CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD


# Virulence Factors Variance Profiling for the *Morganella morganii* Strains in Biofilms

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

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Faculty of Health and Life Sciences
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# CERTIFICATE OF APPROVAL

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# *Abstract*

The comparative genomic analysis of *Morganella morganii* and *Comamonas testosteroni sp.* is the main focus of this study. While *Comamonas testosteroni sp.* is a non-spore-forming bacteria found in soil, particularly in areas rich in organic matter and freshwater habitats like rivers, lakes, and streams, *Morganella morganii* is an opportunistic pathogen of increasing concern due to its resistance to antibiotics. By taking use of virulence features and habitat-driven genetic differences, this study promotes the development of innovative biotechnological and medicinal techniques. We found and characterized resistance genes in both strains' genomes. concentrating on how they are distributed over genomic islands, which are recognized as sites for horizontal gene transfer. The RAST server was used to annotate the genome. Both organisms' resistant antibiotic genes are found using CARD. To detect secondary metabolites, we employ antiSMASH. CG Viewer created a circular gnome that displayed genes linked to many tasks, including antibiotic resistance. Both strains' core genomes were analyzed using antibiotic resistance target finder. In order to comprehend the evolutionary links with other closely related species, a phylogenetic tree was also constructed, offering insights into the emergence and dissemination of resistance genes. *Morganella morganii* has a 4,034,593 bp genome with a 51.1% GC content, 4,242 coding sequences, and 75 RNAs. There are 5,189 coding sequences and 133 RNAs in the 5,497,097 bp genome of *Comamonas testosteroni*, which has a 61.4% GC content. Genes related to protein synthesis, fatty acid metabolism, carbohydrate digestion, and structural regulation were identified by functional annotation. Interestingly, there are 71 genes associated with environmental stress response and 0 genes associated with virulence, illness, and defense in *Morganella morganii*. There are 83 virulence, illness, and deference genes and 76 stress response genes in *Comamonas testosteroni*. *Comamonas testosteroni* has two genes linked to antibiotic resistance, whereas *Morganella morganii* has five genes. In contrast to *Comamonas testosteroni*, which also has three pathways for antibiotic resistance, *Morganella morganii* has three mechanisms, the most significant of which is antibiotic efflux. This thorough analysis improves our knowledge of the genetic elements influencing

the adaptation and resilience of *Morganella morganii*, an emerging opportunistic disease that exhibits antibiotic resistance.

**Keywords:** *Morganella morganii*, *Comamonas testosteroni*, Antibiotic Resistance, resistance Genes, Genomic Islands, antiSMASH, CARD, Horizontal Gene Transfer, Secondary Metabolites, Evolutionary Relationships

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# Abbreviations

| | |
|---|---|
| **AMR** | Antimicrobial Resistance |
| **antiSMASH** | Antibiotic and Secondary Metabolie Analysis Shell |
| **CARD** | Comprehensive Antibiotic Resistant Database |
| **CDC** | Center for Disease Control and Prevention |
| **CROs** | Carbapenem Resistant organisms |
| **ECM** | Extracellular Matrix |
| **EPS** | Extracellular Polymeric Substances |
| **GC** | Guanine Cytosine |
| **GIs** | Genomic islands |
| **HAIs** | Hospital Acquired Infections |
| **HGT** | Horizontal Gene Transfer |
| **MALDI TOF MS** | Matrix Assisted Laser Desorption/Ionization Time Of Flight Mass Spectroscopy |
| **MDR** | Multi Drug Resistance |
| **PDR** | Pan Drug Resistance |
| **R plasmid** | Resistance plasmid |
| **RAST** | Rapid annotation using Subsystem Technology |
| **spp.** | Species |
| **UTIs** | Urinary Tract Infections |
| **WHO** | World Health Organization |
| **XDR** | Extensive Drug Resistance |

# Chapter 1

# Introduction

Nosocomial infections are a widespread and growing problem in healthcare systems around the world, impacting both developed and developing countries and raising morbidity and medical expenses. These infections often start during hospital stays and can continue long after release, especially in environments where invasive procedures and long-term antibiotic usage are common. An rising number of nosocomial infections have been linked to the Gram-negative opportunistic pathogen *Morganella morganii*, particularly in clinical isolates from the respiratory and urinary tracts. Numerous instances of *M. morganii* in healthcare settings have been reported by genomic epidemiology studies utilizing clinical isolates conducted between 2016 and 2023. These studies highlight the importance of *M. morganii* in hospital-acquired illnesses and suggest that there may be clusters of in-hospital transmission [1].

Genomics has basically changed the understanding of bacterial physiology, evolution, and pathogenicity by enabling wide reasoning of an organism's complete DNA content. Whole-genome sequencing (WGS) and subsequent bioinformatic analyses allow researchers to mark gene structure, functional pathways, and evolutionary relationships. In recent large-scale genomic studies of *M. morganii*, WGS disclosed significant genomic diversity among clinical isolates and identified numerous antimicrobial resistance (AMR) genes belonging to multiple drug classes, mark the value of genomic approaches in surveillance and infection control [2].

Plasmids, transposons, integrons, and insertion sequences are examples of mobile genetic elements (MGEs) that are essential to the spread of virulence and resistance factors in bacterial populations. Several plasmid-borne resistance genes, including as those linked to quinolone resistance and $\beta$-lactamases, have been found in *M. morganii*. These elements can be mobilized through horizontal gene transfer methods like as conjugation, transformation, and transduction, and they often colocalize with antimicrobial resistance genes. Positive associations between MGEs and ARGs in *M. morganii* have been shown by genomic analysis, suggesting that resistance determinants are frequently mobilized and that there is a chance for quick expansion in clinical settings [3].

A well-established evolutionary phenomena is the ability of *Morganella morganii* and other bacteria to transfer genetic material, including virulence and resistance determinants, among closely related or even unrelated microbial species. This ability results from the presence of several virulence and antibiotic resistance genes on mobile genetic elements (MGEs), including integrons, transposons, plasmids, and genomic islands. The horizontal gene transfer (HGT) of genetic features between various bacterial populations is made possible by these components, which are a part of the bacterial mobilome. Through conjugation, transformation, or transduction, plasmids encoding numerous antibiotic resistance genes can spread these determinants, enabling recipient bacteria to quickly acquire resistance features that improve their survival under antimicrobial pressure.The evolution of pathogenicity in opportunistic organisms like *M. morganii* and other Enterobacterales is further aided by the fact that mobile elements often carry clusters of functionally related genes, including those encoding toxins and adhesion factors. A major evolutionary process for the establishment and spread of clinically significant bacterial pathogens with improved virulence and multidrug resistance profiles is the extensive involvement of MGEs in gene dissemination [1].

Clinically relevant bacteria, such as *Morganella morganii*, have a notable predominance of virulence genes organized in vast, continuous genomic areas, such as chromosomal inserts or pathogenicity islands. Gene clusters that support pathogenicity and adaptive fitness are frequently seen in these genomic areas. Finding the

minimal gene set needed to sustain cellular activity in the natural environment is made possible by the combination of computational and experimental methods. Given this, genome sequencing has emerged as a vital technique in microbiology, aiding in the identification of virulence factors, prediction of metabolic capacities, and enhanced comprehension of microbial evolution [2].

Numerous search and annotation strategies have been prompted by the growing popularity of genome sequencing techniques, but combining them is still challenging since scientists utilize arbitrary methods to resolve the information [4]. In susceptible circumstances, the bacteria *Morganella morganii* establish themselves as environmental organisms that cause diseases. These microbes typically infect immunocompromised patients, leading to urinary tract and soft tissue infections. Although death is rare, infections in soft tissues or catheter connections are the main causes of bacteremia. In addition to tetracyclines, medical professionals believe that Morganella species are resistant to fluoroquinolones, piperacillin, trimethoprim, and carbapenems [5].

Treating infections caused by Morganella species can be difficult because they are infamously resistant to several medications. Microbiological diagnostics play a major role in halting the spread of nosocomial infections. Understanding the etiological agents of illnesses and their susceptibility to antibiotics enables timely response in the event of a suspected epidemic outbreak [6]. The opportunistic bacteria belonging to the Morganella genus cause infections that are acquired in hospitals and the community and are difficult to treat due to their resistance to antibiotics [7].

The Morganella species is a recently identified pathogen that is mostly non-pathogenic but occasionally causes hospital-acquired UTIs. Morganella is a genus of oxidase-negative, Gram-negative, motile, facultatively anaerobic bacilli that belong to the family Morganellaceae. The genus was named for the bacteriologist H. de R. Morgan, whose name is the source of the name Morganella. Previously, the species was included within the Proteus genus. The most common and important species that infects humans is *Morganella morganii*. *Morganella morganii*

environmental and clinical isolates have been found in soil, water, and animal and human digestive tracts [8].

In addition to clinical specimens, other isolates of *Morganella morganii* have been found in environmental sources as soil, water, and sewage [9]. There are species in the genus Morganella that are motile, facultative anaerobic, Gram-negative bacilli that do not produce pigment or have a unique smell. *Morganella morganii* is the main human pathogen among clinically important species. Previously classified as weak pathogens by medical specialists, the bacteria are common germs that inhabit a variety of habitats. Hospital environmental concerns regarding the management of this difficult infection have been raised by recent case reports involving *Morganella morganii*, which have resulted in cutaneous, urine, and bloodstream infections in immuno-compromised patients [10].

Genes linked to antibiotic resistance have been found in *Morganella morganii*, including chromosomally encoded AmpC $\beta$-lactamase genes that cause $\beta$-lactam resistance, fluoroquinolone resistance-associated genes, tetracycline resistance genes (tet), chloramphenicol resistance genes (cat), intracellular survival-associated genes (katA, clpP, tuf/Ef-Tu, and sodB), iron acquisition-related genes (dnaK, hsp60), and biofilm-associated genes [11]. Antimicrobial resistance is one of the biggest challenges to humanity today and a huge worldwide health concern. Certain bacterial strains have become resistant to nearly every antibiotic. Therefore, in order to combat resistant bacteria, new antibacterial agents must be developed [4].

A list of antibiotic-resistant priority infections that were discovered in 2017 and that urgently need new medications has been made public by the WHO. To direct and promote research and development of novel antibiotics, the list divides them into three priority categories: critical, high, and medium. The majority of the bacterial diseases on the WHO list are Gram negative. Due to their distinct structure, Gram-negative bacteria are more persistent than their Gram-positive counterparts and significantly increase morbidity and mortality worldwide. There are numerous recognized methods for managing and combating resistance brought on by Gram-negative bacteria.

These include developing antimicrobial auxiliary agents, altering the structures of existing antibiotics, and researching chemical structures with fresh targets and modes of action that these resistant bacteria react to. Research efforts have been made to address the urgent need for new treatments, and some have shown success against resistant Gram-negative bacteria by neutralizing the resistance mechanism. One example of this is the use of antibiotic adjuvants that contain $\beta$-lactamase inhibitors [12].

Because of their extensive presence in the environment, the Morganella species are mostly non-pathogenic. However, in recent years, a growing number of cases have been connected to this purportedly aggressive bacteria.

Underlying conditions that can increase a person's risk of contracting Morganella infections include diabetes mellitus, catheter use, and prolonged stays in intensive care units [13]. Biofilm was initially found in tooth plaque by Dutch microscope inventor Anton Van Leeuwenhoek in the 1670s. Before about 50 years ago, there was very little scientific investigation on the characteristics of biofilms. Following its development, electron microscopy demonstrated that biofilm is a microbiological colony of bacteria. In addition to exhibiting resistance to antibacterial drugs at least 500 times stronger than solitary planktonic cells, microorganisms that live in this unique structure exhibit distinct biological behaviors. Bacteria can survive for extended periods of time in a variety of environments thanks to the protective multicellular bacterial habitat, which also acts as a defense mechanism against adverse elements.

Through cooperative growing communities that develop colonies in favorable environments, biofilm formation acts as a common way for surviving challenging situations. Biofilms' key sugar components determine their pathogenic capacity and give rise to their intense structure. This community has extremely high cell densities, ranging from 108 to 1011 cells per gram. At 80% and higher, the primary cause of persistent clinical infections is the formation of biofilms. Numerous types of bacteria exhibit the capacity to form biofilms by attaching themselves to a variety of inanimate objects and living surfaces. Microorganisms use a variety of

technologies in healthcare facilities as attachment surfaces [14]. According to recent studies, most bacteria that cause chronic infections grow into biofilms, which are sessile populations that are inherently resistant to the host's immune system as well as the effects of numerous medications due to their shape and metabolic quiescence. Microbial biofilms may be present in chronic wounds in situ, delaying the healing process, according to several clinical and experimental observations reported in the literature [15].

Biofilms are associated with antibiotic-resistant bacteria. Horizontal gene transfer promotes genetic diversity and evolution in natural microbial communities. Studying gene transfer in natural settings has become more crucial due to the emergence of drug-resistant bacteria. The EPS matrix prevents certain antimicrobial medications from entering the biofilm and restricts the diffusion of chemicals from the surrounding environment. Certain antibiotics, such aminoglycosides, which are positively charged and hydrophilic, are more resistant. When antibiotics are administered at quantities below the minimum inhibitory concentration, a variety of bacterial species can form biofilms. The potential exposure of cells located deep inside the biofilm to antibiotics at sub-minimum inhibitory concentration levels is a serious cause for worry. Instead of preventing biofilm formation, the antibiotic can promote it [16]. Bacteria can develop genomic islands (GIs), which are large chromosomal regions with mobile elements, by horizontal gene transfer (HGT).

GIs may be removed and given to other recipients in specific situations. Pathogenicity islands (PAIs) and antibiotic resistance islands are two of the numerous subclasses into which genomic islands (GIs) can be further subdivided according to the functions of their encoded genes. GIs are thought to play an important role in bacterial diversity and evolution, as well as in primary and secondary metabolism, pathogenicity, and antibiotic resistance. In *Morganella morganii*, several genomic regions have been identified that harbor resistance- and virulence-associated genes and are considered putative genomic islands, including regions linked to $\beta$-lactam resistance and mobile genetic elements. However, it remains unclear whether these genomic regions retain the ability to mobilize or transfer between bacterial lineages [17].

Bacterial species' gene repertoires are frequently very diverse, which is essential for their ability to interact with coevolving eukaryotic hosts, colonize new ecological niches, and adapt to changing environments. Horizontal gene transfer (HGT), a vast evolutionary mechanism that spreads genes among bacterial lineages that may be highly distantly related, is the source of the majority of unique genes in bacterial genomes. Most genes acquired through HGT are thought to be neutral or harmful, and as a result, they are quickly lost. But, especially in nosocomial infection environments, HGT also plays a major role in the development of adaptive characteristics like antibiotic resistance in *Morganella morganii*.

As a result, the balancing processes of gene acquisition and loss impact genome diversity, with many genes facing purifying selection and some enduring positive selection [18].

Antibiotic resistance is a common and well-studied trait associated with GEIs. because it raises questions about how well infectious diseases can be treated. The transformation in bacterial populations from almost universal susceptibility to developing antibiotic resistance worldwide in a matter of decades demonstrates the astounding ability of bacteria to adapt, despite the fact that it is a man-made danger. Many aspects of the evolution of antibiotic resistance are poorly understood. The rate at which this resistance has expanded globally is one noteworthy aspect [19].

Gram-negative, aerobic, motile, rod-shaped bacteria with a modest GC content are found in the genus Comamonas. It is a member of the *Burkholderiales* order's *Comamonadaceae* family. This genus's members are found in a wide range of soil and aquatic settings, such as contaminated industrial sites, freshwater systems, wastewater treatment facilities, and activated sludge. *Comamonas testosteroni* is well known for its exceptional metabolic flexibility and capacity to use a variety of organic molecules as sources of carbon and energy. Notably, this species is well known for its ability to break down xenobiotic contaminants, including phenols, aromatic hydrocarbons, and steroid chemicals like testosterone.Because of its metabolic adaptability, *C. testosteroni* plays a significant role in environmental detoxification and bioremediation procedures.

Genes encoding various dehydrogenases, monooxygenases, dioxygenases, and hydrolases have been found by genomic investigations. These enzymes are essential for the degradation of complex organic compounds and environmental pollutants. Furthermore, some strains show characteristics linked to plant growth promotion, such as the capacity to engage in nutrient cycling and stress reduction, underscoring their possible agricultural significance.

Despite being mainly thought of as an ambient bacteria, *Comamonas testosteroni* has occasionally been known to cause opportunistic infections, usually in those with weakened immune systems. However, when compared to well-known clinical pathogens, comparative genomic studies show that *C. testosteroni* carries comparatively few classical virulence factors and antibiotic resistance genes, indicating its primarily non-pathogenic lifestyle. Overall, *Comamonas testosteroni* is becoming more and more popular for use in biotechnology, wastewater treatment, and synthetic biology due to its metabolic variety, environmental robustness, and low pathogenicity [20].

Whole genome sequencing (WGS) has become the most used method in laboratories worldwide within the last ten years. Its high speed, throughput, and decreasing cost have led to significant advancements in the investigation and surveillance of epidemics caused by a variety of microbial diseases. A "one-stop" solution, WGS provides all the information required for pathogen type and characterisation, including the speedier identification of genes of interest (such as genes for antibiotic resistance, plasmid replicon detection, and sequence typing). This is achieved with previously unheard-of resolution and cost per sample, and it does away with the necessity for multiple consecutive, time-consuming molecular assays [21].

## 1.1 Hypothesis

Biofilms might contribute to the activation of certain genes involved in pathogenicity island.

## 1.2 Aim and Objectives

The main objective of this research is to screen varying virulence factors of *Morganella morganii* and *Comomonas testosteroni* triggered by biofilms

### 1.2.1 Objectives

The research objectives are:

i. To identify genes associated with the virulence properties of two *Morganella morganii* strains originating from biofilm and non biofilms

ii. To examine the variations in potential virulence mechanism of both strains.

iii. To investigate virulence gene clusters and pathogenicity islands.

# Chapter 2

# Literature Review

## 2.1 Nosocomial Infections

Nosocomial infections are caused by bacteria or other infectious organisms that a patient contracts while they are in the hospital. Antibiotics are usually ineffective against microorganisms associated with nosocomial infections, particularly those most frequently used in that hospital setting.Nosocomial infections account for 5% to 10% of all hospitalized patients, and they greatly increase hospital costs, morbidity, and mortality. Common nosocomial illnesses include bloodstream infections, pneumonia, surgical wound infections, UTIs, and infectious diarrhea. In veterinary medicine, the reasons behind the increase in nosocomial infections in human hospitals are being pinpointed more and more. These include longer hospital stays, increased use of invasive equipment, increased use of antimicrobial drugs, and increased use of critical care procedures [22].

A major risk factor for life-threatening illnesses, the phrase "healthcare associated infections" (figure 2.1) refers to the kind of infections caused by prolonged hospital stays [23]. Approximately 75% of the burden of chronic diseases falls on developing countries. Asymptomatic individuals may be considered infected if these germs are found in bodily fluids or at a sterile body site, such as blood or cerebrospinal fluid. Additionally, nosocomial infections can be acquired by hospital staff, visitors,

or other healthcare providers. Infections not considered nosocomial are those that were present at the time of admission and become more complex, but the pathogens or symptoms change, resulting in a new infection.

The infections, such as toxoplasmosis, rubella, syphilis, or CMV, that appear 48 hours after delivery and are acquired transplacentally [24]. Based on biological and clinical criteria, the National Healthcare Safety Network and the CDC classified the 50 nosocomial infection sites into 13 types. The most common ones are meningitis, lung infections, gastroenteritis, surgical and soft tissue infections, and urinary UTIs. Over time, a change in nosocomial infections can be detected. The finest example of this is the case of pneumonia, when over a five-year period, the prevalence of nosocomial pneumonia increased from 17% to 30% [25].

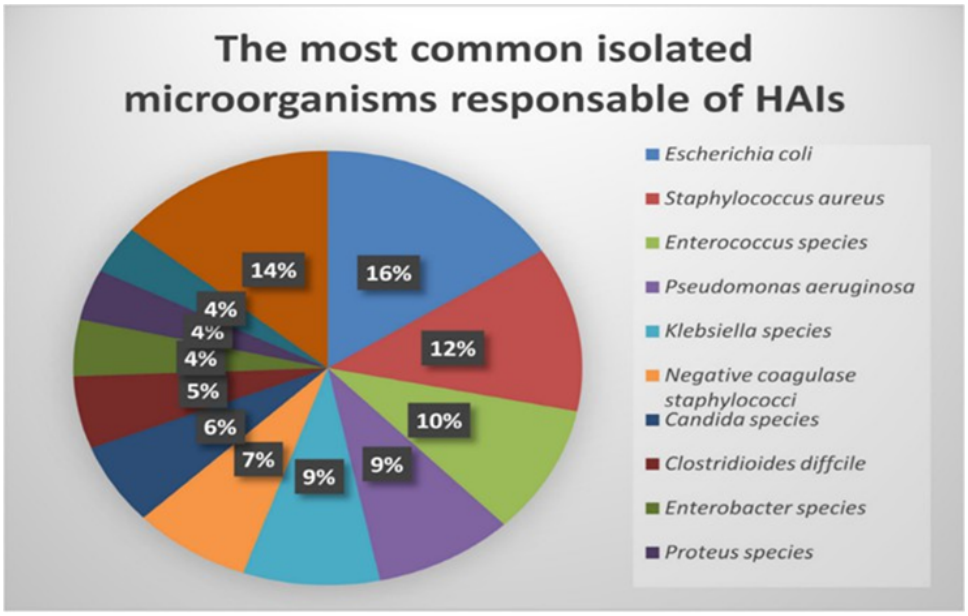


FIGURE 2.1: Most common bacteria associated with hospital acquired infections [26]

## 2.2 Properties of *Morganella* Species

It is frequently found in environmental settings and constitutes a component of the normal human flora, though it is also recognized as a significant opportunistic pathogen, particularly in nosocomial infections. This bacterium, while a common inhabitant of the human gastrointestinal tract, is increasingly recognized

for its role in a diverse array of clinical manifestations, including sepsis, urinary tract infections, and soft tissue infections [27]. Notably, *M. morganii* has also been implicated in severe conditions such as endocarditis, osteomyelitis, and acute postoperative endophthalmitis. Initially considered an uncommon cause of infection, *M. morganii* is now regarded as an increasingly important pathogen due to its virulence and rising drug resistance, which can lead to high mortality rates in certain infections (Liu et al., 2021). A key characteristic contributing to its pathogenicity is its inherent resistance to colistin and its capacity for multidrug resistance often facilitated by various resistance genes and mobile genetic elements (Anfal et al., 2024; Behera et al., 2023). Its intrinsic resistance further complicates treatment strategies, distinguishing it from other members of the Morganellaceae family, such as *Proteus mirabilis*, which is also a significant cause of nosocomial urinary tract infections (Anfal et al., 2024; Li et al., 2022; Liu et al., 2025; Soliman et al., 2024). While both *M. morganii* and *P. mirabilis* are members of the Morganellaceae family and can cause severe infections, Proteus species, including *P. mirabilis*, are intrinsically resistant to polymyxins, nitrofurans, tigecycline, and tetracycline, but susceptible to aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole (Li et al., 2022). However, acquired resistance to imipenem in *P. mirabilis* commonly arises from porin loss, reduced expression of penicillin-binding proteins, or the acquisition of carbapenemase genes, further complicating therapeutic options (Girlich et al., 2020). The intrinsic resistance of *M. morganii* to several crucial antibiotics, such as colistin, and the ubiquitous presence of inducible AmpC enzymes further restrict the therapeutic options for treating *M. morganii* infections (Mbelle et al., 2019).

TABLE 2.1: Description of different properties of M. odoratimimus [28]

| **Property** | **Description** |
|---|---|
| Gram staining | Gram negative |
| Shape | Rod-shaped (bacillus) |
| Colony color | Pale, grayish to off-white colonies on nutrient agar; non-lactose fermenting on MacConkey agar |
| Motility | Motile (peritrichous flagella) |
| Pathogenicity | Opportunistic pathogen |
| Resistance mechanism | Efflux pumps , biofilm production |

**Table 2.1 continued from previous page**

| **Property** | **Description** |
|---|---|
| Biofilm making ability | Form strong biofilm |
| Habitat | Soil, water ,sewage and hospital settings |
| Antibiotic resistance | Multi drug resistance |
| Infections | Nosocomial infections usually UTIs, bacteremia, sepsis |
| Oxygen requirement | Facultative anaerobe |
| Temperature range | 20 to 37 Celsius |
| PH tolerance | Neutral to slightly alkaline |
| GC content | 51% |
| Intrinsic resistance | reduced susceptibility to Carbapenems and pencillins |
| Enzyme production | Urease, phenylalanine deaminase AmpC $\beta$-lactamase |
| Biochemical reactions | Oxidase negaitive, catalase positive and non-lactose fermenting |

## 2.3 Infections Caused by *M. morganii*

The coronavirus pandemic revealed *M. morganii* as one of the pathogens leading to bloodstream infections from catheters when antibiotics provided no treatment success. This emerging threat proves concerning because *M. morganii* causes healthcare acquired bacterial infections that spread to urinary tracts and skin tissues and infect soft tissue. Research has shown that *M. morganii* can cause cellulitis through a pig bite attack on immunocompetent children which demonstrates the pathogen's ability to transmit between animals and humans.

The research proves that aquatic bacteria exist simultaneously as water dwelling organisms while also functioning as opportunistic pathogens that result in important medical conditions which affect humans through aquatic environment interactions. The ability to understand transmission patterns of zoonotic pathogens combined with improved diagnostic procedures stands vital to reduce the health risks associated with zoonotic infection transmission [29].

### 2.3.1 UTIs Cause by *Morganella morganii*

Patients with diabetes mellitus, chronic nephritis, urinary calculi, and urinary retention have all been found to develop UTIs caused by *Morganii spp.* [30]. Multiple risk factors, including immunocompromised conditions and extended urine catheterization, were significant contributors to the development of a MDR Myroides urinary infection. Recurrent hospital stays could be a separate factor for infection and colonization by MDR bacteria like Morganii species [31].

### 2.3.2 Bacteremia Cause by *Morganella morganii*

*Morganella morganii* caused bacteremia is a rare but often reported illness that mostly affects individuals with invasive medical devices or those with impaired immune systems. Usually, especially in hospital settings, the organism enters the bloodstream through surgical wounds or indwelling catheters.

Fever, chills, hypertension, and increased inflammatory markers are among the clinical signs, which, if left untreated, can develop into sepsis. Due to chromosomally encoded metallo-$\beta$-lactamases like MUS-1, it is multidrug resistant, especially to $\beta$-lactams and carbapenems, which is a significant clinical problem. Blood cultures frequently show monomicrobial growth, but sophisticated methods like 16S rRNA sequencing or MALDI-TOF MS are needed for identification. Due to large delayed identification and few available treatments, Morganella-associated bacteremia has a high death rate.Despite strain specific resistance patterns, tigecycline, minocycline, and ciprofloxacin susceptibility has been noted in documented cases. A contributing factor to chronic bacteremia and treatment failure is the development of biofilms on catheters and medical surfaces.Because of its resistance to human defenses and environmental tolerance, the bacterium is a dangerous disease causing agent in critical care units. The existence of many resistance genes and efflux pumps, which make eradication more difficult, has been verified by genomic investigations.There have been reported outbreaks in intensive care units, and infections are frequently acquired in hospitals. Targeted antibiotic treatment,

source management, and early detection are essential for positive results. Due to the general ineffectiveness of standard empirical regimens, susceptibility guided therapy must be used. Preventing bloodstream infections caused by *Morganella morganii* requires strict adherence to infection control procedures and catheter care guidelines [32]. Table 2.2 Illustrate infections caused by *Morganella morganii*.

TABLE 2.2: Illustration of infections caused by *Morganella morganii* [33]

| **Infection** | **Description** |
|---|---|
| Urinary tract infection | Usually occur in immunocompromised and in patients with long stay in hospital with catheter insertion. Many nosocomial outbreaks and cases are reported in literature |
| Skin and soft tissue infections | Occur in patients which are surveying from hyperglycemia and are immunocompromised |
| Bacteremia | *Morganella morganii* also cause bacteremia in patients which are immunocompromised |
| Osteomyelitis | Rare cases but reported in some case studies |
| Pericardial effusion | Most fetal as compared to other infections and cases are reported in India, |
| Wound infection | Associated with wound infections especially after surgery.Cases are reported in hospital settings especially if stay is long |

## 2.4 Clinical Importance of *Morganella morganii*

Advances in molecular microbiology have significantly improved the identification and characterization of bacteria from clinical samples, including *Morganella morganii*. Clinical reports indicate an increasing number of *M. morganii* isolates from various specimens, particularly urine cultures of patients with indwelling urinary catheters. *M. morganii* is a Gram-negative, facultatively anaerobic bacillus that belongs to the Enterobacterales order and is non-lactose fermenting. Environmental reservoirs include soil, water, and the gastrointestinal tract of humans and animals, as well as potential contamination in food processing environments [31].

Despite its widespread environmental occurrence, human infections caused by *M. morganii* are relatively rare but clinically significant. The literature shows that infections predominantly occur in immunocompromised patients or those with comorbid conditions such as diabetes, kidney failure, liver cirrhosis, or chronic pulmonary disease. *M. morganii* acts as an opportunistic pathogen, causing a range of infections including urinary tract infections (UTIs), wound infections, soft tissue infections, bacteremia, and less frequently, osteomyelitis or pericardial infections. Infections are often associated with prolonged ICU stays and the use of invasive devices such as urinary catheters [32].

These infections are complicated by the organism's intrinsic and acquired multidrug resistance. *M. morganii* exhibits resistance to multiple $\beta$-lactams, including penicillins and first-generation cephalosporins, and can acquire resistance to carbapenems through plasmid-encoded carbapenemases. Variable susceptibility is observed to aminoglycosides, fluoroquinolones, and sulfamethoxazole-trimethoprim, making treatment challenging. Reports of multidrug-resistant *M. morganii* isolates have increased across hospitals, especially in isolates recovered from catheter-associated urinary tract infections in intensive care units over recent years [32].

## 2.5 Cases Reported Related to *Morganella morganii*

*Morganella morganii* is an opportunistic pathogen that, although widespread in the environment and gastrointestinal tracts of humans and animals, is considered relatively rare as a cause of clinical infection. Historical reports trace the recognition of *M. morganii* in human infections to early 20th-century microbiology, but systematic documentation has increased in recent decades due to advances in molecular identification techniques. Published literature has reported multiple cases of infections caused by *M. morganii*, including urinary tract infections, bacteremia, wound infections, soft tissue infections, and, in rare instances, central nervous system involvement. Most infections occur in patients with impaired

immune systems or those with underlying comorbidities such as diabetes, renal failure, or chronic pulmonary disease [33].

Clinical management is often complicated by the organism's intrinsic and acquired multidrug resistance. *M. morganii* is naturally resistant to several $\beta$-lactams and may acquire resistance to carbapenems via plasmid-borne carbapenemases. Aminoglycosides, fluoroquinolones, and sulfamethoxazole-trimethoprim show variable efficacy depending on strain-specific resistance profiles. Biofilm formation on medical devices, such as urinary catheters or surgical implants, further contributes to persistent or recurrent infections [33].

Case reports illustrate that *M. morganii* infections can arise from diverse environmental exposures or invasive procedures. For instance, urinary catheterization and prolonged ICU stays are common predisposing factors for bacteremia or catheter-associated UTIs. Timely identification using advanced diagnostic tools such as 16S rRNA sequencing or MALDI-TOF MS, coupled with susceptibility-guided antibiotic therapy, is critical to achieving favorable outcomes. Genomic studies have confirmed the presence of multiple resistance genes, efflux pumps, and virulence determinants, highlighting the pathogen's capacity to survive under selective pressures and evade host defenses. Globally, *M. morganii* infections remain relatively rare, but their frequency is increasing in hospital settings, emphasizing the need for rigorous infection control practices and careful monitoring of high-risk patients [34].

## 2.6 Carbapenem Resistant Organisms

CROs function as a major global threat for infectious diseases which affect both human populations and animals. The CHINET surveillance tracking and pet/animal specialized monitoring demonstrate CROs to be prevalent and important across surveillance networks.

Transmission of multidrug-resistant bacterial strains such as CROs can happen through arthropod species that consume human and animal waste. The research

team obtained a sheep farm fly from Hubei Province China that contained four CROs from various species [35].

The study demands increased research scope for antibiotic resistance which must encompass human subjects as well as animal and environmental factors [36]. Figure 2.2 give an overview of carbapenem resistance.

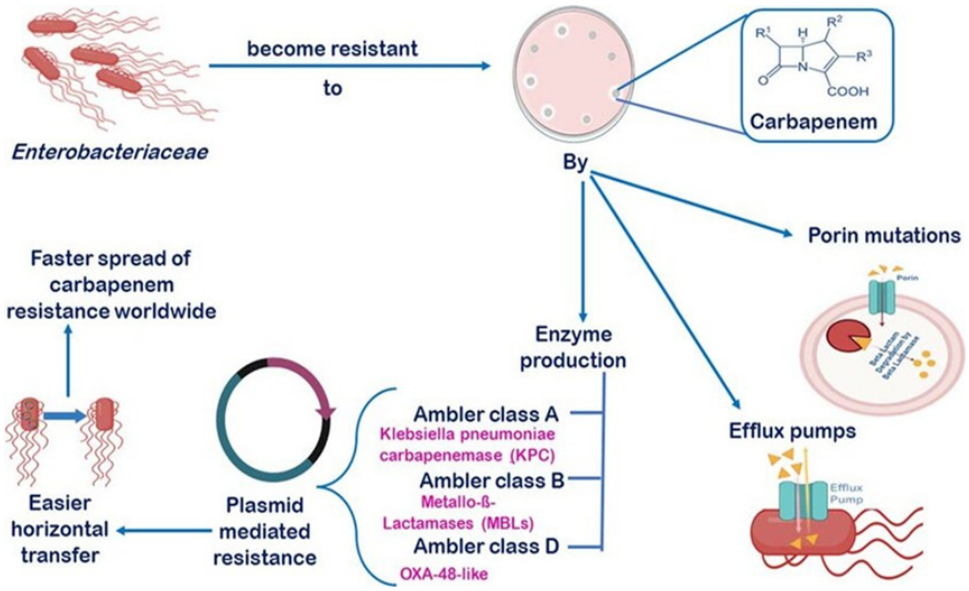


FIGURE 2.2: An overview of carbapenem resistance [37].

## 2.7 Data about Morganella Genus in NCBI

*Morganella morganii* is an opportunistic Gram-negative bacterium that belongs to the family Morganellaceae within the order Enterobacterales. Historically, *M. morganii* was first identified in the early 20th century from human intestinal samples and has since been recognized as a clinically important pathogen, particularly in nosocomial infections. According to the NCBI Taxonomy Browser, Morganella comprises multiple species, with *M. morganii* being the most clinically significant.

The genus name Morganella was proposed in honor of H. L. Morgan, who first described the species, and it reflects the historical classification of this bacterium among the Enterobacteriaceae. *M. morganii* cells are typically rod-shaped, approximately 0.6-0.8 × 1.5-2.5 µm in size, facultatively anaerobic, oxidase-negative,

catalase-positive, and motile via peritrichous flagella. Genome sequencing and NCBI genomic data indicate a GC content of ∼51%, with multiple genes involved in virulence, biofilm formation, and antibiotic resistance.

The availability of whole-genome sequences in public databases facilitates comparative genomics, epidemiological tracking, and identification of resistance determinants and mobile genetic elements, underscoring the clinical and research significance of this species [37].

*Morganella morganii* demonstrates growth on standard culture media such as MacConkey agar, producing pale, smooth, non-lactose fermenting colonies. The organism can survive across a broad temperature range, typically from 20°C to 40°C, reflecting its adaptability to environmental and host conditions.

Among clinical isolates, *M. morganii* is the most frequently encountered species within the Morganella genus, followed by other closely related species such as M. psychrotolerans.

*M. morganii* can cause a wide spectrum of infections, including urinary tract infections, wound and soft tissue infections, bacteremia, and, more rarely, endocarditis, liver abscesses, and sepsis. These infections can occur in both immunocompetent and immunocompromised patients, with severity often influenced by underlying health conditions and invasive procedures.

Multiple resistance mechanisms have been described, including chromosomally encoded AmpC $\beta$-lactamases, acquired carbapenemases, efflux pumps, and biofilm formation, which contribute to its multidrug-resistant phenotype.

Despite the increasing clinical significance of *M. morganii*, microbiological and genomic information remains relatively limited. Comprehensive studies examining the physiological traits, genomic features, phylum classification, pathogenicity, and resistance profiles of clinical *M. morganii* strains are essential for understanding their role in human infections and for guiding effective antimicrobial therapy [37].

## 2.8 *Morganella morganii* as Challenging Infectious Agent

*Morganella morganii* is an opportunistic Gram-negative bacterium belonging to the family Morganellaceae, widely distributed in environmental reservoirs such as soil, water, and the gastrointestinal tracts of humans and animals. Among species in the Morganella genus, *M. morganii* is the most clinically significant, followed by other closely related species. Historically considered a low-virulence organism, *M. morganii* has increasingly been recognized as a serious pathogen, particularly in immunocompromised patients and those with multiple comorbidities.

This bacterium can colonize diverse body sites and cause infections in both hospital and community settings, including urinary tract infections, wound and soft tissue infections, bacteremia, and, rarely, endocarditis or liver abscesses. Its emergence as a clinical concern is compounded by the increasing prevalence of intrinsic and acquired antimicrobial resistance, including AmpC $\beta$-lactamase production, plasmid-mediated carbapenemases, efflux pumps, and biofilm formation. These characteristics make *M. morganii* infections particularly difficult to treat and contribute to high morbidity in critically ill patients.

Healthcare professionals must maintain heightened vigilance for *M. morganii* infections, especially in intensive care units where invasive procedures and prolonged hospitalization increase infection risk. Recent reports have documented cases of extensively drug-resistant *M. morganii* in tertiary care centers, highlighting the need for early detection, susceptibility-guided therapy, and strict infection control measures to prevent transmission and improve patient outcomes [38].

## 2.9 *Morganella morganii* Resistance

*Morganella morganii* is an opportunistic Gram-negative bacillus widely distributed in environmental reservoirs, including soil, water, and the gastrointestinal tract of humans and animals. While generally non-pathogenic in healthy individuals, it can

cause severe infections in immunocompromised patients or those with comorbidities such as diabetes mellitus, chronic kidney disease, or prolonged hospitalization. Common infections include urinary tract infections (UTIs), wound and soft tissue infections, bacteremia, and, less frequently, endocarditis or liver abscesses.

The treatment of *M. morganii* infections is challenging due to its intrinsic and acquired multidrug resistance. Chromosomally encoded AmpC $\beta$-lactamases confer resistance to penicillins, first-generation cephalosporins, and monobactams, while some strains acquire plasmid-mediated carbapenemases, resulting in carbapenem resistance. Variable susceptibility exists to aminoglycosides, fluoroquinolones, and sulfamethoxazole-trimethoprim, making susceptibility-guided therapy essential. Multidrug-resistant isolates have been reported with increasing frequency in tertiary care hospitals, particularly in ICU settings with prolonged urinary catheterization. Biofilm formation on catheters and medical devices contributes to persistent infections and complicates treatment outcomes. Patients with poorly controlled diabetes mellitus or long-term ICU admission are at especially high risk for *M. morganii* UTIs. Modern laboratory systems such as VITEK 2 Compact and MALDI-TOF MS facilitate accurate identification of non-lactose fermenting Gram-negative bacilli at the species level, improving detection and guiding appropriate treatment. Despite advances in diagnostics, the combination of intrinsic $\beta$-lactamase production, acquired resistance genes, efflux pumps, biofilm formation, and environmental persistence makes *M. morganii* a formidable nosocomial pathogen. Prompt detection, strict infection control, and targeted antibiotic therapy are essential to manage infections and prevent outbreaks in high-risk clinical settings.

## 2.10 Antibiotic Resistance Status of Clinical *Morganella morganii* Infections

Infections caused by *Morganella morganii* are relatively uncommon but have been increasingly documented in hospital settings. While immunocompetent individuals rarely develop severe infections, immunocompromised patients-including those

with diabetes mellitus, cancer, chronic kidney disease, or prolonged catheterization- are particularly susceptible.

Clinical presentations include urinary tract infections, wound and soft tissue infections, necrotizing fasciitis, bacteremia, and, in rare cases, ventriculitis. Outbreaks of catheter-associated urinary tract infections caused by *M. morganii* have been reported in tertiary care hospitals, highlighting its significance as a nosocomial pathogen.

The management of *M. morganii* infections is complicated by high levels of antibiotic resistance. Several clinical isolates have demonstrated resistance to a broad range of antibiotics, including penicillins (ampicillin, amoxicillin), $\beta$-lactam/$\beta$-lactamase inhibitor combinations (amoxicillin-clavulanate), cephalosporins, carbapenems (imipenem, meropenem), monobactams (aztreonam), aminoglycosides (amikacin, gentamicin), fluoroquinolones (ciprofloxacin, levofloxacin, norfloxacin), tetracyclines, chloramphenicol, and sulfamethoxazole - trimethoprim.

Certain strains remain susceptible to tigecycline, minocycline, or selected fluoroquinolones, but susceptibility varies depending on the source of isolation and the strain's resistance determinants [39].

Research shows that antibiotic susceptibility can differ based on infection type and patient source. For example, isolates from urinary tract infections may exhibit multidrug resistance patterns distinct from isolates recovered from cellulitis, wound infections, or bloodstream infections.

Treatment failure is frequently reported in the absence of susceptibility-guided therapy, emphasizing the importance of accurate identification using modern diagnostic systems such as VITEK 2 Compact or MALDI-TOF MS.

The presence of chromosomal AmpC $\beta$-lactamases, acquired carbapenemases, efflux pumps, and biofilm formation contribute to the pathogen's intrinsic and acquired resistance, making *M. morganii* infections particularly difficult to manage in high-risk hospital populations.

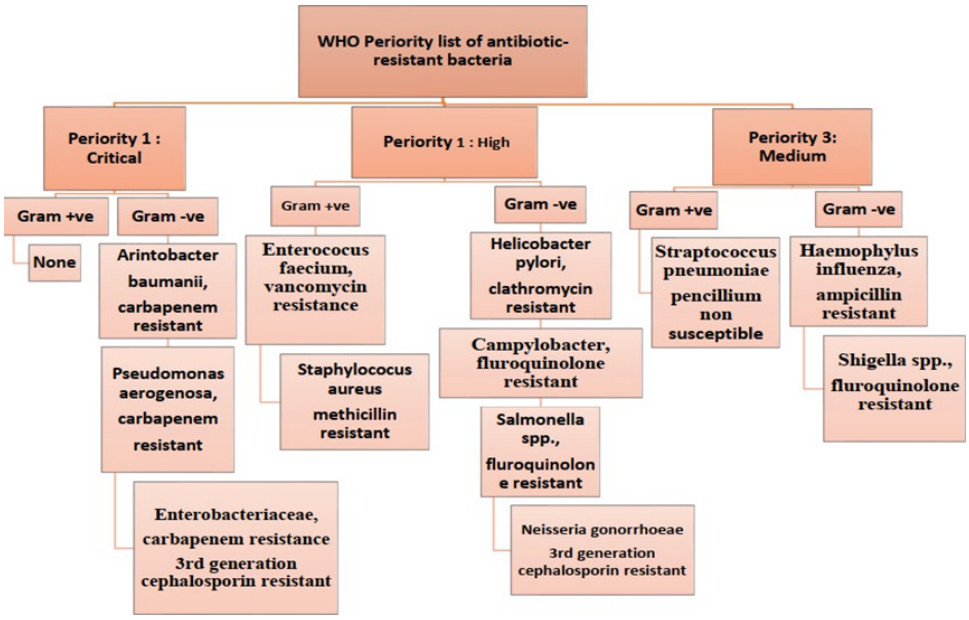


FIGURE 2.3: WHO priority list of antibiotic resistance bacteria [12].

Trauma cases and septicemia patients showed sensitivity to ciprofloxacin treatment [40].*Morganella morganii* is extensive antibiotic resistance. Following table represent list of antibiotics along with their minimal inhibitory concentration against which *Morganella morganii* show resistance.

TABLE 2.3: Antibiotic resistance by *Morganella morganii* [39].

| **Antibiotic** | **MIC (micro g/ml)** | **Interpretation** |
|---|---|---|
| Ticarcillin | 128 | Resistant |
| Piperacillin | 128 | Resistant |
| Tazobactum | 128 | Resistant |
| Ceptazedime | 64 | Resistant |
| Cefepine | 64 | Resistant |
| Aztreonam | 64 | Resistant |
| Imipenem | 16 | Resistant |
| Meropenem | 16 | Resistant |
| Amikacin | 64 | Resistant |
| Gentamicin | 16 | Resistant |
| Ampiciline | 64 - 128 | Resistant |
| Tobramycin | 16 | Resistant |
| Ciprofloxacin | 4 | Resistant |

**Table 2.3 continued from previous page**

| Antibiotic | MIC (micro g/ml) | Interpretation |
|---|---|---|
| Pefloxacin | 8 | Resistant |
| Minocycline | 2 | Susceptible |
| Colistin | 16 | Resistant |
| Trimethoprim | 320 | Resistant |

As a rule, antibiotics work by eliminating bacteria or preventing their proliferation. Chloramphenicol and tetracycline are typical representatives of bacteriostatic antibiotics, whereas $\beta$-lactam antibiotics and fluoroquinolones are classified as bactericidal antibiotics. For antibiotics to strengthen their inhibitory effects, they must disrupt key cellular processes while not causing harm to the patient. This can be accomplished, for instance, by blocking a pathway that is crucial for bacteria [41].

### 2.10.1 Antibiotic Resistance Mechanism of *Morganella morganii*

Antibiotic resistance mechanisms of *Morganella morganii* have been reported worldwide, including in several regions of Asia; however, despite increasing clinical attention, many aspects of its resistance biology remain incompletely understood. Although a number of international studies have investigated antimicrobial resistance in *M. morganii*, gaps still exist regarding the diversity and regulation of its resistance mechanisms. Several *M. morganii* strains exhibit variable resistance patterns to $\beta$-lactam antibiotics and reduced susceptibility to carbapenems, which are primarily associated with the production of $\beta$-lactamase enzymes.

The development and expression of chromosomally encoded AmpC $\beta$-lactamase in *M. morganii* has been identified as a major cause of resistance to $\beta$-lactam antibiotics, as reported in multiple clinical studies involving systemic and nosocomial infections. Resistance to $\beta$-lactam antibiotics in *M. morganii* is therefore largely attributed to the activity of these $\beta$-lactamases. However, research has

demonstrated that the intrinsic resistance of Enterobacterales species, including *M. morganii*, cannot be fully explained by AmpC $\beta$-lactamase production alone, suggesting the involvement of additional mechanisms such as altered membrane permeability and efflux systems [29].

## 2.11 General Mechanisms of Antibiotic Resistance

Figure 2.4 and Figure 2.5 indicate six different mechanisms in bacteria for antibiotic resistance. However efflux pumps play important role in antibiotic resistance. Efflux pumps are proteins responsible for transport.Gram positive and negative bacteria have these proteins.

Pumps can transport different molecules having diverse structure, including antibiotics from different classes, or they can be specialized to a single substrate.

There are four main kinds of efflux transporters in the prokaryotic kingdom: ABC, RND, MATE, and MF. With the exception of the ABC family, which uses ATP hydrolysis to propel the export of substrates, remaining systems use the proton motive force as energy source.

Many additional members of the aforementioned families have been discovered as a result of recent developments in DNA technology and the onset of the genomic age, and efflux pumps are remarkably common.

The presence of multiple distinct efflux pumps in every bacterial genome under study suggests their evolutionary origins. An estimated 5-10% of all bacterial genes are thought to be involved in transport, and many of them encode efflux pumps.

Since both bacteria that are susceptible to and resistant to antibiotics have and express these genes, there is some disagreement over the "normal" physiological function of efflux transporters [42].

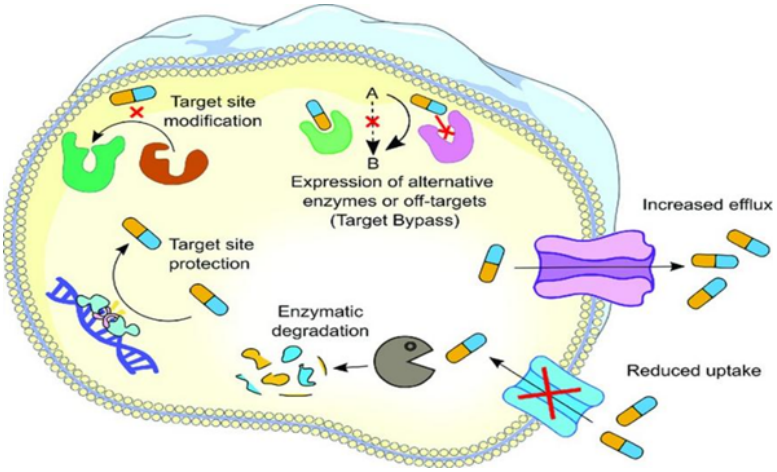


FIGURE 2.4: Schematic diagram of six different mechanisms in bacteria for antibiotic resistance [42].

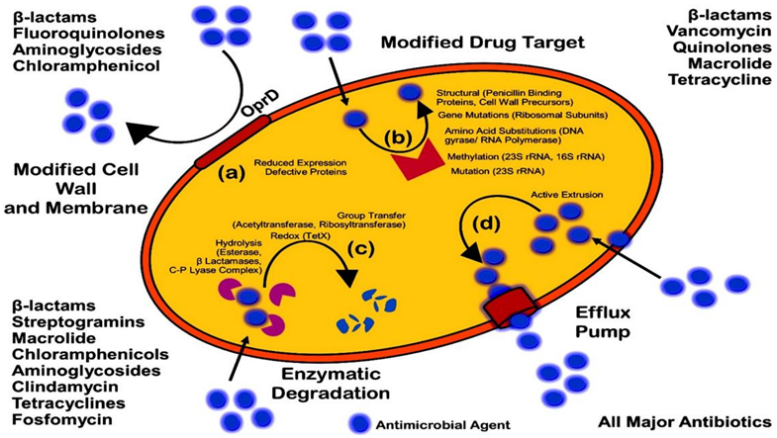


FIGURE 2.5: Schematic diagram of different mechanisms in bacteria for antibiotic resistance [43].

## 2.12 Biofilm Formation

Biofilm is a structured collection of bacteria that are affixed to a surface and live inside a self-produced matrix of extracellular polymeric substances (EPS). Biofilms can strengthen microbial resistance to UV light, high salt, high pressure, severe pH and temperature, inadequate nutrition, different antibiotics, etc. It appears that biofilms' ability to withstand harsh conditions can provide a favorable environment for microbial populations, facilitate easier material and information transmission between microorganisms, and act as a self-defense mechanism for microbial growth [44]. Figure 2.6 illustrate different steps of biofilm formation.

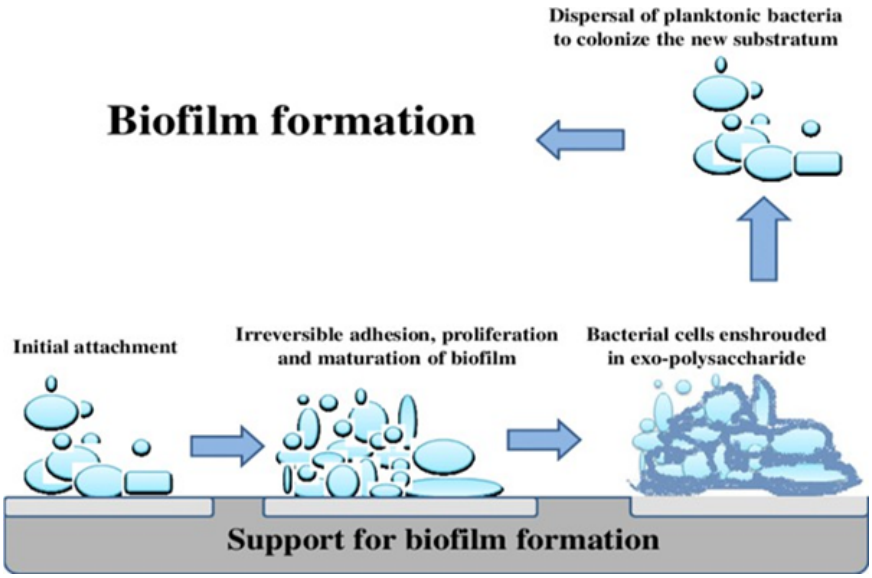


FIGURE 2.6: Steps of biofilm formation [44].

Strong adhesion patterns are shown by *Morganella* species, which prefer to adhere at lower temperatures [45]. The ability of *Morganella morganii* to produce biofilms by auto aggregation and coaggregation may account for their widespread prevalence and capacity to infect people with impaired immune system [46]. Many pathogens exhibit biofilm formation as a key virulence factor; in fact, it is now clear that the sessile bacterial cells in the biofilms exhibit characteristics distinct from those of the planktonic cells, such as the capacity to evade host defense and heightened resistance to antibacterial agent [47]. Strong biofilm formation is a major issue since it makes infections linked to devices more pathogenic and is frequently linked to both infection persistence and treatment failure [48]. The formation of biofilm by *Morganella* species can pose a serious risk to health and frequently results in recurring infections [49].

## 2.13 Biofilm and Antibiotic Resistance

Antibiotic resistance and antibiotic tolerance are two separate processes that make up biofilm recalcitrance (Figure 2.7). Resistance is measured by determining the

minimum inhibitory concentration (MIC), which describes a microorganism's ability to endure and proliferate for extended periods of time at elevated antibiotic doses [50].

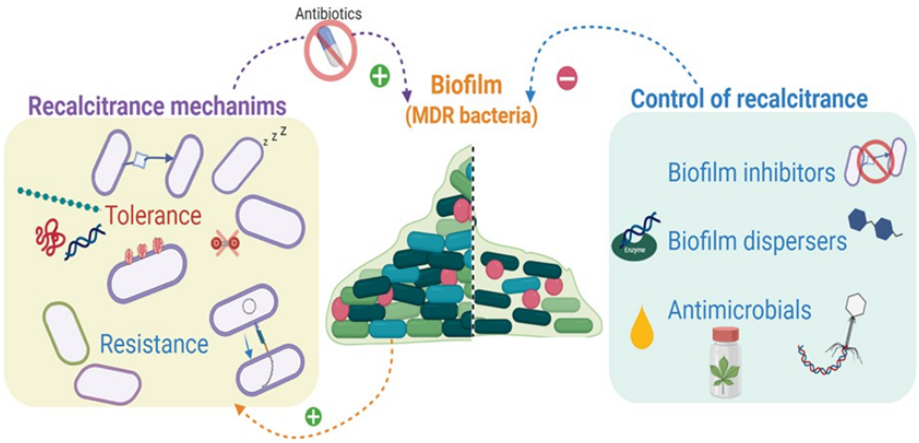


FIGURE 2.7: Schematic diagram of biofilm and its role in antibiotic resistance [51].

In addition to being caused by HGT or mutations, it involves processes that stop an antibiotic from binding to its target, such as enzymatic inactivation, active efflux of a drug once it is in cytoplasm or the cytoplasmic membrane, or decreased inflow. They work together to stop antibiotics from changing how their target works and to stop the synthesis of harmful substances that could harm the cell. There are several types of resistance, including acquired, adaptive, and intrinsic resistance. Antibiotic tolerance, on the other hand, refers to a bacteria's ability to withstand a brief exposure to elevated antibiotic doses, including those over the minimum inhibitory concentration. In order to determine tolerance, the minimum bactericidal concentration that is, the lowest concentration needed to kill 99.9% of the cells using antibiotics [52]. Tolerance, in contrast to resistance, is only transitory [53].

Antibiotic entrapment in the extracellular matrix (ECM), where the antibiotic fails to reach its target, is another factor contributing to tolerance in biofilms. Tolerant cells in the biofilm are unable to proliferate when a bactericidal antibiotic is present, in contrast to resistant cells. One unique aspect of tolerance is persistence. Persistence, unlike tolerance, only impacts a fraction of the population's

cells known as persisters, but it is a phenomenon that increases a population's survival in the presence of bactericidal antibiotics without raising the MIC [54].

The way that antibiotics interact with specific ECM components can also impede their diffusion through the biofilm, which impacts the effectiveness of the antibiotics.

Numerous instances, including *P. aeruginosa*, are used in the literature to demonstrate this. Polyanionic is alginate is aminoglycoside resistant exopolysaccharide that shields Pseudomonas biofilms [55].

With these positively charged antibiotics, ionic interactions are presumably facilitated by the high negative charge of alginate and cyclic glucans. But in strains that don't secrete alginate, the formation of biofilms is aided by the polysaccharides Pel and Psl. Pel offers protection from ciprofloxacin but not from the aminoglycosides tobramycin and gentamicin.

Therapeutic approaches to effectively treat biofilm infections will undoubtedly be guided by an understanding of the mechanisms underlying recalcitrance. These ought to be used in conjunction with techniques for the quick diagnosis of biofilm infections and the in vivo characterization of the biology and composition of biofilms.

Selection of appropriate therapeutic approaches to address specific biofilm infections will be aided by the availability of compounds that inhibit and disperse biofilms [44].

One distinctive feature of polymicrobial biofilms is the way different bacterial species can cooperate to protect one another. As an example, antimicrobial resistant bacteria can secrete protective enzymes or antimicrobial binding proteins that shield neighboring non-antimicrobial resistant bacteria in a biofilm. They can also transfer genes to other bacteria, conferring antimicrobial resistance, even across different species (Figure 2.8) [56].

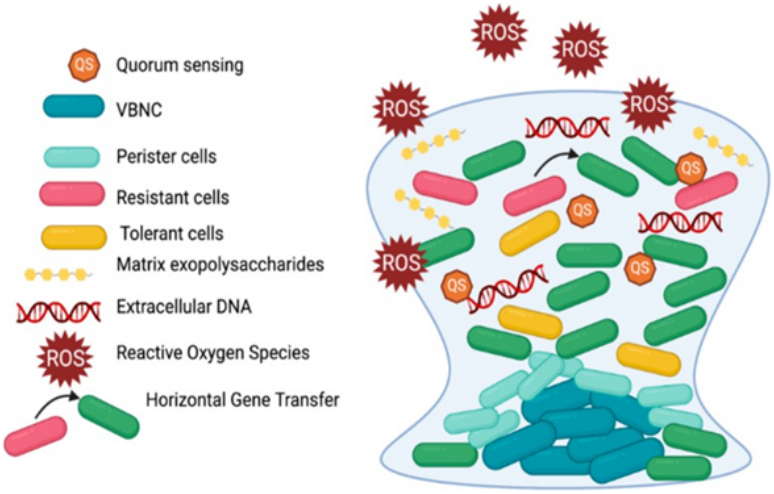


FIGURE 2.8: Role of biofilm in antibiotic resistance [56].

## 2.14 Biosynthetic Gene Clusters and Secondary Metabolites

Co-localized groups of genes known as biosynthetic gene clusters encode the regulatory components and enzymes needed to produce secondary metabolites. These substances have a variety of functions in the physiology and ecology of microorganisms, from host colonization and survival under stress to antimicrobial action. While BGCs are frequently linked to competitive survival strategies like siderophore synthesis or niche adaptation in environmental isolates, they may also increase bacterial fitness during infection or contribute to virulence in clinical strains. An all-inclusive tool for identifying and annotating BGCs in bacterial genomes is the antiSMASH platform. It predicts cluster types based on similarity to experimentally validated reference clusters and enables a comparative evaluation of biosynthetic capacity across strains [57]. Biosynthetic gene clusters control many biosynthetic pathways that create secondary metabolites.

The different mega synthases that are encoded by these biosynthetic gene clusters include Polyketide Synthases and Non-Ribosomal Peptide Synthases, which produce polyketides and non-ribosomal peptides, respectively. These groups of secondary metabolites are the most abundant and comprise a wide range of molecules

with various uses. Antimicrobials, siderophores, pigments, and communication molecules are some of the ways that polyketides and non-ribosomal peptides help defend against stressors [58]. Antimicrobial medicines are one of the main classes of secondary metabolites that bacteria create. The overuse and abuse of antibiotics, which resulted in the maintenance of antibiotic resistant bacteria and antibiotic resistance genes in our environment, made the search for natural antibiotics more urgent. The general public, industrial facilities, hospital facilities, and agricultural wastewater all contain these antibiotics. These antibiotics stay in the treatment plants even after wastewater has been treated, and they are released into the environment shortly after the treated water is discharged. Fluoroquinalones, tetracyclines, and sulfonamides are among the antibiotics that bind to soil particles and prevent their biodegradation. These concerns drive scientists to find natural products in order to prevent environmental and health hazards [59]. .

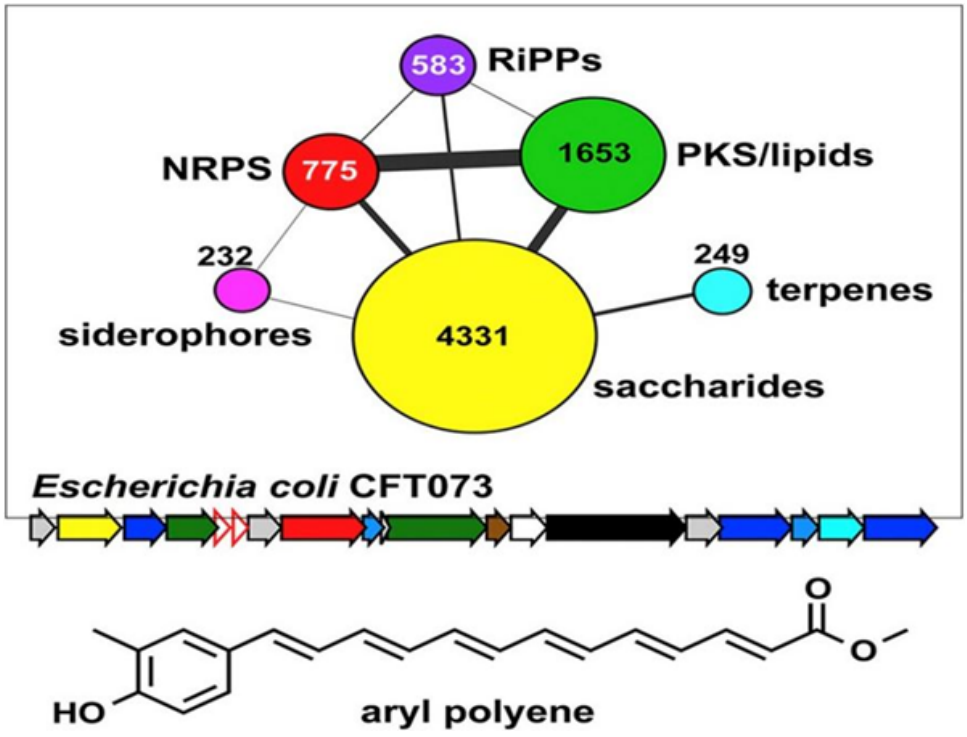


FIGURE 2.9: Biosynthetic gene cluster in *E. coli* CFT 073 [60].

The overall sequence similarity of bacterial TCs is low compared to the domains of core biosynthetic enzymes or other natural product classes (PKSs and NRPSs).

Lack of conservation in primary sequence has impeded the development of efficient genome mining tools for the identification of bacterial terpene BGCs and reduced our understanding of terpene cyclization.

Annotating bacterial terpene BGCs, antiSMASH is the only open source web program available [60]. Figure 2.9 give a general concept of Biosynthetic gene cluster in *E. coli* CFT 073

## 2.15 Resistance Mechanisms for Bacteria

MUS-1 and TUS-1, two metallo-$\beta$-lactamases that share 73% of their amino acid similarity, are the cause of intrinsic resistance to $\beta$-lactamases [59].

Bacterial membranes contain proteins known as efflux pumps, which regulate the movement of harmful substances from the inside to the outside of the cell. The most effective and fastest acting defense mechanism for bacteria against stress is bacterial efflux pumps, which are found in practically all bacteria.

In addition to increasing germs' resistance to antibiotics and other antimicrobial agents, efflux pumps let them survive in harsh environments [61].

Six families of bacterial drug efflux pumps have been found to be involved in the efflux pathway: the proteobacterial antimicrobial complex efflux (PACE) family, the resistance nodulation cytosis (RND) superfamily, the small multidrug resistance (SMR) family, the multidrug and toxin extrusion (MATE) family, the major facilitator superfamily (MFS), and the ATP-binding cassette (ABC) family (Figure 2.10).

Only bacteria that are Gram negative have members of the RND family [62]. Various substrates, frequently antibiotics, can be expelled by an efflux pump, resulting in bacterial phenotypes that display antibiotic resistance. Toxins, waste metabolites, detergents, and dyes can also be expelled by efflux pumps [63].

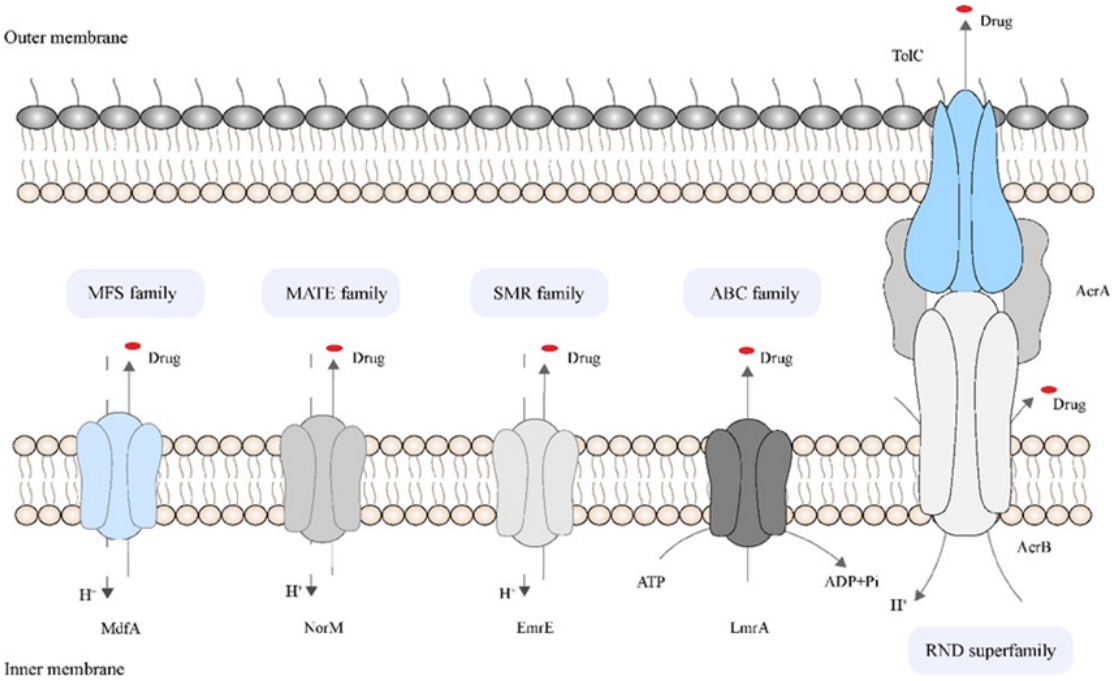


FIGURE 2.10: 5 super families of efflux pumps [64].

An often employed tactic to make antibiotics ineffective is antibiotic modification, particularly when it comes to aminoglycoside antibiotics (kanamycin, streptomycin and gentamycin), $\beta$-lactams, and chloramphenicol. It is known that producer bacteria have a significant number of aminoglycoside modification enzymes (AMEs), such as N-acetyl transferases (AAC), O-phosphotransferases (APH), and O-adenyltransferases (ANT), which acetylate, phosphorylate, or adenylylate the aminoglycoside antibiotic for example.

Even though these enzymes were initially discovered in the early 1970s in the producer Streptomyces species, they carry out biochemical events that are identical to those observed in clinical strains of antibiotic resistant bacteria [65].

The presence of modifying enzymes in producers is directly correlated with the production of aminoglycosides. Not always is Streptomyces visible. Examples include organisms that have modification enzymes but do not produce antibiotics, and vice versa. In the producer S. griseus, streptomycin resistance is caused by the modifying enzyme streptomycin 6-phosphotransferase, which changes streptomycin into the inert precursor streptomycin6-phosphate [66].

## 2.16 Classes of Efflux Pumps

### 2.16.1 RND Family Efflux Pumps

The domains of Archaea, Eukarya, and Eubacteria contain RND efflux pumps, the most significant in Gram negative bacteria from a clinical standpoint (Figure 2.11) [67]. A tripartite complex including an inner RND membrane protein, an outer membrane protein, and a membrane fusion protein makes up the overall structure of RND transporters [68]. Since most other families of efflux pumps only move substrates over one membrane, this tripartite pump spans both membranes of bacteria. The MFP links the OMP and RND protein [69].

In the RND protein, twelve transmembrane segments (TMSs) are predicted [70]. The OMP is a trimer that permits solvents to flow through it by forming a continuous channel that spans the outer membrane and the periplasmic space [71]. The interdependent protomers seen in RND transporters are trimers. With their proximal and distal binding pockets that include a range of substrates they can bind, these protomers cycle between loose, tight, and open conformations [72]. A conserved feature of the RND efflux pump family is the proximal binding pocket. The non-conserved area covers the flexible loop (F-loop) and forms the bottom of the PBP. The inner and outer membranes are brought closer together by the MFP, which helps to stabilize the OMP [71].

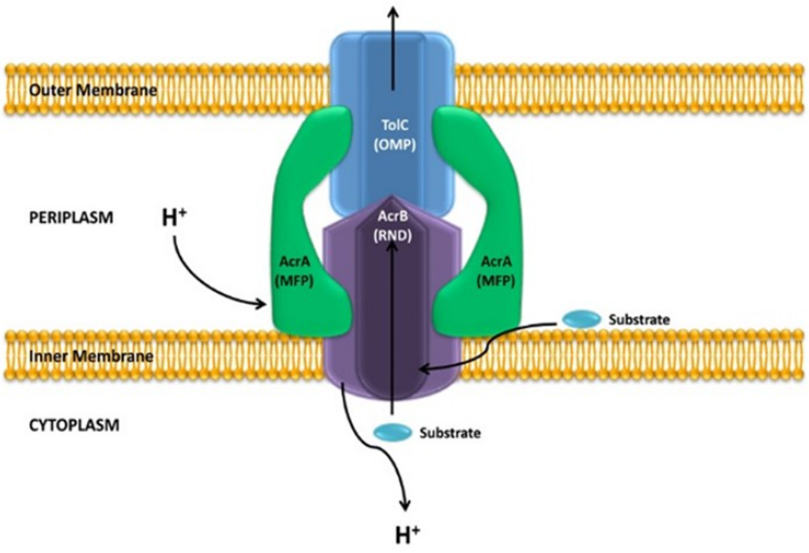


FIGURE 2.11: Representative diagram of RND efflux pump [73].

### 2.16.2 ABC Family Efflux Pump

Utilizing the energy of ATP hydrolysis, the ABC family of efflux pumps moves substrates across membranes or out of cells (Figure 2.12). Two of their four protein domains hydrolyze ATP, and the other two span the membrane. Each domain may be in the either in one or more proteins. For one-way, outward-only substrate transport out of the cell, ABC transporters alternate between open, occluded, and outward open phases. When ATP hydrolysis and conformational changes occur, the binding site's affinity for the substrate decreases.

The substrate is then forced through the channel to pass through the remaining parts of the efflux pump and exit the cell [74]. The outer membrane channel protein TolC, which serves as an exit duct for substrate transport, and MacA, a periplasmic adaptor protein that is activated when ATPase binds specifically with the lipopolysaccharide core, are further elements of this system [74].

In addition to being regulated by the two-component BaeSR system and contributing to tigecycline resistance, TolC is more frequently linked to RND family efflux pumps.

Other research has demonstrated efflux pumps, A1S 0536 and A1S 1242, are involved in virulence, motility, and antimicrobial resistance. Resistance to erythromycin and to chloramphenicol and gentamicin, respectively, was discovered in A1S 1535 [74] .

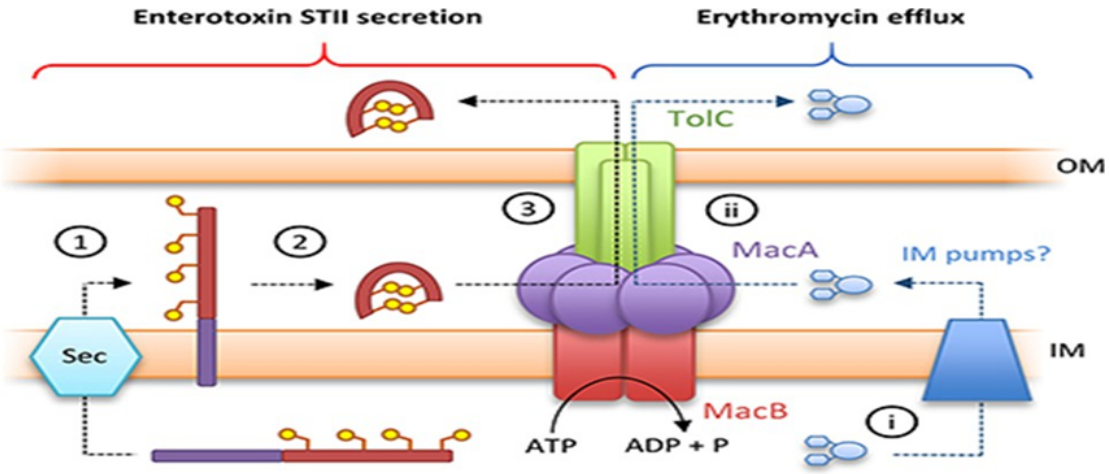


FIGURE 2.12: Antibiotic resistance mediated by ABC efflux pump [75].

## 2.17 Antibiotic Resistance by Target Modification

Another mechanism of antibiotic resistance is target modification. Mechanism of antibiotic resistance by target modification is illustrated in figure 2.13.

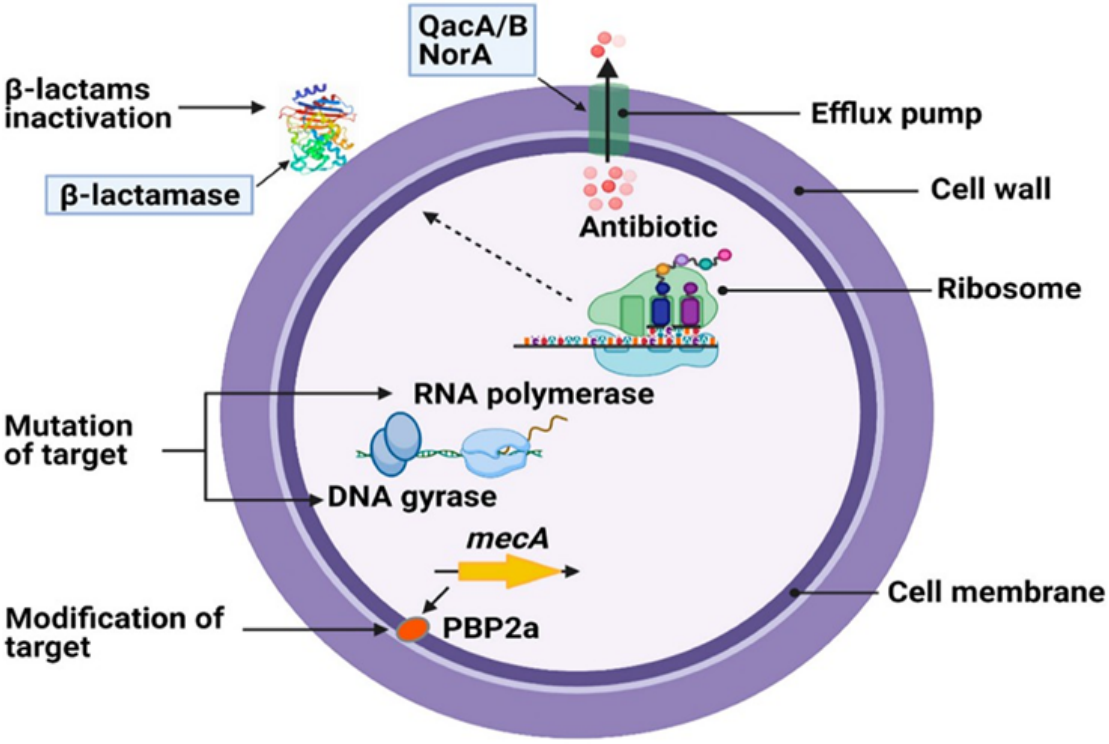


FIGURE 2.13: Schematic diagram for modification of target [76].

Proteins and lipopolysaccharides make up the majority of the cell wall of Gram-negative bacteria (GNB), where hydrophilic substances must be aided by porin channels or outer membrane porins (Omps) to get through the lipid bilayer. Bacterial resistance can arise from the loss or destruction of one or more of the porins (such as OmpF, OmpC, and OmpE) produced by each kind of bacteria.

Natural antibiotic resistance results, for instance, from the ineffectiveness or weakening of many broad-spectrum antibacterial medications against *P. aeruginosa* caused by the lack of OprD porin on the cell's outer membrane, which prevents the antibacterial medications from entering the cell. Changes in the number and

characteristics of porin can decrease the permeability of bacterial membranes after antibiotic exposure, resulting in acquired drug resistance. Typically, OmpF and OmpC form non-specific transmembrane channels with the channel proteins of the bacterial outer membrane, enabling the use of antibiotics and other drugs. However, frequent exposure to antibiotics causes mutations in the structural gene encoding OmpF protein, which reduces or eliminates OmpF channel protein and prevents drugs like $\beta$-lactams or quinolones from entering the bacteria correctly. Gram-positive. Because bacteria lack an exterior membrane that would prevent medications from entering, and because mycobacteria bear one outer membrane with more lipid content, hydrophobic drugs like ciprofloxacin and rifampicin enter cells more readily while hydrophilic drugs are restricted from doing so. Bacteria's membrane permeability can be reduced by inactivating the structural gene of the OmpF protein, which can allow $\beta$-lactams, quinolones, and other medications to enter the bacteria and cause acquired drug resistance [77].

Certain antibiotic targets are protected by bacterial synthetic protein, which removes the bacteriostatic effects of a combination of antibiotics [69]. Target protection involves three types. Tetracycline ribosomal protection proteins (TRPPs) can connect to ribosomes and reverse the deformed ribosomal structure, causing alterations in ribosome structure, and directly disrupting the connection between the 16S rRNA base C1054 and the tetracycline D-ring. Drugs belonging to the tetracycline class are unable to attach to it and separate from the binding site's 30S subunit, safeguarding the ribosome, of which 13 TRPP classes have been found [69]. Antibiotics are indirectly eliminated in Type II target protection by modifications to the target conformation. Clinical resistance to antimicrobials of the ribosome is mostly caused by the ABC-F protein family, which is resistant to antibiotics. The 50S subunits consist of phenols, lincomycins, azadones, macrolides, pleuromutilins, and stroopogramins. In order for antibiotic targets to function while binding to antibiotics, type III target protection proteins cause conformational changes in the targets. Recent years have seen the clinical isolation of *S. aureus* and other staphylococci. The level acquisition of the genes encoding the FusB-type protein is primarily responsible for the notable rise in resistance

to fusidic acid. Because fusB proteins attach to elongation factor G (EF-G) and cause its dissociation from ribosomes (even when fusidic acid is present), they are resistant to fusidic acid. Because of its poor affinity for free EF-G, fusidic acid may detach from EF-G once the elongation factor exits the ribosome [78].

Cell wall-deficient bacteria, including Mycoplasma and related species, are therefore inherently resistant to all medications that target the cell wall [79]. The bacterial cell contains several parts that antimicrobial drugs could target. One way that gram-positive bacteria withstand the $\beta$-lactam medications that they employ virtually exclusively bacteria is through changes to the quantity and/or structure of PBPs (penicillin-binding proteins). Transpeptidases called PBPs aid in the synthesis of peptidoglycan in the cell wall.

The quantity of drug that can bind to that target is affected when the number of PBPs changes, either by an increase in PBPs with a decreased drug binding ability or a decrease in PBPs with normal drug binding. Drug binding may be reduced or completely inhibited by a structural alteration (such as PBP2a in *S. aureus* due to the acquisition of the mecA gene) [72].

Vancomycin and other glycopeptides function by preventing the formation of cell walls, while daptomycin and other lipopeptides depolarize the cell membrane.

The thick layer of LPS in gram-negative bacteria makes them naturally resistant to these medications [80]. Vancomycin resistance has grown to be a significant problem in Staphylococcus aureus (MRSA) and enterococci (VRE vancomycin-resistant enterococci). The acquisition of van genes mediates resistance and causes alterations in the composition of peptidoglycan precursors that reduce vancomycin's capacity to bind [81].

For daptomycin to bind, calcium must be present. Gene mutations, like mprF, cause the cell membrane surface to become positively charged, which prevents calcium and, consequently, daptomycin from binding [82]. For medications that block metabolic processes, resistance occurs through overproduction of resistant DHPS and DHFR enzymes (sulfonamides DHPS, DHFR dihydrofolate reductase,

and DHPS dihydropteroate synthase) involved in the fo-late biosynthesis pathway, as well as mutations in these enzymes.

Trimethoprim DHFR. Both trimethoprim and sulfonamides bind to their corresponding enzymes because they are structural analogues of the natural substrates (trimethoprim dihydrofolate, and sulfonamides p amino benzoic acid).

The way these medications work is by binding to the enzymes' active site and stimulating competition. The active site of these enzymes is frequently where mutations occur, and the ensuing structural alterations in the enzyme prevent drug binding while permitting the natural substrate to bind [83].

Bacteria can inactivate medications in two major ways: either by physically breaking down the drug or by adding a chemical group to it. The $\beta$-lactamases are a broad class of enzymes that hydrolyze drugs (Figure 2.14).

Tetracycline is another medication that can be rendered inactive through hydrolyzation, using the the tetX gene. Acetyl, phosphoryl, and adenyl groups are the most frequently used chemical groups to transfer to the medicine in order to inactivate it.

Many different types of transferases have been found. Acetylation is the most versatile method and can be employed against fluoroquinolones, streptogramins, aminoglycosides, and chloramphenicol. It is well known that phosphorylation and adenylation are mostly employed to combat aminoglycosides [84].

The class of antibacterial compounds known as $\beta$-lactams is the most commonly utilized. All of the drugs in this group have a four-sided $\beta$-lactam ring as their common core structure.

The three main ways that resistance to $\beta$-lactam drugs arises are by blocking the interaction between the drug and the target PBP, typically by changing the drug's capacity to bind to the PBP. Enzymes that hydrolyze the medication by $\beta$-lactamase [85].

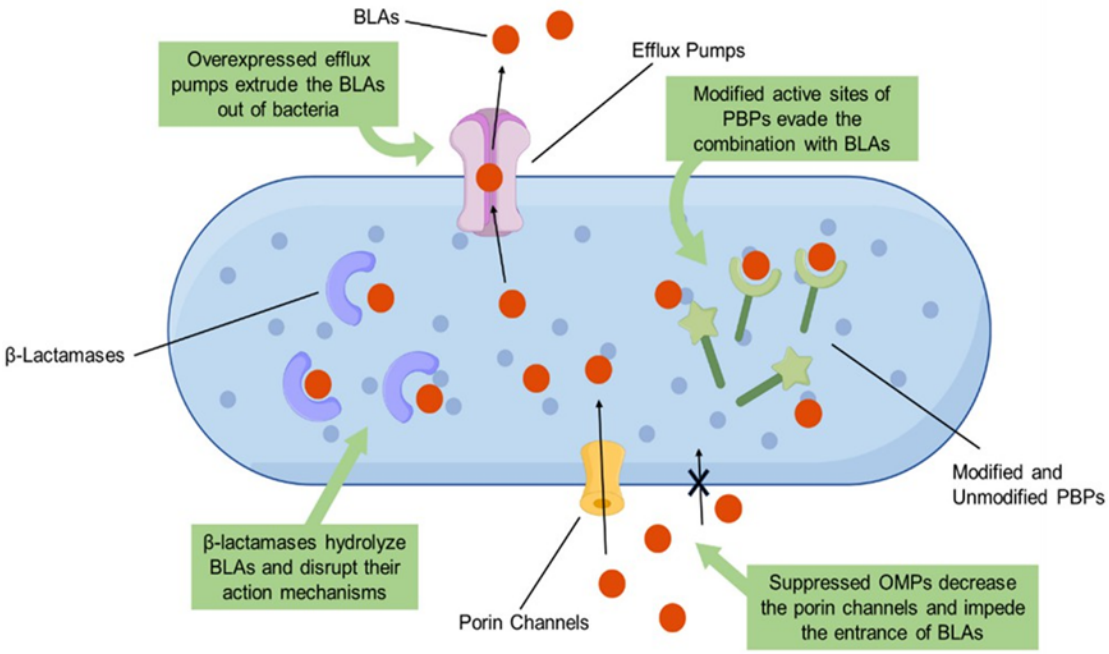


FIGURE 2.14: Beta lactamases and antibiotic resistance [86].

## 2.18 Virulence Factor of *Morganella morganii*

*Morganella morganii* has been shown to harbor not only several well-recognized virulence determinants, including iron-acquisition systems that enable competition with the host for essential nutrients, but also multiple adherence-associated proteins such as DnaK and Hsp60.

In addition, the bacterium possesses genes that facilitate intracellular persistence, including katA, clpP, tuf (EF-Tu), and sodB, allowing survival under oxidative and stress conditions. The presence of urease-associated genes (ureA, ureB, and ureG) further supports its ability to tolerate acidic environments, including the human gastrointestinal tract.

These combined features enhance the organism's capacity for dissemination and contribute to tissue damage during infection.[28].

## 2.19 Mobile Genetic Elements

The collection of all mobile elements found in a bacterial genome, known as "bacterial mobilome," is a key role in bacterial evolution. Through intricate interactions between the mobile element and the host bacteria, the mobile element shapes the host genome.

Numerous things are referred to as mobile elements of genomic sequences that have the capacity to spread either horizontally by transfer or vertically with cell division, including insertion sequences, transposons, restriction and modification systems, pathogenicity islands, plasmids, and props.

Thus, they can shape and co-evolve with chromosomal genomes by moving both within and across the host genome. The insertion location, copy number, novel gene functions, and chromosomal gene expression can all be altered by mobile elements.

It is well recognized that mobile elements can significantly alter bacterial fitness by amplifying gene gain and loss. The fact that Genetic adaptation to novel environments and the formation of diverse bacterial populations that could give rise to evolutionary different species are both facilitated by change.

The processes by which mobile elements interact with the bacterial genome and maintain their persistence have been the subject of increased research since their discovery.

By presenting relevant and significant examples of unique mobile components (genomic islands, pathogenicity), this special issue islands, insertion sequences, prophages, and restriction and modification systems), either by reviewing, demonstrating novel features, or introducing fresh bioinformatics tools, highlights how crucial it is to understand the biology of mobile genomic components [87].

Figure 2.15 indicate Mobile Genetic Elements (MGEs): transposons, integrons and plamid in *E. coli*.

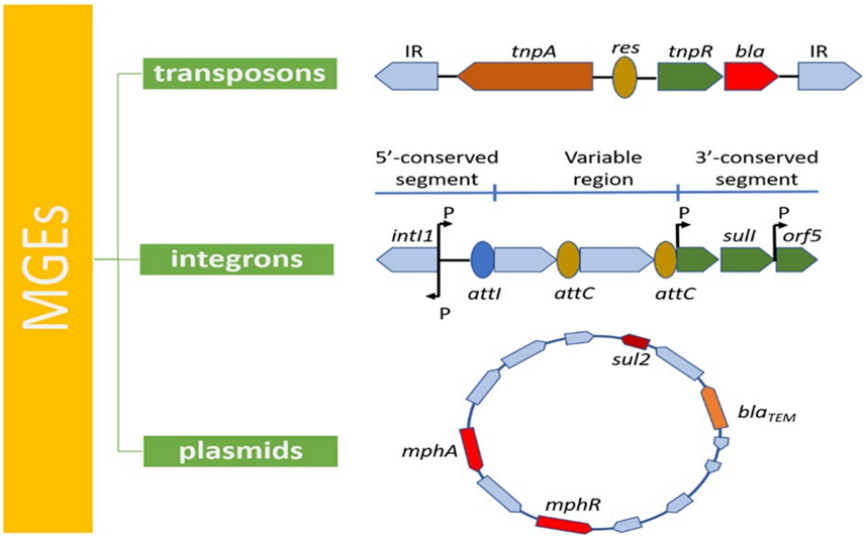


FIGURE 2.15: Mobile Genetic Elements (MGEs): transposons, integrons and plamid in *E. coli* [88].

## 2.20 Horizontal Gene Transfer

For a transferred gene to remain in the recipient lineage for a long time, it usually needs to provide a selection advantage to either itself or the recipient. These genes were the focus of early HGT research. It is now known that many genes identified to be passed down by comparative genomics between close relatives have neutral or almost neutral effects on the recipient in both prokaryotic and eukaryotic animals [89]. One requirement for transferred genes seems to be "first, do no harm." Genes that are successfully integrated often encode peripheral metabolic functions and express themselves at low levels[90].

In some cases, the transferred material eventually gets domesticated and produces a desired phenotype, but these neutral acquisitions may eventually produce new genetic combinations that selection can work upon. In other cases, if the imported genes remain neutral and there is no obvious benefit to retaining them, they are likely to be lost over time.

The evolution of bacteria and archaea was significantly influenced by HGT. However, genetic information transfer across eukaryotes and between eukaryotic hosts

and prokaryotic symbionts suggests that HGT occurs more frequently in eukaryotes than previously thought [91]. A donor cell must physically come into contact with a recipient cell via a conjugation pilus in order for genetic material to be transferred during conjugation. The only cells capable of conjugating as both the donor and the receiver are bacterial cells.[91].

Transformation, or the acquisition of foreign DNA from the environment, has been shown to occur in both bacteria and archaea. Transduction has been demonstrated in both bacteria and archaea. Gene transfer agents (GTAs) and cell fusion are two other gene transfer methods that have been discovered more recently. Chromosome-integrated gene delivery systems, or GTAs, are sometimes controlled by the host.

GTAs use capsids to carry small, random pieces of the host genome to nearby hosts. GTAs are present in both bacteria and archaea. The benefit to the host, which transfers its DNA to others, and the benefit to the GTA-encoding genes are not immediately obvious since the GTA does not preferentially transfer the GTA-encoding genes. How these genes are still chosen for their roles is yet unknown [92]. Membrane-associated protein complexes aid in pushing or pulling ssDNA into the cell, which is a common feature of the classical HGT processes like conjugation and natural transformation.

Since new research has identified other modes of DNA transfer that are independent of the conventional DNA absorption or conjugation machinery, other therapy targets should be considered in the fight against ARGs. By focusing on conserved proteins involved in DNA transport or protection, limiting the transfer of ARGs may lower MDR in bacteria [93]. Nonetheless, the discovery of non-classical HGT mechanisms suggests that controlling the spread of ARGs is more challenging than previously thought[94].

The ability of microbes to use free DNA fragments from their surroundings is known as transformation. Dead cells' foreign DNA is broken up and released from the cell. The free-floating DNA cellules can then be picked up by competent cells.

Through the cell membrane, the receiving cell absorbs exogenous DNA from its surroundings. Through re-association, the external DNA gets incorporated into the host cell's chromosome. The target cell's genetic makeup is altered by transformations.

Bacteriophages are viruses that target bacteria and transfer genetic material between various organisms through the process of transduction. They multiply widely inside a bacterial cell after injecting their genetic material there as viruses. At the same time, some phage genes are still present in the bacterial chromosome. A much greater number of bacteriophages are released into the environment to infect other microbes when the cell eventually bursts.

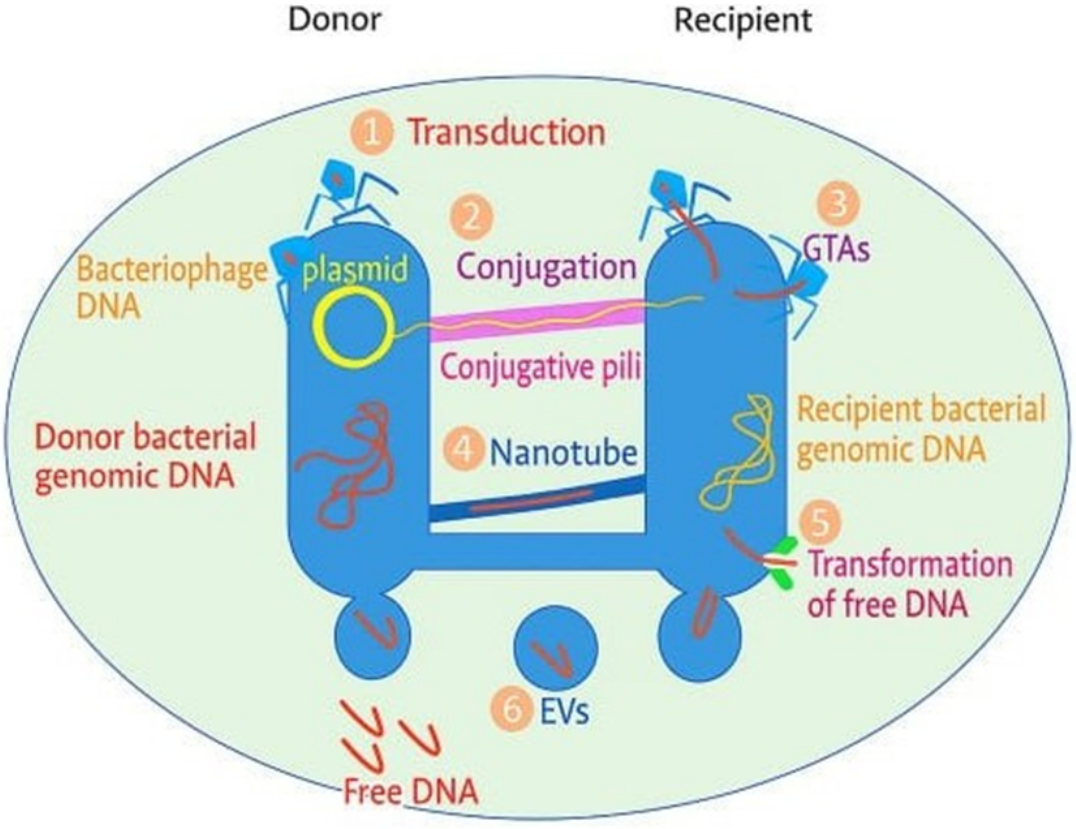


FIGURE 2.16: Methods of HGT in Bactria [81].

The transfer of genetic material between bacterial cells by direct contact or a bridge-like structure connecting the two cells is known as bacterial conjugation (Figure 2.16). Similar to transformation and transduction, which do not necessitate direct cell contact, it is a technique of horizontal gene transfer. [93].

## 2.21 *Comamonas testosteroni*

Aerobic, motile, Gram-negative bacteria from the genus Comamonas in the family Comamonadaceae are found in both soil and water. Among these, *Comamonas testosteroni* stands out for its capacity to metabolize a variety of chemical substances, such as xenobiotics, steroids, and aromatic hydrocarbons.

Numerous strains of *C. testosteroni* have shown their metabolic flexibility by being able to break down environmentally persistent contaminants such phenol, benzoate, toluene, and chlorinated organic compounds.Certain strains of *Comamonas testosteroni* are potential prospects for environmental cleanup applications since they have been shown to use complicated carbon sources under aerobic circumstances.

Their potential relevance in bioremediation solutions is highlighted by their capacity to endure and maintain metabolic activity in contaminated soils and wastewater treatment systems. The metabolic efficiency and physiological adaptability of *C. testosteroni* have drawn more attention for use in the cleanup of contaminated areas due to the ecological persistence and toxicity of numerous industrial contaminants.

In addition to its ability to utilize substrates such as propene, butene, fluoroethene, and nicotine, certain strains of *Comamonas testosteroni* have been proposed as effective biocatalysts for the synthesis of chiral epoxides, owing to their enzymatic specificity and metabolic versatility[95].

*Comamonas testosteroni* is a Gram-negative, environmentally derived bacterium that is commonly found in soil and aquatic environments. It is distinguished by its metabolic adaptability rather than its pathogenic specialization. It is a motile, aerobic bacterium that uses a variety of organic substances as carbon and energy sources to survive in nutrient-limited situations.

Genes encoding enzymes involved in the breakdown of various hydrocarbons and xenobiotic substances, such as oxygenases and dehydrogenases that aid in the

breakdown of environmentally persistent chemicals, have been found through genomic analysis.*Comamonas testosteroni* has a more ecological lifestyle than clinically related bacteria, and its genomes typically lack key antibiotic resistance genes and conventional virulence factors.

Its designation as a low-virulence organism with substantial potential for biotechnological and bioremediation applications is supported by this genomic profile, which shows environmental specialization rather than adaptation to host - associated niches.

Its designation as a low-virulence organism with substantial potential for biotechnological and bioremediation applications is supported by this genomic profile, which shows environmental specialization rather than adaptation to host - associated niches. *Comamonas testosteroni* is widely utilized as a model organism in bioremediation studies due to its exceptional metabolic flexibility and general lack of recognized virulence features. These traits reflect the different evolutionary routes that have shaped the ecological roles and interactions between opportunistic diseases like Myroides odoratimimus and environmentally adapted microorganisms. Public databases like GenBank include the genome sequences of typical strains of *Comamonas testosteroni*, which are useful tools for comparative genomic and functional research [96].

## 2.22 Properties of *Comamonas testosteroni*

The environmentally generated bacterium *Comamonas testosteroni* has a number of advantageous traits, such as a largely nonpathogenic profile, a low potential for pathogenicity, and a restricted capacity to build biofilms under normal circumstances.

Its classification as a noninfectious bacterium that is mostly suited to environmental niches is supported by these characteristics. Table 2.4 summarizes a number of significant physiological, metabolic, and genetic characteristics of *Comamonas testosteroni*.

TABLE 2.4: Description of different properties of *Comamonas testosteroni* [95].

| Characteristic | Description |
|---|---|
| Gram staining | Gram negative |
| Shape | Rod shape |
| Colony color | Grayish white to off-white |
| Motility | Motile, polar flagella |
| Pathogenicity | Rare opportunistic pathogen |
| Resistance mechanism | Low intrinsic resistance; biofilm-forming ability |
| Biofilm formation | Capable of biofilm formation |
| Habitat | Soil, hospital environment |
| Antibiotic resistance | Mostly susceptible |
| Infections | UTIs, wound infection |
| Oxygen requirement | Aerobe |
| Temperature range | 30-37 °C |
| pH tolerance | Neutral to slightly alkaline |
| GC content | 61.1% |
| Biochemical properties | Catalase positive, oxidase positive |

## 2.23 Genomic Features of *M. morganii* and *Comamonas testosteroni*

A strong framework for analyzing how two ecologically different Gram-negative bacteria-the opportunistic human pathogen *Morganella morganii* and the environmentally adapted degrader *Comamonas testosteroni*-have developed unique genomic architectures that represent their divergent lifestyles is provided by comparative genomics. Despite the evolutionary distance between the two animals, functional comparison and direct genomic alignment are made possible by the availability of fully sequenced genomes.Because *Comamonas testosteroni* and *Morganella morganii* have similar genome sizes-typically between 3.8 and 4.5 Mbp meaningful comparisons of gene composition, metabolic pathways, and adaptive features are made possible. Nonetheless, there is a clear difference in GC content between the two species.

While *Morganella morganii* has a lower GC content (about 51%), which is typical of many Enterobacterales members and reflects its adaptation to host-associated and clinical environments, *Comamonas testosteroni* has a moderately high GC content (about 60-62%), consistent with its environmental origin and metabolic versatility.In contrast to opportunistic pathogens that have developed mechanisms for host survival, virulence, and antibiotic resistance, these genetic differences highlight the distinct evolutionary constraints shaping environmental bacteria specialized for biodegradation and nutrient cycling.

Although *Comamonas testosteroni* and *Morganella morganii* both encode between 3,800 and 4,100 protein-coding genes, their functional gene repertoires are very different. The general gene composition also reflects this divergence. *Morganella morganii* is multidrug resistant in clinical settings due to the presence of several antibiotic resistance determinants, such as chromosomally encoded $\beta$-lactamases and efflux pump systems.

Its capacity to endure in hospital environments and complicate antibiotic therapy is partly due to these genetic characteristics.In contrast, *Comamonas testosteroni* lacks extensive antibiotic resistance traits, a characteristic consistent with its primarily environmental origin and low pathogenic potential. Although biosynthetic gene clusters (BGCs) are present in the genomes of both organisms, the predicted metabolites suggest niche-specific adaptations.

In *Morganella morganii*, BGCs associated with siderophore production and other secondary metabolites may support iron acquisition, stress tolerance, and survival during host infection. Conversely, BGCs identified in *Comamonas testosteroni* are more likely linked to metabolic versatility and environmental persistence rather than virulence.

On the other hand, *Comamonas testosteroni*'s biosynthetic gene clusters (BGCs) are mostly linked to environmental functions, such as oxidative stress management and aromatic compound degradation, which reflects its ecological specialization. Comparative antiSMASH investigations show that although both species create

secondary metabolites, their genetic architectures are altered by adaptations to different habitats, such as ambient biodegradation or host-associated infection.

Mobile genetic components also have an impact on these adaptive capacities. The high prevalence of plasmids, prophage regions, and insertion sequences in *Morganella morganii* suggests significant genomic plasticity that promotes the acquisition of virulence-associated genes and antibiotic resistance. In contrast, *Comamonas testosteroni*'s genome has a comparatively stable chromosomal structure with fewer mobile elements, which is consistent with its environmental lifestyle and limited pathogenic potential. *Morganella morganii* and *Comamonas testosteroni* have different evolutionary strategies and ecological niches, as evidenced by the existence of pathogenicity-related genomic islands in *Morganella morganii* that are mostly absent in *Comamonas testosteroni*.

Genomic regions enriched in adhesion factors, secretion system components, and biofilm-associated proteins provide the genetic foundation for *Morganella morganii*'s potential for hospital-acquired infections (HAIs). These regions together improve the bacteria's capacity to colonize hosts and endure in clinical settings. *Comamonas testosteroni*, on the other hand, lacks these virulence-related characteristics, supporting its lifestyle as a nonpathogenic, environmentally adapted bacterium [11].

The genetic characteristics of these environmental and clinical bacteria are compiled in Table 2.5, which highlights the variations in virulence factors, mobility elements, and metabolic capacity that correspond to their distinct ecological niches.

TABLE 2.5: Genomic features of environmental and clinical bacteria [97].

| **Genomic Feature** | ***Morganella morganii*** | ***Comamonas testosteroni*** |
|---|---|---|
| Biofilm-associated genes | Abundant | Weak to moderate |
| Mobile genetic elements | Plasmids, prophages, and insertion sequences are common | Limited mobile genetic elements reported |
| Secondary metabolite function | Contribute to virulence or host survival | Reported as environmental adaptations |
| Genome size | 4.0-4.3 Mbp | ~4.2 Mbp (reported) |

**Table 2.5 continued from previous page**

| **Genomic Feature** | ***Morganella morganii*** | ***Comamonas testosteroni*** |
|---|---|---|
| GC content | 50-51% | 60.62% |
| Virulence genes | Present, mostly related to biofilm formation | Not reported |
| Gene count | 3800-4100 protein-coding genes | ∼4100 protein-coding genes reported |

# Chapter 3

# Material and Methods

## 3.1 Bacterial Isolation

Fifty swab samples were collected from patients in the orthopaedic and general surgical ward of the Rawalpindi-based Benazir Bhutto (tertiary care) hospital. All kinds of bacteria can be transported by the swabs. After being delivered to the university laboratory, each swab was kept in an icebox. In order to determine the infection and variety of germs, the swabs obtained from the patients were classified according to the organ of infection as some (require number) from the foot, upper leg (thy), lower legs, Cather, and chest. The samples include duplicate swabs; one set was sent for metagenomics analysis, and the other duplicate was utilized in the lab for additional research.

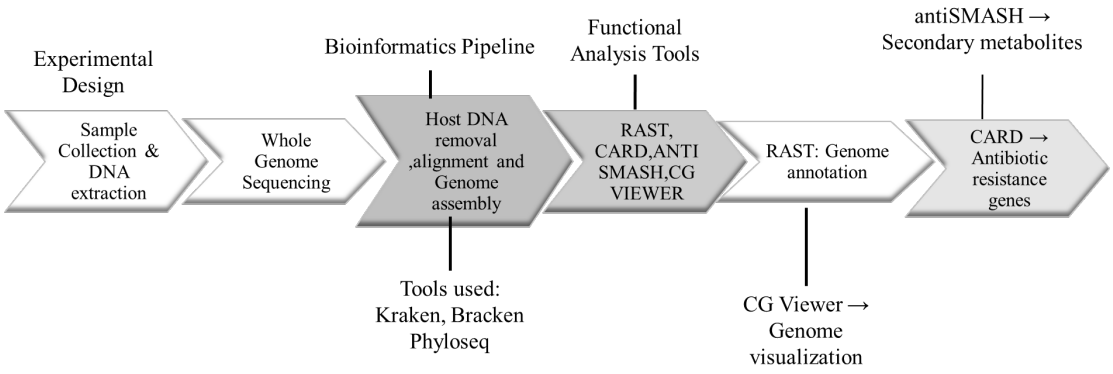


FIGURE 3.1: Research methodology flowchart

## 3.2 DNA Extraction from Bacterial Wound Culture Sample

A well-known method called CTAB was used to extract the DNA using the DNeasy PowerSoil kit (Qiagen). ThermoFisher Scientific's Nano Drop 2000 Spectrophotometer was used to assess the extracted DNA's purity. Additionally, 50 ng of extracted DNA and lambda 46 DNA/HindIII Marker (catalog number SM0102; ThermoFisher Scientific) were loaded onto a 1% agarose gel stained with ultrapure ethidium bromide (ThermoFisher Scientific) in order to validate the quantitative estimation. After that, the gel was run at 80 V for an hour to expose it to electrophoresis. A SmartView Pro 1100 Imager System (Major Science) was then used to visualize the gel [98].

## 3.3 DNA Library Preparation and Sequencing

Covaris targeting was used to enzymatically fragment the 100 nanogram of DNA into 250 base pairs. The overhang is then repaired to become the blunt end. A uracil-specific removal reagent (USER) enzyme is used to ligate and cleave the adenylated portion of the loose adapter.

The materials were further purified using AMPure beads. Six rounds of PCR using the NEBNext Ultra II Q5 master mix, sample-specific octamer primers, and Illumina universal primer increased the amount of DNA. AM pure was used to clear the unused primers from the amplified product.

15 milliliters of 0.1-TE buffer were used to elute the ensuing DNA libraries. The Qubit DNA High Sensitivity (HS) test kit and a Qubit Fluorometer were then used to quantify the quantities.

MEGAHIT v1.2.9 was used to compile these clean reads for microbiome configuration and contig-based ARG analysis. Contigs under 200 bp were eliminated from additional examination.

## 3.4 Genomic Sequencing and Analysis

Using a whole-genome shotgun and a commercial service, the bacterial genome was sequenced. The Illumine Hiseq 2000 platform was used for the sequencing (2 × 150-bp reads), and Covaris 220 was used to generate the DNA library. Genomic data analysis was done using the Rast server oniline. FastQC was used to assess the data's quality. It is a freely accessible bioinformatics application that uses quality control measures to offer all data pertaining to raw genomic sequencing [99].Both reads had an average sequence length of 104 bp, and the poor quality control sequences were eliminated.

## 3.5 Protocol for DNA Extraction

The cotton swab was first resuspended in 1 milliliter of PBS and vortexed for 10 seconds before being removed and centrifuged for 30 seconds at 4°C. The samples were incubated at 37°C for 10 to 60 minutes after 10g of crystalline lysozyme had been completely mixed into the cell solution.

After adding thirty milliliters of SDS (10-20%) and six microliters of Proteinase K (10 mg/ml), the mixture was incubated at 37°C. three till clear and viscous. After adding 100 $\mu$l of NaCI (5 M), the suspension was incubated for two minutes at 65 degrees Celsius. After that, 80$\mu$l of a hot CTAB/NaCI solution was added, thoroughly mixed, and incubated for 10 minutes at 65°C.

10,000 g was centrifuged for five minutes after an equivalent volume (about 800 $\mu$l) of chloroform/isoamyl alcohol (24:1) solution was added. The nucleic acid-containing top (aqueous) phase was transferred to a different Eppendorf tube. The mixture was centrifuged at 15,000 g for five minutes after isoamyl alcohol (25:24:1), phenol, and chloroform were added to the aqueous layer.

An equivalent volume (about 800 $\mu$l) of chloroform:isoamyl alcohol (24:1) solution was added when the upper (aqueous) phase was transferred to a different Eppendorf tube.After that, the samples were centrifuged for five minutes at 10,000g.

The nucleic acids were precipitated by transferring the aqueous phase into a new Eppendorf tube and adding about 560 $\mu$l of isopropanol.

After that, the tube was allowed to stand for five to six hours at room temperature. then a 12,000-15,000g centrifugation stage for 15-30 minutes at room temperature. The isopropanol was progressively removed so as not to disrupt the pellet.

500 $\mu$l of 70% EtOH and 12,000-15,000 g were used for washing, and the mixture was centrifuged for 15-30 minutes at room temperature. The pellet was dried and reconstituted in 40-60 $\mu$l of nuclease-free water after the etOH was removed. Allow the DNA to sit at 37oC so that it can completely resuspend [99].

## 3.6 Agarose Gel Electrophoresis

One gram of agarose was dissolved in one hundred milliliters of 1X TAE buffer (Tris Acetic Acid EDTA) to create a 1% agarose gel for gel electrophoresis. A clear solution was created when the mixture was heated to thoroughly dissolve the agarose. To see the DNA, 7 $\mu$l of ethidium bromide was added to the gel solution.

The gel mixture was then put into a gel casting tray that had combs in it to create wells. The gel caster was carefully placed in a gel tank filled with 1X TAE buffer once the gel had solidified. The wells were then exposed by carefully removing the combs.

To prepare the sample and facilitate tracking during electrophoresis, 2 $\mu$l of extracted DNA and 2 $\mu$l of 6X bromophenol blue dye (loading dye) were mixed together. The agarose gel's wells were filled with the DNA samples and a 1KB ladder for size reference.

The electrophoresis was run for 35 minutes at a current of 500 mA and a voltage of 75 volts. After the run, the gel under a UV trans-illuminator using a Bio Doc Analyzer to determine the size of the DNA fragments.

## 3.7 Approaches for the Analysis of Shotgun Whole Metagenomics Data

Each sample had two files of sequenced data-one for the forward strand and one for the reverse strand-after the full metagenome was sequenced using paired-end shotgun sequencing. The two primary approaches for analyzing metagenomics data are assembly-based and alignment-based.

Both approaches were applied in this study. The reads-based approaches, also called alignment-based methods, align clean readings to carefully chosen databases in order to generate feature tables. The data was quality tested using the FASTQC program. FastQC provides a straightforward method for doing quality control tests on raw sequence data from high-throughput sequencing workflows.

It offers a modular set of analyses to quickly identify any potential problems with the data before carrying out further investigation. The result was an HTML report that included information on a number of quality control variables. This helped identify issues that needed to be resolved to ensure the data was appropriate for additional analysis. The FASTQ format was used for the input files.

The assembly-based approach, which first assembles sequencing reads into larger contiguous sequences (contigs), can be used to reconstruct genomes or important genomic sections from metagenomic data. A number of tools are available, such as SPAdes, MEGAHIT, and Meta Velvet.

Preprocessing (filtering contaminants and low-quality reads), assembly (using assembly software to create contigs from the filtered reads), annotation (identifying genes and functional elements in the assembled contigs), and binning (placing contigs into bins that correspond to distinct genomes or genome fragments) are the steps involved.

The alignment-based approach detects and quantifies the presence of functional genes and known organisms by aligning clean readings using reference databases. This method makes use of carefully chosen databases that are relevant to the

study, such as KEGG, NCBI RefSeq, or specially constructed databases. Preprocessing (trimming and quality filtering raw reads), alignment (mapping reads to reference databases using tools like Bowtie2 or BWA), feature extraction (creating feature tables that quantify the abundance of various taxa or functional genes), and statistical analysis (performing statistical tests and visualization to interpret the feature tables) are some of the procedures.

## 3.8 Removal of Host DNA

Human reads are eliminated after matching with the human genome assembly since they are considered contamination in the bacterial diversity estimation (GRCh38 assembly).

This phase ensures that only microbial sequences are examined, increasing the accuracy of the estimates of bacterial diversity. The raw readings are typically aligned to the human genome using alignment algorithms like as Bowtie2 or BWA. The readings that do not match the human genome are the only ones that remain for further examination.

## 3.9 Alignment of the Reads to Database

Kraken2 and a curated database were used to provide taxonomic information for the readings. Kraken2, the latest version of the Kraken taxonomy classification system, employs accurate k-mer matching for high accuracy and fast classification times. For every k-mer in a query sequence, this classifier determines the lowest common ancestor (LCA) of all genomes that include the given k-mer.

The k-mer assignments are used to inform the classification procedure in order to offer precise taxonomy identification. The Mini Kraken database, a pre-built database in Kraken2, was used for read alignment and taxonomy information assignment. This database provides a comprehensive reference for read classification and contains sequences from bacteria, viruses, and archaea.

## 3.10 Assembly of Metagenome

After quality verification, the assembly was completed using MEGAHIT, an assembler that can effectively handle large and complex metadata data, especially on a single-node server (current maximum memory capacity 768 GB for a 2-socket server). Within the range of k-mer values supported by MEGAHIT, k-mer lengths of 29, 39, 59, 79, 99, 119, and 141 were used for this analysis.

The input files were in the FASTQ format. MEGAHIT offers a variety of parameter presets that can be tailored to suit certain requirements, such as increased sensitivity or the construction of complex and large metagenomes. Each parameter can also be changed independently to enhance the assembly process. The output of the assembly procedure was contigs in FASTA format, which were used for additional analysis.

## 3.11 Abundance Estimation Bracken

Bracken (Bayesian Re estimate of Abundance with KrakEN) is a highly efficient statistical method for determining species abundance in DNA sequences from a metagenomics sample. Bracken determines the number of reads from each species in a sample using the taxonomy labels supplied by Kraken, an accurate metagenomics classification tool.

Kraken, however, assigns readings to the most appropriate location in the taxonomic tree rather than estimating species abundances. Bracken uses the Kraken database to calculate the probability that sequences from one genome are identical to those from other genomes in the database.

This information is used in conjunction with the taxonomic assignments for a particular sample to estimate abundance at other taxonomic levels, such as species and genus. By integrating with the Kraken classifier, Bracken produces accurate species- and genus-level abundance estimates even in samples containing two or more nearly identical species.

## 3.12 Complete Taxonomic Hierarchy Generation

### 3.12.1 Kraken-biom

One or more files from the Kraken-Report utility can be entered into the Bracken program. Each file is parsed to obtain the lineage, database ID (e.g., NCBI), and numbers for each OTU. The retrieved data is subsequently stored in a BIOM (Biological Observation Matrix) table.

Every count is linked to the OTU and sample that it belongs to. The BIOM format was developed to facilitate the transfer of abundance and taxonomic hierarchy data into R for further microbiome data exploration and analysis. This standard format ensures that the data may be used efficiently in downstream analyses such as microbial community visualization, diversity evaluations, and differential abundance tests.

### 3.12.2 Phyloseq

The phyloseq program is a complete tool for importing, storing, analyzing, and visualizing complex sequencing data that has been organized into Operational Taxonomic Units (OTUs). It makes data sharing and reproducibility easier by keeping all pertinent sequencing data as a single experiment-level object utilizing a certain set of S4 classes.

Phyloseq facilitates the use of R for efficient, interactive, and reproducible analysis of OTU-clustered high-throughput sequencing data. The software provides capabilities for diversification analysis, differential abundance testing, and other statistical research to enable seamless data integration.

Additionally, it organizes data in a systematic manner for easy access and modification. Additionally, Phyloseq offers tools for creating publication grade plots, such as ordination plots, heatmaps, bar plots, and phylogenetic trees.

## 3.13 Whole Metagenome Processing

### 3.13.1 Data Processing and Annotation

The *Morganella morganii* genome was recovered from a metagenomic dataset using a method called binning (https://github.com/SEEDtk/.pl), which clusters contigs based on sequence composition and coverage patterns. After binning, MAGpurify (https://github.com/MAGpurify) was used to refine the genome. a program designed to improve the quality of metagenome-assembled genomes (MAGs) by identifying and removing any contamination. Contigs that matched the human genome or other strains were removed. Several filtration approaches, including as GC-content analysis, tetranucleotide frequency evaluation, and phylogenetic marker analysis, were employed to ensure the accuracy and completeness of the genome.

The strain was confirmed using the TYGS server (https://tygs.dsmz.de/) after contamination was removed. Two complementary techniques were used to determine the closest type strain genomes. The MASH methodology, a rapid method of estimating intergenomic relatedness, was initially used to compare the cleaned genome with all type strain genomes in the TYGS database [100]. Based on their smallest MASH distances, ten type strains were selected for every user genome. Second, eleven additional closely related type strains were found using 16S rRNA gene sequences. RNAmmer was used to extract these sequences from the user genomes [100]. They then used BLAST to compare the 16S rRNA gene sequences of the 22,513 type strains in the TYGS database [101].

Utilizing the distance formula d5 and the "coverage" technique [102]. The precise distances between the 50 best-matching type strains were subsequently ascertained using the Genome BLAST Distance Phylogeny (GBDP) method (based on bit score). For every user genome, the ten closest type strain genomes were identified using these distances. GBDP was utilized for all pairwise comparisons between the selected genomes in order to carry out phylogenomic inference. Intergenic distances were computed using the "trimming" procedure and distance formula

d5. A total of one hundred distance replicates were produced. Digital DNA-DNA hybridization (dDDH) values and confidence intervals were estimated using the recommended settings of GGDC 4.0.

Using FASTME 2.1.6 and subtree pruning and regrafting (SPR) postprocessing [103]. The inferred intergeometric distances were used to construct a balanced minimum evolution tree. Branch support was found using 100 pseudo-bootstrap replicates. The trees with midway roots were shown using PhyD3 [104]. Standard protocols were used to cluster species around a 70% dDDH threshold for each of the 14 type strains [105].

As previously stated, a 79% dDDH threshold was used for subspecies grouping. The cleaned Morganella genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://github.com/ncbi/pgap) to provide a high-quality annotated genome for additional research. Genes, functional components, and other genomic properties are systematically predicted by the process. CRISPR, mobile genetic elements, and resistance gene identities were predicted and shown on a circos plot using proksee.ca.

## 3.14 Functional Analysis

PROKKA v1.14.5 was utilized to annotate the wound metagenome sample assembly file [106]. PROKKA is an effective method for rapidly annotating bacterial genomes, offering detailed information on the characteristics and functions of genes. The annotated sequences in the Virulence Factors Database (VFDB) were then analyzed to identify potential virulence factors present in the wound samples.

Several databases were used to confirm the existence of antibiotic resistance genes (ARGs). These included the Comprehensive Antibiotic Resistance Database (CARD), which offers a comprehensive and up-to-date resource for finding ARGs, and Resfinder, a tool designed specifically for identifying ARGs in metagenomic data.

### 3.14.1 ARGs Identification

The metagenomic data was examined to discover antibiotic resistant genes (ARGs) using the Comprehensive Antibiotic Resistance Database (CARD). CARD, an integrated and up-to-date resource that gathers information from several sources into an extensive database, can be used to locate ARGs.

The assembled contigs in FASTA format were used as the input for the ARG prediction in this study. The assembled sequences must be compared to the CARD database in order to identify potential ARGs.

This step is crucial for determining the prevalence and diversity of antibiotic resistance in the microbial communities present in the samples [100].

### 3.14.2 CG Viewer

The circular genome map was generated by using the online bioinformatics tool CGviewer (http://cgview.ca/) and GenSKew (https://genskew.csb.univie.ac.at/) were used to calculated the nucleotide skew.

The comprehensive antibiotics resistance database CARD and their virulence factors were used to determine by (https://card.mcmaster.ca/), Virulence Finder (cge.cbs.dtu.dk/VirulenceFinder/), as well as antibiotics resistance seeker respectoively [101].

### 3.14.3 Secondary Metabolite Identification by antiSMASH

For identification of secondary metabolite in both strains that is *Morganella morganii* and *Comamonas testosteroni*, online web server antiSMASH (anti-smash.secondarymetabolite.org) was used.

Clean FASTA file of *Morganella morganii* was upload while for *Comamonas testosteroni*, NCBI accession number was entered. Input format that antiSMASH accept are FASTA, gbk files, embl files and accession number [102].

### 3.14.4 Core Genome Analysis by ARTS

Antibiotic resistance target seeker (artz.ziemertlab.com) was use for core genome analysis of *Morganella morganii* and *Comamonas testosteroni*. gbk file for both strais were uploaded (one by one separately).

ARTS also accept NCBI accessiom number but preferred files are gbk [101].

### 3.14.5 Mobile Genetic Elements Identification

For mobile genetic elements identification online Island Viewer (islandviewer.org) was used. FASTA file for *Morganella morganii* was uploaded while in case of *Comamonas testosteroni* gbk file was uploaded. Output file was a circular diagram representing major categories of different genes having different functions [99].

# Chapter 4

# Results

## 4.1 Results of RASTserver

RAST server findings indicate major difference in genomic characteristics of *Morganella morganii* and *Comamonas testosteroni*, (GC content and number of coding sequences). Table 4.1 explain RAST results.

TABLE 4.1: Comparative Genomic Characteristics of *Morganella morganii* and *Comamonas testosteroni* based on RAST server

| **Genome Feature** | **Wound Source Strain** | **Soil Source Strain** |
|---|---|---|
| Taxonomy | *Morganella morganii* | *Comamonas testosteroni* |
| Genome size (bp) | 4,034,593 | 5,293,685 |
| GC content (%) | 51.0 | 71.4 |
| N50 (bp) | 34,562 | — |
| L50 | 39 | 1 |
| Number of contigs (with PEGs) | 210 | 1 |
| Number of subsystems | 340 | 335 |
| Number of coding sequences (CDS) | 4,242 | 5,189 |
| Number of RNAs | 75 | 133 |

## 4.2 Whole Genome Testing of *Morganella morganii* and *Comamonas testosteroni*

Genomic assembly of this genome show that *Morganella morganii* consists of 4,034,593 bp genome with 51.1% GC content and contain 4242 coding sequencing with 75 RNAs. The genome of *Morganella morganii* by RAST functional annotation showed that 22 genes for nitrogen metabolism, 191 for protein, 195 for carbohydrates,51 for fatty acid, lipid and isoprenoids,25 for phosphorus,6 for sulfur metabolism and 15 for potassium. The structural regulation gene observed to be 49 for membrane transport, 42 for cell wall and capsule. Moreover 71 for environmental stress response and 45 for virulence, disease and defense, 109 for nucleotides and nucleoside, 35 for resistance to antibiotic and toxic compounds,58 for RNA metaboslism. Additionally, *Comamonas testosteroni*i consisted of 5,497,097 bp genome with 61.4% GC content and contains 5189 coding sequences with 133 RNAs. RAST annotation showed that it had 13 genes for nitrogen metabolism 257 genes for proteins, 220 genes for carbohydrates, 151 genes for fatty acid, lipid and isoprenoids, 21 for phosphorus, 11 potassium and 26 genes for sulfur metabolism. Genes involved in structural regulation included 25 for cell wall and capsule, 96 for motility and chemotaxis and 128 for membrane transport. Furthermore, total genes for virulence, disease and defense were 83 and 76 for environmental stress response. All results are explained in Table 4.2.

TABLE 4.2: Comparative Genomic of *Morganella morganii* and *Comamonas testosteroni* based on RAST server

| **Function** | **Number of Genes in *Morganella morganii*** | **Number of Genes in *Comamonas testosteroni*** |
|---|---|---|
| Nucleosides and nucleotides | *109* | *107* |
| Cell division and cell cycle | 6 | 0 |
| Protein metabolism | 191 | 257 |
| Sulfur metabolism | 6 | 26 |
| Secondary metabolism | 6 | 0 |
| Amino acids and derivatives | 310 | 325 |
| DNA metabolism | 84 | 73 |

**Table 4.2 continued from previous page**

| **Function** | **Number of Genes in *Morganella morganii*** | **Number of Genes in *Comamonas testosteroni*** |
|---|---|---|
| Iron acquisition and metabolism | 18 | 30 |
| Number of RNAs | 75 | 133 |
| Nitrogen metabolism | 22 | 13 |
| Phosphorus metabolism | 25 | 21 |
| Miscellaneous | 12 | 24 |
| Potassium metabolism | 15 | 11 |
| Fatty acids, lipids, and isoprenoids | 51 | 151 |
| Phages, prophages, transposable elements, plasmids | 23 | 8 |
| Cell wall and capsule | 42 | 25 |
| Regulation and cell signaling | 45 | 34 |
| Respiration | 104 | 120 |
| Stress response | 71 | 76 |
| Metabolism of aromatic compounds | 7 | 41 |
| Cofactors, vitamins, prosthetic groups, pigments | 164 | 212 |
| RNA metabolism | 58 | 50 |
| Virulence, disease and defense | 0 | 83 |
| Motility and chemotaxis | 14 | 96 |
| Carbohydrates | 195 | 220 |

## 4.3 Genomic Islands and Resistance Gene based on Island Viewer

Resistance genes annotation was performed to visualize the resistance genes in *Morganella morganii*. Red highlighted inner circle showed GC rich region, while blue and orange highlighted regions indicate the predicted island. These results

did not show any significant pathogenic island (Figure 4.1 and Table 4.3). The comprehensive antibiotic resistance database (CARD) showed that *Morganella morganii* contained 4genes which are adef 4.5 mbp, adeF 1.75 mbp, fosAB and QAC-3.*Comamonas testosteroni* have fourresistance genes which are adef 4.5 mbp, adeF 1.75 mbp, fosAB and QAC-3. according to card results (Table 4.5).

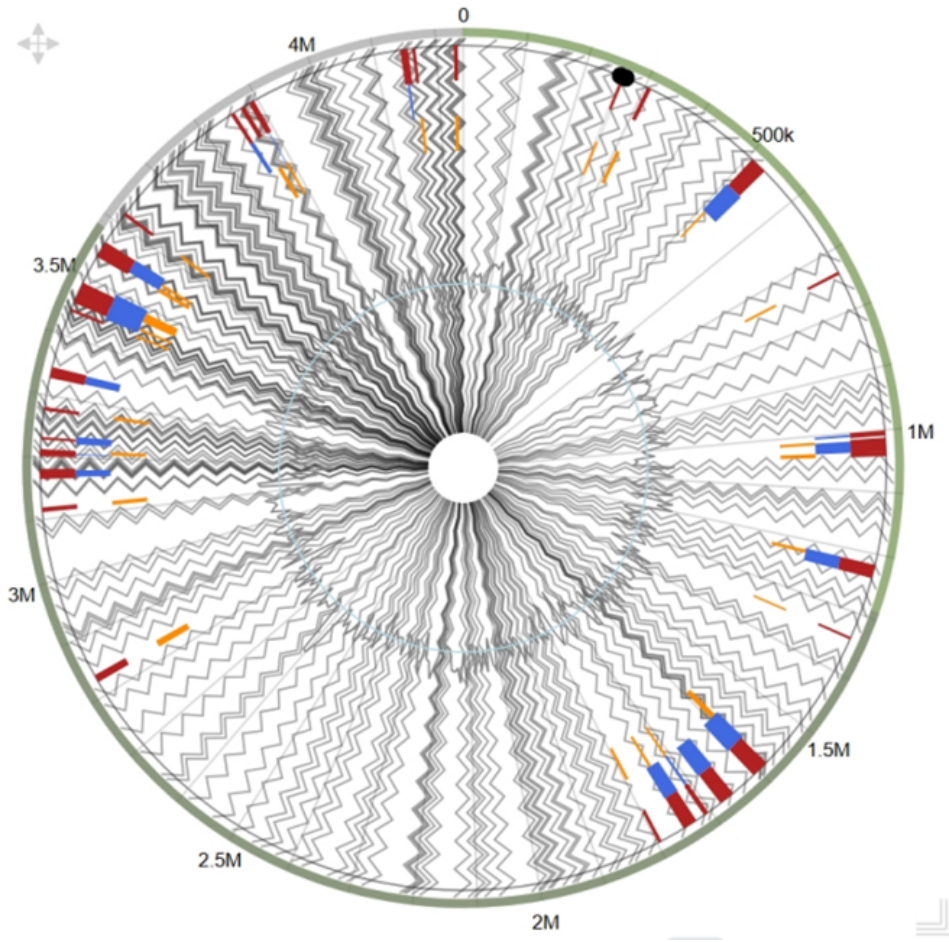


FIGURE 4.1: Color scheme of island viewer Circular diagram showing resistance genomic island (red for integrated prediction, orange for SIGI-HMM and blue for island path) for *Morganella morganii*

TABLE 4.3: Explanation of different segments of circular diagram generated by Island viewer

| **Segment** | **Description** |
|---|---|
| Red color | Predicted genomic islands (high degree of certainty) |
| Blue color | Predicted genomic islands (medium confidence) |
| Green color | Indicates mobile genes |
| Wavy lines | Indicate atypical genomic regions |
| Labelled coordinates | Indicate positions along the circular genome |

**Table 4.3 continued from previous page**

| Segment | Description |
|---|---|
| Inner concentric rings | Comparative alignment with reference genome |

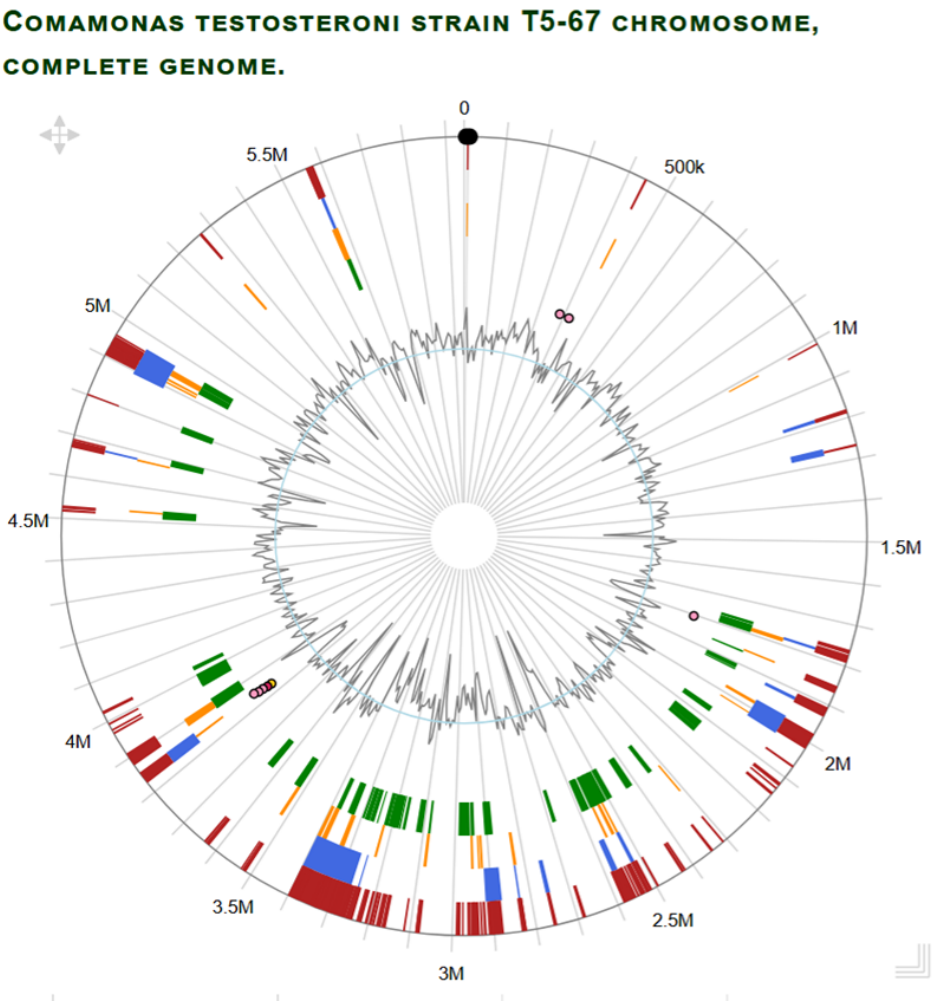


FIGURE 4.2: Genomic island of *Comamonas testosteroni*

TABLE 4.4: Resistance genes annotated on CARD for *Morganella morganii*

| ARO Term | RGI Criteria | Detection Criteria | AMR Family | Drug Class | Resistance Mechanism |
|---|---|---|---|---|---|
| AadA | Perfect | Protein homolog | ANT(6) | Aminoglycoside antibiotic | Antibiotic inactivation |
| tet(A) | Strict | Protein homolog | Tetracycline inactivation enzyme | Glycylcycline, tetracycline | Antibiotic inactivation |

**Table 4.4 continued from previous page**

| ARO Term | RGI Criteria | Detection Criteria | AMR Family | Drug Class | Resistance Mechanism |
|---|---|---|---|---|---|
| qac(G) | Strict | Protein homolog | Small multidrug resistance (SMR) efflux pump | Disinfecting agents and antiseptics | Antibiotic efflux |
| blaDHA-1 | Strict | Protein homolog | AmpC $\beta$-lactamase | Carbapenem, penicillin, $\beta$-lactam | Antibiotic inactivation |
| sul1 | Strict | protein homology | Sulfonamide-resistant dihydropteroate synthase | Sulfonamide antibiotics | Antibiotic target alteration |

TABLE 4.5: Resistance genes annotated on CARD for Nocardioides sp. JS614

| ARO Term | RGI Criteria | Detection Criteria | AMR Gene Family | Drug Class | Resistance Mechanism |
|---|---|---|---|---|---|
| HelR | Strict | Protein homology model | Helicase-like RNA polymerase protection protein | Rifamycin antibiotic | Antibiotic target protection |
| *vanW gene (vanI cluster)* | Strict | Protein homology model | *vanW glycopeptide resistance gene cluster* | Glycopeptide antibiotic | Antibiotic target alteration |

## 4.4 Prediction of Core Genome and Cluster Gene for *Morganella morganii* and *Comamonas testosteroni* by Antibiotic Resistance Target Seeker

Gene associated with core function belonged to 7% of the total genes. The color coded segments represented different functional categories, along with percentage

of the total core functions. The largest segment accounts for 28%, followed by other segments representing 12%, 9%, 8%, 6% of the core functions for *Morganella morganii* (Figure 4.3). additionally, for *Comamonas testosteroni* Gene associated with core function belonged to 6% of the total genes. The color coded segments represented different functional categories, along with percentage of the total core functions. The largest segment accounts for 31%, followed by other segments representing 10%, 9%, 6%, 4% and 3% of the core functions.


FIGURE 4.3: Antibiotic Resistance Target Seeker (ARTS) tool version 2 result for *Morganella morganii*

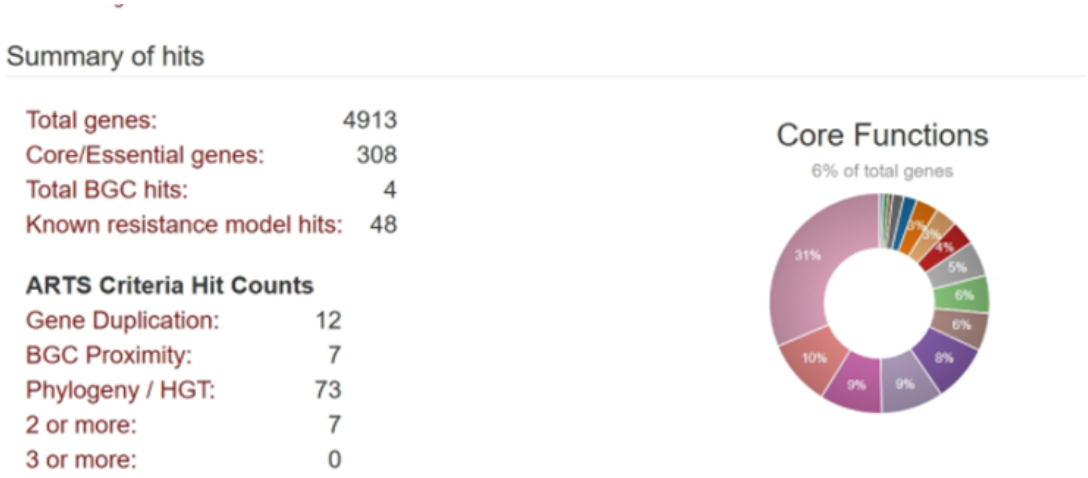


FIGURE 4.4: Antibiotic Resistance Target Seeker (ARTS) tool version 2 results for *Comamonas testosteroni*

The Table 4.6 presents an analysis of biological functions or processes identified using the Antibiotic Resistance Target Seeker version 2. The most prominent category is protein synthesis, with 86 sequences, indicating that this process is heavily targeted or involved in antibiotic resistance mechanisms. DNA metabolisms 28 sequences, highlighting their significant roles in the survival and replication of resistant organisms. Other key functions include protein fate and biosynthesis of cofactors, which also play essential roles in maintaining cellular functions under antibiotic pressure. Categories such as amino acid biosynthesis and purine, pyrimidine, nucleotide, and nucleoside metabolism are moderately represented, reflecting their importance in fundamental cellular processes. However, certain functions like fatty acid and phospholipid metabolisms are underrepresented, suggesting they might be less critical in the context of antibiotic resistance. 17% unclassified and 14% unknown function categories.

TABLE 4.6: Distribution of Genes Involved in Various Cellular Functions in *Morganella morganii* based on data obtained from ARTS

| **Cellular Functions** | **Genes** |
|---|---|
| Protein synthesis | 86 |
| Energy metabolisms | 36 |
| DNA metabolisms | 28 |
| Protein fate | 24 |
| Biosynthesis of cofactor, prosthetic group | 28 |
| Unclassified | 17 |
| Amino acid biosynthesis | 17 |
| Purine, pyrimidine, nucleotide and nucleoside | 15 |
| Transcription | 14 |
| Unknown function | 14 |
| Cell envelope | 11 |
| Cellular process | 6 |
| center intermediary metabolism | 5 |

The table 4.7 shows the distribution of genes across different functional categories, with the highest numbers found in protein synthesis (86), followed by energy metabolisms (36), DNA metabolisms (28), and protein fate (24). Other categories include biosynthesis of cofactors, nucleotide metabolism, transcription.

TABLE 4.7: Distribution of Genes Involved in Various Cellular Functions in *Comamonas testosteroni* based on data obtained from ARTS.

| **Cellular Functions** | **Genes** |
|---|---|
| Protein synthesis | 91 |
| Energy metabolisms | 23 |
| DNA metabolisms | 26 |
| Protein fate | 25 |
| Biosynthesis of cofactor, prosthetic group | 28 |
| Unclassified | 15 |
| Amino acid biosynthesis | 14 |
| Purine, pyrimidine, nucleotide and nucleoside | 10 |
| Transcription | 13 |
| Unknown function | 12 |
| Cell envelope | 9 |
| Cellular process | 5 |
| center intermediary metabolism | 3 |
| Hypothetical protein | 3 |
| Fatty acid and phospholipid metabolism | 2 |

The table 4.8 shows the distribution of genes across different functional categories, with the highest numbers found in protein synthesis (91), followed by biosynthesis of cofactor, prosthetic group (28) DNA metabolisms (26), energy metabolisms (23), and protein fate (25). Other transcription, cellular envelop, hypothetical proteins, fattay acid and phospholipid metabolism etc.

TABLE 4.8: Comparison of Genes Involved in Various Cellular Functions in *Morganella morganii* and *Comamonas testosteroni* based on data obtained from ARTS

| **Cellular Functions** | **Number of Genes in *Morganella morganii*** | **Number of Genes in *Comamonas testosteroni*** |
|---|---|---|
| Protein synthesis | 86 | 91 |
| Energy metabolisms | 36 | 23 |
| DNA metabolisms | 28 | 26 |
| Protein fate | 24 | 25 |
| Biosynthesis of cofactor, prosthetic group | 28 | 28 |
| Unclassified | 17 | 15 |

**Table 4.8 continued from previous page**

| **Cellular Functions** | **Number of Genes in *Morganella morganii*** | **Number of Genes in *Comamonas testosteroni*** |
|---|---|---|
| Amino acid biosynthesis | 17 | 14 |
| Purine, pyrimidine, nucleotide and nucleoside | 15 | 10 |
| Transcription | 14 | 13 |
| Unknown function | 14 | 12 |
| Cell envelope | 11 | 9 |
| Cellular process | 6 | 5 |
| Center intermediary metabolism | 5 | 3 |
| Hypothetical protein | 2 | 3 |
| Fatty acid and phospho-lipid metabolism | 1 | 2 |

## 4.5 Identification of BGCs in *Morganella morganii* and *Comamonas testosteroni*

The antiSMASH analysis of *Morganella morganii* revealed terpene precursor bio synthetic gene clusters, suggesting that the organism may produce terpenebased secondary metabolites (Figure 4.5).

Terpenes, a vast group of organic compounds originating from isoprene units, are well-known for their various biological functions.

These compounds are often key players in microbial interactions, including antimicrobial activity, signaling, and adaptation to the environment. Identifying terpene biosynthetic genes in *Morganella morganii* indicated that the bacterium might use these metabolites to improve its ecological fitness, compete with other microorganisms, or interact with host organisms.

Moreover, these metabolic capabilities might play a role in the organism's survival in clinical or environmental niches and could be associated with its virulence or resistance traits seen in pathogenic strains.

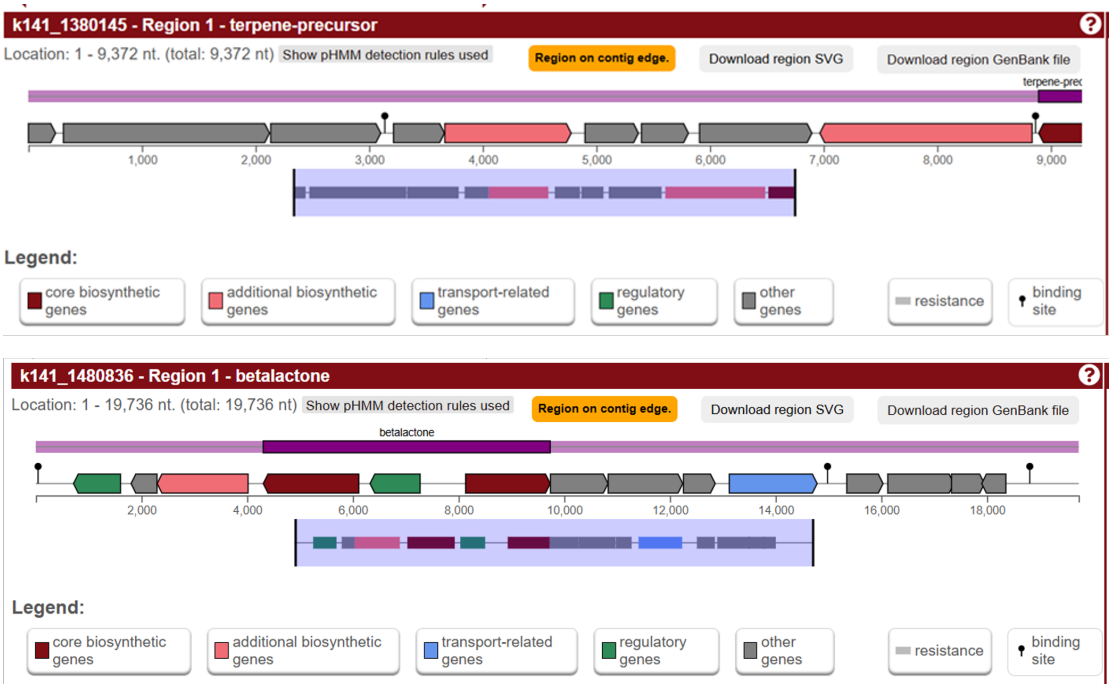


FIGURE 4.5: BGC of terpene and betalactone by antiSMASH for *Morganella morganii*

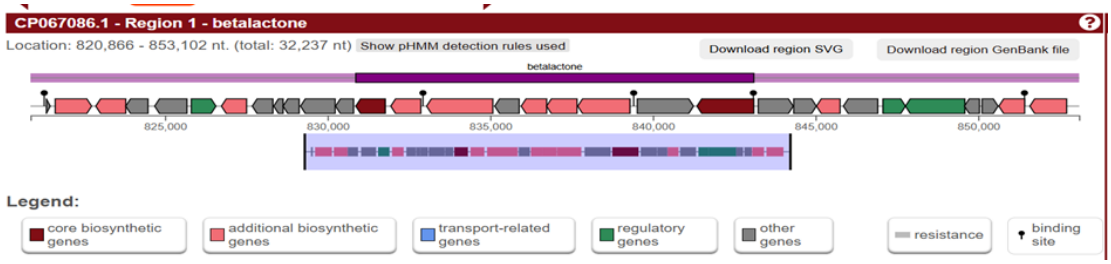


FIGURE 4.6: BGC of betalactone by antiSHASH for *Comamonas testosteroni*

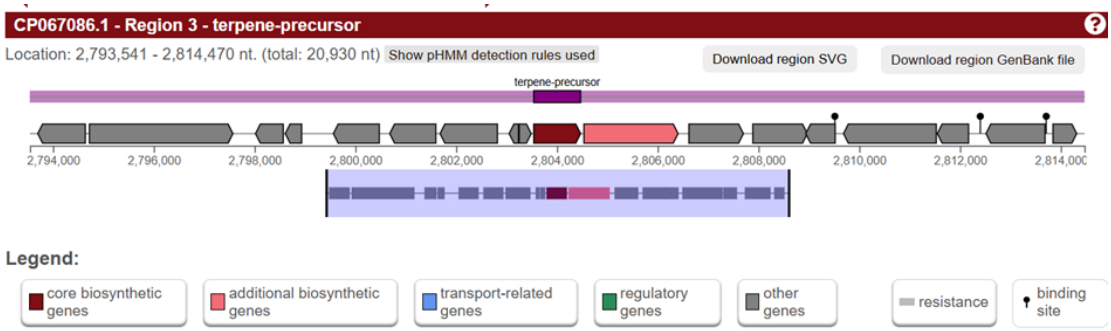


FIGURE 4.7: BGC tarpenel precursor or *Comamonas testosteroni*

In *Comamonas testosteroni* betalactone (secondary metabolite) biosynthetic gene cluster is present, having total length of 32,237 nucleotides. Start point is nucleotide number 82,500 and end point is nucleotide number 850,000.

Red color indicates core biosynthetic genes that produce enzymes which are directly involve in betalactone production.

Pink color indicate genes that produce such enzymes which help in biosynthesis of betalactone. Blue color points out genes related to transport and help in transport of metabolites through membrane.

Green color represents to genes that are responsible for regulation and act as suppressor or activator during gene expression.

Gary color indicate other genes which are hypothetical that is there is no evidence about protein product of these genes. Similarly, resistance genes are linked with host protection from its own toxic products.

TTA codons are associated with specialized genes related to metabolism. Finally binding side indicate point of transcription regulation (Figure 4.6).

Furthermore, biosynthetic gene clusters for terpene precursors were identified in *Morganella morganii*, indicating the strain's potential to produce a diverse range of terpene compounds. Terpenes, a varied collection of bioactive secondary metabolites originating from isoprene units, are often linked to ecological roles such as communication, defense, and adaptation (Figure 4.7).

In microbial communities, terpenes serve as antimicrobial agents and quorum sensing molecules in response to environmental stressors.

These gene clusters suggest that *Morganella morganii* generate specialized metabolites for competition with nearby microorganisms, to improve their colonization capabilities, or to foster advantageous relationships with plants or other hosts. This metabolic versatility enhances the organism's ecological fitness.

## 4.6 Island Viewer Results for Functional Categories of Genes within Mobile Genetic Elements

Within mobile genetic elements of *Morganella morganii* five major categories found that are involved in different functions (Figure 4.8). These functions are integration, replication, repair, stability and protein coding for conjugation.

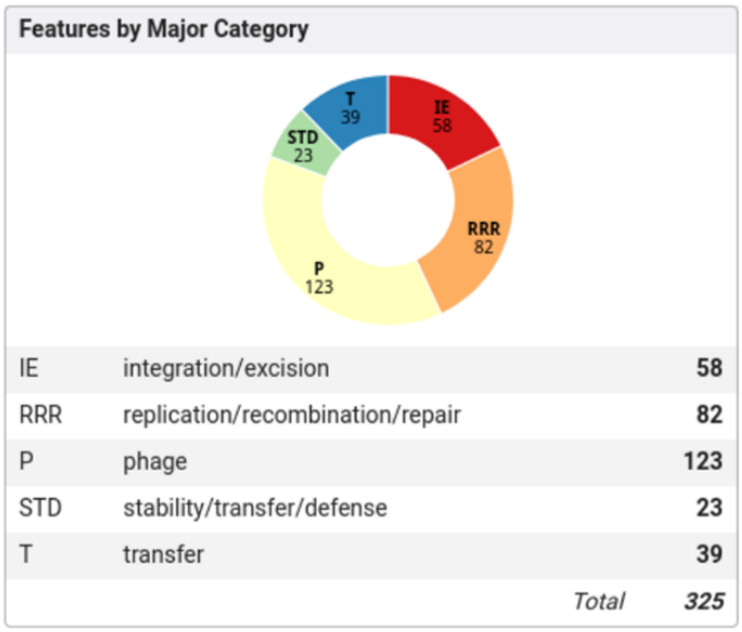


FIGURE 4.8: MGEs in *Morganella morganii*

TABLE 4.9: Illustrate code, number and functional of gene with in MGEs

| Code | Number | Function |
|---|---|---|
| IE | 58 | Help in insertion or removal of MGEs into host genome |
| RRR | 82 | Ensure replication, recombination and repair of MGEs |
| P | 123 | Indicate origin of MGEs |
| STD | 23 | Provide stability, ease of transfer and protection from foreign DNA |
| T | 39 | Encode proteins that help in bacterial conjugation and transfer of MGEs by HGT |

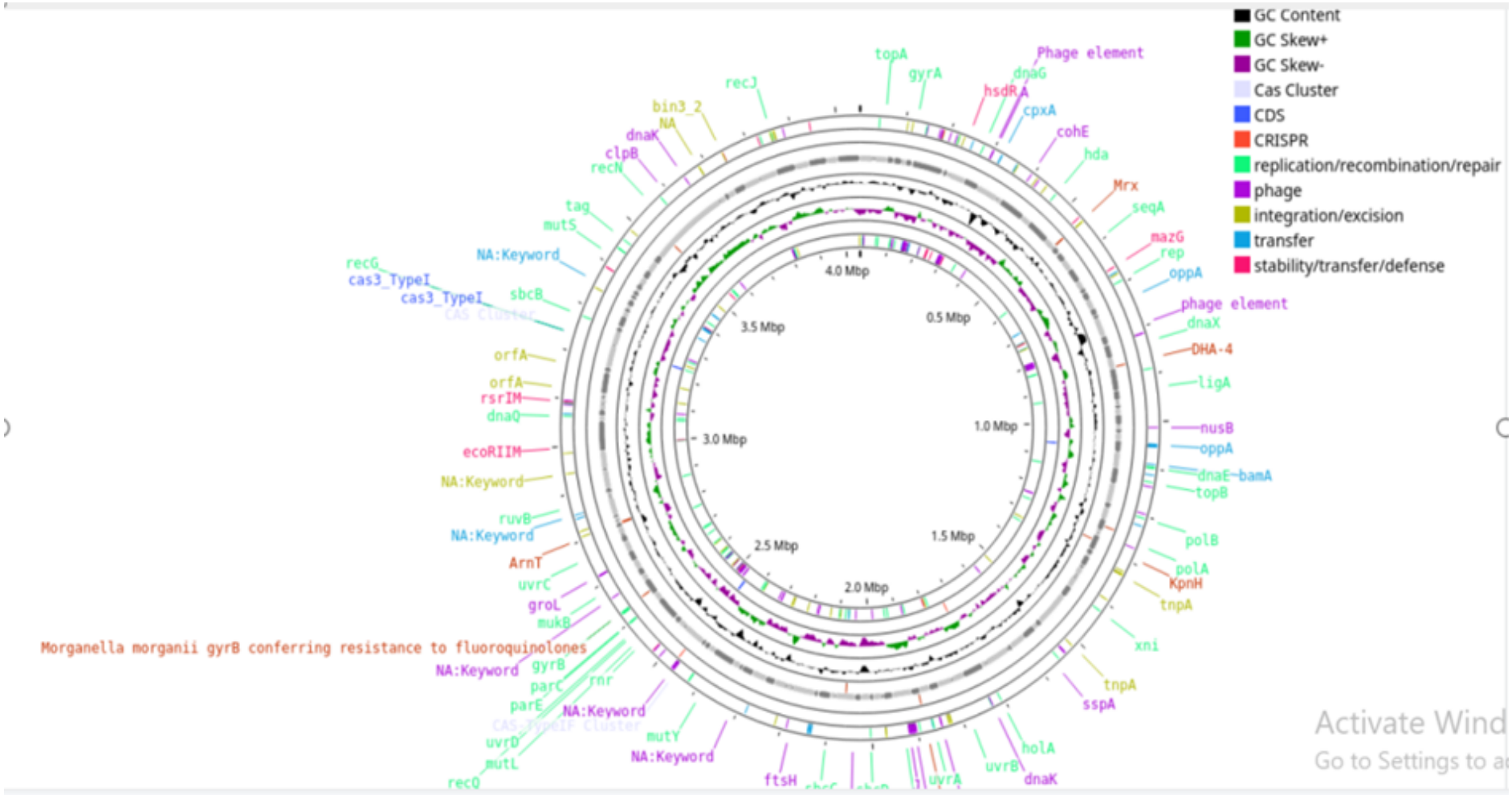


FIGURE 4.9: Graphical representation of genome (*Morganella morganii*) by CG Viewer

## 4.7 Results of CG Viewer

Circular genome map of *Morganella morganii* shows a genome size of about 3.9 Mbp with a consistent GC skew and content, indicating stable replication regions. Multiple antibiotic resistance genes, including ArnT, kpnH, DHA-4, and Mrx (in the vanG cluster), which indicate multidrug resistance and *Morganella morganii* gyr8 conferring resistance to fluroquinolones, are important characteristics (Figure 4.9.

Both defense capabilities and prior viral exposure are suggested by CRISPR-Cas systems and phage-related regions. Genomic integrity is maintained by the presence of DNA repair genes (orfA, tnpA, and bin3). Horizontal gene transfer potential is indicated by transposase (tnpA) and plasmidrelated genes. Clusters pertaining to defense, stability, replication, and transfer are found throughout the genome. Overall, this genetic composition reflects clinical significance, environmental resilience, and adaptability, particularly in nosocomial settings where antibiotic resistance is a significant concern.

TABLE 4.10: Data analysis of CG Viewer and identification of important genes along with their function

| **Gene** | **Function** |
|---|---|
| ArnT | Provides tetracycline resistance via enzymatic inactivation |
| gyrB | Encodes DNA gyrase subunit B; mutations are associated with fluoroquinolone resistance |
| parC / parE | Encode subunits of topoisomerase IV; involved in DNA replication and quinolone resistance |
| recA | Central role in DNA repair, homologous recombination, and the SOS response |
| recG | Helicase involved in recombination and DNA repair pathways |
| mutS | Component of the DNA mismatch repair system, maintaining genome stability |
| uvrA, uvrB, uvrD | Components of nucleotide excision repair, protecting DNA from UV-induced damage |
| dnaK | Heat-shock protein involved in protein folding and stress response |

**Table 4.10 continued from previous page**

| Gene | Function |
|---|---|
| mutL | Works with MutS in the mismatch repair mechanism |
| Cas cluster | Part of the CRISPR-Cas adaptive immune system protecting against foreign DNA |
| CpxA | Sensor kinase involved in envelope stress response and virulence regulation |
| TnpA | Transposase enzyme involved in DNA transposition and horizontal gene transfer |

# Chapter 5

# Discussion

The importance of host-microbe interactions in both health and disease is being increasingly acknowledged. By comprehending host-pathogen interactions at the genetic level, scientists may be able to create focused infection management and prevention plans. Nonetheless, the majority of research has concentrated on microbial communities, giving greater weight to microbial diversity than gene function [107]. In recent years, *Morganella morganii* has emerged as opportunistic pathogen, as evidenced by the rise in nosocomial outbreaks and cases reported, particularly in developing nations [108].

*Morganella morganii* found in soil, water, sewage and hospital settings and show extensive drug resistance due to resistance genes such as ArnT, kpnH, DHA-4, and Mrx [29]. In hospital setting *Morganella morganii* form strong biofilm that also contribute as a major factor in antibiotic resistance [29]. Genomic comparison of *Morganella morganii* with an environmental strain that is *Comamonas testosteroni* reveals significant insights that indicate difference in gene expression occur as habitat change [29]. RAST results indicated that GC content of *Morganella morganii* is 51.1% while for *Comamonas testosteroni* GC content is 61.4 which indicate *Morganella morganii* genome undergo continuous mutations and *Comamonas testosteroni* genome is stable due to environmental adaptations [109]. Subsystem of RAST indicated that there are 6 genes in *Morganella morganii* responsible for Sulphur metabolism while *Comamonas testosteroni* contain 26 genes

for Sulphur metabolism. These findings indi- cate that *Comamonas testosteroni* enhance metabolic pathways associated with Sul- phur metabolism in nutrient limited ecosystem while *Morganella morganii* is adopted to nutrient rich host dependent environment [110]. Another interesting finding was number of genes responsible for iron acquisition and metabolism. *Morganella morganii* have 18 genes for iron acquisition and metabolism while *Comamonas testosteroni* have 30 genes for iron acquisition and metabolism.

The significance difference in genes responsible for iron acquisition and metabolism indicate that soil bacteria does not face host level sequestration and have free iron availability in different oxidizes forms so they do not complex iron obtaining and metabolizing system while host dependent *Morganella morganii* face problem regarding host level sequestration so multiple iron acquisition and metabolism evolve [111]. RAST results indicated that there are 23 genes associated with Phages, Prophages, Transposable elements, Plasmids in *Morganella morganii* while are 8 genes are identified for Phages, Prophages, Transposable elements, Plasmids in *Comamonas testosteroni*. significant difference in genes as- sociated with Phages, Prophages, Transposable elements, Plasmids indicate that *Morganella morganii* have well developed mechanism for horizontal gene transfer, spread of antibiotic resistance genes and virulence factors, while 8 genes associated with Phages, Prophages, Transposable elements, Plasmids in *Comamonas testosteroni* stable genome shaped by long term adaptive environment with low stress [112].

For stress response *Morganella morganii* have 71 genes while *Comamonas testosteroni* have 76 genes which indicate *Comamonas testosteroni* is adaptive to broad range environmental stresses such as temperature variations, UV radiation, heavy metals exposure while *Morganella morganii* is adopted according to host related stress such as immune response and antibiotic stress [113]. According to a RAST analysis, *Morganella morganii* has 27 genes linked to defense, illness, and virulence, while *Comamonas testosteroni* have 83 genes in the same category. Despite having a greater number of genes, *Comamonas testosteroni* genes are probably linked to broad-spectrum environmental defense mechanisms such heavy metal detoxi- fication, oxidative stress protection, and general antimicrobial resistance,

which are necessary for survival in a variety of soil conditions. On the other hand, Morganella's genes are more specialized and tailored to clinical settings, which helps with antibiotic resistance, biofilm development, and immune evasion. With Morganella evolving compact, host-focused virulence strategies and Comamonas maintaining a broader defensive capacity appropriate for changing environmental challenges, this contrast demonstrates how ecological pressures impact the functional genome content of bacteria [114].

For carbohydrates *Morganella morganii* have 195 genes while *Comamonas testosteroni* contain 220 genes. Significant difference in carbohydrates metabolism indicate that *Comamonas testosteroni* require broad spectrum of active enzymes for carbohydrate metabolism to degrade and use different organic matter in contrast *Morganella morganii* depend upon host for carbon source and adapted to host dependent mode [115]. Resistance gene identifier indicate 5 genes associ- ated with antibiotic resistance in *Morganella morganii*. Several antibiotic resistance genes, including as ArnT, DfrA, and sul1, were found in *Morganella morganii*. By decreasing binding affinity to the bacterial outer membrane, lipid A modification—which is facilitated by the ArnT gene—contributes to resistance against polymyxins. The sul1 gene is linked to resistance to sulfonamides via changing the target enzyme dihydropteroate synthase, whereas the DfrA gene encodes dihydrofolate reductase, which confers resistance to trimethoprim. Given that *Morganella morganii* is an opportunistic clinical infection, the existence of these genes suggests strong selective pressure for antibiotic resistance.

*Morganella morganii* uses a variety of antibiotic resistance mechanisms, such as efflux-mediated resistance, antibiotic target modification, and antibiotic inactivation, according to CARD-RGI research. The confluence of several pathways indicates a strong capacity for adaptation, allowing the organism to endure high levels of antibiotic exposure, which are frequently found in hospital settings. The clinical significance of *Morganella morganii* and its ability to acquire and retain resistance determinants are highlighted by these genetic characteristics. *Comamonas testosteroni*, on the other hand, showed a relatively small number of antibiotic resistance genes. Efflux pump-related genes and target protection mechanisms were

among the genes mostly linked to intrinsic resistance found by Resistance Gene Identifier (RGI) analysis. Rather than reflecting the organism's adaptability to clinical circumstances, these genes are mostly associated with resistance against $\beta$-lactams and other broad-spectrum antibiotics.

*Comamonas testosteroni* exhibits two primary resistance mechanisms, according to CARD-RGI results: antibiotic efflux pumps and antibiotic target protection. Compared to *Morganella morganii*, there appears to be less selection pressure for antimicrobial resistance due to the restricted variety of resistance mechanisms. *Comamonas testosteroni*'s classification as an ambient bacterium with sporadic opportunistic pathogenicity is supported by this.*Comamonas testosteroni* showed greater metabolic versatility than *Morganella morganii*, according to core genome analysis utilizing ARTS. Because *Comamonas testosteroni* may thrive in a variety of ecological niches, genes related to fatty acid metabolism, amino acid biosynthesis, and environmental stress responses were more prevalent in this species. On the other hand, *Morganella morganii*'s adaptation to host-associated and opportunistic lifestyles is supported by its comparatively smaller metabolic gene repertoire.*Comamonas testosteroni* contains several biosynthetic gene clusters, including those involved in the synthesis of secondary metabolites, which may enhance environmental competitiveness, according to antiSMASH study. *Morganella morganii*, on the other hand, has fewer biosynthetic gene clusters, suggesting genome streamlining linked to host environment survival. Overall, the genomic and resistance profiles indicate that *Comamonas testosteroni* maintains traits of an ecologically adaptable bacterium, but *Morganella morganii* is more suited to clinical settings.

# Chapter 6

# Conclusion, Limitations and Future Directions

## 6.1 Conclusion

This study highlights the increasing threat posed by *Morganella morganii* in healthcare settings, particularly in chronic wound infections. The whole genome sequencing of the strain isolated from wound infections revealed significant differences in genomic content when compared with *Comamonas testosteroni*. Particularly presence of efflux for antibiotic resistance mechanism contribute to the organism's resilience and resistance to antibiotics. The discovery of antibiotic resistance genes, especially related to efflux pumps like qac(G), underscores the complexity of resistance mechanisms in *Morganella*. Genomic data collected from different bioinformatics tools prove that *Morganella morganii* adopted as opportunistic pathogen. This comparative genomic investigation provides clear evidence that *Morganella morganii* possesses genetic traits associated with virulence, resistance, and adaptation to hospital environments. In contrast, *Comamonas testosteroni* is an environmentally specialized strain that does not exhibit pathogenic characteristics or significant resistance.

## 6.2 Limitations

Using bioinformatics tools like CARD, RAST, and antiSMASH, functional annotations were predicted, including resistance genes and secondary metabolite biosynthetic gene clusters. These predictions, however, were not validated through experimentation, and their real expression under relevant conditions has yet to be confirmed.

## 6.3 Future Directions

Experimental studies, including antimicrobial susceptibility testing, gene knockout/overexpression experiments, and biofilm assays, should be conducted to establish the functional relevance of identified genes. Further, detailed investigation of mobile genetic elements and horizontal gene transfer mechanisms could provide insight into the acquisition of resistance in *Morganella spp.*

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