxacin is excreted unchanged in the urine within 48 hours of administration.	FDA, 2019
	Ciprofloxacin	CIP	There is a lack of available data on the enzymes and types of reactions involved in forming these metabolites.	RUSCH et al., 2018
	Norfloxacin	NOR	Is eliminated by metabolism, biliary excretion, and renal excretion.	GOLDSTEIN, 1987
	Enoxacin	ENX	After a single dose, over 40% was recovered in the urine within 48 hours as an unchanged drug.	YOSHIDA et al., 1993

	Enrofloxacin	ENR	Excretion not described.	-
Sulfonamides	Sulfamethizole	SMZ	With rapid absorption, its metabolization takes place in the liver	FDA, 2019
	Sulfathiazole	STZ	No longer used in humans.	FDA, 2019
	Sulfamerazine	SMR	They lack information regarding their absorption and metabolism.	ANGELAKO et al., 2011
	Sulfaquinoxaline	SQX	Veterinary uses, Only.	DE ASSIS et al., 2016
	Sulfapyridine	SPY	Veterinary uses, Only.	LESCH, 2007
	Sulfamethoxazole	SMX	About 20% of sulfamethoxazole in urine is the unchanged drug.	FDA, 2019
	Sulfadiazine	SDZ	Excretion not described.	-
Amphenicols	Chloramphenicol	CHP	Metabolization occurs via the liver, with 90% conjugated to inactive glucuronide.	FDA, 2019
	Thiamphenicol	TAP	Is predominantly excreted unchanged.	FDA, 2019
	Florfenicol	FF	Excretion not described.	-
β -Lactams	Amoxicillin	AMO	Regarding its route of elimination, doses of 125mg to 1g of amoxicillin are 70-78% eliminated in the urine after 6 hours.	BODEY & NANCE, 1972; GORDON et al., 1972
	Ampicillin	AMP	Ampicillin is excreted largely unchanged in the urine, and its excretion can be delayed by concurrent administration of probenecid.	PADARI et al., 2021
	Cephalexin	CEP	It is excreted in the urine by more than 90% after 6 hours by glomerular	SULLIVAN et al.,

			filtration and tubular secretion, with a mean urinary recovery of 99.3%. Cephalexin remains unchanged in urine.	1969; THORNHILL et al., 1969
	Cefazolin	CEZ	It is excreted unchanged in the urine within the first six hours. Approximately 60% of the drug is excreted in the urine and increases to 70%-80% within 24 hours.	FDA, 2019
	Penicillin G	PEN	The kidneys eliminate penicillin G. Non-renal clearance includes hepatic metabolism and, to a lesser extent, biliary excretion.	FDA, 2019

Source: The Author.

Macrolides

The word macrolide is derived from “macro,” meaning large, and “olideo”, meaning lactone. They are compounds of the second most crucial antibacterial class used in human treatment after the β -lactamases family. For 40 years, they have been used to treat different types of infections. It has been used, especially in patients allergic to penicillins (HUB and HUA, 2005).

This class of antibiotics has activity against Gram-positive, Gram-negative, and anaerobic bacteria. Due to their intracellular concentration in several types of cells, such as macrophages, they can treat infections caused by intracellular pathogens. This class of antibiotics is used in human and veterinary medicine (WANG, 2019; HUB and HUA, 2005).

Macrolides are subdivided into two groups: those of natural origin and those of semi-synthetic origin. The latter can still be divided into three subgroups according to the chemical modification carried out in the core of Erythromycin. In the first subgroup, we have Roxithromycin and Clarithromycin. In the second subgroup, there is Azithromycin. Finally, in the third subgroup, the ketolides (KANFER et al., 1998).

Erythromycin is a bacteriostatic antibiotic produced by a strain of *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*) and belongs to the macrolide group of antibiotics. It was initially discovered in 1952. Erythromycin is widely used to treat various infections, including those caused by gram-positive and gram-negative bacteria. It is available for administration in several forms, including intravenous, topical, and eye drop preparations.

Erythromycin is indicated for treating infections caused by susceptible strains of various bacteria. Erythromycin indications were summarized by the following body systems: respiratory infections, skin infections, gastrointestinal infections, and genital infections/sexually transmitted infections.

In patients with normal liver function, erythromycin concentrates in the liver and is then excreted in the bile. Less than 5% of an orally administered erythromycin dose is excreted in the urine. A high percentage of absorbed erythromycin is not accounted for but is likely metabolized. (KAVI et al., 1988).

Azithromycin is a semi-synthetic macrolide derived from erythromycin by inserting nitrogen into the lactone ring. It is considered the first antibiotic of the azalide subclass (ZUCKERMAN, 2004; WANG, 2009).

It has a broader spectrum of action against Gram-positive microorganisms, high participation in the tissue, stability in an acid medium, and low metabolism rate, which make it more widely used than erythromycin.

Since most azithromycin is eliminated unchanged in the feces, significant active amounts of this compound can appear in sewage treatment plants. Its slow metabolism indicates poor degradation in treatment plants. Its active presence, along with its incomplete degradation in water treatment plants, is a facilitating factor in the development of antibiotic resistance in these stations and in the environment in general and its susceptibility to bind to the soil. Biliary excretion of azithromycin, mainly as an unchanged drug, is the primary route of elimination. Approximately 6% of the administered dose is found as a whole drug in the urine for one week (ZUCKERMAN, 2004).

Clarithromycin, a semi-synthetic macrolide antibiotic derived from erythromycin, inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit. Binding inhibits peptidyl transferase activity and interferes with amino acid translocation during protein translation and assembly. Clarithromycin can be bacteriostatic or bactericidal, depending on the organism and drug concentration.

Because it is more stable in an acid medium, its bioavailability is better than erythromycin, and gastrointestinal intolerance is less (ZUCKERMAN, 2004). Its metabolism takes place in the liver. Between 30 and 40% of an oral dose of clarithromycin is excreted unchanged through the urine, and the rest is excreted in the bile.

After a 250 mg tablet every 12 hours, approximately 20% of the dose is excreted in the urine as clarithromycin. In contrast, after a 500 mg tablet every 12 hours, urinary excretion of clarithromycin is slightly higher, approximately 30%. (MALHOTRA-KUMAR et al., 2007).

Roxithromycin is a semi-synthetic macrolide that derives from and resembles erythromycin, differing in its prolonged half-life. It is active against Gram-positive and Gram-negative (LANGE et al., 2006). It has excellent stability in an acid medium. Its metabolization in animals and humans and biotransformation have not yet been well elucidated (ZHONG et al., 2000).

Roxithromycin is a semi-synthetic macrolide antibiotic structurally and pharmacologically similar to erythromycin, azithromycin, or clarithromycin. It is most effective against certain Gram-negative bacteria, particularly *Legionella pneumophila*. Roxithromycin exerts antibacterial action by binding to the bacterial ribosome and

interfering with bacterial protein synthesis. Used to treat respiratory, urinary tract, and soft tissue infections. Roxithromycin is only partially metabolized in the liver, with more than half of the parent compound excreted unchanged.

Tetracyclines

Tetracyclines were discovered in 1948 as the natural fermentation products of a soil bacterium, *Streptomyces aureofaciens*. The first chemically purified tetracycline was chlortetracycline (1954). Currently, three groups of tetracyclines are available: natural tetracycline products, semisynthetic tetracycline compounds, and chemically modified tetracyclines (CMTs). The literature suggests tetracyclines acting as antibiotics may also affect inflammation, immunomodulation, cell proliferation, and angiogenesis.

Due to their broad spectrum of action and the ease of administering them orally, tetracyclines were widely used in all countries. This led to the development of resistance by microorganisms, mainly through efflux mechanisms and modification in the ribosome.

The widespread production and use of natural tetracyclines in human and animal medicine in the decades following their discovery led to resistance mechanisms and decreased efficacy as first-line antibiotics (LEVY et al., 1989).

Tetracyclines are drugs indicated mainly in the treatment of rickettsial diseases, chancroid, lymphogranuloma venereum, non-gonococcal urethritis, psittacosis, trachoma, cholera, atypical pneumonia caused by mycoplasma and chlamydia, recurrent fevers, tularemia, rat-bite fever, and plague.

Tetracyclines are broad-spectrum antibiotics used to treat infections caused by many aerobic gram-positive and gram-negative bacteria. Urethral and prostatic diseases due to chlamydia and mycoplasma have, in tetracyclines, the drug of choice for treatment.

Urethral and prostatic infections due to chlamydia and mycoplasma have, in tetracyclines, the drug of choice for treatment. This drug is not metabolized but is concentrated by the liver in the bile and excreted in the urine and feces in high concentrations in a biologically active form.

Doxycycline is a member of the tetracycline class of antibiotics and has been used clinically for over 40 years. It is a well-tolerated bacteriostatic drug that acts by inhibiting bacterial ribosomes. It is well absorbed and generally has good tissue penetration. The main side effects are gastrointestinal and dermatological, and it is usually contraindicated in pregnancy or childhood due to concerns about discoloration of developing teeth and possible impact on growing bones (HOLMES and CHARLES, 2009).

It has activity against many organisms, including Gram-positive, Gram-negative, and atypical bacteria. Furthermore, it appears to have some potentially clinically useful anti-inflammatory properties. (HOLMES and CHARLES, 2009).

Tetracyclines, including doxycycline, are concentrated in the bile by the liver and excreted in the urine and feces in high concentrations and a biologically active form. Kidney excretion of doxycycline is about 40%/72 hours in subjects with creatinine clearance of about 75 mL/min. This percentage can drop as low as 1-5%/72 hours in individuals with creatinine clearance below 10 mL/min. (FDA, 2019).

Oxytetracycline is a broad-spectrum antibiotic that works by inhibiting protein synthesis in bacteria. Like other tetracyclines, it treats many common and rare infections. In penicillin-sensitive patients, it is sometimes used to treat spirochetal infections, clostridial wound infections, and anthrax. Oxytetracycline treats respiratory and urinary tract infections, skin, ear, eye, and gonorrhea. However, its use for these purposes has declined recently because of significantly increased bacterial resistance to this class of drugs. The drug is beneficial when penicillins and macrolides cannot be used due to allergy. It can be used to treat Legionnaires' disease as a replacement for a macrolide or quinolone (WHO, 2021).

Chlortetracycline was the first tetracycline to be identified. It was discovered in 1945 at Lederle Laboratories under the supervision of Subbarow and Duggar, who recognized the antibiotic as the product of an actinomycete grown from a soil sample collected at Sanborn Field at the University of Missouri. The organism was named *Streptomyces aureofaciens*, and the isolated drug, Aureomycin, because of its golden color. (JUKES, 1985).

It is combined with a triamcinolone acetonide cream for treating infectious, allergic dermatitis in humans. In veterinary medicine, chlortetracycline is commonly used to treat conjunctivitis in cats, dogs, and horses. It also treats infected wounds in cattle, sheep, and pigs and respiratory tract infections in calves, pigs, and chickens.

Chlortetracycline is not known to undergo significant metabolism. Chlortetracycline is mainly eliminated in the feces. Renal function does not affect the elimination rate (AGWUH and MACGOWAN, 2006).

Fluoroquinolones

Fluoroquinolones are highly effective antibiotics with many advantageous pharmacokinetic properties, including high oral bioavailability, large volume of

distribution, and broad-spectrum antimicrobial activity. With widespread use, antimicrobial resistance to fluoroquinolones has increased. In addition, fluoroquinolones risk serious adverse effects (e.g., *Clostridioides difficile* infection, tendinopathy, neuropathy) and have multiple drug interactions. Thus, fluoroquinolones are usually reserved for cases where the benefits outweigh the risks. (HOOPER, 1999)

Ofloxacin is a quinolone antibiotic used for the treatment of various bacterial infections. When taken by mouth or injected into a vein, they treat pneumonia, cellulitis, urinary tract infections, prostatitis, plague, and infectious diarrhea. Other uses, along with other medications, include the treatment of MDR-TB. An eye drop can be used for a superficial bacterial infection of the eye, and an eye drop can be used for otitis media when a hole in the eardrum is present (WHO, 2009).

Ofloxacin is also sometimes used to treat other types of infections, including Legionnaires' disease (a type of lung infection), certain sexually transmitted diseases, bones and joints, and disorders of the stomach and intestines. Ofloxacin can also treat or prevent anthrax or plague (serious infections that can be transmitted intentionally as part of a bioterrorist attack) in people who may have been exposed to the germs that cause these airborne infections. Ofloxacin may also treat or prevent traveler's diarrhea in certain patients (FDA, 2019).

Its metabolization takes place in the liver. Ofloxacin is eliminated primarily by renal excretion, where between 65% and 80% of an orally administered dose of ofloxacin is excreted unchanged in the urine within 48 hours of administration. About 4-8% of an ofloxacin dose is excreted in the feces, and the drug is minimally subject to biliary excretion (FDA, 2019).

Ciprofloxacin is a broad-spectrum antibiotic of the fluoroquinolone class. It is active against some Gram-positive bacteria and many Gram-negative bacteria. It works by inhibiting a type II topoisomerase (DNA gyrase) and a topoisomerase IV, which is needed to separate bacterial DNA, thereby inhibiting cell division. Fragmentation of bacterial DNA occurs as a result of enzyme inhibition (ZHAO, 2016; POMMIER et al., 2010).

Ciprofloxacin was patented in 1980 and introduced in 1987. It is on the World Health Organization's List of Essential Medicines. The World Health Organization classifies ciprofloxacin as extremely important for human medicine. It is available as a generic drug (WHO, 2019).

Ciprofloxacin is used to treat various infections, including bone and joint

infections, endocarditis, gastroenteritis, malignant external otitis, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, and chancroid.

CYP1A2 primarily metabolizes Ciprofloxacin. The primary metabolites oxociprofloxacin and sulciprofloxacin comprise 3-8% of the total dose. Ciprofloxacin is also converted to the minor ethylene metabolites ciprofloxacin and formylciprofloxacin. These four metabolites represent 15% of the entire oral amount. There is a lack of available data on the enzymes and types of reactions involved in forming these metabolites (VAN GEIJLSWIJK et al., 2006; RUSCH et al., 2018; MACK, 1992).

Norfloxacin is a synthetic broad-spectrum fluoroquinolone antibiotic with variable activity on gram-positive and gram-negative bacteria. It is indicated in the treatment of uncomplicated urinary tract infections (including cystitis), complicated urinary tract infections (restricted use), uncomplicated urethral and cervical gonorrhea (however, this indication is no longer considered adequate by some experts due to bacterial resistance), and *E. coli* prostatitis (NABER, 1991) (EFFA et al., 2011).

Its metabolism is via the liver and kidney. Norfloxacin is eliminated by metabolism, biliary excretion, and renal excretion. It is expected to undergo glomerular filtration and tubular secretion during renal excretion, as demonstrated by its high renal clearance rate of approximately 275 mL/min (GOLDSTEIN, 1987).

Enoxacin is a broad-spectrum fluoroquinolone oral antibacterial agent structurally related to nalidixic acid used to treat urinary tract infections and gonorrhea. It has recently been shown to have a cancer-inhibiting effect (MELO et al., 2011).

It is indicated for the treatment of adults (≥ 18 years of age) with the following infections caused by susceptible strains of the designated microorganisms: uncomplicated urethral or cervical gonorrhea due to *Neisseria gonorrhoeae*, uncomplicated urinary tract infections (cystitis) due to *Escherichia coli*, *Staphylococcus epidermidis* or *Staphylococcus saprophyticus* and complicated urinary tract infections due to *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* or *Enterobacter cloacae*. (WEISSER & WIEDEMANN, 1986).

Its metabolism is hepatic. Some isoenzymes of the cytochrome P-450 hepatic microsomal enzyme system are inhibited by enoxacin. After a single dose, over 40% was recovered in the urine within 48 hours as an unchanged drug (YOSHIDA et al., 1993).

Enrofloxacin is an antibiotic agent of the fluoroquinolone family developed by Bayer Corporation. The FDA approves Enrofloxacin for veterinary use. Due to the

identification of fluoroquinolone-resistant strains of *Campylobacter*, in September 2005, the FDA withdrew approval of enrofloxacin for use in water to treat poultry flocks (DICK, 1993).

The bactericidal activity of enrofloxacin is concentration-dependent, with cell death of susceptible bacteria occurring within 20 to 30 minutes after exposure. Enrofloxacin has demonstrated a significant post-antibiotic effect for Gram-negative and Gram-positive bacteria and is active in both the stationary and growth phases of bacterial replication (WETZSTEIN et al., 1997).

Sulfonamides

Sulfonamide is a functional group (a part of a molecule) that is the basis of several groups of drugs called sulfonamides or sulfa. The original antibacterial sulfonamides are synthetic antimicrobial agents (not antibiotics) that contain the sulfonamide group. Some sulfonamides are also devoid of antibacterial activity, for example, the anticonvulsant sultiame. Sulfonylureas and thiazide diuretics are newer groups of drugs based on antibacterial sulfonamides (HENRY, 1943).

Sulfonamides were the first widely effective antibacterials to be used systemically and paved the way for the antibiotic revolution in medicine. The first sulfonamide, trade name Prontosil, was a prodrug. Experiments with Prontosil began in 1932 in the laboratories of Bayer AG, at the time a component of the substantial German chemical fund IG Farben. The Bayer team believed that coal tar dyes, which can preferentially bind to bacteria and parasites, could be used to attack harmful organisms in the body (OTTEN, 1986).

Many thousands of molecules containing the sulfanilamide structure have been created since its discovery (by one account, over 5,400 permutations in 1945), producing improved formulations with greater efficacy and less toxicity. Sulfa drugs are still widely used for conditions like acne and urinary tract infections and are receiving renewed interest in treating infections caused by bacteria resistant to other antibiotics.

Sulfamethizole is a sulfonamide antibiotic. Sulfonamides are synthetic bacteriostatic antibiotics with a broad spectrum against gram-positive and gram-negative organisms. However, many strains of an individual species can be resistant. It is indicated for treating infections and inflammations in the urinary tract, cystitis, prostatitis, and gonorrhoea. With rapid absorption, its metabolization takes place in the liver. Sulfathiazole is a short-acting sulfonamide drug. It was a common oral and topical

antimicrobial until less toxic alternatives were discovered. It is still used occasionally, sometimes in combination with sulfabenzamide and sulfacetamide.

Except for those formulated for vaginal use, the FDA has withdrawn its approval for all sulfathiazole-containing medications. (Mertschenk et al., 2000) (Rouf et al., 2015) Sulfathiazole is effective against a wide range of gram-positive and gram-negative pathogenic microorganisms. Although it is no longer used in humans, it is used in cattle and is associated with treating bacterial pneumonia and infections of the eyes and hearing aids.

Sulfamerazine is a sulfonamide that is an antibacterial agent used to treat various bacterial infections such as bronchitis, prostatitis, and urinary tract infections (ANGELAKO et al., 2011). They lack information regarding their absorption and metabolism.

Sulfaquinoxaline is an antimicrobial for veterinary use, with activity against a broad spectrum of Gram-negative and Gram-positive bacteria. Sulfaquinoxaline is used to prevent coccidiosis and bacterial infections (DE ASSIS et al., 2016).

This antimicrobial is widely used in poultry production as a therapeutic or prophylactic agent because broiler chickens are raised in intensive industrial farming systems, whose stressful conditions can make these animals more susceptible to infectious diseases (MIRANDA et al., 2008).

Sulfapyridine is a sulfonamide antibacterial drug. At the same time, it was commonly referred to as M&B 693. Sulfapyridine is no longer prescribed for treating human infections, so much so that it has been withdrawn from the market in several countries. However, it can treat linear IgA disease and has use in veterinary medicine. It is an excellent antibacterial drug, but its water solubility is very pH-dependent. Thus, there is a risk of crystallization in the bladder or urethra, which can cause pain or obstruction. It was indicated for treating dermatitis herpetiformis, benign pemphigoid of the mucous membranes, and pyoderma gangrenosum. Its metabolization takes place in the liver (LESCH, 2007).

Sulfamethoxazole is a bacteriostatic sulfonamide antibiotic that interferes with folic acid synthesis in susceptible bacteria. It is usually given in combination with trimethoprim, which inhibits a sequential step in the bacterial synthesis of folic acid.

These agents act synergistically to block two consecutive steps in the biosynthesis of nucleic acids and proteins needed for bacterial growth and division and use them together to help delay the development of bacterial resistance. In this combination,

sulfamethoxazole helps treat various bacterial infections, including those of the urinary, respiratory, and gastrointestinal tracts (FDA, 2019).

Sulfamethoxazole is indicated in combination with trimethoprim, in various formulations, for the following infections caused by bacteria with documented susceptibility: urinary tract infections, acute otitis media in pediatric patients (when clinically indicated), acute exacerbations of chronic bronchitis in adults, enteritis caused by susceptible *Shigella*, prophylaxis and treatment of pneumonia by *Pneumocystis jiroveci* and travelers' diarrhea caused by enterotoxigenic *E. coli* (Goodman et al., 2011). Sulfamethoxazole is metabolized in the human liver to at least five metabolites. These metabolites are N4-acetyl-, N4-hydroxy-, 5-methyl-hydroxy-, N4-acetyl-5-methyl-hydroxy-sulfamethoxazole and an N-glucuronide conjugate. The CYP2C9 enzyme is responsible for the formation of the N4-hydroxy metabolite. In vitro studies suggest that sulfamethoxazole is not a P-glycoprotein transporter substrate (FDA, 2019).

Sulfamethoxazole is mainly excreted really by glomerular filtration and tubular secretion. About 20% of sulfamethoxazole in urine is the unchanged drug, 15–20% is the N-glucuronide conjugate, and 50–70% is the acetylated metabolite. Sulfamethoxazole is also excreted in human milk.

Sulfadiazine is an antibiotic of the sulphamide class. Used in conjunction with pyrimethamine, a dihydrofolate reductase inhibitor, it is the treatment of choice for toxoplasmosis caused by a protozoan parasite. It is a second-line treatment for otitis media prevention of rheumatic fever, chancroid, chlamydia, and *Haemophilus influenzae* infections. It is also an adjunctive therapy for chloroquine-resistant malaria and various forms of bacterial meningitis. It is taken orally and excreted mainly in the urine (FDA, 2019).

Sulfadiazine was approved for medical use in the United States in 1941. It is on the World Health Organization's List of Essential Medicines. Sulfadiazine is available as a generic drug (WHO, 2019).

Amphenicols

Amphenicols are synthetic antibiotics with a broad spectrum of activity used mainly for treating severe bacterial infections in human and veterinary medicine. They exhibit a wide range of movement against gram-negative and gram-positive organisms, including *Streptococcus spp.*, *Staphylococcus spp.*, *Pasteurella spp.*, *Escherichia coli*, *Salmonella spp.* and *Bordetella bronchiseptica* that cause respiratory and enteric diseases

in cattle, through the inhibition of protein biosynthesis by binding to the ribosomal subunits of susceptible bacteria (THOMPSON et al., 2017).

Amphenicols have been used in livestock for many years, being marketed under brands such as Nuflor®, Resflor Gold®, Florocol®, and Selectan®. However, its use has been questioned since the mid-1980s due to concerns related to hematological toxicity and the risk associated with human health by ingesting animal products contaminated with amphenicol. Furthermore, growing global concerns regarding the emergence of drug-resistant bacteria due to overprescribing antibiotics have led to their strictly regulated use in many countries, including the European Union, China, and the United States (THOMPSON et al., 2017).

Chloramphenicol is a broad-spectrum antibiotic that is effective against various serious and susceptible bacterial infections but is infrequently used because of the high risk of bone marrow toxicity. It was discovered after being isolated from *Streptomyces venezuelae* in 1947. Its chemical structure was identified, and it was first synthesized in 1949. It is on the World Health Organization's List of Essential Medicines. It is available as a generic drug (HANH, 1979).

Chloramphenicol is a valuable antibiotic for treating various bacterial infections. This includes use as an eye ointment to treat conjunctivitis. It treats meningitis, plague, cholera, and typhoid fever by mouth or injection into a vein. It is only recommended orally or by injection when safer antibiotics cannot be used. Monitoring medication and blood cell levels every other day is recommended during treatment (ROSENFELD et al., 2009).

The FDA has withdrawn all oral medications containing chloramphenicol due to the high risk of fatal aplastic anemia associated with this specific route of administration (FDA). Metabolization occurs via the liver, with 90% conjugated to inactive glucuronide.

Thiamphenicol (also known as thiophenicol or dextrosulfenidol) is an amphenicol antibiotic. It is the methylsulfonyl analog of chloramphenicol and has a similar spectrum of activity but is 2.5 to 5 times more potent. Like chloramphenicol, it is insoluble in water but highly soluble in lipids. It is used in many countries as a veterinary antibiotic but is available in China, Morocco, and Italy for human use. Its main advantage over chloramphenicol is that it has never been associated with aplastic anemia (RAYMOND et al., 2004). Thiamphenicol is also widely used in Brazil, mainly for treating sexually transmitted infections and pelvic inflammatory disease (FUCHS, 2004).

Unlike chloramphenicol, thiamphenicol is not readily metabolized in cattle,

poultry, sheep, or humans but is predominantly excreted unchanged. The drug is excreted in pigs and rats as parent drugs and as thiamphenicol glucuronate.

Florfenicol is a synthetic fluorinated analog of thiamphenicol, used mainly in veterinary medicine. As a generic, it is now available worldwide (SYRIOPOULO et al., 1981). In the United States, florfenicol is currently indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*, for the treatment of bovine interdigital phlegmon (foot rot, acute interdigital necrobacillosis, infectious pododermatitis) associated with *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*. Florfenicol is also used in aquaculture and is licensed in the United States to control enteric septicemia in catfish. (GAUNT et al., 2013)

Since the early 2000s, it has been used in Europe, mainly treating primary or secondary colibacillosis in broilers and parent flocks. Not allowed in laying hens due to residues in the eggs. It is also indicated in Türkiye. (SHEN et al., 2002) The use of florfenicol in horses, and probably other equines, commonly causes diarrhea. This has been reported to progress to lethal cases of acute colitis. Therefore, using this antimicrobial in the equine patient should be limited to issues where other safer options are unavailable (ROBINSON et al., 2009).

β -lactam

β -lactam antibiotics (beta-lactam antibiotics) contain a beta-lactam ring in their chemical structure. Primarily, they act by inhibiting cell wall biosynthesis in the bacterial organism and are the most widely used group of antibiotics. By 2003, when measured by sales, more than half of all commercially available antibiotics were β -lactam compounds (ELANDER, 2003).

The first β -lactam antibiotic discovered, penicillin was isolated from a strain of the fungus *Penicillium rubens* called *Penicillium notatum* at the time (HOUBRAKEN et al., 2011). β -lactam antibiotics are indicated for preventing and treating bacterial infections caused by susceptible organisms. At first, β -lactam antibiotics were primarily active only against Gram-positive bacteria. Still, the recent development of broad-spectrum β -lactam antibiotics active against many Gram-negative organisms has increased their usefulness (PANDEY and CASCELLA, 2023).

Bacteria usually develop resistance to β -lactam antibiotics by synthesizing a β -lactamase, an enzyme that attacks the β -lactam ring. To overcome this resistance, β -

lactam antibiotics can be administered with β -lactamase inhibitors such as clavulanic acid (PANDEY and CASCELLA, 2023).

Amoxicillin is in the family of beta-lactam antibiotics, a medication used to treat many bacterial infections. Amoxicillin was discovered in 1958 and was used in medicine in 1972. It was approved for medical use in the United States 1974 and the United Kingdom in 1977. It is on the World Health Organization's List of Essential Medicines. It is one of the most commonly prescribed antibiotics for children. Amoxicillin is available as a generic drug. In 2020, it was the 40th most prescribed drug in the United States, with over 15 million prescriptions (FISCHER and GANELLIN, 2010) (WHO, 2021).

Amoxicillin alone is indicated to treat susceptible bacterial infections of the ear, nose, throat, genitourinary tract, skin, skin structure, and lower respiratory tract. Amoxicillin is given with clavulanic acid to treat acute bacterial sinusitis, community-acquired pneumonia, respiratory tract infections, acute bacterial otitis media, infections of the skin and skin structure, and urinary tract infections. Amoxicillin is given with omeprazole to treat the disease of *Helicobacter pylori* (*H. pylori*) (BODEY and NANCE, 1972).

Incubation with human liver microsomes led to the detection of 7 metabolites. Metabolite M1 underwent hydroxylation, M2 underwent oxidative deamination, M3 to M5 underwent aliphatic chain oxidation, M6 underwent decarboxylation, and M7 underwent glucuronidation. Regarding its route of elimination, doses of 125mg to 1g of amoxicillin are 70-78% eliminated in the urine after 6 hours (BODEY and NANCE, 1972; GORDON et al., 1972).

Ampicillin is a semi-synthetic derivative of penicillin that functions as an orally active broad-spectrum antibiotic. It was discovered in 1958 and began to be used commercially in 1961. It is on the World Health Organization's List of Essential Medicines, classifying it as extremely important for human medicine. It is available as a generic drug (WHO, 2019).

Ampicillin is used to treat infections with many gram-positive and gram-negative bacteria. It was the first "broad-spectrum" penicillin with activity against gram-positive bacteria; activity against gram-negative bacteria includes *Neisseria meningitidis*, some *Haemophilus influenzae*, and some of the *Enterobacteriaceae* (although most *Enterobacteriaceae* and *Pseudomonas* are resistant). Its spectrum of activity is increased by co-administration of sulbactam, a drug that inhibits beta-lactamase, an enzyme

produced by bacteria to inactivate ampicillin and related antibiotics (MAGDESIAN, 2017; PAPICH, 2016; HAUSER, 2013).

It is indicated for the treatment of infections - respiratory, gastrointestinal, genitourinary, and meningitis - caused by *E. coli*, *P. mirabilis*, *enterococci*, *Shigella*, *S. typhosa*, and other Salmonella, *N. gonorrhoeae* that do not produce penicillinase, *H. influenzae*, *staphylococci*, *streptococci*. Ampicillin is excreted largely unchanged in the urine, and its excretion can be delayed by concurrent administration of probenecid (PADARI et al., 2021).

Cephalexin is an antibiotic that can treat many bacterial infections. It kills gram-positive and some gram-negative bacteria by stopping bacterial cell wall growth. It is a beta-lactam antibiotic of the first-generation cephalosporin class (GOODMAN et al., 2011; HEY, 2007).

Cephalexin was developed in 1967. It was first marketed in 1969 and 1970 under Keflex and Ceporex, among others. Generic drug versions are available under other brand names and are inexpensive. It is on the World Health Organization's List of Essential Medicines. It is among the most prescribed drugs in the US, Canada, and Australia (SITTING, 2007; WHO, 2019).

Cephalexin is indicated for the treatment of certain infections caused by sensitive bacteria. These infections include respiratory tract infections, otitis media, infections of the skin and skin structures, bone infections, and genitourinary tract infections (FDA). Cephalexin is not metabolized in the body. It is excreted in the urine by more than 90% after 6 hours by glomerular filtration and tubular secretion, with a mean urinary recovery of 99.3%. Cephalexin remains unchanged in urine (SULLIVAN et al., 1969; THORNHILL et al., 1969).

Cefazolin is a first-generation cephalosporin antibiotic used to treat various bacterial infections. Cefazolin was patented in 1967 and began to be used commercially in 1971. It is a generic drug (FISCHER and GANELLIN, 2006).

Cefazolin is a broad-spectrum cephalosporin antibiotic used primarily to treat bacterial skin infections. It can also treat moderately severe bacterial infections involving the lungs, bones, joints, stomach, blood, heart valve, and urinary tract. It is clinically effective against infections caused by staphylococci and streptococci species of Gram-positive bacteria (WHO, 2019)

This drug is not metabolized. Cefazolin is present in deficient concentrations in the milk of nursing mothers. It is excreted unchanged in the urine within the first six

hours. Approximately 60% of the drug is excreted in the urine and increases to 70%-80% within 24 hours.

Benzylpenicillin (penicillin G) is a narrow-spectrum antibiotic used to treat infections caused by susceptible bacteria. It is a natural antibiotic given intravenously or intramuscularly due to oral malabsorption. Penicillin G can also be used in some cases as prophylaxis against susceptible organisms (FDA, 2019).

Natural penicillins are considered the drugs of choice for many infections caused by susceptible aerobic gram-positive organisms, such as *Streptococcus pneumoniae*, Group A, B, C, and G Streptococci, Group D non-enterococcal Streptococci, Viridans group Streptococci, and Non-enterococcal staphylococci.

Natural penicillins can also be used as first- or second-line agents against susceptible aerobic gram-positive bacilli such as *Bacillus anthracis*, *Corynebacterium diphtheriae*, and *Erysipelothrix rhusiopathiae*. Natural penicillins have limited activity against gram-negative organisms; however, they can be used in some cases to treat infections caused by *Neisseria meningitidis* and *Pasteurella*. They are generally not used to treat anaerobic conditions.

About 16-30% of an intramuscular dose is metabolized to penicilloic acid, an inactive metabolite. Small amounts of 6-aminopenicillins acid have been recovered in patients taking penicillin G. A small percentage of the drug appears to be hydroxylated to one or more active metabolites, which are also excreted in the urine. The kidneys eliminate penicillin G. Non-renal clearance includes hepatic metabolism and, to a lesser extent, biliary excretion (FDA, 2019).

Explaining the resistance

The increase in bacterial resistance is associated with the number of antibiotics prescribed and the fact that the prescription instructions are not followed precisely (the treatment is not followed to the end). Underprescribing antibiotics occurs for various reasons, including patients asking doctors to prescribe them, doctors prescribing them without explaining why they are not needed, or why they define them cautiously and for medical or legal reasons (PECHÈRE, 2001; ARNOLD and STRAUSS, 2005).

Resistance occurs in three ways: natural resistance of certain bacteria, genetic mutation, or a species acquiring resistance from another (Horizontal Gene Transfer –

THG). Resistance can arise spontaneously through random mutations or, more often, after a gradual buildup over time and due to the misuse of antibiotics or antimicrobials. Resistant microbes are increasingly difficult to treat, requiring higher doses or alternative drugs that can be more expensive or toxic. Microbes resistant to multiple antimicrobials are called "multidrug-resistant" (MMR), or sometimes superbug.

Natural Resistance

Some bacteria are naturally resistant to antibiotics. One of the defenses of some bacteria is a substance that destroys the antibiotic molecule. When the antibiotic is close to the bacteria, they release their chemical defenses, so the antibiotic stops working. Another way to protect yourself is to change your proteins so that the antibiotic no longer touches the outside of the body, which are the cells. For example, many antibiotics try to bind to bacterial receptors, molecules that are outside the bacteria and receive signals. Some of these bacteria can change the shape of their receptors so that the antibiotic cannot attach to them. If it can't bind, it can't kill the bacteria.

This type of resistance is caused by the structural characteristics of bacteria and is not associated with the use of antibiotics. It has no hereditary property. It develops as a result of natural resistance, or microorganisms not embracing the structure of the target antibiotic, or antibiotics not reaching their target due to their characteristics. For example, the Gram-negative bacteria vancomycin does not pass through the outer membrane, so Gram-negative bacteria are naturally resistant to vancomycin. Likewise, L-shaped bacteria, which are wallless forms of the bacteria, and bacteria such as *Mycoplasma* and *Ureaplasma* are naturally resistant to beta-lactam antibiotics that inhibit cell wall synthesis (CEZUR & DEMÍROZ, 2013).

Genetic Mutation

The likelihood that a mutation will confer an antibiotic resistance phenotype (variability) affects the mutation rate. The variability depends on the structure and number of genes where mutations can produce a selectable phenotype. The DNA structure of a gene is essential for variability. If all positions mutate equally, long genes may be more susceptible to mutation than short ones. However, the size of the gene is not the most critical factor in terms of its variability, as not every transformation in the gene encoding

the antibiotic target leads to resistance. Resistance only occurs through permissive mutations (non-lethal, or more broadly, that do not cause an unacceptable loss of fitness) and can produce the resistance phenotype. The probability of an effective resistance mutation is proportional to the number of such positions. For example, in *Escherichia coli*, changes in at least seven places in the *gyrA* gene have led to a quinolone resistance phenotype. Still, changes in as few as three positions in the *parC* gene lead to a resistance phenotype (HOOPER, 1999).

The mutation rate of the *gyrA* gene is expected to be higher than that of the *parC* gene; indeed, *gyrA* mutants are found more frequently than *parC* mutants. In contrast, in *Streptococcus pneumoniae*, resistance to quinolones is due to changes in two positions of *gyrA*. Still, it is due to changes in five parts of *parC*, and *parC* mutants are the most common. In the same organism, the gene encoding 2× PBP, whose mutations can confer resistance to β -lactams, has 13 positions for cefotaxime resistance but only 1 for penicillin resistance. This, together with the number of different targets for both antibiotics, may contribute to the higher mutation rate of cefotaxime resistance compared to penicillin resistance (HAKENBECK et al., 1999).

Furthermore, resistance to streptomycin is caused by an alteration of one or a few nucleotides in the gene encoding the ribosomal protein S12 (Bonny et al., 1991), and the rate of spontaneous mutations is low. The stability of the sequences containing the nucleotides associated with the mutant phenotype is also essential regarding variability. It has been reported that a single base-specific mutation can vary more than 10,000-fold, and long stretches of repeating bases are prone to deletions or additions due to sliding strand mismatch (DRAKE, 1991) (BZYMEK et al., 1999). Even the location of a gene on the bacterial chromosome affects the mutation rate, as genes located farther from the reproductive origin of the Enterobacteriaceae chromosome can have a mutation rate approximately twice as high as genes closer to it (SHARP et al., 1989).

Since the antibiotic resistance phenotype can result from mutations at different bacterial loci, the emergence of an antibiotic-resistant mutant depends on the total mutation rate resulting from the combination of independent mutation rates in these genes. Classical genetic analysis shows that when mutations in one gene or another can cause antibiotic resistance, the total variation is the sum of the independent mutation values. On the other hand, if mutations in both genes are required to achieve the antibiotic resistance phenotype, the total variability results from separate mutation rates for each gene. We considered two genes that contribute independently to the antibiotic resistance

phenotype with independence values of 10^{-8} . If mutations in any gene can produce an antibiotic resistance phenotype (see independent conversions below), the total variability is 2×10^{-8} ; thus, it is only slightly larger than if only one gene contributing to the resistance phenotype were mutated. On the other hand, when mutations in both genes are needed for resistance (see Cooperative Mutations below), the total mutation rate is 10 to 16, much lower than when only one gene is necessary (MARTÍNEZ & BAQUERO, 2000).

List of the main factors that increase antibiotic resistance mutation rates according to Martínez & Baquero, 2000:

- Unstable sequences around bases relevant to the resistance phenotype
- Long distance from the R gene to the origin of replication
- Large number of sites in the R gene that can give rise to a resistant and permissive phenotype
- Wide variety of R genes
- Low or high copy number of each R gene, if the mutation is recessive or dominant, respectively
- Few independent antibiotic targets or targeted access routes
- Various independent protection mechanisms
- Multiple cooperative targets for antibiotic action or cooperative access routes
- Few cooperative elements in target protection mechanisms
- Bacteria under stress (starvation, antibiotic stress, pathogenic stress)
- Contingency R genes (hypermutable)
- Transposable elements in bacteria
- Bacteria with a hypermutable phenotype (mutator)
- Low-level expression of bacterial programmed cell death
- Pre-existing low-level R gene mutations
- Low biological cost of R gene mutations
- Antibiotic concentrations in the selective window
- Low concentrations of antibiotics or short exposure time
- Slow-killing ability of the antibiotic
- Small phenotypic delay for expression of R gene mutations
- Compartmentalized (structured) physical structure of the selective habitat

Horizontal Gene Transfer

Horizontal gene transfer (THG) or lateral gene transfer (TLG) is the movement of genetic material between unicellular and multicellular organisms other than by ("vertical") transmission of DNA from parent to offspring (reproduction). Factor in the evolution of many organisms. THG influences the scientific understanding of higher-order evolution while significantly changing perspectives on bacterial evolution (KEELING & PALMER, 2008) (VAUX et al., 2017).

Horizontal gene transfer is the primary mechanism for the spread of antibiotic resistance in bacteria. It plays an essential role in the evolution of bacteria that can degrade novel compounds (HUDDLESTON, 2014), such as artificial pesticides, and in developing, maintaining, and transmitting virulence (KEEN, 2012). It often involves bacteriophages and temperate plasmids (VARGA et al., 2016). The genes responsible for antibiotic resistance in one species of bacteria can be transferred into another through various HGT mechanisms such as transformation, transduction, and conjugation, subsequently arming the recipient of the antibiotic-resistant genes against antibiotics. The rapid spread of antibiotic-resistant genes in this way is becoming a challenge in the medical field. Ecological factors may also play a role in the HGT of antibiotic-resistant genes (CAIRNS et al., 2018).

Horizontal Gene Transfer Mechanisms:

- Transformation is a cell's genetic alteration resulting from the introduction, uptake, and expression of foreign genetic material (DNA or RNA). This process is relatively common in bacteria but less so in eukaryotes. Transformation is often used in laboratories to insert new genes into bacteria for experiments or industrial or medical applications (RENNER and BELLOT, 2012)
- Transduction, the process in which bacterial DNA is moved from one bacterium to another by a virus (a bacteriophage or phage) (STEARNS and HOEKSTRA, 2005).
- Bacterial conjugation involves the transfer of DNA via a plasmid from a donor cell to a recombinant recipient cell during cell-to-cell contact (STEARNS and HOEKSTRA, 2005).
- Gene transfer agents are host-encoded virus-like elements in the alphaproteobacteria order Rhodobacterales (MAXMEN, 2010).

Occurrence of antibiotics in water bodies

Antibiotic compounds have different mechanisms in cells, such as suppression of

cell wall synthesis, inhibition of nucleic acid synthesis, modification of cell membranes, suppression of protein synthesis, and DNA inhibition, depending on the different functions of the molecule (PAGE, 2012). Some antibiotic molecules are metabolized in the bodies of humans or animals, while the majority (70-90%) is excreted unchanged through feces and urine (VÄLITALO et al., 2017; GIEDRAITIENĖ et al., 2011).

Antibiotic molecules enter wastewater as major compounds or metabolites of effluents from hospitals, pharmaceutical companies, wastewater treatment plants (WWTPs), aquaculture, and livestock farms. The low removal capacity of WWTPs led to the transfer of large amounts of antibiotics to surface water, groundwater, and even drinking water. Marine environments are the main sites of antibiotic accumulation (HEUER et al., 2002).

The widespread presence of antibiotics in high concentrations in surface water, groundwater, sediments, and biota around the world has made these polluting compounds an emerging concern; these concentrations have been reported as 'ng/L and µg/L' and 'ng/g and µg/g' (CHEN et al., 2017; OHORE et al., 2020).

The presence of these compounds in water bodies - especially in developing countries, where management practices for antibiotics and antibiotic-related waste are not addressed - raises concerns (LIU et al., 2021).

In different regions of the world, the spatial and temporal distribution of antibiotics in water sources varies significantly, and this difference is closely related to the local industrial structure, the mode of disposal of antibiotics in the pharmaceutical industry, and the way of use of antibiotics in livestock. (LIU et al., 2021).

Generally, 50-80% of the total parent compounds are excreted via urine and feces. The highest excretion rates are observed for ciprofloxacin (50-80%) and tetracycline (80-90%), while the lowest excretion rates are observed for erythromycin (5 to 10%), 4 sulfamethoxazole (15 to 30%) or clarithromycin (25%) (Mompelat et al., 2009).

Occurrence in rivers

Antibiotic contamination in small rivers and streams is mainly due to wastewater discharge. Many rivers face severe problems due to antibiotic contamination. Antibiotics in aquatic environments cause the accumulation of these contaminants in the biota (DÍAZ-CRUZ and BARCELÓ, 2007; LARSSON, 2014).

The concentrations of pollutants (including antibiotics) in rivers that pass through urban and rural areas have increased, caused by sewage discharge into these sources. In

addition, population density downstream of the river compared to upstream, dehydration, and insufficient continuous flow of seasonal runoff for dilution are the main factors of high concentrations of antibiotics in the lower reaches of the rivers (DING et al., 2017). Studies on the cognition and mechanisms of action of antibiotics in humans show that their function differs in fish, algae, birds, and other species living in rivers (LU et al., 2013).

The reduction in antibiotic concentrations in rivers may be linked to dilution effects. Variable concentrations in wastewater and effluents are due to the chemical transformation of pollutants – which become metabolites – along with purification systems that have little impact on antibiotic removal (ZHANG et al., 2020).

Occurrence in Lakes

The entry of antibiotics into aqueous media occurs through several sources. Most wastewater treatment plants cannot remove antibiotics effectively, so their production can contaminate surface waters with antibiotics (CHEN et al., 2018).

Lakes, unlike rivers with high water exchange, have low water circulation and are, therefore, more exposed to antibiotic contamination. Although lakes have a high potential for long-term antibiotic storage, information on antibiotic contamination in lakes is much scarcer than in rivers. The entry of effluents from human activities and aquaculture increases the concentrations of these pollutants in lakes (ZHAO et al., 2016).

Quantity vs. Legislation (BR, USA and EU)

According to the United Nations World Report on the Development of Water Resources, in all countries except for the most developed ones, most wastewater is released directly into the environment without adequate treatment, causing harmful effects on human health and the environment. The inadequate disposal of solid waste, the inefficiency of Sewage Treatment Stations, and the release of raw sewage into water bodies are the factors that most contribute to the entry of emerging pollutants into the environment (ANA, 2017; WWAP, 2017).

According to the United Nations (UN) for the environment, conventional sewage treatment processes cannot remove all antibiotics and water-resistant bacteria. There is scientific evidence that microorganisms resistant to several drugs are found in wastewater and may represent foci for *developing* microbial resistance (UNEP, 2017).

In Brazil, CONAMA resolutions n° 357/2005 and CONAMA n° 430/2011

establish conditions and standards for effluent discharge into water bodies to protect aquatic life. The current potability standard is *set* by Ordinance MS No. 2914 of December 12, 2011, which provides for the control and surveillance procedures for *water quality* for human consumption in the country. The preparation of this Ordinance considered the advances in technical-scientific knowledge in the area, international experiences, and the principles advocated in the World Health Organization Guides on Water Quality for Human Consumption as a systemic and integrated vision in water quality control. However, despite the current legislation listing several chemical and microbiological parameters, it does not establish standard limits for drugs, especially antibiotics, which are in low concentrations (in the order of micrograms and nanograms/L) and are constantly present in aquatic environments.

Brazil

In Brazil, the Ministry of the Environment and Climate Changes, represented by the National Council for the Environment (CONAMA), is responsible for the *primary* legal references *for* water and environmental quality. This body draws up a series of resolutions at the national level that must be followed to preserve the quality of water resources in Brazil.

CONAMA Resolution No. 430 of 2011 completes and amends CONAMA No. 357 of 2005 and contains conditions, parameters, standards, and guidelines for *releasing* effluents. In general, these norms determine several physical-chemical parameters, organic and inorganic chemical substances, algae, and microorganisms in *monitoring* water quality, changing the concentrations established for each substance according to the category to which the water body belongs.

The Ordinance of the Ministry of Health - MS No. 2,914 of 2011 and the Consolidation Ordinance n° 5 of 2017, provides for the control and surveillance procedures for water quality for human consumption and its potability standard. Established parameters: microbiological standard; turbidity standard for post-filtration or pre-disinfection water; and potability standard for chemical substances that pose a health risk – Inorganic; organoleptic pattern of potability; pesticides; disinfectants and by-products of disinfection; cyanotoxins; radioactivity. This Ordinance was based on the World Health Organization (WHO) recommendations and several international standards. The guidelines that define the quality of water suitable for human consumption are established by an ordinance published by the Ministry of Health, and the monitoring

of the quality of this water is also an attribution of that same Ministry through Environmental Health Surveillance.

Among the various activities that fall into the domain of water quality monitoring, there are gaps in legislation that threaten mandatory measures, such as monitoring of contaminants in drinking water - especially antimicrobials - and the release of sewage into water bodies, which is a problem substantial threat to water quality and a danger to human health and the environment.

European Union

In recent decades, the European Union (EU) has implemented various environmental regulations, significantly reducing air, water, and soil pollutants. Chemical legislation has been updated, and many hazardous or toxic substances have been restricted. This has led EU citizens to enjoy some of the highest quality water in the world, and over 18% of the land has been designated as protected areas for nature. The European Parliament and the Council of the EU work together to develop Directives that set quality standards for Member States to follow. In addition to the hydrological and ecological characteristics, several physical-chemical parameters are monitored to ensure compliance with European rivers' "good condition" standards. Standards have been defined to ensure that all these parameters are within competitive ranges.

Directive 60/2000/EC of the European Parliament and the Council established a framework for Community action in the field of water policy, and the EU Water Framework Directive, also known as the Water Framework Directive (WFD), was adopted. This Directive aims to safeguard and restore European water quality while ensuring its long-term sustainable use.

As a first step in implementing the strategy elaborated by the WFD, Decision 2455/2001/EC established the initial priority list of 33 substances, which had no concentration limit protected at the time. It was only in 2008 that the boundaries of the Environmental Quality Standards (EQL) were defined for these substances in discharge waters through Directive 105/2008/CE. These substances were selected based on a prioritization process based on scientific evidence and supported by extensive consultation with experts from Commission services, Member States, stakeholders, and the Scientific Committee on Health and Environmental Risks – SCHER. Substances included on the watch list should be chosen from the available information that suggests

they may pose a significant risk to or via the aquatic environment and for which monitoring data are insufficient.

The first watch list of ten substances or groups of substances was established by Implementing Decision (EU) 495/2015, which included certain medicines such as the macrolide antibiotics Azithromycin, Clarithromycin, and Erythromycin.

The European Commission has adopted a strategic approach to environmental pharmaceuticals, focusing on ecotoxicity - the presence of antimicrobials in the background; and the potential consequences of prolonged exposure in humans. The primary tool to guarantee the quality, safety, and efficacy of medicines for human and animal use and safeguarding the environment is the legislation on drugs.

In the EU, effective monitoring relies on surveillance programs implemented by Member States, with relevant data shared with the Commission. The guidelines were designed to reduce misuse, encourage responsible use of antimicrobials, and apply to all stakeholders involved in their use or prescription in Europe.

U.S

The Environmental Protection Agency (EPA) is responsible for observing and enforcing US drinking water laws and regulations to protect the environment and human health. The Clean Water Act (CWA) is the primary federal law governing water contamination (EPA, 2018).

The EPA/CWA has implemented programs to control pollution, including establishing industry wastewater standards and creating national recommendations for water quality criteria for pollutants in surface water. Concerning drinking water quality, the Safe Drinking Water Act (SDWA) was passed by Congress in 1974 and amended in 1986 and 1996 to protect the quality of all water currently or potentially used for human consumption., either from surface or groundwater sources. This Act authorizes the EPA to establish safe purity standards and obliges all owners or operators of public water systems to comply with standards set to protect human health (EPA, 2018).

The EPA oversees the release of hazardous substances from municipal and industrial facilities that treat wastewater and collect sewage, as well as stormwater discharges from industrial and municipal facilities. The objective is to protect human health and the environment, as required by the National System for the Elimination of Pollutant Discharges (NPDES) - National System for the Elimination of Pollutant Discharges (EPA, 2018).

The SDWA directs the Agency to assess the health implications and prevalence data of unregulated pollutants when determining which contaminants to include on the list. The SDWA also specifies that the list should contain substances that present the most significant risk to public health in drinking water. The EPA uses the Drinking Water Contaminant Candidate List (CCL) to assist in regulatory decision-making and data collection to identify priority contaminants (EPA, 2018).

The Environmental Protection Agency (EPA) implemented the Unregulated Contaminant Monitoring Rule (UCMR) to track contaminants in drinking water that are not yet regulated under the Safe Drinking Water Act (SDWA). This program aims to collect data on suspected pollutants in drinking water, estimate the level of exposure, and identify the number of people at risk. By collecting this information, EPA can make informed decisions to protect public health. In addition, the EPA publishes a Contaminant Candidate List (CCL) of contaminants that are not currently regulated by national drinking water standards but may be found in public water systems. This list is updated periodically, with the last version published in 2016 (CCL4). The CCL includes a variety of waterborne pathogens, chemicals, and pharmaceuticals, such as erythromycin, which was also included in the previous version. Contaminants in the CCL are evaluated to determine if they meet SDWA regulatory criteria.

The CCL only includes a limited number of harmful substances found in drinking water that require further investigation into their potential health effects and the levels at which they occur. If sufficient data and information are available, a Regulatory Determination will be made to increase the level of a specific contaminant. The SDWA also requires the Agency to select at least five contaminants from the current CCL and determine whether they should be regulated with a Core National Drinking Water Regulation (NPDWR). In addition, the EPA must publish a new list of up to 30 unregulated contaminants to be monitored by public water systems every five years, which serves as the basis for future regulatory actions to protect public health (EPA, 2018).

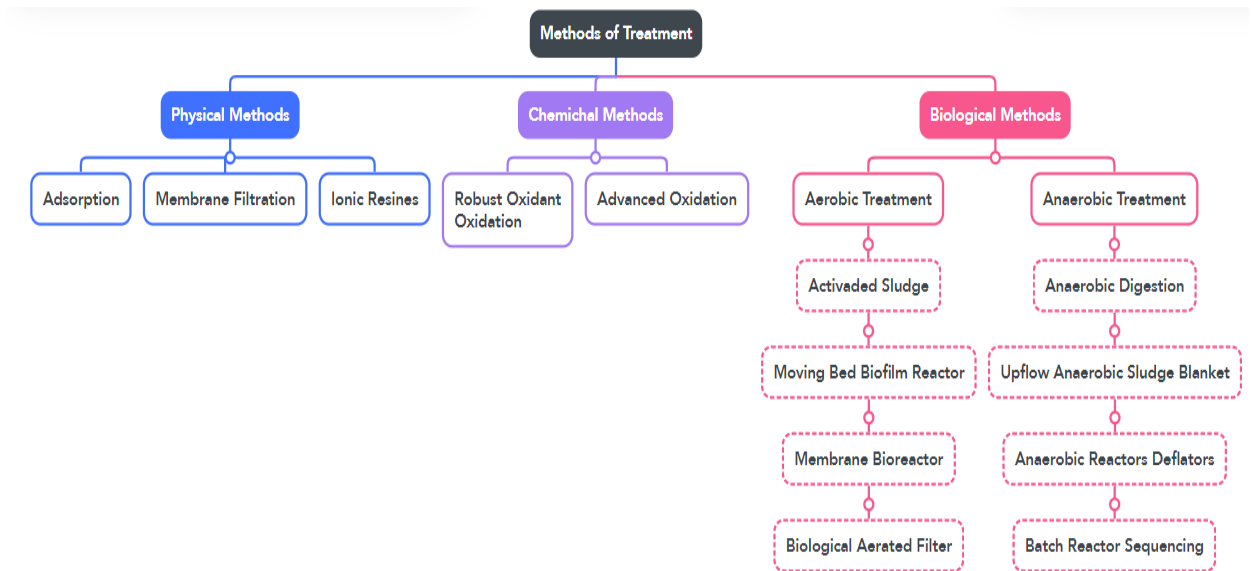
Degradation of Antibiotics

Antibiotic pollution is becoming increasingly serious globally. Although countries with severe antibiotic pollution have introduced corresponding policies, they have come too late and are inadequate to address the problem.

Researchers are developing environmentally friendly alternatives to antibiotics to reduce

their use. At the same time, due to their excessive content in aquatic environments, researchers have developed various treatment techniques to degrade antibiotics. These methods can be divided into the following categories: physical treatment, chemical treatment and biological treatment (LIU et al., 2021). To better understand the types of treatment, the flowchart shown in the Figure 3 was developed.

Figure 3. Flowchart of the methods of treatment of water.



Source: The Author.

Physical Methods

Physical treatment is a water purification method based on the enrichment and transfer of pollutants by physical action. This section reviews the research applications of three approaches, adsorption, membrane filtration, and ionic resins, for removing antibiotics from water, including treatment efficiency, removal mechanisms, and influencing factors.

Adsorption

The term adsorption is the accumulation of matter from a gaseous or liquid phase to the surface of a solid phase and may involve physical and chemical adsorption. The components that bind to the surface are called adsorbates, while the solid phase that retains the adsorbate is called an adsorbent. The removal of substances from the surface is called desorption.

Adsorbent materials are fast, efficient, and economical in treating antibiotics. Due to the unique and superior physical properties of most adsorbent materials, such as higher specific surface area and higher porosity, they can provide more active adsorption sites

for adsorbates, combined with van der Waals forces between adsorbents and adsorbates, electrostatics, bonding of hydrogen, Π - Π bonds and hydrophobic forces, which can efficiently adsorb and remove pollutants in water (XIANG et al., 2019).

The most common process is the application of activated carbon to remove organic substances. Activated carbon can be granular or powder-based, and its application depends on the treatment needs and expectations of final water quality. Activated carbon filtration (ACF) is primarily used as a polishing step in water treatment processes and is gaining renewed focus in recent years for its effectiveness in removing emerging contaminants called “micropollutants” (LAHNSTEINER, 2020).

Obtained from burning some types of wood, activated carbon is a form of carbon that contains pores between atoms. The material, mainly of plant origin, can be used in powder or granules and aims to remove impurities through its pores.

Powdered activated carbon has a large surface area and well-distributed pore structure, with a predominance of medium pores and macropores. It is mainly used in batch filtration processes. Among its applications, the purification of substances such as caffeine, fats, sodium carbonate, wines, fruit juices, alcoholic beverages, municipal water, sugar refining, soft drinks, and antibiotics stands out.

Granulated activated carbon, on the other hand, is used in columns with fixed or mobile beds through which the fluid is filtered and purified, being responsible for adsorption processes in the liquid or gaseous phase. Applications include filters to remove chlorine from drinking and industrial water.

Despite numerous applications, activated carbon retains impurities with carbon and chlorine at the base of the composition. Therefore, other substances like sodium and nitrates can pass directly through the product. In addition, its potential is limited. When all the pores are filled, an activated carbon filter stops working. This is because the adhesion area is compromised and does not allow impurities to settle, so changing the filter to a new one is recommended.

Granular activated carbon (GAC) has been widely used to remove organic contaminants from water and wastewater (SABIO et al., 2006; SOTELO et al., 2012; SOTELO et al., 2014). It has high adsorption capacity and removal efficiency of certain organic substances. In some applications, the removal efficiency can reach 100%. In actual application, adsorption processes using activated carbon are carried out in column mode (PUTRA et al., 2009). Furthermore, the United States Environmental Protection Agency (USEPA) has designated GAC as the best technology for treating many regulated

organic pollutants (SOTELO et al., 2012).

Membrane Filtration

Membrane filtration is a green, harmless, and efficient treatment method, widely used in the zero-emission research field and without harmful additives. Membrane separation technology uses micro and nanoporous membranes to trap or reverse the osmosis of antibiotics in water for purification purposes. In practice, membrane separation is commonly used in conjunction with other methods to remove antibiotics from the aquatic environment (LIU et al., 2021)

The membrane filtration method has the advantages of high separation efficiency, wide application range, and simple operation. Standard membrane treatment processes include microfiltration membranes, ultrafiltration membranes, nanofiltration membranes, and reverse osmosis membranes (HUANG et al., 2022)

Membrane technology is considered one of the most promising water treatment methods due to its high separation selectivity, low energy consumption, lack of additional chemical requirements, relatively easy sizing, and the possibility of continuous operation (YING et al., 2017; GOH and ISMAIL, 2018). However, the membrane process also has some disadvantages. During filtration, the accumulation of contaminants on the surface and within the membrane's pores can lead to fouling of the membrane by blocking and rejecting the membrane (MILLER et al., 2017). Thus, its prolonged use can lead to a decrease in its performance. Furthermore, membrane separation is a physical process, so although contaminants such as antibiotics may be concentrated, they are not decomposed, making it difficult to remove the impurities (JHAVERI and MURTHY, 2016).

Although membrane filtration effectively retains antibiotics, the retention efficiency depends on the antibiotic type and the solution's pH. Changes in pH can affect the degree of protonation of amine and antibiotic groups, the strength of membrane surface charge, and the presence of antibiotics.

Ion Exchange

Ion exchange is a chemical process to remove unwanted ions dissolved in water and wastewater. To remove the ions, they are exchanged for ions of similar charge. When positive ions are exchanged during water treatment, the cations that come into contact with the ion exchange resin are exchanged for other available positively charged ions (usually sodium) on the surface of the resin. During anion exchange, negatively charged

ions are exchanged for other negative ions on the surface of the resin. These ions are usually chloride ions. Anion exchange is essential as the ions can remove contaminants such as nitrate, arsenic, sulfate, and fluorine. Ion exchange in water treatment involves removing unwanted ionic pollutants from water by replacing them with another ionic substance. The four ion exchange methods are water softening, deionization, demineralization, and dealkalinization.

It is essential to remove contaminants from water to make it safe. When removing impurities, the contaminant and the exchanged substance must be dissolved and carry the same type of electrical charge. The most common ion exchange is water softening, which reduces calcium and magnesium in the water.

Bound antibiotics also exist in the form of ions, and research with magnetic ion exchange resins has also begun to be used. The structure of magnetic ion exchange resin contains a polyacrylic acid matrix, quaternary amine functional group, and magnetization components, which can act as a weak magnet (LUO et al., 2022)

Unlike traditional ion exchange resins, magnetic ones have smaller particle sizes and larger specific surface areas, which can quickly adsorb pollutants.

It was found by Wang et al. that compared with powdered activated carbon, magnetic ion exchange resin has a better adsorption effect on antibiotics, which can have 2 to 7 times greater impact than powdered activated carbon in certain antibiotics. In addition, it is noteworthy that anion exchange is the primary mechanism of the adsorption of antibiotics on the anionic resin, and the hydrogen bond formed between the antibiotics and the resin also enhances the adsorption (WANG et al., 2017).

In addition, the removal performance of the magnetic ion resin for antibiotics is also related to factors such as pH value and coexisting anions. The effect of the pH value is mainly based on the different degrees of protonation of the antibiotics under other pH conditions, which will affect the ion exchange with the ionic resin.

Chemical Treatment

The chemical treatment method is based on the chemical reaction between chemical oxidizing agents or reactive oxides generated in the reaction process and pollutants, thus destroying the chemical molecular structure of the contaminants, further converting the pollutants into non-toxic and harmless small molecular substances or performing the complete mineralization and removal, and finally achieve the goal of pollutant degradation or harmless treatment. Standard chemical treatment methods

include strong oxidizing oxidation and advanced oxidation.

The robust oxidant oxidation method mainly relies on the solid oxidizing property of the oxidant itself to attack the electrophilic group structure in the antibiotic, destroy the chemical structure of the antibiotic, and carry out the oxidative degradation of the antibiotic. Common strong oxidants include chlorination and ferrate oxidation. In addition to water disinfection, chlorination has been used to study antibiotic degradation (HUANG et al., 2022).

Advanced Oxidation Method

Also known as the Advanced Oxidation Process (AOP), they depend on the in situ production of highly reactive hydroxyl radicals ($\cdot\text{OH}$). These reactive species are the most potent oxidants that can be applied to water and can oxidize virtually any compound in the aqueous matrix, often at a diffusion-controlled reaction rate. Consequently, OH reacts non-selectively once formed, and contaminants will be quickly and efficiently broken down and converted into small inorganic molecules. Hydroxyl radicals are produced with the help of one or more primary oxidants (e.g., ozone, hydrogen peroxide, oxygen) and energy sources (e.g., ultraviolet light) or catalysts (e.g., titanium dioxide). Accurate and pre-programmed dosages, sequences, and combinations of these reagents are applied to obtain a maximum yield of $\cdot\text{OH}$. In general, when used under proper conditions, POAs can reduce the concentration of contaminants from several hundred ppm to less than five ppb and, therefore, significantly reduce Chemical Oxygen Demand (DOQ) and Total Organic Carbon (TOC), which earned him the credit of "21st-century water treatment processes" (MUNTER, 2001).

Although oxidation processes involving $\cdot\text{OH}$ have been in use since the late 19th century - such as Fenton's reagent, which was used as an analytical reagent at the time - the use of such oxidative species in water treatment did not receive adequate attention until Glaze et al. al. suggest the possible generation of $\cdot\text{OH}$ "in sufficient quantity to affect water purification" and defined the term "Advanced Oxidation Processes" for the first time in 1987 (GLAZE et al., 1987).

Biological Treatment

Biological treatment is an improved artificial natural treatment technology based on environmental self-purification. It uses the metabolic action of organisms in the environment to oxidize and decompose organic pollutants in water and convert them into

stable and harmless inorganic substances. The mechanism for removing micropollutants is a process of biosorption and biodegradation.

Aerobic Treatment

Aerobic water treatment is a biological technique that uses oxygen to break down organic impurities and other pollutants, such as nitrogen and phosphorus. A mechanical aeration device, such as an air blower or compressor, continuously mixes oxygen into wastewater or sewage. The organic material in the water is subsequently consumed by aerobic bacteria, which convert it into carbon dioxide and biomass, which can be removed.

Aerobic wastewater treatment is a reliable, easy, and effective method of producing high-quality secondary effluents. The resulting sludge is odorless and can be sold as a high-quality agricultural fertilizer. Aerobic treatment systems ensure the total removal of pollutants and nutrients when used in conjunction with anaerobic treatment.

Aerobic treatment of wastewater and sewage can be accomplished using various technologies. These are some: Conventional activated sludge, Moving bed biofilm reactor, membrane bioreactor, and biological aerated filter.

Activated sludge is a collective term for communities of microorganisms and the organic and inorganic materials attached to them. It is used for antibiotic biosorption, antibiotic biodegradation, and flocculation. The complex organisms in the activated sludge form a complex food chain with organic nutrients in the effluent, and the degradation of antibiotics is achieved through the action of the microbial community. Composting with activated sludge removes contaminants through microbial adsorption and biodegradation (LIU et al., 2021).

Activated sludge for antibiotic degradation is usually derived from biopharmaceutical or hospital effluents and significantly reduces post-degradation toxicity after activated sludge treatment. However, this method is highly dependent on environmental factors such as pH, temperature, dissolved oxygen, nutrients, and toxic substances, as well as on the composition and proportion of microorganisms, which influence the degradation time. Under low dissolved oxygen conditions, irritating gases such as ammonia or sulfur dioxide are quickly produced, and the nutrient ratio in wastewater needs to be adjusted frequently; otherwise, the degradation efficiency is low (LIU et al., 2021). Furthermore, the performance of the activated sludge process in removing antibiotics is influenced by the chemical structure of the antibiotics, the nature

of the sludge, and the operational conditions of the biological treatment process (HUANG et al., 2022).

As for the operation of activated sludge, the organic matter is purified through a colony of heterogeneous microorganisms that form activated sludge in the presence of oxygen during the process, which occurs in different stages.

Coarse solids are removed and passed through the equalization tank and into the aeration tank. In this last tank, the whole process is aerobic, and the aeration serves to add oxygen that helps the aerobic bacteria to change the biochemical form of the organic matter, converting them into simple molecules (H_2O , CO_2 ...), thus forming a homogeneous mixture with the effluent undergoing treatment. Continuing, the effluent is sent to a secondary decanter that separates treated effluent and sludge through decantation; the solids settle due to the action of gravity, with the deposition of flakes at the bottom of the tank the effluent clarifies. The flakes decanted with live microorganisms can still decompose organic matter and, therefore, can be recirculated to the aerobic reactor. An activated sludge system has three modes: conventional aeration, extended aeration, and batch.

- **Conventional Aeration:** This modality has a primary treatment system, stabilizing the biodigester sludge. Part of the organic matter is removed before the aeration tank in a primary decanter. This system is designed to be able to handle a large BOD load.
- **Prolonged Aeration:** The biomass remains longer in the system in this mode and receives the same BOD load as in the previous way. There is less food available for the microorganisms, which results in less organic matter per unit volume of the aeration tank and per unit of reactor biomass.
- **Batch:** In this mode, aeration and decantation are carried out in the same tank, and the biological mass is sedimented at the bottom, treating the effluent. A part of the resulting sludge is discarded, and the other part is kept for new treatments.

The Moving Bed Bioreactor (MBBR) offers a solution for wastewater treatment if the "bulk" of the pollutant load needs to be disposed of (as a means of reducing costs) or if the applicable discharge regulations are not as strict. This process is used to remove organic substances, nitrification, and denitrification.

The MBBR system consists of an aeration tank (similar to an activated sludge tank) with special plastic carriers that provide a surface where a biofilm can grow. There is a wide variety of plastic carriers used in these systems. These conveyors vary in surface area and shape, offering different advantages and disadvantages. The surface area plays

a significant role in biofilm formation. Floating carriers allow biofilms to form on the surface, so a large internal surface area is crucial for contact with water, air, bacteria, and nutrients (DI BIASE et al., 2019). The carriers will be mixed in the tank by the aeration system and thus have good contact between the substrate in the influent wastewater and the biomass in the carriers. Today's most preferred material is high-density polyethylene (HDPE) due to its plasticity, density, and durability.

To achieve higher biomass concentration in bioreactors, hybrid MBBR systems have been used where suspended and adhered biomass coexist, contributing to biological processes (MAZIOTI et al., 2017).

The membrane bioreactor (MBR) combines membrane processes such as microfiltration or ultrafiltration with a biological wastewater treatment process, the activated sludge process (JUDD, 2010). The two basic MBR configurations are a submerged membrane bioreactor (SMBR) and a lateral flow membrane bioreactor. In the SMBR configuration, the membrane is located inside the bioreactor and submerged in the effluent. In contrast, in a side-flow membrane bioreactor, the membrane is located outside the reactor as an additional step after the biological treatment.

Two main types of membrane materials are available on the market: organic-based polymeric membranes and ceramic membranes. Polymeric membranes are the most commonly used materials in water and wastewater treatment. In particular, polyvinylidene difluoride (PVDF) is the most popular due to its long service life and chemical and mechanical resistance (ZHEN et al., 2019). The following tables demonstrate the types of materials that membranes can be made from and a comparison between the two types.

The main advantages of this type of process are:

- High-quality effluent: With the small pore size of the membrane, the effluent is clear and free of pathogens;
- Independent Control of Solids Retention Time (SRT) and Hydraulic Retention Time (HRT): Since all biological solids are contained in the bioreactor, the SRT can be controlled independently of the HRT;
- Small footprint: Thanks to membrane filtration, there is a high concentration of biomass contained in a small volume;
- Robust to load variations: MBRs can be operated with a wide range of operating conditions;
- Compact process: Compared to conventional activated sludge (CAS), MBRs are more

compact.

The main disadvantages of an MBR are the complexity of the operating process and the cost, in addition to the membrane life, permeate flux, and the membrane air cleaning rate (air cleaning energy).

The BAF system is one of the latest advances in biofiltration treatment techniques. It is considered a future technology and the most promising development in biological processes for drinking water treatment. The media used for a BAF include fixed or aerated, submerged or floating film reactors, where microorganisms in the form of biofilms are used to remove organic and inorganic matter, after which the suspended solids are filtered through the medium (KLOC & GONZÁLEZ, 2012).

The BAF system design consists of a column or tank as the main reactor, the filter medium, and a set of aeration, backwash, and feed systems (MARSIDI et al., 2018). BAFs are widely used in European countries such as the Netherlands and Germany and are particularly advantageous for treating Fe, Mn, and ammonia in untreated water.

The BAFs are operated with a carrier medium, and the water flows into the reactor after the primary treatment (coagulation/flocculation). The carrier medium is used to accumulate microbial growth in biofilms. The treated water from the immediate treatment can pass through the BAF system from above or below, depending on the design of the water treatment plant. During operation, the air is diffused upwards using a compressor or air blower to oxygenate the biofilm for growth purposes (KLOC & GONZÁLEZ, 2012), as the process depends on autotrophic bacterial activity for the oxidation of Fe and Mn ions (THOMPSON et al., 2012). In addition, the air supply also supports the reaction of Fe and Mn ions with oxygen to form precipitates. Backwashing is necessary to ensure the BAF system's sustainability when maximum pressure loss is increasingly detected so that system performance can return to the original operating condition (RAY, 2012).

The BAF system is a flexible reactor that can remove chemical and physical pollutants (suspension solids), requiring little space for treatment. According to Zhang et al., a BAF system also offers high specific volumetric removal rates compared to a conventional activated sludge process. Previous studies by Fu et al. on the simultaneous removal of Fe and Mn ions showed that 90% and 99% removal rates were achieved using a BAF system. Hasan et al. studied Mn ion removal from heavily and lightly polluted drinking water using a BAF system and found Mn ion removal efficiency of 99.1% and 82.9% for both conditions, respectively. The Fe ion removal efficiency for biological aeration filters was greater than 98% from the beginning and throughout the experiment.

Anaerobic wastewater treatment is a biological process in which microorganisms break down organic pollutants without oxygen. In a basic anaerobic treatment cycle, wastewater is fed into a bioreactor. The bioreactor contains a thick, semi-solid substance called sludge, which comprises anaerobic bacteria and other microorganisms. These anaerobic, or “anaerobic,” microorganisms digest the biodegradable materials present in wastewater, resulting in sewage with lower biological oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS), and by-products, of biogas.

Anaerobic Treatment

Anaerobic wastewater treatment is used to treat a variety of industrial wastewater streams from the agriculture, food and beverage, dairy, pulp and paper, and textile industries, as well as municipal sewage sludge and wastewater. Anaerobic technologies are typically used for wastewater with high concentrations of organic material (measured as BOD, COD, or TSS), usually before aerobic treatment. Anaerobic treatment is also used for specialist applications, such as treating waste streams containing inorganic or chlorinated organics, and is suitable for treating hot industrial wastewater.

Anaerobic methods include Anaerobic Digestion, Upflow Anaerobic Sludge Blanket, Anaerobic Deflector Reactor, and Sequencing of the Batch Reactor.

Anaerobic digestion (AD) is a series of biochemical steps in which microorganisms break down organic matter such as sewage sludge, manure, and food waste in the absence of oxygen (hence the word “anaerobic”), producing primarily gases such as methane, carbon and carbon dioxide and the wet or residual organic mixture known as “digestate”. Anaerobic digestion treats or stabilizes food and other organic wastes, reduces greenhouse gas emissions from waste that would otherwise go to landfills, and produces renewable energy in biogas.

The anaerobic digestion process is used by several industries, including agriculture for processing manure, energy crops, and agro-industrial waste; food and food waste processing industries, slaughterhouse waste, pulp and paper waste, and biochemical waste; and waste and wastewater industries for treatment or management of municipal organic waste and sewage sludge.

In addition to anaerobic digestion, there are other methods to stabilize sewage sludge, such as alkaline stabilization (generally by adding lime), aerobic digestion, composting, and autothermal thermophilic digestion. However, anaerobic digestion is considered one of the most sustainable options, as it generates renewable energy and

reduces the volume of sludge or organic matter.

During the anaerobic fermentation process, four main steps occur: Hydrolysis, Acidogenesis, Acetogenesis, and Methanogenesis, all carried out by a diverse microbial community without oxygen. While these are the main biochemical reactions that take place in a fermenter, it should be noted that other biochemical reactions take place in the fermenter that are not discussed below.

- Hydrolysis – the organic starting material contains compounds that must be accessed/broken down before they are made available as food for microorganisms. Complex polymers such as proteins, carbohydrates (polysaccharides), and lipids must first be broken down into their simplest forms. This “breaking down” of polymers in a digester is usually accomplished by hydrolases secreted by the hydrolytic bacteria in the digester. Hydrolysis is often referred to as the rate-determining step in anaerobic digestion, meaning that it is the slowest step and plays an essential role in determining the residence time of the feedstock in the digester. For this reason, pretreatment methods for anaerobic digestion, such as heat treatment, focus on optimizing this step. The steps of acidogenesis and acetogenesis co-occur.

- Acidogenesis (fermentation) - In this step, acidogenic or fermentative bacteria present in the fermenter absorb some of the hydrolysis products and form volatile fatty acids or VFAs (also called short-chain volatile organic acids) such as propionate, butyrate, and alcohols. Protein-rich raw materials such as sewage sludge also produce a lot of ammonia when amino acids are broken down, which is known to hamper anaerobic digestion. In addition to ammonia, carbon dioxide and other gases, such as hydrogen sulfide, can also be produced.

- Acetogenesis - Acetate is formed from volatile/short-chain fatty acids in acidogenesis along with hydrogen and carbon dioxide.

- Methanogenesis - Methanogenic microorganisms in the digester consume the accessible intermediates produced during acidogenesis and acetogenesis (acetate, hydrogen, and carbon dioxide) to produce methane. This mainly occurs in two pathways: acetoclastic methanogenesis and hydrogenotrophic methanogenesis. Next to methane, carbon dioxide is the second most abundant gas produced in this last stage.

Upflow anaerobic sludge blanket (UASB) technology, often called a UASB reactor, is a form of anaerobic digestion used for wastewater treatment.

The UASB uses an anaerobic process, forming a blanket of granular sludge flooring in the tank. Wastewater flows upwards through the blanket and is processed

(broken down) by anaerobic microorganisms. Upflow combined with gravity keeps the sludge blanket suspended with the help of flocculants. After about three months, the slime mat begins to mature. Small slime granules are formed, covering the surface with bacterial accumulations. Without a supporting matrix, flow conditions create a selective environment where only microorganisms capable of binding to one another survive and multiply. Eventually, the aggregates transform into dense, compact biofilms called "granules".

The by-product is biogas with a high concentration of methane, which can be captured and used as an energy source to generate electricity for export and domestic use. The technology must be constantly monitored as it is deployed to ensure the sludge blanket is maintained and not washed away (causing the effect to be lost). The heat generated as a by-product of electricity generation can be reused to heat the digesters. The screening of the sludge allows a double solid and hydraulic (liquid) residence time in the digesters. Solids that require a high degree of digestion can remain in the reactors for up to 90 days. Sugars dissolved in the liquid waste stream can be rapidly converted to gas in the liquid phase, which can leave the system in less than a day (Finstein et al., 2004)

UASB reactors are generally suitable for dilute wastewater streams (3% TSS with a particle size > 0.75 mm). Anaerobic baffle reactors (ABR) are upgraded septic tanks with baffles along the treatment chamber. Upflow chambers provide enhanced organic matter removal and digestion. Like septic tanks, ABRs are based on physical (decantation) and biological (anaerobic digestion) treatments.

An ABR consists of a tank and reciprocating suspended and permanent baffles that divide the reactors and force the liquid to flow up and down from one chamber to the other, allowing better contact between the fresh effluent entering the reactor and the waste sludge containing the microorganisms responsible for the anaerobic digestion of organic pollutants. Compartmentalization separates solids residence time from hydraulic residence time, allowing anaerobic wastewater treatment with residence times as short as a few hours (EPA 2006). Solids treatment rates are high, while total sludge production is characteristically low (FOXON et al. 2004). They are simple to build and operate and robust to hydraulic and organic shocks (SASSE 1998). However, both sludge and effluent require additional treatment.

ABRs are suitable for a wide range of wastewater, including highly polluted industrial effluents, but their efficiency increases with higher organic load. Therefore,

ABRs are ideal for influents with high levels of unsettled suspended solids and a narrow COD/BOD ratio (SASSE 1998). ABRs are typically used in DEWATS, often in combination with various other treatment stages. A typical DEWATS might be a five-component system consisting first of three anaerobic steps, i.e., a biogas decanter, an ABR, and an anaerobic filter, followed by an aerobic treatment unit such as a built-in swamp (Free-Water Surface CV, Horizontal Subsurface Flow CV or Vertical Flow CV) and a maturation pond (WHO, 2009). BOD can be reduced by up to 90%, which is far superior to removal in a conventional septic tank.

The anaerobic digestion in an ABR consists of different groups of organisms. The first group of organisms is the hydrolytic-fermentative (acidogenic) bacteria that hydrolyze the complex polymeric substrate into organic acids, alcohols, sugars, hydrogen, and carbon dioxide. The second group is hydrogen-producing and acetogenic organisms, which convert the fermentation products of the previous step (hydrolysis and acidogenesis) into acetate and carbon dioxide. The third group is methanogens, which convert simple compounds like acetic acid, methanol, carbon dioxide, and hydrogen into methane. The four main steps that generally cause the reaction of organisms in an anaerobic process are Hydrolysis, Acidogenesis, Acetogenesis, and Methanogenesis (NGUYEN et al., 2010).

The most significant advantage of ABR is its ability to separate acidogenesis and methanogenesis longitudinally of the reactor, allowing different groups of bacteria to grow under the most favorable conditions. This specific advantage also enables the reactor to behave as a two-phase system without the associated high costs and control problems. The two-stage operation allows acidogenesis to dominate in the first compartment and methanogenesis in the subsequent section. This can increase acidogenesis and methanogenesis activity by a factor of four, as the separation of the two phases results in better protection against toxic substances and more excellent resistance to changes in environmental parameters (i.e., pH, temperature, and organic loads) (NGUYEN et al. al., 2010).

Batch Reactor Sequencing (SBR) is an activated sludge process for wastewater treatment. In batches, SBR reactors treat wastewater, such as faecal matter or effluent from anaerobic digesters or mechanical-biological treatment plants. Oxygen is blown through the mix of wastewater and activated sludge to reduce organic matter (measured as biochemical oxygen demand (BOD) and chemical oxygen demand (COD)). Treated wastewater may be suitable for discharge into surface water or for application to land.

While there are different SBR configurations, the basic process is similar. The system consists of one or more tanks, which can be plug-flow or thoroughly mixed. The tanks have a “flow-through” system, with raw sewage (influent) entering on one side and treated water (effluent) leaving on the other. In multi-tank systems, one tank is in settling/draining mode while the other is aerating and filling. In some scenarios, tanks contain a section known as a selector, consisting of walls or baffles that direct flow from one side of the tank to the other or over successive baffles. In this way, the influent and returned activated sludge (RAS) are mixed, and the biological digestion process is started before the liquid enters the main body of the tank (IRVINE & BUSCH, 1979).

In its simplest form, the SBR system consists of a series of tanks that operate on a "fill and remove" principle. Each tank in the SBR system is filled for a certain period and then used as a batch reactor. After the desired treatment, the mixed liquid is allowed to settle, and the clarified supernatant is removed from the tank. The cycle of each tank in a typical SBR is divided into five distinct periods: Several filling and reaction periods vary depending on the aeration and mixing processes. Sludge removal can occur at the end of the reactor phase or during the sedimentation, discharge, or idle phases. A vital feature of the SBR design is using a single vessel for multiple aspects of wastewater treatment.

SBR technology is much more flexible than conventional activated sludge processes in adapting reaction times to the concentration and degree of treatment required for a given effluent. Thus, in addition to adjustments that can be made in an equivalent conventional process (sludge age and solids concentration in the operating mix liquid).

Conclusion

Therefore, antibiotics in water have evolved from a subtle environmental concern to an urgent global challenge. The presence of antibiotics in water sources, with their potential for ecological disruption and the development of antibiotic resistance, is alarming. As reviewed, several removal and treatment methods have been developed to mitigate this problem, each with advantages and limitations.

Activated carbon, advanced oxidation processes, membrane filtration techniques, and other methods have demonstrated their effectiveness in reducing antibiotic concentrations in water, offering promising solutions to reduce the spread of these pharmaceuticals in our ecosystems. Furthermore, combining multiple treatment methods can increase

removal efficiency, providing a comprehensive approach to solving this complex problem.

However, it is essential to recognize that no method is a definitive solution. Choosing the most appropriate treatment approach must consider the specific antibiotics present, the source of contamination, and local environmental conditions. Furthermore, continued research and development efforts are needed to refine existing methods and explore new, more sustainable approaches to solving this problem.

The implications of antibiotic contamination in water go far beyond the limits of aquatic ecosystems. It extends to public health, agriculture, and the global fight against antibiotic resistance. Therefore, multidisciplinary collaboration and a holistic perspective are imperative to address this challenge effectively.

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