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The effect of the discharged wastewater from Tlemcen's university hospital on multi-drug resistance bacteria

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thank you to those who have been part of my life from the very beginning,

Thank you for whom I love .

My precious parents

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Dedication

To Grandma

Daoudi Zoulikha

Though you never got to see this

You're in every page

To my beloving ones

To every dreamer . To every believer

To whom belived in me and to whom who didn't

I MADE IT

To the strongest women the ones who love beyond all

faults , cry behind closed doors

and fights battles that nobody knows about

To whom ever crossed paths in my life

I love you , i cherish you

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ABBREVIATIONS LISTE

ABC: ATB binding cassette

***A .baumannii* :** *Acinetobacter baumannii*

AMR: Antimicrobial resistance

ASS : Aerobic Anaerobic Sequence

ARB: Antibiotic resistant bacteria

ARGs: Antibiotic resistant genes

Amp: ampicillin

BOD: Biochemical Oxygen Demand

BOD5: Biochemical Oxygen Demand 5 days of incubation

BMW:biomedical waste

CAS: conventional/ classic activated sludge

COD: Chemical oxygen Demand

CPE: Carbapenemase-producing Enterobacteriaceae

CRAB: Carbapenem-resistant *A. baumannii*

CTX: Cefotaxime resistante

CW : Constructed wetlands

DNA:Deoxyribonucleic acide

E.coli* :Escherichia coli*

Ecs: Emerging contaminants

***E. casseliflavus* :** *Enterobacter casseliflavus*

E. Faecium: *Enterobacter Faecium*

E. Gallinarum:*Enterobacter Gallinarum*

EHS: Environmental, Health, and Safety

Eps : Efflux pump

ESBLs: extended-spectrum β-lactamases

ESKAPE: *Enterococcus faeciumi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp.*

HGT: Horizontal gene transfer

HWW: Hospita WasteWater

IMP: imipenemase

IS: insertion sequences

KPC: *klebsiela pneumoniae carbaprnemase producing bacteria*

***K. pneumoniae* :** *klebsiela pneumoniae*

LAMP: Loop-mediated isothermal amplification

MRSA: Methicillin-Resistant Staphylococcus Aureus

MATE: Multidrug and Toxic Compound Extrusion

MBR :membran bioreactor

MDR :multidrug resistance

MERS:middle east respiratory syndrome

MFS: Major Facilitator Superfamily

MGEs: Mobil Genetic Elements

MIC : minimum inhibitory concentration

MWW: Municipal Wastewater

NDM: New Delhi metallo -beta -lactamase

NSAIDs: Nonsteroidal anti-inflammatory drugs

OXA : oxacillinase

PACE: Proteobacterial Antimicrobial Compound Efflux superfamily

P. aeruginosa : *Pseudomonas aeruginosa*

PBP : Penicillin Binding Protein

PCR: polymerase chain reaction

PDR : Pan Drug Resistance

PFC : poly ferric chloride

PhACs: Pharmaceutically active compounds

PMQR:plasmid-mediated quinolone resistance

qPCR: quantitave polymerase chain reaction

RNA: Ribonucleic acide

rRNA: Ribosomal ribonucleic acide

RND: Resistance Nodulation-division

RT-PCR : Real Time polymerase chain reaction

SARS:severe acute respiratory syndrome

SARS-CoV2: severe acute respiratory syndrome coronavirus 2

S. aureus :*Staphylococcus aureus*

SMR: Small Multi-drug Resistance

TSS: Total Suspended solid

UV : Ultraviolet

VAN: vancomycine

VIM: Verona integron encoded metallo β-lactamase

VRE: vancomycine resistant Enterococci

WGS:whole genome sequence

WHO : World Health Organization

WWTP: Wastewater treatment plants

XDR: extensive Drug Resistance

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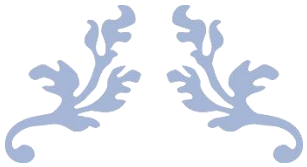


INTRODUCTION

Introduction

Wastewater includes any type (e.g., from agriculture, domestic means, industries, human excretion, commercial sectors, pharmaceuticals, healthcare units) of water which quality has been deteriorated under anthropogenic influence (Buelow et *al*., 2018).

Hospitals serve an important role in human welfare and the growth of medical knowledge and research. They contribute to health care by providing ongoing services to meet complex health problems . These activities, however, are associated with the generation of large amounts of wastewater . Furthermore, hospitals create a significant amount of biological waste (BMW) . The size of the hospital has a significant impact on the nature and quantity of HWW and BMW released, the services and facilities provided, and the waste management strategies used (Patel et *al*., 2019).

Hospital wastewater (HWW) is quite different from the wastewater discharged from other sources and is hazardous and infectious. It consists of a wide range of several micro-and macro pollutants, discharged from operation (surgery) rooms, wards, laboratories, laundry, polyclinics, research units, radiology, and medicine and nutrient solutions used in microbiology laboratories(Kaur et *al*., 2020).

The expansion of resistance to antibiotics in the clinical setting and the environment resulting from reckless use has become a global issue. And aquatic environments are the main receptacles of waste of human activities and hospital effluents are potentially the most dangerous given the nature and significance of xenobiotics and microorganisms that they convey (Anssour et *al*., 2013).

The COVID-19 outbreak has also resulted in a significant increase in the generation of solid waste over the last two years (Agamuthu et Barasarathi, 2021; Das et *al*., 2021). The COVID-19 pandemic, according to Kalantary et *al*. (2021), resulted in a 102.2% rise in BMW generation in Iranian hospitals. Several studies have reported that the incorrect disposal or mistreatment of BMW can have serious consequences for both humans and the environment . As a result, hospitals' solid waste must be properly handled to eliminate any health problems.

Several research on HWW focused on a small number of pharmaceutical substances (mostly antibiotics and anti-inflammatory medications), their destiny in the water management cycle, and their impact on the environment. To control these effluents.

Only a few nations have reference criteria and specialized treatment procedures. However, specific reference standards and treatment methods for industrial effluents are placed at regional or municipal levels by competent authorities, with regard to direct discharge (in surface waters), the reuse after appropriate treatment, and discharge in a municipal wastewater treatment plant (WWTP) (indirect discharge) (Carraro et *al*., 2016).

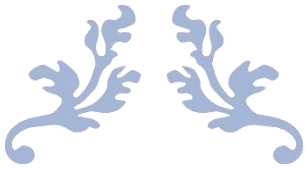
In reality, several nations consider hospital wastewater to be domestic and hence release it straight into the municipal sewer network without any pre-treatment or quality requirements. Typically, reference standards and quality control are applied only after the WWTP effluent

has been treated. Only in a few countries hospital effluents are considered industrial and pretreated before discharge into the municipal sewer network (Carraro et *al*., 2016).

The goal of this study was to identify and describe the resistance mechanisms of multidrug-resistant bacteria detected in hospital effluents, as well as to get a better knowledge of the function of hospital effluents as a source of a wide range of antibiotic resistance genes.

There are few contemporary investigations on the influence of hospital effluents on antimicrobial resistance in aquatic ecosystems, and almost no data on antibiotic resistance in non-clinical settings in Algeria or other African nations.

The objectives of this review are to explain the qualitative features of hospital effluent, estimate their potential effect depending on quantity, and give guidance about main treatment processes and their efficiency worldwide.



CHAPTER 01: HOSPITAL WASTEWATER

1 Definition

Hospital effluents are an example of anthropogenic pollution in action. Hospital wastewaters (HWW) are, in fact, complex mixes of chemical and biological constituents that are released on a regular basis. This mixture is made up of active principles from pharmaceutical drugs and their metabolites, chemicals, disinfecting agents, specific detergents, radioactive markers, iodinated contrast media, nutrients, and bacteria and their antimicrobial resistance genes excreted during diagnostic laboratory and research activities**. (Verlicchi et *al*., 2010).**

2 Hospital wastewater characteristics

2.1 physio-chemical characteristics

According to The Environmental, Health, and Safety (EHS) Guidelines .Table01 summarizes the basic physio-chemical indicators generally prescribed by legislation for establishing the quality of a generic wastewater sample:Temperature (typically around 40 C), pH .Chemical oxygen Demand (COD), BOD also known as Biochemical Oxygen Demand

(most commonly expressed in milligrams of oxygen consumed per litre of sample during 5 days of incubation at 20°C (BOD5))and Total Suspended solid (TSS) **(Carraro et *al*., 2016).**

When a wastewater sample is considered to be a specific effluent, such as that from a hospital, measurements of other specific macropollutants, as total and free chlorine, detergents, disinfectants, oil and grease, total nitrogen, heavy metals, and microbiological indicators (total coliform, faecal coliform, or *Escherichia coli*), as well as toxicity, are required**(Carraro et *al*., 2016).**

BOD is listed as a conventional pollutant under the U.S. Clean Water Act. Typical maximum values range from 10 mg/l for direct environmental disposal and 300 mg/l for disposal to sewer systems **(Biochemical Oxygen Demand (BOD) in Wastewater Treatment | AOSTS, 2018).**

Many studies compared HWW with the Municipal Wastewater (MWW) of different countries to check whether there is a correlation between the wastewater quality parameters, and it was observed that the parameters including BOD, COD, TSS in the hospital effluents were 2–3 times higher than MWW **(Verlicchiet *al*., 2010b).**

The measured concentrations indicate the importance of hospital effluents as a source of organic and inorganic matter when compared to urban effluents, where BOD5 ranges from 100 to 400 mg/l, COD ranges from 43 to 270 mg/l, and total nitrogen ranges from 30 to 100 mg/l. **(El-Ogri et *al*., 2016).**

Table 1:Limitation of health-care facilities wastewater guidelines https://www.ifc.org/wps/wcm/connect/topics_ext_content/ifc_external_corporate_site/sustainability-at-ifc/policies-standards/ehs-guidelines

Polluants	Units	Guideline value
pH	S.U	6-9
Biochemical oxygen demand (BOD5)	Mg/L	50
Chemicol oxygen demand (COD)	mg/l	250
Oil and grease	mg/l	10
Total suspended solid (TSS)	mg/l	50
Cadmium(Cd)	mg/l	0.05
Chromium (Cr)	mg/l	0.5
Lead (Pb)	mg/l	0.1
Mercury(Hg)	mg/l	0.01
Chroline, total residual	mg/l	0.2
Phenols	mg/l	0.5
Total coliform bacteria	MPN[a]/100ml	400
Polychlorinated dibenzodioxin and dibenzofuran (PCDD/F)	Ng/L	0.1
Temperature increase	℃	<3[b]

Note :

[a]MPN= most probable number

[b]At the edge of a scientifically established mixing zone which takes into account ambient water quality , receiving water use, potential receptors and assimilative capacity

2.2 Heavy metals

A wide range of heavy metals are also present in HWW Amongst, They have a wide range of dangerous effects that differ from one metal to another. They may be acute fatal poisons such as (As) and (Cr6+) or may produce chronic diseases such as (Cadimium, Mercury Hg, Lead Pb, and Tl) **(Hassan Omer, 2020).**

Hg has been continuously detected because of its use in diagnostic agents, diuretic agents in treatment, and as an active ingredient of disinfectants. Also, Pt has been found in hospital effluents resulting from excretions by oncological patients treated with cis-platinum and carbo-platinum .It was reported that approximately 98% of unmetabolized metals discharged in HWW within 24 h of usage **(Verlicchi et *al*., 2010b; Khan et *al.*,2020).**

2.3 Emerging contaminants

Excessive medication use has resulted in high concentrations of emerging contaminants (ECs) in diverse water matrices, posing a hazard to aquatic life and humans.

Pharmaceutically active compounds (PhACs) have been found in most water matrices among the various ECs due to their widespread use in medical institutions **(Majumder et *al*., 2021a).** In diverse HWWs, more than 300 PhACs, including their metabolites and trans products, have been discovered **(Khan et *al*., 2021).**

Antibiotics and non-steroidal anti-inflammatory medicines (NSAIDs) such as carbamazepine, an antiepileptic drug, have been reported to be more commonly discovered in HWW.

Furthermore, X-ray contrast media such as iopromide and iomeprol have been discovered in hospital effluents from various nations, according to a few investigations. **(Parida et *al*., 2021).**

Aside from PhACs and contrast media, hospital effluents include a variety of chemical pollutants such as surfactants and disinfectants, which can be hazardous to biotic components . The majority of the ECs found in hospital wastewater had concentrations higher than the no-effect concentration thresholds expected **(Parida et *al*., 2021).**

2.4 Microbiological composition:

Furthermore, wastewater from a variety of hospital services activities, which contains high amount of organic and inorganic debris, transforms sewage into an ecological niche that is well-suited to the development and spread of microorganisms such as viruses, fungi, and a wide range of bacteria.

The most prevalent human-transmitting infectious viruses, such as severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS), are spread through water and are known to cause serious illnesses. These viruses are frequently dangerous to the entire community, resulting in an epidemic or pandemic. The SARS-coronavirus 2 (sars-cov-2) epidemic is the most recent example of virus severity **(Revilla Pacheco et *al*., 2021).**

HWW includes a wide range of numerous bacteria: high levels of anaerobic bacteria including genera such as Bifidobacteriales, Clostridales, Bacteroidales that were likely to originate from the human gut.

The research of HWW microbiology revealed the presence of a high concentration of pathogens; the most predominant pathogenic bacteria found are of genus Bacillus, which count for 80%–90% with Staphylococcus and Streptococcus varying from 5% to 10% **(Oyeleke et *al*., 2009).**

Many studies have reported the presence of a high concentration of coliform species and other bacteria species from HWW in different countries across the world such as *Escherichia coliform (E. coli)*, total coliform, thermotolerant coliform, Streptococcus, Mycobacterium, *Pseudomonas aeruginosa*, etc **(Khan et *al*., 2020; Majumder et *al*., 2021a)** .

Although hospital effluents contain significant concentrations of microorganisms, such as *E. coli* and total coliform, they should not be seen as harmless indicators of fecal contaminations but rather as pathogens that propagate antibiotic resistance due to their exposure to high concentrations of drugs and antibiotics.

The wastewater coming from the laboratories and hospitals also consists of multiple microbes with drug resistance propreties including Acinetobacter, Enterococcus, and Pseudomonas specie and *Staphylococcus aureus* is the most common pathogenic Gram-positive bacterium with high level of multidrug resistance (MDR with drug resistance properties).

3 Pathway of hospital wastewater contaminants into the aquatic ecosystem:

Figure 01 depicts the processes involved in transferring HWW pollutants to aquatic bodies. Hospitals discharge unmetabolized fractions of ECs and other pollutants along with their wastewater **(Verlicchi et *al*., 2010b; Khan et *al*., 2021).**

The total organic components and solids content of HWW are influenced by these pollutants. Most low- and middle-income nations dump hospital effluents straight into freshwater streams without treatment, resulting in high levels of organic debris, pathogens, and ECs in aquatic environments **(Parida et *al*., 2022).**

Co-treatment is used in several countries, including Iran, Japan, Egypt, Australia, South Africa, India, and Thailand, as well as Algeria, where HWW runs into household sewers and is treated alongside MWW at WWTPs **(Parida et *al*., 2022).** The majority of municipal wastewater treatment plants are not designed to handle such complex organic pollutants **(Patel et *al*., 2019).** As a result, municipal WWTPs are only able to remove a portion of the ECs. Therefore, municipal WWTPs frequently become a main source for releasing these ECs into various water matrices **(Patel et *al*., 2019).**

Furthermore, treated sewage from these municipal WWTPs is frequently used in agriculture as fertilizer. As a result, a portion of ECs may leak from the soil into the groundwater **(Khan et *al*., 2021).** Most high-income nations, on the other hand, have on-site hospitals WWTPs that pre-treat HWW before discharging it into municipal sewers **(Verlicchi, 2018).**

Organics, pathogens, and ECs may be removed at much higher levels because this treatment is focused to HWW specific pollutants. On-site treatment of HWW, on the other hand, is costly and takes a lot of energy to operate and maintain**(Parida et *al*, 2022).**

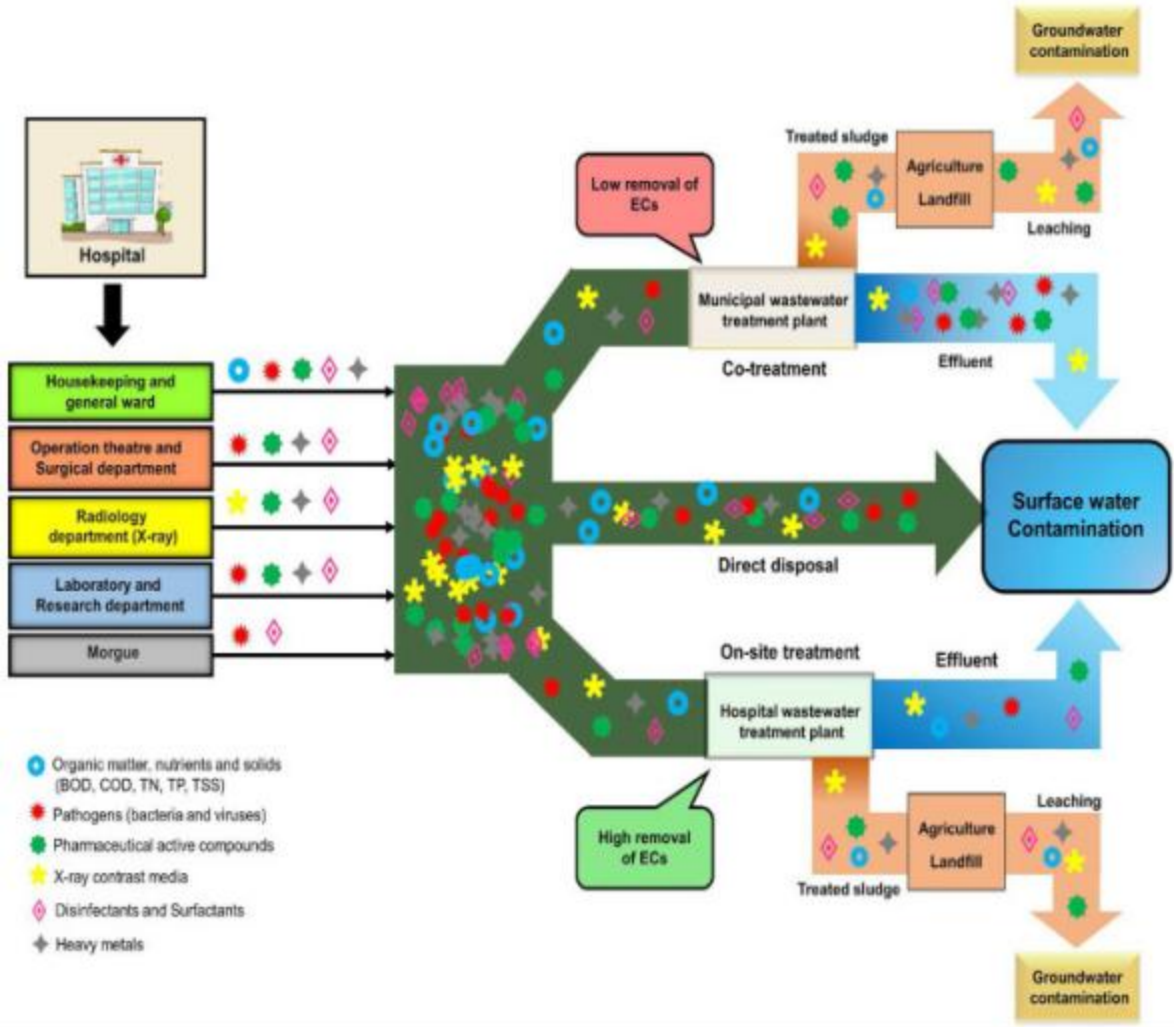


Figure 1: Generation of diffrent contaminants from the hospital facilities and their subsequent pathway into different aqueous environments **(Parida et *al.*, 2022).**

4 The associated hazards with HWW discharge into the aquatic ecosystems

Antibiotics, analgesics and anti-inflammatories, psychiatric medicines, b-blockers, anaesthetics, disinfectants, chemicals from laboratory operations, developer and fixer solutions from photographic film processing, and X-ray contrast media are the most common contaminants detected in HWWs **(WHO, 2013).**

These compounds are eliminated mostly in the urine (55-80%) and less so in the feces (4-30%), as unmetabolized substances, metabolites, or conjugated with inactivating substances. Because of their differences in solubility, volatility, molecular weight, adsorbility and biodegradability, polarity, stability, half-life, and persistency, these substances may behave

differently in the WWTP, and if they are not neutralized in the wastewater treatment, they are released in surface waters with treated effluents **(Verlicchi et *al*., 2010a).**

5 Health risks :

The HWW can act as an ideal growth medium for various pathogenic microbes including bacteria, viruses, fungi, and parasites.

The flashed water from hospital facilities used by patients is most responsible often to urinary tract and lung infections due to the presence of bacteria in biological liquids or urins such as Salmonella, Shigella, Coliforms, Vibrios,Streptococci, Enterobacteria..., and other bacteria responsible for nosocomial infections as Staphylococci, Streptococci, Pseudomonas.... All these pathogens has the ability of acquiring resistance to antibiotics **(Hocquet et *al*., 2016).**

The direct discharge of HWW into municipal wastewater containing disease-causing parasites has also increased the risk of skin infections and other harmful diseases in humans.

The presence of pathogens in treated water poses little risk since humans rarely drink surface water without additional treatment. However, we cannot rule out the risk of human contamination with antibiotic-resistant Pathogens. after consumption of vegetables grown in fields watered with contaminated rivers or direct reuse of treated water for irrigation.

(Blaak et *al*., 2014; Becerra-Castro et *al*., 2015).

4.2 Ecotoxicological risks

Ecotoxicology is the study of the integration of ecological and toxicological effects of chemical polluants on population , communities and ecosystems combined with the fate (transport , transformation and breakdown) of polluants in the environment .

Contamination of natural aquatic ecosystems by hospital wastewater is a major environmental problem since the release of chemical compounds from hospital activities into the natural environment can lead to pollution of water resources **(Carob et *al*., 2016).**

Furthermore, ARBs and ARGs discharged from the human body may return to their original habitats (e.g., food, water, soil) and offer a greater environmental danger **(Wang et *al*., 2018).**

The risk of resistant pathogen dissemination with sewage sludge is also relevant, since this WWTP byproduct may be used as fertilizer in agriculture.

The contraceptive-rich pharmaceuticals present in HWW were reported to be associated with effects of endocrine disruption, for instance, exposure to pharmaceutical waste containing estrogen or androgen caused sex reversals in fishes and thus, reproductive impairment.

The hospital effluent discharged directly into the ponds has caused eutrophication, which is evident by oxygen depletion, dense algal blooms, and death of aquatic animals **(Obasi et *al*, 2014).**

CHAPTER 02: ANTIBIOTIC RESISTANCE

1 Introduction to Antibiotic resistance

Basic Mechanisms of Antibiotic Action against Bacterial Cells

Antibiotics inhibit fundamental bacterial physiology and biochemistry, resulting in microbial cell death or growth cessation. The bacterial cell wall, cell membrane, protein synthesis, DNA and RNA synthesis, and folic acid (vitamin B9) metabolism are the five principal antibiotic targets. Penicillins, cephalosporins, and carbapenems are examples of -lactam antibiotics that inhibit bacterial cell wall formation. This structure is not seen in higher species but is necessary for bacterial survival. Tetracycline, aminoglycoside, macrolide, and other antibiotics target the bacterial ribosome, which is sufficiently distinct from the eukaryotic ribosome to prevent cross-inhibition **(Kırmusaoğlu, 2019).**

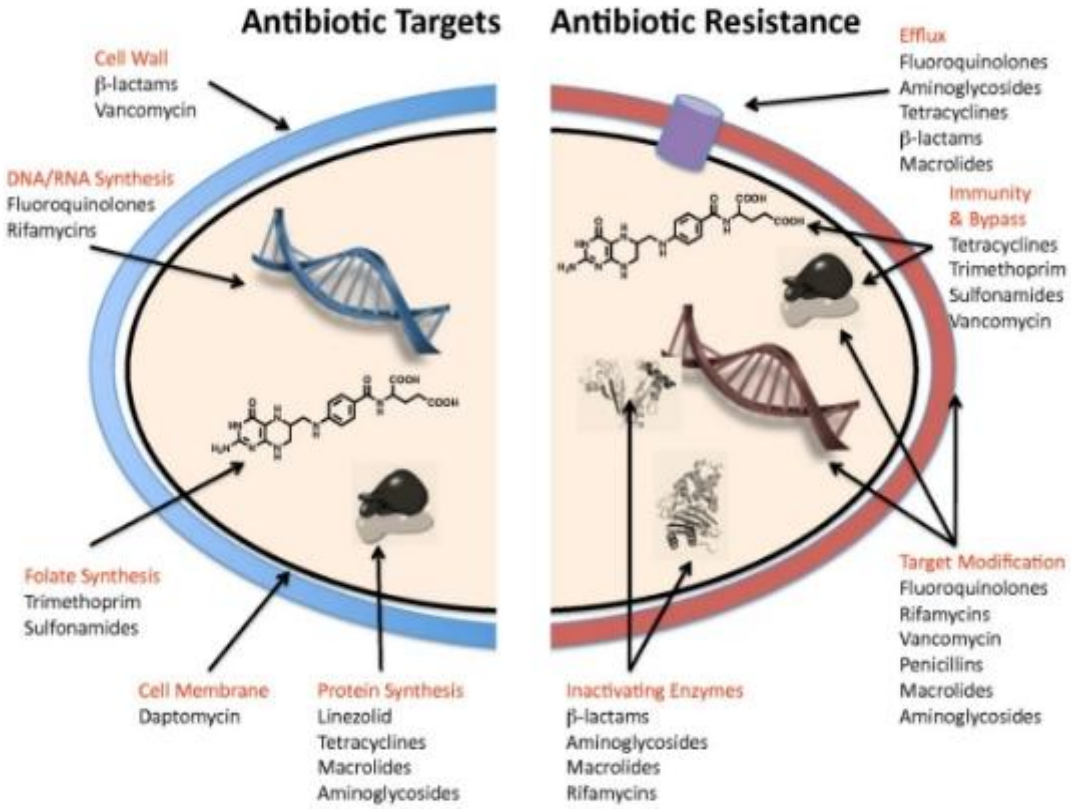


Figure 2: antibiotic targets and mechanisms of resistance **(Wright, 2010).**

1.1 History

The majority of antibiotics are derived from natural molecules created by microorganisms that have been battling with bacteria for millions of years. Antibiotic resistance is as old as drugs themselves. Antibiotic-resistant bacteria have been discovered for more than 4 million years from Lechuguilla Cave in New Mexico, including some strains that were resistant to over 14 antimicrobial drugs **(Bhullar et *al*., 2012).**

Unfortunately, practically all antibiotics which have already been produced have acquired resistance. As seen in Figure 02, penicillin resistance was discovered in 1940, before the antibiotic was even available on the market. Vancomycin was first used in the treatment of methicillin resistance in *S. aureus* and coagulase-negative staphylococci in 1972 **(Sengupta et *al*., 2013).**

Because it was so difficult to establish vancomycin resistance, it was thought unlikely to occur in a clinical environment. However, incidences of vancomycin resistance in coagulase-negative staphylococci were documented in 1979 and 1983 **(Sengupta et *al*., 2013).**

1.2 Resistance categories

Natural Resistance genetically inherited " intrinsic resistance": all strains of the same bacterial species which have a wild-type resistance phenotype.

If the MIC of all strains of a bacterial species exceeds a previously determined breakpoint, the species is deemed inherently resistant to that antibiotic. However, Natural resistance occurred prior to the introduction of antibiotics; this was often attributed to a lack of a vulnerable target or decreased permeability to the antibiotic under consideration **(Martinez, 2014).**

Acquired resistance is characterized by the appearance of resistance to one or more antibiotics in certain bacteria that were previously susceptible. The acquisition of resistance is explained by two processes: mutation and horizontal gene transfer (HGT) **(Pontes et al., 2018**).

MDR (Multi-Drug Resistance) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

XDR (extensive Drug Resistance) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories).

PDR (Pan Drug Resistance) was defined as non-susceptibility to all agents in all antimicrobial categories. Ex : *klebsiella pneumoniae* **(Rex, 2021).**

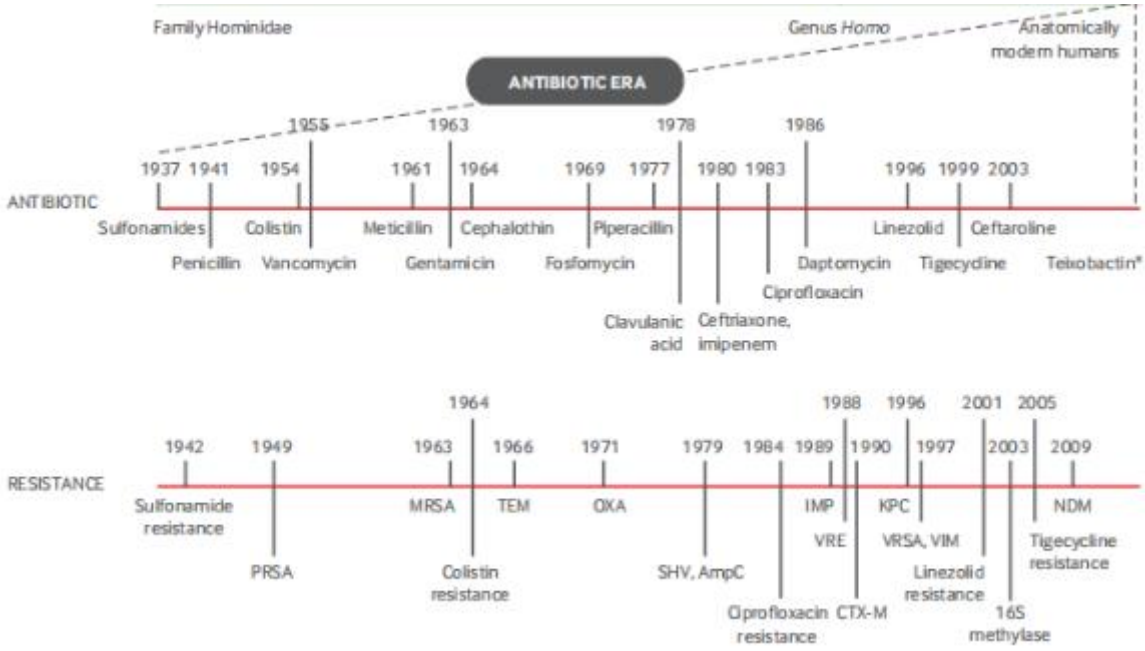


Figure 3: Developing antibiotic resistance a timeline of key events (Iredell et *al*., 2016).

2 Genetic and molecular mechanisms of antibiotic resistance

2.1 Genetics of bacterial resistance

Intrinsic resistance is associated with the innate ability of almost all prokaryotes to resist specific drugs; naturally occurs in bacterial genomes :It has the chromosome as its genetic support and is transmitted vertically to offspring **(Pontes et *al*., 2018).**

Acquired resistance is a consequence of spontaneous chromosomal mutations or gene gain through mobile genetic elements (MGE) , the spread of these MGEs through the Horizontal gene transfer (HGT) is crucial to prokaryotic genome evolution, but has serious clinical implications **(Hu et *al*., 2016).**

According to studies, HGT has transmitted roughly 75% of the genes in each genome over evolution. Many resistance genes are found on MGEs such as plasmids, transposons (also known as jumping genes), and integrons, which are susceptible of capturing and expressing gene cassettes and serve as vectors for the spread of antibiotic resistance **(Kaur et *al.*, 2020).**

2.1.1 Mobile genetic elements

MGEs are DNA segments that can capture genes and mediate their movement within the genome (intracellular mobility) or between cells (intercellular mobility)**(Partridge et *al.*, 2018).**

Integrons: genetic elements composed of an integrase-encoding intI gene, an attI recombination site, and a Pc promoter. These elements present a variable region flanked by two conserved regions (5′CS and 3′CS). The integrase mediates site-specific recombination responsible for the acquisition or excision of gene cassettes harboring AR genes. Several gene cassettes may be captured by the same integron into the variable region and their expression will be further regulated by the Pc promoter. **(Domingues et *al.*, 2012; Partridge et *al.*, 2018b).**

Plasmids: are circular, double-stranded, self-replicating DNA molecules that are easily vertically transmitted in a growing population of both Gram- and Gram-positive bacteria. Conjugative plasmids are primarily responsible for the spread of resistance genes.

Plasmids are classified into a number of incompatibility (Inc) groups, with Inc group types F, I, H (HI1 and HI2), L, C, and N being the most commonly linked with multidrug resistance**(Partridge et *al.*, 2018).**

Transposons: Are linear DNA sequences that can migrate from one chromosomal position to another or from the chromosome to a transmissible plasmid. Transposons are made up of insertion sequences (IS), intervening DNA, and the transposase enzyme, which is responsible for the transposition. This "jumping" group of genes contains the enzymes and genetic sequences essential for mobility within the transposon, allowing it to "jump" from one genomic site to another at random **(Mc Carlie et *al.*, 2020).**

2.1.2 Horizontal gene transfer

HGT can occur via bacterial recombination , three major mechanisms employed **(García-Aljaro et *al*., 2017)** : conjugation, transformation, and transduction presented in figure 03 where :

- Conjugation : is a direct transfer of genetic material between two bacteria that occures through sex pillus. The conjugation machinery encoded by the gene on plasmide or by integrative conjugative elements on chromosome **(Bennett, 2008)** .

The conjugation of plasmids has caused dissemination of ARGs worldwide that encode resistance to β-lactamases, carbapenemases, quinolones, aminoglycosides, colistin, sulfonamide, tetracyclines, and other classes of drugs **(Berglund, 2015)** . Many species are capable of conjugation, in particular enterobacteria such as *Escherichia coli* .

- Transformation: in which the cells picks up the naked DNA from the surrounding .Natural transformation can be seen in a limited number Gram-positive (Streptococcus and Bacillus) or Gram-negative (Neisseria, Branhamella, Acinetobacter, Haemophilus). This is the historical mode of resistance to penicillin described for pneumococcus.
- Transduction : is the process by which DNA is transferred with the help of bacteriophages. between different species or among different taxonomic groups Bacteriophages can transfer DNA sequences like chromosomal DNA, MGEs such as plasmids, transposons and genomic islands that are advantageous to their bacterial hosts as well as serves in improving the survival of bacteriophages **(Kaur et *al*., 2020).**

ARGs transfer by transduction has also been reported in various bacteria, for example, β-lactamase gene transfers by P1-like bacteriophages in *E. coli* .tetracycline and gentamycin resistance between enterococci **(Kaur et *al*., 2020).**

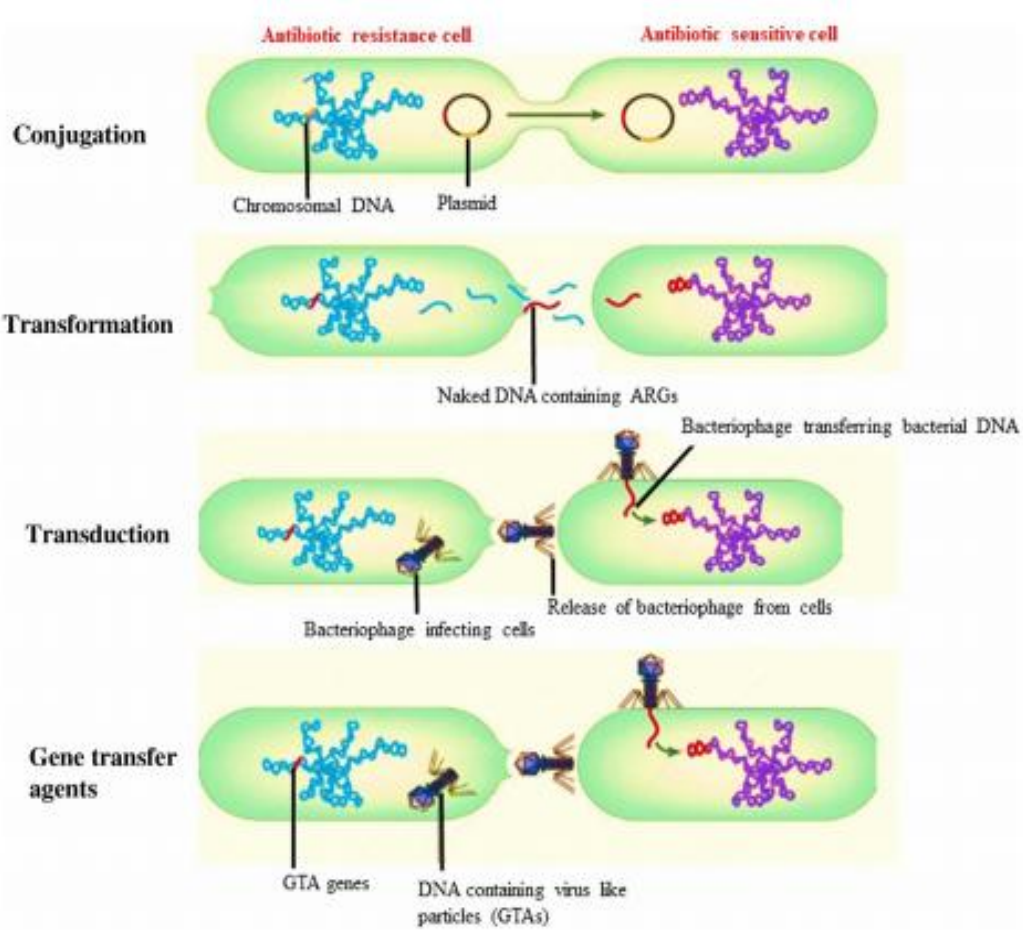


Figure4: Diffrent mechanisms of horizontal gene transfer shown by bacterial cell for transferring ARGs.**(Kaur et al., 2020).**

2.2 Antibiotic molecular resistance mechanisms

The development or the accumulation of resistance is commonly mediated by several mechanisms, which fall into four main groups.Plus biofilm formation ***(Nishizawa & Suzuki, 2014).***

- Reduction of bacterial envelope permeability
- Increases in both expression and activity of efflux pump systems
- Synthesis of enzymes which are able to destroy or modify the drug
- Bypass /modification, of antibiotic bacterial targets

In order to provide examples for the mechanisms aforementioned, figure 04 demonstrated the major resistance mechanisms applied by microorganisms to bypass the acts of different class of antibiotics . Underlying their importance in multi-resistance, for clinical pathogens.

that it seems like the multi-drug resistance is generated by diverse combination of mechanisms and accumulation of genes **.*(Pontes et al., 2018)***

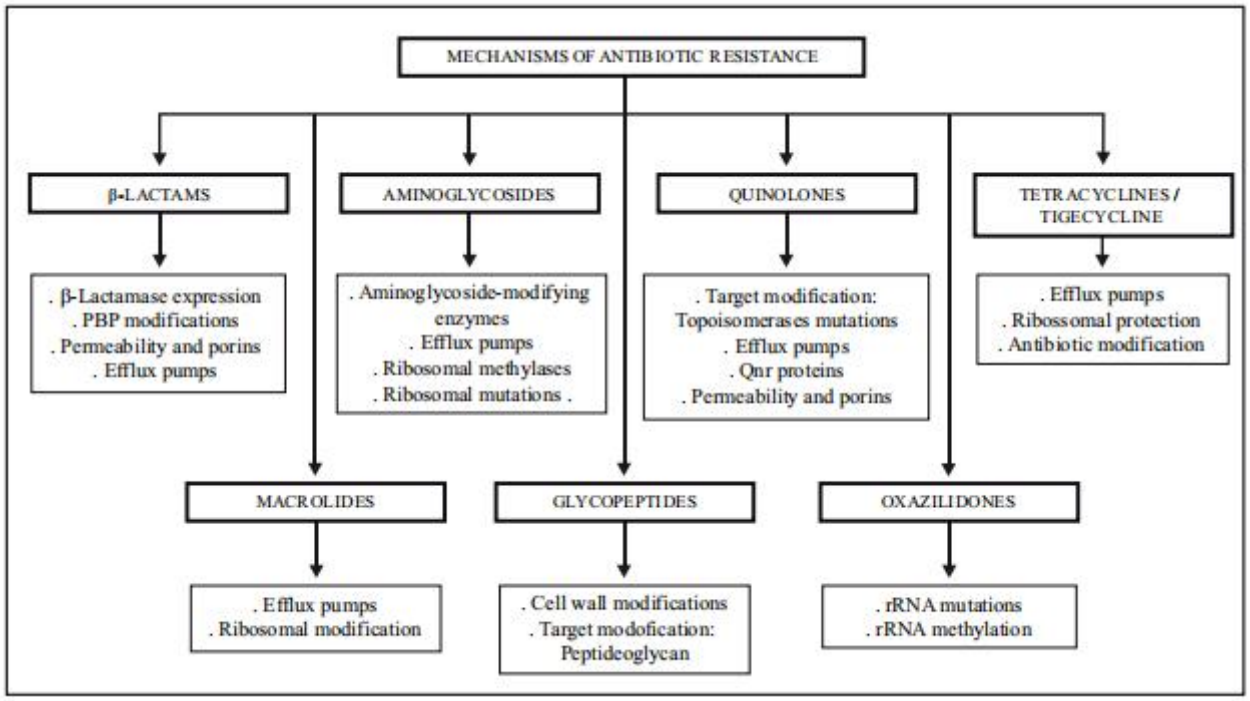


Figure 5: Diagram representing the main classes of antibiotics and their respective resistance mechanisms **(Pontes et *al*., 2018).**

2.2.1 Reduction of Bacterial Envelope Permeability

Microorganisms have cellular envelopes with inherent permeability, which facilitates nutrient intake while acting as the first barrier to external agents. Gram-negative bacteria are fundamentally less permeable to many antibiotics than Gram-positive bacteria because their outer membrane provides a permeability barrier.**(Ghai & Ghai, 2018).**

Hydrophilic antibiotics pass through the outer membrane via diffusing through porin proteins. The major porins in most Enterobacteriaceae, such as the outer-membrane proteins OmpF and OmpC of *E.coli*, are thought to function as non-specific channels; thus, reducing the permeability of the outer membrane and limiting antimicrobial drugs entry into the bacterial cell is achieved by downregulation of porins or by replacing porins with more-selective channels***(Blair et al., 2014).***

Recent data , however, suggest that in Enterobacteriaceae, Pseudomonas spp., and Acinetobacter spp., decreases in porin expression significantly contribute to resistance to newer antibiotics like carbapenems and cephalosporins, where resistance is often mediated by enzymatic degradation.**(Blair et *al*., 2014)**.

2.2.2 Synthesis of Enzymes which are Able to Destroy or Modify the Drug

- **Inactivation of antibiotics by hydrolysis:**

The enzymecatalysed modification of antibiotics is a major mechanism of antibiotic resistance that has been relevant since the first use of antibiotics, with the discovery of penicillinase (a β-lactamase), in 1940.Classes of β lactamas and their genetic support are presented in the following table **(Abraham & Chain, 1940).**

The early β-lactamases, which were active against first-generation β-lactams, were followed by extended-spectrum β-lactamases (ESBLs) that have activity against oxyimino-cephalosporins. The carriage of diverse ESBLs and carbapenemases, including the IMP (imipenemase), VIM (Verona integron encoded metallo β-lactamase), *K. pneumoniae* carbapenemase (KPC), OXA (oxacillinase) and NDM enzymes in Gram-negative bacteria such as *K. pneumoniae, E. coli, P. aeruginosa* and *A. baumannii,* has underpinned the emergence of isolates that are resistant to all β-lactam antibiotics**.(Nishizawa & Suzuki, 2014)** .

The CTX-M genes which encode ESBLs that are notable for their greater activity against cefotaxime than other oxyimino-β- lactams is presented by the plasmid. ***(Nishizawa & Suzuki, 2014).***

Table 2 : classes of beta lactamases and thier genetic support .

B lactamas	Microorganism	Gentetic support	Refrences
Penicillinases (Ambler class A) TEM, SHV	*E coli* *Klebsiella spp*	Plasmid chromosomally encoded.	Iredell et *al.*, (2016)
AmpC (class c)	Enterobacteriaceae (p) *Enterobacter*, *Serratia*, *Citrobacter*,*Providencia*, *Morganella*, *Pseudomonas*, *Acinetobacter (c)*	Plasmid chromosomally encoded.	Iredell et *al.*, (2016)
ESBLs			
ESBLs:extended-spectrum β-lactamases (Ambler class A) CTX-M SHV and TEM b-lactamases. OXA	*E.coli* *K.pneumoniae* *E.cloacae* Pseudomonas sp.	IncFII Plasmids holding hundreds of variants of the CTX-M gene coded : *bla* $_{CTX}$ *bla* $_{SHV}$	Miyagi & Hirai (2019)
Carbapenemas			
CPE: Carbapenemase-producing Enterobacteriaceae **Classes : ABC and D**	**Specific for each specie**	**Specific for each specie**	Piedra-Carrasco et *al*. (2017)
IMI class **A**	*Enterobacter cloacae*	Plasmid coded *bla* $_{IMI-2}$	Piedra-Carrasco et *al*. (2017)
VIM (Verona integron encoded metallo β-lactamase), class **B**	*Klebsiella oxytoca*	Integron coded *bla* $_{VIM-1}$	Piedra-Carrasco et *al*. (2017)
KPC class **A**	*K. pneumoniae* *Enterobacter cloacae*	plasmid-borne *kpc* gene *bla* $_{KPC-2}$	Iredell et *al.*, (2016)

NDM (New Delhi metallo -beta - lactamase 1)	Gram - *E.coli* *K.pneumoniae* *A.baumannii*	conjugative plasmids (IncA, IncC IncF, IncHI1 and IncL–IncM). *bla* NDM	Iredell et *al*., (2016)

- **Inactivation of antibiotic by transfer of a chemical group.**

Antibiotic resistance is caused by the addition of chemical groups to susceptible sites on the molecule by bacterial enzymes, which prevents the antibiotic from binding to its target protein due to steric hindrance. Various chemical groups, including acyl, phosphate, nucleotidyl, and ribitoyl groups, can be transferred, and the enzymes involved comprise a wide and varied family of antibiotic-resistance enzymes. Aminoglycoside side-modifying enzymes are one of the most well-known examples: Aminoglycoside antibiotics are particularly vulnerable to modification because they are big molecules with numerous exposed hydroxyl and amide groups. **(Blair et *al*., 2014).**

Aminoglycoside-modifying enzymes increase resistance to the antibiotic (or antibiotics) that they change. Aminoglycoside-modifying enzymes are classified into three types: acetyltransferases, phosphotransferases, and nucleotidyltransferase.

Acetylation of aminoglycosides, chloramphenicol and streptogramins. Phosphorylation of aminoglycosides, chloramphenicol and finally, adenylation of aminoglycosides, lincosamides .these are the most common biochemical events they catalyze.

(Blair et *al*., 2014).

2.2.3 Bypass /modification, of Antibiotic Bacterial Targets

Camouflage or target modification can take place through mutation of the targets themselves - such as the topoisomerases that fluoroquinolone antibiotics target - or by the creation of enzymes that change antibiotic targets, as in ribosomal methylation. Reprogram the target, as for Vancomycin-resistant bacteria make a different cell wall compared to susceptible bacteria which has a high affinity to the antibiotic .**(ReAct, 2021).**

For the MRSA (methicillin-resistant Staphylococcus aureus) they present the alteration of proteins to overcome the antibiotic effect. *Staphylococcus aureus* have the ability to acquire mecA resistance gene and then create a novel penicillin -binding protein which has a poor affinity to β lactam antibiotics, consequently resist it , and survive treatment.**(ReAct, 2021).**

2.2.4 Efflux pump system

EPs are proteins found in all bacterial plasma membranes that recognize and eject antibiotics into the environment before they reach their targets. they are categorized into six families cited in table 02. The Eps classification is based on their composition, substrates, transmembrane spanning regions, and energy sources. **(Pontes et *al.*, 2018b).**

Table 3: efflux pumps classification .

Efflux family	Exemple	Bacteria	Antibiotic resistance	Refrences
RND: Resistance Nodulation-division	AcrAB-TolC MexAB-OprM	*E.coli* *Pseudomonas aeruginosa*	Quinolones, Chloramphenicol-florfenicol, Tetracyclines	(Pontes et al., 2018)
MFS: Major Facilitator Superfamily	NorA, QacA, TetA(K) and Tet38 EmeA	Both Gram + /Gram- Chiefly *Staphylococcus aureus* *Enterococcus faecalis*	Tetracycline and fluoroquinolone	
ABC: ATP (adenosine triphosphate)-binding cassette superfamily	MacA, LmrA MacB in MsrD	Gram +/Gram- *E. coli* *S. pneumonia*		
MATE: Multidrug and Toxic Compound Extrusion (MATE)	VmrA, YdhE AbeM	Gram+/Gram- *A. baumannii*	chloramphenicol, kanamycin, norfloxacin, ciprofloxacin, streptomycin and ampicillin	
SMR: Small Multidrug Resistance Noted: qac / sugE	EmrE, EbrAB QacE	*E. coli* *Bacillus subtilis* *Klebsiella aerogenes*	(participat in biofilm formation)	
PACE: Proteobacterial Antimicrobial Compound Efflux superfamily	AceI	Gram – Recently *A.baumannii*	acriflavine, proflavine, benzalkonium, acriflavine and chlorhexidine	

2.2.5 Biofilm formation

One of the most significant virulence aspects of pathogenic bacteria is biofilm development, which allows germs to bypass the host defensive mechanism. Furthermore, bacteria in biofilm are 1000 times more resistant to medicines due to sluggish drug penetration and changed microenvironment **(Sabir et *al*., 2017).**

Furthermore, due to physical closeness and cell density, a biofilm matrix can boost intracellular communication as well as horizontal gene transfer, and conjugation can be up to 700 times more successful than in wild-type bacterial cells**.(Flemming et *al*., 2016).**

Biofilms have improved survival and resistance to chemical and environmental stresses (e.g., antibiotics), owing mostly, but not exclusively, to the extracellular polysaccharide matrix. Bacterial cells in biofilms are 10 to 1,000 times less susceptible to certain antimicrobial agents than planktonic counterparts. This decreased susceptibility is caused by a combination of factors, including: I poor antibiotic penetration into the polysaccharide matrix; (ii) the arbitrary presence of cells with a resistant phenotype (referred to as "persisters"); and (iii) the occurrence of either non-growing cells or cells that triggered stress responses under unfavorable chemical conditions within the biofilm matrix**.(Balcázar et *al*., 2015).**

Sub-inhibitory doses of aminoglycosides were discovered to stimulate biofilm formation as part of a defensive response in *E. coli* and *P. aeruginosa*. **Salcedo et *al*. (2014)** found that sub-inhibitory doses of tetracycline and cephradine cause biofilm formation and increase the rate of transfer of the pB10 plasmid among biofilm biomass (*E. coli* and *P. aeruginosa*) at rates 2–5 times quicker than without antibiotic treatment. Because most bacterial pathogens generate biofilms, the increased resistance of biofilms to antibiotics is a severe problem for human health, as many chronic infections are connected to biofilm growth on either natural surfaces (e.g., teeth, lungs) or foreign-body implants (e.g., pacemakers, catheters, prosthetic heart valves).

3 Multi-drug resistance bacteria

In 2017, the World Health Organization (WHO) released a list of antibiotic-resistant 'priority pathogens,' which covers 12 bacteria families that represent the greatest threat to human

health. Based on the urgency and need for novel antibiotics, the list has been classified into three primary priority (critical, high, and medium). **(World Health Organization, Antibiotic-Resistant Bacteria Global Priority Pathogens List, 2021).**

Comes in first place among the most dangerous bacteria: multidrug-resistant bacteria, particularly the most common human pathogens, which belong to the ESKAPE group (*Enterococcus faeciumi, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.* **(Pontes et *al*., 2018).**

Their resistance to a wide range of antibiotics is a worldwide public health threat , as it frequently leads to the spread of bacterial illnesses with high morbidity and fatality rates).

Table 4: World health organization (WHO) list of antibiotic-resistant 'priority pathogens' (WHO ; 2017).

BACTERIA	ANTIBIOTIC RESISTANCE
PRIORITY 1: CRUTICAL	
Acinetobacter baumannii	Carbapenem
Pseudomonas aeruginosa	Carbapenem
Enterobacteriaceae	Carbapenem, ESBLa-producing
PRIORITY 2: HIGH	
Enterococcus faecium	Vancomycin
Staphylococcus aureus	Methicillin, vancomycin-intermediate, and resistant
PRIORITY 3: MEDIUM	
Streptococcus pneumoniae	Penicillin-non-susceptible

3.1 Principal isolated multi-drug resistant bacteria :

3.1.1 Enterobacteriaecae

The family Enterobacteriaceae are Gram-negative non-spore-forming bacteria, including *Escherichia coli* (*E. coli*), Citrobacter, Salmonella, *Yersinia pestis*, Shigella, Proteus, Enterobacter, Salmonella, and Klebsiella, motile by peritrichous cilia expect for Klebsiella, Shigella and *Yersinia pestis* are immobile.

They are natural hosts of the digestive tract of humans and animals. *Escherichia coli, Klebsiella pneumonia*, and *Proteus mirabilis* are the most important because they are responsible for 80% of human infections caused by this family **(Nnadozie & Odume, 2019).**

Antibiotic resistance in Enterobacteriaceae is evolving over the world, with bacteria becoming resistant to current extended-spectrum cephalosporin antibiotics, as well as penicillins and monobactams. Their antibiotic resistance is owing to plasmid-mediated synthesis of enzymes that hydrolyze the antibiotic compounds' β-lactam ring. A significant number of point mutation variants of existing wide spectrum β-lactamases, also known as extended-spectrum β-lactamases, create this sort of resistance (ESBLs) **(Miyagi & Hirai, 2019).**

Furthermore, carbapenemases are a vast set of b-lactamases that are classified into clases ABC and D and are responsible for the inactivation of carbapenem antibiotics. Carbapenem antibiotics, including imipenem and meropenem, are used as last-resort treatments for severe infections caused by ESBL producers. Because of the scarcity of antibiotic alternatives, treating infections caused by pathogens resistant to carbapenem poses significant hurdles and is linked with extremely high mortality rates **(Khalili & Izadpanah, 2015).**

ESBL , plasmidic AmpC, and associated quinolone resistance determinants in coliforms isolated from hospital effluent were first reported in Algeria in 2010.

The identification of extended-spectrum beta-lactamases, plasmidic AmpC (pAmpC), and associated plasmid-mediated quinolone resistance (PMQR) determinants in cefotaxime-resistant coliforms isolated from hospital effluent in Algiers revealed blaCTX-M genes in 89 % of isolates, blaTEM-1 genes in 79.8 %, and pAmpC genes (blaCIT) in 2.7 All CTX-Mz isolates had ISEcp1B-blaCTX-M association, and 97.2 % had class 1 integrons. The genes blaCTX-M-15, blaCTX-M-3, and blaCMY-4 were discovered by sequencing. blaCTX-M-3 Inc L/M conjugative plasmids included blaCTX-M-15 and blaCTX-M-15. The PMQR determinants discovered wereqnrB1, qnrB2, qnrB9, qnrB19, qnrS2, and aac(6')-Ib-cr are all examples. The genes qnrB2, qnrB9, qnrB19, and blaCMY-4 are described **(Anssour et *al*., 2013).**

3.1.2 Methicillin-Resistant Staphylococcus Aureus

Staphylococci are Gram-positive bacteria belonging to the Staphylococcaceae family. Coagulation-negative staphylococci are commonly seen in staphylococcus epidermidis and staphylococcus haemolyticus, and are typically isolated in hospitals.

The second group of staphylococci is coagulase-positive staphylococci, the most common of which is *Staphylococcus aureus*, the most dangerous species responsible for nosocomial and community infections caused by its complex toxins.

Staphylococcus aureus secretes penicillinase in 70% to 90% of cases, resulting in resistance to penicillin G, penicillin A (ampicillin, amoxicillin, etc.), ureidopenicillin (piperacillin), and carboxypenicillins (ticarcillin) **(Bekhti & belhadi.,2019).**

The presence of a novel penicillin-binding protein PLP2a encoded by the gene mecA makes Staphylococci resistant to methicillin (oxacillin), and all beta-lactams.**(Bekhti & belhadi.,2019).**

MRSA is resistant to penicillin-like beta-lactam drugs. However, glycopeptides (e.g., vancomycin and teicoplanin), linezolid, tigecycline, daptomycin, and even certain novel beta-lactams, such as ceftaroline and ceftobiprole, continue to be active against MRSA. MRSA, on the other hand, has demonstrated remarkable adaptability in originating and spreading in a variety of epidemiological contexts across time (in hospitals, the community, and, more recently, in animals) **(Bekhti & belhadi.,2019).**

The emergence of a vancomycin-resistant strain as a consequence of Staphylococcus gaining a resistance plasmid by horizontal transfer between environmental enterococci strains that already have natural vancomycin resistance**(Bekhti & belhadi.,2019).**

3.1.3 Vancomycin-Resistant Enterococci

One notable feature of the genus Enterococcus is its proclivity to acquire and spread antibiotic resistance determinants. Enterococci have developed resistance to a wide range of antibiotics, including penicillin. Enterococci are naturally resistant to cephalosporins and aminoglycosides, whereas resistance to chloramphenicol, erythromycin, tetracycline, fluoroquinolones, and vancomycin is acquired **(Iweriebor et *al.*, 2015) .**

E. faecalis and *E. faecium* were the first clinical isolates of vancomycin-resistant pathogenic strains.

Vancomycin resistance Enterococci (VRE) has multiple described phenotypes, including *vanA, vanB, vanC, vanD, and vanE* . Resistance phenotypes *vanA* and *vanB* have mostly been reported in *E. faecalis* and *E. faecium*. *VanC* resistance phenotype has been reported in *E. casseliflavus* and *E. gallinarum* **(Iweriebor et *al*., 2015).**

Cherak et *al*.,(2022) reported the first environmental vanA positive Enterococcus faecium isolates from Algeria. Between November 2018 and October 2019, a program to screen for carbapenem- and colistin-resistant Gram-negative bacteria in hospital sewage was underway. The strains were obtained from hospital effluent using matrix-assisted laser desorption and ionisation time-of-flight mass spectrometry. The disc diffusion technique was used to determine antibiotic susceptibility. Vancomycin resistance genes were identified using conventional PCR, and the clonality of the isolates was determined using multilocus sequence typing. Five highly vancomycin-resistant Gram-positive bacteria, identified as *Enterococcus faecium*, were isolated. The isolates were classified to the clonal complex 17 because they had the *vanA* gene.

The initial finding, published in 2013, described the isolation of vanA-positive *E. faecium* from a surgical wound infection being treated at Algiers University Hospital. The second study revealed a probable epidemic involving three VREfm clonally related strains (same pulsotype) identified at Batna University Hospital during a six-month period. Surprisingly, the three causative strains were assigned to the ST80 sequence type, which was also detected in the **Cherak et *al*.,(2022)** investigation. The third investigation discovered the vanA gene in a collection of clinical *E. faecium* isolates from several Algerian locations, all of which belonged to the CC17 group. The most recent study found a significant incidence of vanA positive *E. faecium* isolates in hospitalized patients at Tlemcen University Hospital.

3.1.4 MDR Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram-negative bacillus .It is characterized by its large nutritional flexibility allowing it to adapt to hostile environments, **(Botelho et *al*., 2019).**

According to **Magiorakos et al.,(2012)** *P. aeruginosa* strains are defined as multidrug-resistant (MDR) if non-susceptible to ≥1 agent in ≥3 antimicrobial categories, extensively

drug-resistant (XDR) if non-susceptible to ≥1 agent in all but ≤2 categories and pandrug-resistant (PDR) if non-susceptible to all antimicrobial agents listed , it's distinguished by its high level of natural resistance to a wide rang of antibiotics including : penicillins G, A and M, the first and second cephalosporins generations and some third generation cephalosporins, cotrimoxazole, kanamycin, macrolides, cyclins, chloramphenicol, first generation quinolones, rifampicin, **(Botelho et *al*., 2019).**

glycopeptides, fusidic acid and its ability to acquire and accumulate a wide range of resistance mechanisms in hospitals and the environment as a result of antibiotic selection: secretion of beta-lactamases : OXA-50 class D Mettalo β lactamase PIB-1, modification of membrane permeability : efflux pump constitutive expression (efflux , impermeability), and modification of target, particularly topoisomerases . **(Botelho et *al*., 2019).**

3.1.5 Acinetobacter spp :

belongs to the Moraxellaceae family and is a Gram-negative ubiquitar bacterium . Natural resistance of Acinetobacter strains had been observed against molecules available in the early 1970s: ampicillin, cephalotin, tetracyclines, chloramphenicol; however, with the development of carboxypenicillins, cephalosporins of 2nd and 3rd generation, carbapenems, aminoglycosides, fluoroquinolones, It is difficult to locate active molecules on these bacteria since Acinetobacter spp are known for their great ability to develop resistance mechanisms to the majority of new antibiotics.**(Kyriakidis et al., 2021).**

In the hospital environment, it is *Acinetobacter baumanni* the most frequently encountered in the most severe Acinetobacter infections belongs to ESKAPE pathogen that threatens public health by generating severe and invasive infections (mainly nosocomial) with high fatality rates. This microorganism has developed multidrug resistance (MDR) in recent years, owing mostly to widespread antibiotic misuse and inadequate stewardship.**(Kyriakidis et al., 2021)**

Carbapenem-resistant A. *baumannii* (CRAB) was declared the WHO's top priority for antibiotic research and development in 2018. Carbapenem was chosen as a marker because carbapenem resistance is commonly linked with a wide variety of antibiotic co-resistance.

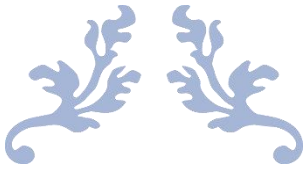
- Antibiotic resistance mechanisms in A. baumannii Control of antibiotic transportation through decreasing membranes permeability (reduced porin permeability or enhanced efflux), to prevent the access of antibiotics to the target.
- Alteration of antibiotic targets: Through genetic mutation or post-translational change, the antibiotic target is modified
- Enzymatic inactivation of antibiotics : By hydrolysis or modification, antibiotics can be rendered inactive.

These are the three basic ways by which antibiotic resistance can be acquired.**(Kyriakidis et al., 2021)**

Table 5 : Intrinsic and acquired resistance for some microorganisms examples

Organism	**Intrinsic resistance**	**Acquired resistance**	**references**
All the Gram+	Aztreonam Colistin	Specific for each gener	Reygaert, 2018
All the Gram -	Glycopeptides, lipopeptides	Specific for each gener	
Anaerobes	Aminoglycosides, fluoroquinolones ,b lactams , macrolides	Specific for each gener	
Escherichia coli	Macrolides	Aminopenicillins, fluoroquinolones, aminoglycosides, and 3rd generation cephalosporins Polymyxin, colistin	Reygaert, 2018 liu et *al*., 2016
The ESKAPE group			
Enterococcus faecium	Aminoglycosides, cephalosporins, lincosamides, , ertapenem , tmp-smx , clindamycine	Chloramphenicol, erythromycin, tetracycline, fluoroquinolones, and vancomycin	Nnadozie and Odume; 2019 Reygaert, 2018
Staphylococcus aureus	Macrolide , fluoroquinolones	Vancomycin resistance through HGT . Methicillin	Nnadozie and Odume ;2019 Reygaert, 2018

Klebsiella pneumoniae	Penicillin (β lactame class a shv-1)	Cephalosporin- carbapenem thought : Hgt, mutation of esbl : Tem-1 tem-2 , ctx-m genotype : Bla ctx-m-n	Reygaert, 2018
Acinetobacter baumannii	Ampicillin, glycopeptides, ertapenem	Colistin	Reygaert, 2018
Pseudomonas aeruginosa	Tetracyline , many b lactams , ertapenem Sulfonamides, cephalosporins, chloramphenicol, tmp-smx	Polymyxin- and carbapenem	Reygaert, 2018 Nnadozie and Odume; 2019
Enterobacter species	Similar to Enerobacteriaceae	Carbapenem,colistin	Reygaert, 2018



CHAPTER 03: AQUATIC ENVIRONMENTS AS MULTIDRUG RESISTANT BACTERIA RESERVOIRS

1 Antibiotics in natural water surfaces:

Bacteria that naturally exist in a variety of habitats, including waterbodies, are extremely essential. They have an important role in various ecological processes, including the conservation of water quality, due to their engagement in biogeochemical cycling and the decomposition of organic contaminants **(Grenni et *al*., 2018).**

Antibiotics, due to their inherent bioactivity, can have a variety of impacts on environmental bacteria, notably changes in community structure and function, with both direct (short-term, e.g., bactericide and bacteriostatic activities) and indirect (long-term) effects. In the short run, the impacts might lead to the extinction of several essential microbial communities that perform important ecological roles **(Grenni, 2022).**

The emergence of ARB is one of the secondary consequences. The excessive use of antibiotics not just in human therapeutics but also in veterinary, agricultural, and aquaculture applications, as well as their long-term persistence throughout many aquifers, pressures selection at sub-inhibitory concentrations upon pathogens, has contributed to the emergence of ARGs and ARB compromising or shrinking the effect of antibiotic compounds as they become resistant to multiple drugs, causing a major concern**(Kaur et *al*., 2020).**

Antibiotics are released into the environment on a daily basis through urine and feces as a combination of unaltered xenobiotic chemicals and bioactive forms that have been partially decomposed by people and animals**(Amador et *al*., 2014; Manyi-Loh et *al*., 2018; Zagui et *al*., 2020).**

This release, along with antibiotic-resistant bacteria (ARB), can occur via several routes, the most significant of which being the municipal sewer network and soil farm fertilization using manure or sewage sludge . Aside from the chemical pollution caused by AB **(Amador et *al*., 2014)** , the environment has become a reactor of ARB and ARGs, promoting the development and transmission of resistant genes, such that 70% of all hospital-acquired infections demonstrate resistance to at least one antibiotic family**(Kaur et *al*., 2020).**

2 Metabolic versatility

Antibiotic susceptibility is naturally low in some bacterial species. Such bacteria originate in settings with low antibiotic loads, and those responsible for hospital infections (such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, or *Stenotrophomonas maltophilia*) are not antibiotic producers. Large genomes are common in free-living opportunistic pathogens, allowing them to colonize a variety of settings through metabolic plasticity, which aids in the degradation and resistance to toxicity of substances found in these ecosystems. This can comprise a huge number of biodegradative enzymes working together to modify and use antibiotics as a food source **(MartíNez, 2008).**

In fact, studies have demonstrated that different bacteria, including commonly studied *Pseudomonas* strains and *E.coli* can utilize various antibiotics, including the β-lactam (penicillin) antibiotics, as a source of carbon. or use it's respiratory metobolisms to overcome antibiotics susubtibility .

A team of scientists led by Gautam Dantas of Washington University in St. Louis (2018) has discovered evidence for a route that bacteria may employ to soak up β-lactams into their core metabolism .That when given penicillin as a food supply, the bacteria all activated three distinct sets of genes, but not when given sugar as an alternate. These genes regulated three phases of penicillin catabolism by bacteria, the primary of which is that the deactivation of the antibiotic's hazardous component.**(Crofts et *al*., 2018).**

First, in response to penicillin, β-lactamase is selectively increased. Following that, a putative penicillin utilization operon is expressed (put). Finally, it appears that the method is completed by overexpression of the phenylacetic acid catabolon (paa). according to the researchers , " the scheme below offers a catabolic pathway composed of the subsequent phases."

Detoxification of penicillin: the bacteria first cleave the β-lactam ring to provide benzylpenicilloic acid.

Import of the benzylpenicilloic acid product: That compound acts as a substrate for an amidase enzyme, which hydrolyzes the amide bond to release the phenylacetic acid side chain.

Phenylacetic acid may be metabolized into acetyl-CoA and succinyl-CoA, which feed into the bacteria's central metabolism (Writer, 2018).

The researchers inserted a group of those penicillin-processing enzymes into *Escherichia coli*, leading to a strain that may only survive on penicillin. They suggest that such modified bacteria is also utilized to bioremediate antibiotic-contaminated waterways. However, the researchers agree that bioremediation has the potential to transfer resistance and degradation genes to other species **(Writer, 2018).**

3 Prevalence and persistance of ARB and ARGs in freshwater ecosystems

Freshwater environments are an important reservoir of ARBs and ARGs. . The level of ARBs is extremely high in most of the river systems (up to 98% of the total detected bacteria), followed by lakes (up to 77% of the total detected bacteria). This indicates that rivers and lakes serve as a significant means of the transport and the spread of antibiotic resistance to opportunistic pathogens.

Different factors such as dilution, degradation, adsorption, and transport have been found to play major significant role in the control of the fate and persistence of microbial contaminants freshwater and are fundamental for predicting the actual risk of antibiotic resistance dissemination from freshwater reservoirs **(Yoon et *al*., 2018).**

The ARBs and ARGs can be transported, experience decay through biological and non-biological processes, adsorbed onto particulate matter, diluted, uptake by aquatic microorganisms through HGT **(Nnadozie & Odume, 2019).**

Transport it means the movement of water by advection or diffusion in streams or ponds, and ARGs and ARBs can be transported over considerable distances in water **(Pote et *al*., 2003).**

Adsorption :Adsorption refers to the deposition and trapping of biofilms in substrate fissures by stream biofilms colonizing substrate surfaces.In the freshwater environment, ARGs interact with minerals and humid acid, which explains why the bulk of ARGs remain fixed in sediments. The immobilization of ARGs will result in greater persistence in water.**(Nnadozie & Odume, 2019).**

Degradation : Has the most significant process that influences the fate of ARBs and ARGs in the freshwater environment.

Enzymatic degradation:Environmental ARGs are susceptible to the biotic degradation process, which includes DNAses. DNA degradation in aquatic environments has been shown in studies to be caused by increased biocatalytic activity. Sediments, on the other hand, preserve ARGs from biological destruction. Previous research has explained DNA conservation from degradation after adsorption onto sediments in several ways. It has been proposed that adsorption onto sediments inhibits nuclease accessibility.

Sunlight / UV degradation: Bacteria are capable to heal sunlight damage, and there is a potential for survival and regrowth if the injury is sublethal **(Nelson et *al*., 2018).** Worse, several nosocomial infections (Serratia marcescens, Pseudomonas putida, and Stenotrophomonas) are sun UV resistant.

Therefore, ARGs in freshwater are not completely eradicated by sunlight-mediated degradation. Even if the original donor ARB cell is not present, the ARGs can be picked up by a competent bacteria and integrated into its genome. For example, UV-depleted plasmid-borne ARG can be repaired by competent bacterial cells in the environment during transformation.. The significant repair was observed in *E. coli* recipient strain (DH5a) **(Yoon et *al*., 2018).**

Dilution : Research indicates that it is directly proportional to the flow regime of the water and commonly in high flowing rivers the ARBs and ARGs are diluted . While dilution lowers a contaminant's concentration, it does not remove it from the environment, according to the law of conservation of mass, polluants in aquifer get to be a part of the hydrological cycle with a long-term concern of persistence. **(Yoon et *al*., 2018).**

Biofilm formation as a factor of ARGs and ARBs persistance and prevalence fact in aquifers

The macro- and microorganisms that live in these bodies of water are subjected to a low but consistent concentration of a wide variety of chemical contaminants. At these sub-MIC concentrations, antibiotics appear to serve as signaling molecules, mediating a wide range of cell activities (gene transcription and expression, quorum sensing, inter- or intra-species communication, biofilm formation, among others).

Because biofilms serve a significant function as ARG reservoirs, they might be regarded as biological indicators of antibiotic resistance pollution in the same way as river ecologists utilize streambed biofilms to assess the overall "ecological condition" of the river ecosystem. Chronic antibiotic exposure at sub-MIC concentrations exerts a selection pressure on biofilm bacterial populations, which may promote the formation and spread of antibiotic resistance. ARGs have been found in river biofilm, sediments, and water column along anthropogenically altered riverine systems **(Balcázar et *al*., 2015).**

Several studies have shown that WWTP effluents have an influence on the prevalence of ARGs in river biofilms. The abundance of genes conferring resistance to major antibiotic families, including beta-lactams (blaCTX-M), fluoroquinolones (qnrS), sulfonamides (sul I), and macrolides (ermB), was determined in biofilm samples collected from WWTP discharge points in small Mediterranean streams using quantitative PCR (qPCR) **(Proia et *al*., 2016).**

Wastewater treatment plants : Antibiotics and ARGs of various classes are discharged from hospitals and urban wastewaters treated by WWTPs . Throughout addition, the WWTP serves as a hotspot for the emergence and dissemination of ARGs and ARBs in the ecosystem . Even after treatment, certain antibiotics and ARGs are still detectable and are being released into recipient water bodies, converting aquatic ecosystems into an excellent location for the acquisition and spread of such genes **(Rodriguez-Mozaz et *al*., 2015).**

4 Antibiotic-resistance gene identification tools

There are several detection techniques available for both phenotypic and genotypic determination of ARGs in isolates.

4.1 Phenotypical detection :

Antibiotic resistance is a selected phenotype that may be identified utilizing disc diffusion growth inhibition experiments.broth dilution, gradient strips,or other ways to detect antibiotics' minimum inhibitory concentration (MIC).

However, there are several issues with this approach, such as resistance gradation, time-consuming (may take several weeks for slow-growing bacteria), and culture-dependent method, which only relates to concentration of antibiotics (difficult to identify low-level resistance) and provide no information on resistance dissemination via MGEs **(Anjum, 2015).**

4.2 Genotypical detection :

To circumvent the phenotipical detection methods drawbacks, genotypic or molecular characterisation methods such as PCR, hybridization techniques such as microarray, and whole genome sequence (WGS) are widely employed to identify particular resistance genes and provide findings within hours.

PCRs (real-time, multiplex PCR) can detect resistance to aminoglycoside, β-lactum chloramphenicol, macrolide, penicillin, trimethoprim, and tetracycline.

In wastewater, surface water, and drinking water biofilms, Schwartz et al.(2003) utilized PCR to identify vanA (vancomycin-resistance gene), mecA (methicillin-resistant gene), and ampC (ampicillin-resistant gene). PCR and RT-PCR have recently been employed in Europe to detect plasmid-mediated mcr-1 genes containing colistin resistance.

Loop-mediated isothermal amplification PCRs are highly fast, done at a constant temperature, and have been created to identify ESBLs and carbapenemases genes in bacteria; however, they cannot be utilized in multiplex to detect many genes at the same time. A paper-based device integrated with LAMP and a switch molecule for fluorescence detection of ARGs was recently created to enable for more easy and efficient detection, particularly in resource-limited settings**(B. Li et *al*., 2018).**

DNA microarray is another technology that has been effectively utilized to identify ARGs in the test organism in contrast to a reference strain is high-throughput DNA microarray, which operates at great speed and delicacy. Probes unique to the gene are detected using this approach.On a firm foundation (e.g., glass slide). The particular targetprobe duplexes are recognized after labeling and hybridizing the DNA.

It can identify a huge number of single genes, mutations, and MGEs, as well as describe strains at the molecular level.**(Anjum et *al*., 2017).**

WGS is another promising tool for detecting antibiotic resistance genes and mutations. The key benefit is its capacity to employ and identify many targets at the same time, as well as subtype-specific gene variations**(Kaur et *al*., 2020).**

Yet , there are limits to each of the phenotypic and genotypic techniques, and one can use a mix of these screening methods to monitor resistance.

Since, the need for gene-specific primers and the species-specific strategy utilized by these instruments restrict their efficacy for detecting certain targeted bacteria, providing insufficient information on microbial communities found in wastewater**(Chu et *al*., 2018).**

Recently, **metagenomics** analysis was established, which overcomes the constraints of traditional molecular analysis techniques and may provide hundreds to thousands of results **(Rowe et *al*., 2017).**

Sequences provide a full picture of the microbial communities present in unknown samples, detecting a high abundance of possible microorganisms . The metagenomics analysis consists of four steps: genetic material isolation, cloning, library formation, and analysis of genetic material from the metagenomics library.

Metagenomics, in collaboration with the Search Engine for AMR , may examine unknown samples acquired from the environment and offer full-length ARG data**(Rowe et *al*., 2017).**

5 The most detected ARGs in hospital wastewater and other water bodies

As previously suggested. Antibiotic-resistant bacteria and their accompanying antibiotic resistance genes are progressively becoming recognized as a wide range of environmental pollutants. These antibiotic resistance genes are no longer restricted to point sources such as hospitals, sewage, and farms, but were also detected in relatively pristine habitats such as rivers, lakes, and soils.

The emergence of extended-spectrum b-lactamase producing Enterobacteriaceae, Carbapenemase-producing Enterobacteriaceae, methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant Enterobacteriaceae, (VRE) all of which can cause potentially highly epidemic infections, is of particular concern to public health. Their prevalence in freshwater habitats has been documented in countries like as Portugal, Finland,

France, Pakistan, Switzerland, the United States of America, China, and Brazil.**(Nnadozie & Odume, 2019).**

The environmental distribution of these ARBs carrying ARGs is depicted in Table. mentioning the most common antibiotic classes and the relevant resistant ARB group with species examples and their occurence in diffrent waterbodies .

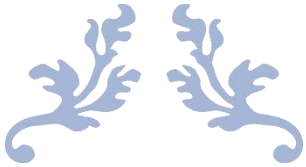
Table 6 : detected ARGs in water surfaces

Antibiotic class	ARB group	ARG carried	Specie	Type of freshwater	Refrence
β-lactams	Extended-spectrum b-lactamase producing Enterobacteriaceae	*blaTEM, blaCTX-M, blaSHV and blaOx*	*Klebsiella pneumoniae* *Citrobacter freundii,* *Enterobacter cloacae* *Citrobacter koseri* *Salmonella choleraesuis* *Serratia liquefaciens*	River WWTP effluent, drinking water, and sewage water	Nnadozie and Odume ; 2019
		blaVEB	Aeromonas spp	River	
	Carbapenemase-producing Enterobacteriaceae	*integrase genes intI1*	*Escherichia coli* Pseudomonas sp Acinetobacter sp	River sediments	

		blaKPC-2	*Escherichia coli* *Klebsiella pneumoniae* *Enterobacter cloacae*	River	
		blaIMI-2	*Enterobacter cloacae*		
		blaKPC-2 and blaVIM-1	*Klebsiella oxytoca*		
methicillin	methicillin resistant Staphylococcus aureus	*mecA gene*	*Staphylococcus aureus*	River freshwater drinking water biofilms	
Vancomycin	Vancomycin resistant Enterococci	*vanA;vanB* *Van c* *VanC1 vanC2/C3*	*Enterococci faecalis* *E. casseliflflavus*	River drinking water biofilms	
aminoglycoside	-	*aac, aad, aph)*	*Gram negative bacilli* *Pseudomonas aeruginosa,*	Rivers and lake Sediments	
Quinolone	-	*qnrA, qnrB*	*E. coli* and Klebsiella spp	Sediments	

		; qnr C qnrD ; qnrS		and river water	
Sulfonamides	-	*sul(I), sul (II) sul(III), sulA*	*E.coli* *shigella* *Klebsiella pneumoniae* *Acinetobacter baumannii* Salmonella spp Staphylococcus sp. Enterobacter sp.	WWTP effluent Groundwater	
Colistin	-	*mcr-1 to mcr-10* *mcr-1to mcr-5* *mcr-1*	serratia spp., Proteus spp. and Burkholderia spp *Pseudomonas aeruginosa, Acinetobacter baumanni*i, Enterobacteriaceae members, such as *Escherichia coli*, Salmonella spp., Klebsiella spp.	Hospital wastewater	
Macrolides	*E.coli* as a resrvoir Spreading it to shigella spp	erm A ermB ermC ermD mph A mphB	*E.coil*	HWW WWTP	

		ermF, ermB, ermA	*Streptococcus pneumoniae* *streptococcus pyogenes* *Staphylococcus aureus*	effluents Groundwater	
Tetracyline	Both Gram +and Gram-	*tetA, tetB, tetC, tetD, tetE, tetG, tetH, tetJ, tetY, tetZ* *tetA tetB tetC tetK*	Salmonella sp *E.coli* *Staphylococcus aureus* Acinetobacter sp.	HWW, farms, untreated sewage, WWTP effluent Groundwater	



CHAPTER 04: ANTIBIOTIC RESISTANCE MANAGEMENT IN WASTEWATER TREATMENT PLANTS

The goal of this chapter is to evaluate various options for hospital effluent treatments prior to discharge into public sewage or the environment, to improve the biodegradability of hospital effluents, to avoid the spread of pathogenic microorganisms, viruses, antibiotic resistant bacteria, pharmaceuticals, and chemical pollutants, to reduce the organic load, and finally, to meet the requirements of discharge standards in different countries. Only four of the research address the presence of PhCs in hospital effluents, whereas the rest studies address pathogenic bacteria and conventional pollutants such as COD, BOD, and SS.

1 Treatment strategies :

Efforts to counteract ARGs are listed in Table 6 and are examined further in this section.

1.1 Anaerobic and/or aerobic treatment reactors

Aerobic and anaerobic treatment techniques are low-energy, environmentally friendly solutions that are commonly used to reduce chemical oxygen demand (COD); moreover, they may effectively eliminate ARB and ARGs **(Christgen et *al.*, 2015)**. Aerobic treatment methods take place in the presence of air and microorganisms that utilize oxygen to convert organic pollutants to carbon dioxide, water, and biomass (aerobes). Anaerobic treatment techniques, on the other hand, occur in the absence of air and use microorganisms that do not require air to convert organic pollutants to methane, carbon dioxide gas, and biomass (anaerobes).

According to **Du et *al.*, (2014)**, anaerobic and anoxic treatments are far more effective than aerobic treatments in removing ARGs because microorganisms have reduced bioactivity in anaerobic conditions and the propagation of resistance genes are inhibited.

Anaerobic–aerobic sequence (AAS) bioreactors removed more than 85% of ARGs in the influent, making them more effective than aerobic and anaerobic units, which removed 83 and 62 %, respectively **(Christgen et *al.*, 2015).**

To summarize, biological treatment approaches may successfully remove antibiotics, ARBs, and ARGs if anaerobic and aerobic reactors run in succession, but aerobic reactors alone are ineffective. If membrane-based technologies, such as Membran biorector (MBR), can be employed in conjunction with biological treatment, ARG removal efficiency will improve.

1.2 Constructed wetlands

Constructed wetlands (CW) are tiny semi-aquatic habitats in which a diverse microbial community thrives and many physical-chemical processes occur. Because of their simplicity, cost effectiveness, and efficacy on eliminating ARGs, they have been devised and are known as appealing municipal, industrial, and agricultural wastewater treatment techniques **(Barancheshme & Munir, 2018)**. The characteristics of a manmade wetland can impact the efficacy of ARB and ARG removal. These features are flow configuration, plant species, and flow kinds, among others (surface flow, horizontal subsurface flow, and vertical subsurface flow). Although biodegradation, substrate adsorption, and plant uptake all have a role in reducing nutrient, antibiotic, and ARG loadings in artificial wetlands, biodegradation is the most important mechanism in the removal of these pollutants.**(Chen et *al.*, 2016).**

The elimination of ARB and ARGs in raw residential wastewater by variously designed wetland has been researched, as have 8 antibiotics, 12 genes, and 16S rRNA (bacteria) in various matrices. Total antibiotic aqueous removal efficiencies varied from 75.8 to 98.6%, while total ARG aqueous removal efficiencies ranged from 63.9 to 84.0 % by the built wetland. The presence of plants aided in pollutant removal, and the subsurface flow created wetland removed more pollutants than the surface flow constructed wetlands, notably antibiotics. **(Chen et *al.*, 2016).**

1.3 Coagulation

Coagulation is an active approach for treating turbidity, color, natural organic matter, and heavy metals in water **(Zainal-Abideen et *al.*, 2012)**. Colloidal particles have predominantly negative electrical charges, whereas coagulants contain positive charges.

When particles come into touch with a coagulant, they get neutralized and cling together. Coagulation, as a tertiary treatment process in WWTPs, is widely used to improve water quality and remove pollutants.

Coagulation technology's efficiency in removing ARGs from treated wastewater has been studied in recent years. **(Li N. et *al.* 2017)**. Coagulation is a proactive way of removing ARG from WWTP effluent. **Li H. et *al.* (2017)** investigated the eradication of sul, tet, and integrase genes using the inorganic coagulant FeCl3 and the inorganic polymer coagulant poly ferric chloride (PFC).

There were significant removal associations between dissolved NH3-N, DOC1, and ARGs. The efficacy of ARG removal varied from 0.5-log to 3.1-log reductions **(Li N. et *al*., 2017).**

1.4 Disinfection

Water and wastewater disinfection destroys a considerable percentage of dangerous organisms that might cause bacterial, viral, or parasite disorders. Because it is both accessible and effective, chlorination is the most often used disinfection technique in wastewater treatment. **(Barancheshme & Munir, 2018).**

Chlorination has previously been studied for its influence on the elimination of antibiotics such as cephalexin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, rifampicin, sulfadiazine, tetracycline, and vancomycin, as well as its effect on the inactivation of ARB and ARGs **(Sharma et *al*., 2016).**

Table 7: Removal of ARGs by diffrent treatment processes **(Barancheshme & Munir, 2018).**

Target	Log removal	References
ANAEROBIC AND/OR AEROBIC TREATMENT REACTORS		
tet(G), tet(W), tet(X), sul(1), and intI(1)	–	Du et *al.*, 2015
BIOCHAR		
sul genes	1.21	Ye et *al.*, 2016
CONSTRUCTED WETLANDS		
sul(1), sul(2), sul(3), tet(G), tet(M), tet(O), tet(X), erm(B), erm(C), cml(A) and flo®	0.44 to 0.80	Chen et *al.*, 2016
sul(1), tet(A), tet(C), tet(E), qnr(S), sul(1), sul(3), tet(A), tet(C), tet(E), and qnr(S)	0.39 to 0.65 removal rates of total 14 targeted ARGs	Fang et *al.*, 2017
DISINFECTION		
tet(C), tet(G), tet(W), tet(X), sul(2), drfA1, drfA7, erm(B), erm(F), erm(Q), and erm(X)	0.1 to 2.3	Li H. et *al.*, 2017
ere(A), ere(B), erm(A), erm(B), tet(A), tet(B), tet(M), and tet(O)	0.42 and 0.10 removal of erm and tet genes,	Yuan et *al.*, 2015

sul(1), tet(X), tet(G), intI(1), and 16S rRNA	respectivel 1.30 to 1.49	Sharma et *al*., 2016
COAGULATION		
sul, tet, and integrase genes	0.5 to 3.1	Li N. et *al*., 2017
Note :sulfonamide(*sul*), tetracycline (*tet*), fluoroquinolone (*qnr*), macrolide (*erm*), chloramphenicol (*cml, flo*), methicillin (*mec*), and b-lactam (*bla*). (drf)		

2 Treatment sequences for hospital waste water from different countries :

Table 8 shows the sequences used in various countries for the particular treatment of hospital wastewater.

Table 8: treatment seqquences for hospital wastewater from different countries

country	treatment	PILOT	FULL scale	References
Algeria	**Direct disposal in the environment**			Ly et *al*., 2017
Ethiopia	Direct disposal in the environment		**ponds**	al Aukidy et *al*., 2017
China	Specific treatment	-	MBR + chlorination Biological contact oxydization + MBR + sodium hypochlorite disinfection	J. Li et *al*., 2014
Republic of Korea	Specific treatment	-	**Floc- activated carbon floc-CAS**	al Aukidy et *al*., 2017
India	Direct disposal/co-tratment/specific treatment	**CAS- SF - chlorination**	**CAS**	Lv et *al*., 2015 Rowe et *al*., 2017
Nepal	Direct disposal in the environment	-	**septic tank+H-SSF bed +V-SSF bed**	al Aukidy et *al*., 2017

Note :

CAS : Classical activated sludge *H-SSF:* horizontal subsurface flow
Floc : flocculation MBR: Membrane bioreactor
SF: sand filtration *V-SSF:* vertical subsurface flow

3 Efficiency of the adopted HWW treatment plants :

The removal efficiencies of conventional parameters as well as PhCs from HWWs utilizing various techniques are depicted in the figure below.

As previously stated, many methods were evaluated for the treatment of HWWs as primary, secondary, and tertiary phases.

Primary treatment (Coagulation + filtration + disinfection; Photo Fenton) and secondary treatment (CW; Ponds; CAS; MBR; Biological contact oxidation + MBR + NaClO disinfection; Anaerobic aerobic fixed film reactor, and Aerated fixed film bioreactor + O3)

Figure 06 clearly shows how MBR technology is capable of attaining high removal efficiency (80%) of all macropollutants, with the exception of NH3–N, whose removal was determined to be 71 % **(al Aukidy et *al*., 2017).**

TSS and BOD5 removal efficiencies were very good (97–99 percent), COD (94–97 percent), N–NH4 (80–99 percent), total coliform (99.87–99.999 percent), *E. coli* (99.98–99.999 percent), and Streptococcus (99.3–99.99 percent) were observed using a septic tank followed by an H-SSF and a V-SSF bed specifically designed for the treatment of HW **(al Aukidy et *al*., 2017).**

The results reveal that the pilot-scale system integrated anaerobic–aerobic fixed film reactor for HWW treatment efficiently eliminated 95, 89, and 86 percent of the COD, BOD, and NH4, respectively. When 200 mg/L of ferric chloride was added to an Indian raw hospital effluent, COD removal rose to more than 98 percent, and removal increased to more than 98 percent when the coagulant was applied to settle HWW. Following that, a disinfection process with calcium hydrochloride decreases not just bacteria but also COD **(Rowe et *al*., 2017).**

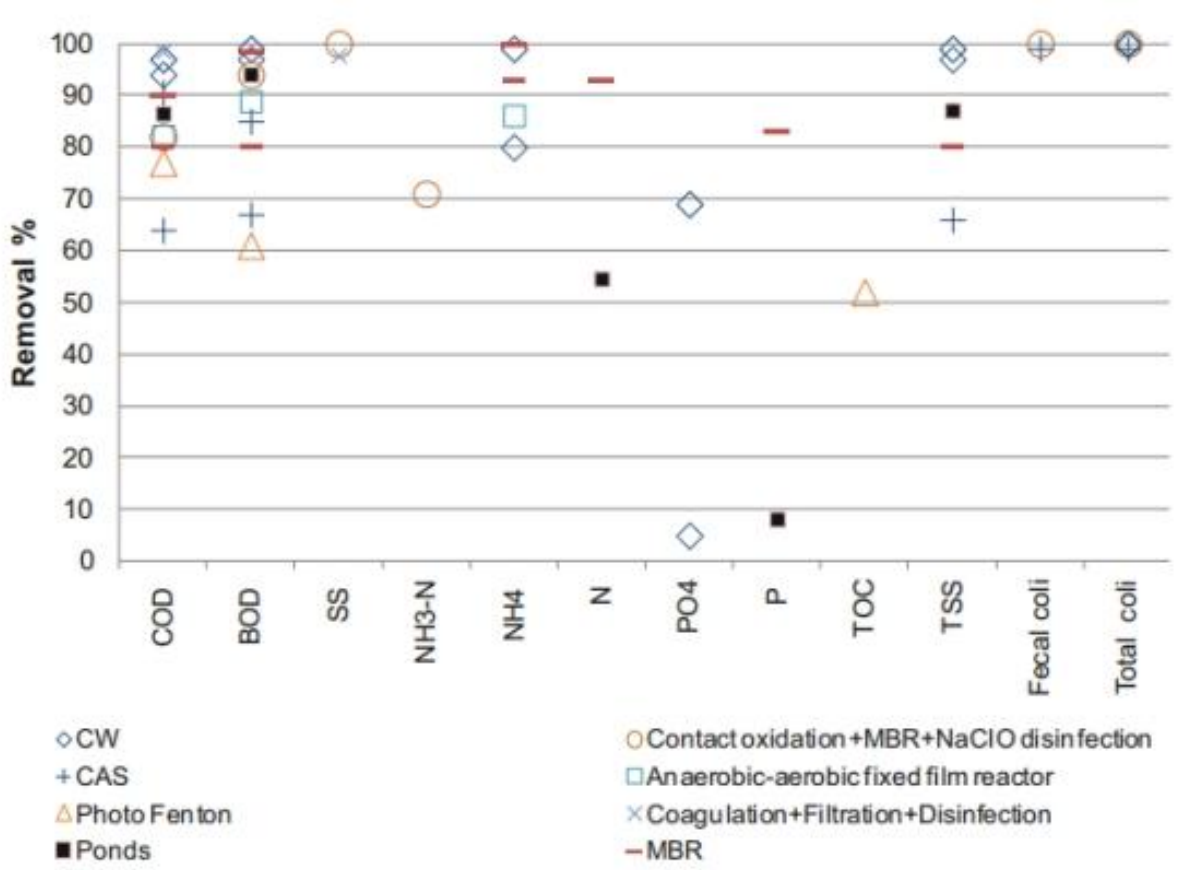


Figure 6: Removal efficiencies from WWTPs for conventional polluants in different primary and secondary treatments **(al Aukidy et *al*., 2017).**

CONCLUSION

Conclusion

This study has demonstrated that the high spread of multidrug-resistant bacteria detected is a consequence of the anthropogenic activities, particularly in urban and clinical environments.

Moreover, The recent years have witnessed the emphasis toward management of HWW and various studies focused on the microbial communities present in aquatic ecosystems.

Antibiotics resistant bacteria and resistance genes are prevalent in freshwater environments, and their presence have increased immensely , that anticipative natural processes of degradation and dilution are not able to completely eradicate them.

ARGs are resistant to natural innactivators and even if they got innactivated their ARG-containing DNA can still be intact and capable of transferring resistance to non-resistant strains through HGT process .

Hospital wastewater are highly complex effluents acting as a hotspot for antibiotic resistant bacteria. and antibiotic resistance genes as it's discharged to the sewer systems, rivers, lakes, and seas without prior treatment .This problem is more severe in developing countries because no wastewater treatment facility is available in most of the cases. Hospital wastewaters are subjected to different treatment scenarios in various countries .but still there is no single practice which could be considered a solution to the problem of managing HWWs.

Indeed, in many cases, a number of sequences are used in combination. Each practice has its own strengths and weaknesses. More effective disinfection processes coupled with activated sludge , membrane filtration and disinfectants should be adopted for better removal of harmful bacteria and ECs.

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ABSTRACT :

Hospitals release significant quantities of wastewater (HWW) and biomedical waste (BMW), which hosts a wide range of contaminants that represent a chemical, biological and physical risk for public and environmental health if left untreated. The HWW can act as an ideal growth medium for various pathogenic microbes. The wastewaters from hospitals also consist of several resistant bacteria and antibiotic residues, which could inhibit the growth of susceptible bacteria, thereby increasing the population of resistant bacteria in the receiving water. The rapid emergence of resistant bacteria is occurring worldwide, one of the most critical multidrug resistance is due to

iuiAntimicrobial-resistant ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and Enterobacter species) pathogens that represent a global threat to human health. The acquisition of antimicrobial resistance genes by ESKAPE pathogens has reduced the treatment options for serious infections, increased the burden of disease, and increased death rates due to treatment failure and requires a coordinated global response for antimicrobial resistance surveillance.

In this context, a thorough literature study was carried out to reveal the negative implications of untreated hospital waste and delineate the proper ways to handle them. Conventional treatment methods can remove only 50%–70% of the emerging contaminants (ECs) present in the HWW. Still, many countries have not implemented suitable treatment methods to treat the HWW in-situ. This review presents an overview of worldwide HWW management and highlights the various treatment techniques for efficiently removing ECs from HWW. As a conclusion when combined with advanced oxidation processes, biological or physical treatment processes could remove around 90% of polluants

Key words :Hospital wastewater , health and environmental risks , antibiotic resistant genes, antibiotic resistant bacteria, multidrug resistant bacteria . WWTPs , treatment strategies .

Resumé :

Les hôpitaux rejettent des quantités importantes d'eaux usées et de déchets biomédicaux , qui hébergent un large éventail de contaminants qui représentent un risque chimique, biologique et physique pour la santé publique et environnementale s'ils ne sont pas traités. Les eaux de rejets hospitalière peut agir comme un milieu de croissance idéal pour divers pathogènes. Les eaux usées des hôpitaux sont également constituées de plusieurs bactéries résistantes et de résidus d'antibiotiques, qui pourraient inhiber la croissance de bactéries sensibles, augmentant ainsi la population de bactéries résistantes dans les eaux réceptrices. L'émergence rapide de bactéries résistantes se produit dans le monde entier, l'une des résistances multidrogues les plus critiques est due à agents pathogènes ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* et les espèces des Enterobacter) résistants aux antimicrobiens qui représentent une menace mondiale pour la santé humaine. L'acquisition de gènes de résistance aux antimicrobiens par les agents pathogènes ESKAPE a réduit les options de traitement pour les infections graves, augmenté le fardeau de la maladie et augmenté les taux de mortalité dus à l'échec du traitement et nécessite une réponse mondiale coordonnée pour la surveillance de la résistance aux antimicrobiens.

Dans ce contexte, une étude approfondie de la littérature a été réalisée pour révéler les implications négatives des déchets hospitaliers non traités et définir les bonnes façons de les gérer. Les méthodes de traitement conventionnelles ne peuvent éliminer que 50 % à 70 % des contaminants émergents (CE) présents dans les les eaux de rejets hospitalières . Pourtant, de nombreux pays n'ont pas mis en

œuvre de méthodes de traitement appropriées pour traiter les déchets in situ. Cette revue présente un aperçu de la gestion mondiale des HWW et met en évidence les différentes techniques de traitement pour éliminer efficacement les CE . En conclusion, combinés à des procédés d'oxydation avancés, les procédés de traitement biologique ou physique pourraient éliminer environ 90 % des polluants.

Les mots clé :

Eaux usées hospitalières, risques sanitaires et environnementaux, gènes résistants aux antibiotiques, bactéries résistantes aux antibiotiques, bactéries multirésistantes. Stations d'épuration, stratégies de traitement.

ملخص

تنتج المستشفيات كميات هائلة من مياه الصرف الصحي ،و النفايات الحيوية الطبية التي تحتوي على مجموعة واسعة من الملوثات التي تمثل خطرا كيميائيا و بيولوجيا على الصحه العامة و البيئية اذا ما تركت دون معالجة.

يمكن ان تعمل مياه صرف المستشفيات كوسيط نمو المثالي للميكروبات المسببه للامراض، و تتكون هذه الاخيرة من العديد من البكتيريا المقاومة و بقايا المضادات الحيوية التي يمكن ان تمنع نمو البكتيريا الحساسة، و بالتالي زيادة عدد البكتيريا المقاومه في المكورات البرازيل المعوية،) ESKAPE المياه المستقبلة . تنتشر البكتيريا بشكل سريع في جميع انحاء العالم، و تعتبر مجموعة المكورات العنقودية الذهبية، كلبسيلل الرئوية، الراكدة البومانية،ازائفة الزنجارية، وانواع البكتيريا الأمعائية) واحدة من اهم اسباب انتشار المقاومه المتعددة للمضادات الحيوية البكتيرية، والامراض التي تمثل تهديدا عالميا لصحة الانسان. أدى اكتساب الجينات إلى تقليل خيارات العلاج للعدوى الخطيرة و زيادة عبء ESKAPE المقاومة للمضادات الميكروبات من قبل مسببات الامراض المرض و معدلات الوفيات ، بسبب فشل العلاج و يتطلب هذا الوضع استجابة عالمية منسقة لمراقبة مقاومة مضادات الميكروبات.

في هذا السياق، تم اجراء دراسة شاملة للكشف عن الاثار السلبية لنفايات المستشفيات الغير المعالج ،و تحديد الطرق المناسبة للتعامل معها حيث يمكن ان تزيل طرق المعالجة التقليدية من 50 إلى 70 ٪ فقط من الملوثات الموجودة في النفايات الخطيرة ، ومع ذلك لم تنفذ العديد من البلدان أيا من هذه التقنيات .

تقدم هذه المراجعة لمحة عامة عن ادارة مخلفات المستشفيات في جميع انحاء العالم، وتسلط الضوء على تقنيات الاصطلاح المختلفة لازاله الملوثات بكفاءة.

كخلاصة ،عند دمج هذه الاخيرة مع عمليات الاكسدة المتقدمة، يمكن لتقنيات المعالجة البيولوجية او الفيزيائية ان تزيل حوالي 90 ٪ من الملوثات.

الكلمات المفتاحية : مياه الصرف الصحي ، المخاطر الصحية والبيئية ، الجينات المقاومة للمضادات الحيوية ، البكتيريا المقاومة للمضادات الحيوية ، البكتيريا المقاومة للأدوية المتعددة. محطات معالجة مياه الصرف الصحي واستراتيجيات العلاج..