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Habitat's Impact on Genomic Variations and Virulence

by

Tabassum Shahzad

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I would like to dedicate this thesis to Allah Almighty, my teachers and my parents.

CERTIFICATE OF APPROVAL

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Tabassum Shahzad

(MBS233011)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Waseem Haider	CUI, Islamabad
(b)	Internal Examiner	Dr. Sami Ullah Jan	CUST, Islamabad
(c)	Supervisor	Dr. Arshia Amin Butt	CUST, Islamabad

Dr. Arshia Amin Butt
Thesis Supervisor
September, 2025

Dr. Syeda Marriam Bakhtiar
Head
Dept. of Bioinfo. & Biosciences
September, 2025

Dr. Sahar Fazal
Dean
Faculty of Health & Life Sciences
September, 2025

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Abstract

This study focuses on the comparative genomic analysis between *Myroides odoratimimus K6* and *Nocardioides sp.JS614*. *Myroides odoratimimus K6* is an opportunistic pathogen of increasing concern due to its antibiotic resistance while *Nocardioides sp.JS614* is nonpathogenic bacteria found in soil. This study supports development of novel biotechnological and therapeutic approaches by exploiting habitat driven genomic variations and virulence traits. We identified and characterized resistance genes within the genome of both strains, specifically focusing on their distribution across genomic islands, which are known to be hotspots for horizontal gene transfer. Genome annotation was done by using RAST server. CARD is used for detection of resistant antibiotic genes in both strains. We use antiSMASH for secondary metabolite detection. Circular gnome presenting antibiotic resistance genes and other important gene associated with different functions was generated by CG Viewer. Antibiotic resistance target seeker was used for core genome analysis for both strains. Furthermore, a phylogenetic tree was constructed to understand the evolutionary relationships with other closely related species, providing insights into the origin and spread of resistance genes. The genome of *Myroides odoratimimus K6* consists of 3,619,586 bp with 34.1% GC content, containing 3,300 coding sequences and 76 RNAs. *Nocardioides sp.JS614* genome contain 5,293,685 bp with 71.4% GC content, containing 5,222 coding sequences and 52 RNAs. Functional annotation revealed genes involved in protein synthesis, fatty acid metabolism, carbohydrate processing, and structural regulation. Notably, 27 genes are linked to virulence, disease, and defense in *Myroides odoratimimus K6* and 21 related to environmental stress response. In *Nocardioides sp.JS614* genes for virulence, disease and deference are 47 and stress response genes are 29. Five genes are identified in *Myroide odoratomimus K6* which are related to antibiotic resistance while two genes are identified in *Nocardioides sp.JS614* for antibiotic resistance. *Myroide odoratomimus K6* have three mechanisms for antibiotic resistance most important is antibiotic efflux, in contrast *Nocardioides sp.JS614* have two mechanisms for antibiotic resistance. This comprehensive analysis enhances our understanding of the genetic factors contributing to the resilience

and adaptability of *Myroides odoratomimus K6* which is emerging opportunistic pathogen and show antibiotic resistance.

Keywords: *Myroides odoratimimus K6*, *Nocardioides sp.JS614*, Antibiotic Resistance, Rresistance 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 rapidly evolving issue in the modern world, impacting nearly every hospital worldwide, in both developed and developing nations. They typically arise in nearly every healthcare setting and persist even after a patient is released from the hospital [1]. Bacterial physiology, evolution, and pathogenicity have all been transformed by genomics, the thorough study of an organism's whole DNA content. The research method requires genomic sequencing followed by analytical examinations that focus on gene structure alongside gene functionality and evolutionary patterns and genetic location [2]. Many plasmid encoded pathogenicity factors (genes for antibiotic resistance) are known to be found next to one another, frequently flanked by transposable elements or repetitive sequences. They can spread to other bacteria by bacterial conjugation, transformation, and transduction. Mobile genetic elements contain a disproportionate number of critical virulence determinants, especially poisons and adhesion factors [3].

The ability to transmit to other microbes or, more likely, to nonpathogenic members of the same or closely related species is theoretical possibility because many virulence genes are encoded on "mobile DNA" genetic components. The transmission of antibiotic resistance genes and proliferation of R plasmids serve as clear examples of this type of genetic spread [4]. In the last ten years, a commonality among several different microbial pathogens has been found, which points to a significant evolutionary mechanism for the emergence of harmful bacteria.

The prevalence of virulence genes in big, continuous blocks, such as pathogenicity islands or chromosomal inserts, is surprisingly high [5].The integration of computational and experimental methods leads to identifying a minimal set of genes that maintains a functional cell within its natural state. Genome sequencing stands as a vital element in microbiology by predicting metabolic functions and virulence factor detection and microbial evolutionary understanding [6].

The increasing popularity of genome sequencing techniques has triggered numerous search and annotation approaches yet their combination remains difficult because scientists resolve the information by using arbitrary methods [7]. The bacteria *Myroides odoratimimus K6* establish themselves as environmental organisms that cause infections during vulnerable situations. Immunocompromised patients most often get infected by these microorganisms which result in soft tissue and urinary tract infections. Bacteremia primarily develops due to infections in soft tissues or catheter relationships although death occurs only sporadically. Medical experts consider *Myroides spp.* resistant to fluoroquinolones, piperacillin and trimethoprim, carbapenems alongside tetracyclines [8]. Because *Myroides* species are infamously resistant to a number of medicines, treating infections brought on by this bacterium can be challenging. Reducing the spread of nosocomial illnesses greatly depends on microbiological diagnostics. In the event of a suspected epidemic outbreak, prompt action is made possible by knowledge of the etiological agents of diseases and their antibiotic susceptibility [9]. The opportunistic bacteria from the *Myroides* genus lead to community and hospital acquired infections which prove resistant to treatment because of their antibiotic resistance ability [10].

The *Myroides* species is a newly discovered pathogen that sometimes causes hospital acquired urinary tract infections but is otherwise nonpathogenic. The family Flavobacteriaceae includes the genus *Myroides*, which is a group of oxidase positive, gram-negative, non-motile, non-fermentative bacilli. *Myroides* name is derived from the Greek word "Myron," literally means "perfume," because of its distinct fruity scent. Previously known as *Flavobacterium odoratum. Myroides odoratimimus* and *Myroides odoratus* are the two most frequent species that infect people. *Myroides pelagicus, Myroides profundi*, and *Myroides marinus* are among

the other species that have been found in seawater [11]. The genus *Myroides* contains species that function as obligate aerobic, Gram-negative bacilli with yellow pigmentation and particular smell. Among clinical relevant species exist *M. odoratus* and *M. odoratimimus* with *M. injenensis* and *M. phaeus*. The bacteria occupy a wide range of environments making them common microorganisms while medical experts previously labeled them as weak pathogens. Recent case reports involving *M. odoratus* or *M. odoratimimus* led to skin, urinary and bloodstream infections in immunocompromised patients thus causing hospital environmental concern about dealing with this challenging infection. It has been observed that *Myroides spp.* have genes which are associated with antibiotic resistance, such as beta lactamase production gene (MUS-1, TUS-1, bla-OXA-347, bla-OXA-209), tetracycline resistance gene (tetX), chloramphenicol resistance gene (cat), intracellular surviving gene (katA, clpP, Ef-Tu, and sodB), iron competing gene (DnaK, Hsp60), and biofilm production genes [12]. The global health crisis posed by antimicrobial resistance is enormous, and it constitutes one of the gravest threats to humanity today. Some strains of bacteria have developed resistance to almost all antibiotics. As a result, it is essential to develop new antibacterial agents in order to tackle resistant bacteria. The WHO has released a catalog of antibiotic resistant priority pathogens identified in 2017, these pathogens pose a significant threat to humans and require new antibiotics urgently. The list categorizes them based on the urgency of new antibiotics needed: critical, high, and medium priority, to guide and encourage research and development of new antibiotics. The WHO list comprises mainly Gram negative bacterial pathogens. Gram-negative bacteria are more resistant than their Gram positive counterparts due to their unique structure, and they contribute to considerable global morbidity and mortality. Various strategies have been documented to combat and manage resistant caused by Gram negative bacteria. These include creating antimicrobial auxiliary agents, modifying the structures of current antibiotics, and investigating chemical structures with new mechanisms of action and novel targets that these resistant bacteria respond to. To address the pressing requirement for novel therapies, research endeavors have been undertaken, with some achieving results that demonstrate effectiveness against resistant Gram negative bacteria by neutralizing the resistance mechanism,

such as the effects of β-lactamase inhibitor antibiotic adjuvants [13].

The *Myroides* species are generally non-pathogenic due to their widespread presence in the environment. This supposedly a virulent microbe has, however, been linked to an increasing number of cases in recent years. Diabetes mellitus, the use of catheters, and extended intensive care unit stays are among underlying causes that can make people more susceptible to *Myroides* infections [14]. During the 1670s Dutch microscope inventor Anton Van Leeuwenhoek discovered biofilm for the first time within dental plaque. Scientific research into biofilm characteristics had been minimal before approximately 50 years ago. Electron microscopy revealed after its development that biofilm represents a microbial community of bacteria. Microorganisms that inhabit this exclusive structure display cellular behaviors different from solitary planktonic cells while demonstrating resistance to antibacterial agents at least 500 times greater. The protective multicellular bacterial environment helps bacteria survive prolonged times in various conditions and serves as a defensive mechanism against unfavorable factors. The formation of biofilm serves as standard method for surviving difficult conditions through cooperative growing communities which establish colonies under suitable environments. The intense structure of biofilms arises from its primary sugar components which also decide its virulent potential. The cell density in this community exists at very high levels between 108 and 1011 cells per gram. The development of biofilms serves as the main cause for sustained clinical infections to a degree of 80% and above. The bacteria belonging to several species demonstrate the ability to bind themselves to various inanimate materials together with different living surfaces while creating biofilms. Multiple devices within healthcare facilities serve as attachment surfaces for microorganisms [15].

Recent research has shown that the majority of bacteria that causes persistent infections develop into biofilms, which are sessile populations that because of their form and metabolic quiescence, they are naturally resistant to both the host's immunological response and the effects of many drugs. There are a number of clinical and experimental findings in the literature that suggest microbial biofilms

are present in chronic wounds in situ, which delays the healing process [16]. Bacteria that are resistant to antibiotics are linked to biofilms. In natural microbial communities, horizontal gene transfer, fosters genetic diversity and evolution. The rise of microorganisms that are resistant to drugs has increased the importance of studying gene transfer in natural contexts. The EPS matrix limits the diffusion of substances from the environment into the biofilm and blocks the entry of some antimicrobial drugs. More resistance exists to certain kinds of antibiotics, such as aminoglycosides, which are hydrophilic and positively charged. Numerous bacterial species can develop biofilms when antibiotics are given at concentrations lower than the minimum inhibitory concentration. Significant concern arises from the possibility that cells deep inside the biofilm may be exposed to antibiotics at sub minimal inhibitory concentration levels. The antibiotic may encourage biofilm formation rather than prevent it [17]. By using horizontal gene transfer (HGT), bacteria can acquire genomic islands (GIs), which are sizable chromosomal areas with mobile elements. GIs may be removed and given to other recipients in specific situations. Pathogenicity islands (PAI) and antibiotic resistance islands are two of the numerous subclasses into which GIs can be further subdivided according to the roles of their expressed genes. GIs are thought to have role in variety and evolution of bacteria, as well as in primary and secondary metabolism, pathogenicity, and antibiotic resistance. The PR63039 strain's MY-63039-RR area is the sole GI identified in *M. odoratimimus* and is regarded as an incomplete antibiotic resistance island. However, it is unclear whether the MY-63039-RR region still has the ability to migrate [18]. The gene repertoires of bacterial species are often highly diverse, which is crucial for their adaptation to changing environments, new ecological niches, and coevolving eukaryotic hosts. Most novel genes in bacterial genomes arise through HGT, an extensive evolutionary process that disseminates genes among bacterial lineages that may be very distantly related. It is widely believed that most genes obtained through HGT are neutral or harmful and therefore quickly lost. However, HGT also contributes to the acquisition of many adaptive traits, such as antibiotic resistance in nosocomial infections. Consequently, genome diversification is influenced by the balancing processes of gene acquisition and loss, with some genes undergoing positive selection and many

others experiencing purifying selection [19].

A prevalent and extensively researched characteristic linked to GEIs is antibiotic resistance. Since it casts doubt on the effectiveness of treating infectious diseases. Although it is a man-made threat, the astonishing ability of bacteria to adapt is demonstrated by the transition in bacterial populations from nearly universal susceptibility to rising antibiotic resistance globally in a matter of decades. There is a lack of knowledge on several areas of antibiotic resistance development. One notable feature is the speed with which this resistance has spread around the world [20].

The genus *Nocardioides* comprises Gram positive, aerobic, non-motile bacteria with a high GC content. It belongs to the family Nocardioidaceae and the order Propionibacteriales. Bacteria of this kind are mainly obtained from soil and environmental settings, including activated sludge, freshwater, plant roots, and even extreme environments like deserts and cold ecosystems. *Nocardioides* species, distinguished by their rod to coccoid shape and lack of mycelial structures, are recognized for their metabolic versatility, especially in breaking down complex and xenobiotic compounds like polycyclic aromatic hydrocarbons, herbicides, and industrial waste products. This capability renders them important for ecological processes of detoxifying the environment and bioremediation. Studies of the genome have shown that genes exist for a range of hydrolases and oxygenases that play a role in the degradation of hydrocarbons. Some strains of *Nocardioides* show potential plant growth promoting characteristics by generating indole 3 acetic acid (IAA) or solubilizing phosphate, indicating possible agricultural uses. While not conventionally linked to clinical infections, there have been rare instances of opportunistic pathogenicity, especially among those with weakened immune systems. Genomic comparisons of *Nocardioides* strains with clinical actinobacteria such as Myroides or Nocardia have revealed a lower occurrence of traditional virulence and resistance genes, underscoring their mainly environmental and nonpathogenic lifestyle. Even so, their possible uses in biotechnology, spanning enzyme production and pollution reduction have led to an increasing interest in *Nocardioides* within the fields of environmental microbiology and synthetic biology [21].

In the past ten years, whole genome sequencing (WGS) has emerged as the most widely used technique in labs across the globe. Significant advances in the study and surveillance of outbreaks produced by a wide range of microbial pathogens have resulted from its declining cost as well as its high speed and throughput. WGS offers a "one-stop" approach, offering all the data needed for pathogen type and characterization, including the quicker detection of genes of interest (such as genes for antibiotic resistance, plasmid replicon detection, and sequence typing). This eliminates the need for numerous consecutive, time consuming molecular tests and is accomplished with previously unheard of resolution and cost per sample [22].

1.1 Hypothesis

Different microbial strains obtained from different origins exhibit unique affiliations between antibiotic resistance genes and antibiotic resistance mechanisms.

1.2 Aim and Objectives

The main goal of this research is to analyze variations in virulence islands of two strains from varying habitats.

Objectives

1. To find genes connected to AMR development in pathogenic bacteria from both types of species.
2. To study possible virulence mechanisms of *M. odoratimus K6* while assessing these factors relative to *Nocardioides sp.JS614* virulence features.
3. To investigate whether virulence gene clusters and islands are present in similar forms between these two bacterial species.

Chapter 2

Literature Review

2.1 Nosocomial Infections

Bacteria or other infectious organisms that a patient acquire while in the hospital are known as nosocomial infections. Antibiotics, especially those most commonly used in that hospital context, are typically ineffective against bacteria linked to nosocomial infections. Between 5% and 10% of all hospitalized patients have nosocomial infections, which significantly raise hospital expenses, morbidity, and mortality. Infectious diarrhea, pneumonia, surgical wound infections, UTIs, and bloodstream infections are common nosocomial diseases. The causes of the rise in nosocomial infections in human hospitals are increasingly being identified in the field of veterinary medicine. These include lengthening hospital stays, using invasive equipment more frequently, using antimicrobial medications more frequently, and using critical care procedures more frequently [23]. The term "healthcare associated infections," (figure 2.1) which has recently been coined, refers to the type of infections brought on by extended hospital stays and is a significant risk factor for life threatening illnesses [24]. Developing nations bear around 75% of the burden of these illnesses. If these bacteria are detected in bodily fluids or at a sterile body location, like blood or cerebrospinal fluid, asymptomatic people may be deemed infected. Nosocomial infections can also be those contracted by hospital employees, guests, or other medical professionals. The infections that

were present at the time of admission and grow complicated, but the pathogens or symptoms change, leading to a new infection, are the cases where infections are not thought to be nosocomial. The infections that manifest 48 hours after birth that are contracted transplacentally as a result of certain illnesses, such as toxoplasmosis, rubella, syphilis, or cytomegalovirus [25]. There are 50 nosocomial infection sites, and the National Healthcare Safety Network, in collaboration with the CDC, categorized them into 13 kinds based on biological and clinical criteria. These sites are Meningitis, lung infections, gastroenteritis, surgical and soft tissue infections, and urinary UTIs are the prevalent ones. A shift in nosocomial infections can be identified through time. This is best illustrated by the case of pneumonia, when the prevalence of nosocomial pneumonia rose from 17% to 30% over a five year period [26].

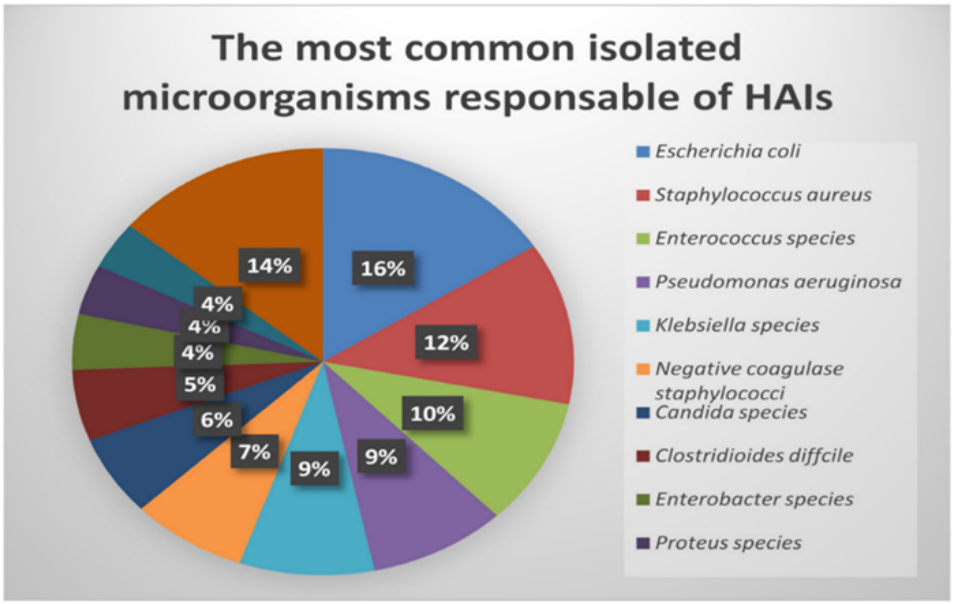


FIGURE 2.1: Most common bacteria associated with hospital acquired infections [27]

2.2 Properties of *Myriodes* Species

The bacterial specie *Myroides spp.* functions as aerobic organisms that appear yellow and resist lactose fermentation with positive oxidase test to show their identification as gram- negative rods that exist throughout the natural environment.

Members of the *Flavobacteriaceae* genus together with *Myroides spp.* naturally inhabit soil environments but also exist in water systems. The number of *Myroides spp.* infections documented in medical journals has grown substantially because the pathogens demonstrate considerable antibiotic resistance toward Beta lactams and aminoglycosides thus interfering with healthcare treated infections [28]. Table 2.1 describe different properties of *M. odoratimimus*.

TABLE 2.1: Description of different properties of *M. odoratimimus* [29]

Property	**Description**
Gram staining	Gram negative
Shape	Road shape
Colony color	Yellow to orange
Motility	Non motile
Pathogenicity	Opportunistic pathogen
Resistance mechanism	Efflux pumps , biofilm production
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	Strictly anaerobe
Temperature range	18 to 37 Celsius
PH tolerance	Neutral to slightly alkaline
GC content	30-35%
Intrinsic resistance	Carbapenems and beta lactams
Enzyme production	MUS-1 and TUS-1
Biochemical reactions	Oxidase positive, catalase positive and non-lactose fermenting

2.3 Infections caused by *M. odoratimimus*

The coronavirus pandemic revealed *M. odoratimimus* as one of the pathogens leading to bloodstream infections from catheters when antibiotics provided no treatment success. This emerging threat proves concerning because *M. odoratimimus* causes healthcare acquired bacterial infections that spread to urinary tracts and skin tissues and infect soft tissue. Research has shown that *M. odoratimimus*

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 [30].

2.3.1 UTIs cause by *Myroides odoratimimus*

Patients with diabetes mellitus, chronic nephritis, urinary calculi, and urinary retention have all been found to develop UTIs caused by *Myroides spp.* [31]. 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 *Myroides* species [32].

2.3.2 Bacteremia cause by *Myroides odoratimimus*

Myroides odoratimimus 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-β-lactamases like MUS-1, it is multidrug resistant, especially to β-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, *Myroides*-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 *Myroides odoratimimus* requires strict adherence to infection control procedures and catheter care guidelines [33]. Table 2.2 Illustrate infections caused by *Myroides odoratimimus*.

TABLE 2.2: Illustration of infections caused by *Myroides odoratimimus* [34].

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	Myroide odoratimimus 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

Molecular microbiology advances have enabled bacteria from clinical samples to start receiving identification typology. Medical reports indicate rising *Myroides* species encounters in both clinical samples and particular urine cultures obtained from patients using urinary catheters.

Myroides species exhibit yellow pigment as they belong to the category of non-fermentative Gram negative bacilli that were historically known as *Flavobacterium* species. Environmental sources contain *Myroides spp.* in particular the species are commonly detected both in soil and water and also exist in seafood production and meat processing. Since the human medical community discovered *Myroides spp.* in the 1920s very few documented human infections have occurred in spite of their wide occurrence in marine and soil habitats.

The literature shows that *Myroides spp.* only infect immunocompromised patients very frequently. Reports exist that document *Myroides spp.* as causes of multiple dangerous health conditions including meningitis, pneumonia, septicemia and urinary tract infections and soft tissue infections in recent medical literature. Patients with kidney failure, liver cirrhosis and lung disease, and prolonged care in intensive care unit experience infections from *Myroides spp.* which function as low grade opportunistic pathogens. Infrequent Myroides species detection occurs from multiple clinical specimens of human infection that include urine together with wounds and blood samples. The media reports *Myroides spp.* infections as urinary tract infections (UTIs) as their primary type. The use of urinary catheters identifies as an essential condition that leads to such infections.

These infections from *Myroides spp.* are uncommon yet show resistance against several antibiotics including carbapenems, beta-lactams and demonstrate different reaction patterns to aminoglycosides, quinolones as well as sulfamethoxazole. The number of multi-drug resistant *Myroides spp.* isolate reports rose at different institution. *Myroides spp.* strains recovered from urinary catheter cultures of ICU patients regarding infection versus colonization during the period from January 2018 to December 2022 [35].

2.5 Cases Reported Related to *Myroides odoratimimus*

Myroids odoratum exists as a rare infection under the previous name *Flavobacterium odoratum* which medical science identified during 1929. Medical literature has documented 104 confirmed cases so far that show various signs such as infections involving the urinary tract, lungs, soft tissues and brain as well as sepsis primarily in patients with impaired immune systems. The paucity of available data along with recorded antibiotic resistance makes it difficult to pick the right antibiotics for treatment.

The subject presented *Myroids odoratum* cellulitis and bacteremia which received successful treatment from dual antibiotic prescription. Her leg displayed indicators of redness and warmth with swelling. Symptoms developed first after a dog scratch before the patient got into contact with swimming pool water. The urgent care clinic observed and initiated Keflex treatment for the patient before emergency room attendance. Her condition worsened with the development of whole body tiredness and weakening in her legs along with an ongoing mild fever. The patient arrived at the emergency department in a stable condition according to their vital signs. The team performing the assessment observed left lower limb edema with warmth and redness that did not have distinct borders. Other parts of her physical examination returned normal results [36]. The bacteria *Myroides odoratimimus* falls under the grouping of extremely rare according to worldwide reports. Reports from around the world class bacterial species into five extremely rare, seven rare, and eight common genera.

The medical field considers *Globicatella, Myroides, Elizabethkingia, Pandoraea* and *Cedecea* among the most infrequently detected bacterial genera. *Gemella* and *Sphingomonas* and *Brevundimonas* and *Sphingobacterium* and *Ralstonia* and *Stenotrophomonas* and *Sarcina* together with the rare genera represented the bacterial species in this case. Within the Flavobacteria class there exist two genera which represent the same rare occurrence group. Six parasitic species were discovered through a thorough research analysis in India. The parasites belonged to

the Cestoda, Chromadorea and Enoplea and Trematoda classes. Global analysis confirmed that *Dipylidium caninum* and *Bertiella studeri* exist as rare parasitic species.

The origin of *D. caninum* and *Bertiella* stems from animals in their natural habitat as reservoir hosts where *D. caninum* lives in breeds of wild canids as well as cats and dogs and felids and *Bertiella* resides in animal reservoir monkeys. The beta-lactam antibiotic and aminoglycoside antibiotic resistance of *Ralstonia pickettii* arises from its possession of two inducible beta-lactamases and aminoglycoside acetyl-transferases.

Experimental evidence shows that *Pandoraea pnomenusa* develops antimicrobial resistance due to its production of biofilms. Different antimicrobial susceptibilities existed between *Gemella morbillorum* and *Gemella haemolysans* although they belonged to the same genera.

The fatality of drug-resistant fungal infections was caused by the combination of amphotericin B, 5-flucytosine and fluconazole in one patient whereas the other case lacked any recorded drug connection.

Two studies explored MDR tuberculosis and antibiotic-resistant pulmonary infiltration from rare fungal co infections in immunocompromised patients [37] .

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.

The study demands increased research scope for antibiotic resistance which must encompass human subjects as well as animal and environmental factors [38]. Figure 2.2 give an overview of carbapenem resistance.

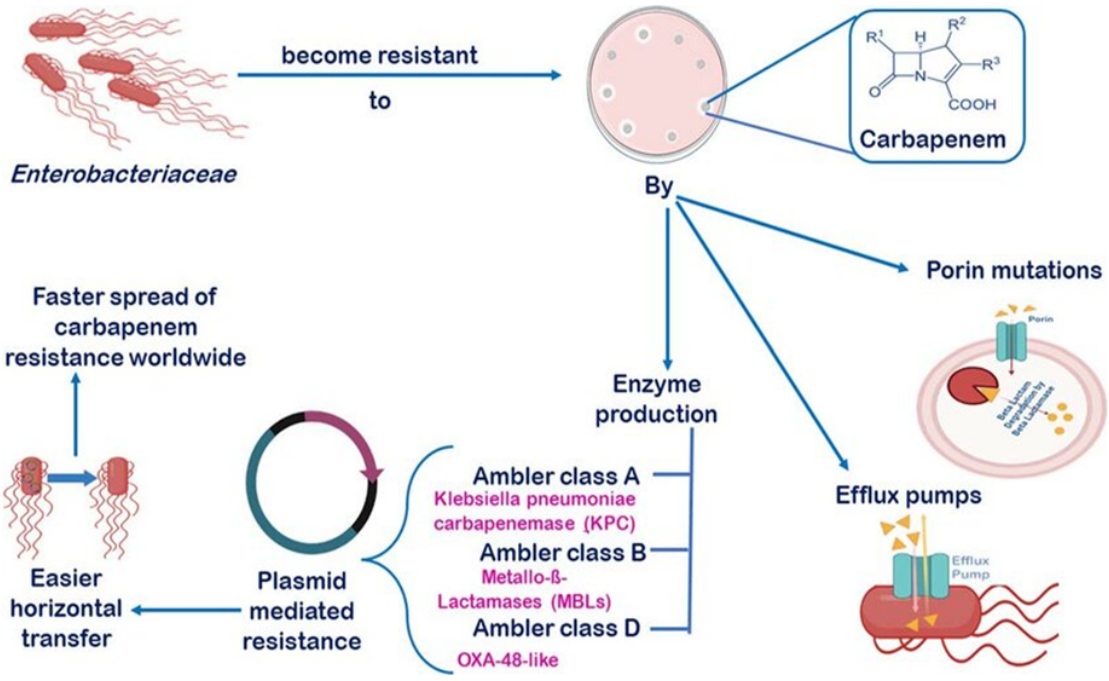


FIGURE 2.2: An overview of carbapenem resistance [39].

2.7 Data about *Myroides* Genus in NCBI

The intestinal bacteria *Myroides* originated in feces from gastrointestinal infected patients during 1923 and today scientists classify the genus *Myroides* in Bacteroidota phylum as members of Flavobacteriia class in the Flavobacteriales order and within the Flavobacteriaceae family. NCBI Taxonomy Browser includes 13 *Myroides* species. Most important are *Myroides indicus*, *Myroides marinus*, *Myroides odoratimimus*, and *Myroides odoratus*, Vancanneyt and coworkers established *Myroides* as a fresh bacterial genus. As the genus name *Myroides* originated from the Greek mýron which means perfume and the Latin oides which symbolizes something resembling or similar to perfume *Myroides* acquires its name because of the genus' smell that resembles fragrance. The bacterium is aerobic yet strict and shows positive results for oxidase and catalase activities together with these Gram-negative rod-shaped properties: 0.5 × 1–2 μm in size with non-motility and

gliding abilities. *Myroides* shows its growth capability through MacConkey agar and produces non-hemolysis while it survives in temperature ranges spanning from 18– 22^{o}C and 37^{o}C. Yellow or orange circular colonies usually form from *Myroides*. Among clinical reports *Myroides odoratimimus* takes the first position followed by *M. odoratus* and behind them are *M. injenensis* and *M. phaeus*. Pathogens belonging to *M. injenensis* and *M. phaeus* continue to cause endocarditis in combination with pericarditis and urinary tract infections and skin and soft tissue infections and ventriculitis and liver abscesses and bacteremia in either immunocompetent and immunocompromised patient. Several resistant mechanisms relate to this bacterial species. The increasing amount of data requires additional species classifications for different forms of infections yet microbiological and genetic information about *Myroides spp.*, infections remains insufficient which results in poor documentation of infection roles. This study aimed to explain both microbiological traits and genetic features of seven clinical *Myroides spp.* strains together with evaluating their phylum classification and pathogenicity and resistance properties [40].

2.8 *Myroides odoratimimus* as Challenging Infectious Agent

Myroides genus consists of environmental bacteria from Flavobacteriaceae which exist universally throughout the environment. *Myroides odoratus* stands as the main clinically important species among *M. odoratimimus, M. injenensis. M. phaeus* and *M. odoratus*. *Myroides* related infections used to be classified as less threatening but they can now prove fatal to immunocompromised patients with multiple health issues. These pathogenic agents establish themselves in all locations and they appear in both hospital and community patient populations and they infect numerous sites in their host. Healthcare professionals need to recognize this pathogen since automated medical technology detection methods have become more widespread. Recent medical reports prove this infection exhibits native drug resistance in addition to developing acquired resistance qualities which creates one of the toughest infections for medical professionals to treat. Healthcare

professionals need special attention for *M. odoratimimus* infections in intensive care units due to higher risks of pathogen detection and drug resistance. The study reports five cases of extensively drug-resistant M. odoratimimus infection that appeared at a tertiary care center [41].

2.9 *Myroides odoratimimus* Resistance

The *Myroides* genus comprises aerobic yellow-colored bacteria that are immobile gram- negative non-fermenters which were previously identified as *Flavobacterium odoratum*. There are two notable species within this grouping; the first one is *Myroides odoratus* and the second one is *Myroides odoratimimus*.

Genetic members of *Myroides* occur naturally in environmental settings particularly water ecosystems. The environment hosts these microbes abundantly yet they generally do not infect human patients. Immunocompromised patients who are severely diseased develop urinary tract infections and endocarditis and ventriculitis and cutaneous infections by *Myroides spp.*

Treatment of these microorganisms is difficult because they show resistance to many antibiotics. A total of 32 cases were documented within the literature review performed in 2010.Medical reports of *Myroides spp.* infections have recorded a rising number during recent time periods. We report about the nosocomial urinary tract infection outbreak of *Myroides spp.* that developed in tertiary intensive care units while discussing this fourth outbreak based on the available literature. *Myroides spp.* exist primarily as environmental microorganisms which fail to inhabit normal human body flora yet they represent pathways for opportunistic infections when conditions are favorable. Severely suppressed patients educated by medical experts may develop opportunistic *Myroides spp.* infections.

The literature reveals *Myroidess spp.* outbreaks are very uncommon among patients with normal immune systems and doctors have reported only three such outbreaks. The fourth outbreak report exists in the medical record. Doctors reported the first outbreak where patients developed stones in their urinary system

with four patients having neoplasms in their urinary tracts. Six of seven patients who experienced *M.odrathimimus* urinary tract infection in the second outbreak presented with urinary stones while one additional subject had bladder cancer. The only exception was one case while all others experienced long term hospitalization combined with endourological intervention history.

The article by Licker *et al.* showed that every patient experienced *M.odoratimimus* UTI because they needed a permanent urinary catheter. The research team identified that all their cases spent time in long term hospitalization until their recovery except for one patient. We believe patients with extended urinary catheterization face a severe risk because of illnesses developed from this bacterium. The immune system suffers damage from diabetes mellitus which causes infection development through opportunistic pathogens while making the patient especially vulnerable to these pathogens. The existence of diabetes mellitus stands as a notable risk element for *Myroides spp.* UTI infections.

The medical literature contains only small documentation regarding *Myroides spp.* UTI occurrences in diabetic patients. Seven reported cases of *Myroides spp.* infections are available in literature to date. The long-standing and poorly controlled diabetes in all of our patients created significant conditions that promoted these infections. LABS such as VITEK 2 Compact and MALDI-TOF make it simple to identify non fermenting gram- negative bacilli (NFGNB) at their species level in current laboratory testing. NFGNB demonstrates growing frequency as a bacteriuria pathogen when these identification systems are used.

Patients received identification through our implemented MALDI-TOF device. Studies show *Myroides spp.* demonstrates multi-drug resistance properties and displays pan- resistance in specific infections. Research has not yet fully defined the antibiotic resistance mechanism that occurs in *Myroides spp. Myroides spp.* infections need appropriate treatment because these bacteria can create severe hospital-acquired infections and their broad drug resistance ability. *M.odoratus* and *M. odoratimimus* demonstrate intrinsic B lactam immunity stemming from chromosomal B lactamase proteins MUS-1 and TUS-1. Multiple strains show resistance to beta-lactams together with monobactams and carbapenems [42].

2.10 Antibiotic Resistance Status of Clinical *Myroides spp.* Infections

Myroides spp. infections are rare. Only limited research material could be retrieved when using the PubMed with either "Myroides" or "*Flavobacterium odoratum*" as the search terms. Research shows *Myroides spp.* primary infection occurrences are infrequent in immunocompetent subjects with *M. odoratimimus* cellulitis being reported after pig bite in a healthy child. Human immunity impairment due to various conditions enables secondary infections to occur commonly after catheterization and among patients with cancer or diabetes mellitus and also affects newborns. Surface tissue infections together with necrotizing fasciitis, ventriculitis and urinary tract infections are treated *Myroides spp.* infections. Residents of the hospital experienced an outbreak of urinary tract infections because of *M. odoratimimus*. Primary healthcare structures have encountered treatment failure and patient deaths because *Myroides spp.* demonstrate resistance to numerous antibiotics.

A July 2009 patient experienced *M. odoratimimus* urinary tract infection after injury requiring antibiotic sensitivity testing which demonstrated resistance to thirteen antibiotics including ampicillin and amoxicillin and their combination drug clavulanate as well as amikacin. Aztreonam and chloramphenicol and the antibiotic cephalosporin and imipenem and gentamycin also failed to work alongside levofloxacin and meropenem and sulfamethoxazole and tetracycline and ciprofloxacin and tazobactam (Figure 2.3). The patient received multiple antibiotics including cefazolin oxime, amikacin, tetracycline but still did not recover from the infection. Research found *Myroides spp.* antibiotic resistance to change across different types of bacterial strains obtained from different origins. The research showed norfloxacin sensitivity in strains originating from liver hydatid cyst patients yet this property did not exist in strains from pulmonary infection patients. Among the isolated strains from cellulitis patients and those from patients with a leg amputation the bacteria displayed ciprofloxacin resistance while strains isolated from

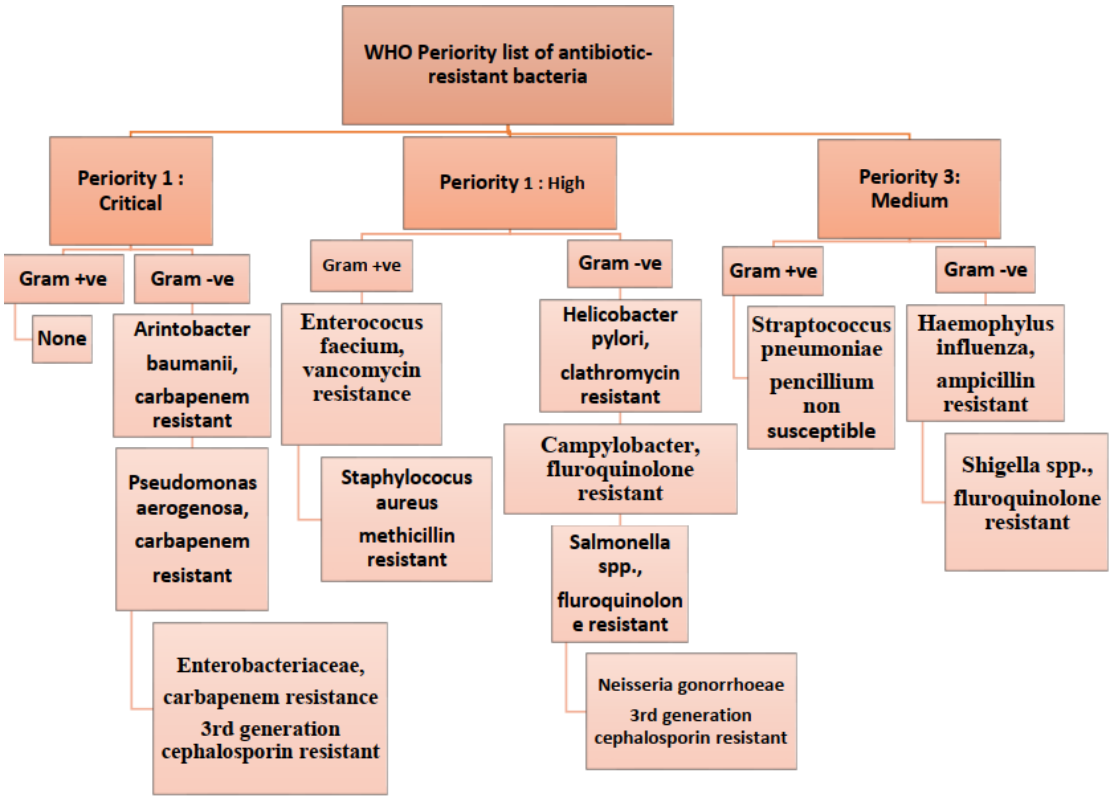


FIGURE 2.3: WHO priority list of antibiotic resistance bacteria [13].

trauma cases and septicemia patients showed sensitivity to ciprofloxacin treatment [43].

Myriodes odoratimimus is extensive antibiotic resistance. Following table represent list of antibiotics along with their minimal inhibitory concentration against which *Myroides odoratimimus* show resistance.

TABLE 2.3: Antibiotic resistance by *Myriodes odoratimimus* [44].

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

Table 2.3 continued from previous page

Antibiotic	MIC (micro g/ml)	Interpretation
Tobramycin	16	Resistant
Ciprofloxacin	4	Resistant
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 β-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 [45].

2.11 Antibiotic Resistance Mechanism of *Myroides odoratomimus*

Myroides spp. antibiotic resistance mechanisms have not been reported in China. Despite the fact that a number of international scholars have studied this subject, not much is known about it. The diverse patterns of resistance to β-lactam antibiotics and reduced sensitivity to carbapenems of several *Myroides spp.* strains were caused by the β-lactamase gene. The development of the chromosome encoded β-lactamases TUS-1 and MUS-1 in *M. odoratus* and *M. odoratimimus* was cited as the cause of resistance to β-lactams in a study examining several clinical cases involving systemic infections. Resistance to β-lactam antibiotics is largely attributed to the β-lactamases. According to research, Flavobacteriaceae species' inherent resistance to β-lactams can only be partially explained by the β-lactamases TUS-1 and MUS-1 [30].

2.12 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 [46].

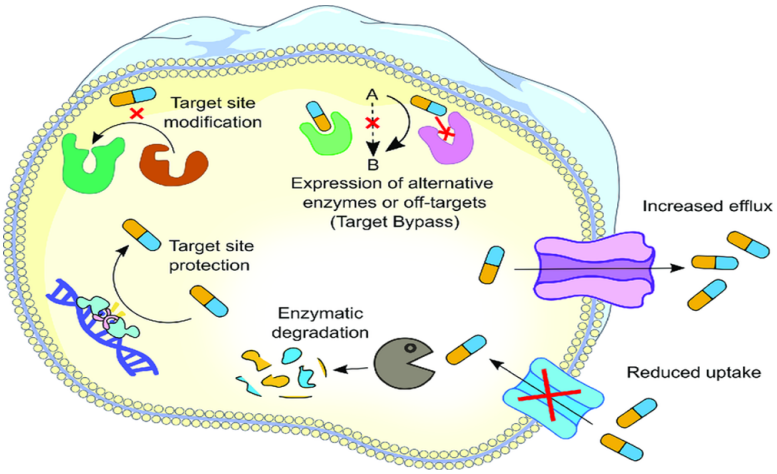


FIGURE 2.4: Schematic diagram of six different mechanisms in bacteria for antibiotic resistance [46].

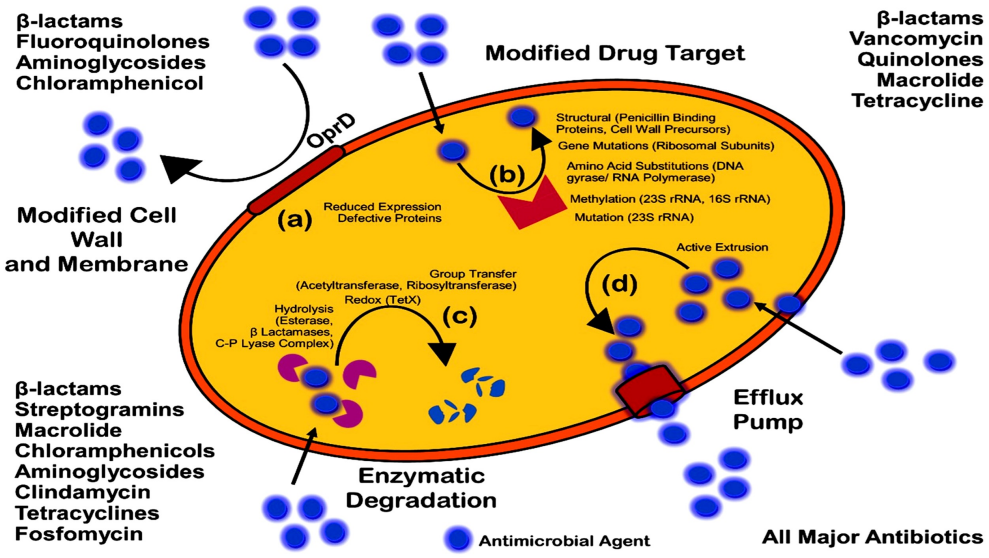


FIGURE 2.5: Schematic diagram of different mechanisms in bacteria for antibiotic resistance [47].

2.13 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 [48]. Figure 2.6 illustrate different steps of biofilm formation.

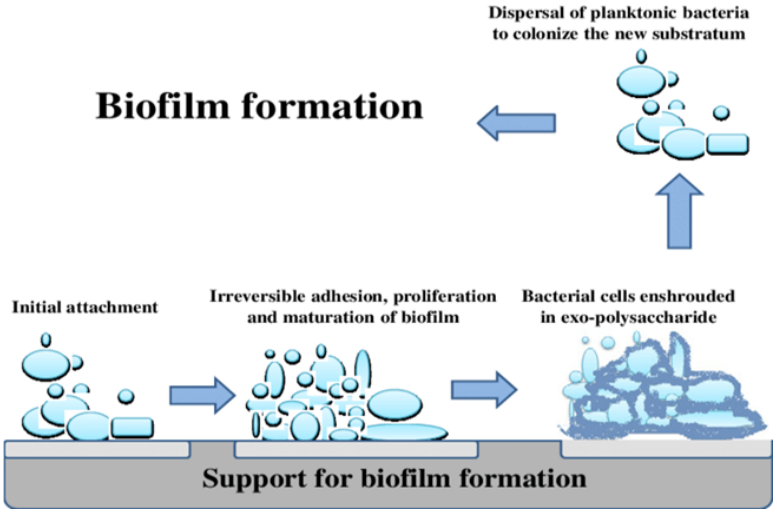


FIGURE 2.6: Steps of biofilm formation [48].

Strong adhesion patterns are shown by *Myroides* species, which prefer to adhere at lower temperatures [49]. The ability of *Myroides odoratomumus* to produce biofilms by auto aggregation and coaggregation may account for their widespread prevalence and capacity to infect people with impaired immune system [50]. 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 [51]. 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 [52]. The formation of biofilm by *Myroides* species can pose a serious risk to health and frequently results in recurring infections [53].

2.14 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 [55].

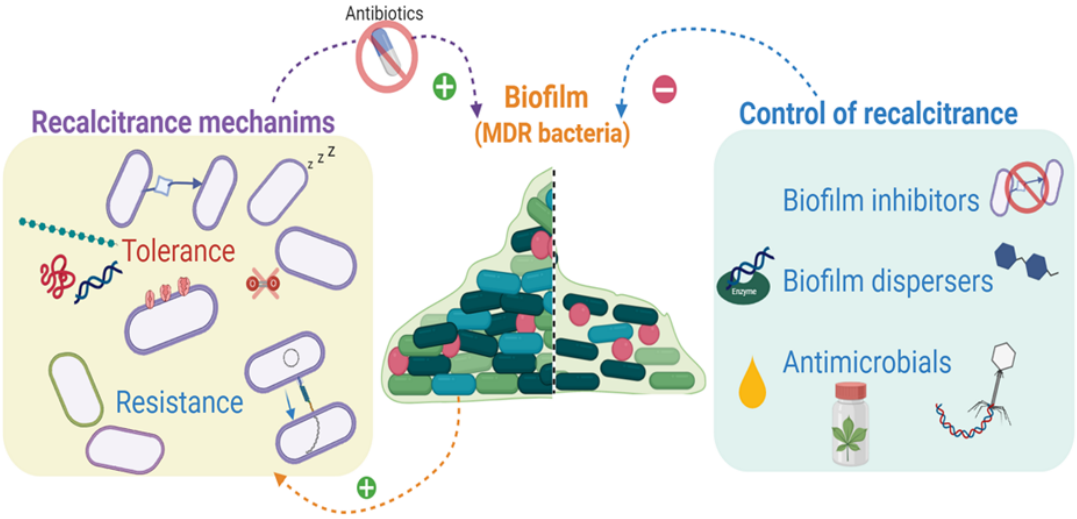


FIGURE 2.7: Schematic diagram of biofilm and its role in antibiotic resistance [54].

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 [56]. Tolerance, in contrast to resistance, is only transitory [57].

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 [58].

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 [59].

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 [48].

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) [60].

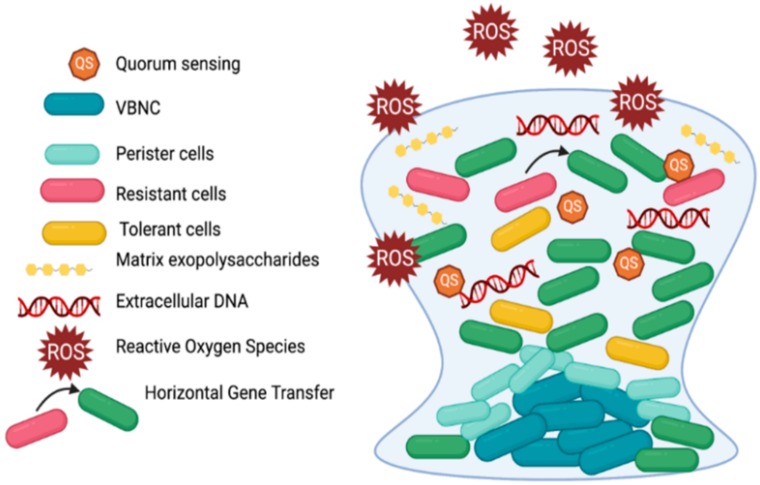


FIGURE 2.8: Role of biofilm in antibiotic resistance [60].

2.15 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 [61]. 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 [62]. 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 [63]. 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 [64]. Figure 2.9 give a general concept of Biosynthetic gene cluster in *E.coli* CFT 073.

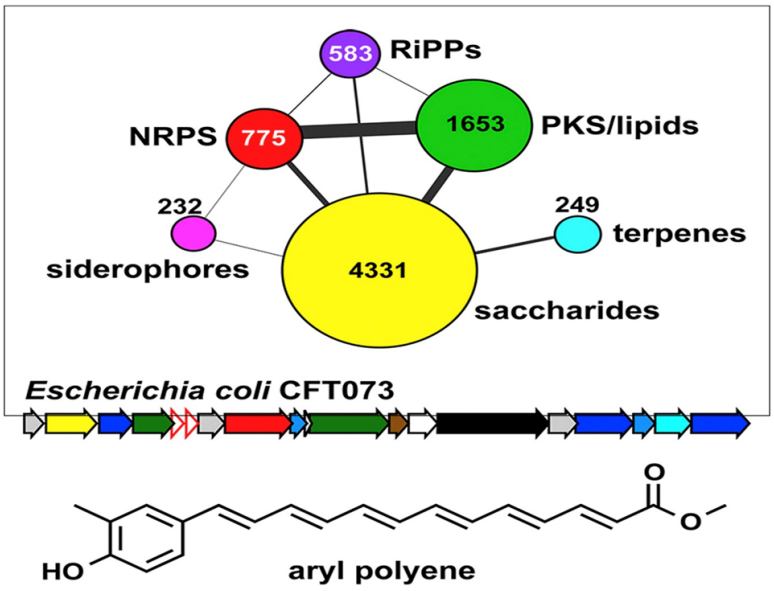


FIGURE 2.9: Biosynthetic gene cluster in *E.coli* CFT 073 [64].

2.16 Resistance Mechanisms for Bacteria

MUS-1 and TUS-1, two metallo-β-lactamases that share 73% of their amino acid similarity, are the cause of intrinsic resistance to β-lactamases [63]. 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 [65]. 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 [66]. 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 [67].

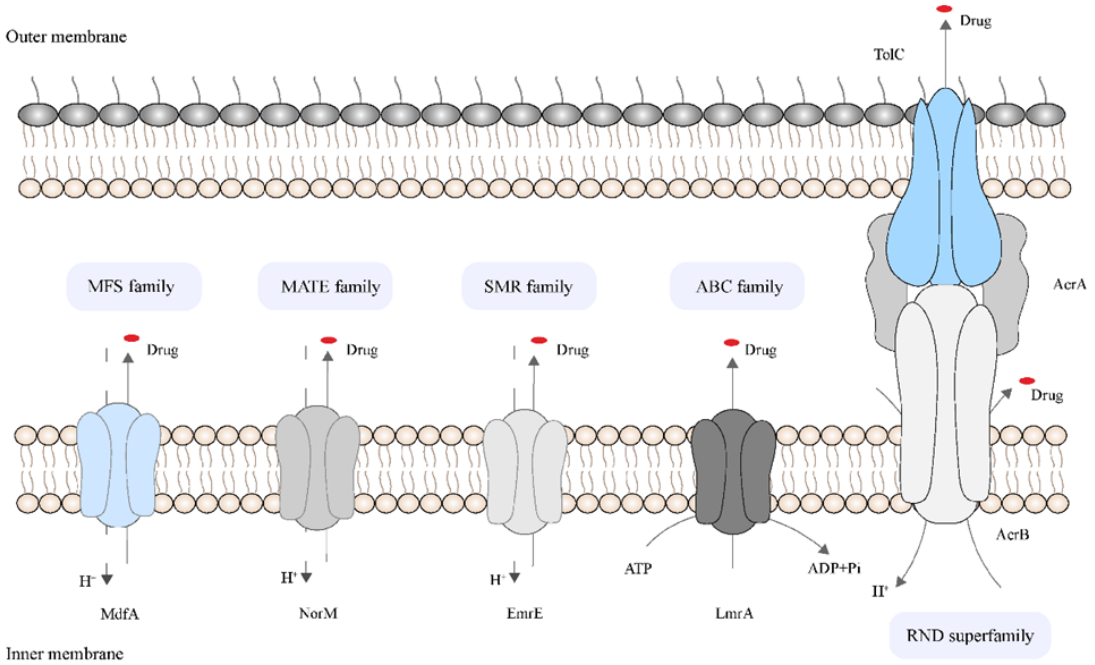


FIGURE 2.10: 5 super families of efflux pumps [68].

An often employed tactic to make antibiotics ineffective is antibiotic modification, particularly when it comes to aminoglycoside antibiotics (kanamycin, streptomycin and gentamycin), β-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 [69]. 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 [70].

2.17 Classes of Efflux Pumps

2.17.1 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) [71]. 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 [72]. 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 [80].

In the RND protein, twelve transmembrane segments (TMSs) are predicted [73]. 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 [74]. 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 [83]. 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 [74].

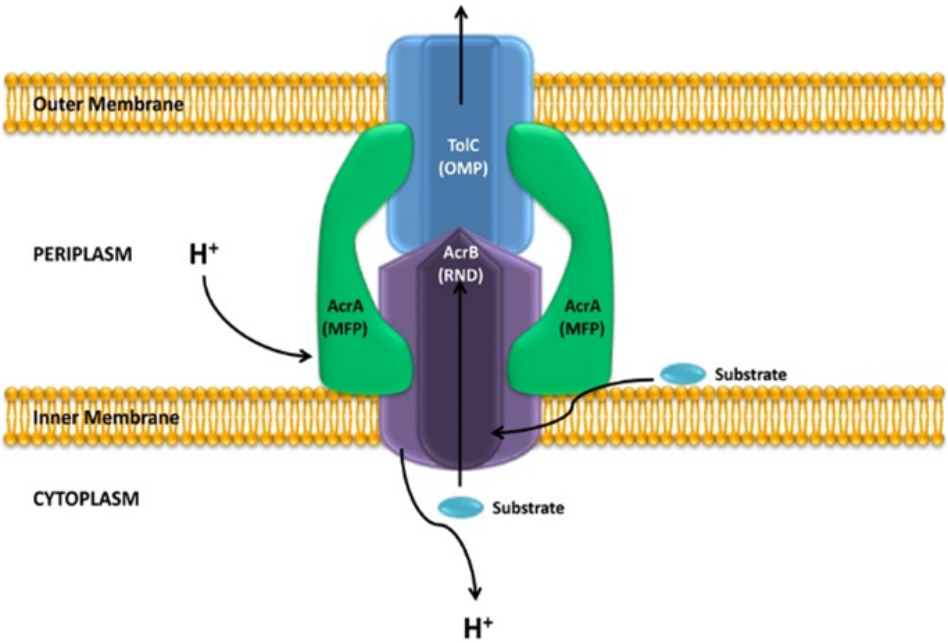


FIGURE 2.11: Representative diagram of RND efflux pump [75].

2.17.2 ABC Family Efflux Pum

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 [76]. 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 [76].

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 [76] .

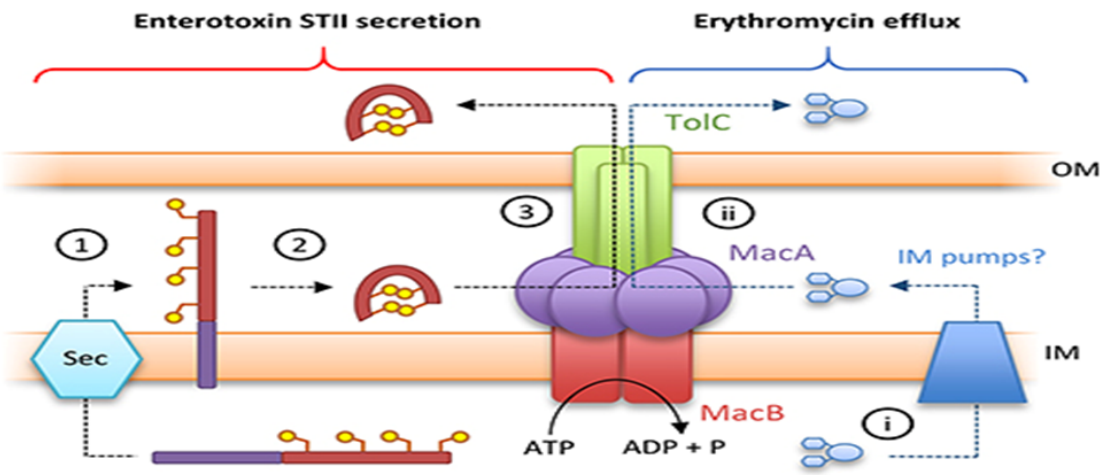


FIGURE 2.12: Antibiotic resistance mediated by ABC efflux pump [77].

2.18 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.

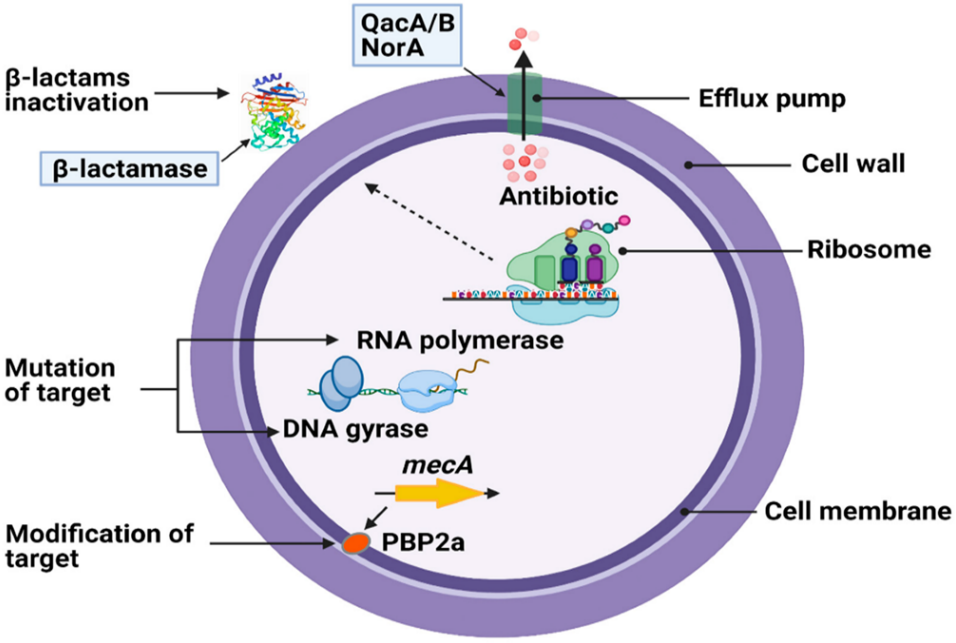


FIGURE 2.13: Schematic diagram for modification of target [78].

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 β-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 β-lactams, quinolones, and other medications to enter the bacteria and cause acquired drug resistance [79].

Certain antibiotic targets are protected by bacterial synthetic protein, which removes the bacteriostatic effects of a combination of antibiotics [80]. 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 [80]. 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 [81].

Cell wall-deficient bacteria, including Mycoplasma and related species, are therefore inherently resistant to all medications that target the cell wall [82]. The bacterial cell contains several parts that antimicrobial drugs could target. One way that gram-positive bacteria withstand the β-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) [83]. 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 [84]. 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 [98]. 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 [85]. 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 folate 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 [86].

Bacteria can inactivate medications in two major ways: either by physically breaking down the drug or by adding a chemical group to it. The β-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 [87]. The class of antibacterial compounds known as β-lactams is the most commonly utilized. All of the drugs in this group have a four-sided β-lactam ring as their common core structure. The three main ways that resistance to β-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 β-lactamase [88].

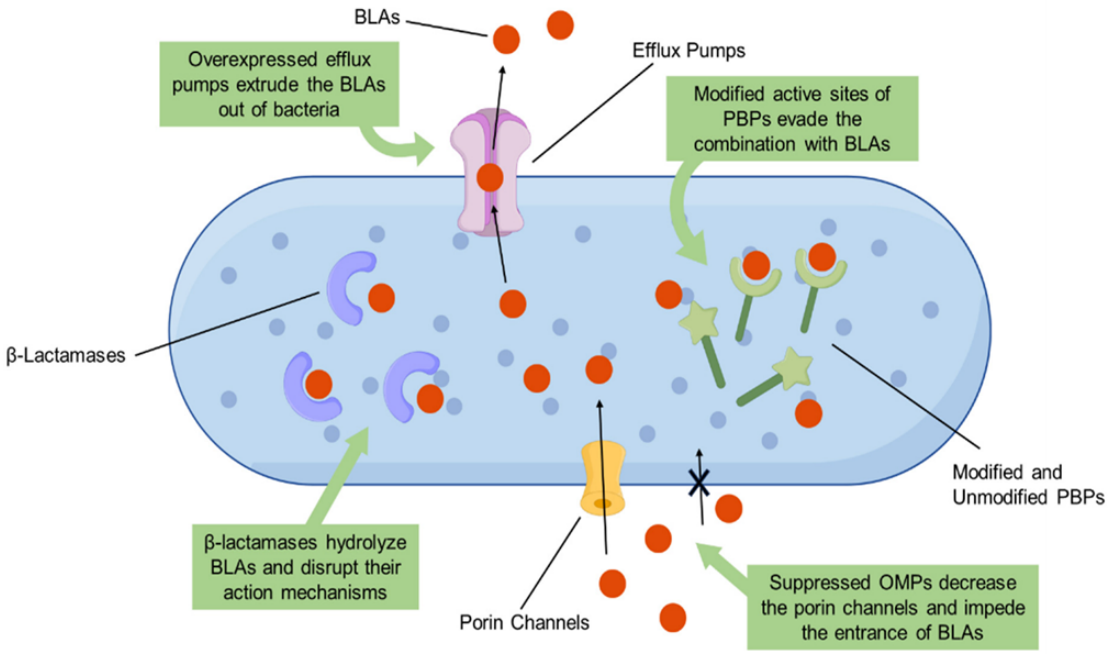


FIGURE 2.14: Beta lactamases and antibiotic resistance [89].

2.19 Virulence Factor of *Myroides odoratomimus*

Myroides odoratimimus has been discovered to possess not only common virulence factors, such as the bauE gene to acquire iron, which competes with the host,

and adherence factors, such as DNAK and Hsp60, but also the ability to survive intracellularly (katA, clpP, EF-Tu, and sodB), even in the human stomach (ureA, ureB, and ureG), spread readily, and degrade human tissues [29].

2.20 Mobile Genetic Elements (MGEs)

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

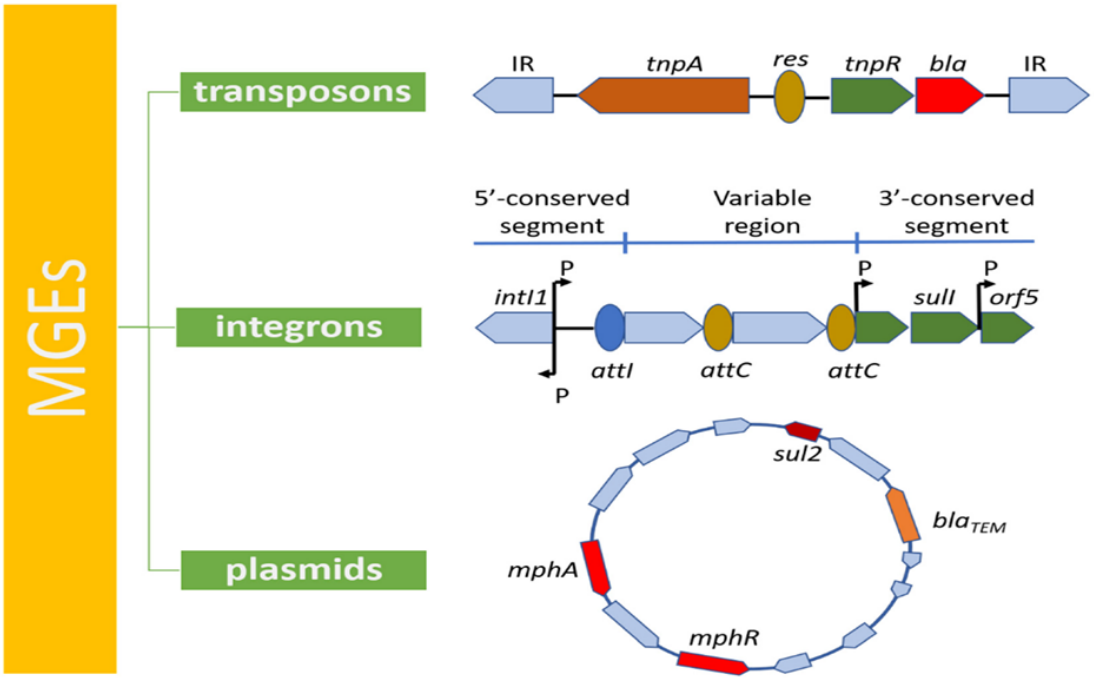


FIGURE 2.15: Mobile Genetic Elements (MGEs): transposons, integrons and plamid in *E. coli* [91].

2.21 Horizontal Gene Transfer (HGT)

A transferred gene typically needs to offer a selection benefit to the receiver or to itself in order to persist in the recipient lineage for extended periods of time. Initially, HGT research concentrated on these genes. In both prokaryotic and eukaryotic organisms, it is now understood that a large number of genes that have been found to be passed down by comparative genomics between close relatives have neutral or virtually neutral effects on the receiver [92]. "First, do no harm" appears to be one criterion for transferred genes. Successfully incorporated genes frequently express themselves at modest levels and encode peripheral metabolic functions [93].

However, these neutral acquisitions may eventually yield new genetic combinations that selection can work upon; in certain situations, the transferred material eventually becomes domesticated and results in a desirable phenotype. In other situations, the imported genes are likely to be lost over time if they stay neutral and there is no clear advantage to keeping them. It has long been acknowledged that

HGT had a significant role in the evolution of bacteria and archaea. Nonetheless, the genetic information sharing between eukaryotic hosts and prokaryotic symbionts, as well as between eukaryotes, indicates that HGT in eukaryotes happens more frequently than previously believed [94]. In order for genetic material to be transferred during conjugation, a donor cell must physically touch a recipient cell through a conjugation pilus. Bacterial cells are the only cells that can conjugate as the donor and the recipient [94].

It has been documented that both bacteria and archaea undergo transformation, which is the acquisition of exogenous DNA from the environment. Both bacteria and archaea have been shown to exhibit transduction. More recently, additional gene transfer pathways have been identified, including cell fusion and gene transfer agents (GTAs). GTAs are chromosome-integrated gene delivery systems that are occasionally regulated by the host.

GTAs transport little, arbitrary fragments of the host genome to neighboring hosts in capsids. Both bacteria and archaea have GTAs. Since the GTA does not preferentially transfer the GTA-encoding genes, neither the benefit to the host, which transfers its DNA to others, nor the benefit to the GTA-encoding genes are immediately apparent. It's still unclear how these genes continue to be selected for their functions [95]. A common characteristic of the classical processes of HGT, such as natural transformation and conjugation, is that membrane-associated protein complexes help push or pull ssDNA into the cell. Other treatment targets should be taken into consideration in the fight against ARGs, as recent research have discovered alternative forms of DNA transfer that are not dependent on the traditional DNA absorption or conjugation machinery. Limiting the transfer of ARGs may reduce MDR in bacteria by targeting conserved proteins involved in DNA protection or transport [96]. However, the finding of non-classical HGT mechanisms implies that regulating the spread of ARGs is more difficult than previously believed [97].

Transformation denotes the capability of microorganisms to employ snippets of free DNA from their environment. Foreign DNA from dead cells is cut into pieces and exits the cell. Competent cells can then pick up the free-floating DNA cellules.

Exogenous DNA is absorbed by the recipient cell from its environment via the cell membrane. The external DNA is integrated into the chromosome of the host cell through re-association. Transformations yield the genetic modification of the target cell.

The process of transduction allows bacteriophages, which are viruses that target bacteria, to convey genetic material between different organisms. As viruses, they inject their genetic material into a bacterial cell and replicate extensively within it. Concurrently, certain phage genes remain in the bacterial chromosome. When the cell finally bursts, it releases a significantly larger quantity of bacteriophages into the environment to infect other microorganisms.

Bacterial conjugation (Figure 2.16) refers to the transfer of genetic material between bacterial cells through direct contact or via a bridge-like structure connecting the two cells. It is a mechanism of horizontal gene transfer, like transformation and transduction, though the latter two do not require direct cell contact [96].

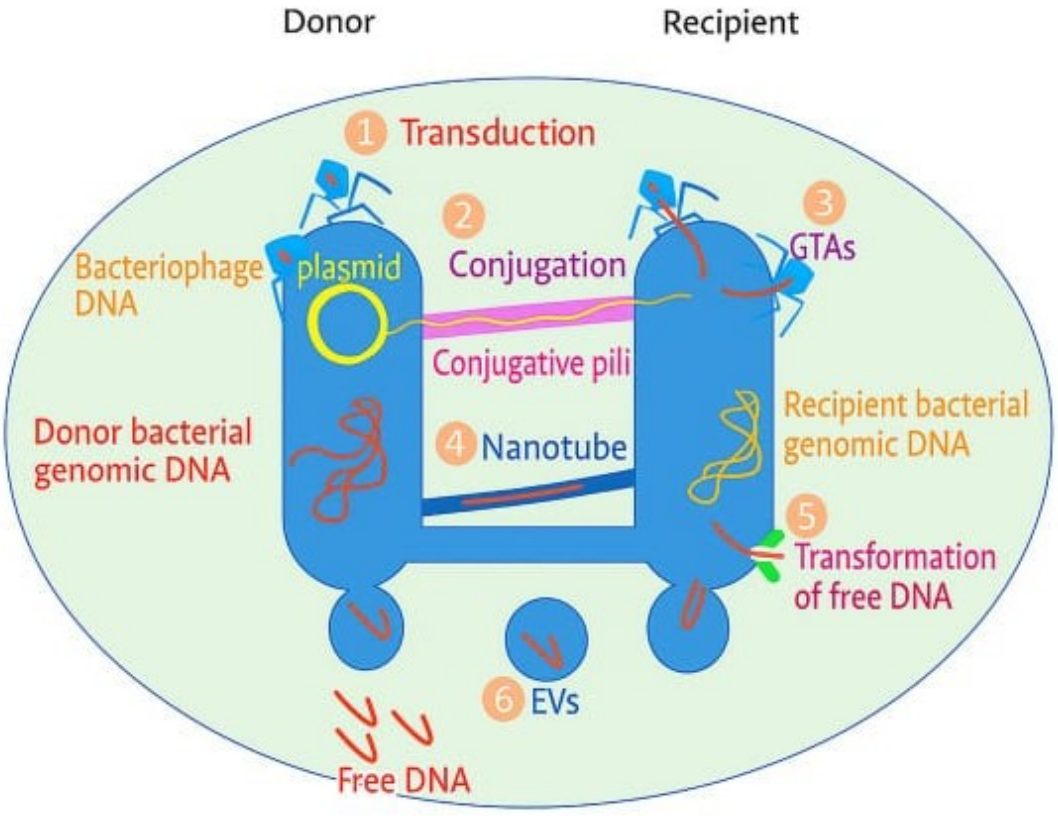


FIGURE 2.16: Methods of HGT in Bactria [98].

2.22 *Nocardioides sp. JS 614*

Soil contains aerobic, nonmotile, Gram-positive rods of bacteria belonging to the genus *Nocardioides* (phylum Actinobacteria). Certain strains of *Nocardioides* are capable of breaking down uncommon substrates such as butane, jet fuel, phenanthrene, and p-nitrophenol, vinyl chloride (VC), ethene, trinitrophenol, and atrazine. The JS614 strain of *Nocardioides sp..* thrives on ethene and VC and could be helpful for bioremediation, or cleaning up VC-contaminated locations. VC's carcinogenicity and enduring status as a contaminant make this of special interest. Strain JS614's high activity on VC and unique physiology set it apart from other VC degraders.

In addition to growing on propene and butene, fluoroethene, and nicotine. strain JS614 has been suggested as a biocatalyst for the chiral epoxide synthesis process [99].

Nocardioides sp.. JS614 is a soil-isolated actinobacterium with a high GC content (71.4%) that is remarkable at breaking down environmental contaminants, especially ethene and vinyl chloride (VC).

It is an aerobic, gram-positive, non-motile creature that has evolved to live in nutrient-poor terrestrial habitats. Its genome shows that it contains genes that encode epoxy alkane, coenzyme M transferase and alkene monooxygenase, which enable it to oxidize harmful halogenated chemicals.

In contrast to clinical bacteria, JS614 exhibits ecological specialization rather than host connection by lacking common virulence proteins and genes for antibiotic resistance.

It is a model organism for research on bioremediation because of its metabolic adaptability and absence of harmful characteristics. Comparing ambient bacteria to opportunistic infections such as *Myroides odoratimimus*, these characteristics demonstrate how different their evolutionary histories are The sequences for *Nocardioides JS614* have been added to GenBank with accession numbers CP000509 and CP000508 [100].

2.23 Properties of *Nocardioides sp. JS 614*

Nocardioides sp.JS 614 a soil dwelling bacteria show diverse properties such as nonpathogenic nature, absence of biofilm making ability and noninfectious nature. Along with these properties some important properties related to *Nocardioides sp.JS 614* in Table 2.4.

TABLE 2.4: Description of different properties of *Nocardioides sp.. JS 614* [99].

Colony color	**Description**
Gram staining	Gram positive
Shape	Road shape
Colony color	White to creamy
Motility	Non motile
Pathogenicity	Non pathogenic
Resistance mechanism	Minimal intrinsic resistance
Biofilm making ability	Does not form biofilm
Habitat	Soil
Antibiotic resistance	Mostly susceptible
Infections	Not reported
Oxygen requirement	Strictly anaerobe
Temperature range	28 to 30 Celsius
PH tolerance	Neutral to slightly alkaline
GC content	71.40%
Biochemical properties	Catalase positive, oxidase positive

2.24 Genomic Features of *M. odoratimimus* and *Nocardioides sp.. JS614*

Comparative genomics provides a strong framework for analyzing how two ecologically different bacteria, the Gram-positive environmental degrader *Nocardioides sp. JS614* and the Gram negative opportunistic pathogen *Myroides odoratimimus* have developed different genomic architectures that reflect their different lifestyles. Because both strains' genomes are fully sequenced (∼4.0–4.3 Mbp), functional comparison and direct alignment are made possible. However, a notable variation

in GC content is apparent: JS614 has a high GC level (∼71%), a characteristic of Actinobacteria that reflects long-term adaptation to soil conditions, whereas *M. odoratimimus* maintains a relatively low GC content (∼34%).

Both encode roughly 3,800–4,100 protein-coding sequences, but their functional repertoires differ significantly. This difference also applies to gene composition. Multiple antibiotic resistance genes, such as efflux transporters and chromosomal β-lactamases (MUS-1, TUS-1), are present in *M. odoratimimus*, highlighting its multidrug-resistant character in clinical settings.

In contrast, JS614 does not have these resistance characteristics, which is indicative of its environmentally specific and non-pathogenic nature. Though biosynthetic gene clusters (BGCs) are present in both genomes, the projected products indicate adaptations driven by niches. *M. odoratimimus* encodes terpene clusters, siderophores, and non-ribosomal peptide synthetases (NRPS), which may enhance virulence and survival in host conditions.

In contrast, the BGCs of JS614 appear to be focused on environmental processes such oxidative stress control and pyridine metabolism. Although both species generate secondary metabolites, comparative antiSMASH research confirms that their genetic configurations show adaptability to either infection or biodegradation niches. Their adaptive mechanisms are further characterized by mobile genetic components.

The abundance of insertion sequences, plasmids, and prophage regions found in *M. odoratimimus* suggests that the organism has great genomic flexibility, which facilitates the acquisition of resistance genes as compared to *Nocardioides sp. JS 614*. The fixed chromosomal structure and lack of horizontal gene transfer associated with virulence are consistent with *Nocardioides sp.JS614* reduced mobile elements. A further significant distinction is the existence of genomic islands linked to pathogenicity.

The genetic foundation of *M. odoratimimus*'s nosocomial infection, now called hospital acquired infection (HAIs) potential is provided by the genomic areas it

encodes that are rich in adhesions, secretion system genes, and biofilm-related proteins. Since JS614 lacks these attributes, it reiterates its exclusively eco-friendly way of living [12]. Table 2.5 explain Genomic features of environmental and clinical bacteria.

TABLE 2.5: Genomic features of environmental and clinical bacteria [101].

Genomic Feature	***Myroides odoratimimus***	***Nocardioides***
Biofilm associated genes	Abundant	Not reported
Mobile genetic elements	Plasmid, prophage and insertion sequences are common	Limited mobile genetic elements are reported
Secondary metabolite function	Contribute to virulence or survival of host	Reported as environmental adaptations
Genome size	4-4.2 Mbp	4.3 Mbp as reported
GC count	34-35%	71.40%
Virulence genes	Present mostly for biofilm	Not reported
Gene count	3700-4000 protein coding genes	4100 protein coding genes reported in literature

Chapter 3

Material and Methods

3.1 Bacterial Isolation

A total of 50 swab samples were taken from the patients from the ward of the orthopaedic and general surgical ward in Benazir Bhutto (tertiary care) hospital which is located in Rawalpindi. The swabs contain the transportation material for all types of microorganisms. All swabs were stored in an icebox and transported to the university laboratory. The swab taken from the patients were categorised based on the organ of infection as some (need number) from the foot, upper leg (thy), lower legs, Cather and chest to know the infection and diversity of bacteria. The samples contain duplicate swab one set of sample send for metagenomics study and other one duplicate were used in laboratory for further purposes.

3.2 DNA Extraction from Bacterial Wound Culture Sample

The DNA extraction was performed by a well renowned technique which ls CTAB by using DNeasy PowerSoil kit (Qiagen). The quality of extracted DNA checked by using Nano Drop 2000 Spectrophotometer (ThermoFisher Scientific). Moreover, to validate the quantitative estimation, 50 ng of extracted DNA along with lambda

DNA/HindIII Marker (catalog number SM0102; ThermoFisher Scientific) loaded onto a 1% agarose gel stained with ultrapure ethidium bromide (ThermoFisher Scientific). The gel was then exposed to electrophoresis by running it at 80 V for 1 hour. Next, the gel was visualized using a SmartView Pro 1100 Imager System (Major Science) [102].

3.3 DNA Library Preparation and Sequencing

The DNA size containing one hundred nanogram was enzymatically fragment into 250- bp by using Covaris targeting. The fragment then repairs to change the overhang into the blunt end. The adenylated fragment the loose adapter is ligated cut with a uracil-specific removal reagent (USER) enzyme.

AMPure beads were used to further purify the samples. DNA was higher by PCR with six cycles using NEBNext Ultra II Q5 master mix, sample-specific octamer primers and Illumina universal primer. The unused primers were cleaned by AM pure from amplified product. The subsequent DNA libraries were eluted in 15 mL of 0.1-TE buffer. Next, quantification of the concentrations was performed using the Qubit DNA High Sensitivity (HS) assay kit and a Qubit Fluorometer.

For microbiome configuration and contig based ARG analyses these clean reads were assembled using MEGAHIT v1.2.9. The contigs shorter than 200 bp were removed from further analysis.

3.4 Genomic Sequencing and Analysis

The genome of bacteria was sequenced by using whole-genome shotgun via commercial service. The sequencing was performed with illumine Hiseq 2000 platform (2 x 150-bp reads) and DNA library were prepared with covaris 220.The Rast server oniline were used for genomic data analysis. The quality of data was evaluated by using FastQC. It is freely available bioinformatics tool which is used to provide all information related to raw genomic sequencing by quality control

metrics [103].The average sequence length of both reads were 104bp and the poor quality control sequenced were excluded.

3.5 Protocol for DNA Extraction

Prior to being removed and centrifuged for 30 seconds at 4^{o}C, the cotton swab was first resuspended in 1 milliliter of PBS and vortexed for 10 seconds. After thoroughly mixing 10g of crystalline lysozyme into the cell solution, the samples were incubated at 37^{o}C for 10 to 60 minutes.

Thirty milliliters of SDS (10-20%) and six microliters of Proteinase K (10 mg/ml) were then added, and the mixture was incubated at 37 ^{o}C. three until viscous and clear. 100 μl of NaCI (5 M) was then added, and the suspension was incubated at 65 degrees Celsius for two minutes. Next, 80μl of a preheated CTAB/NaCI solution was added, mixed well, and incubated at 65 ^{o}C for 10 minutes.

Following the addition of an equivalent volume (about 800 μl) of chloroform/isoamyl alcohol (24:1) solution, 10,000 g was centrifuged for five minutes. The top (aqueous) phase containing the nucleic acids was moved to another Eppendorf tube. After adding isoamyl alcohol (25:24:1), phenol, and chloroform to the aqueous layer, the mixture was centrifuged at 15,000 g for five minutes.

Following the transfer of the upper (aqueous) phase to a separate Eppendorf tube, an equivalent volume (about 800 μl) of chloroform: isoamyl alcohol (24:1) solution was added.The samples were then centrifuged at 10,000 g for five minutes.

After moving the aqueous phase into a fresh Eppendorf tube, approximately 560 μl of isopropanol was added to precipitate the nucleic acids. The tube was then left to stand at room temperature for five to an hour. then a 12,000-15,000g centrifugation stage at ambient temperature for 15-30 minutes. To avoid disturbing the pellet, the isopropanol was gradually eliminated.

Washing was done using 500 μl of 70% EtOH, 12,000-15,000 g, and centrifuged for 15-30 minutes at room temperature. After removing the etOH, the pellet was

dried and reconstituted in 40-60 μl of water devoid of nuclease. In order for the DNA to thoroughly resuspend, let it rest at 37^{o}C [103].

3.6 Agarose Gel Electrophoresis

A 1% agarose gel was used for gel electrophoresis, which was made by dissolving one gram of agarose in one hundred milliliters of 1X TAE buffer (Tris Acetic Acid EDTA). After the agarose was completely dissolved by heating the mixture, a clear solution was produced. Ethidium bromide (7 μl) was added to the gel solution in order to see the DNA. After that, combs were placed into a gel casting tray to form wells, and the gel mixture was poured into it. After the gel had set, the gel caster was carefully placed to a gel tank that had been filled with 1X TAE buffer. The combs were then carefully removed to reveal the wells.

2 μl of extracted DNA and 2 μl of 6X bromophenol blue dye (loading dye) were combined to prepare the sample and make tracking easier during electrophoresis. The agarose gel's wells were filled with the DNA samples and a 1KB ladder for size reference. For 35 minutes, the electrophoresis was carried out with a current of 500 mA and a voltage of 75 volts. Following the run, a Bio Doc Analyzer was used to observe the gel under a UV trans-illuminator. To ascertain the size of the DNA fragments, representative DNA bands from the resultant gel picture were compared to the 1KB ladder.

3.7 Approaches for the Analysis of Shotgun Whole Metagenomics Data

Following paired-end shotgun sequencing of the entire metagenome, each sample had two files of sequenced data: one for the forward strand and one for the reverse strand. Assembly-based and alignment-based methods are the two main methods used in metagenomics data analysis.

In this investigation, both methods were used. To create feature tables, the reads-based approaches—also referred to as alignment-based methods align clean readings to carefully selected databases. Utilizing the FASTQC tool, the data was quality checked. FastQC offers a simple way to perform quality control tests on high-throughput sequencing pipelines' raw sequence data.

Prior to conducting additional research, it provides a modular collection of analyses to provide a rapid sense of any possible issues with the data. The output was an HTML report with details on several quality control factors, which helped to detect problems that needed to be fixed to guarantee the data was suitable for further analysis. The input files were in the FASTQ format.

Genomes or significant genomic regions can be reconstructed from metagenomic data using the assembly-based method, which first assembles sequencing reads into larger contiguous sequences (contigs). One can use a variety of tools, including Meta Velvet, MEGAHIT, and SPAdes.

The processes are as follows: preprocessing (filtering contaminants and low-quality reads), assembly (building contigs from the filtered reads using assembly software), annotation (finding genes and functional elements in the assembled contigs), and binning (putting contigs into bins that correspond to distinct genomes or genome fragments).

The alignment-based method uses reference databases to align clean readings in order to detect and measure the existence of functioning genes and known organisms.

This approach uses carefully selected databases like KEGG, NCBI RefSeq, or specially created databases that are pertinent to the research. The processes include preprocessing (raw reads are trimmed and quality filtered), alignment (reads are mapped to reference databases using tools like Bowtie2 or BWA), feature extraction (feature tables are created that quantify the abundance of different taxa or functional genes), and statistical analysis (statistical tests and visualization are performed to interpret the feature tables).

3.8 Removal of Host DNA

Following matching with the human genome assembly, human reads are eliminated since they are regarded as contamination in the bacterial diversity estimation (GRCh38 assembly). The accuracy of the estimations of bacterial diversity is increased by this step, which guarantees that only microbial sequences are examined. With alignment programs like Bowtie2 or BWA, the raw reads are usually aligned to the human genome. The only reads left for additional analysis are those that do not match the human genome.

3.9 Alignment of the Reads to Database

The readings were given taxonomic information using Kraken2 and a curated database. For high accuracy and quick classification times, Kraken2, the most recent iteration of the Kraken taxonomy classification system, uses exact k-mer matching. This classifier finds the lowest common ancestor (LCA) of all genomes that include the specified k-mer for each k-mer in a query sequence.

To provide accurate taxonomy identification, the classification method is informed by the k-mer assignments. For read alignment and taxonomy information assignment, a pre-built database in Kraken2 called the Mini Kraken database was utilized. This database, which includes sequences from viruses, bacteria, and archaea, offers a thorough reference for read classification.

3.10 Assembly of Metagenome

MEGAHIT, an assembler that can efficiently handle big and complicated metagenomics data, particularly on a single-node server (current maximum memory capacity 768 GB for a 2-socket server), was used to complete the assembly after quality checking. For this analysis, k-mer lengths of 29, 39, 59, 79, 99, 119, and 141 were employed within the range of k-mer values that MEGAHIT supports.

FASTQ was the format of the input files. Multiple parameter presets are available in MEGAHIT that can be customized to meet specific needs, such heightened sensitivity or the assembly of intricate and sizable metagenomes. To improve the assembly process, each parameter can also be set separately. Contigs in FASTA format constituted the assembly process's output and were utilized for further analysis.

3.11 Abundance Estimation Bracken

A highly effective statistical technique for calculating species abundance in DNA sequences from a metagenomics sample is called Bracken (Bayesian Re estimate of Abundance with KrakEN). Bracken uses the taxonomy labels that Kraken, an accurate metagenomics classification tool, assigns to determine how many reads from each species are in a sample.

However, Kraken does not estimate species abundances; instead, it classifies readings to the best matching place in the taxonomic tree. In order to determine the likelihood that sequences from one genome are identical to those from other genomes in the database, Bracken uses the Kraken database. Abundance at other taxonomic levels, such as species and genus, is estimated using this data in conjunction with the taxonomic assignments for a specific sample. Even in samples with two or more virtually identical species, Bracken generates precise species- and genus-level abundance estimates by integrating with the Kraken classifier.

3.12 Complete Taxonomic Hierarchy Generation

3.12.1 Kraken-biom

The Bracken software accepts one or more files from the Kraken-Report utility as input. Every file is parsed to capture the counts for every OTU, as well as the lineage and database ID (e.g., NCBI). A BIOM (Biological Observation Matrix)

table containing the extracted data is then used to store the data. Each count is associated with the sample and OTU to which it belongs. To make it easier to import abundance and taxonomic hierarchy data into R for additional microbiome data exploration and analysis, the BIOM format was created. In downstream analysis like diversity evaluations, differential abundance testing, and microbial community visualization, this consistent format guarantees that the data may be effectively used.

3.12.2 Phyloseq

A comprehensive tool for importing, storing, analyzing, and visually representing complicated sequencing data that has been grouped into Operational Taxonomic Units (OTUs) is the phyloseq program. By storing all relevant sequencing data as a single experiment-level object t using a specific set of S4 classes, it simplifies data sharing and reproducibility.

Phyloseq makes it easier to utilize R for effective, interactive, and repeatable analysis of high-throughput sequencing data that is OTU-clustered. To facilitate smooth data integration, the software offers tools for diversity analysis, differential abundance testing, and other statistical studies. It also arranges data in a structured style for convenient access and manipulation. Phyloseq also has tools for making plots of publication quality, including phylogenetic trees, bar plots, heatmaps, and ordination plots.

3.13 Whole Metagenome Processing

3.13.1 Data Processing and Annotation

By clustering contigs according to sequence composition and coverage patterns, a technique known as binning (https://github.com/SEEDtk/.pl) was used to recover the Myroides odoratimimus genome from a metagenomic dataset. Following binning, the genome was refined using MAGpurify (https://github.com/MAGpurify),

a program made to detect and eliminate any contamination in metagenome-assembled genomes (MAGs) in order to enhance their quality. Contigs that matched other strains or the human genome were eliminated. To make sure the genome was accurate and complete, a number of filtration techniques were used, including as GC-content analysis, tetranucleotide frequency assessment, and phylogenetic marker analysis.

After contamination was eliminated, the strain was verified using the TYGS server (https://tygs.dsmz.de/). To identify the nearest type strain genomes, two complimentary methods were applied. The cleaned genome was first compared with all type strain genomes in the TYGS database using the MASH technique, which is a quick way to estimate intergenomic relatedness [104]. Ten type strains were chosen for each user genome based on their shortest MASH distances. Second, using 16S rRNA gene sequences, eleven more closely related type strains were identified. These sequences were taken out of the user genomes using RNAmmer [104]. and then compared to the 16S rRNA gene sequences of the 22,513 type strains in the TYGS database using BLAST [105].

Using the 'coverage' algorithm and distance formula d5 [106]. The Genome BLAST Distance Phylogeny (GBDP) approach was then utilized to determine exact distances between the 50 best-matching type strains (based on bit score). These distances were used to determine the ten closest type strain genomes for each user genome. In order to perform phylogenomic inference, GBDP was used for all pairwise comparisons between the chosen genomes. The 'trimming' procedure and distance formula d5 were used to calculate intergenic distances. One hundred distance replicates were created in all. GGDC 4.0's suggested settings were used to estimate digital DNA-DNA hybridization (dDDH) values and confidence intervals.

Utilizing subtree pruning and regrafting (SPR) postprocessing and FASTME 2.1.6 [107]. A balanced minimal evolution tree was built utilizing the inferred intergenomic distances. Through the use of 100 pseudo-bootstrap replicates, branch support was identified. PhyD3 was used to visualize the trees, which had midpoint roots [108]. Using standard procedures, species clustering was carried out around

each of the 14 type strains using a 70% dDDH threshold [109]. As previously mentioned, subspecies clustering was carried out with a 79% dDDH threshold. In order to produce a high-quality annotated genome for further analysis, the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://github.com/ncbi/pgap) was used to annotate the cleaned Myroides genome. The pipeline systematically predicts genes, functional elements, and other genomic features. Using proksee.ca, CRISPR, mobile genetic elements, and resistance gene identities were predicted and displayed on a circos plot.

3.14 Functional Analysis

In order to annotate the wound metagenome sample assembly file, PROKKA v1.14.5 was used [110].Providing comprehensive details about the properties and activities of genes, PROKKA is a powerful tool for quickly annotating bacterial genomes. Potential virulence factors found in the wound samples were subsequently identified by analyzing the annotated sequences in the Virulence Factors Database (VFDB). The presence of antibiotic resistance genes (ARGs) was verified using a variety of databases. Among these were Resfinder, a program created especially for identifying ARGs in metagenomic data, and the Comprehensive Antibiotic Resistance Database (CARD), which provides an extensive and current resource for identifying ARGs.

3.14.1 ARGs Identification

The metagenomic data was analyzed to identify antibiotic resistant genes (ARGs) using the Comprehensive Antibiotic Resistance Database (CARD). ARGs can be found using CARD, an integrated and current resource that compiles data from multiple sources into a comprehensive database.

In this investigation, the FASTA-formatted assembled contigs served as the input for the ARG prediction. Identifying possible ARGs entails matching the assembled sequences to the CARD database.

This stage is essential for figuring out how common and varied antibiotic resistance is among the microbial communities found in the samples [104].

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 [105].

3.14.3 Secondary Metabolite Identification by antiSMASH

For identification of secondary metabolite in both strains that is *Myroides odoratimimus K6* and *Nocardioides sp.JS614*, online web server antiSMASH (antismash.secondarymetabolite,org) was used.

Clean FASTA file of *Myroides odoratimimus K6* was upload while for *Nocardioides sp.JS614*, NCBI accession number CP 000509.1 was entered. Input format that antiSMASH accept are FASTA, gbk files, embl files and accession number [106].

3.14.4 Core Genome Analysis by ARTS

Antibiotic resistance target seeker (artz.ziemertlab.com) was use for core genome analysis of *Myroides odoratimimus K6* and *Nocardioides sp.JS614*. gbk file for both strais were uploaded (one by one separately).

ARTS also accept NCBI accessiom number but preferred files are gbk [105].

3.14.5 Mobile Genetic Elements Identification

For mobile genetic elements identification online Island Viewer (islandviewer.org) was used.FASTA file for *Myroides odoratimimus K6* was uploaded while in case of *Nocardioides sp.JS614* gbk file was uploaded. Output file was a circular diagram representing major categories of different genes having different functions [103].

Chapter 4

Results

4.1 Results of RASTserver

RAST server findings indicate major difference in genomic characteristics of *Myroides odoratimimus K6* and *Nocardioides spp. JS614*, (GC content and number of coding sequences). Table 4.1 explain RAST results.

Table 4.1: Comparative Genomic Characteristics of *Myroides odoratimimus K6* and *Nocardioides sp. JS614* based on RAST server

Genome	***Wound Source Strain***	***Soil Source Strain***
Taxonomy	*Myroides odoratimimus*	*Nocardioides sp*
Size	3,619,586	5,293,685
GC Content	34.1	71.4
N50	118278	4985871
L50	10	1
Number of Contigs (with PEGs)	75	2
Number of Subsystems	230	300
Number of Coding Sequences	3300	5222
Number of RNAs	76	52

4.2 Whole Genome Testing of *Myroides odoratimimus K6* and *Nocardioides sp. JS614*

Genomic assembly of this genome show that *Myroides odoratimimus K6* consists of 3,619,586 bp genome with 34.1% GC content and contain 3300 coding sequencing with 76 tRNA. The genome of *Myroides odoratimimus K6* by RAST functional annotation showed that 7 genes for nitrogen metabolism, 89 for protein, 63 for carbohydrates,28 for fatty acid, lipid and isoprenoids,12 for phosphorus,5 for sulfur metabolism and 4 for potassium. The structural regulation gene observed to be 54 for membrane transport, 25 for cell wall and capsule. Moreover 21 for environmental stress response and 27 for virulence, disease and defense. Additionally, *Nocardioides sp.* JS614 consisted of 5,293,685 bp genome with 71.4% GC content and contains 5222 coding sequences with 52 tRNAs. RAST annotation showed that it had 21 genes for nitrogen metabolism 148 genes for proteins, 324 genes for carbohydrates, 152 genes for fatty acid, lipid and isoprenoids, 24 for phosphorus, 3 potassium and 29 genes for sulfur metabolism. Genes involved in structural regulation included 12 for cell wall and capsule, 5 for motility and chemotaxis and 58 for membrane transport. Furthermore, total genes for virulence, disease and defense were 47 and 29 for environmental stress response. All results are explained in Table 4.2.

TABLE 4.2: Comparative Genomic of *Myroides odoratimimus K6* and *Nocardioides sp. JS614* based on RAST server

Function	***Number of Genes in Myroides odoratimimus K6***	***Number of Genes in Nocardioides sp. JS614***
Nucleosides and Nucleotides	73	111
Cell Division and Cell Cycle	0	0
Protein Metabolism	89	148
Sulfur Metabolism	5	29
Secondary Metabolism	7	0
Amino Acids and Derivatives	161	355
DNA Metabolism	38	96
Iron acquisition and metabolism	11	1

Table 4.2 continued from previous page

Function	***Number of Genes in Myroides odoratimimus K6***	***Number of Genes in Nocardioides sp. JS614***
Nitrogen Metabolism	7	21
Phosphorus Metabolism	12	24
Miscellaneous	14	37
Potassium metabolism	4	3
Fatty Acids, Lipids, and Isoprenoids	28	152
Phages, Prophages, Transposable elements, Plasmids	10	0
Cell Wall and Capsule	25	12
Regulation and Cell signaling	5	20
Respiration	54	112
Stress Response	21	29
Metabolism of Aromatic Compounds	7	55
Cofactors, Vitamins, Prosthetic Groups, Pigments	138	198
RNA Metabolism	31	36
Virulence, Disease and Defense	27	47
Motility and Chemotaxis	0	5
Carbohydrates	63	324

4.3 Genomic Islands and Resistance/Virulence Gene based on Island Viewer

Resistance genes annotation was performed to visualize the resistance genes in *Myroides odoratimimus K6*. 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 *Myroides odoratimimus K6* contained 5 which are aadS, tet(X), qac(G), vanT, and MUS-1.

Nocardioides sp. JS614 have two resistance genes which are HeIR and vanW gene according to card results (Table 4.5).

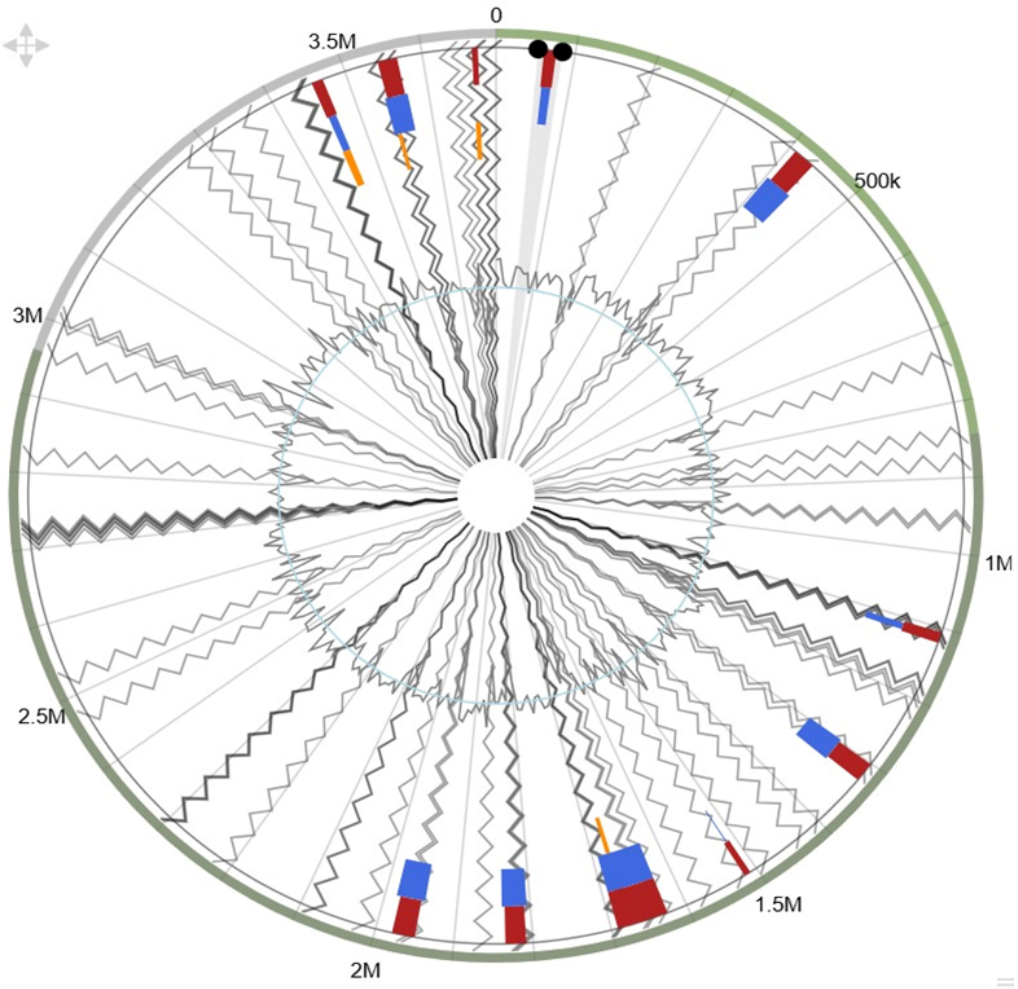


FIGURE 4.1: Color scheme of island viewer Circular diagram showing resistance genomic island (red for integrated prediction, orange for SIGI-HMM and blue for island path) for *Myroides odoratimimus K6*

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

Segment	**Description**
Red color	Predicted genomic islands (high degree certainty)
Blue color	Also predict genomic island (medium confidence)
Green color	Indicate mobile gene
Wavy lines	Indicate atypical regions
Labelled coordinates	Indicate positions along circular genome
Inner concentric rings	Comparative alignment with reference genome

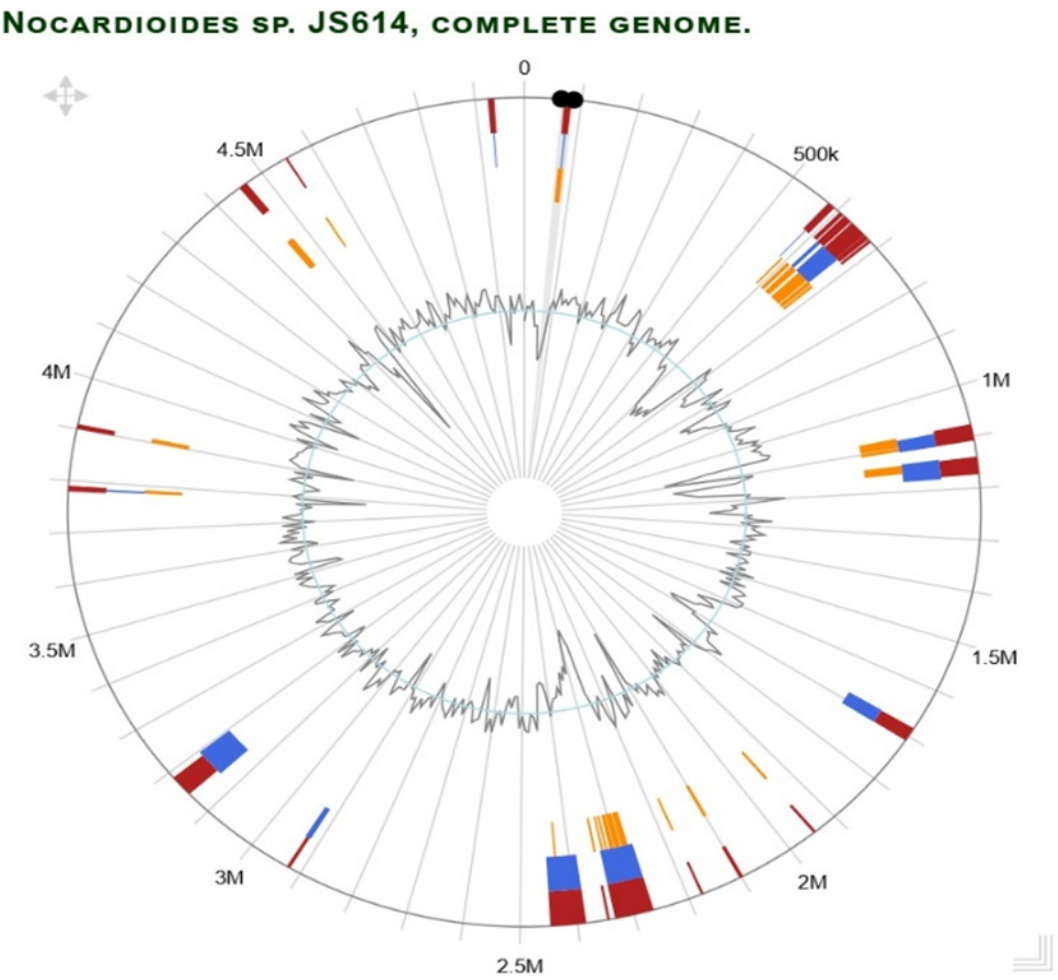


FIGURE 4.2: Genomic island of *Nocardioides sp.JS614*

TABLE 4.4: Resistance genes annotated on CARD for *Myroides odoratimimus K6*

ARO Term	**RGI Criteria**	**Detection Criteria**	**AMR Family**	**Drug Class**	**Resistance Mechanism**
AadS	Perfect	protein homolog	ANT(6)	Aminoglycoside antibiotic	Antibiotic inactivation
tet(X)	Strict	protein homolog	Tetracycline inactivation enzyme	Glycylcycline, tetracycline antibiotic	Antibiotic inactivation
qac(G)	Strict	protein homolog	Small multidrug resistance (SMR) Efflux pump	Disinfecting agents and antiseptics	Antibiotic efflux
MUS-1	Strict	protein homolog	MUS beta lactamase	Carbapenem, penicillin, beta-lactam	Antibiotic inactivation

Table 4.4 continued from previous page

ARO Term	RGI Criteria	Detection Criteria	AMR Family	Drug Class	Resistance Mechanism
vanT gene in vanG cluster	Strict	protein homolog	Glycopeptide resistance gene cluster, vanT	Glycopeptide 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 in vanl 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 *Myroides odoratimimus K6* and *Nocardioides sp. JS614* by Antibiotic Resistance Target Seeker (ARTS)

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 35%, followed by other segments representing 10%, 8%, 5%, 4% of the core functions for *Myroides*

odoratimimus K6 (Figure 4.3). additionally, for *Nocardioides sp.JS614* 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 32%, followed by other segments representing 10%, 8%, 5%, 4% and 3% of the core functions.

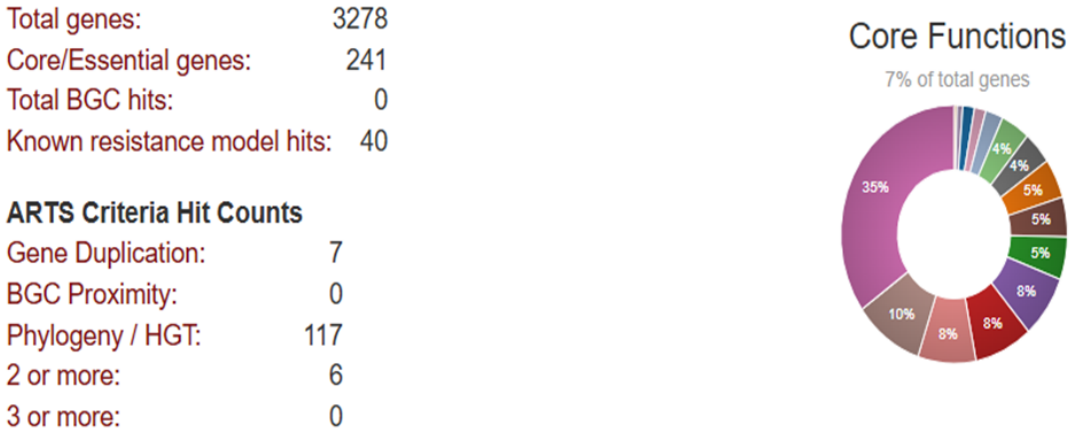


FIGURE 4.3: Antibiotic Resistance Target Seeker (ARTS) tool version 2 result for *Myroides odoratimimus K6*

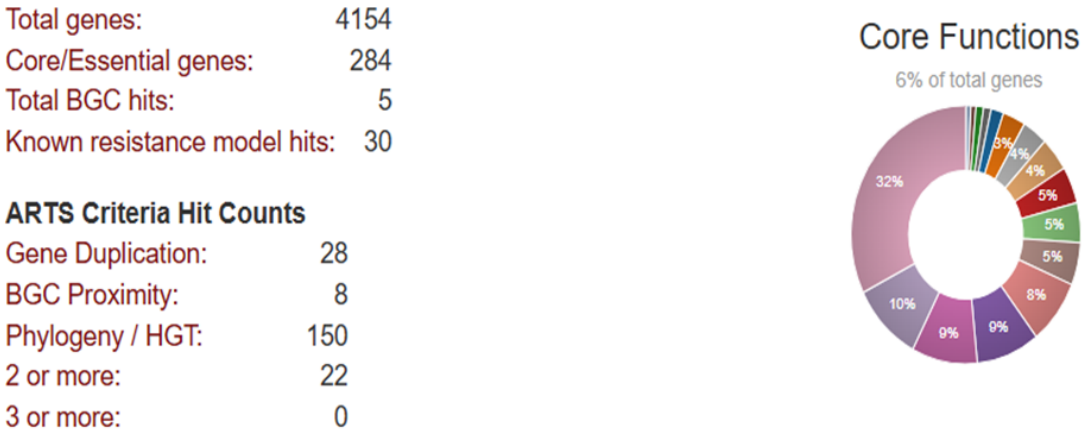


FIGURE 4.4: Antibiotic Resistance Target Seeker (ARTS) tool version 2 results for for *Nocardioides sp.JS614*

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 84 sequences, indicating that this process is heavily targeted or involved in antibiotic resistance mechanisms. DNA metabolisms 24 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. 5% unclassified and 5% unknown function categories.

TABLE 4.6: Distribution of Genes Involved in Various Cellular Functions in *Myroides odoratimimus K6* based on data obtained from ARTS

Protein synthesis	**84**
Energy metabolisms	20
DNA metabolisms	24
Protein fate	20
Biosynthesis of cofactor, prosthetic group	19
Unclassified	12
Amino acid biosynthesis	13
Purine, pyrimidine, nucleotide and nucleoside	10
Transcription	10
Unknown function	12
Cell envelope	6
Cellular process	4
center intermediary metabolism	4

The table 4.7 shows the distribution of genes across different functional categories, with the highest numbers found in protein synthesis (84), followed by DNA metabolisms (24), energy metabolisms (20), and protein fate (20). Other categories include biosynthesis of cofactors, nucleotide metabolism, transcription.

TABLE 4.7: Distribution of Genes Involved in Various Cellular Functions in *Nocardioides sp.* JS614 based on data obtained from ARTS.

Data obtained from ARTS	
Protein synthesis	91
Energy metabolisms	23
DNA metabolisms	26

Table 4.7 continued from previous page

Data obtained from ARTS	
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 *Myroides odoratimimus K6* and *Nocardioides sp.JS614* based on data obtained from ARTS

Cellular function	*Number of genes in* ***Myroides odoratimimus K6***	*Number of genes in* ***Nocardioides sp.JS614***
Protein synthesis	84	91
Energy metabolisms	20	23
DNA metabolisms	24	26
Protein fate	20	25
Biosynthesis of cofactor, prosthetic group	19	28
Unclassified	12	15
Amino acid biosynthesis	13	14
Purine, pyrimidine, nucleotide and nucleoside	10	10

Table 4.8 continued from previous page

Cellular function	*Number of genes in* ***Myroides odoratimimus K6***	*Number of genes in* ***Nocardioides sp.JS614***
Transcription	10	13
Unknown function	12	12
Cell envelope	6	9
Cellular process	4	5
center intermediary metabolism	4	3
Hypothetical protein	0	3
Fatty acid and phospholipid metabolism	0	2

4.5 Identification of BGCs in *Myroides odoratomimus K6* and *Nocardioides sp.JS614*

The antiSMASH analysis of *Myroides odoratimimus K6* revealed terpene precursor biosynthetic gene clusters, suggesting that the organism may produce terpene-based 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 *M. odoratimimus K6* 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.

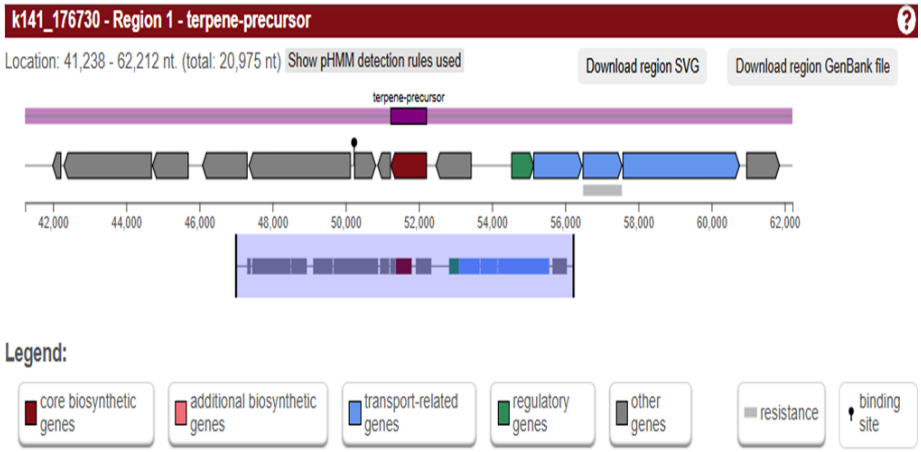


FIGURE 4.5: BGC of terpene by antiSMASH for *Myroides odoratimimus K6*

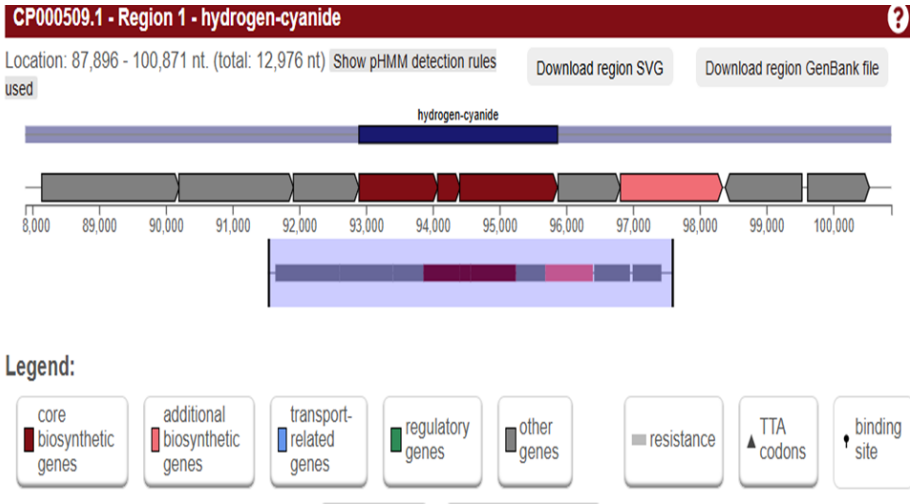


FIGURE 4.6: BGC of hydrogen cyanide by antiSHASH for *Nocardioides sp.JS614*

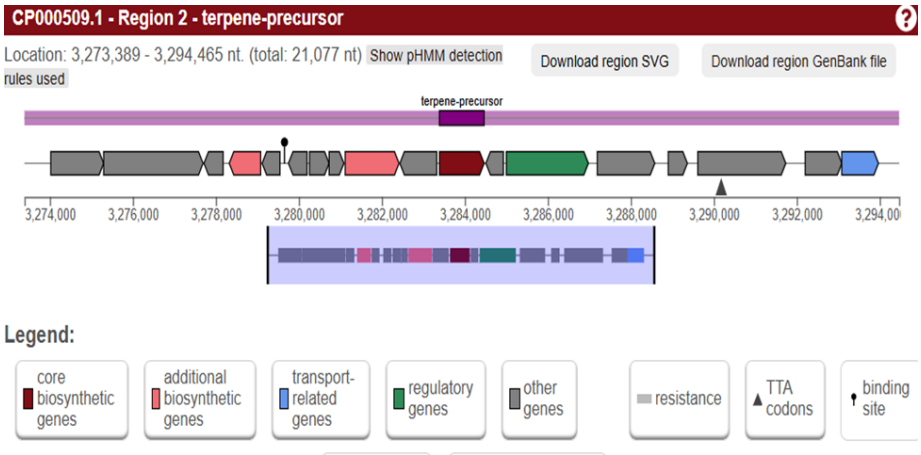


FIGURE 4.7: BGC terpene precursor or *Nocardioides sp. JS614*

In *Nocardioides sp. strain 614* hydrogen cyanide (secondary metabolite) biosynthetic gene cluster is present, having total length of 12,976 nucleotides. Start point is nucleotide number 87,896 and end point is nucleotide number 12,976.

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

Pink color indicate genes that produce such enzymes which help in biosynthesis of hydrogen cyanide. 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).

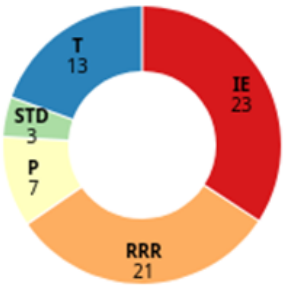
Furthermore, biosynthetic gene clusters for terpene precursors were identified in Nocardioides, indicating the strain's potential to produce a diverse range of terpene compounds.

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 Nocardioides 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 (MGEs)

Within mobile genetic elements of *Myroides odoratomomis K6* 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.



IE	integration/excision	**23**
RRR	replication/recombination/repair	**21**
P	phage	**7**
STD	stability/transfer/defense	**3**
T	transfer	**13**
	Total	***67***

FIGURE 4.8: MGEs in *Myroides odoratimimus K6*

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

Code	Number	Function
IE	23	Help in insertion or removal of MGEs into host genome
RRR	21	Ensure replication, recombination and repair of MGEs
P	7	Indicate origin of MGEs
STD	3	Provide stability, ease of transfer and protection from foreign DNA
T	13	Encode proteins that help in bacterial conjugation and transfer of MGEs by HGT

4.7 Results of CG Viewer

Circular genome map of *Myroides odoratimimus* shows a genome size of about 3.8 Mbp with a consistent GC skew and content, indicating stable replication regions. Multiple antibiotic resistance genes, including aadS, tet(X), qacG, and vanT (in the vanG cluster), which indicate multidrug resistance, 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 (recA, mutS, and ruvB).

Horizontal gene transfer potential is indicated by transposase (tnpA) and plasmid-related 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.

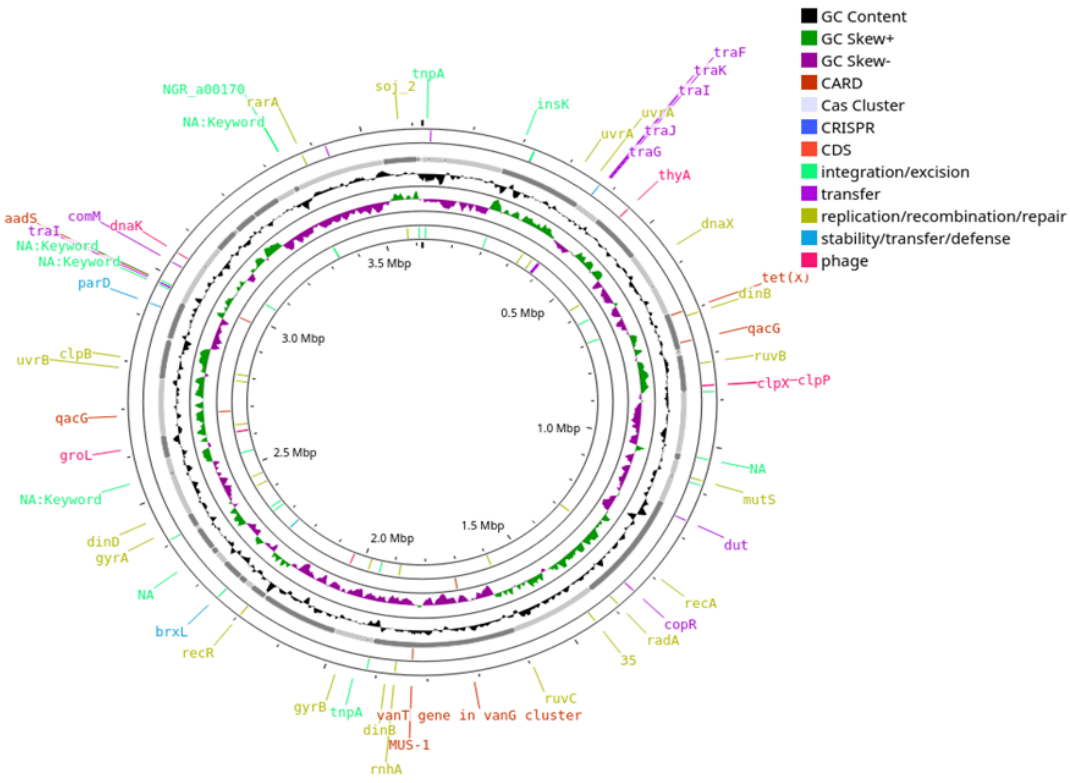


FIGURE 4.9: Graphical representation of genome (*Myroides odoratimimus K6*) by CG Viewer

In Table 4.8 data analysis of CG Viewer is presented. This analysis examine important genes and their function.

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

Gene	**Function**
tet(X)	Provide tetracycline resistance by inactivating enzyme
DfrA	Responsible for synthesis of dihydrofolate reductase which provide resistance against trimethoprim
sul1	Responsible for the production of dihydropteroate synthase that give resistance against sulfonamide
AadS	Provide resistance against streptomycin by the production of aminoglycoside adenylyl transferase
QacE	Encode efflux pump which are present in membrane and play vital role in antibiotic resistance
tnpA	Key regulator in DNA movement
traF, traN, traL, traK	Involve in bacteria conjugation
recA	Involve on mechanism which are associated with DNA repair
mut S	Encode enzymes that are involved in mechanism that are associated with mismatch repair
copR	Provide resistance against copper
MUS 1	Involve in resistance as well as mismatch repairing pathway
vanT	Provide resistance against vancomycin by altering target site

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 [111]. In recent years, *Myroides odoratimimus K6* has emerged as opportunistic pathogen, as evidenced by the rise in nosocomial outbreaks and cases reported, particularly in developing nations [112].

Myroides odoratimimus K6 found in soil, water, sewage and hospital settings and show extensive drug resistance due to resistance genes such as aadS, tat(X), qacG, vanT and MUS-1 [30]. In hospital setting *Myroides odoratimimus* K6 form strong biofilm that also contribute as a major factor in antibiotic resistance [30]. Genomic comparison of *Myroides odoratimimus* K6 with an environmental strain that is *Nocardioides sp.JS614* reveals significant insights that indicate difference in gene expression occur as habitat change [30]. RAST results indicated that GC content of *Myroides odoratimimus* K6 is 34.1% while for *Nocardioides sp.JS614* GC content is 71.4 which indicate *Myroides odoratimimus* K6 genome undergo continuous mutations and *Nocardioides sp.JS614* genome is stable due to environmental adaptations [113]. Subsystem of RAST indicated that there are 5

genes in *Myroides odoratimimus* responsible for Sulphur metabolism while *Nocardioides sp.JS614* contain 29 genes for Sulphur metabolism. These findings indicate that *Nocardioides sp.JS614* enhance metabolic pathways associated with Sulphur metabolism in nutrient limited ecosystem while *Myroides odoratimimus* K6 is adopted to nutrient rich host dependent environment [114]. Another interesting finding was number of genes responsible for iron acquisition and metabolism. *Myroides odoratimimus* K6 have 11 genes for iron acquisition and metabolism while *Nocardioides sp.JS614* just have one gene 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 *Myroides odoratimimus K6* face problem regarding host level sequestration so multiple iron acquisition and metabolism evolve [115]. RAST results indicated that there are 10 genes associated with Phages, Prophages, Transposable elements, Plasmids in *Myroides odoratimimus K6* while no gene is identified for Phages, Prophages, Transposable elements, Plasmids in *Nocardioides sp.JS614*. significant difference in genes associated with Phages, Prophages, Transposable elements, Plasmids indicate that *Myroides odoratimimus K6* have well developed mechanism for horizontal gene transfer, spread of antibiotic resistance genes and virulence factors, while absence of genes associated with Phages, Prophages, Transposable elements, Plasmids in *Nocardioides sp.JS614* stable genome shaped by long term adaptive environment with low stress [116].

For stress response *Myroides odoratimimus K6* have 21 genes while *Nocardioides sp.JS614* have 29 genes which indicate *Nocardioides sp.JS614* is adaptive to broad range environmental stresses such as temperature variations, UV radiation, heavy metals exposure while *Myroides odoratimimus K6* is adopted accoeding to host related stress such as immune response and antibiotic stress [117]. According to a RAST analysis, *Myroides odoratimimus K6* has 27 genes linked to defense, illness, and virulence, while *Nocardioides sp. JS614* have 47 genes in the same category. Despite having a greater number of genes, Nocardioides's genes are probably linked

to broad-spectrum environmental defense mechanisms such heavy metal detoxification, oxidative stress protection, and general antimicrobial resistance, which are necessary for survival in a variety of soil conditions. On the other hand, *Myroides* genes are more specialized and tailored to clinical settings, which helps with antibiotic resistance, biofilm development, and immune evasion. With *Myroides* evolving compact, host-focused virulence strategies and *Nocardioides* maintaining a broader defensive capacity appropriate for changing environmental challenges, this contrast demonstrates how ecological pressures impact the functional genome content of bacteria [118].

For carbohydrates *Myroides odoratimimus K6* have 63 genes while *Nocardioides sp.JS614* contain 324 genes. Significant difference in carbohydrates metabolism indicate that *Nocardioides sp.JS614* require broad spectrum of active enzymes for carbohydrate metabolism to degrade and use different organic matter in contrast *Myroides odoratimimus K6* depend upon host for carbon source and adapted to host dependent mode [119]. Resistance gene identifier indicate 5 genes associated with antibiotic resistance in *Myroides odoratimimus*. These genes are aadS, tat(X), qacG, vanT and MUS-1 while in case of *Nocardioides sp.JS614* two genes were identified by resistant gene identifier. There genes are HeIR and vanW. aadS gene is associated with aminoglycoside resistance, tat(X) give resistance against tetracycline, vanT provide resistance against vancomycin, qacT gene provide resistance against quaternary ammonium compounds and MUS-1 give carbapenem resistance in *Myroides odoratimimus K6*. In case of *Nocardioides sp.JS614* HeIP is associated with rifamycin resistance and vanW is responsible for vancomycin resistance. These results indicate that *Nocardioides sp.JS614* have lower selective pressure for antimicrobial resistance as compared to clinical isolate that is *Myroides odoratimimus K6* which show higher selective pressure for antimicrobial resistance [119].

According to CARD RGI results for antibiotic resistance *Myroides odoratimimus K6* have three mechanisms which are inactivation of antibiotics, antibiotic target alteration and antibiotic efflux pump. in contrast *Nocardioides sp.JS614* have two mechanisms for antibiotic resistance. These mechanisms are target alteration of

antibiotics and antibodies target protection. Presence of three different mechanisms for antibiotic resistance indicate that *Myroides odoratimimus K6* have more potential of antibiotic resistance and adapted to survive in clinical environment. *Nocardioides sp.JS614* show limited or limited capacity of antibiotic resistance [120]. Analysis of core genes by antibiotic resistance target seeker (ARTS) indicate that *Myroides odoratimimus K6* limited metabolic versatility as compared to *Nocardioides sp.JS614* which indicate broad versatility for metabolic activity. Absence of genes in *Myroides odoratimimus K6* related to fatty acid metabolism indicate opportunistic nature of *Myroides odoratimimus K6* [121]. Results of antiSMASH indicate *Myroides odoratimimus K6* has one biosynthetic gene cluster for terpene while *Nocardioides sp.JS614* have two biosynthetic gene cluster, one for hydrogen cyanide and other for terpene. Difference in number of biosynthetic gene clusters especially presence of hydrogen cyanide biosynthetic gene cluster in *Nocardioides sp.JS614* indicate expression of genes that help to compete other microbes in diverse and fluctuating environment. *Myroides odoratimimus K6* in contrast streamlined its genome [121]. Genes linked to transcriptional regulation and metabolism are more abundant in environmental bacteria than in host-dependent bacteria. This shows that in complex and dynamic environments, these bacteria need more adaptable metabolic and regulatory mechanisms to cope with environmental survival demands [121].

Chapter 6

Conclusion, Limitations and Future Directions

6.1 Conclusion

This study highlights the increasing threat posed by *Myroides odoratimimus K6* 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 *Nocardioides spp.JS614*. 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 *Myroides odoratimimus K6*. Genomic data collected from different bioinformatics tools prove that *Myroides odoratimimus K6* adopted as opportunistic pathogen. This comparative genomic investigation provides clear evidence that *Myroides odoratimimus K6* possesses genetic traits associated with virulence, resistance, and adaptation to hospital environments. In contrast, *Nocardioides sp. JS614* 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 *Myroides spp.*

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