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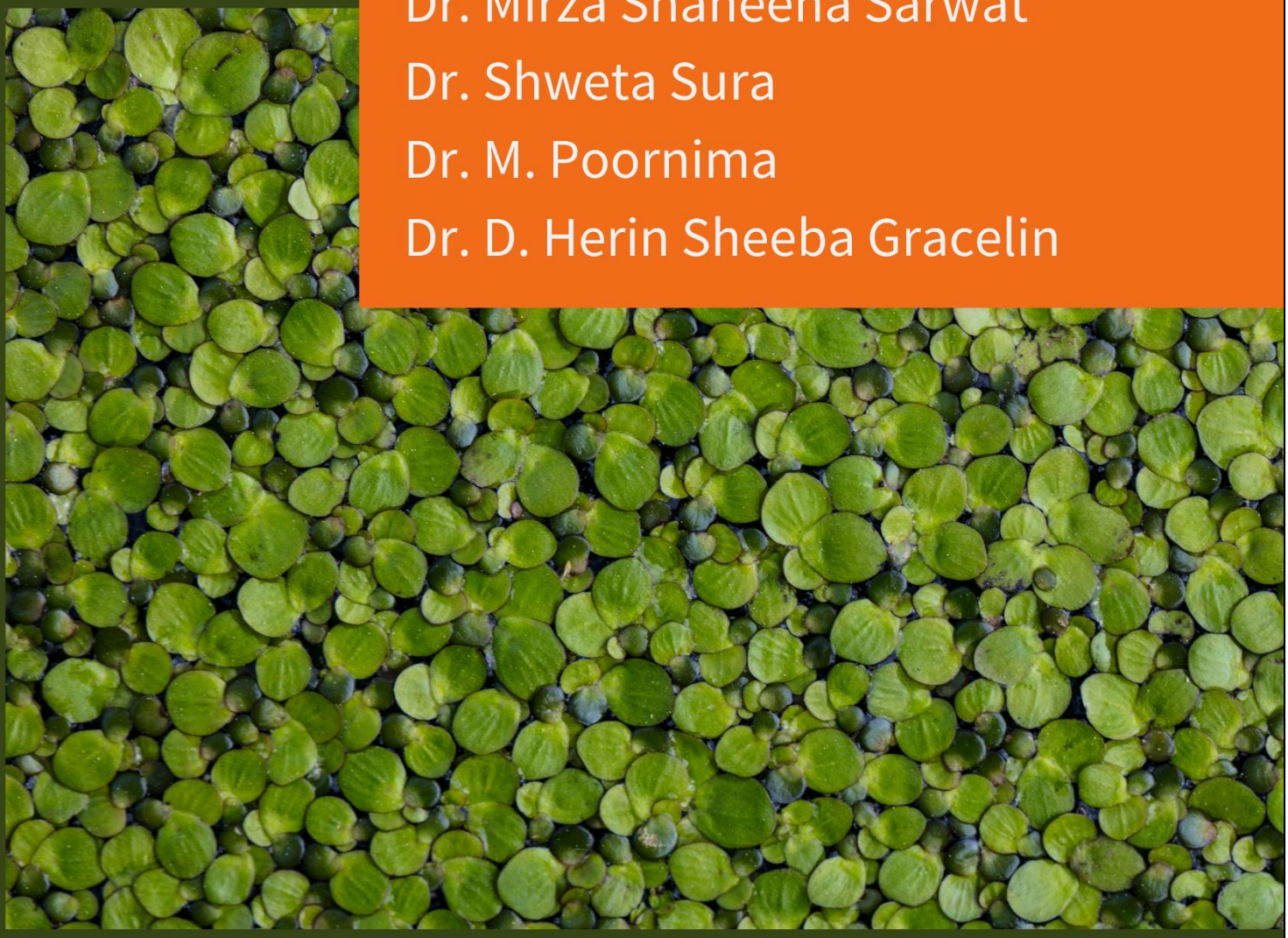
Integrative Approaches in Modern Life Science

Volume I

Editors:
Dr. Mirza Shaheena Sarwat
Dr. Shweta Sura
Dr. M. Poornima
Dr. D. Herin Sheeba Gracelin



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PREFACE

The life sciences have entered a new era marked by convergence—where once-disparate disciplines now collaborate to unlock deeper insights into the complexities of living systems. The book Integrative Approaches in Modern Life Science is a testament to this evolving landscape, where traditional boundaries between biology, chemistry, physics, mathematics, and computational science are increasingly blurred to foster innovation, discovery, and real-world applications.

This volume brings together a collection of scholarly contributions that reflect the spirit of integration across multiple domains of life science. Whether it is the amalgamation of molecular biology and bioinformatics to decode the genome, the fusion of pharmacology and nanotechnology in therapeutic design, or the interplay of systems biology and ecological modeling to address environmental challenges, each chapter underscores the value of interdisciplinary thinking.

Modern scientific challenges—ranging from understanding the molecular basis of disease to tackling global sustainability—demand holistic approaches. As such, this book not only highlights theoretical and methodological advancements but also focuses on applied research that has the potential to revolutionize health care, agriculture, environmental science, and biotechnology.

The contributors to this volume include established researchers and emerging scholars whose work exemplifies the cutting-edge of integrative life science. The book is designed to serve a broad audience—students, academicians, industry professionals, and policy makers—who are committed to advancing knowledge through collaborative inquiry and innovation.

We hope this book inspires readers to explore new intersections within their own work, encourages cross-disciplinary collaborations, and contributes to a more unified and impactful scientific endeavor. The integration of diverse perspectives and techniques is not only the hallmark of modern life sciences but also the pathway to addressing the complex biological questions of our time.

- Editors

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CHELATION-DRIVEN BIOREMEDIATION OF LEAD AND CADMIUM: STRATEGIES AND MECHANISMS

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Abstract:

The persistent contamination of ecosystems by heavy metals, notably lead (Pb) and cadmium (Cd), poses a severe environmental and public health threat. These metals are non-biodegradable, accumulate in biological systems, and disrupt physiological functions across trophic levels. Chelation-driven bioremediation, an integrative approach combining chelating agents with microbial or plant-based strategies, offers a promising solution for the detoxification of Pb and Cd in contaminated sites. This chapter examines the chemistry of chelation, categorizes natural and synthetic chelators, and explores microbial, phytoremediation, and engineered systems that leverage chelation for bioremediation. By presenting case studies, mechanistic insights, and emerging innovations such as nano-chelation and omics-guided remediation, this work highlights the transformative potential of chelation-based strategies. While challenges related to chelator toxicity and metal mobilization remain, the evolving field of chelation-assisted bioremediation stands poised to redefine sustainable environmental cleanup.

Keywords: Chelation, Bioremediation, Lead, Cadmium, Strategy

1. Introduction:

Heavy metal pollution, driven by industrialization, mining, and agricultural runoff, is one of the most intractable environmental challenges facing ecosystems globally. Lead (Pb) and cadmium (Cd), in particular, have been designated as priority pollutants by the United States Environmental Protection Agency (USEPA) due to their high toxicity, environmental persistence, and bioaccumulation in food chains (ATSDR, 2022).

Unlike organic pollutants that can be degraded enzymatically or through oxidation, heavy metals do not degrade naturally. Therefore, their remediation requires either physical removal or chemical transformation into less toxic forms. Traditional remediation techniques, such as soil excavation and chemical washing, are expensive, invasive, and unsustainable (Kumar *et al.,* 2020). In contrast, bioremediation — the use of living organisms or biologically inspired processes — offers a more ecologically balanced and cost-effective alternative.

Among bioremediation strategies, chelation-driven bioremediation is gaining momentum as a synergistic and targeted approach. Chelation involves the binding of metal ions by organic molecules to form stable complexes, which can then be removed, immobilized, or assimilated by organisms (Saha *et al.,* 2021). Natural chelators such as Phytochelatin and siderophores, along with synthetic ones like EDTA and DTPA, have been employed in both microbial and plant systems for enhancing metal solubility, transport, and detoxification.

This chapter explores the mechanistic foundations and practical applications of chelation-assisted remediation. It provides an in-depth examination of chelation chemistry, delineates the types of chelators, and discusses microbial and phytoremediation pathways augmented by chelation. The chapter also investigates field applications, novel developments, and the ecological implications of deploying chelation-based systems.

Through this comprehensive analysis, we aim to underscore the critical role of chelators in advancing sustainable solutions for heavy metal detoxification, particularly for the pressing cases of lead and cadmium contamination.

2. Heavy Metal Toxicity: A Global Environmental Crisis

2.1 Sources of Lead and Cadmium

Lead (Pb) and cadmium (Cd) enter ecosystems from both natural and anthropogenic sources. Though trace amounts exist in the Earth's crust, the rise in industrial activities has resulted in unprecedented environmental loading.

Major Anthropogenic Sources Include:

- Lead**:**
    - Lead-acid battery recycling
    - Leaded gasoline (still used in some countries)
    - Paint and pigment industries
    - Ammunition and e-waste
    - Plumbing and soldering materials
- Cadmium**:**
    - Non-ferrous metal smelting (especially zinc)
    - Cadmium-nickel batteries
    - Phosphate fertilizers
    - Waste incineration
    - Paints and coatings

In agricultural areas, cadmium contamination is frequently attributed to long-term use of phosphate-based fertilizers, leading to soil buildup and plant uptake (Alloway, 2013). Similarly,

battery industries and illegal e-waste dumping have led to alarming concentrations of lead in peri-urban and informal industrial zones (Li *et al.,* 2019).

2.2 Environmental and Human Health Effects

Lead and cadmium exhibit severe ecological toxicity and bioaccumulate in food chains. Their effects range from sub-lethal cellular dysfunctions in microbes and plants to irreversible neurological damage in humans.

Metal	Affected System	Effects in Humans	Ecological Impact
Lead (Pb)	Nervous, hematopoietic, renal	Neurotoxicity, cognitive deficits, anaemia, hypertension	Soil fertility decline, inhibition of plant enzymatic systems
Cadmium (Cd)	Renal, skeletal, reproductive	Itai-Itai disease, kidney failure, bone demineralization, carcinogenicity	Disruption of microbial diversity, chlorosis, inhibition of photosynthesis

Children and foetuses are particularly susceptible to lead toxicity, which can impair IQ, attention span, and neurobehavioral development at levels <5 µg/dL (WHO, 2021). Cadmium, on the other hand, is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC), associated with lung and prostate cancer due to chronic exposure (IARC, 2012).

In aquatic environments, both metals impair gill function and ionic regulation in fish and amphibians. Terrestrial effects include reduced crop yields, inhibition of rhizosphere microbes, and reduced soil enzymatic activities.

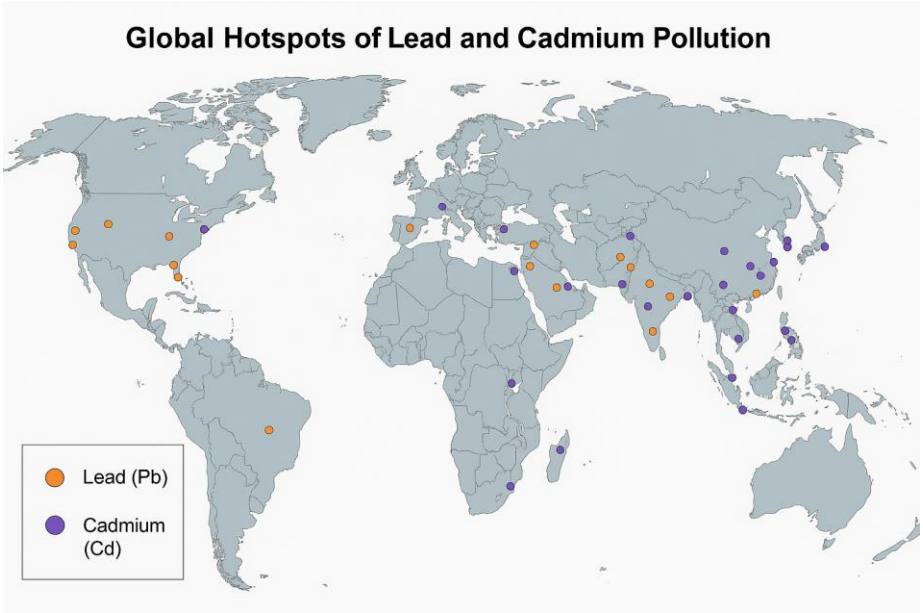

Figure 1: Global Hotspots of Lead and Cadmium Pollution

This map highlights global lead and cadmium hotspots based on WHO and UNEP datasets, overlaying industrial corridors and contaminated zones.

3. Principles of Bioremediation

3.1 Microbial Bioremediation

Microorganisms have evolved diverse mechanisms to resist, transform, or immobilize heavy metals. Microbial bioremediation utilizes specific bacteria, fungi, or archaea capable of tolerating and detoxifying lead and cadmium through bioaccumulation, enzymatic transformation, or sequestration.

Mechanisms include**:**

- Biosorption: Passive adsorption of metals on microbial cell walls (e.g., *Bacillus subtilis*)
- Bioaccumulation: Active transport and intracellular binding (e.g., *Pseudomonas aeruginosa*)
- Biotransformation: Enzymatic conversion of metals into less toxic or immobile forms
- EPS-mediated binding: Extracellular polymeric substances trap metal ions in biofilms

Some metal-tolerant microbes synthesize metal-binding peptides or chelators like siderophores, which enhance metal solubility and facilitate uptake.

3.2 Phytoremediation

Plants offer a cost-effective and aesthetic alternative for heavy metal removal. Several species have evolved natural capabilities to hyperaccumulate or immobilize toxic metals in their tissues.

Key strategies include:

- Phytoextraction: Uptake and accumulation in aerial parts (e.g., *Brassica juncea* for Pb and Cd)
- Phyto stabilization: Immobilization in root zones, reducing leaching
- Rhizo-filtration: Absorption or precipitation by root systems in aquatic environments

The presence of root exudates, including organic acids and chelating agents (e.g., citrate, oxalate), significantly influences metal solubility and microbial associations in the rhizosphere.

3.3 Role of Chelating Agents in Bioremediation

Chelators are critical facilitators in both microbial and phytoremediation by:

- Increasing metal solubility in soils and water
- Enhancing uptake efficiency via transport complexes
- Reducing metal toxicity by stabilizing free ion activity
- Mobilizing metals for translocation in plant shoots or microbial compartments

Chelators can be natural (e.g., siderophores, phytochelatins) or synthetic (e.g., EDTA, DTPA). Their application must be optimized to avoid excessive leaching of mobilized metals into water bodies.

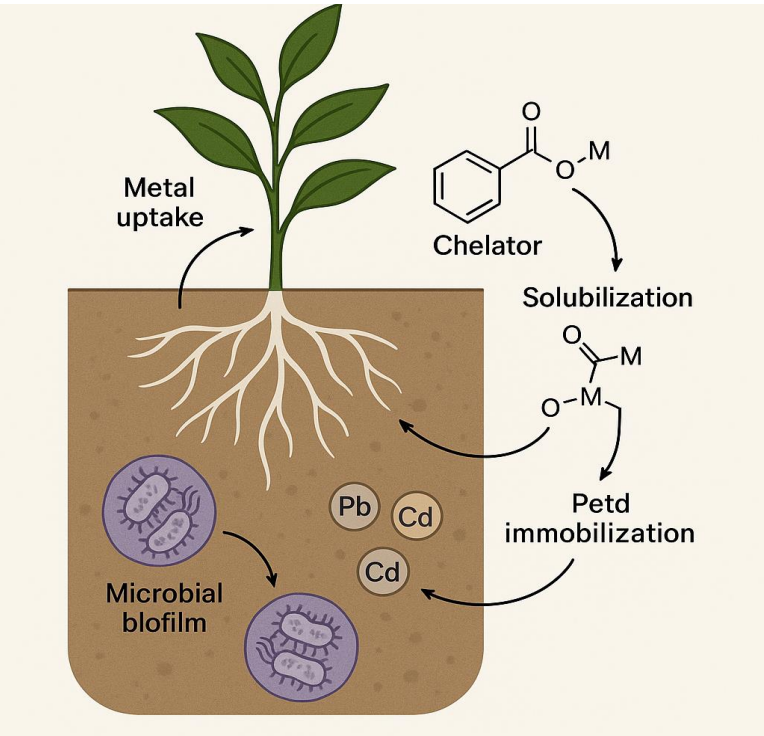

Figure 2: Schematic Representation of Chelation-Enhanced Bioremediation

This diagram illustrates the synergistic interaction between microbial biofilms, plant roots, and chelators facilitating lead and cadmium remediation in contaminated soil.

4. Chelation Chemistry of Lead and Cadmium

4.1 Chelating Agents: Classification and Mechanisms

Chelating agents are organic molecules that can form multiple bonds with a single metal ion, creating a ring-like metal complex known as a *chelate*. These agents increase the solubility, mobility, and detoxification of metal ions by forming thermodynamically stable and kinetically inert complexes.

Type	**Examples**	**Source**
Natural Chelators	Phytochelatin, metallothionein, siderophores	Microbes, plants
Synthetic Chelators	EDTA, DTPA, NTA, EGTA	Man-made
Biologically Derived	Glutathione, citric acid, oxalic acid	Endogenous

Chelation Mechanism**:**

Most chelators operate via electron-donating atoms (e.g., oxygen, nitrogen, sulfur) forming coordinate bonds with empty orbitals of the metal ion. This process:

- Neutralizes or reduces the metal ion's reactivity

- Enhances its bioavailability (or sequestration)
- Modulates its transport in microbial and plant systems

For example, EDTA (ethylenediaminetetraacetic acid) is a hexadentate ligand, forming highly stable octahedral complexes with divalent and trivalent metal ions.

4.2 Stability Constants and Metal Specificity

The stability of metal-chelate complexes is expressed by their *formation constants (K_e)*, which indicate the strength of the interaction. Higher values reflect more stable complexes.

Chelator	Metal	Log K_e (Stability Constant)
EDTA	Pb^{2+}	18.0
EDTA	Cd^{2+}	16.5
DTPA	Pb^{2+}	20.3
DTPA	Cd^{2+}	18.4
Phytochelatin	Pb^{2+}	~13–15 (approx.)
Siderophores	Cd^{2+}	~12–14

From this data, DTPA exhibits superior affinity for both lead and cadmium compared to EDTA. However, natural chelators like phytochelatins and metallothioneins are more environmentally benign, though their specificity is lower.

4.3 Comparative Chelation of Pb vs. Cd

- Lead (Pb^{2+}): Forms stable complexes with oxygen and nitrogen donors. More likely to bind EDTA, DTPA, or NTA. Less mobile in soil due to stronger adsorption to particles.
- Cadmium (Cd^{2+}): Prefers sulphur and oxygen ligands. It is more mobile and bioavailable, especially in the presence of low-molecular-weight chelators (e.g., glutathione, oxalic acid).

The speciation behaviour of both metals affects how and where chelation is most effective — Pb tends to remain in the rhizosphere, whereas Cd is more prone to translocation within plants.

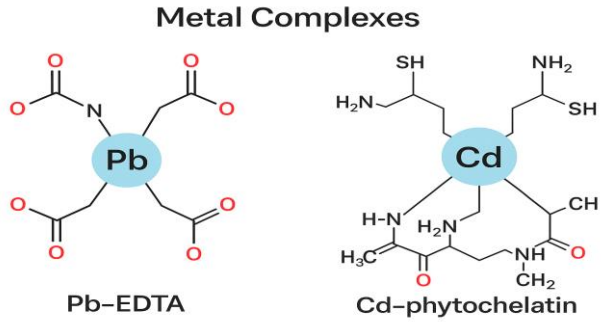

Figure 3: Representative Structures of Chelator-Metal Complexes

This illustration shows example metal complexes of Pb-EDTA and Cd-Phytochelatin.

5. Chelation-Based Bioremediation Strategies

5.1 Natural Chelators

Natural chelators are produced by organisms to regulate metal homeostasis and defend against metal toxicity. They are biodegradable, environmentally benign, and often metal-specific. Major classes**:**

- Phytochelatin (PCs): Peptides synthesized in plants from glutathione that bind metals through thiol (-SH) groups. Particularly effective for Cd^{2+} sequestration.
- Siderophores**:** Low-molecular-weight iron-scavenging molecules secreted by microbes. Some can bind Cd^{2+}, Pb^{2+}, and other divalent cations due to structural versatility.
- Metallothionein**:** Cysteine-rich proteins in plants, animals, and microbes that bind heavy metals via thiolate bonds.

These chelators are critical in intracellular detoxification and can be engineered into transgenic plants or used to stimulate native microbial populations.

5.2 Synthetic Chelators

Synthetic chelators are widely used in remediation due to their high metal-binding affinity and known structure–function relationships.

Chelator	Structure	Application	Limitations
EDTA	Hexadentate	Soil flushing, enhanced phytoextraction	Non-biodegradable, risk of leaching
DTPA	Pentadentate	Metal solubilization in alkaline soils	Expensive, moderate persistence
NTA	Tridentate	Used in rhizoremediation	Less toxic than EDTA but weaker stability
EGTA	Selective for Ca^{2+}	Biomedical and aquatic remediation	Not widely applied in soils

Although EDTA is effective in Pb^{2+} extraction, its persistence and potential to leach metals into groundwater require tight control and post-treatment measures (Wenzel, 2009).

5.3 Microbial Chelation Mechanisms

Microorganisms contribute to metal chelation and mobilization through:

- Siderophore secretion**:** Enhancing Pb^{2+}/Cd^{2+} solubility and microbial uptake
- Organic acid production**:** Citric, oxalic, and gluconic acids bind metals and reduce soil pH, increasing solubility
- EPS binding**:** Extracellular polymeric substances from biofilms bind and immobilize metal ions

Species like *Pseudomonas putida*, *Bacillus subtilis*, and *Aspergillus niger* are commonly studied for these traits.

5.4 Plant-Assisted Chelation in Phytoextraction

Chelation enhances phytoextraction by facilitating metal translocation from roots to shoots. Key processes:

- Chelators mobilize Pb^{2+}/Cd^{2+} from soil particles
- Chelate-metal complexes enter the root via transporters
- Metals are translocated through the xylem, reducing root retention

Hyperaccumulators like *Thlaspi caerulescens*, *Helianthus annuus*, and *Brassica juncea* show increased uptake when EDTA or natural chelators are applied.

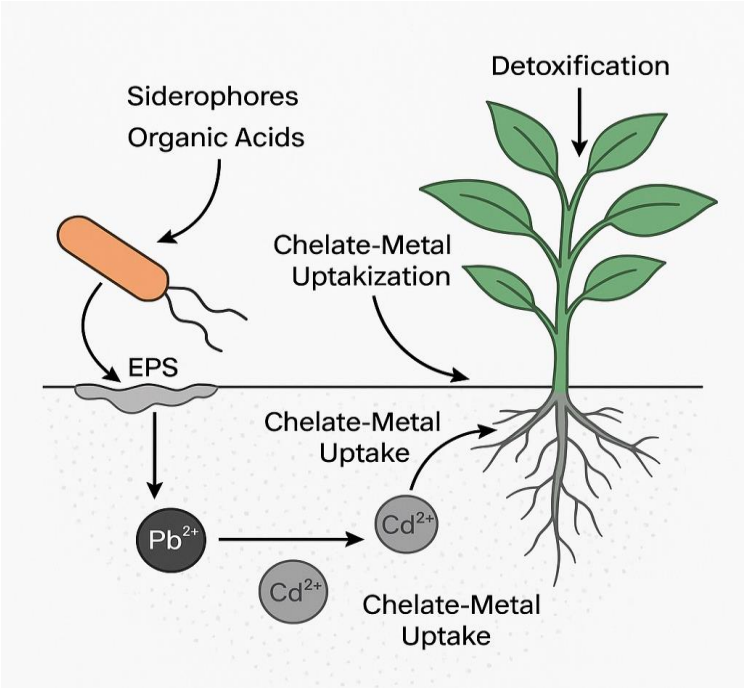

Figure 4: Mechanism of Chelation-Based Bioremediation: Microbial and Plant Synergy

This schematic illustrates a coordinated system involving microbial chelator production, chelate-metal mobilization, plant uptake, and detoxification.

6. Case Studies in Lead and Cadmium Chelation

6.1 Soil Remediation Using Microbial Chelators

Case Study A: Lead Remediation in Industrial Soils Using *Pseudomonas fluorescens*
In a contaminated site near an abandoned battery recycling facility in Maharashtra, India, soil Pb levels exceeded 1,000 mg/kg. Inoculation with *P. fluorescens*, a siderophore-producing bacterium, resulted in:

- 60% reduction in bioavailable Pb within 60 days
- Enhanced solubilization via siderophore chelation

- Stabilization of Pb through biosorption on extracellular polysaccharides

Reference: Sharma *et al.* (2020), *Journal of Hazardous Materials*

6.2 Rhizosphere Engineering for Metal Uptake

Case Study B: Cd Phytoextraction Using *Brassica juncea* and EDTA

A greenhouse trial in Nanjing, China, evaluated the synergistic use of *B. juncea* and low-concentration EDTA (1.5 mmol/kg). The study found:

- 3.2× higher Cd uptake in shoots compared to control
- No observed phytotoxicity at low chelator dose
- Residual Cd in soil reduced by 47% after 8 weeks

Reference: Liu *et al.* (2019), *Chemosphere*

6.3 Combined Chelation and Bioaugmentation Approaches

Case Study C: Field Bioremediation of Pb/Cd in Urban Garden Soils (Poland)

An integrated setup used:

- Chelators: EDDS (readily biodegradable)
- Microbes: *Rhizobium leguminosarum* + *Trichoderma harzianum*
- Plant: *Helianthus annuus* (sunflower)

Results:

- Total metal content reduced by 38% (Pb) and 42% (Cd)
- Chelator + microbe co-treatment improved uptake efficiency by 2.7×
- Soil health indicators (enzyme activity, microbial biomass) improved significantly

Reference: Kowalska *et al.* (2022), *Environmental Science and Pollution Research*

Table 1: Summary of Selected Case Studies in Chelation-Based Remediation

Location	Contaminant	Chelator	Organism(s)	Outcome
Maharashtra, India	Lead (Pb)	Siderophores	*P. fluorescens*	60% Pb reduction
Nanjing, China	Cadmium (Cd)	EDTA	*Brassica juncea*	3.2× Cd uptake
Kraków, Poland	Pb, Cd	EDDS	*Rhizobium*, *Trichoderma*, sunflower	38–42% reduction, improved soil health

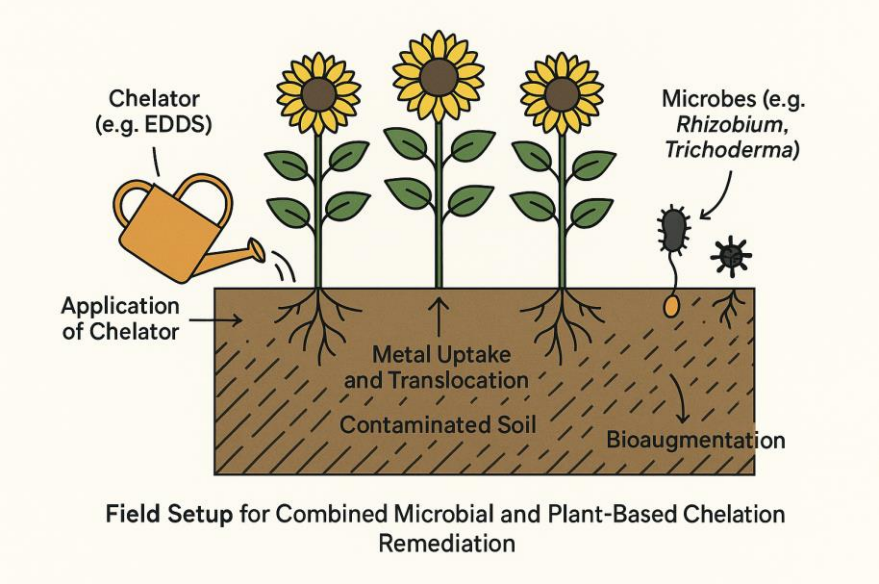

Figure 5: Field Setup for Combined Microbial and Plant-Based Chelation Remediation

This schematic shows the layout of a bioremediation plot integrating chelator application, microbial bioaugmentation, and hyperaccumulator crops.

7. Challenges and Risks in Chelation-Based Remediation

7.1 Chelator Toxicity and Persistence

The use of synthetic chelators like EDTA and DTPA has raised serious environmental concerns:

- Persistence: EDTA is highly resistant to biodegradation and may remain in soil and water for extended periods.
- Aquatic toxicity: Mobilized metal-chelate complexes can leach into groundwater or surface waters, increasing bioavailability and posing risks to aquatic life.
- Metal redistribution: Improper dosage or timing can lead to unintentional dispersion of metals beyond the remediation site.

Example: EDTA–Pb^{2+} complexes can travel through soil profiles, contaminating downstream ecosystems (Tandy *et al.,* 2006).

7.2 Risk of Metal Mobilization and Leaching

One of the paradoxes of chelation is its success in solubilizing metals, which, if not properly controlled, leads to:

- Enhanced leaching into groundwater
- Secondary contamination in adjacent areas
- Reduced remediation efficiency due to loss of chelated metals

Risk mitigation approaches include:

- Application of biodegradable chelators like **EDDS**

- Use of immobilization barriers (e.g., biochar or zeolite)
- Controlled irrigation and drainage systems to limit downward metal movement

7.3 Regulation and Environmental Policy Concerns

Most countries lack specific legislation regulating the use of chelators in environmental remediation. Key issues include:

- Lack of chelator-specific guidelines in national remediation protocols
- No monitoring framework for chelator residues in soil or water
- Difficulty in balancing cost-effective cleanup with long-term ecological safety

Case in point: The EU restricts widespread agricultural use of EDTA, encouraging natural alternatives like citric acid and EDDS (European Chemicals Agency, 2020).

Table 2: Risk-Benefit Matrix for Chelation Strategies in Pb/Cd Remediation

Factor	Benefit	Risk	Mitigation
Metal Solubilization	Increases uptake	Groundwater leaching	Timed irrigation
Chelator Selection	High affinity chelation	Toxicity (e.g., EDTA)	Use of EDDS, natural chelators
Translocation	Aids phytoextraction	Metal redistribution	Immobilization barriers
Cost-effectiveness	Reduces excavation need	Variable effectiveness	Microbial support systems

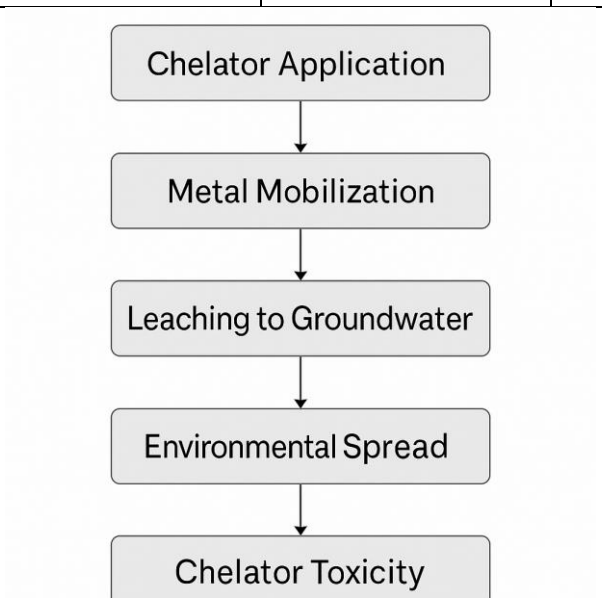

Figure 6: Risk Pathways in Chelation-Based Remediation

This flowchart outlines potential unintended outcomes of chelator use, including mobilization, leaching, toxicity, and environmental spread.

8. Advancements and Future Prospects

8.1 Smart Chelators and Bioengineered Systems

Recent advances in biotechnology have enabled the design of smart chelators that combine specificity, biodegradability, and functional programmability.

- Synthetic biology has enabled microbes to overexpress siderophores or Phytochelatin.
- Engineered rhizobacteria can simultaneously mobilize metals and enhance root metal uptake.
- Functionalized chelators can be pH-responsive or metal-specific, minimizing ecological disruption.

Example: Bioengineered *E. coli* strains capable of secreting metallothionein-fused peptides show increased Cd^{2+} binding in wastewater settings (Patel *et al.,* 2021).

8.2 Nano-Chelates and Nano remediation

Nanotechnology introduces highly reactive and tenable materials for precision bioremediation:

- Nano-chelates: Nanoscale particles conjugated with chelating ligands allow for high surface area binding of Pb and Cd
- Magnetic nanocarriers: Enable recovery of chelated metal complexes via external magnets
- Nanozymes: Mimic enzymatic chelation and facilitate in-situ detoxification

Example: EDTA-functionalized Fe_3O_4 nanoparticles have demonstrated >90% removal of Pb^{2+} from contaminated river sediments (Zhang *et al.,* 2022).

8.3 Omics Tools in Chelation-Based Remediation Research

Genomics, proteomics, and metabolomics are being applied to:

- Identify microbial strains with superior chelating potential
- Decode plant-metal interaction pathways
- Engineer high-performing microbial consortia for tailored remediation

Metagenomics reveals shifts in soil microbiomes during chelation, guiding adaptive management strategies (Singh & Narayan, 2020).

8.4 Policy, Commercialization, and Scale-Up Potential

Key enabling factors for widespread adoption include:

- Government incentives for bioremediation R&D and low-toxicity chelator manufacturing
- International guidelines (e.g., UNEP, FAO) for safe deployment of synthetic chelators
- Public-private partnerships for scaling field trials in mining belts, e-waste zones, and industrial regions

Pilot projects in the EU and India are already validating nano-chelation and engineered bioremediation consortia at >10-hectare scale.

Table 3: Emerging Strategies for Next-Gen Chelation-Driven Bioremediation

Technology	**Mechanism**	**Advantage**	**Status**
Smart chelators	Metal-specific release	Reduced toxicity	Lab-scale validated
Nano-chelates	Surface-enhanced adsorption	Rapid kinetics, recoverability	Field trials
Synthetic microbes	Overexpression of chelators	Controlled release & uptake	Proof-of-concept
Multi-omics	Targeted strain selection	Precision remediation	Active research
Policy frameworks	Incentive-based adoption	Scalable and regulated	Country-specific

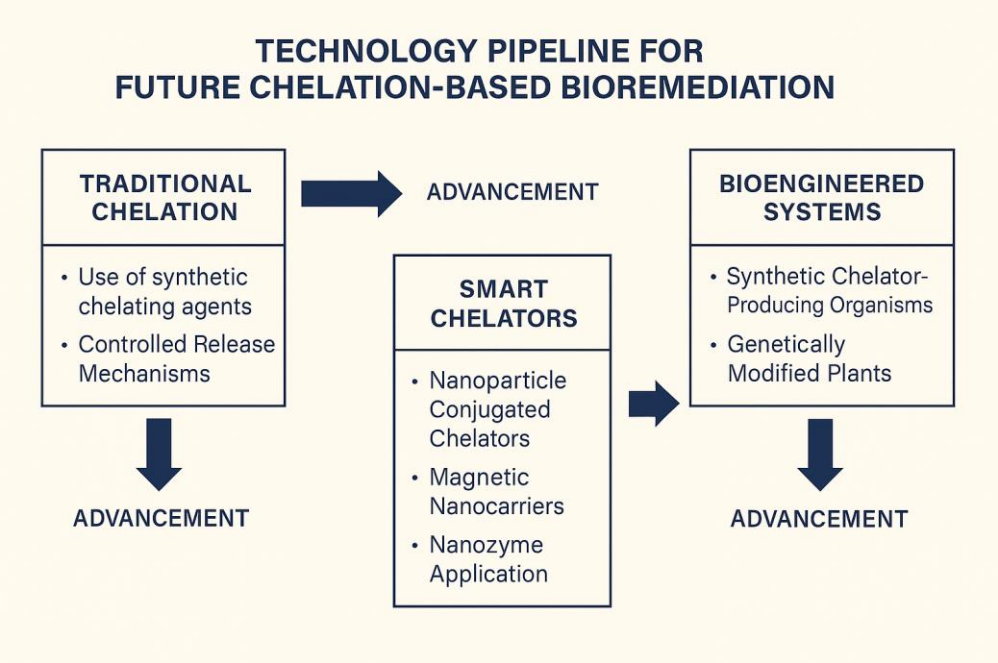

Figure 7: Technology Pipeline for Future Chelation-Based Bioremediation

This infographic shows the evolution from traditional chelation to advanced smart, nano, and bioengineered systems.

Conclusion:

The growing burden of lead and cadmium contamination in terrestrial and aquatic environments demands innovative, sustainable, and scalable remediation strategies. Chelation-driven bioremediation has emerged as a scientifically robust and ecologically responsible approach for detoxifying these persistent heavy metals. By leveraging the powerful chemistry of

chelators—both synthetic and natural—combined with the biological potential of microorganisms and hyperaccumulator plants, this strategy offers a synergistic solution for environmental restoration.

From foundational principles of metal chelation to practical field applications and emerging nanotechnologies, this chapter has demonstrated how chelators function not only as facilitators of metal solubilization but also as critical agents in metal mobilization, uptake, sequestration, and detoxification. While synthetic chelators like EDTA and DTPA offer high binding affinities, their environmental persistence necessitates caution. In contrast, natural chelators such as Phytochelatin and siderophores, when paired with bioaugmentation and phytotechnology, hold immense promise for low-impact remediation.

Real-world case studies from industrial zones, agricultural fields, and urban soils show that integrated approaches—especially those combining microbial, plant-based, and chemical strategies—can yield significant reductions in bioavailable Pb and Cd. Innovations in synthetic biology, nano-chelation, and omics-guided bioremediation are paving the way for next-generation remediation platforms with enhanced precision, safety, and efficiency.

However, challenges such as chelator toxicity, groundwater leaching, and lack of regulatory frameworks remain. Overcoming these barriers will require interdisciplinary collaboration, policy reform, and public–private partnerships. Moving forward, chelation-assisted remediation is poised not just to clean polluted lands but to set the foundation for smarter, more responsive environmental biotechnology systems.

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IMPORTANCE OF END-FEEL EVALUATION IN MUSCULOSKELETAL AND SPORTS REHABILITATION

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Introduction:

End-feel is a term used in musculoskeletal assessment that refers to the sensation a clinician feels when a joint reaches its limit of motion. It is an essential part of the physical examination, helping to determine the integrity of the joint, tissues, and structures around it. Evaluating end-feel gives insight into joint pathology, the type of restriction (whether mechanical or pathological), and guides clinical decision-making in rehabilitation protocols [1][2].

Purpose of End-Feel Evaluation

Diagnosing Joint Dysfunction: End-feel is often evaluated during physical assessments to differentiate between normal and abnormal joint movement [3]. Guiding Treatment Plans: By identifying the type of end-feel, physiotherapists can tailor interventions such as joint mobilizations, stretching, or strengthening exercises [4]. Monitoring Rehabilitation Progress: As rehabilitation progresses, changes in end-feel may indicate improvements in joint mobility or the development of complications [5].

Definition and Types of End-Feel

End-feel can be categorized into normal and abnormal types. Understanding the different types of end-feels is essential for the clinician in interpreting findings during joint mobility tests.

I. Normal End-Feel Types

a. Hard End-Feel: A sudden, hard stop often felt in joint like the elbow when bone contacts bone (e.g., full extension of the elbow) [6][7].

b. Soft End-Feel: Felt when soft tissues (muscle or fat) are compressed, such as during flexion at the knee joint [8].

c. Firm End-Feel: A resistance felt due to tension in the joint capsule, ligaments, or muscles (e.g., hip rotations) [9].

II Abnormal End-Feel Types

a. Bony End-Feel: An abnormal hard stop often caused by pathological bone changes like osteophytes or joint deformities (e.g., in arthritis) [10][11].

b. Capsular End-Feel: A restriction in motion due to capsule stiffness, often seen in conditions like adhesive capsulitis [12].

c. Springy Block: Occurs when there is a rebound effect in the joint, usually due to intra-articular meniscal tears [13].

d. Empty End-Feel: When no resistance is felt due to pain limiting the movement, common in acute inflammatory conditions or infections [14].

Clinical Significance of End-Feel Evaluation

A. Differentiating between pathologies: End-feel helps to differentiate between joint and soft tissue pathologies. A hard end-feel could indicate joint osteoarthritis, whereas a soft end-feel may indicate a muscle spasm or inflammation [15][16].

B. Determining the nature of tissue involvement: End-feel evaluation can be used to identify whether the limitation in motion is capsular, ligamentous, or muscular in origin. Understanding the specific tissue involved helps direct appropriate interventions [17].

C. Guiding joint mobilization firm end-feel: Indicating the presence of capsular restriction, clinicians might apply joint mobilizations to improve movement [18][19]. Hard End-Feel: Suggests bone-to-bone contact, limiting mobilization interventions [20].

D. Monitoring Rehabilitation Progress: As part of ongoing assessment, changes in end-feel can indicate improvements in joint mobility, allowing the clinician to adjust treatment protocols. For example, a shift from a bony to a firm end-feel after an injury may suggest that the joint is regaining its normal range of motion [21].

Techniques for Assessing End-Feel

A. Palpation and Manual Muscle Testing: Clinicians often use palpation in conjunction with manual muscle testing to identify and assess end-feel. A systematic approach to manual examination ensures accurate evaluation [22][23].

B. Joint Range of Motion Measurement: Goniometers and inclinometers are common tools to measure range of motion, while end-feel is assessed manually during these measurements [24].

C. Active vs. Passive Motion: Passive range of motion provides a more accurate assessment of end-feel as it removes muscle contraction from the equation [25].

D. Testing for Pathological End-Feel: Special tests and palpation techniques can help distinguish between abnormal end-feels, including assessments for joint effusion (e.g., in the knee joint) and joint instability [26][27].

End-Feel in Sports Rehabilitation

Sports injuries often involve joint and soft tissue damage, and end-feel evaluation is critical in assessing the severity of the injury and the stage of recovery.

A. Acute Injuries: In the acute phase of an injury, end-feel may be limited by pain (empty end-feel), and this guides the clinician to avoid aggressive joint mobilizations or stretches [28].

B. Chronic Injuries: For chronic conditions, like tendinopathies, end-feel changes may be indicative of muscle tightness, capsular restrictions, or degenerative changes [29].

C. Post-Surgical Rehabilitation: End-feel evaluation is crucial in the rehabilitation of post-surgical joint replacements or ligament reconstructions, as it assists in determining when a patient is ready for more challenging rehabilitation exercises [30][31].

Limitations and Considerations

A. Patient Factors Pain Tolerance: Patient discomfort can alter the perceived end-feel, making accurate assessment challenging [32].

Anxiety: A patient's emotional state may influence joint mobility and lead to an inaccurate assessment [33].

B. Examiner Skill: The evaluator's experience plays a significant role in accurately assessing end-feel. Variability in skill can lead to inconsistent interpretations, so continuous professional development is essential for clinicians [34].

Case Studies and Clinical Applications

Case Study 1: Post-Operative Shoulder

In a patient recovering from a shoulder rotator cuff repair, assessing the end-feel at the glenohumeral joint reveals a firm end-feel, indicating proper tissue healing and capsular restriction. This guides the clinician to introduce joint mobilizations and stretching [35].

Case Study 2: Hip Osteoarthritis

A patient with hip osteoarthritis presents with a bony end-feel during hip flexion, indicating bone-on-bone contact. The clinician then focuses on pain management, strength exercises, and joint protection techniques [36][37].

Conclusion:

The evaluation of end-feel is an indispensable skill for physiotherapists, providing valuable information about joint pathology, guiding treatment plans, and monitoring

rehabilitation progress. Mastery of end-feel assessment allows for more effective and targeted interventions in musculoskeletal and sports rehabilitation [38][39].

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RETURN TO SPORTS AFTER ACL INJURY

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One of the most frequent sports-related injuries, anterior cruciate ligament (ACL) injuries have a major impact on sportsmen and physically active people. A patient's capacity to carry out everyday tasks and preserve their quality of life may be seriously hampered by these impairments.(1) High-demand activities that require quick turning, sudden direction changes, cutting, and jumping are typical causes of ACL injuries, especially in sports like football, basketball, and soccer.(2) Resuming physical activity after suffering an ACL tear raises the risk of additional damage to the knee's intra-articular structures, such as meniscus tears and cartilage degradation, which can exacerbate long-term functional impairments and joint instability if treatment is not received.(3)

An ACL repair is usually recommended for athletes who want to resume pivoting sports after 4–8 weeks, if full range of motion has been established and there is no joint swelling. An ACL repair aims to provide the athlete with a mechanically stable knee and, by limiting antero-posterior joint motion, lower the chance of further damage to the menisci and cartilage.(4)

An important part of the healing process following anterior cruciate ligament restoration (ACLR) is rehabilitation. The athlete's main objective is to minimize the chance of re-injury while returning to sport as soon as possible, ideally at their pre-injury level of performance. About 80% of individuals who have had an ACL reconstruction resume some form of athletic activity, but only 65% get back to their pre-injury level and 55% resume competitive sports. In addition to graft failure, two short-term (e.g., muscular muscles) and long-term (such as osteoarthritis, meniscal or chondral injuries, and knee-related quality of life) A person's rehabilitation may also be adversely affected by five to eight ACLR comorbidities.(5)

Despite advances in surgical techniques and rehabilitation science, the risk of re-injury remains high. Evidence shows that athletes who return to sport before meeting objective criteria are up to 4 times more likely to suffer a secondary ACL injury. A pivotal study by Grindem *et al.* reported that each month of delayed return (up to 9 months) reduced re-injury risk by 51%. ACL

rehabilitation has gradually transitioned from time-based protocols to criteria-based decision-making, emphasizing a more holistic view of readiness that includes strength, biomechanics, and psychological factors. [6,7]

Furthermore, psychosocial readiness is now recognized as a critical factor. Athletes with higher confidence and lower fear of reinjury—as measured by tools like the **ACL-RSI (Return to Sport after Injury) scale**—are more likely to achieve successful return and maintain participation without reinjury.

Criteria-Based vs. Time-Based Return to Sport

Returning to sport (RTS) following anterior cruciate ligament reconstruction (ACLR) is a critical phase of rehabilitation that influences an athlete's long-term performance, health, and risk of reinjury. Traditionally, RTS decisions have been guided by **time-based protocols**, where clearance is given after a set number of months post-surgery, usually ranging from six to nine months. However, emerging evidence and clinical consensus increasingly support a **criteria-based approach** that focuses on the athlete's functional recovery, rather than simply elapsed time.

Time-Based Approach: A Traditional Standard with Limitations

The time-based model of RTS is rooted in early rehabilitation protocols, which presumed that biological healing and graft maturation follow predictable timelines. Many clinicians and surgeons have used the "6-month rule" as a benchmark, assuming that most athletes would be ready to resume sport-specific activities by this time. However, this method has several limitations:

- **Biological variability:** Healing rates vary significantly between individuals based on graft type, surgical technique, preoperative status, and postoperative rehabilitation adherence.
- **Risk of reinjury:** Returning to sport solely based on time without functional readiness increases the likelihood of reinjury. Studies have shown that athletes returning to sport within 6 months have a **sevenfold greater risk of ACL graft rupture** compared to those who delay RTS until at least 9 months post-surgery.
- **Lack of individualized assessment:** Time-based approaches do not account for neuromuscular deficits, movement asymmetries, or psychological readiness—factors known to affect outcomes after ACLR.

Phases of Rehabilitation Before Return

Phase	Timeline	Goals	Key Interventions	Progression Criteria
Preoperative Phase	~3–6 weeks before surgery	- Reduce pain & swelling- Restore ROM, especially extension- Initiate muscle activation (quads)- Educate patient	- Cryotherapy- NMES for quad activation- ROM exercises- Isometrics- Gait training- Patient education	- Full knee extension- Minimal swelling- Volitional quad control- Mental readiness for surgery
Phase 1: Early Post-op Phase	0–6 weeks	- Protect the graft- Reduce inflammation- Regain ROM (esp. extension)- Activate quadriceps- Normalize gait	- Cryotherapy & compression- Patellar mobilization- Heel slides, active-assisted ROM- NMES- Gait training with crutches (WBAT)- Core stabilization	- Full extension- Flexion ≥ 90° by week 2- SLR without lag- Pain & swelling controlled
Phase 2: Intermediate Phase	6–12 weeks	- Improve lower limb strength- Enhance neuromuscular control- Begin proprioception work- Initiate cardiovascular conditioning	- Closed kinetic chain (CKC) exercises- Step-ups, leg press, mini squats- Balance training (Y-balance)- Stationary cycling, elliptical- Light resistance OKC (from 6 weeks onward for HS graft)	- LSI > 70% in quads- Proper movement control- Single-leg balance 30 sec- No swelling after activity
Phase 3: Advanced Strengthening	12–20 weeks	- Build muscular strength & endurance- Enhance dynamic stability- Progress proprioception & plyometric drills	- Progressive resistance training- Plyometrics: box jumps, tuck jumps- Agility drills: ladder, cones- Single-leg balance & perturbation training- BFR (if indicated)	- LSI > 80% in strength- Symmetric hop test performance- No instability with dynamic tasks

Phase 4: Return to Running & Impact	4–6 months	- Initiate controlled impact loading- Restore neuromuscular coordination- Gradual running progression	- Return to running protocols- Hops: single, triple, crossover- Jump-landing mechanics (LESS)- Progressive agility & directional drills	- Pass hop test battery (LSI ≥ 85%)- Proper biomechanics- Pain-free with activity- ACL-RSI ≥ 70
Phase 5: Sport-Specific Training	6–9 months	- Prepare for sport demands- Simulate game-specific activities- Improve confidence & skill level	- Cutting, pivoting, sport drills- High-speed running, deceleration- Fatigue-based drills- RTS psychological assessment	- LSI ≥ 90% on all tests- ACL-RSI ≥ 80- No effusion- Sport-specific clearance
Phase 6: Return to Sport / Performance	9–12+ months	- Return to pre-injury performance level- Prevent reinjury- Maintain strength & conditioning	- Full practice sessions- Competitive games- Injury prevention programs (e.g. FIFA 11+, PEP)- Maintenance strength & neuromuscular control	- Surgeon + therapist clearance- Full participation in training- No symptoms post-exercise- Completed prevention education

Criteria-Based Approach: A Functional and Individualized Model

The **criteria-based model** emphasizes the assessment of **functional recovery milestones** rather than a fixed postoperative timeline. Progression through rehabilitation and eventual RTS are based on the athlete meeting specific clinical, physical, and psychological benchmarks. These commonly include:

- **Quadriceps and hamstring strength** ≥90% limb symmetry index (LSI)
- **Functional hop test battery** (single hop, triple hop, crossover hop, timed hop) ≥90% LSI
- **Y-Balance Test** and other balance/proprioceptive measures
- **Movement quality** assessments, including jump landing mechanics (e.g., LESS score)
- **Psychological readiness**, using tools like the ACL-RSI (Return to Sport after Injury) scale, with a recommended score ≥80

This approach allows for a **personalized and evidence-informed pathway** to safely return to sport. Athletes who meet objective RTS criteria have significantly lower reinjury rates and improved long-term outcomes

Functional Testing for Return to Play

Return to sport (RTS) after an anterior cruciate ligament (ACL) injury or reconstruction is a high-stakes decision requiring objective evidence of physical, neuromuscular, and psychological readiness. Functional testing provides critical benchmarks for evaluating an athlete's ability to safely resume sport-specific activities. A battery of tests is often used to assess symmetry, strength, stability, and control, with a typical criterion being a **limb symmetry index (LSI) of ≥90%**.

- **Strength Testing**
- **Isokinetic Testing**

Isokinetic dynamometry remains the gold standard for quantifying lower limb muscle strength. It allows the measurement of **peak torque** across different speeds and helps detect side-to-side deficits post-ACLR.

RTP Criterion: LSI ≥90% for both quadriceps and hamstrings at multiple angular velocities (e.g., 60°/s and 180°/s).

Hamstring-to-Quadriceps Ratio (H:Q)

The H:Q ratio provides insight into muscle balance and dynamic knee stability. A low ratio may increase strain on the ACL during high-demand movements.

Normal H:Q Ratio: ≥0.6 is considered acceptable, though sport-specific targets may vary.

Hop Test Battery

The hop test battery evaluates power, dynamic balance, and limb symmetry in a functional and sport-relevant context.

- **Single Hop for Distance**: One maximal hop on the involved leg.
- **Triple Hop for Distance**: Three consecutive maximal hops in a straight line.
- **Crossover Hop for Distance**: Three hops while crossing over a center line.
- **6-Meter Timed Hop**: Time taken to hop 6 meters on one leg.

RTP Criterion: LSI ≥90% on all four tests, performed with controlled landings and without compensation.

Y-Balance Test

The Y-Balance Test is used to assess **dynamic balance and proprioception**. It challenges the athlete to maintain single-leg stance while reaching in three directions (anterior, posteromedial, posterolateral).

RTP Criteria:

- Anterior reach difference <4 cm between limbs
- Composite score ≥94% of limb length

Agility and Change of Direction (COD) Tests

Agility tests simulate the dynamic, unpredictable movements common in sports and assess an athlete's ability to **accelerate, decelerate, and change direction safely**.

Commonly used tests include:

- **T-Test**
- **Illinois Agility Test**
- **Modified 505 Agility Test**
- **Pro Agility Shuttle (5-10-5)**

RTP Criterion: Performance should be within 90–95% of the uninjured limb or match pre-injury benchmark times, with correct technique and no signs of instability [6].

Plyometric and Jump-Landing Assessments

Drop Vertical Jump (DVJ) Test

This test evaluates landing mechanics and neuromuscular control. Athletes drop from a box and land in a squat position, which is analyzed for valgus collapse, trunk lean, and ground reaction asymmetries.

Landing Error Scoring System (LESS)

LESS is a validated scoring system to assess quality of movement during jump landings. It focuses on knee alignment, trunk position, and foot placement.

- **RTP Criterion**: Low LESS scores (ideally ≤5 errors) indicate safe, efficient landing mechanics.

Psychological Readiness

Psychological recovery is equally important as physical readiness. Many athletes returning from ACL injury experience fear of reinjury, loss of confidence, and performance anxiety.

ACL-RSI SCALE

The **Anterior Cruciate Ligament–Return to Sport after Injury (ACL-RSI)** scale is a validated questionnaire that assesses an athlete's:

- Confidence in performance
- Fear of reinjury
- Emotional response to returning
- **RTP Criterion**: Score ≥80 is generally recommended for safe return.

Psychological barriers have been shown to significantly delay RTS and increase the risk of reinjury if left unaddressed.

Return to Sport Criteria and Guidelines after ACL Reconstruction

Domain	Criterion	Purpose/Justification
1. Strength Testing	LSI ≥ 90% for quadriceps and hamstrings (isokinetic or handheld dynamometry)	Ensures sufficient force production and dynamic joint stability
2. Hamstring-to-Quadriceps Ratio (H:Q)	H:Q ratio ≥ 0.6	Prevents dominance of quadriceps, reducing anterior tibial translation and graft stress
3. Hop Test Battery	LSI ≥ 90% on:• Single hop• Triple hop• Crossover hop• Timed hop	Evaluates limb power, neuromuscular control, and functional symmetry
4. Balance & Proprioception	Y-Balance Test:• Composite score ≥ 94%• Anterior reach difference <4 cm	Detects asymmetries and deficits in dynamic postural control
5. Landing Mechanics	• LESS score ≤ 5• No dynamic valgus or trunk lean	Assesses movement quality during jump-landing tasks
6. Agility & COD	• Symmetrical, stable performance• Within 90–95% of pre-injury values	Validates sport-specific readiness and control during directional changes
7. Psychological Readiness	ACL-RSI score ≥ 80	Ensures confidence, low fear of reinjury, and mental readiness
8. RTP Checklist (Composite)	All of the above passed	Reduces reinjury risk by up to 84%; failure in one domain increases risk 4x
9. Medical Clearance	• Surgeon: joint stability, graft integrity• Physiotherapist: functional criteria met	Ensures multidisciplinary consensus and athlete safety
10. Sport-Specific Performance	Full participation in training and drills without pain or instability	Confirms practical readiness for competitive environments

Risk Factors for Re-Injury

Despite advances in surgical techniques and rehabilitation protocols, **anterior cruciate ligament (ACL) re-injury** remains a significant concern, particularly among young, active individuals returning to high-demand sports. The risk of **graft rupture or contralateral ACL injury** is influenced by multiple intrinsic and extrinsic factors. Recognizing and addressing these risk factors is crucial in reducing recurrence and improving long-term functional outcomes.

1. Graft Choice

The choice of graft used in ACL reconstruction can significantly influence re-injury risk. Common graft types include:

- **Bone–Patellar Tendon–Bone (BPTB)**: Offers strong fixation and low failure rates, especially in high-demand athletes.
- **Hamstring Tendon (HT)**: Associated with **greater laxity** post-op and potentially higher graft rupture rates in younger athletes.
- **Quadriceps Tendon (QT)**: Emerging option with promising strength and graft size.
- **Allografts**: Linked to a **higher failure rate**, especially in younger, active populations, due to delayed biological incorporation.

2. Age and Sex

- **Young age (<25 years)** is one of the **strongest predictors** of ACL re-injury, particularly in pivoting sports.
- **Adolescents and early adults** are more likely to return to high-risk activities and may exhibit greater movement asymmetries and risk-taking behaviors.
- **Female athletes** demonstrate a higher incidence of initial ACL injuries, attributed to anatomical and hormonal differences, as well as biomechanical patterns (e.g., greater knee valgus and reduced hamstring activation).

3. Premature Return to Sport

Returning to sport **before 9 months post-op** is associated with a **significantly elevated risk** of ACL graft rupture and contralateral injury.

Grindem *et al.* (2016) found that **each month of delay in RTS up to 9 months reduced the risk of reinjury by 51%**. Premature RTS often occurs before achieving adequate strength, proprioception, and movement quality, placing the joint at greater biomechanical risk.

4. Inadequate Strength or Neuromuscular Control

Failure to restore **quadriceps and hamstring strength (LSI ≥90%)** and deficits in **neuromuscular control** are linked to poor movement mechanics during dynamic activities such as landing, cutting, or pivoting.

Common deficits include:

- Quadriceps inhibition
- Poor trunk control
- Increased knee valgus
- Asymmetrical loading during landing

5. Psychological Factors

Psychological readiness plays a critical role in RTS and injury prevention. Athletes who return to sport with **high fear of reinjury, low confidence, or emotional stress** are more likely to modify their movement patterns, hesitate during high-speed play, or avoid full effort—paradoxically increasing injury risk.

- **ACL-RSI (Return to Sport after Injury) scale** is commonly used to assess psychological readiness.
- Athletes with low ACL-RSI scores (<70) are **less likely to return** and **more likely to suffer re-injury.**

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PRE AND POST-OPERATIVE PHYSIOTHERAPY MANAGEMENT – AN OUTLINE

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- **Benefits of Pre-operative physiotherapy management**
  - ✓ Examination and evaluation of a patient's preoperative impairments and functional status to establish a baseline for documenting postoperative improvement
  - ✓ Opportunity to identify and prioritize a patient's needs and understand a patient's goals and functional expectations after surgery
  - ✓ A basis for establishing rapport for enhanced continuity of care after surgery
  - ✓ A mechanism for patient education about the scheduled surgery and the components of postoperative rehabilitation
- **Components of Preoperative Patient Education**
  - ✓ Overview of the plan of care.

    Explain the general physiotherapy plan of care the patient can expect during the postoperative period.
  - ✓ Postoperative precautions.

    Advise the patient of any precautions or contraindications to positioning, movement, or weight bearing that must be followed postoperatively.
  - ✓ Bed mobility and transfers.

    Teach the patient how to move in bed or perform wheelchair transfers safely, incorporating necessary postoperative precautions.
  - ✓ Wound care.

    Explain or reinforce postoperative care of the incision for optimal wound healing.
  - ✓ Initial postoperative exercises.

    Teach the patient any exercises that will be started during the very early post-operative period.

    Example:
    - ➢ Breathing exercise
    - ➢ Splinted coughing

- Incentive spirometer etc

Post-operative physiotherapy management

A well-planned physiotherapy program, composed of a carefully progressed sequence of therapeutic exercise and functional training and ongoing patient education, is fundamental to the patient's postoperative care.

Appropriate physiotherapeutic management takes many factors into consideration, any of which may affect the components and progression of a patient's postoperative program.

To design a safe, effective, efficient rehabilitation program for a patient,

A therapist must

- Understand the indications and rationale for a particular surgical procedure,
- Become familiar with the procedure itself,
- Be aware of special precautions related to the surgery, and
- Communicate effectively with the patient and surgeon.

Every individually designed postoperative rehabilitation program must be based on initial and ongoing examinations of a patient. In addition to the components of a pre-operative examination noted previously in this section, an assessment of integumentary integrity is important after surgery.

The incision should be inspected before and after each exercise session to identify any evidence of wound infection or delayed healing.

Inspection of the Surgical Incision

- ✓ Check for signs of redness or tissue necrosis along the incision(s) and around sutures.
- ✓ Palpate along the incision and note signs of tenderness and oedema.
- ✓ Palpate to determine evidence of increased heat.
- ✓ Check for signs of drainage; note colour and amount of drainage on the dressing.
- ✓ Note the integrity of an incision across a joint during and after exercise.
- ✓ As the incision heals, check the mobility of the scar

Aims of post-operative physiotherapy management

- ✓ To minimize / prevent post-operative pulmonary complications
- ✓ To reduce incision pain
- ✓ To minimize or prevent post-operative circulatory complications
- ✓ To maintain functional mobility while protecting the operative site.
- ✓ Incision or wound care
- ✓ To prevent development of pressure sore
- ✓ To restore exercise tolerance

To minimize / prevent post-operative pulmonary complications

- Breathing exercises:

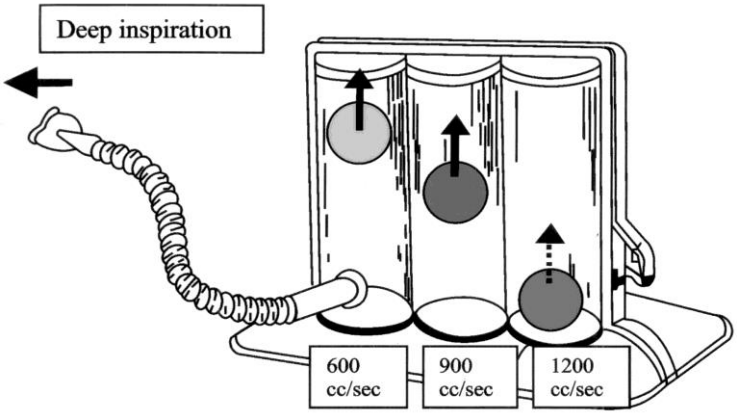
  Diaphragmatic breathing

  Segmental expansion (Focus on Lateral costal breathing, posterior basal breathing)

  Thoracic mobility

- Incentive Spirometer

  Incentive Spirometer enhances lung expansion via spontaneous and sustained decrease in pleural pressure. The basic manoeuvre of incentive spirometry is sustained maximal inspiration. Sustained maximal inspiration is a slow, deep inhalation from Functional Residual Capacity up to Total Lung Capacity followed by 5 to 10 second of breath hold (breath hold to improve collateral circulation).



  Two types of incentive spirometer available, volume and flow and it provide visual cues to patients when desired inspiratory flow or volume achieved.

- Frequent re-positioning and
- Early Mobilization also has important role in preventing post-operative pulmonary complications.
- If patient have a secretion
  - ✓ Postural drainage (with all precautions)
  - ✓ Splinted Coughing
  - ✓ ACBT

To reduce incision pain

- Trans Electrical Nerve Stimulation (TENS):

  High frequency TENS at least for 20 minutes has beneficial effects in pain reduction.

  Note: Dosage and method of application needs to mention as given for high TENS

  (From TENS note)

- Relaxed positions
- Patient educations regarding any precautions or contraindications to positioning, movement, or weight bearing or splinted coughing that must be followed postoperatively.

To minimize or prevent post-operative circulatory complications

- Ankle – Toe movement
- Early or bed site mobilizations
- Compressive stockings / elevations etc for oedema

To maintain functional mobility while protecting the operative site

- Passive / Active assisted or Active ROM exercises according to patient condition

To prevent development of pressure sore

- Frequent change in positions
- Bed site mobilization

To restore exercise tolerance

- Early mobilization
- Aerobic exercises according patient condition

Establish a mobile scar

- Gentle massage across and around the maturing scar

Home advice

- Educational counselling
- Importance of exercise & to be continued at home also
- Mobility and posture maintenance
- Energy conservation during ADL's
- Ergonomics Advice

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NUTRITION AS A PILLAR OF SUPPORT IN HEPATIC TRANSPLANTATION: FROM MALNUTRITION TO METABOLIC STABILITY

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Introduction:

Malnutrition is a prevalent and serious complication of end-stage liver disease (ESLD), affecting 50%–90% of patients and worsening with disease severity[1]. Sarcopenia—loss of skeletal muscle mass and function—affects 30%–70% of ESLD patients[2]. These conditions increase waitlist and post-transplant mortality, with pooled hazard ratios of 1.72 and 1.84, respectively[2]. Malnourished candidates show reduced albumin, BMI, grip strength, and higher rates of complications such as infections and hepatic encephalopathy[4–5].

The metabolic environment in ESLD is marked by "accelerated starvation," driven by depleted glycogen, increased gluconeogenesis, fatty acid oxidation, and muscle catabolism[6]. Hypermetabolism, insulin resistance, systemic inflammation, and hormonal changes (e.g., low testosterone) worsen muscle breakdown[7–8]. Nutritional assessment is critical but often overlooked due to confounding factors like ascites and edema. Tools like the Royal Free Hospital-Nutritional Prioritizing Tool (RFH-NPT), Subjective Global Assessment (SGA), and CT-based muscle indices are more accurate than traditional BMI[9–10].

Clinical societies, including ESPEN, EASL, ASPEN, and AASLD, recommend early nutritional intervention, targeting 30–40 kcal/kg/day and 1.2–1.5 g/kg/day protein[11–12]. Strategies such as frequent small meals and late-night snacks help reduce protein catabolism. Post-transplant, continued challenges—sarcopenia, weight gain, diabetes, and metabolic syndrome—demand sustained dietary and physical interventions for metabolic stability[15–16].

Pathophysiology of Malnutrition and Sarcopenia in ESLD

Malnutrition in ESLD stems from disrupted macronutrient metabolism, inflammation, hormonal imbalances, and gastrointestinal dysfunction[17]. ESLD induces hypermetabolism, with resting energy expenditure (REE) 120%–140% of predicted, particularly in those with ascites and inflammation[18]. Hepatic glycogen depletion shifts metabolism to amino acid-driven gluconeogenesis, accelerating muscle loss[19].

Insulin resistance—present in nearly 80% of cirrhotic patients—impairs glucose oxidation and muscle protein synthesis, compounding sarcopenia[20]. Inflammatory cytokines (TNF-α, IL-6, TGF-β) activate muscle degradation via the ubiquitin–proteasome pathway[21]. Hormonal deficits (testosterone, growth hormone) further reduce muscle anabolism[22–23].

Cholestasis limits bile acid secretion, reducing fat and fat-soluble vitamin absorption. Portal hypertension causes gut edema and bacterial overgrowth, impairing nutrient assimilation[24]. Hypoalbuminemia, ascites, anorexia, and taste alterations also reduce oral intake[25]. Reduced branched-chain amino acids (BCAA) and elevated aromatic amino acids disturb nitrogen balance and neurotransmission, especially in hepatic encephalopathy[26].

These factors create a vicious cycle of frailty and decompensation, requiring a multifaceted intervention including nutrition, exercise, and early transplantation referral[27].

Nutritional Assessment in Liver Transplant Candidates

Due to fluid overload and altered metabolism, conventional markers of nutrition are inadequate in ESLD. A combined approach using clinical, anthropometric, biochemical, and radiologic parameters offers better assessment.

1. Clinical and Functional Assessment

SGA and RFH-NPT integrate weight history, intake, GI symptoms, and signs of wasting. RFH-NPT has superior predictive validity; severely malnourished scores correlate with higher post-transplant complications[3]. Handgrip strength (HGS), a proxy for muscle function, predicts mortality and is included in the Liver Frailty Index (LFI)[4]. Gait speed and chair-stand tests assess fall risk and functional capacity.

2. Anthropometry and Body Composition

BMI is unreliable due to ascites and edema. Mid-arm muscle circumference (MAMC) and triceps skinfold (TSF) are alternatives but less precise[5]. CT or MRI at L3 vertebra quantifies skeletal muscle index (SMI), the gold standard for sarcopenia diagnosis[6–7]. Sarcopenia is defined as SMI $<50\ cm^2/m^2$ (men) or $<39\ cm^2/m^2$ (women)[6]. Myosteatosis (fat infiltration in muscle) independently predicts poor outcomes[8].

3. Biochemical Markers

Albumin and prealbumin reflect hepatic function more than nutritional status[9]. Micronutrient deficiencies—especially vitamin D, zinc, selenium, and B-vitamins—are prevalent, leading to fatigue, cognitive dysfunction, and falls[10]. Up to 77% of liver transplant candidates have vitamin D deficiency, associated with sarcopenia and osteopenia[11].

4. Frailty and Sarcopenia Indexes

Frailty tools like the LFI, Fried Frailty Phenotype, and Clinical Frailty Scale are now integral to transplant evaluation. AASLD recommends routine frailty assessment to guide nutrition and rehabilitation strategies[12–13].

Pre-Transplant Nutritional Optimization Strategies

1. Energy and Macronutrient Needs

Hypermetabolism in 30%–60% of cirrhotics necessitates 30–35 kcal/kg/day and 1.2–1.5 g/kg/day protein (dry weight)[2–3]. Protein restriction is no longer indicated, even in hepatic

encephalopathy, as it exacerbates sarcopenia[4]. Frequent meals and late-night snacks rich in carbohydrates and proteins reduce nocturnal catabolism[5]. One study found that a carbohydrate-rich night snack improved nitrogen balance and reduced ammonia levels[6].

2. Route of Nutritional Support

Oral nutrition is preferred. Oral nutritional supplements (ONS), especially BCAA-enriched formulas, benefit those with reduced intake and hepatic encephalopathy[7]. If intake is <60% of requirements, enteral nutrition (EN) is advised, preserving gut integrity and reducing infection risk[8]. In cases of gastric varices or gastroparesis, nasojejunal feeding is safer[9].

Parenteral nutrition (PN) is a last resort when EN is not feasible. Though effective, it carries risks of infection, hyperglycemia, and fluid overload, warranting cautious use[10].

3. Micronutrient Repletion

Targeted correction of deficiencies is essential:

- **Vitamin D**: Supplement 1000–4000 IU/day; improves muscle and bone health[11].
- **Zinc**: Reduces ammonia and improves hepatic encephalopathy symptoms[12].
- **Thiamine & Folate**: Crucial in alcoholic liver disease to prevent Wernicke's encephalopathy and anemia[13].
- **Selenium, Magnesium, Phosphate**: Needed for muscle, neurologic, and cardiac function[14].

Post-Transplant Nutritional Challenges

Post-transplant, patients often gain excessive weight and develop metabolic syndrome due to immunosuppressants and reduced activity. Persistent sarcopenia also occurs, especially in those with preoperative muscle wasting[15].

To address this, nutritional surveillance, individualized exercise plans, and adherence to a Mediterranean-style diet are recommended for long-term health and graft success[16]. Diets rich in fruits, vegetables, whole grains, healthy fats, and lean proteins help counteract metabolic derangements.

Addressing Sarcopenia and Frailty

Sarcopenia, a prevalent complication in liver transplant candidates, cannot be reversed by nutrition alone. A multimodal approach that includes physical rehabilitation is essential. Supervised resistance training and aerobic exercises have been proven to improve muscle mass, functional capacity, and transplant eligibility[15]. Lai *et al.* demonstrated that a structured prehabilitation program significantly improved the Liver Frailty Index and reduced waitlist dropout[16]. Emerging strategies such as creatine supplementation and testosterone therapy show potential in enhancing muscle mass in hypogonadal cirrhotic men, but their routine use requires further validation[17].

Nutrition in Special Populations

Certain subgroups, such as those with refractory ascites or hepatorenal syndrome, require tailored nutrition plans to avoid fluid overload and manage electrolyte imbalances[18]. Patients with cholestatic liver diseases often face fat-soluble vitamin deficiencies, necessitating specialized oral nutrition supplements and multivitamin preparations[19]. For alcohol-related liver disease, nutritional therapy must be integrated with addiction counseling. Alcohol abstinence alone markedly improves nutritional status and transplant readiness[20].

Perioperative and Post-Transplant Nutrition

During the perioperative phase, early enteral nutrition (within 12–24 hours) is advised for hemodynamically stable patients, as it reduces infections, maintains gut integrity, and shortens ICU stays[1–3]. If enteral feeding is not feasible, parenteral nutrition may be considered briefly, but carries infection and metabolic risks[4]. A balanced PN formula (60:40 glucose:lipid) with tight glucose control is critical[5]. Energy needs in this phase are elevated, requiring 30–35 kcal/kg/day and 1.3–1.5 g protein/kg/day[6].

Post-transplant, many patients remain sarcopenic due to immunosuppressants and prolonged inactivity[7]. Resistance exercise and high-protein diets can accelerate muscle recovery[8]. Immunosuppressants like corticosteroids and tacrolimus can induce hyperglycemia, weight gain, and dyslipidemia, contributing to post-transplant diabetes and metabolic syndrome in over 40% of recipients[9]. Up to 70% gain more than 10% body weight in the first year[10], raising cardiovascular risks[11]. Caloric moderation, low glycemic diets, and Mediterranean-style nutrition help manage these complications[12].

Long-Term Nutritional Strategies

Post-transplant, many patients shift from undernutrition to overnutrition. Corticosteroid-induced appetite increases lead to fat gain, often resulting in sarcopenic obesity[4]. Structured nutrition counseling should promote portion control, macronutrient balance, and regular physical activity[5]. Metabolic syndrome affects 50% of recipients and increases cardiovascular risk[6–7]. Mediterranean diets rich in fiber and omega-3s have shown positive outcomes[8–9]. Glycemic control is vital, particularly for those with post-transplant diabetes mellitus, affecting up to 30% of patients[10–11].

Bone health requires attention, as corticosteroids and vitamin D deficiency contribute to osteoporosis. Supplementation, DEXA monitoring, and resistance training are recommended[12]. Muscle regeneration remains slow, needing long-term protein-rich diets and exercise[13].

Multidisciplinary and Psychosocial Support

Successful long-term care relies on a team of hepatologists, dietitians, physiotherapists, and psychologists. Regular assessments and behavioral support improve dietary adherence and

outcomes. Psychosocial barriers such as taste changes or cultural preferences must be addressed sensitively[14]. Telehealth and mobile apps are emerging tools for sustained support[15].

Conclusion:

Nutrition is a foundational component of liver transplant care, from prehabilitation to lifelong metabolic stability. Individualized and multidisciplinary strategies are crucial in optimizing graft survival, reducing complications, and improving patient quality of life.

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PRESERVING QUALITY: TRACING THE CAUSES AND CURBING THE CONSEQUENCES OF FOOD SPOILAGE

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1. Introduction to Food Spoilage

Food spoilage is a critical process that affects the quality, safety, and availability of food, with far-reaching implications for human health, economic stability, and environmental sustainability. This section defines food spoilage, explores its significance in food safety and sustainability, and outlines the scope of the chapter, drawing on both historical and recent research to provide a comprehensive foundation for understanding this complex phenomenon.

1.1 Definition of Food Spoilage

Food spoilage refers to the deterioration of food that renders it unsafe, unpalatable, or unsuitable for consumption due to changes in its sensory, nutritional, or safety characteristics. These changes are evident through off-odors (e.g., sour smells in milk or putrid odors in meat), altered textures (e.g., sliminess in fish or softening in fruits), discoloration (e.g., browning in apples or greening in potatoes), or visible microbial growth, such as mold on bread or cheese (Jay *et al.,* 2005). Spoilage is driven by biological, chemical, and physical processes. Biologically, microorganisms like bacteria (Pseudomonas, Lactobacillus), yeasts (Candida), and molds (Aspergillus, Penicillium) proliferate under favorable conditions, producing metabolites like volatile organic compounds that alter sensory properties (Jay *et al.,* 2005). For example, Pseudomonas species are notorious for causing slime and off-odors in refrigerated meats, while molds can produce mycotoxins that compromise safety.

Historically, spoilage was defined primarily by sensory changes and microbial activity, as described by Mossel *et al.* (1995), who emphasized the role of microbial metabolism in quality degradation. Recent research expands this definition to include subtler forms of spoilage, such as nutrient loss without visible signs. For instance, Wang *et al.* (2023) found that oxidative processes degrade essential nutrients like vitamin C in stored fruits and vegetables before sensory changes become apparent, highlighting the need for early detection methods. Spoilage mechanisms vary by food type: high-moisture foods like dairy or seafood spoil rapidly due to bacterial growth, often within days if not refrigerated, while low-moisture foods like grains or

nuts degrade more slowly through chemical reactions like lipid oxidation (Labuza, 1971). The Food and Agriculture Organization (FAO, 2011) notes that spoilage affects both perishable and non-perishable foods, with factors like water activity (typically >0.85 for microbial growth), pH (optimal 4.5–7 for bacteria), and storage conditions determining the rate and type of deterioration. Understanding these diverse pathways is essential for developing effective spoilage prevention strategies.

1.2 Importance of Understanding Spoilage in Food Safety and Sustainability

Understanding food spoilage is vital for ensuring food safety, reducing economic losses, and promoting environmental sustainability. From a safety perspective, spoilage often signals the presence of harmful microorganisms or toxins that can pose health risks. For example, molds like Aspergillus flavus produce aflatoxins in grains and nuts, which are carcinogenic and can cause severe illness even in small quantities (Pitt & Hocking, 2009). Early studies by Gram *et al.* (2002) highlighted that conditions favoring spoilage, such as improper storage at temperatures above 4°C, also enable pathogens like Salmonella or Listeria to proliferate, increasing the risk of foodborne illnesses. By identifying spoilage mechanisms, food producers can implement controls like temperature regulation, preservatives, or modified atmosphere packaging to mitigate these risks, ensuring consumer safety.

Economically, spoilage contributes to significant global food losses, impacting farmers, retailers, and consumers. According to the FAO (2011), approximately 1.3 billion tons of food—roughly one-third of global production—are lost or wasted annually, with spoilage being a major contributor, particularly in developing countries where cold chain infrastructure is often inadequate. For instance, postharvest spoilage of fruits and vegetables can account for up to 50% of losses in tropical regions due to poor storage facilities (Prusky, 2011). Recent research by Zhang *et al.* (2023) estimates that optimizing spoilage prevention strategies, such as improved cold chain logistics, could save 20–30% of perishable goods, reducing economic losses across the supply chain. These losses not only affect profitability but also drive-up food prices, disproportionately impacting low-income communities.

From a sustainability perspective, spoilage exacerbates resource waste and environmental degradation. Producing food requires substantial inputs of water, energy, and land, and when food spoils, these resources are wasted. The FAO (2011) reports that food waste, including spoilage, contributes to 8% of global greenhouse gas emissions, equivalent to the emissions of entire countries like Russia or Japan. Spoiled food often ends up in landfills, where it decomposes and releases methane, a greenhouse gas 25 times more potent than carbon dioxide (Porat *et al.,* 2021). Reducing spoilage through better storage practices or consumer education

could significantly lower these environmental impacts. Moreover, spoilage undermines food security, particularly in regions where access to fresh food is limited. Recent studies by Li *et al.* (2024) warn that climate change, with rising global temperatures, accelerates spoilage by enhancing microbial and chemical processes, necessitating innovative solutions like smart sensors or sustainable packaging. Addressing spoilage aligns with the United Nations' Sustainable Development Goal 12.3, which aims to halve food waste by 2030, making it a cornerstone of sustainable food systems.

1.3 Overview of the Chapter's Scope

This chapter provides a comprehensive examination of food spoilage, addressing its causes, methods for tracing it, contributing factors, and mitigation strategies to equip readers with a holistic understanding. The causes of spoilage are categorized into microbial, chemical, and physical factors. Microbial spoilage, driven by bacteria, yeasts, and molds, is explored through foundational research by Jay *et al.* (2005) and recent insights into microbial biofilms, which enhance resistance to preservatives (Odeyemi *et al.,* 2020). Chemical spoilage, including lipid oxidation and enzymatic browning, is analyzed using early work by Labuza (1971) and recent studies on reactive oxygen species in high-fat foods (Wang *et al.,* 2023). Physical factors, such as temperature fluctuations and mechanical damage, are discussed with reference to transportation challenges (James *et al.,* 2008) and light-induced degradation in dairy products (Li *et al.,* 2022).

Tracing spoilage involves both traditional and modern approaches. Sensory indicators, like off-odors or texture changes, are reliable markers, as studied by Dainty (1996), while chemical markers, such as biogenic amines in fish, are validated by recent research (Kim *et al.,* 2024). Advanced technologies, including time-temperature indicators (Taoukis, 2010), Internet of Things (IoT) sensors for real-time monitoring (Bouzembrak *et al.,* 2019), and blockchain for supply chain traceability (Feng *et al.,* 2022), are transforming spoilage detection. Environmental and human factors, such as improper storage conditions or consumer mishandling, are examined using insights from Walker and Betts (2000) and household waste studies (Porat *et al.,* 2021).

Mitigation strategies focus on practical solutions to extend shelf life and reduce waste. These include advanced packaging techniques like vacuum sealing (McMillin, 2017), robust cold chain management (Mercier *et al.,* 2017), and consumer education initiatives, such as the USDA's FoodKeeper app (USDA, 2020). The chapter concludes with future directions, emphasizing emerging technologies like AI-driven spoilage prediction (Li *et al.,* 2024) and sustainable packaging innovations to enhance food security and minimize environmental impacts. By integrating historical and recent research, this chapter aims to provide actionable

insights for food industry professionals, researchers, and policymakers, while fostering awareness among consumers to reduce food waste.

2. Causes of Food Spoilage

Food spoilage is driven by a complex interplay of microbial, chemical, and physical factors that degrade food quality, safety, and nutritional value. This section explores these causes in detail, focusing on the role of microorganisms, chemical reactions, and physical influences, supported by historical and recent research. Tables and a chart are included to enhance understanding of key spoilage mechanisms and their contributing factors.

2.1 Microbial Factors

Microbial activity is the primary driver of food spoilage, with bacteria, yeasts, and molds altering sensory properties, nutritional content, and safety. These microorganisms thrive under specific environmental conditions, producing off-odors, undesirable textures, and sometimes toxins, making food unpalatable or hazardous.

2.1.1 Role of Bacteria, Yeasts, and Molds

Bacteria are the most significant contributors to spoilage in high-moisture foods such as meat, dairy, and seafood. Pseudomonas species, for example, are notorious for causing sliminess and sour or putrid odors in refrigerated meats stored under aerobic conditions (Jay *et al.,* 2005). These bacteria break down proteins and lipids, producing volatile compounds like amines that result in off-flavors. Lactic acid bacteria, such as Lactobacillus and Leuconostoc, spoil dairy products like milk and yogurt by producing lactic acid, leading to sour tastes and curdling (Gram *et al.,* 2002). In seafood, bacteria like Shewanella contribute to fishy odors through the reduction of trimethylamine oxide to trimethylamine, a hallmark of spoilage (Gram *et al.,* 2002).

Yeasts play a significant role in spoiling sugary or acidic foods, such as fruits, juices, and fermented beverages. Candida and Saccharomyces species ferment sugars, producing alcohol and carbon dioxide, which cause off-flavors and fizziness in products like fruit juices (Pitt & Hocking, 2009). For example, yeast spoilage in soft drinks can lead to container swelling or bursting due to gas production. Molds, including Aspergillus, Penicillium, and Rhizopus, are major spoilage agents in grains, nuts, and baked goods. They produce visible mycelia and, in some cases, mycotoxins like aflatoxins, which are carcinogenic and pose significant health risks (Pitt & Hocking, 2009). For instance, Aspergillus flavus contamination in peanuts can render them unsafe for consumption.

Recent research highlights the role of microbial biofilms in exacerbating spoilage. Biofilms are structured communities of microorganisms encased in a protective matrix, adhering to food surfaces or processing equipment (Odeyemi *et al.,* 2020). These biofilms enhance

microbial resistance to cleaning agents and preservatives, leading to persistent spoilage issues in food processing environments, such as seafood or dairy facilities. A study by Odeyemi *et al.* (2020) found that biofilms in fish processing plants contribute to recurrent Pseudomonas contamination, reducing shelf life and increasing waste. Advances in microbial detection, such as polymerase chain reaction (PCR), have improved identification of spoilage organisms, enabling targeted interventions (Settanni & Corsetti, 2007).

2.1.2 Conditions Promoting Microbial Growth

Microbial growth is heavily influenced by environmental conditions, including temperature, moisture, and pH. Most spoilage bacteria thrive in the temperature range of 4–60°C, with optimal growth between 20–40°C, known as the "danger zone" (Mossel *et al.,* 1995). Refrigeration below 4°C slows bacterial growth, but psychrotrophic bacteria like Pseudomonas and Listeria can still proliferate, causing spoilage in chilled foods like milk or meat (Jay *et al.,* 2005). For example, improper refrigeration at 7°C can reduce the shelf life of milk from weeks to days due to bacterial proliferation.

Moisture, measured as water activity (a_w), is another critical factor. Most bacteria require a_w > 0.85 to grow, making high-moisture foods like seafood and fresh produce particularly susceptible (Pitt & Hocking, 2009). Yeasts and molds, however, can tolerate lower water activity, with some molds growing at a_w as low as 0.6, enabling spoilage in drier foods like bread or nuts (Pitt & Hocking, 2009). pH also plays a significant role: bacteria prefer neutral to slightly acidic conditions (pH 4.5–7), while yeasts and molds thrive in more acidic environments, spoiling foods like fruits and pickles (Mossel *et al.,* 1995). Recent studies by Kim *et al.* (2024) highlight that temperature abuse during transportation, such as exposure to ambient conditions, accelerates microbial spoilage in perishable goods, particularly fish, leading to significant quality losses.

Table 1: Conditions Promoting Microbial Spoilage

Factor	Bacteria	Yeasts	Molds
Temperature	4–60°C (optimal 20–40°C)	10–35°C	10–35°C
Water Activity	>0.85	>0.80	>0.60
pH Range	4.5–7	3–8	2–8
Example Foods	Meat, dairy, seafood	Fruits, juices, beverages	Grains, nuts, bread

2.2 Chemical Factors

Chemical reactions, independent of microbial activity, significantly contribute to food spoilage by altering flavor, texture, color, and nutritional quality. These reactions primarily

involve oxidation and enzymatic processes, leading to phenomena like lipid rancidity and browning.

2.2.1 Oxidation and Enzymatic Reactions

Oxidation occurs when oxygen reacts with food components, particularly lipids, proteins, and vitamins, leading to quality degradation. Lipid oxidation is a primary spoilage mechanism in foods containing fats, such as oils, nuts, and fatty fish. It produces reactive compounds like peroxides and aldehydes, resulting in rancid odors and off-flavors (Labuza, 1971). For example, in fish oils, oxidation of unsaturated fatty acids creates a characteristic "fishy" smell that renders the product unpalatable. Recent research by Wang *et al.* (2023) highlights the role of reactive oxygen species (ROS) in accelerating oxidative spoilage, particularly in high-fat foods stored under warm conditions. ROS can degrade not only lipids but also vitamins like ascorbic acid, reducing nutritional value before sensory changes are evident.

Enzymatic reactions, driven by naturally occurring enzymes in food, also cause spoilage. Polyphenol oxidase (PPO) in fruits and vegetables, such as apples and potatoes, catalyzes enzymatic browning when tissues are cut or bruised, exposing them to oxygen (McEvily *et al.,* 1992). This reaction produces melanins, causing brown discoloration and off-flavors. In seafood, enzymes like proteases break down proteins post-mortem, leading to texture softening and amine production, contributing to spoilage odors (Jay *et al.,* 2005). Recent studies by Fang *et al.* (2023) suggest that antioxidant additives, such as ascorbic acid or rosemary extract, can inhibit oxidative and enzymatic reactions, extending shelf life in processed foods.

2.2.2 Lipid Rancidity and Browning Reactions

Lipid rancidity encompasses two main types: hydrolytic and oxidative. Hydrolytic rancidity occurs when lipases, either endogenous or microbial, break down triglycerides into free fatty acids, producing unpleasant odors and tastes, as seen in rancid butter (Labuza, 1971). Oxidative rancidity, more common in unsaturated fats, results from oxygen reacting with double bonds in fatty acids, forming peroxides and secondary compounds like aldehydes (Labuza, 1971). This process is particularly problematic in foods like nuts and fried snacks, where rancidity reduces shelf life and consumer acceptability.

Browning reactions, both enzymatic and non-enzymatic, further contribute to spoilage. Enzymatic browning, as studied by McEvily *et al.* (1992), occurs in fruits like bananas and vegetables like lettuce, where PPO catalyzes the oxidation of phenolic compounds, leading to brown pigments. Non-enzymatic browning, such as the Maillard reaction, occurs during storage of processed foods like baked goods or dried milk, involving reactions between sugars and amino acids (Wang *et al.,* 2023). While Maillard reactions can enhance flavor in controlled

settings (e.g., baking), uncontrolled reactions during storage produce off-flavors and reduce nutritional quality by degrading essential amino acids. Recent advancements in packaging, such as oxygen scavengers, show promise in mitigating these reactions (Fang *et al.,* 2023).

Table 2: Chemical Spoilage Mechanisms

Reaction Type	Mechanism	Affected Foods	Outcome
Oxidation	Oxygen reacts with lipids/proteins	Oils, nuts, fish	Rancid odors, nutrient loss
Enzymatic Browning	PPO oxidizes phenolics	Apples, potatoes	Brown discoloration, off-flavors
Maillard Reaction	Sugars react with amino acids	Baked goods, dried milk	Off-flavors, nutrient degradation

2.3 Physical Factors

Physical factors, including environmental conditions and handling practices, exacerbate spoilage by creating conditions that favor microbial and chemical degradation. These factors are often preventable through proper storage and handling techniques.

2.3.1 Temperature Fluctuations and Improper Storage

Temperature fluctuations during storage or transportation significantly accelerate spoilage by promoting microbial growth and chemical reactions. Perishable foods like dairy, meat, and seafood require consistent storage below 4°C to minimize bacterial proliferation (James *et al.,* 2008). For example, milk stored at 7°C can spoil within days due to Pseudomonas growth, compared to weeks at 2°C (Jay *et al.,* 2005). Temperature abuse, such as exposure to ambient conditions during transportation, is a major issue in supply chains, particularly in developing countries with limited cold chain infrastructure (Zhang *et al.,* 2023). High humidity (>70%) also promotes mold growth in grains and baked goods, as molds thrive in moist environments (Pitt & Hocking, 2009). Recent data by Zhang *et al.* (2023) estimate that temperature-related spoilage accounts for 20–30% of global food losses, highlighting the need for improved cold chain logistics.

Improper storage conditions, such as inadequate refrigeration or poor ventilation, exacerbate spoilage. For instance, storing fruits in sealed plastic bags can trap ethylene gas, accelerating ripening and spoilage (Prusky, 2011). Recent advancements in smart sensors, which monitor temperature and humidity in real time, are helping to address these issues by alerting suppliers to storage deviations (Bouzembrak *et al.,* 2019).

2.3.2 Physical Damage and Exposure to Light or Air

Physical damage, such as bruising, crushing, or cutting during harvest or transportation, compromises food integrity, creating entry points for microorganisms. Bruised apples or tomatoes are more susceptible to fungal infections like Penicillium expansum, which causes blue mold rot (Prusky, 2011). Mechanical damage also triggers enzymatic reactions, such as PPO activity, leading to browning and quality loss (McEvily *et al.,* 1992). Exposure to light degrades photosensitive compounds, such as riboflavin in milk or chlorophyll in oils, causing off-flavors and nutrient loss (Li *et al.,* 2022). For example, milk exposed to fluorescent light develops a "cardboard" flavor due to riboflavin degradation.

Exposure to air accelerates oxidation, particularly in high-fat foods, exacerbating lipid rancidity (Labuza, 1971). Packaging failures, such as non-airtight seals, allow oxygen and moisture ingress, promoting both microbial and chemical spoilage (Robertson, 2012). Recent studies by Li *et al.* (2022) demonstrate that UV light exposure in dairy products reduces shelf life by degrading pigments and nutrients. Innovations like vacuum sealing and modified atmosphere packaging (MAP) reduce oxygen exposure, mitigating these effects (McMillin, 2017).

3. Tracing Food Spoilage

Tracing food spoilage is essential for identifying quality degradation, ensuring food safety, and reducing waste in the food supply chain. This section explores traditional and modern methods for detecting spoilage, focusing on sensory and chemical indicators and advanced technologies like sensors, molecular techniques, and blockchain systems. Historical and recent research are integrated to provide a comprehensive understanding, with tables and a chart to enhance clarity.

3.1 Indicators of Spoilage

Detecting food spoilage relies on observable and measurable indicators that signal quality deterioration. Sensory changes and chemical markers are the primary methods used traditionally, offering practical and accessible ways to assess food condition for producers, retailers, and consumers.

3.1.1 Sensory Changes (Odor, Texture, Color, Taste)

Sensory changes are the most intuitive indicators of spoilage, as they can be detected by human senses without specialized equipment. Odor is often the first sign of spoilage, with off-odors like sourness in milk, putridity in meat, or fishy smells in seafood signaling microbial activity (Dainty, 1996). For example, Pseudomonas bacteria in refrigerated meats produce volatile sulfur compounds, resulting in a rotten egg smell (Jay *et al.,* 2005). Texture changes, such as sliminess in fish or softening in fruits, are also common. Lactic acid bacteria in dairy

products cause curdling, altering texture and making products like yogurt grainy or lumpy (Gram *et al.,* 2002). Color changes, such as browning in apples or greening in potatoes, indicate enzymatic or oxidative spoilage, while mold growth on bread appears as visible green or white patches (Pitt & Hocking, 2009). Taste alterations, like bitterness in rancid oils or sourness in spoiled milk, further confirm spoilage, though tasting is less common due to safety concerns.

Historically, sensory evaluation has been a cornerstone of spoilage detection, as outlined by Dainty (1996), who emphasized the reliability of sensory panels in identifying spoilage in meats through odor and appearance. However, sensory methods are subjective and depend on trained evaluators or consumer perception, which can vary. Recent studies by Kim *et al.* (2024) validate sensory indicators by correlating them with microbial counts in fish, noting that fishy odors appear when bacterial levels exceed 10^7 CFU/g. Sensory changes are particularly valuable for consumers, who rely on visual and olfactory cues to assess food freshness at home. For example, the USDA (2020) provides guidelines for consumers to recognize spoilage in perishable goods, such as checking for off-odors in poultry or sliminess in seafood. Despite their accessibility, sensory methods are limited by their lack of precision and inability to detect early spoilage before sensory changes are evident, necessitating complementary chemical markers.

3.1.2 Chemical Markers (pH Changes, Volatile Compounds)

Chemical markers provide objective measures of spoilage, often detecting changes before sensory signs are apparent. pH changes are a reliable indicator, particularly in protein-rich foods like meat and dairy. Microbial metabolism, such as lactic acid production by Lactobacillus in milk, lowers pH, causing souring and curdling (Gram *et al.,* 2002). In seafood, bacterial breakdown of proteins produces ammonia, increasing pH and indicating spoilage (Jay *et al.,* 2005). Early research by Mossel *et al.* (1995) established pH as a key spoilage marker, with normal pH ranges for fresh meat (5.5–6.0) shifting to 6.5 or higher in spoiled products.

Volatile organic compounds (VOCs) are another critical chemical marker. These compounds, produced by microbial or chemical processes, include ethyl acetate (fruity odors in spoiled fruits), trimethylamine (fishy odors in seafood), and sulfur compounds (rotten odors in meat) (Dainty, 1996). Gas chromatography-mass spectrometry (GC-MS) has been used to identify VOC profiles associated with spoilage, as demonstrated by Dainty (1996) in meats. Recent advancements by Kim *et al.* (2024) highlight biogenic amines, such as histamine and cadaverine, as precise spoilage indicators in fish, detectable through simple chemical assays. These amines accumulate as bacteria like Morganella degrade amino acids, correlating with sensory spoilage. Chemical markers are particularly useful in industrial settings, where rapid

tests can assess spoilage without relying on subjective sensory evaluation. However, these methods require specialized equipment, limiting their use for consumers.

Table 3: Common Spoilage Indicators

Indicator Type	Examples	Associated Foods	Detection Method
Sensory	Off-odors (sour, fishy, putrid)	Meat, fish, dairy	Human senses (smell, sight)
Sensory	Texture (slimy, soft, curdled)	Fish, fruits, dairy	Human senses (touch, sight)
Sensory	Color (browning, mold growth)	Apples, bread	Human senses (sight)
Chemical	pH changes (acidic or alkaline shift)	Meat, dairy, fish	pH meters
Chemical	VOCs (amines, sulfur compounds)	Fish, meat, fruits	GC-MS, chemical assays

3.2 Modern Tracing Technologies

Advancements in technology have revolutionized spoilage detection, enabling real-time monitoring and precise identification of spoilage causes. Sensors, IoT, molecular techniques, and blockchain systems offer innovative solutions for tracking and managing spoilage across the food supply chain.

3.2.1 Use of Sensors and IoT for Real-Time Monitoring

Sensors and Internet of Things (IoT) technologies enable continuous monitoring of environmental conditions that contribute to spoilage, such as temperature, humidity, and gas levels. Time-temperature indicators (TTIs) are simple, cost-effective devices that change color to indicate cumulative temperature exposure, signaling potential spoilage in perishable goods like dairy or meat (Taoukis, 2010). For example, TTIs on milk packaging turn red when exposed to temperatures above 4°C for extended periods, alerting retailers to potential quality issues. IoT-enabled sensors take this further by providing real-time data on storage conditions. These sensors, embedded in packaging or storage facilities, monitor temperature, humidity, and VOCs, transmitting data to cloud-based systems for analysis (Bouzembrak *et al.,* 2019). A study by Bouzembrak *et al.* (2019) found that IoT sensors in cold chains reduced spoilage losses by 15% by detecting temperature deviations early.

Recent advancements include smart packaging with gas sensors that detect VOCs like ammonia or ethyl acetate, indicating spoilage in real time (Kim *et al.,* 2024). For instance, smart labels on seafood packaging change color when amine levels rise, providing a visual cue for spoilage. These technologies are particularly valuable in supply chains, where real-time

monitoring can prevent spoilage during transportation. However, high costs and technical complexity limit their widespread adoption, particularly in developing regions (Bouzembrak *et al.,* 2019).

3.2.2 Molecular Techniques (e.g., PCR for Microbial Detection)

Molecular techniques, such as polymerase chain reaction (PCR), enable precise identification of spoilage microorganisms, offering higher specificity than sensory or chemical methods. PCR amplifies DNA from bacteria, yeasts, or molds, detecting species like Pseudomonas or Aspergillus in food samples (Settanni & Corsetti, 2007). Early work by Settanni and Corsetti (2007) showed PCR's effectiveness in identifying Lactobacillus in dairy, allowing early intervention before sensory spoilage. Recent advancements in real-time PCR provide rapid quantification of microbial loads, critical for high-risk foods like seafood (Kim *et al.,* 2024). For instance, real-time PCR can detect Morganella in fish within hours, compared to days for traditional culturing.

Next-generation sequencing (NGS) further enhances detection by profiling entire microbial communities in spoiled foods, revealing complex spoilage dynamics (Odeyemi *et al.,* 2020). A study by Li *et al.* (2024) suggests that portable PCR devices could enable on-site testing in processing facilities, reducing reliance on laboratory-based methods. While highly accurate, molecular techniques require specialized equipment and expertise, limiting their use to industrial and research settings. Ongoing developments aim to make these tools more accessible for smaller operations.

3.2.3 Blockchain and Traceability Systems in Supply Chains

Blockchain technology enhances spoilage tracing by providing transparent, tamper-proof records of food movement and storage conditions. By tracking data from harvest to retail, blockchain systems identify spoilage causes, such as temperature abuse or delays, enabling rapid corrective actions (Feng *et al.,* 2022). For example, a blockchain system in seafood supply chains can trace a spoiled shipment to a specific refrigeration failure, facilitating targeted recalls. Feng *et al.* (2022) found that blockchain reduced spoilage-related losses in fresh produce by 10–20% through improved traceability.

Blockchain integrates with IoT sensors to provide real-time data on temperature, humidity, and handling, creating a comprehensive monitoring system (Bouzembrak *et al.,* 2019). Platforms like IBM's Food Trust demonstrate blockchain's potential to enhance accountability and reduce waste (Feng *et al.,* 2022). However, challenges include high implementation costs, data standardization, and the need for industry-wide adoption, particularly in fragmented supply chains. Recent initiatives are addressing these barriers to make blockchain more accessible.

Table 4: Modern Tracing Technologies

Technology	Function	Applications	Advantages	Limitations
TTIs	Monitor temperature exposure	Dairy, meat, seafood	Cost-effective, visual	Limited to temperature
IoT Sensors	Real-time condition monitoring	Cold chains, packaging	Continuous data, early detection	High cost, technical complexity
PCR	Detect specific spoilage microbes	Seafood, dairy	High specificity, rapid	Requires equipment, expertise
Blockchain	Track supply chain conditions	Produce, seafood	Transparency, rapid traceback	High cost, adoption barriers

4. Environmental and Human Factors Contributing to Spoilage

Environmental and human factors play a significant role in food spoilage, exacerbating microbial, chemical, and physical degradation processes. This section examines how storage conditions, supply chain inefficiencies, and consumer behavior contribute to spoilage, drawing on historical and recent research to provide a comprehensive understanding.

4.1 Impact of Storage Conditions

Storage conditions, including humidity, temperature, and packaging, are critical environmental factors that influence the rate and extent of food spoilage. Improper management of these conditions accelerates microbial growth, chemical reactions, and physical deterioration, leading to significant food losses.

- **Humidity**

High humidity levels promote microbial growth, particularly for molds and yeasts, which thrive in moist environments. Foods with high water activity ($a_w > 0.85$), such as fresh produce, dairy, and seafood, are especially susceptible to spoilage when stored in humid conditions (Walker & Betts, 2000). For example, bread stored in humid environments develops mold growth from Aspergillus or Penicillium within days, as molds can grow at water activity as low as 0.6 (Pitt & Hocking, 2009). Conversely, low humidity can dehydrate certain foods, like fruits and vegetables, causing wilting and texture loss, which indirectly promotes spoilage by creating entry points for microorganisms (Prusky, 2011). Early research by Walker and Betts (2000) established that maintaining humidity below 70% in storage facilities reduces mold growth in grains and baked goods. Recent studies by Zhang *et al.* (2023) highlight that humidity control in refrigerated storage can extend the shelf life of leafy greens by up to 30%, emphasizing the need for precise environmental management.

- **Temperature**

Temperature is a primary driver of spoilage, as it directly affects microbial proliferation and chemical reaction rates. Most spoilage bacteria, such as Pseudomonas and Lactobacillus, thrive between 4°C and 60°C, with optimal growth at 20–40°C (Mossel *et al.,* 1995). Perishable foods like meat, dairy, and seafood require storage below 4°C to slow bacterial growth, but even slight temperature increases can accelerate spoilage. For instance, milk stored at 7°C spoils within days due to Pseudomonas proliferation, compared to weeks at 2°C (Jay *et al.,* 2005). High temperatures also accelerate chemical reactions like lipid oxidation, reducing the shelf life of oils and nuts (Labuza, 1971). Recent research by Zhang *et al.* (2023) indicates that temperature fluctuations in storage facilities, often due to inadequate refrigeration, account for 20–25% of global food losses, particularly in developing countries with limited cold chain infrastructure. Maintaining consistent cold storage is critical for minimizing spoilage.

- **Packaging**

Packaging significantly influences spoilage by controlling exposure to oxygen, moisture, and light. Inadequate packaging, such as non-airtight containers, allows oxygen ingress, promoting oxidative rancidity in high-fat foods and microbial growth in perishable items (Robertson, 2012). For example, improperly sealed meat packages enable Pseudomonas growth, leading to sliminess and off-odors (Jay *et al.,* 2005). Poor packaging also fails to protect against light-induced spoilage, such as riboflavin degradation in milk, which causes off-flavors (Li *et al.,* 2022). Early studies by Robertson (2012) emphasized the role of packaging materials, like high-barrier plastics, in extending shelf life by reducing oxygen and moisture exposure. Recent advancements in active packaging, incorporating oxygen scavengers or antimicrobial agents, have shown promise in reducing spoilage rates by up to 15% in fresh produce and meat (Fang *et al.,* 2023). Proper packaging design is thus essential for mitigating environmental impacts on food quality.

Table 5: Impact of Storage Conditions on Spoilage

Condition	Effect on Spoilage	Affected Foods	Prevention Strategy
Humidity	Promotes mold/yeast growth ($a_w > 0.6$)	Bread, grains, produce	Maintain <70% humidity
Temperature	Accelerates microbial/chemical reactions	Meat, dairy, seafood	Store below 4°C, avoid fluctuations
Packaging	Allows oxygen/moisture/light exposure	Oils, dairy, meat	Use airtight, light-blocking materials

4.2 Supply Chain Inefficiencies

Supply chain inefficiencies, particularly in transportation and handling, significantly contribute to food spoilage by exposing products to suboptimal conditions and physical damage. These inefficiencies are prevalent across global food systems, especially in regions with underdeveloped infrastructure.

- **Transportation**

Transportation challenges, such as inadequate refrigeration or prolonged transit times, accelerate spoilage, particularly for perishable goods. Cold chain disruptions, where products are exposed to temperatures above 4°C, promote microbial growth and chemical degradation (James *et al.,* 2008). For example, seafood transported without consistent refrigeration can develop high levels of biogenic amines, leading to spoilage within hours (Kim *et al.,* 2024). Early research by James *et al.* (2008) highlighted that temperature abuse during transportation accounts for significant losses in meat and dairy supply chains, particularly in developing countries. Recent data by Zhang *et al.* (2023) estimate that 20–30% of global food losses occur due to transportation-related inefficiencies, with tropical regions facing higher risks due to ambient heat. IoT-enabled temperature sensors are increasingly used to monitor cold chains, reducing spoilage by alerting suppliers to deviations in real time (Bouzembrak *et al.,* 2019).

Delays in transportation, such as customs holdups or logistical bottlenecks, further exacerbate spoilage. For instance, fresh produce like bananas can overripen during extended transit, becoming susceptible to fungal infections (Prusky, 2011). Recent advancements in blockchain technology improve traceability, enabling rapid identification of delays and their impact on spoilage (Feng *et al.,* 2022). These technologies are critical for optimizing transportation and minimizing losses in global supply chains.

- **Handling**

Improper handling during harvesting, loading, or unloading introduces physical damage that accelerates spoilage. Bruising or crushing of fruits and vegetables, such as apples or tomatoes, creates entry points for microorganisms like Penicillium expansum, leading to mold growth (Prusky, 2011). In meat processing, rough handling can damage muscle tissue, promoting bacterial contamination and enzymatic breakdown (Jay *et al.,* 2005). Early studies by Barth *et al.* (2009) noted that poor handling practices, such as inadequate sanitation during processing, introduce spoilage organisms, reducing shelf life. Recent research by Zhang *et al.* (2023) highlights that improper handling accounts for 10–15% of postharvest losses in produce, emphasizing the need for standardized handling protocols.

Training programs for workers and automated handling systems are emerging solutions to reduce damage. For example, robotic sorting systems in fruit packing facilities minimize bruising, extending shelf life by up to 20% (Prusky, 2011). Improved training and mechanization are essential for reducing spoilage in supply chains.

Table 6: Supply Chain Inefficiencies and Spoilage

Factor	Effect on Spoilage	Affected Foods	Prevention Strategy
Transportation	Temperature abuse, delays	Seafood, dairy, produce	Cold chain monitoring, blockchain
Handling	Physical damage, contamination	Fruits, vegetables, meat	Automated systems, worker training

4.3 Consumer Behavior and Improper Food Handling Practices

Consumer behavior and improper food handling practices at the household level significantly contribute to food spoilage, leading to substantial waste. Missteps in storage, preparation, and disposal practices exacerbate spoilage, particularly in developed countries.

- **Storage Practices**

Improper storage by consumers, such as storing perishable foods at incorrect temperatures or in unsuitable containers, accelerates spoilage. For example, storing milk at room temperature rather than below 4°C promotes Pseudomonas growth, causing souring within hours (Jay *et al.,* 2005). Similarly, keeping fruits like bananas in sealed plastic bags traps ethylene gas, accelerating ripening and spoilage (Prusky, 2011). Early research by Walker and Betts (2000) noted that consumer misunderstanding of storage requirements, such as not refrigerating opened canned goods, contributes to spoilage. Recent studies by Porat *et al.* (2021) estimate that 40% of household food waste in developed countries results from improper storage, with dairy and produce being the most affected. Consumer education campaigns, such as the USDA's FoodKeeper app, provide guidance on optimal storage conditions, reducing waste by up to 25% in households that follow recommendations (USDA, 2020).

- **Preparation and Disposal Practices**

Improper preparation, such as inadequate cleaning or cross-contamination, introduces spoilage organisms. For instance, using the same cutting board for raw meat and vegetables without proper sanitation can transfer Pseudomonas or Lactobacillus, accelerating spoilage (Barth *et al.,* 2009). Over-preparation, such as peeling fruits long before consumption, exposes them to oxygen, triggering enzymatic browning (McEvily *et al.,* 1992). Disposal practices also contribute to waste; consumers often discard food based on "best before" dates without checking for spoilage, leading to unnecessary losses (Porat *et al.,* 2021). Recent research by Porat *et al.*

(2021) found that 30–50% of household food waste in the U.S. and Europe is due to premature disposal, driven by confusion over date labels.

Education and awareness are key to addressing these issues. Initiatives like the USDA's FoodKeeper app and campaigns promoting "smell and see" checks before discarding food help reduce waste (USDA, 2020). Recent studies by Li *et al.* (2024) suggest that smart kitchen technologies, such as IoT-enabled refrigerators, could guide consumers on proper storage and reduce spoilage by alerting them to expiring foods.

Table 7: Consumer Practices Contributing to Spoilage

Practice	Effect on Spoilage	Affected Foods	Prevention Strategy
Storage	Incorrect temperature, poor containers	Dairy, produce	Refrigerate below 4°C, use airtight containers
Preparation	Cross-contamination, premature peeling	Meat, vegetables	Proper sanitation, timely preparation
Disposal	Premature discarding based on date labels	All foods	Education on sensory checks

5. Strategies to Mitigate Food Spoilage

Mitigating food spoilage is essential for enhancing food safety, reducing economic losses, and promoting sustainability. This section explores effective strategies to combat spoilage, focusing on advanced storage and packaging technologies, robust cold chain management, and education for consumers and industry stakeholders. Drawing on historical and recent research, the section provides actionable insights, supported by tables and a chart to illustrate key approaches.

5.1 Improved Storage and Packaging Technologies

Advanced storage and packaging technologies play a critical role in extending food shelf life by controlling environmental factors that contribute to spoilage, such as oxygen, moisture, and microbial growth. Innovations like vacuum sealing and modified atmosphere packaging (MAP) have transformed food preservation, offering practical solutions for both industry and consumers.

- **Vacuum Sealing**

Vacuum sealing removes air from packaging, reducing oxygen levels that promote microbial growth and oxidative spoilage. By limiting oxygen, vacuum sealing inhibits aerobic bacteria like Pseudomonas, which cause sliminess and off-odors in meats, and slows lipid oxidation in high-fat foods like nuts (McMillin, 2017). Early research by Robertson (2012) demonstrated that vacuum-sealed meats maintain quality up to three times longer than those stored in air-permeable packaging. For example, vacuum-sealed beef can last 30–40 days at 0–

4°C, compared to 10–14 days in traditional packaging (Jay *et al.,* 2005). Vacuum sealing is widely used for meats, cheeses, and processed foods, offering a cost-effective solution for retailers and households.

Recent advancements have made vacuum sealing more accessible for home use, with compact devices allowing consumers to preserve leftovers or bulk purchases (Porat *et al.,* 2021). A study by Fang *et al.* (2023) found that vacuum sealing reduces household food waste by up to 20% for perishable items like meats and vegetables. However, vacuum sealing is less effective for high-moisture produce, as anaerobic conditions can promote Clostridium growth, necessitating complementary strategies like refrigeration (McMillin, 2017).

- **Modified Atmosphere Packaging (MAP)**

MAP involves altering the gas composition inside packaging to extend shelf life, typically by reducing oxygen and increasing carbon dioxide or nitrogen levels. This inhibits microbial growth and slows chemical reactions like oxidation. For example, MAP with 20–40% CO_2 is used for fresh meats to suppress Pseudomonas and Lactobacillus, extending shelf life by 1–2 weeks at 0–4°C (McMillin, 2017). MAP is also effective for produce, where low oxygen (2–5%) and elevated CO_2 (5–10%) slow respiration and ethylene production, delaying ripening in fruits like apples (Robertson, 2012). Early studies by McMillin (2017) established MAP as a standard for perishable goods, particularly in retail settings.

Recent innovations include active MAP systems incorporating antimicrobial agents or oxygen scavengers. Fang *et al.* (2023) reported that MAP with essential oil-based antimicrobials reduces mold growth in berries by 30%, enhancing shelf life without chemical preservatives. Smart packaging, integrating sensors that monitor gas levels, further optimizes MAP by detecting spoilage early (Bouzembrak *et al.,* 2019). While MAP is highly effective, its cost and complexity limit adoption in small-scale operations, particularly in developing regions (Fang *et al.,* 2023). Continued research aims to develop affordable MAP solutions to broaden accessibility.

Table 8: Storage and Packaging Technologies

Technology	**Mechanism**	**Affected Foods**	**Benefits**	**Limitations**
Vacuum Sealing	Removes oxygen, inhibits microbes	Meats, cheeses, nuts	Extends shelf life, cost-effective	Less effective for produce
MAP	Alters gas composition (CO_2, N_2)	Meats, produce, bakery	Inhibits microbes, slows ripening	High cost, technical complexity

5.2 Cold Chain Management and Temperature Control

Effective cold chain management and temperature control are foundational strategies for mitigating food spoilage, particularly for perishable goods. Maintaining consistent low temperatures throughout the supply chain slows microbial growth and chemical reactions, preserving food quality.

- **Cold Chain Management**

The cold chain encompasses refrigeration and freezing systems used during storage, transportation, and retail to maintain food at optimal temperatures (typically 0–4°C for fresh foods, -18°C for frozen). Perishable foods like dairy, meat, and seafood are highly susceptible to spoilage when exposed to temperatures above 4°C, where bacteria like Pseudomonas and Listeria proliferate rapidly (Jay *et al.,* 2005). Early research by Mercier *et al.* (2017) highlighted that cold chain disruptions, such as inadequate refrigeration during transportation, contribute to 20–30% of global food losses, particularly in developing countries. For example, seafood transported without consistent refrigeration can spoil within hours due to biogenic amine production (Kim *et al.,* 2024).

Recent advancements in cold chain technology include IoT-enabled temperature sensors that monitor conditions in real time, alerting suppliers to deviations (Bouzembrak *et al.,* 2019). A study by Zhang *et al.* (2023) found that IoT-based cold chain monitoring reduces spoilage losses by 15% in meat and dairy supply chains. Insulated packaging and refrigerated trucks have also improved cold chain reliability, particularly for long-distance transport. However, cold chain infrastructure remains limited in low-income regions, where ambient temperatures exacerbate spoilage (Mercier *et al.,* 2017). Investments in affordable refrigeration and renewable energy-powered cold storage are critical for global adoption.

- **Temperature Control**

Precise temperature control at every stage—production, storage, and retail—is essential for minimizing spoilage. Refrigeration below 4°C slows microbial growth, while freezing at -18°C halts it entirely, preserving foods like meat and fish for months (Jay *et al.,* 2005). For produce, controlled atmosphere storage (low temperature combined with reduced oxygen) extends shelf life by slowing respiration, as seen in apples stored for up to a year (Prusky, 2011). Early studies by Labuza (1971) established temperature thresholds for various foods, noting that a 10°C increase doubles microbial growth rates, significantly reducing shelf life.

Recent research emphasizes the importance in the context of climate change, where rising ambient temperatures challenge cold chain systems. Li *et al.* (2024) warn that global warming

could increase spoilage rates by 20% without improved temperature control measures. Innovations like phase-change cooling systems, which maintain stable temperatures during power outages, are addressing this challenge (Zhang *et al.,* 2023). Retail and household refrigeration must also be optimized; for instance, setting refrigerators to 4°C or below prevents spoilage of dairy and meats, yet many consumers store foods at higher temperatures, accelerating deterioration (Porat *et al.,* 2021).

Table 9: Cold Chain and Temperature Control Strategies

Strategy	Mechanism	Affected Foods	Benefits	Limitations
Cold Chain	Maintains refrigeration/freezing	Meat, dairy, seafood	Extends shelf life, reduces losses	Limited infrastructure in some regions
Temperature Control	Precise cooling, slows reactions	Produce, dairy	Preserves quality, scalable	Energy-intensive

5.3 Education and Awareness for Consumers and Industry Stakeholders

Educating consumers and industry stakeholders about spoilage prevention is a powerful strategy to reduce food waste and enhance safety. Awareness campaigns and training programs address improper handling, promote best practices, and foster sustainable behaviors.

- **Consumer Education**

Consumers often lack knowledge about proper food handling and storage, leading to significant household waste. For example, storing milk at room temperature or misinterpreting "best before" dates results in premature spoilage or discard (Porat *et al.,* 2021). Early studies by Walker and Betts (2000) noted that consumer misunderstanding of refrigeration requirements contributes to 40% of household spoilage in developed countries. The USDA's FoodKeeper app educates consumers on optimal storage conditions, such as refrigerating dairy at 0–4°C or freezing meat within 24 hours of purchase, reducing waste by up to 25% (USDA, 2020). Campaigns promoting sensory checks (e.g., smelling or inspecting food before discarding) address confusion over date labels, preventing unnecessary waste (Porat *et al.,* 2021).

Recent initiatives leverage digital platforms to enhance consumer education. Social media campaigns and apps like Too Good To Go encourage consumers to use leftovers creatively, reducing spoilage (Li *et al.,* 2024). A study by Porat *et al.* (2021) found that households educated on spoilage prevention reduce food waste by 20–30%, particularly for dairy and produce. Providing education in schools further embeds sustainable practices, fostering long-

term behavior change. However, reaching diverse populations, especially in low-literacy areas, requires tailored, multilingual resources.

- **Industry Stakeholder Education**

Industry stakeholders, including farmers, processors, and retailers, benefit from training on spoilage prevention. Improper handling, such as rough handling of produce or inadequate sanitation, introduces spoilage organisms, reducing shelf life (Barth *et al.,* 2009). Early training programs by Barth *et al.* (2009) emphasized hygiene and storage protocols to prevent contamination in fruit and vegetable processing, extending shelf life by 10–15%. Recent training on IoT and blockchain technologies equips stakeholders to monitor supply chain conditions and identify spoilage risks, such as temperature abuse during transport (Bouzembrak *et al.,* 2019). For example, retailers trained in IoT sensor use reduce losses by ensuring products remain within safe temperature ranges (Zhang *et al.,* 2023).

Workshops and certification programs, such as those offered by the Global Food Safety Initiative, promote best practices in cold chain management and packaging (Mercier *et al.,* 2017). Recent research by Li *et al.* (2024) highlights the role of AI-driven training, where simulations teach workers to predict spoilage risks based on environmental data, improving efficiency. Industry adoption of these practices, however, varies by region, with cost and access to training posing barriers in developing countries (Bouzembrak *et al.,* 2019). Public-private partnerships are essential to scale these efforts globally.

Table 10: Education and Awareness Initiatives

Target Group	Action	Focus Areas	Benefits	Challenges
Consumers	Learn storage, sensory checks	Dairy, produce	Reduces household waste	Reaching diverse populations
Industry Stakeholders	Training on hygiene, technology	All foods	Improves supply chain efficiency	Cost, access in developing regions

6. Conclusion and Future Directions

6.1 Summary of Key Points

Food spoilage remains a critical challenge in the global food supply chain, contributing significantly to food waste and economic losses. The primary causes of spoilage include microbial growth, enzymatic reactions, and environmental factors such as temperature, humidity, and oxygen exposure. Effective prevention strategies encompass improved storage techniques, advanced packaging technologies, and stringent quality control measures. Technologies like

modified atmosphere packaging (MAP), active packaging, and cold chain logistics have proven instrumental in extending shelf life and maintaining food quality. Additionally, regulatory frameworks and industry standards ensure safety and compliance, while consumer education promotes better handling practices to minimize waste. The integration of these approaches has led to significant advancements in reducing spoilage, but challenges persist, particularly in resource-constrained regions where infrastructure limitations hinder implementation. Collaboration among stakeholders—producers, distributors, retailers, and consumers—is essential to address these challenges holistically. By leveraging scientific advancements and fostering sustainable practices, the food industry can mitigate spoilage, ensuring safer and more accessible food supplies globally.

The table below summarizes the key strategies and their impact on spoilage prevention:

Strategy	**Description**	**Impact on Spoilage Prevention**
Modified Atmosphere Packaging	Alters gas composition to slow microbial and oxidative processes	Extends shelf life by up to 50% for perishable goods
Cold Chain Logistics	Maintains consistent low temperatures during storage and transport	Reduces microbial growth by 70–80% in temperature-sensitive foods
Active Packaging	Incorporates agents (e.g., oxygen scavengers) to control spoilage factors	Decreases spoilage rates by 20–30% in high-moisture foods
Consumer Education	Promotes proper storage and handling practices	Reduces household food waste by 10–15%

6.2 Emerging Trends in Spoilage Prevention

The food industry is witnessing transformative trends in spoilage prevention, driven by technological advancements and sustainability goals. Two prominent trends are AI-driven monitoring systems and sustainable packaging innovations, which promise to revolutionize food preservation.

- **AI-Driven Monitoring**

Artificial intelligence (AI) is reshaping food spoilage prevention by enabling real-time monitoring and predictive analytics. AI-powered sensors and IoT devices can track environmental conditions such as temperature, humidity, and gas levels in storage facilities and during transportation. Machine learning algorithms analyze this data to predict spoilage risks, allowing proactive interventions. For instance, AI systems can detect early signs of microbial

growth by analyzing volatile organic compounds emitted by food, enabling timely corrective actions. Companies like IBM and Microsoft are developing AI platforms that integrate with supply chain systems to optimize storage conditions and reduce waste. Studies suggest that AI-driven monitoring can reduce spoilage losses by up to 25% in perishable supply chains. Additionally, blockchain integration with AI ensures traceability, enhancing transparency and accountability. However, challenges such as high implementation costs and the need for skilled personnel limit adoption, particularly in developing regions. Future advancements in affordable AI solutions and cloud-based platforms could democratize access, making these technologies viable for small-scale producers.

- **Sustainable Packaging**

Sustainable packaging is gaining traction as an eco-friendly solution to spoilage prevention. Innovations include biodegradable films made from natural polymers like chitosan and starch, which offer antimicrobial properties and reduce reliance on plastic. Edible coatings, such as those derived from whey protein or seaweed, create protective barriers that extend shelf life while being environmentally friendly. Nanotechnology is also emerging, with nanoparticle-infused packaging that inhibits microbial growth and scavenges oxygen. For example, silver nanoparticle-based films have shown a 30% reduction in spoilage for fresh produce. Additionally, smart packaging with embedded sensors provides real-time spoilage indicators, empowering consumers to make informed decisions. These innovations align with global sustainability goals, as they reduce plastic waste and carbon footprints. However, scalability remains a challenge due to high production costs and regulatory hurdles for novel materials. Ongoing research aims to address these barriers, with a focus on cost-effective, biodegradable alternatives that maintain efficacy.

6.3 Importance of Reducing Food Waste for Global Food Security

Reducing food waste is a cornerstone of global food security, addressing both immediate hunger and long-term sustainability. Approximately one-third of food produced globally—about 1.3 billion tons annually—is wasted, exacerbating food insecurity in vulnerable populations. By minimizing spoilage, we can ensure more food reaches consumers, particularly in regions facing scarcity. Food waste also has significant environmental implications, contributing 8–10% of global greenhouse gas emissions due to landfill decomposition and resource-intensive production processes. Effective spoilage prevention strategies, such as those discussed, directly support food security by preserving resources and reducing economic losses, estimated at $1 trillion annually.

From a social perspective, reducing food waste ensures equitable food distribution. In low-income countries, spoilage often occurs during storage and transport due to inadequate

infrastructure. Investments in cold chain systems and affordable preservation technologies can bridge this gap, enabling smallholder farmers to deliver fresh produce to markets. In high-income countries, consumer-level waste, driven by over-purchasing and improper storage, accounts for a significant portion of losses. Education campaigns and smart packaging can empower consumers to minimize waste, redirecting surplus food to those in need through food banks and redistribution networks.

Economically, reducing spoilage enhances supply chain efficiency, lowering costs for producers and consumers. For instance, extending shelf life through advanced packaging can reduce retail losses by 15–20%, stabilizing food prices. Environmentally, it conserves resources like water, energy, and land used in food production. For example, preventing the waste of one ton of grain saves approximately 1,000 cubic meters of water. These savings are critical in the context of a growing global population, projected to reach 9.7 billion by 2050, which will intensify pressure on food systems.

The table below highlights the multifaceted benefits of reducing food waste:

Aspect	**Impact of Reducing Food Waste**	**Quantitative Benefit**
Food Security	Increases food availability for vulnerable populations	Feeds 1–2 billion people annually
Environmental	Lowers greenhouse gas emissions from food waste	Reduces emissions by 8–10% globally
Economic	Decreases production and retail losses	Saves $1 trillion annually
Resource Conservation	Preserves water, energy, and land used in food production	Saves 1,000 m^3 water per ton of grain preserved

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HERBAL GARDENS:
CONSERVING INDIA'S RICH MEDICINAL PLANT HERITAGE

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Abstract:

The medicinal plants or herbs lead to the thought of miraculous and supernatural cures used in traditional system of medicine such as Ayurveda, Naturopathy, Sidda, Unanai etc. our ancient literature not only has references of medicinal preparation cation. As rightly said by Charka father of Modern but also the plant species and their classification. Medicine, there is no plant on earth which does not have medicinal value. Generally speaking, all plants provide food and all of them have one or the other medicinal properties. India is one the world's richest source of herbal wealth. Due to varied climatic conditions and soil types India gifted with heritage of medicinal plants. Due to indiscriminate collection of medicinal plants from its natural sources led to become endangered and vulnerable species. Therefore, it is duty of every human being to save the plants in natural habitat which is only the source of diversity and also new drugs. In this view government has taken the initiative to establish herbal gardens in every state, region, even at community level to conserve the native medicinal plants and also to multiply the plants to make it available for commercial cultivation. One of such herbal gardens has been established at ICAR-National Institute of Abiotic stress management, Baramati, District Pune, Maharashtra in an area of 2.0 hectare comprising of 65 Species of Trees, Shrubs and Climbers.

Keywords: Medicine, Ayurveda, Drug, Plants

Introduction:

Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in Ayurveda. The Rigveda (5000 BC) has recorded 67 medicinal plants, Yajurveda 81 species, Atharvaveda (4500-2500 BC) 290 species, Charak Samhita (700 BC) and Sushrut Samhita (200 BC) had described properties and uses of 1100 and 1270 species respectively, in compounding of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine. Unfortunately, much of the

ancient knowledge and many valuable plants are being lost at an alarming rate. With the rapid depletion of forests, impairing the availability of raw drugs, Ayurveda, like other systems of herbal medicines has reached a very critical phase. About 50% of the tropical forests, the treasure house of plant and animal diversity have already been destroyed. In India, forest cover is disappearing at an annual rate 1.5mha/yr. What is left at present is only 8% as against a mandatory 33% of the geographical area. Many valuable medicinal plants are under the verge of extinction. The Red Data Book of India has 427 entries of endangered species of which 28 are considered extinct, 124 endangered, 81 vulnerable, 100 rare and 34 insufficiently known species (Thomas, 1997). Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practised in India. Ayurveda dating back to 1500-800 BC has been an integral part of Indian culture. The term comes from the Sanskrit root Au (life) and Veda (knowledge). As the name implies it is not only the science of treatment of the ill but covers the whole gamut of happy human life involving the physical, metaphysical and the spiritual aspects. Ayurveda recognises that besides a balance of body elements one has to have an enlightened state of consciousness, sense organs and mind if one has to be perfectly healthy.

Ayurveda by and large is an experience with nature and unlike in Western medicine, many of the concepts elude scientific explanation. Ayurveda is gaining prominence as the natural system of health care all over the world. Today this system of medicine is being practised in countries like Nepal, Bhutan, Sri Lanka, Bangladesh and Pakistan, while the traditional system of medicine in the other countries like Tibet, Mongolia and Thailand appear to be derived from Ayurveda. Phytomedicines are also being used increasingly in Western Europe. Recently the US Government has established the “Office of Alternative Medicine” at the National Institute of Health at Bethesda and its support to alternative medicine includes basic and applied research in traditional systems of medicines such as Chinese, Ayurvedic, etc. with a view to assess the possible integration of effective treatments with modern medicines. The development of systematic pharmacopoeias dates back to 3000 BC, when the Chinese were already using over 350 herbal remedies. Ayurveda, a system of herbal medicine in India, Sri Lanka and South-East Asia has more than 8000 plant remedies and using around 35,000-70,000 plant species. China has demonstrated the best use of traditional medicine in providing the health care. China has pharmacologically validated and improved many traditional herbal medicines and eventually integrated them in formal health care system. Green plants synthesise and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are

commercially important and find use in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, selection of the superior plant stock and over exploitation by pharmaceutical industry.

Some Important Medicinal Plants and Their Uses

Drug	**Plant**	**Use**
Vinblastine	*Catharanthus roseus*	Anticancer
Rescinnamine	*Rauvolfia serpentina*	Tranquilizer
Quinine	*Cinchona sp.*	Antimalarial
Pilocarpine	*Pilocarpus jaborandi*	Antiglucoma
Morphine	*Papaver somniferum*	Painkiller
Cardiac glycosides for congestive	*Digitalis sp.*	Heart failure
Taxol,	*Taxus baccata T. brevifolia*	Breast and ovary cancer
Gossypol	*Gossypium sp.*	Antispermatogenic
Allicin Antifungal	*Allium sativum*	Amoebiasis
Glycyrrhizin	*Glycyrrhizia glabra*	Antiulcer
Digitoxin	*Digoxin Digitalis*	Thevetia Cardio tonic
Codeine	*Papaver somniferum*	Anticough
Quassinoids	*Ailanthus*	Antiprotozoal
Magnolol	*Magnolia bark*	Peptic ulcer
Forskolin	*Coleus forskohlii*	Hypotensive, cardiotonic
Allicin,	*Allium sativum Antifungal*	Amoebiasis

Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and /or reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine among others. In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important. Where the active molecule cannot be synthesised

economically, the product must be obtained from the cultivation of plant material. About 121 (45 tropical and 76 subtropical) major plant drugs have been identified for which no synthetic one is currently available (table 1). The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance. Table 1. Major plant drugs for which no synthetic one is currently available (Kumar *et al.,* 1997).

Cultivation of Medicinal Plants

Most of medicinal plants, even today, are collected from wild. The continued commercial exploitation of these plants has resulted in receding the population of many species in their natural habitat. Vacuum is likely to occur in the supply of raw plant materials that are used extensively by the pharmaceutical industry as well as the traditional practitioners. Consequently, cultivation of these plants is urgently needed to ensure their availability to the industry as well as to people associated with traditional system of medicine. If timely steps are not taken for their conservation, cultivation and mass propagation, they may be lost from the natural vegetation for ever. In situ conservation of these resources alone cannot meet the ever increasing demand of pharmaceutical industry. It is, therefore, inevitable to develop cultural practices and propagate these plants in suitable agroclimatic regions. Commercial cultivation will put a check on the continued exploitation from wild sources and serve as an effective means to conserve the rare floristic wealth and genetic diversity. It is necessary to initiate systematic cultivation of medicinal plants in order to conserve biodiversity and protect endangered species. In the pharmaceutical industry, where the active medicinal principle cannot be synthesised economically, the product must be obtained from the cultivation of plants. Systematic conservation and large scale cultivation of the concerned medicinal plants are thus of great importance. Efforts are also required to suggest appropriate cropping patterns for the incorporation of these plants into the conventional agricultural and forestry cropping systems. Cultivation of this type of plants could only be promoted if there is a continuous demand for the raw materials. There are at least 35 major medicinal plants that can be cultivated in India and have established demand for their raw material or active principles in the international trade (table). It is also necessary to develop genetically superior planting material for assured uniformity and desired quality and resort to organised cultivation to ensure the supply of raw material at growers end. Hence, small scale processing units too have to be established in order that the farmer is assured of the sale of raw material. Thus, cultivation and processing should go hand in hand in rural areas. In order to initiate systematic cultivation of medicinal and aromatic plants high yielding varieties have to be selected (table 8). In the case of wild plants, their

demonstration would require careful development work. Sometimes high yielding varieties have also to be developed by selective breeding or clonal micropropagation. The selected propagation materials have to be distributed to the farmer either through nurseries or seed banks. Systematic cultivation needs specific cultural practices and agronomical requirements. These are species specific and are dependent on soil, water and climatic conditions. Hence research and development work has to be done to formulate Good Agricultural Practices (GAP) which should include proper cultivation techniques, harvesting methods, safe use of fertilizers and pestisides and waste disposal.

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THE SCIENCE OF AQUATIC REHABILITATION IN PHYSIOTHERAPY

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Abstract:

Aquatic rehabilitation, a vital subset of physiotherapy, utilizes the unique properties of water to facilitate movement, reduce pain, and promote recovery in various patient populations. The physical characteristics of water, including buoyancy, viscosity, hydrostatic pressure, and thermodynamics, provide an environment conducive to functional training, especially for individuals with musculoskeletal, neurological, and cardiopulmonary conditions. This chapter explores the scientific principles underpinning aquatic rehabilitation, outlines its physiological and psychological benefits, and discusses clinical applications, safety considerations, and evidence-based protocols. Emphasis is placed on integrating aquatic therapy within broader rehabilitation strategies to optimize patient outcomes.

Keywords: Aquatic Rehabilitation, Physiotherapy

Introduction:

Aquatic rehabilitation, also known as hydrotherapy or aquatic physiotherapy, refers to the use of water for therapeutic exercise and recovery. This intervention has gained widespread acceptance in clinical practice for its ability to reduce joint loading and promote functional recovery in a low-impact environment. The chapter delves into the science behind this approach and its relevance in contemporary physiotherapy.

Physical Properties of Water and Their Therapeutic Implications

Buoyancy is the upward thrust exerted by water, which counters the effects of gravity on the body. This property reduces the effective body weight, making movements easier and less painful for individuals with joint issues or post-operative limitations. It reduces gravitational forces, thereby decreasing stress on weight-bearing joints, alleviates joint compression, and encourages early mobilization and safe participation in weight-bearing exercises during the initial stages of rehabilitation.

Hydrostatic pressure is the force exerted by water on immersed objects, increasing with depth and evenly distributed. This enhances venous return by compressing peripheral veins,

aiding in circulatory efficiency and reducing limb swelling or edema. It also offers consistent and uniform support to all submerged body parts, improving postural stability and balance.

Viscosity refers to the thickness and internal friction of water molecules, creating resistance against movement. It provides natural resistance that is directionally adjustable and safe, promoting muscle engagement without external weights. It enables progressive and graded strengthening by varying movement speed, range, and surface area and enhances proprioceptive feedback due to water's resistance, aiding in neuromuscular control.

Water's temperature can significantly influence physiological responses. Therapeutic pools are typically maintained between 31–35°C. Warm water promotes muscle relaxation, reduces spasticity, and enhances circulation. It aids in pain relief through modulation of sensory nerve endings and increases connective tissue extensibility, thus improving joint range of motion and flexibility.

Physiological and Psychological Effects of Aquatic Therapy

Aquatic therapy positively impacts the musculoskeletal system through its supportive and resistive environment. It diminishes muscle spasms and alleviates joint stiffness, increases range of motion (ROM) through low-impact stretching and mobilization, and facilitates muscular endurance and strength development via water resistance training.

Water provides a safe environment for individuals with neurological conditions to explore movement and regain motor function. It offers proprioceptive stimulation crucial for sensory integration and motor planning, enhances balance and coordination by challenging equilibrium in a controlled setting, and supports gait training by reducing fall risk, particularly in patients with stroke, cerebral palsy (CP), and Parkinson's disease.

The pressure and temperature of water influence cardiovascular and respiratory performance. Hydrostatic pressure assists in venous return, thereby increasing cardiac output without significantly elevating heart rate. It improves lung function by providing resistance during breathing, strengthening respiratory muscles, and supports aerobic conditioning in individuals with limited land-based exercise capacity.

Aquatic environments provide mental and emotional benefits, enhancing overall therapy outcomes. It promotes relaxation by reducing stress and anxiety levels, increases patient motivation and compliance through enjoyable and novel therapeutic experiences, and facilitates social interaction and confidence-building, especially in group therapy settings.

Clinical Indications and Contraindications

Aquatic therapy is indicated for a wide range of conditions, particularly when land-based therapy is limited. These include degenerative joint diseases like osteoarthritis and rheumatoid

arthritis, post-operative recovery such as total joint replacements and ACL reconstruction, neurological impairments including stroke, multiple sclerosis (MS), and Parkinson's disease, as well as chronic pain syndromes like fibromyalgia and low back pain.

Despite its benefits, aquatic therapy may not be suitable for all individuals. Contraindications include the presence of open wounds or active infections, severe or decompensated cardiac conditions, history of uncontrolled seizures or epilepsy, and incontinence due to the risk of contamination and infection.

Assessment and Treatment Planning

A comprehensive assessment helps determine the patient's suitability for aquatic therapy. This includes a review of medical history, diagnosis, and current medications, a physical examination focusing on joint mobility, strength, and functional limitations, and functional tests adapted to water settings such as balance and gait assessment.

Establishing clear goals helps guide the treatment plan and measure outcomes. The SMART framework ensures goals are Specific, Measurable, Achievable, Relevant, and Time-bound.

Therapy sessions are structured to include all phases of exercise and individualized techniques. This involves warm-up and cool-down sessions to prevent injuries, selection of appropriate aquatic methods such as Halliwick for balance, Watsu for relaxation, and Ai Chi for mindfulness, and incorporation of equipment like noodles, dumbbells, kickboards, and underwater treadmills for progressive training.

Techniques and Methodologies

The Halliwick Concept is a technique emphasizing mental adjustment to water, postural control, and independent movement. It is effective for patients with neurological conditions such as cerebral palsy or multiple sclerosis.

The Bad Ragaz Ring Method utilizes floatation rings and is based on PNF principles to enhance muscular coordination and strength. It is often applied in a supine position to support spine alignment and promote core engagement.

Ai Chi integrates slow, flowing movements with deep breathing to improve trunk control and mental focus. It is ideal for patients with balance issues, stress-related conditions, or chronic fatigue.

Evidence-Based Practice in Aquatic Rehabilitation

Research supports aquatic therapy's role in reducing pain, improving function, and enhancing quality of life in arthritis and neurological rehabilitation. Comparative trials indicate

that aquatic therapy provides equal or superior outcomes in terms of balance, gait, and pain reduction compared to traditional land-based therapy.

Integration with Multidisciplinary Care

Aquatic therapy should be implemented in coordination with other disciplines such as medicine, occupational therapy, and psychology. It facilitates smoother transitions to land-based therapy and holistic recovery planning.

Safety Considerations and Facility Requirements

Maintaining water between 31–35°C is essential for optimal therapeutic benefits. Ensuring water hygiene through routine testing and maintenance is also critical. The presence of certified aquatic therapists and life-saving equipment is necessary, along with regular safety drills and patient orientation sessions.

Future Directions in Aquatic Rehabilitation

The future of aquatic therapy includes incorporation of virtual reality interfaces for real-time feedback and motivation, as well as development of underwater robotics and AI-based motion tracking to personalize treatment.

Conclusion:

Aquatic rehabilitation presents a versatile and evidence-supported modality within physiotherapy. Its scientific foundation, combined with wide-ranging therapeutic effects, makes it a valuable tool for treating diverse clinical populations. Continued research and innovation will further enhance its scope and efficacy.

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ORBITING NUTRITION:
THE SCIENCE AND ART OF SPACE FOOD FOR ASTRONAUTS

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Abstract:

This review paper explores innovative space food design concepts aimed at enhancing the gastronomic experience for astronauts in microgravity environments. The first design concept, "Spice Bomb Mixing," draws inspiration from the concept of an "emotional" cleanser, seeking to intensify flavors and make food preparation interactive and enjoyable. The second concept, the "Flavor Journey 3D Printer," focuses on providing personalized flavors through a customizable food printing system, enabling on-demand tastes and nutrients using advanced 3D printing technology. The third concept, "Earth Memory Bites," introduces small, self-contained bites with distinct flavors representing various Earth regions, cultures, or food-related experiences. These bites, prepared as 3D print recipes, are accompanied by multisensory environments, offering a unique and immersive dining experience on the spaceship. This paper highlights the key aspects of each design concept, including functionality, sensorial elements, emotional aspects, and social dynamics. The proposed concepts aim to revolutionize space food by addressing challenges related to taste perception and enhancing overall sensory aspects in microgravity.

Keywords: Space Food, Gastronomic Experience, Innovative Design Concepts, Spice Bomb Mixing, Flavor Journey 3D Printer, Earth Memory Bites, Microgravity, Astronaut Nutrition, 3D Food Printing, Multisensory Dining.

Introduction:

As space exploration advances, ensuring the well-being and satisfaction of astronauts during extended missions becomes a paramount consideration. A critical aspect of this concern revolves around space food – its taste, variety, and the overall sensory experience it offers. This review paper delves into innovative space food design concepts aimed at transforming the culinary experience for astronauts. Three distinct design concepts are explored, each offering unique approaches to address the challenges of perceived blandness and monotony associated with space cuisine.

The first concept, "Spice Bomb Mixing," takes inspiration from the idea of enhancing the emotional aspect of eating by introducing a flavor-enhancing seasoning design. It not only seeks

to intensify flavors but also aims to make food preparation in microgravity interactive, social, and enjoyable. The paper examines how this concept considers functionality, sensorial elements, emotional aspects, and social dynamics to create a diverse and appealing gastronomic experience in space.

Moving forward, the second concept, "Flavor Journey 3D Printer," explores the realm of on-demand tastes and nutrients through customizable food printing technology. Users can design their flavor profiles or order them from various sources, and the recipes are transmitted directly to the printer. The review paper assesses the functionality, sensorial elements, emotional aspects, and social dynamics associated with this innovative approach, emphasizing its potential to turn food preparation into a collaborative and satisfying experience despite communication delays.

Lastly, the "Earth Memory Bites" concept introduces small, self-contained bites that encapsulate flavors representing various Earth regions and cultures. These bites are integrated into specific dining environments, offering a multisensory experience. The paper investigates how this concept provides comfort through familiar flavors and assesses its unique approach to space food design, considering factors like flavor profiles, multisensory experiences, and the one-size-fits-all approach to minimize logistical challenges in space.

Through a comprehensive exploration of these innovative space food design concepts, this review paper aims to contribute insights into the future of space cuisine, addressing the physical and psychological well-being of astronauts during prolonged space missions.

1. What is Space Food?

Space food refers to a range of specially designed and processed food products intended for Use by space crew members during their missions in the expanse of space. Nutrition, the act of providing or obtaining the necessary food for health and growth, is a crucial aspect of space exploration. Historically, explorers, including those in space, have grappled with the challenge of carrying sufficient food due to limited storage space. In long-duration spaceflight, it becomes imperative to ensure the right nutrient balance for maintaining vitality and safeguarding against the impacts of weak gravity conditions. Sustaining adequate nutrient intake during space travel is essential not only to meet the astronauts' nutritional needs but also to counteract the adverse effects of space flight on the human body and prevent deficiency diseases. Edibility throughout the entire voyage is a key consideration, and space food must provide all the necessary nutrients. For instance, the loss of calcium, nitrogen, and phosphorus in microgravity requires replenishment through food sources. Space foods typically possess specific characteristics such as being nutritious, lightweight, compact, easily digestible, palatable, physiologically appropriate, well-packed, quick to serve, easy to clean up, and having high acceptability with

minimal preparation. These features ensure that astronauts receive the essential nutrients, enjoy their meals, and can manage the challenges of space conditions effectively (Oluwafemi, 2022)**.**

2. Culinary Delights of Astronauts in Zero Gravity

Since Yuri Gagarin's inaugural space meal in 1961, the landscape of eating in space has evolved significantly. In the early days, astronauts consumed food from toothpaste-like tubes and bite-sized cubes (M. Perchonok & Bourland, 2002).

Presently, astronauts enjoy individually packaged, almost ready-to-eat meals such as beef tips, ravioli, and chicken teriyaki. These meals can be conveniently microwaved, opened with scissors, and eaten with utensils. International space missions bring a diverse array of foods from various countries, expanding the culinary options for astronauts. Russian crews on the ISS, for instance, have access to over 300 dish options, including mashed potatoes with nuts and goulash with buckwheat. The international menu extends to include Chinese yuxiang pork, eight treasures rice, Japanese ramen, sushi, rice with ume, as well as Korean kimchi and bulgogi (Pultarova, 2014).

To counter the effects of microgravity and prevent food from floating away, astronauts utilize special trays equipped with Velcro, magnets, and bungee cords. Small food warmer trays facilitate in-flight heating, while a potable water dispenser warms food or prepares beverages. The preparation of modern space food occurs on Earth in laboratories using commercially available raw materials. These foods are periodically sent to the space station, with new shipments arriving approximately six times a year. Bonus containers, containing fresh produce and astronauts' favorite meals, are dispatched every six months from Earth (Lupo, 2015; Preston, 2015).

3. Designing Astronaut Diets: Key Considerations and Nutritional Guidelines

Regarding nutritional specifications, Dobrovolsky V.F. (2016) suggests a daily caloric intake of 3000 ±150 kcal for astronauts. This caloric distribution should include 12–15% from proteins, 30–35% from fat, and 50–55% from carbohydrates. Adhering to these guidelines is critical to ensure astronauts receive the essential nutrients and energy required for maintaining optimal health and performance throughout space missions (Dobrovolsky, 2016).

The current crew members aboard the International Space Station (ISS) and the Space Shuttle receive around 1.8 kg of food per person per day, including packaging. They show a preference for thermostabilized foods over freeze-dried options due to taste considerations. Unlike the Apollo missions, the ISS relies on solar panels, eliminating the previous mass advantage of freeze-dried foods produced through water by fuel cells. Water is now transported separately. To meet the increased caloric needs of individuals, the average caloric delivery has risen to 3000 kcal, compared to the 2500 kcal for Apollo crew members. NASA is actively

exploring options to reduce the mass of the food system while ensuring an adequate caloric intake and maintaining an acceptable diet (NASA Human Research Program Advanced Food Technology, 2016).

Initially, the food system prioritized mass and volume constraints. However, there has been a shift in focus towards palatability, driven by concerns about crew intake as observed by flight doctors. In the Gemini Food System, for instance, bite-size cubes containing various components were designed to provide 21.3 J/g. The entire system delivered 12100 J (approximately 2890 cal) within a packaged food weight of 0.73 kg (M. C. Smith *et al.,* 1975).

In the study conducted by Smith *et al.* (2014), the vital considerations for designing astronauts' diets include several factors. Space food needs to achieve a delicate balance of essential nutrients, ensuring high energy provision while minimizing weight and volume. It must also be resilient to temperature variations and mechanical exposure, possessing a prolonged shelf life suitable for extended missions. In the microgravity environment, the food should be easily consumable and possess a familiar taste to enhance palatability. Additionally, there is a focus on minimizing food waste and facilitating straightforward preparation in space (S. M. Smith *et al.,* 2014).

4. Exploring the Latest in Astronaut Nutrition

The International Space Station (ISS) faces challenges in water use due to its mass (approximately 420,000 kg) and limited cargo capacity of the Space Shuttle (approximately 19,000 kg, with a launch cost of $25,000 for 0.5 kg of water to the ISS). To overcome these constraints, the ISS employs recycling methods, converting 70% of the daily 9 kg of urine Generated by six crew members and converted into water, resulting in significant cost savings (NASA Johnson Space Center, 2017).

The space foods prepared for the International Space Station (ISS) include a wide range of options such as frozen, refrigerated, or heat-treated items. These consist of beverages, fresh produce, irradiated meats, intermediate-moisture products, natural foods, rehydratable meals, thermostabilized items, and extras like salt, pepper, condiments, and tortillas (Cooper & Douglas, 2015).

Powdered beverages including coffee, tea, lemonade, and orange drink are contained in vacuum-sealed, pliable packaging. Rehydrated beverages are consumed using a straw with a clamp, and empty beverage pouches are provided for drinking water from the potable water dispenser. These practices contribute to resource optimization and sustainability in the space environment (Getsov & colleagues, 2020).

Fresh fruits and vegetables prepared for space missions receive minimal processing and are sanitized using a chlorine rinse to ensure safety. As these perishable items are sent via

resupply missions, they must be consumed within the first two days of the journey to avoid spoilage. To extend shelf life and maintain food quality in space, various preservation methods such as irradiation, thermal processing, freeze-drying, and dehydration are utilized. Meats like barbecued beef brisket, beef steak, beef tips with mushrooms, and smoked turkey are sterilized through irradiation, packaged in flexible pouches, and reheated when ready to eat (M. Perchonok & Bourland, 2002).

Intermediate moisture foods, containing 15–30% water to inhibit microbial growth, consist of items like dried peaches, pears, apricots, and naturally shelf-stable snacks such as candy-coated peanuts, trail mix, and granola bars. These products are stored in hygienic, flexible pouches that are opened using scissors. Rehydratable foods—including soups like chicken consommé and cream of mushroom, casseroles such as macaroni and cheese or rice with chicken, and breakfast options like scrambled eggs and cereals—are heat-treated, dehydrated, and vacuum-sealed. Just before consumption, hot or cold water is added using the onboard potable water dispenser. Thermostabilized foods, which are heat-processed to destroy harmful microbes and enzymes, are typically packaged in retort pouches for main dishes like beef tips with mushrooms, grilled chicken, and ham with tomatoes and eggplant. Fruits and seafood, including tuna and salmon, are thermostabilized in cans, while puddings are stored in plastic cups. Additional items such as oily pepper paste, liquid salt, and condiments like mayonnaise, ketchup, and mustard are included, along with shelf-stable tortillas, which serve as a common bread substitute(Lupo, 2015; Pultarova, 2014).

5. Crafting Menus and Optimizing Appliances in Space Food Formulations

The final objective of the Lunar/Planetary Food System involves food preparation in the galley, where a menu will be crafted to incorporate both processed crops and minimal resupply items. The focus is on devising recipes that demand minimal crew time while ensuring a secure, nutritious, and agreeable food system. The menu's goal is to provide sufficient variety to prevent crew "burnout." Various appliances, including a combination microwave/convection oven, dehydrator, bread maker, pasta maker, juicer/pulper, food processor, bagel maker, blender, rice cooker, scale, and dryer oven, are under consideration for potential use in the galley. These appliances may undergo minor modifications to suit long-duration exploratory missions (NASA Advanced Food Technology Team, 2003).

Crucial aspects include determining storage conditions and selecting suitable packaging for menu items, aiming for minimal weight and volume with extended shelf life and usability. Efficiency is of utmost importance, with efforts directed at minimizing crew time spent on galley procedures, encompassing food preparation, cleaning, and sanitizing. The assessment and integration of potable water needs, waste-water production, and solid-waste production during

food preparation will be conducted in coordination with other advanced life-support elements (M. Perchonok & Bourland, 2002).

6. Innovations in Space Food Preservation and Packaging

In preparation for extended spaceflights like those to Mars lasting up to 2.5 years, the Transit Food System aims to integrate prepackaged foods similar to those utilized on the Shuttle and the ISS. In addition to existing preservation techniques, the focus will be on technologies that enhance food quality, providing extended shelf lives, improved acceptability, and enhanced nutrition (NASA Johnson Space Center, 2009). The main challenge is to present palatable food with a shelf life of 3 to 5 years, where shelf life is defined as the duration until a product no longer maintains its quality, with safety as the top priority. The endpoint of shelf life may be determined by nutrition loss. Factors like changes in appearance, texture, and odor also play a role in defining shelf life (NASA Advanced Food Technology Team, 2005). The packaging system must align with processing and storage conditions, volume constraints, and solid-waste management requirements (NASA Environmental Systems Branch, 2006). The evaluation of biodegradable, reusable, or edible packaging materials is underway to minimize the impact on the solid-waste management system, given that waste from food packaging is expected to be a significant contributor to total transit waste. Ensuring the food maintains its shelf life is crucial to guarantee both safety and acceptability throughout the entire mission duration (Getsov & others, 2023).

The existing packaging system faces a notable drawback concerning mass and volume, as it employs two distinct packages simultaneously for specific products. The primary packaging material, which lacks sufficient oxygen and moisture barrier properties necessary for an 18-month shelf life mandated by the ISS flight food system, is utilized for freeze-dried and natural form foods. This primary packaging allows for tray molding and visual inspection. However, to meet the required protection standards and attain the desired shelf life, these foods undergo an additional layer of wrapping with a second package containing foil, possessing more stringent barrier properties (NASA Johnson Space Center, 2012).

Ensuring the safety of the food system involves subjecting packaged foods to processes that achieve commercial sterility. However, this rigorous level of processing, while guaranteeing safety, can have implications for food quality, impacting both nutritional content and acceptability.

The provided food items for space missions are diverse in their forms. NASA utilizes a retort process for thermostabilized foods, heating them to eliminate pathogens, spoilage microorganisms, and enzyme activity. This includes various products like pouched soups, sides, desserts, puddings, and entrees. Although not commonly used for commercial sterility, NASA

has FDA approval for nine irradiated meat items. Rehydratable foods, both commercially and internally processed freeze-dried, are part of NASA's provisions and are rehydrated during missions using potable water. Examples include spicy green beans, cornbread dressing, or cereals, typically serving as side dishes and rehydrated with ambient or hot water. Shelf-stable natural form foods with reduced water activity inhibit microbial growth, providing familiar menu options with no preparation time. Extended shelf life bread products, like scones and tortillas, formulated for an 18-month shelf life, offer menu variety. Despite limited provision due to a short shelf life, fresh fruits and vegetables on the ISS and Space Shuttle contribute more to psychological support than meeting strict dietary requirements. Beverages, including freeze-dried coffee and tea mixes, flavored drinks like lemonade and orange, are vacuum-sealed in pouches with options for adding sugar or powdered cream, and empty pouches are provided for drinking water(NASA Advanced Food Technology, 2014).

6.1 Edible Film

Edible films are generally made from starches, polysaccharides, proteins, fats, or their combinations. They are used to preserve fresh produce, meats, frozen and baked goods, and also serve as packaging for flavoring powders in fast food applications (Zhang *et al.,* 2011).

The main purpose of edible packaging films is to preserve the taste and texture of food during storage and transport. They function by blocking the transfer of gases, moisture, solutes, and aromatic compounds, thereby maintaining food quality and prolonging shelf life (M. Perchonok & Bourland, 2002).

However, current edible film technology faces performance limitations, such as poor tensile strength, sealing performance, water resistance, and high-temperature resistance. Furthermore, its barrier performance falls short of meeting the demands for extended manned missions, making it ineffective for achieving the required 3-5 years shelf life of spaceflight food. Nevertheless, edible films may find application in short-term storage of dry materials like flour during long-term missions or the establishment of a Mars base due to their degradable nature and minimal waste production (Sun *et al.,* 2016).

6.2 Metal Can

Metal can packaging materials, including tinplate and aluminum alloy, offer excellent barrier properties, ensuring a food shelf life of up to 3 years. During the Skylab program, aluminum cans were predominantly used, maintaining a 2-year shelf life (Klicka and Smith 1982). This packaging technology is still employed in manned missions, like the International Space Station food provided by Russia, due to its effective barrier properties. However, the drawback lies in its weight, making it unsuitable for long-duration manned flight missions due to challenges in both weight and waste disposal (Sun *et al.,* 2016).

6.3 Retort Pouch

Retort-processed products effectively ensure food safety, nutritional value, and high acceptability. This packaging method, utilizing a quad-laminate of polyolefin/aluminum foil/polyamide/polyester has substantial potential to maintain the sensory texture acceptability of food over a storage period of 3–5 years (S. M. Smith *et al.,* 2014). The package exhibits nearly zero oxygen and moisture permeation. NASA's research indicates that a metalized film overwrap significantly slows the rancidity progression of butter cookies compared to non-metalized films with the highest barrier (Cooper & Douglas, 2015). Catauro and Perchonok (2012) conducted a 36-month accelerated shelf life study on 13 typical retort pouch products to assess their suitability for long-term space flight (M. H. Perchonok *et al.,* 2012).

6.4 High Barrier Packaging

High water vapor and oxygen barrier properties were developed in packaging material during the Gemini mission (1965–1966) to safeguard food flavor (Perchonok and Bourland 2002). These packaging materials incorporate barrier layers like EVOH, SiOx, alumina, and titanium oxide (Sun *et al.,* 2016).

7. Innovative Space Food Design Concepts

Design Concept 1: Spice Bomb Mixing

The Spice Bomb Mixing concept draws inspiration from the notion of an "emotional" cleanser, aiming to enhance the food experience for astronauts by setting an appropriate mood before eating. Addressing the challenge of space food being perceived as less intense and sometimes bland, this concept introduces a flavor-enhancing seasoning design. It allows individuals to mix foods together, intensifying flavors in a convenient microgravity setting. The goal is to make food preparation interactive, social, and enjoyable, considering functionality, sensorial elements, emotional aspects, and social dynamics. The aim is to provide a variety of taste experiences and improve the overall sensory aspects of eating in space (Spence, 2017).

Design Concept 2: Flavor Journey 3D Printer

The Flavor Journey 3D Printer concept focuses on providing individuals with their preferred flavors through a customizable food printing system. Users can design their flavor profiles or order them from various sources, and the recipes are transmitted directly to the printer, eliminating the need for physical delivery. This concept aims to offer on-demand tastes and nutrients through the reconstruction of ingredients using 3D food printing technology. Key aspects considered include functionality, sensorial elements, emotional aspects, and social dynamics. The concept enables collaboration with individuals on Earth, turning food preparation into a social and personally satisfying experience, despite communication delays (Cappellini & others, 2019).

Design Concept 3: Earth Memory Bites

The Earth Memory Bites concept introduces small bites encapsulating distinct flavors representing various Earth regions, cultures, or specific food-related experiences. The aim is to provide comfort through familiar flavors, integrating each Earth Memory Bite into a specific dining environment. Users can order a combination of flavor profiles and multisensory experiences, choosing between receiving the suggested dining environment generated by an automated algorithm or selecting a specific one. The flavors, prepared as a 3D print recipe, are sent along with a carefully chosen multisensory environment. Individuals can then print and experience the flavors in the immersive dining environment on the spaceship, either alone or with others. Each self-contained and edible bite is one-size, avoiding the need for different items to move around the spaceship, and mimics the form and texture of the actual food item (S. M. Smith *et al.,* 2014).

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MONOGENIC DIABETES:
AN OVERVIEW OF MODY AND RELATED CONDITIONS

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Introduction:

Monogenic diabetes is a type of diabetes caused by a change in a single gene. One common form is called maturity-onset diabetes of the young (MODY), a term introduced by Fajans and Tattersall. MODY usually appears in young people under 30 years old and runs in families in an autosomal dominant pattern, meaning it often affects two or three generations. People with MODY are usually not obese and may respond well to certain medications like sulfonylureas. Research by Fajans and others helped discover the first MODY genes through studies of affected families[1,2].

To date, more than 20 genes have been identified as associated with monogenic diabetes. In the past, many of these genes—especially those linked to MODY—were named using numbers based on the order in which they were discovered, often without meaningful reference to the actual gene involved. As the list of MODY-related genes expanded and some previously assigned MODY numbers were later disproven, it became more appropriate to use the actual gene names, such as GCK-MODY instead of MODY 2 or HNF1B-MODY instead of MODY 5, to reduce confusion. Therefore, gene names are now commonly used throughout the text, though both gene names and older MODY numbers are included in tables for clarity. This naming system is particularly important because some newer MODY genes were given the same number by different researchers, and a few listed genes may either be extremely rare or not directly cause diabetes. Adopting a clear and consistent naming method is essential for improving the recognition and diagnosis of these conditions[3,4]. The glucokinase gene (GCK) is the most common gene linked to monogenic diabetes, followed by the HNF1A gene in many cases. People with monogenic diabetes may be noticed during a urine test that shows sugar (glucosuria), even if their blood sugar and HbA1c levels are normal[5]. This article discusses the common signs and treatment approaches for monogenic diabetes.

Presentation of Monogenic forms of Diabetes:

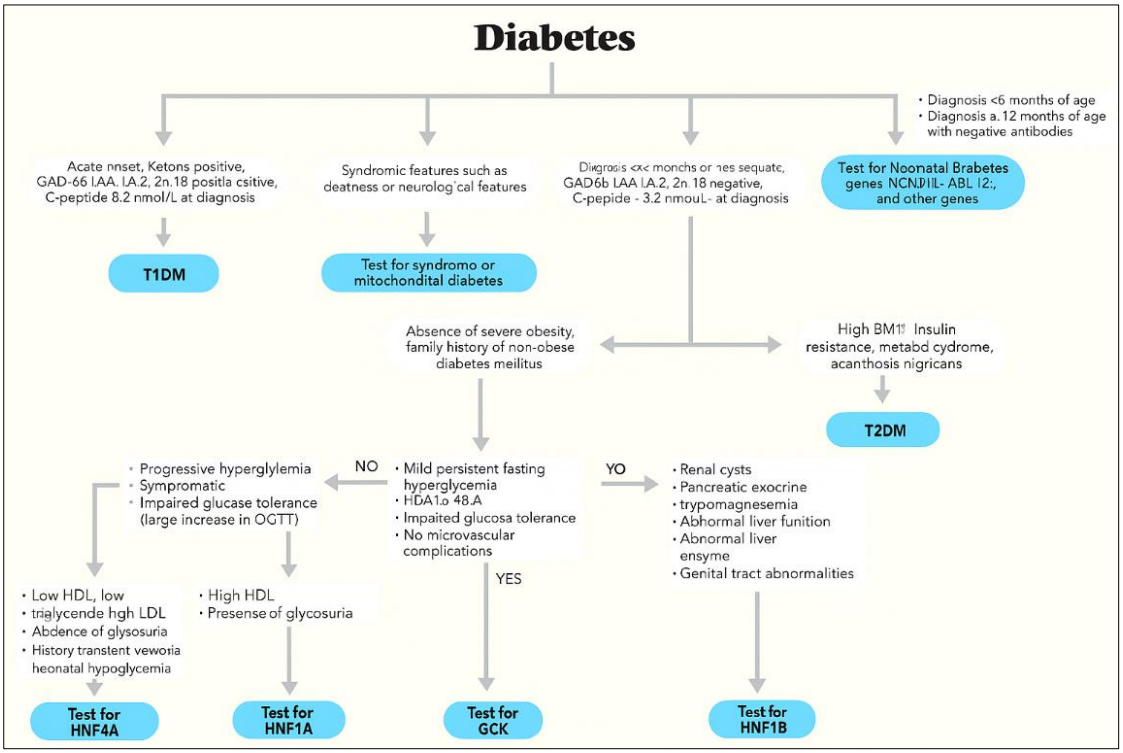

Figure 1

Algorithm for the Diagnosis of Monogenic Diabetes.
Diagnosis of monogenic diabetes is a stepwise process (clinical assessment, diabetes-specific test, and genetic testing). Commercial panels for monogenic diabetes typically include the following genes to be sequenced: GCK, HNF1A, HNF4A, HNF1B. Conversion formulas for C-peptide and HbA1c values are provided in the Conversions section. ABCC8, ATP-binding cassette transporter subfamily C member 8; BMI, body mass index; GAD-65, glutamic acid decarboxylase 65 autoantibodies; GCK, glucokinase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HNF, hepatocyte nuclear factor; IA-2A, tyrosine phosphatase-related islet antigen-2 autoantibodies; IAA, insulin autoantibodies; INS, insulin; KCNJ11, potassium inwardly rectifying channel subfamily J member 11; LDL, low density lipoprotein; OGTT, oral glucose tolerance test; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; ZnT8, zinc transporter 8 autoantibodies.

Monogenic diabetes includes types of diabetes that start in newborns, children, or young adults, usually before the age of 35. It is often mistaken for type 1 or type 2 diabetes at first. However, people with monogenic diabetes usually test negative for autoantibodies (which are found in type 1 diabetes). Even if a person has a slightly positive result for the anti-GAD test, it doesn't always mean they have autoimmune diabetes, because some healthy people can also test positive. Also, autoantibodies can fade over time and may not show up later. Testing for several types of autoantibodies at the time of diagnosis—like anti-GAD, anti-IA2, anti-ZnT8, and anti-insulin—can help, but a negative result doesn't fully rule out type 1 diabetes. A family or personal history of autoimmune diseases might still suggest type 1 diabetes. For research, a special type 1 diabetes genetic risk score (T1DGRS) can help predict the likelihood of type 1 diabetes. Measuring C-peptide (which shows how much insulin the body makes) isn't very useful in the early years after diagnosis. In people with type 1 diabetes, C-peptide levels usually

drop after 3–5 years. A newer method that measures C-peptide in urine—developed by researchers in Exeter—is gaining interest because it gives a more stable and average picture of how well the pancreas is working[6-8].

Figure 1 shows a step-by-step guide for diagnosing monogenic diabetes. Looking for certain health features can be very helpful when deciding if genetic testing is needed. These features can vary and may include developmental delays, hearing loss, eye muscle or vision problems, liver growths, kidney or other organ cysts, urinary issues, chemical imbalances like alkalosis, and low magnesium levels. It is also important to check the person's birthweight (whether it was too high or low) and if they had low blood sugar as a newborn. Taking a detailed family history is also useful. If any close family member has diabetes—especially if diagnosed at a young age—it could be monogenic, even if labeled as type 1 or type 2. A family history of other related health problems or autoimmune diseases can also give helpful clues[9].

Figure 2 Some types of diabetes caused by a single gene defect (monogenic diabetes) are passed down through families. Most are dominant, meaning you only need one copy of the faulty gene from a parent to inherit the condition. However, some are recessive (you need two copies) or inherited from the mother. If a family has GCK-, HNF1A-, HNF4A-, or HNF1B-MODY, you might see diabetes in three or more generations, indicating a dominant inheritance pattern, with many relatives affected[10].

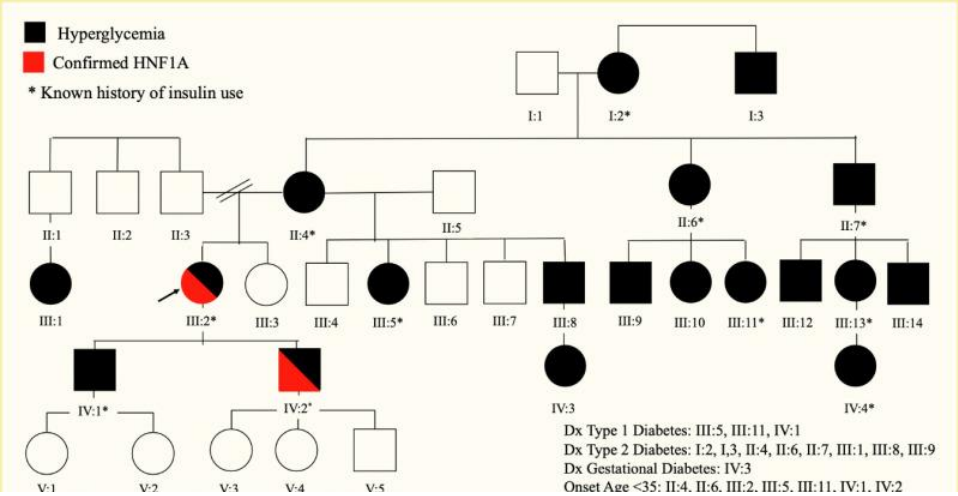

Figure 2

HNF1A Pedigree Shows Diabetes in Multiple Generations (Autosomal Dominant).
A 58-year-old female was initially found to be hyperglycemic at age 19 years with fasting blood glucose of 130 mg/dL. BMI was 19 kg/m2. Blood glucose was retested at age 23 years during pregnancy, and the individual was diagnosed as having gestational diabetes and then type 2 diabetes mellitus. Initially diet-controlled, but transitioned between oral agents (metformin and troglitazone) and insulin due to fluctuating diagnoses of gestational, type 1, and type 2 diabetes. Presented to a new endocrinologist at age 58 years, weight 230 pounds, BMI 40.7 kg/m2, using 90 units/day via an insulin pump. Sulfonylureas were started; A1c improved to the 6% range. Insulin was withdrawn as she lost weight (over 70 pounds). She had euglycemic diabetic ketoacidosis (DKA) after SGLT2i treatment. Conversion formulas for A1c and glucose levels are provided in the Conversions section. A1c, glycated hemoglobin; BMI, body mass index; HNF1A, hepatocyte nuclear factor 1A; SGLT2i, sodium-glucose cotransporter 2 inhibitor.

Figure 3: Once a gene change (variant) causing monogenic diabetes is found, the person should be referred for genetic counseling. The counselor will explain the results to the patient and their family, and give them information to share with other family members who may also need testing. Since most of these gene changes are inherited in a dominant way, each child of an affected person has a 50% chance of having the same type of diabetes[10].

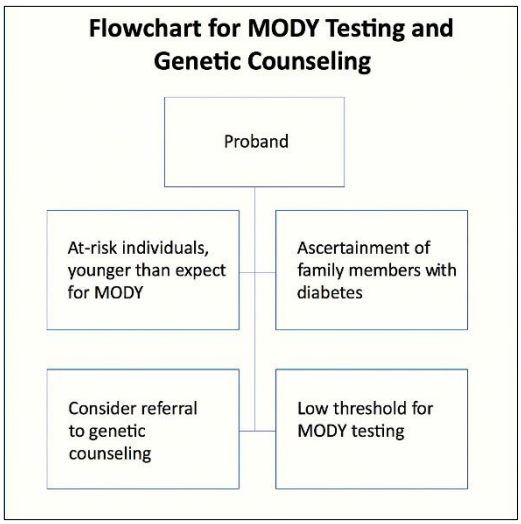

Figure 3
Cascade Testing for Monogenic Diabetes.
In any autosomal dominant disease, the diagnosis in a proband should prompt the identification of affected members. In the case of MODY, any family member should be clinically assessed to decide whether testing is needed. MODY, maturity-onset diabetes of the young.

Common Forms of Monogenic Diabetes:

GCK was the first confirmed gene linked to MODY, although it was once called MODY 2 because it was the second gene mapped through family studies. GCK-MODY is caused by a loss-of-function mutation in the GCK gene, located on chromosome 7, which affects how beta cells sense glucose. This leads to mild, stable high blood sugar (fasting levels between 99–144 mg/dL) starting from birth. It is fairly rare—found in about 0.2% of the general population and 1.3% of people with diabetes. It is often found by accident during routine blood tests in children or adults, or during pregnancy check-ups. People with GCK-MODY usually test negative for islet cell antibodies, and glucose tolerance tests show mild increases in blood sugar, rarely going over 200 mg/dL after two hours. Their blood sugar then slowly returns to slightly high levels (90–130 mg/dL) (5.00–7.22 mmol/L) (Figure.4). Despite different levels of gene mutation severity, patients with GCK-MODY usually have similar symptoms. This may be because the normal copy of the gene works harder to make up for the faulty one[11-15].

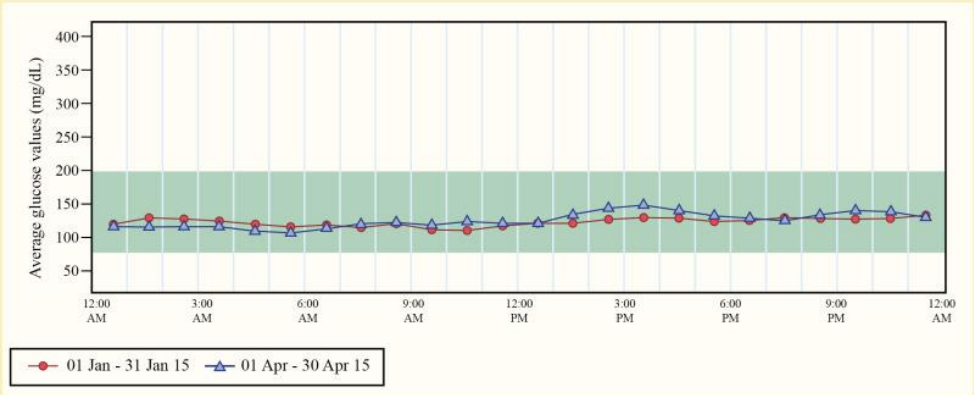

Figure 4

Insulin Treatment is Not Required for GCK-MODY.
Average glucose levels of a patient with insulin (red, filled circles) and without insulin (blue, open triangles) are shown. After 13 years of diabetes treatment, the patient unplugged his insulin pump. A conversion formula for glucose values is provided in the Conversions section. GCK, glucokinase; MODY, maturity-onset diabetes of the young.

If insulin levels are tested, they are usually normal but are released when blood sugar levels are higher than normal. A1c levels in these patients are typically between 5.6% and 7.6% (38–60 mmol/mol)[16,17]. When there is no family history, people with GCK-MODY are often mistakenly diagnosed with type 1 diabetes and started on insulin. Others may be treated as if they have type 2 diabetes and given tablets or insulin sensitizers. However, these patients usually do not respond to diabetes tablets or low-dose insulin. Their beta cell function stays mostly stable with age, similar to the general population. Diabetes-related complications are rare. The only known complication is mild eye damage (nonproliferative retinopathy), which happens only slightly more often than in people without diabetes[18].

Medicines used to treat GCK-MODY usually don't work well because the problem comes from reduced glucokinase activity in the pancreas, liver, and brain, which also affects how the body controls blood sugar. One study showed that a single dose of dapagliflozin lowered fasting blood sugar, but more research is needed to understand this effect. Long-term studies are also needed for SGLT2 inhibitors, as they may increase the risk of a serious condition called euglycemic diabetic ketoacidosis (DKA). However, current research suggests that these drugs are not necessary, since GCK-MODY rarely causes long-term complications. In fact, glucose-lowering treatments may even be harmful in these patients. Many physicians are still unsure about this advice, but it may be because people with GCK-MODY usually aren't obese and tend to have normal cholesterol and blood pressure, unlike many with type 2 diabetes. If a patient with GCK-MODY has a high A1c level, the diagnosis should be reviewed, and other factors such as obesity, steroid use, or type 1 diabetes should be considered. Rarely, a person with GCK-MODY may also develop type 1 diabetes, in which case insulin treatment would be needed.

Pregnancy is one special case where treatment may be required. If the baby inherits the GCK mutation, no treatment is needed because the baby's blood sugar levels will match the mother's. But if the baby does not inherit the mutation, the mother's high blood sugar could harm the baby. In that case, insulin is given to the mother to keep her blood sugar at normal pregnancy levels. Often, high insulin doses are needed, which can increase the risk of low blood sugar in the mother[19-21].

HNF 1A-MODY

HNF1A-MODY (MODY 3) is caused by a heterozygous dominant mutation in the HNF1A gene, which codes for a transcription factor mainly found in the liver, pancreatic beta cells, and kidneys. This gene was the third to be identified in MODY families, so it is also called MODY 3. The HNF4A gene partly controls HNF1A expression, linking these two types of monogenic diabetes. HNF1A and HNF1B are transcription factors that bind DNA and work as dimers (pairs). Most mutations occur in the DNA-binding region, but can also be insertions or deletions anywhere in the gene. Diabetes occurs mainly due to haploinsufficiency, where one non-functioning copy of the gene leads to disease. Proper timing and location of HNF1A expression are important for the development and maintenance of organs like the pancreatic islets. While there is no clear link between the specific mutation and how diabetes presents, some studies suggest that mutation type and location may influence the age at diagnosis [22-24].

In individuals with heterozygous HNF1A mutations, birth weight is typically normal. Diabetes usually develops between the late childhood years and early adulthood (from around age 10 to 30). Clinical presentation can range from very mild to more obvious symptoms. In some cases, diabetes is discovered by chance, such as during a routine urine test. Others may present with more typical symptoms of diabetes and sometimes ketosis, though diabetic ketoacidosis (DKA) is rare. These patients may be given insulin temporarily, especially at diagnosis. Most individuals with HNF1A-MODY respond very well to sulfonylureas, often even at low doses, which may cause hypoglycemia. If a patient has severe low blood sugar after starting a usual dose of sulfonylurea, a HNF1A mutation should be suspected. Over time, some patients may no longer respond well to sulfonylureas and might need insulin therapy. Weight gain has been linked to this treatment failure. However, newer medications like GLP-1 receptor agonists and DPP-4 inhibitors, when used along with sulfonylureas, may help maintain good blood sugar control without insulin. There can be variations in the age of diabetes onset and response to sulfonylureas, even within the same family, and these differences do not seem to depend on the specific gene mutation[15,25].

Another possible feature of HNF1A-MODY is the presence of liver adenomas, which are usually benign (non-cancerous) but can sometimes be large and may cause bleeding. Patients may also have mild changes in cholesterol and lipid levels, which increases their risk of cardiovascular disease. Therefore, early use of lipid-lowering medications may be considered. The HNF1A gene also plays a role in regulating SGLT2, a protein in the kidneys that helps reabsorb glucose. In people with HNF1A-MODY, reduced HNF1A function leads to lower SGLT2 levels, which causes glucose to spill into the urine (glucosuria) even when blood glucose levels are normal. This explains why some young individuals with normal A1c and glucose levels but glucosuria are later diagnosed with HNF1A-MODY. These cases are sometimes mistaken for type 1 or type 2 diabetes. Additionally, C-reactive protein (CRP), a marker of inflammation made in the liver, is partly regulated by HNF1A. Patients with HNF1A mutations often have low levels of high-sensitivity CRP (hsCRP) compared to people with other types of diabetes. However, not all studies have confirmed this finding[(26)]. If not properly managed, HNF1A-MODY can lead to serious diabetes complications, similar to those seen in uncontrolled type 2 diabetes. Although many patients initially respond well to sulfonylurea treatment, this effect may decrease over time in some individuals. In such cases, GLP-1 receptor agonists (GLP-1 RAs) may be helpful. Some small studies and case reports suggest that GLP-1 RAs could either improve blood sugar control or restore the patient's response to sulfonylureas[(27)]. A large systematic study on HNF1A-MODY treatments has not yet been conducted. Some smaller studies suggest that SGLT2 inhibitors might be helpful. However, since patients with HNF1A-MODY already have reduced SGLT2 expression, further lowering it with medication may cause a condition called euglycemic diabetic ketoacidosis (DKA)—where ketones increase despite normal blood sugar levels. Although this has not been reported in HNF1A-MODY, it has been seen in type 1 and type 2 diabetes with low insulin levels. Therefore, this treatment should be used with caution and preferably within a clinical trial, as it may increase the risk of complications. Additionally, genome-wide association studies have linked HNF1A gene variants to common type 2 diabetes, suggesting that a range of mutations with different effects may exist. The Framingham Heart Study found that not everyone who carries an HNF1A-MODY variant develops diabetes, a concept known as incomplete penetrance. This suggests that other genes might influence or compensate for the loss of HNF1A function in some people[(28)].

HNF4A-MODY

HNF4A-MODY, also called MODY 1, is caused by mutations in the HNF4A gene, which produces the HNF4α transcription factor. This gene was the first to be mapped in MODY families but was actually the third gene identified as causing MODY. HNF4A is mainly active in

the liver, kidneys, intestines, and pancreatic islets, and is present at lower levels in many other tissues. Like HNF1A-MODY, this form of MODY involves a gradual loss of insulin secretion with age. Symptoms of HNF4A-MODY can begin in early childhood and may appear any time up to the third decade of life. Many patients respond well to sulfonylureas, which help increase insulin secretion and lower blood sugar, but some may eventually need insulin therapy due to a progressive decline in insulin production. Two unique features have been reported in some individuals with HNF4A-MODY: Higher birth weight—about 800 grams more than unaffected siblings. Neonatal hypoglycemia caused by high insulin levels at birth, which usually resolves on its own. In some cases, diazoxide is used to manage this. These observations suggest that some HNF4A mutations may initially cause excess insulin production, but later lead to beta-cell failure and diabetes. It is still unclear whether all babies with high birth weight and neonatal hypoglycemia due to HNF4A mutations will develop diabetes, but long-term monitoring is advised, especially approaching puberty. People with HNF4A-MODY also tend to have lower HDL cholesterol and lipoprotein A2, patterns often seen in type 2 diabetes. Unlike HNF1A-MODY, these patients do not consistently show low CRP levels, so hsCRP testing is not a reliable screening tool for diagnosing HNF4A-MODY[29-34].

HNF1B-MODY:

HNF1B-MODY, also known as MODY 5, is caused by mutations in the HNF1B gene, which was the fifth MODY gene to be discovered. About 50% of cases are due to single nucleotide variants, and the other 50% are due to large deletions in the gene. Both types of mutations can lead to a multi-organ disorder, sometimes affecting only one organ (such as the pancreas or kidneys), or several. The disease is thought to result from haploinsufficiency, where having only one working copy of the gene is not enough. There is no clear difference in the diabetes symptoms between people with small mutations and those with large deletions in this gene. HNF1B also regulates HNF4A, linking the three major MODY genes: HNF1A, HNF4A, and HNF1B. Mutations in HNF1B most often cause abnormal kidney development. A common condition seen is Renal Cysts and Diabetes (RCAD) syndrome. Other related issues may include, Underdeveloped pancreas and pancreatic enzyme deficiency, which can worsen over time and may need fecal elastase testing for monitoring, Reproductive system abnormalities, which may affect fertility, Liver function abnormalities, high uric acid levels, low magnesium due to kidney magnesium loss, and alkalosis, Early proteinuria not related to diabetic kidney disease and Progressive kidney failure, which may lead to dialysis or kidney transplant. Sometimes, HNF1B-MODY is diagnosed when a person with diabetes has a family history of kidney cysts or early kidney failure in siblings or parents[35-37]. The underlying mechanism of HNF1B-MODY

involves both beta cell dysfunction and insulin resistance. Unlike patients with HNF1A-MODY or HNF4A-MODY, most individuals with HNF1B-MODY do not respond well to sulfonylureas, and many require insulin therapy. Other diabetes medications have not been well studied in this group.

A report by Ray *et al.* described three patients with diabetes and persistent low magnesium levels (hypomagnesemia). Two of them had confirmed HNF1B mutations, while a deletion could not be ruled out in the third. The study found that SGLT2 inhibitors improved magnesium levels in these patients by possibly enhancing kidney reabsorption of magnesium. However, the study did not include data on blood glucose levels or kidney function (glomerular filtration rate) after treatment with SGLT2 inhibitors[38,39].

In addition to single nucleotide variants and large deletions in the HNF1B gene (which may be due to chromosome microdeletions), chromosome 17q12 deletions—which remove HNF1B and 14 nearby genes—are also known causes of HNF1B-MODY. These 17q12 deletions are often linked with neurodevelopmental disorders, including autism spectrum disorder, ADHD, and cognitive impairments. It is still uncertain whether neurocognitive problems are present in people with only intragenic HNF1B mutations (mutations within the gene itself). One study by Dubois-Laforgue *et al.* found these issues were more common in patients with the 17q12 deletion, though they also appeared—less frequently—in patients with intragenic mutations. Clissold *et al.* identified differences in DNA methylation patterns in individuals with HNF1B-related disease, especially in those with 17q12 deletions. These changes suggest that epigenetic mechanisms may try to compensate for the gene loss. However, they did not study the link between methylation and psychiatric symptoms due to the lack of mental health data. Interestingly, newer research suggests a genotype-phenotype correlation for kidney disease: individuals with 17q12 deletions may have better kidney function than those with HNF1B intragenic mutations, which cause complete loss of function. Because HNF1B-associated disease can affect many organs and systems, care for these patients and their families is often complex. It may require input from specialists in diabetes, nephrology, gynecology/urology, gastroenterology, and neurology[40-44].

Neonatal Diabetes Mellitus:

Neonatal diabetes mellitus (NDM) is a rare form of diabetes that begins very early in life, typically before 6 months of age. In these cases, the cause is usually genetic (monogenic). Although rare, some monogenic forms of diabetes can also appear between 6 and 9 months, or even later. However, after 6 months of age, the majority of diabetes cases (more than 95%) are due to autoimmune type 1 diabetes. Because of this, genetic testing for known NDM-related

genes is recommended for all infants diagnosed with diabetes before 6 months, and also for those diagnosed between 6 and 12 months—especially if islet autoantibody tests are negative or if there are other signs suggesting a monogenic cause. NDM is classified into two main types: Permanent neonatal diabetes mellitus (PNDM) – the condition is lifelong and Transient neonatal diabetes mellitus (TNDM) – the diabetes may disappear in infancy but can return later in life. Some of the same genes involved in NDM are also associated with later-onset MODY and type 2 diabetes, showing some genetic overlap between these forms.

Table 1: Classification of Neonatal Diabetes Mellitus, 2022.

Gene Name	**Mode of Inheritance***	**Relative incidence/frequency (percent)†**		**Phenotypic features**
		Non-Consanguineous	Consanguineous	
KATP Channel				Diabetes responds well to high-dose sulfonylurea treatment in most cases. Spectrum of neurodevelopmental dysfunction that depends on specific mutation.
KCNJ11	Dominant	30	5	
ABCC8	Dominant/Recessive	15	10	
6q24	Variable	15	5	Intrauterine growth restriction (IUGR), macroglossia, umbilical hernias are common; other features are rare. Diabetes is always transient, with median remission by age 4 months, with recurrence of diabetes around puberty or later.
INS	Dominant/Recessive	10	10	Progressive insulin deficiency clinically like type 1 diabetes

GATA6 *GATA4*	Dominant	5	<2.5	Pancreatic agenesis/hypoplasia and corresponding exocrine pancreatic insufficiency; cardiac malformations; developmental delay; other features less common.
EIF2AK3	Recessive	2.5	25	The most common recessive cause, especially in populations where consanguinity is more common. Wolcott-Rallison syndrome: spondyloepiphyseal dysplasia, recurrent episodic liver failure, renal failure, neurocognitive dysfunction.
FOXP3	X-Linked	1.5	1.5	IPEX syndrome or IPEX-like syndrome: autoimmune enteropathy, eczema, and other autoimmune manifestations (similar manifestations may be seen in other autoimmune causes, such as STAT3, LRBA, IL2RA, and others). Severe cases often require stem cell transplant.
GCK‡	Recessive	1	10	Both parents will have GCK-MODY.
PTF1A	Recessive	<2.5	10	Pancreatic agenesis; cerebellar agenesis; developmental delay.
Rare				
HNF1B‡, *GATA4,* *STAT3*	Dominant	5	10	
GLIS3, *PDX1*‡, *ZFP57,*	Recessive			

RFX6, NEUROG3, NKX2-2, MNX1, SLC2A2, SLC19A2, IER3IP1, CNOT1, IL2RA, LRBA, WFS1, PAX6				
Unknown				
Fraction of cases in which no monogenic cause has yet been identified		15	10	A significant proportion of cases may represent type 1 diabetes. Several cases in this category have Down syndrome

Table: 1

ABCC8, ATP-binding cassette transporter subfamily C member 8; CNOT1, CCR4-NOT transcription complex subunit 1; EIF2AK3, eukaryotic translation initiation factor 2-alpha kinase 3; FOXP3, forkhead box P3; GATA4/6, GATA-binding factor 4/6; GCK, glucokinase; GLIS3, GLI-similar zinc finger protein family member 3; HNF, hepatocyte nuclear factor; IER3IP1, immediate early response 3 interacting protein 1; IL2RA, interleukin-2 receptor alpha chain; INS, insulin; IPEX, immunodysregulation, polyendocrinopathy, enteropathy, X-linked; KATP, ATP-regulated potassium channel; KCNJ11, potassium inwardly rectifying channel subfamily J member 11; LRBA, lipopolysaccharide-responsive and beige-like anchor protein; MNX1, motor neuron and pancreas homeobox 1; MODY, maturity-onset diabetes of the young; NEUROG3, neurogenin 3; NKX2-2, NK2 homeobox 2; PAX6, paired box 6; PDX1, pancreatic and duodenal homeobox 1; PTF1A, pancreas transcription factor 1 subunit alpha; RFX6, regulatory factor X6; SLC19A2, solute carrier family 19 member 2; SLC2A2, solute carrier family 2 member 2; STAT3, signal transducer and activator of transcription 3; WFS1, wolframin endoplasmic reticulum transmembrane glycoprotein; ZFP57, zinc finger protein 57.

**For the more common dominant causes of neonatal diabetes mellitus, including KATP channel genes and INS, approximately 80%–85% of cases have de novo/spontaneous mutations that were not inherited but thereafter could be passed on to future generations.*

† Reference[45]

‡Heterozygous mutations in GCK, PDX1, and HNF1B may also cause MODY 2, MODY 4, and MODY 5, respectively.

SOURCE: Original table constructed by S. Greeley, M. Salguero, and R. Naylor.

KATP Channel Diabetes: KCNJ11 and ABCC8

The most common cause of neonatal diabetes mellitus (NDM) is activating mutations in one of the two genes that code for the KATP channel, accounting for nearly 50% of all cases. The KATP channel is an important ion channel that helps regulate insulin secretion by maintaining a resting membrane potential when open. When glucose levels rise, ATP production increases, causing the channel to close, leading to membrane depolarization and insulin release. In NDM, activating mutations (usually heterozygous, but sometimes biallelic) keep the KATP channels open, even when glucose levels are high, preventing normal insulin secretion. About 95% of patients with these mutations respond well to high doses of oral sulfonylureas (most commonly glyburide, at doses of 0.5 to 2 mg/kg/day or higher), which work by closing the channels and restoring insulin secretion.Since KATP channels are also present in the brain, these mutations can lead to a range of neurodevelopmental problems, from mild learning difficulties and ADHD to severe developmental delays and uncontrolled seizures. The severity of brain symptoms is linked to how strongly the mutation affects the channel's function[46,47,48,49].

Most children with KATP channel-related neonatal diabetes have de novo mutations, meaning the mutation occurred spontaneously and was not inherited from their parents. However, in about 10–15% of cases, there is a family history of neonatal diabetes in a parent or other relatives. Genetic testing can confirm whether the mutation is present in affected family members, who should be offered a trial of high-dose oral sulfonylurea therapy (such as glyburide up to 2 mg/kg/day or more). This treatment is often effective even in adults who have been on insulin for many years. Parents of children with an apparent de novo mutation should be counseled about the risk of recurrence in future pregnancies. This is due to the possibility of germline mosaicism, where the mutation exists in the reproductive cells (eggs or sperm) but is not found in the parent’s blood. High-dose sulfonylurea therapy improves insulin secretion not only by closing KATP channels but also by enhancing non-KATP pathways, such as those activated by the incretin response to oral feeding[50].

While severe hypoglycemia is very rare in patients treated with sulfonylureas for KATP channel diabetes, mild to moderate hypoglycemia may occur, especially after meals that are low in carbohydrates. This is because insulin is still released in response to protein and fat, even when blood glucose levels are not high—since insulin secretion is no longer controlled by the glucose-sensitive KATP channel. Clinical reports suggest that neurological symptoms may improve after starting sulfonylurea therapy. However, the extent of improvement depends on how early treatment is started, ideally during early brain development. If brain dysfunction begins in the womb, the chance for full recovery may be limited[10].

Transient Neonatal Diabetes Mellitus Due to Overexpression of imprinted Genes on chromosome 6q24 (6q24-TNDM)

Children with 6q24-related transient neonatal diabetes mellitus (6q24-TNDM) are typically born underweight, with an average birth weight of about 2,000 grams, which is low for near-term infants. Diabetes is usually diagnosed within the first week of life, though in some cases it may be identified a few weeks later. Most newborns show significant high blood sugar (hyperglycemia) and are initially treated with insulin, although a few may respond to sulfonylureas. Unlike other forms of neonatal diabetes caused by insulin deficiency, diabetic ketoacidosis (DKA) is rare in 6q24-TNDM. This suggests that there is some residual beta cell function, which tends to improve gradually over the first few months. As blood sugar levels stabilize, treatment can often be reduced and stopped, usually by about 4 months of age, though a few cases may require therapy for up to a year. During this time, remission of diabetes occurs in almost all patients. Some children with 6q24-TNDM may also have macroglossia (enlarged tongue), umbilical hernia, or, less commonly, neurodevelopmental delay, especially when the diabetes is related to a global maternal methylation defect (such as from ZFP57 gene mutations or unexplained causes). During remission, insulin production often appears normal, and glucose metabolism is close to normal. However, a significant number of individuals experience relapse of diabetes during puberty or young adulthood. In many of these cases, oral diabetes medications may be effective, and insulin may not be required.

The cause of 6q24-TNDM involves overexpression of maternally imprinted genes on chromosome 6q24, due to three known mechanisms:

- Paternal uniparental disomy (both copies of 6q24 are inherited from the father),
- Paternal duplication of the 6q24 region (which can be inherited)
- Defects in maternal imprinting, which are usually idiopathic, but in rare cases may be caused by biallelic mutations in the ZFP57 gene. These may also affect other imprinted regions beyond 6q24[51-53].

Insulin Gene Mutation:

Mutations in the insulin gene (INS) are the third most common cause of neonatal diabetes mellitus (NDM), following KATP channel mutations and 6q24 defects. Most INS mutations are heterozygous and dominant and occur in critical regions of the preproinsulin molecule, such as near disulfide bridges. These mutations cause the insulin molecule to misfold, leading to problems in processing and secretion, and triggering endoplasmic reticulum (ER) stress and beta cell death (apoptosis). People with INS mutations usually do not have neurological symptoms,

but the diabetes is typically permanent and caused by a progressive loss of beta cell function, requiring insulin therapy—similar to type 1 diabetes. In some cases, diagnosis may be delayed until 6–12 months of age or later. Certain milder mutations can even present later in life with a MODY-like phenotype. Recessive mutations in INS have also been reported. Compared to dominant mutations, these homozygous recessive forms usually cause more severe insulin deficiency, with:

- Lower birth weight (average of –3.2 SD vs. –2.0 SD),
- Earlier onset (median diagnosis at 1 week vs. 10 weeks).

Some patients with recessive INS mutations may have a transient form (TNDM), while others may not develop diabetes until adolescence or adulthood. These mutations can affect mRNA splicing, promoter function, or cleavage sites in the proinsulin molecule. Interestingly, some heterozygous carriers of recessive INS mutations may develop MODY-like diabetes later in life, suggesting a milder degree of insulin deficiency[54-58]. (See Table 1, where INS is listed as a rare cause of MODY.)

Monogenic Autoimmune Neonatal Diabetes: IPEX and IPEX-Like Syndromes:

Mutations in the FOXP3 gene, located on the X chromosome, can cause a rare condition called IPEX syndrome. IPEX stands for immune dysregulation, polyendocrinopathy, and enteropathy, X-linked. It is typically marked by severe diarrhea, skin rashes, and immune system dysfunction. In many cases, the condition is life-threatening and requires a stem cell transplant, although some patients may have milder symptoms. Similar symptoms can also be caused by mutations in other genes involved in immune regulation, such as STAT3, LRBA, IL2RA, and others[59].

Other Causes of Neonatal Diabetes Mellitus:

Table 1 summarizes the known causes of neonatal diabetes mellitus (NDM). In addition to the more common causes already discussed, mutations in genes like GATA6, GATA4, PDX1, and PTF1A can lead to pancreatic agenesis or underdevelopment (hypoplasia). Most of the other causes are very rare recessive syndromes, with EIF2AK3 mutations being the most common among them. These rare genetic conditions help researchers better understand how the pancreas develops and how insulin is produced in humans. Several review articles are available that provide more detail on this topic. In some NDM cases, the exact genetic cause remains unknown. Interestingly, a few of these unexplained cases have been found in children with Down syndrome[60].

Genetic Defects in Insulin Action: Insulin Receptor and Post-receptor Defects:

The INSR gene encodes the insulin receptor, a protein made up of two alpha (α) subunits that bind insulin and two beta (β) subunits that span the cell membrane and trigger insulin signaling through a tyrosine kinase domain. Both parts of the receptor are produced from the same gene. Mutations in the INSR gene can lead to different syndromes, depending on how much they disrupt insulin signaling. It is estimated that 0.1%–1% of the population may carry variants that affect insulin action, though many of these cases are managed by the body through increased insulin secretion. Three rare syndromes caused by INSR mutations that impair insulin action and lead to diabetes are: Type A insulin resistance syndrome – a milder and more common condition, marked by insulin resistance, hyperinsulinemia, acanthosis nigricans (dark, thickened skin), and hyperandrogenism (excess male hormones). Donohue syndrome – the most severe form, caused by almost complete loss of insulin receptor function. Affected individuals show severe growth restriction before birth, distinctive facial features, very high insulin levels, and abnormal glucose regulation. They experience high blood sugar after meals (due to no insulin action to promote glucose uptake) and low blood sugar during fasting (due to failure of insulin-dependent glucose storage). Rabson-Mendenhall syndrome – an intermediate form where some insulin action remains. These patients have abnormal teeth and nails, and may show pineal gland enlargement. They typically survive into adulthood, later developing signs of severe insulin resistance, such as acanthosis nigricans and hirsutism. Like Donohue syndrome, they show high blood sugar after meals and low blood sugar during fasting. These syndromes are rare, and there is no precise data on how common they are[61-63].

Gaps in Monogenic Diabetes Diagnosis:

Several barriers can delay or complicate the diagnosis of diabetes, especially in cases such as MODY. A 2020 study on patient perspectives highlighted challenges at three main levels. Patient-related barriers include the mild or atypical symptoms of MODY, uncertainty about the value of genetic testing, and personal factors such as fear, denial, or low motivation. Provider-related barriers involve limited awareness or insufficient knowledge of MODY among healthcare professionals, as well as poor communication with patients. At the healthcare system level, barriers include the high cost of genetic testing, limited access to specialists familiar with monogenic diabetes, and a lack of adequate patient education and support. Together, these obstacles contribute to delayed or missed diagnoses and may impact the delivery of appropriate, personalized treatment[64].

Conclusion:

Monogenic diabetes is rare but contributes to approximately 2%–3% of diabetes cases diagnosed before the age of 35. Neonatal diabetes occurs in about 1 in 1Lakh births, with syndromic forms being even less common. Despite this rarity, clinicians who frequently manage diabetes are likely to encounter individuals with monogenic forms. Mitochondrial diabetes, in particular, may be underdiagnosed due to limited clinical awareness. Advances in the understanding of the genetic causes of monogenic diabetes have enhanced knowledge about pancreatic development, insulin production, and insulin signaling. These discoveries have also clarified patterns of inheritance, distinguishing between dominantly and recessively inherited forms of early-onset diabetes. Crucially, genetically informed treatments—such as the use of sulfonylureas for KATP channel mutations—have led to significant improvements in patient outcomes. Some of the same genes implicated in monogenic diabetes are also associated with type 1 and type 2 diabetes, and varying penetrance of these gene variants contributes to differences in disease onset and severity, even within the same family. Accurate diagnosis requires molecular confirmation, which allows for personalized treatment, avoids unnecessary use of insulin or other inappropriate therapies, supports family member testing, and enables genetic counseling. In autosomal dominant conditions like MODY, identification of one affected individual should prompt clinical evaluation of family members. Although next-generation sequencing has made genetic testing more affordable, barriers such as insurance coverage and limited provider familiarity continue to restrict its widespread use. Nonetheless, these advances are expected to significantly influence future diabetes care.

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THE ROLE OF BOTANICAL PESTICIDES IN SUSTAINABLE AGRICULTURAL PRACTICES

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Abstract:

Sustainable agriculture seeks to balance productivity with environmental stewardship, economic viability, and social responsibility. In this context, botanical pesticides—natural compounds derived from plants—have emerged as eco-friendly alternatives to synthetic chemical pesticides. These plant-based agents, extracted from species such as neem (*Azadirachta indica*), garlic (*Allium sativum*), and chrysanthemum (*Chrysanthemum cinerariaefolium*), offer targeted pest control with reduced impact on non-target organisms, human health, and ecosystems. This chapter explores the classification, mechanisms of action, benefits, limitations, and future prospects of botanical pesticides in promoting sustainable agriculture. While they contribute to reducing environmental contamination and promoting biodiversity, their limited field efficacy, high production costs, and regulatory challenges remain significant obstacles. Nevertheless, advances in biotechnology, extraction techniques, and precision agriculture offer new avenues for enhancing their effectiveness and adoption. Botanical pesticides thus represent a critical tool in the transition toward more resilient and environmentally sound agricultural systems.

Keywords: Botanical Pesticides, Sustainable Agriculture, Plant-Derived Compounds, Integrated Pest Management

Introduction:

Sustainable agriculture is an integrated system of plant and animal production practices that meets current and future societal needs, including food and fiber, while enhancing environmental quality and natural resource bases. It emphasizes the conservation of biodiversity, economic viability for farmers, and the well-being of rural communities. One of the most pressing challenges faced by modern agriculture is the widespread and intensive use of synthetic pesticides. Although these chemicals have historically played a critical role in enhancing crop

yields and reducing pest-associated losses, they have also been associated with numerous adverse effects. These include the contamination of soil and water resources, bioaccumulation in the food chain, the emergence of pesticide-resistant pest populations, and the decline of beneficial insects and pollinators (Muhie, 2022).

Against this backdrop, there has been a growing shift toward sustainable and environmentally responsible pest management strategies. Botanical pesticides—natural substances derived from plant materials—have emerged as a promising solution. These biopesticides are typically biodegradable, target-specific, and exhibit low toxicity to humans and non-target organisms. Derived from plants with known pesticidal properties, such as neem (*Azadirachta indica*), garlic (*Allium sativum*), pyrethrum (*Chrysanthemum cinerariaefolium*), and black walnut (*Juglans nigra*), botanical pesticides are increasingly being integrated into agricultural practices worldwide (Acheuk *et al.,* 2022; Lengai *et al.,* 2020).

The appeal of botanical pesticides lies in their potential to be harmoniously incorporated into Integrated Pest Management (IPM) systems and organic farming frameworks. They offer a means of maintaining crop productivity while reducing dependence on synthetic inputs that threaten environmental and human health. Additionally, as consumer awareness and demand for pesticide-free produce rise globally, the market for natural and organic pest control methods continues to expand.

This chapter seeks to provide a comprehensive examination of the role of botanical pesticides in sustainable agriculture. It will explore their definition, classification, and mechanisms of action, while also addressing their numerous benefits, the practical limitations that impede their widespread adoption, and the innovative strategies that may shape their future development. Through this analysis, the chapter aims to highlight the vital contribution of botanical pesticides to creating a more ecologically sound and resilient agricultural landscape.

Definition and Classification of Botanical Pesticides

Botanical pesticides, also referred to as biopesticides or phytopesticides, are naturally derived compounds extracted from various parts of plants such as leaves, seeds, roots, barks, and flowers. These plant-derived compounds exhibit insecticidal, fungicidal, bactericidal, or herbicidal activities, making them effective tools for pest management. The major categories based on their target activity include:

- **Insecticides**: These include compounds such as azadirachtin from *Azadirachta indica* (neem), pyrethrins from *Chrysanthemum cinerariaefolium*, and rotenone from *Derris* species. They interfere with the growth, reproduction, and feeding behavior of insect pests (Acheuk *et al.,* 2022).

- **Fungicides and Bactericides**: Derived from plants such as garlic (*Allium sativum*), cinnamon (*Cinnamomum zeylanicum*), and eucalyptus (*Eucalyptus globulus*), these compounds inhibit the growth of fungi and bacteria affecting crops (Mohd Israfi *et al.,* 2022).
- **Herbicides**: Plants such as *Juglans nigra* produce allelochemicals like juglone that suppress the growth of neighboring plants, providing a natural form of weed management (Islam and Widhalm, 2020).

Mechanisms of Action

Botanical pesticides act through a variety of biochemical and physiological mechanisms:

- **Neurotoxicity**: Pyrethrins and nicotine target the nervous system of insects, causing paralysis and death (Araújo *et al.,* 2023).
- **Feeding Deterrence and Repellency**: Compounds such as azadirachtin act as repellents and antifeedants, making the treated plants unattractive to pests (Chatterjee *et al.,* 2023).
- **Growth Regulation**: Some phytochemicals function as insect growth regulators (IGRs), interrupting developmental stages like molting and pupation (Rembold *et al.,* 1982).
- **Systemic Protection**: Certain botanical compounds, such as pyrethrins, can be absorbed by plant tissues, offering systemic protection against pests (Hodoșan *et al.,* 2023).

Benefits in Sustainable Agriculture

Botanical pesticides offer several benefits aligned with the goals of sustainable agriculture:

- **Environmental Friendliness**: Due to their biodegradable nature, these pesticides do not persist in the environment, thus avoiding contamination of soil, water, and air (Aktar *et al.,* 2009).
- **Low Toxicity to Humans and Animals**: Most botanical pesticides are considered safer for human and animal health, making them suitable for use in community and household agriculture.
- **Resistance Management**: Their diverse modes of action make it difficult for pests to develop resistance, thus enhancing long-term pest management (Iqbal *et al.,* 2021).
- **Support for Biodiversity**: By selectively targeting pests, botanical pesticides help conserve natural predators and pollinators (Ndakidemi *et al.,* 2016).
- **Compatibility with Organic Farming**: Their natural origin makes them ideal for organic farming systems that restrict the use of synthetic chemicals (Toan *et al.,* 2021).

4. Limitations and Challenges

Despite their potential, botanical pesticides face several limitations:

- **Short Residual Effect**: They degrade quickly, necessitating frequent applications which can increase labor and costs (Mitra *et al.,* 2024).
- **Limited Efficacy**: Compared to synthetic pesticides, they may show reduced efficacy under certain environmental conditions (Ayilara *et al.,* 2023).
- **Production Costs**: The cost of extraction and standardization of active ingredients remains high.
- **Regulatory Hurdles**: Complex and inconsistent regulations can delay their registration and commercialization (Damalas and Koutroubas, 2020).

Future Prospects

Botanical pesticides are expected to play a growing role in future agriculture, especially with increasing demand for organic and residue-free produce. Innovations in biotechnology, green extraction technologies, and precision agriculture are paving the way for improved efficacy, longer shelf life, and cost-effective production. Integration into digital farming platforms for targeted application could further enhance their utility (Guo *et al.,* 2024; Karunathilake *et al.,* 2023).

Conclusion:

Botanical pesticides represent a promising, eco-friendly alternative to synthetic pesticides, offering significant benefits for sustainable agriculture. Their biodegradability, lower toxicity, and compatibility with organic farming systems make them ideal for addressing the environmental and health challenges posed by chemical pesticides. They contribute to biodiversity conservation, reduce the risk of resistance development, and support the overall resilience of agroecosystems (Lengai *et al.,* 2020).

However, their broader adoption is hindered by factors such as limited residual activity, variable efficacy, high production costs, and regulatory barriers. Overcoming these challenges requires coordinated efforts in research, policy-making, and farmer education. With advancements in formulation technologies, biotechnology, and data-driven farming approaches, botanical pesticides are poised to become a cornerstone of modern, sustainable agricultural practices.

By investing in these natural solutions and integrating them into pest management frameworks, the agricultural sector can move toward a future that is not only productive but also environmentally responsible and health-conscious (Reddy and Chowdary, 2021; Getahun *et al.,* 2021).

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BIOFILM AND ANTIMICROBIAL RESISTANCE ~ THE DUAL THREAT

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Abstract:

For decades now, biofilms have been subjected to intense study and is thought to be the greatest adaptable microbial trait present in nature. Biofilms may be found adhering to sundry surfaces in diverse environments ranging from mammalian teeth to medical devices and implants, from rocks in streams to water pipes, from roots of plants to food products and many more. When the associated bacteria are pathogenic, such biofilms become a major virulence factor and a serious public health hazard. Indeed, it has been shown that a huge number of human, livestock and plant infections are actually biofilm mediated. Being mostly polymicrobial, they are difficult to treat. Moreover, the protective mechanisms of biofilms can impact both host immune response and antimicrobial efficacy, resulting in antimicrobial resistance (AMR), thus leading to persistent/chronic or even non-treatable infections. AMR is a significant global health menace and, coupled with biofilms, may contribute to more and more drug-resistant bacteria. Thus, together they pose an aggravated dual threat.

Keywords: Biofilm, Extracellular Polymeric Substances/EPS, Antimicrobial Resistance/AMR, Drug Resistant Bacteria, Infection

Introduction:

A biofilm is a dense assembly of microbial cells closely adherent to each other, and is irreversibly associated with a surface. It cannot be removed simply by rinsing. The term ‘biofilm’ is, strictly speaking, a misnomer. Biofilms are not deposited as a continuous monolayer, although the name misleadingly implies so. Rather, a biofilm is extremely heterogeneous, usually multi-layered in nature, and comprises of microcolonies of microbial cells embedded in a self-produced matrix of extracellular polymeric substances or EPS. Biofilms are rich in interstitial voids (water/fluid channels), that form a huge network and play a critical role in the transport of both nutrients and waste materials within the biofilm (Donlan, 2002). The most unique feature is that bacteria in biofilms display a set of ‘emergent properties’ and a distinctive ‘smart lifestyle’, that differ markedly from planktonic or free-living bacterial cells. Biofilms represent one of the most extensively distributed modes of life on the planet and epitomize a highly efficient and evolved ecological success as a novel habitat former (Flemming

et al., 2016). The natural biofilm is almost invariably a multi-species microbial community and resembles a complex, multicultural commune like a modern city (Watnick and Kolter, 2000). Bacteria are known to be incredible decision makers that can fittingly respond and adapt to a vast array of environmental cues and challenges, often through differential gene regulation and other mechanisms. In a biofilm, such decision-making often surpasses the conventional paradigm of isolated planktonic bacteria. Thus, biofilms are well-known predominating micro-ecosystems that are highly adapted to survive harsh environmental conditions or stress situations, including starvation, high temperature/pH/salinity, UV radiation, and antimicrobials (Mirghani *et al.,* 2022).

Biofilms are responsible for 70% of infectious diseases clinically reported, and are a formidable contributor to nosocomial or healthcare-associated infections (HAIs) in humans (Sharma *et al.,* 2023). They have been shown to be clinically relevant in a variety of persistent infections (characterised by chronic inflammation and prolonged tissue damage), in non-healing chronic wounds, related systemic diseases and most alarmingly, in many medical device-related infections (Høiby *et al.,* 2010; Donlan, 2002). Moreover, bacteria in biofilms are resistant to broad spectrum antibiotics in their standard doses or even in higher concentrations. Hence, early detection is the key. At the same time, multidisciplinary research on new and alternative treatments are essential for treating and suppressing conventional drug-resistant biofilm-associated infections (Zhao *et al.,* 2023).

Antimicrobial resistance (AMR) is world-wide the foremost health hazard. Increasing incidences of multi-drug resistance in biofilms, thus, pose a dual threat to public health (Mirghani *et al.,* 2022). Biofilm consists of major classes of macromolecules e.g., polysaccharides, nucleic acids, lipids, proteins, enzymes, and also ions and humic substances, all of which are responsible for its remarkable resilience (Vasudevan, 2014; Schilcher and Horswill, 2020). The EPS acts as a shield and helps in AMR (Zhao *et al.,* 2023).

Our present antibiotic resource forms the foundation of modern medicine to treat infectious diseases. However, their continued usefulness has already been endangered by extensive misuse, underuse, overuse in community, clinical, farming and agricultural settings. The inherent drug resistance offered by biofilms is a grave public concern. For example, rise and spread of various drug resistant types and strains of *M. tuberculosis* have greatly hindered the goal of building a 'TB-free' world declared by WHO (Sharma *et al.,* 2019).

What are Biofilms

A very common misconception is that bacteria exist in individual 'planktonic state', while the truth is that they prefer to form close, diverse communities amongst themselves, and also with other microbial life forms (as in biofilms), where the different species can support each

other. Biofilms may be found adhering to sundry surfaces in diverse environments ranging from mammalian teeth to medical devices and implants, from rocks in streams to water pipes, from roots of plants to food products and many more. Biofilms are complicated, multifaceted systems with high cell densities, stretching from 10^8 - 10^{11} cells / gram of wet weight and typically encompasses multiple species. A further basis of heterogeneity in biofilms is the ability of the component cells to undergo differentiation (prompted by local conditions), stage-specific and need-based gene expression, physical and social interactions, genetic exchange, etc., all resulting in novel structures, patterns, activities and properties including increased resistance and/or tolerance to antimicrobials (Flemming *et al.,* 2016).

The biofilm is made of 10% microbial mass and 90% water. The prevalent organic component in the EPS is the group of closely interacting polysaccharide chains that are woven together in a dense, mesh-like structure (Chiba *et al.,* 2022). The biofilm architecture can have positively or negatively charged ions, which further provide structural integrity (Sharma *et al.,* 2023). Other components of EPS include nucleic acids (viz., eDNA), lipids, enzymes, structural proteins, cell debris, etc.

A few interesting case studies suggest that sometimes erythrocytes and fibrin may accumulate as biofilms form in human tissues. For example, biofilms on native heart valves exhibit interaction in which bacterial microcolonies develop in a matrix of platelets, fibrin and EPS. The fibrin capsule, thus formed, will protect the biofilm from host leukocytes, leading to infective endocarditis. On other occasions, biofilms on urinary catheters may be able to hydrolyse urea in the urine to produce free ammonia via the action of urease. The ammonia may subsequently increase the pH, resulting in mineral precipitation (e.g., calcium phosphate), which may then get stuck in the biofilm and cause encrustation and blocking of the catheter (Donlan, 2002).

Multi-species biofilms exhibit a number of original characteristics and show different forms of interactions including synergy, cooperation, mutual benefit, as also antagonism and competition (Liu *et al.,* 2016). Among them, synergistic interactions play the most crucial role, where one species may enhance the stability of the other. Multispecies biofilms are found in many natural environments, e.g., from mammalian intestines and human oral cavity to medical devices and implants (Donlan 2002).

Biofilm Formation and Propagation:

Biofilm formation can be divided into five stages, as shown in Figure 1. The first stage is Initial Reversible Attachment, followed by Irreversible Attachment (stage 2), Microcolony formation and EPS secretion (stage 3), Maturation (stage 4) and Dispersion (stage 5) (Zhao *et al.,* 2023).

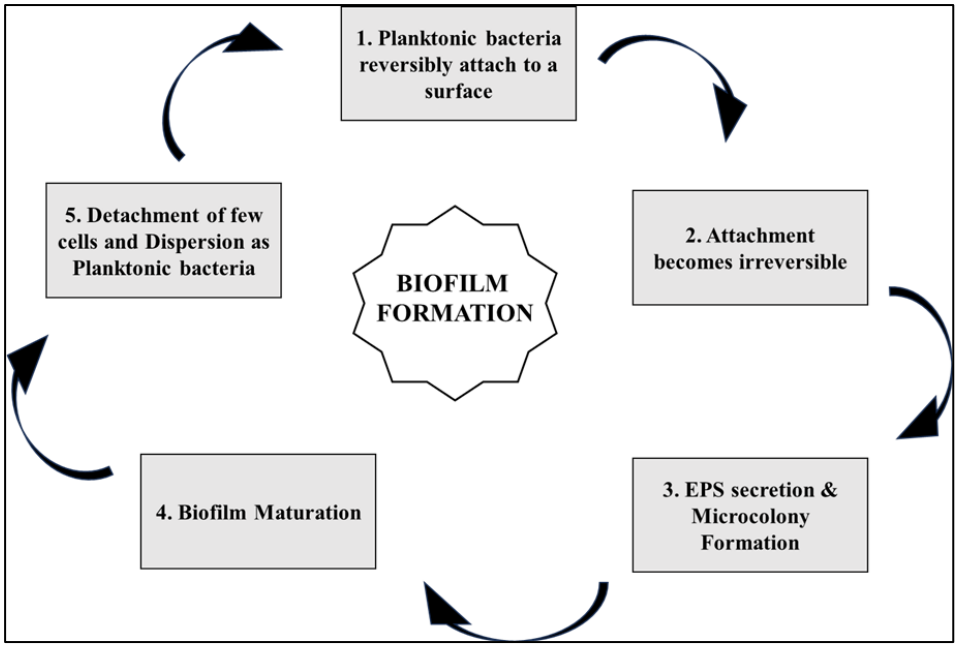

Figure 1: Formation of Biofilm: Biofilm formation can be divided into five stages, as briefly summarized in the figure. The first stage is Initial Reversible Attachment, followed by Irreversible Attachment (stage 2), Microcolony formation and EPS secretion (stage 3), Maturation (stage 4) and Dispersion (stage 5).

In the first stage, the starting point consists of the initial contact (via Brownian motion, convection or sedimentation) of moving planktonic bacteria with a suitable surface (Palmer *et al.,* 2007). Chemotaxis supports specific connection between planktonic bacterial cells and the target surface. Planktonic bacteria adhere to the surface by non-specific physical forces e.g., electrostatic and hydrophobic interactions and/or van der Waals forces (Carniello *et al.,* 2018; Zhao *et al.,* 2023). Attachment is still reversible and weak at this stage, but, eventually, stimulates the colonization and growth of planktonic bacteria on the surface.

The second phase is irreversible attachment, in which cell surface adhesion structures, flagella and fimbriae play important roles. Forces at play include bacterial cell surface hydrophobicity, ionic bonding, covalent bonding, hydrogen bonding, and dipole-dipole interactions.

In the third stage, bacteria will begin to form a monolayer and will produce an extracellular matrix or protective "slime" ~ the sticky EPS. EPS can guide maximum aspects of the process including competent surface adhesion, efficient biofilm formation, cell maintenance, nutrient acquisition. It can shape biofilm internal architecture and can affect mutual recognition and communication processes between cells, as well as genetic exchange. It can influence the signal transduction machinery too (Costa *et al.,* 2018). The formation of microcolonies is thus initiated, with notable growth, cell-cell communication and Quorum

Sensing (QS). The latter can help microbes sense their population density and accordingly influence biofilm formation and maturation, community and host interactions as well as antibiotic resistance. Through QS, bacteria can produce and secrete first chemical messengers, to facilitate signal transmission (Zhao *et al.,* 2023). The second messenger, bacterial cyclic di-GMP, can accelerate the process of irreversible bacterial adhesion.

The maturation of bacterial biofilms is the fourth stage of biofilm development, when fluid channels start acting as circulatory system and colonization continues, forming well-structured "smart communities".

In the last stage, some cells of the mature biofilm start to detach (actively or passively), and disperse into the surroundings as planktonic cells which may potentially pioneer a fresh, new cycle of biofilm formation (Zhao *et al.,* 2023).

A biofilm is a 3-dimensional structure that acts as a microbial battlefront, highly skilled in responding to external threats and accordingly switches between passive, protective, or attack modes of action. This is decided through QS which only allows specific collective behaviour once a certain size has been reached and a sufficiently large, well-coordinated assembly of microbes established (Garde *et al.,* 2015).

Role of Biofilm in Infections

Biofilms are often associated with infections in human, animal and plant kingdom. Multispecies biofilms have been especially linked to various human diseases, ranging from genetic disorder like Cystic Fibrosis (CF) to tooth decay, diabetic foot ulcers, chronic wounds, skin infections, otitis media, etc. Biofilm infections are also frequently found in medical devices and implants (e.g. contact lenses, catheters, knee replacements, breast implants, prosthetic valves and joints, screws and pins) (Zhao *et al.,* 2023).

Cystic fibrosis (CF) patients suffer long term from infections by persistent CF-adapted *Pseudomonas aeruginosa* species. This is due to the overproduction of the matrix polysaccharide alginate, leading to the formation of a mucoid biofilm that evades the host innate and adaptive immune responses, resists phagocytosis and tolerates standard antibiotics. This leads to chronic inflammation and severe damage to the lung tissue of CF patients (Garde *et al.,* 2015).

Another classic example can be found in relation to human oral microbiome which is dynamic and diverse (may comprise of *Archaea*, bacteria, viruses, fungi, mycoplasmas and protozoa). It exists as multispecies microbial communities in structurally and functionally organized biofilms on oral surfaces and is normally in harmony with the host, but may become potentially damaging (dysbiotic), depending on local conditions and lead to dental plaque and caries or periodontitis and gingivitis and tooth decay (Marsh and Zaura, 2017). The consumption of fermentable sugary carbohydrates causes an increase in organic acid secretion by the plaque

bacteria which may eventually result in demineralisation of the enamel and the formation of dental caries. Research work from different laboratories has shown that dental biofilms can even cause various systemic diseases such as infective endocarditis, diabetes mellitus, rheumatoid arthritis. For instance, bacterial pathogens in the mouth can enter the circulation, reach the heart and adversely affect its function, or may bind fatty plaque in the coronary arteries (Larsen and Fiehn, 2017; Marsh and Zaura, 2017; Zhao *et al.,* 2023).

Bacterial keratitis is a cornea infection in which bacterial biofilm forms on the eye's surface. If not treated timely and effectively, such infection may lead to vision loss (Zhao *et al.,* 2023).

Women are more frequently susceptible to urinary tract infections or UTI, than men. The most common pathogenic bacteria implicated in urinary tract infections in adult women are *E. coli* and *Proteus* spp., who can form biofilms.

Otitis media is a complex inflammatory disease, that can lead to hearing loss. Bacteria capable of forming biofilm in the middle ear are the chief causal organisms and include *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* (Zhao *et al.,* 2023).

Biofilms that form in the open wounds of diabetic patients is believed to cause chronic infections. While aerobic bacteria make biofilms on the exterior of deep wounds, anaerobic bacteria invade the interior, resulting in serious persistent complications (Sharma *et al.,* 2023).

In addition, microorganisms can adhere to and develop biofilms in various other tissue surfaces in the body which may lead to severe chronic inflammatory and autoimmune diseases in the host (Zhao *et al.,* 2023).

The use of medical devices/implants can significantly improve quality of life in patients by addressing various health challenges and by enabling individuals to lead fuller, more independent lives. However, medical device-associated chronic infections are of great concern and are closely linked to biofilm formation. The hazard is intensified by the fact that in some cases, the component bacterial cells get released from the bacterial biofilm and can be carried via bloodstream leading to infection or damage elsewhere in the body. Device-associated infections are majorly caused by *Staphylococcus aureus* and *Staphylococcus epidermidis* and may come from the environment, or the patient's own skin, or the healthcare worker's skin. Research suggests that *S. epidermidis* accounts for approximately 80% of such infections in the hospital setting (Zhao *et al.,* 2023). However, the list is long and diverse. Multidrug-resistant gram-negative bacteria like *Escherichia coli*, *Enterococcus faecalis, Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* have emerged as significant threats in CAUTI or catheter-associated urinary tract infection. More recently, fungi like *Candida auris* have also been implicated in such device-linked infections (Zhao *et al.,* 2023).

Cardiac implants e.g., artificial heart valves, pacemakers, etc., are found to be associated with higher morbidity and mortality due to infections. Similarly, PEGs (percutaneous endoscopic gastrostomy tubes or cannulas) are a risk factor for colonization of the gastrointestinal tract by drug-resistant bacterial biofilms. Post-operative complications in case of orthopaedic implant surgery (to restore hip and knee function), arise mostly due to artificial joint infection, where formation of combined bacterial biofilm results in osteomyelitis. Likewise, in case of neurosurgical implants e.g., cerebrospinal fluid shunts, infection is often caused by biofilm producers, the most common perpetrator being coagulase-negative *Staphylococcus* (CONS), including *S. epidermidis* and *S. aureus*. *P. acnes*, *Streptococcus* sp., *Lactobacillus* sp., *Bacillus* sp., and *Mycobacterium* sp. can grow as biofilms in the environment around breast implants (Zhao *et al.,* 2023). Intrauterine Devices (IUDs) also exhibit instances of biofilm formation by *S. aureus*, *S. epidermidis*, beta-haemolytic *Streptococci*, *Enterococcus* sp., *E. coli*, *Micrococcus* sp., *Corynebacterium* sp., *Candida albicans* (Sharma *et al.,* 2023).

It should be noted that EPS produced by bacteria in biofilms serve as a formidable shield, blocking the effectiveness of host immune system and antimicrobial agents. In addition, QS also plays vital roles in mounting a successful infection, in producing host immunomodulatory effects and in limiting the efficacy of antimicrobial drugs (Garde *et al.,* 2015).

How Biofilm perpetuates AMR:

Bacteria, as integral part of a biofilm, display 10 to 1,000-fold rise in antibiotic resistance compared to similar bacteria living in a planktonic state. For instance, in a study on antibiotic resistance of *Staphylococcus epidermidis* in biofilms, 100% of isolates were found to be susceptible to the antibiotic vancomycin when tested in a planktonic state. But, almost 75% of them remained fully resistant to the same antibiotic when tested from a biofilm. The same trend has been seen in others, e.g., *Klebsiella pneumoniae*, which appears to be quite vulnerable to certain antibiotics when tested from an aqueous solution but becomes highly resistant when tested from a biofilm (Sharma *et al.,* 2023).

Much higher resistance and/or superior tolerance to antimicrobial agents (e.g., antibiotics), compared with planktonic bacterial cells, are hallmarks of the emergent properties of bacterial biofilm, which can be viewed as an almost impenetrable fortress (Flemming *et al.,* 2016; Uruén *et al.,* 2020). With regards to biofilms, many scientists prefer to use the term ‘resistance’ to denote a genetic, stably heritable characteristic that is gained either by genetic exchange or by mutation and that remains unchanged even when cells in the biofilm are detached and dispersed. By contrast, the term ‘tolerance’ is used to denote a trait which is temporary and is specific to biofilms only and that is lost following dispersal to free-living bacterial cells (Flemming *et al.,* 2016). It is well established that the success of the biofilm lifestyle is largely

due to the protective matrix that not only shields the microcolonies from a range of environmental stress factors, but also hinders antibiotic absorption and access to the biofilm via entrapment and inactivation strategies (Liu *et al.,* 2024). Antibiotic tolerance can also be a resultant of microbial slow growth and dormancy that can occur inherently in biofilms. Biofilms exhibit both nutrient and oxygen concentration gradients and consequently prompt bacterial populations to display different growth rates (Dincer *et al.,* 2020). Close scrutiny of environmental as well as *in vitro* biofilms has shown that oxygen concentration may be high at the surface but low towards the centre of the biofilm where anaerobic conditions may predominate. Studies indicate many antibiotics exhibit selective aerobic or anaerobic spectrum and accordingly have different degrees of therapeutic efficacy. Some antibiotics, such as β-lactams and fluoroquinolones are futile in their action towards the inner anaerobic biofilm because they are useless in the absence of oxygen and nutrients (Mirghani *et al.,* 2022). Similarly, pH changes can negatively impact aminoglycoside action. Along with factors like anoxia and nutrient deficiencies, other features like phenotypic diversity, antibiotic modification enzymes, and oxidative stress can also lead to antibiotic resistance in bacterial biofilm (Hall and Mah, 2017; Sharma *et al.,* 2019), making it difficult to eradicate.

Growth, metabolic activity, protein synthesis are all stratified in biofilms, displaying a high level of activity at the surface and a low level and slow or no growth in the centre. This is another plausible explanation for the strikingly diminished susceptibility of biofilms to antibiotics (Høiby *et al.,* 2010). Slower growth results in sluggish uptake of antimicrobial agents that leads to sub-optimal intracellular drug concentrations that is largely inadequate for bacterial killing. In fact, younger biofilms with faster-growing cells have indeed been found to be more vulnerable than older biofilms, when exposed to the same antimicrobials (Sharma *et al.,* 2023). In addition, biofilms contain substantial numbers of cells in stationary phase. Since antimicrobials rely on the active metabolism of bacterial cells for their effective function, such stationary cells have a dramatically lowered drug susceptibility. Biofilms also have special spore-like ‘persister cells’ in a state of dormancy, which are multidrug tolerant subpopulations and are phenotypic, rather than genetic, variants. This implies that they are not genetically resistant, but their dormant state is characterized by extremely reduced metabolic activity, allowing them to survive antibiotic exposure even when the majority of the biofilm population is killed. Thus, they exhibit high tolerance to antibiotics, contributing to the recalcitrance of biofilm infections, as they can serve as the initiator cells to further resume the process when conditions become favourable (Vasudevan, 2014; Rather *et al.,* 2021). High levels of persistent cells have been reported in chronic urinary tract infections and also in the lungs of patients with cystic fibrosis (Dincer *et al.,* 2020).

Many virulent biofilm phenotypes are capable of expressing periplasmic glucans that bind to the antibiotics and physically sequester them which, in turn, reduces treatment efficacy (Vasudevan, 2014).

Intuitively it may appear that the EPS matrix represents an absolute physical shield and diffusion barrier, thus explaining AMR in biofilms. However, this factor alone is not nearly large enough to fully account for such remarkable resistance phenomenon. Its contribution is, nonetheless, monumental. EPS usually contains lipopolysaccharide and alginate, both of which can act together as a barrier for antibacterial drug diffusion. Moreover, numerous anionic and cationic molecules are also present (such as proteins, glycoproteins, and glycolipid), that can bind charged antimicrobial agents and shelter resident microorganisms from them, as in the case of *Pseudomonas aeruginosa* (Dincer *et al.,* 2020). EPS can deny permeability to large molecules of aminoglycosides (Mirghani *et al.,* 2022). Additionally, a special form of inhibition known as 'diffusion–reaction inhibition' is an exceptional property of EPS. This encompasses 'antibiotic chelation' by complex formation, selective enzymatic degradation of antimicrobials (exemplified by presence of high β-lactamases in the biofilm matrix), or other defence mechanisms e.g., delayed penetration that might provide enough time for an adaptive phenotypic response, which would reduce susceptibility (Dincer *et al.,* 2020; Sharma *et al.,* 2023). It is also interesting and important to note that by decreasing the effective concentration of antimicrobials to sublethal doses, diffusion–reaction inhibition may actually further promote selection for AMR in biofilm cells that are subjected to, but can withstand, antimicrobial stress. Numerous other protective means can also support drug tolerance, including extracellular signalling, metabolic heterogeneity, genetic mutations and phenotypic variations. One well-recognized mechanism by which antimicrobial resistance can be acquired, enhanced or spread in biofilms is the uptake of antimicrobial resistance genes by horizontal gene transfer (HGT). HGT has been proved to be a major contributor to the global AMR crisis (Dincer *et al.,* 2020; Uruén *et al.,* 2020; Liu *et al.,* 2024). Biofilms act as reservoirs of genetic diversity which is crucial for bacterial adaptation and evolution, providing the raw material for natural selection and allowing bacteria to thrive in diverse and changing environments. A very common mechanism of HGT in biofilms is plasmid conjugation, which has been shown to be about 700-fold more efficient in biofilms compared with free-living bacterial cells. Factors like elevated cell density, enhanced genetic competence and accumulation of mobile genetic elements are all simultaneously present in biofilms and together they provide just the ideal set of elements needed for extremely efficient HGT. Also, plasmid maintenance is much higher in biofilm populations relative to planktonic counterparts. Persister cells, which are common in biofilms, can act as plasmid reservoirs (Liu *et al.,* 2024). In *Vibrio cholerae* biofilms, type VI secretion systems provide an alternative mechanism of HGT.

Extracellular DNA (eDNA) that is almost ubiquitous in the matrix, is prompting unparalleled scientific focus and contemplation for its vital role in drug-resistant biofilm pathogenesis in persistent infections (Flemming *et al.,* 2016). eDNA can also be a source of DNA for HGT (Campoccia *et al.,* 2021). Antibiotics, such as positively charged aminoglycosides, can bind to negatively charged eDNA in the matrix, slowing down antibiotic penetration. During chronic infection, polymorphonuclear leukocytes are recruited to biofilms which eventually undergo bacteria-induced necrosis, releasing host eDNA. Investigations in the lung of CF patients suggest that, *P. aeruginosa* eDNA together with the host eDNA, can protect the biofilm from detrimental effects of host immune system and antibiotics alike (Liu *et al.,* 2024). eDNA boosts AMR by sequestering cations, e.g., Ca^{++} and Mg^{++}, as observed in *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *A. baumannii*, *Salmonella enterica*, *Enterococcus faecium*, *Enterobacter* sp. All these pathogens are known to be responsible for drug-resistant biofilm associated infections (Mirghani *et al.,* 2022).

Interestingly, *P. aeruginosa* biofilms can also be protected by formation of host neutrophil extracellular trap (NET), whose real purpose is to prevent bacterial dissemination, but it also ends up blocking entry of antibiotics (Liu *et al.,* 2024).

The number of target cells present within a biofilm (cell density) can impact susceptibility to drugs, a phenomenon known as the 'inoculum effect'. The inoculum effect describes a phenomenon where the effectiveness of some antibiotics decreases as the initial bacterial population (inoculum) increases. *P. aeruginosa* biofilms have indicated that different beta-lactams express such inoculum effect (Liu *et al.,* 2024).

Recent reports indicate a close link between biofilm antibiotic tolerance/survival and efflux machinery, with cooperative effects leading to biofilm AMR (Vareschi *et al.,* 2025). Efflux pumps contribute significantly to biofilm formation and also help bacteria counteract several classes of antibiotics (Dincer *et al.,* 2020; Uruén *et al.,* 2020). The physiological heterogeneity within biofilms explains the observed patterns of differential efflux pump gene expression. For instance, in some cases, upregulation of specific antibiotic resistance pumps was noted in the upper region of biofilms, while downregulation or no change was observed in the middle of the biofilm. In the case of *Pseudomonas aeruginosa*, it has been demonstrated that hypoxia augments antibiotic resistance by altering the composition of multidrug efflux pumps. Furthermore, efflux pump inhibitors have been shown to effectively block antibiotic tolerance of biofilms and even completely abolish biofilm formation (Grooters *et al.,* 2024).

As is evident from the above discussions, various components work in tandem or in combination within a biofilm in order to reduce or fully prevent antibiotic effectiveness and further augment resistance and recalcitrance (Sharma *et al.,* 2023). A few factors that enhance

antimicrobial resistance in biofilms are summarised in Figure 2. Scientists have reported that antimicrobial resistance/tolerance and survival in multiple-species biofilms are far greater than those found in biofilms formed of single species. For instance, *in vivo* it has been found that *P. aeruginosa* growing in a mono-species biofilm is two times more vulnerable to actions of gentamicin than that growing in multispecies biofilm consisting of *Enterococcus faecalis*, *S. aureus*, and *Finegoldia magna*. In another study, *C. albicans*, an opportunistic fungal pathogen, and *S. aureus* were found to exhibit higher resistance to vancomycin in a dual species setting. The molecular mechanism of this is not yet fully known (Dincer *et al.,* 2020).

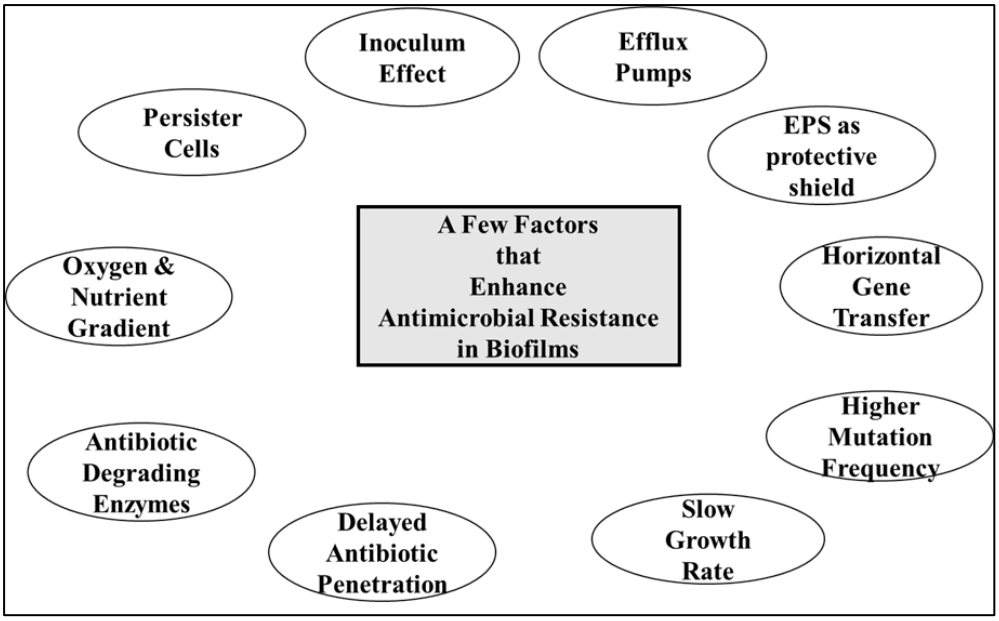

Figure 2: A few factors that work together to enhance antimicrobial resistance in biofilms are summarised in this figure

In addition, genetic mutations can also lead to biofilm AMR (Høiby *et al.,* 2010). The mutation rate of biofilm bacteria is notably higher than its free-living counterpart. This can be explained by the increased production of endogenous reactive oxygen species (ROS) and a deficient antioxidant system that leads to oxidative stress and enhanced mutability in biofilms (Uruén *et al.,* 2020).

In a highly interesting case study, *Salmonella* biofilms were shown to rapidly adapt and evolve resistance when exposed to sub-lethal concentrations of antibiotics or non-antibiotic antimicrobials, like copper. But while resistance appeared promptly, mutants showed significantly less ability to form a biofilm, indicating 'trade-offs' in adaptation (Liu *et al.,* 2024).

Conclusion:

Antonie Philips van Leeuwenhoek, using his hand-made ancient microscopes, first observed aggregates of microorganisms adhering to tooth surfaces and can be credited with the discovery of microbial biofilms which later refuted the deep-seeded concept of only planktonic

bacterial life forms (Donlan, 2002). The vast importance of biofilms was however acknowledged by the scientific community only in 1990s (Sharma *et al.,* 2023).A couple of recent initiatives have been instrumental in helping understand and appreciate biofilm properties. This includes the use of the confocal laser scanning microscope to characterize biofilm ultrastructure, and an initiative for detailed investigation of the genes involved in cell adhesion and biofilm formation (Donlan, 2002). The emergence of the role that biofilms play in the pathogenesis of recalcitrant and persistent clinical infections has made it the focus of intense research.

Clinically, most chronic infections are associated with biofilms, which are resistant to scavenging actions of conventional antibiotics, can evade host immune measures and can grow and mature even under harsh conditions. Currently, methods for detecting, inhibiting, treating and managing biofilm-associated resistant infections are wanting, thus throwing the scientific community and the health sector a colossal challenge. Since biofilm-associated infections cannot be treated with traditional antibiotics (both as standalone and in combination), researchers must come up with new antimicrobial agents or novel anti-biofilm-associated therapeutic strategies (Zhao *et al.,* 2023; Liu *et al.,* 2024).

Scientists are only now starting to scratch the surface of the properties of the biofilm matrix which provides unparalleled protection from antimicrobials, but how this protection occurs is not yet fully understood. Currently there is renewed interest in finding alternate novel therapeutic approaches for biofilm suppression, healing of active infections and impeding new biofilm formation. A few of such tactics (e.g., use of antimicrobial peptides and lipids, anti-cancer drugs, nanoparticles, bacteriophage therapy, immunotherapy, etc.), offer new possibilities and hopes to combat and control pathogenic biofilms and mitigate the associated AMR threat (Dutt *et al.,* 2022; Grooters *et al*, 2024). A recent study has revealed that vitamin C weakens biofilm formation and restores the antimicrobial susceptibility of multidrug-resistant (MDR) isolates (Rahim *et al.,* 2025). Future research endeavours should emphasize on illuminating the exact mode of action of these novel therapeutic strategies and evaluating sustainable clinical efficacy in diverse microbial biofilm contexts, to alleviate this dual threat.

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IBD (INFLAMMATORY BOWL DISEASE) SCREENING MODELS: FROM BENCH TO BEDSIDE

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Abstract:

Inflammatory Bowel Disease (IBD), encompassing Crohn's disease and ulcerative colitis, is a chronic, immune-mediated gastrointestinal disorder with multifactorial etiology involving genetic, environmental, microbial, and immunological components. Animal models have proven indispensable in elucidating the complex pathogenesis of IBD and in evaluating therapeutic strategies. This review outlines both the pathophysiological basis of IBD and the experimental models used to simulate disease in vivo. Chemically induced models, including dextran sulfate sodium (DSS), trinitrobenzene sulfonic acid (TNBS), oxazolone, acetic acid, and indomethacin, are widely used due to their reproducibility and ability to mimic specific disease phenotypes. Additionally, immune-based approaches such as adoptive T-cell transfer models and genetically engineered models (e.g., IL-10 and IL-2 knockout mice) provide insights into the molecular and cellular mechanisms of chronic intestinal inflammation. Spontaneous and microbiota-driven models further highlight the interplay between host immunity and the gut microbiome. Collectively, these models are critical for translational research and the development of novel IBD therapies.

Keywords: Inflammatory Bowel Disease (IBD), Crohn's Disease, Ulcerative Colitis, Animal Models, Dextran Sulfate Sodium

Introduction:

Inflammatory Bowel Disease (IBD) encompasses chronic, relapsing inflammatory conditions of the gastrointestinal tract, primarily affecting the colon and small intestine. It includes two major subtypes: ulcerative colitis (UC) and Crohn's disease (CD). UC is characterized by continuous mucosal inflammation restricted to the colon and rectum, presenting with symptoms such as abdominal pain, anorexia, fatigue, rectal bleeding, and anemia. Its pathogenesis involves a dysregulated immune response to intestinal microbiota in genetically

predisposed individuals.[1] Crohn's disease, in contrast, may affect any segment of the gastrointestinal tract from mouth to anus and is marked by transmural inflammation, often involving the ileum and colon. Clinical features include abdominal pain, diarrhea with blood and mucus, bloating, anemia, and extraintestinal manifestations such as nausea, vomiting, and anal fissures.[2,3] Both forms of IBD involve multifactorial etiologies, including genetic susceptibility, environmental triggers, immune dysregulation, and microbiome alterations. Crohn's disease is currently more prevalent than UC and continues to show a rising global incidence, alongside expanding therapeutic modalities**.**[1-5]

Etiology

The etiology of Inflammatory Bowel Disease (IBD) is multifactorial, with genetics playing a key role—individuals with a family history or certain gene variants are at increased risk. Dysregulated immune responses also contribute significantly, as the body erroneously targets the intestinal lining, often triggered by environmental stimuli or infections.[6,7] Environmental and lifestyle factors such as a Westernized diet, smoking, pollution, prolonged infections, and chronic stress further exacerbate susceptibility.[8,9] Smoking, in particular, is strongly linked to Crohn's disease, with nicotine inducing immune modulation, DNA damage, and epigenetic changes.[10] Advancing age, especially between 40 and 55, is associated with higher prevalence, likely due to weakened immunity. Certain medications, especially NSAIDs and broad-spectrum antibiotics, can damage the gut mucosa and disturb microbial balance, increasing the risk of IBD. Gender differences also exist, with some evidence suggesting higher susceptibility among females, potentially influenced by hormonal or genetic factors. Additionally, pathogenic microorganisms—including *Mycobacterium paratuberculosis*, *Fusobacterium spp.*, *Clostridium difficile*, and adherent-invasive *E. coli*—are frequently implicated in triggering or aggravating IBD through chronic inflammation and immune system disruption.[6,7,11-14]

Sing and Symptoms

Ulcerative colitis is a chronic inflammatory condition that primarily targets the gastrointestinal tract, particularly the colon; however, it can also extend its effects to various extraintestinal organ systems. Approximately 25% of individuals diagnosed with inflammatory bowel disease (IBD) experience extraintestinal manifestations (EIMs) at some point during their disease progression, even though fewer than 10% present with these manifestations initially. EIMs generally parallel the clinical course of intestinal inflammation, with exceptions such as ankylosing spondylitis, uveitis, and primary sclerosing cholangitis, which may develop independently of bowel disease activity. Among the extraintestinal features, cutaneous

involvement is relatively common, with pyoderma gangrenosum and erythema nodosum being the most frequently encountered dermatologic conditions associated with IBD. The manifestations of IBD in the skin and their therapeutic strategies are addressed extensively in the literature. Clinically, patients with ulcerative colitis may present with a range of symptoms including abdominal pain, diarrhea accompanied by rectal bleeding, unintentional weight loss, and low-grade fever. Extraintestinal symptoms often include arthralgia and joint swelling, anemia secondary to malabsorption, mucocutaneous ulcerations (including those affecting the skin and oral cavity), ocular inflammation, stomatitis, and hepatic involvement. [15,16]

Numerous Methods of Inducing IBD

In the animal, like the rodents, generally there are the various methods that are also used to create an inflammation in the intestine. Animal models are important tools for intestinal inflammation and rely on both intrinsic and extrinsic factors as well as therapeutic strategies. Experimental models of IBD are helpful in identifying new targets for therapeutic therapies. Owing to their genetic similarities with humans, rodents are the most commonly used animal model due to their repeatability, affordability, and ease of handling.[17,18]

1. Chemically Induced Colitis

- Dextran sodium sulfate Induce colitis model
- Acetic acid-induced colitis model
- Oxazolone induce model
- Indomethacin-induced colitis model
- Peptidoglycan-polysaccharide (PG-PS) model**:**

2. Spontaneous animal model

- Adoptive transfer models

3. Genetic animal model

Chemically Induced Colitis

The study of inflammatory bowel disease (IBD) frequently employs the chemically induced colitis model in rats. In order to cause inflammation in the colon, substances such as trinitrobenzene sulfonic acid (TNBS) or dextran sulfate sodium (DSS) are administered. These substances harm the intestinal lining, which sets off an immunological reaction and mimics human IBD-like disorders. Researchers can examine illness pathways and assess possible remedies with the aid of this model. Animal models, particularly rodents, are invaluable tools in studying intestinal inflammation and developing treatments for inflammatory bowel disease (IBD). Researchers can choose therapeutic targets and comprehend illness mechanisms by using

these models, which are influenced by both internal and external influences. Because of their ease of handling, affordability, and genetic resemblance to humans, rats are favored. The hallmark of IBD, which includes disorders like Crohn's disease and ulcerative colitis, is ongoing gastrointestinal tract inflammation.[19,20]

Dextran sodium sulfate Induce colitis model

Many people utilize the dextran sodium sulfate (DSS) model to induce colitis by damaging the intestinal epithelium, disrupting the mucosal barrier, and triggering immune responses. Histological alterations include mucosal edema, goblet cell loss, and disruption to the crypt architecture, while clinical signs include diarrhea, weight loss, and bloody stools. The severity of colitis is influenced by DSS concentration and duration, with, for example, 3.5% DSS inducing colitis in 14 days. One popular animal model for researching inflammatory bowel disease (IBD), especially ulcerative colitis, is colitis caused by dextran sodium sulfate (DSS). In this model, rodents (usually mice or rats) are given DSS, a sulfated polysaccharide, through their drinking water, which causes colonic inflammation and damage that closely resembles important aspects of IBD in humans. Generally, Dextran sodium sulfate is damaged; also, intestinal barrier disruption is how dextran sodium sulfate causes colitis.[21,22] DSS causes direct harm to the colon's epithelial cells. Intestinal permeability increases as a result of this disruption to the intestinal epithelial barrier. Toxins, antigens, and luminal microorganisms can enter the underlying tissue through the breached barrier, inciting an immunological reaction. Activation of the immune system by gut bacteria and other antigens penetrates the lamina propria, the tissue beneath the epithelial layer, and the local immune system is activated. This mechanism activates immune cells like macrophages, dendritic cells, and T-cells. The release of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) by these immune cells that worsen inflammation. Th1/Th17 Reactions and T-cell Activation The T-helper cell activation is the main factor driving the immunological response in the colitis caused by DSS. Th1 and Th17 cells are drawn in and activated during the acute phase. The inflammatory cascade is exacerbated by these cells' secretion of extra pro-inflammatory cytokines like IL-17 and interferon-gamma (IFN-γ). This is very crucial for causing the chronic illness.[23]Colon damage generally causes ulceration and necrosis in the colonic tissue. The hallmark lesions of DSS-induced colitis are caused by the inflammatory infiltration and epithelial destruction. The model displays symptoms that are comparable to those of ulcerative colitis in humans, including diarrhea, weight loss, and rectal bleeding. DSS colitis can resolve after stopping DSS administration, but repeated exposure can lead to chronic inflammation. This

chronic inflammation may cause persistent immune cell infiltration, fibrosis, and potentially cancer, offering insights into the progression of IBD. [21,23]

Trinitrobenzene Sulfonic Acid (TNBS) model

TNBS disrupts the epithelial barrier and initiates immune responses characterized by cytokine production (e.g., TNF-α, IL-12). TNBS-induced colitis is another frequently used animal model for studying Crohn's disease and other forms of inflammatory bowel disease (IBD). This model closely resembles the features of IBD in humans. TNBS is a substance that causes colonic inflammation by inducing an immunological response. The TNBS-induced colitis model and its mechanism are described in detail below:First Administration and Chemical Reaction: In order to directly interact with the colon, TNBS is usually given to rodents (mice or rats) via an enema. After entering the colon, TNBS modifies intestinal epithelial cells by binding to proteins on their surface. Pro-inflammatory, activated immune cells release cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), interferon-gamma (IFN-γ), and TNF-α. The inflammatory response, which damages tissue and attracts more immune cells to the affected location, is triggered by these cytokines. Additionally, this causes tissue injury and ulceration. The intestinal lining is directly damaged by immune cell activation and inflammatory cytokine release, which leads to ulceration, bleeding, and rupture of the mucosa. This leads to the usual colitis symptoms caused by TNBS, such as diarrhea, blood in the stool, and weight loss. Symptoms include diarrhea, mucosal damage, and weight loss, typically showing up 3–7 days after therapy.[24,25]

Oxazolone induce model

One popular animal model for researching inflammatory bowel disease (IBD), especially ulcerative colitis, is the oxazolone-induced colitis model. This model is well-known for its capacity to elicit an immunological response and acute colonic inflammation, which is comparable to what is seen in human ulcerative colitis. The model is frequently used to investigate the involvement of particular immune pathways in IBD, assess possible treatment agents, and investigate the processes of inflammation. Oxazolone, a synthetic substance administered chemically, causes colitis by inducing an immunological reaction. In rodents (mice or rats), it is usually applied topically or given intrarectally. The substance enters the colon and alters proteins on the surface of colonic epithelial cells, which the immune system subsequently interprets as foreign antigens.and induces inflammation through hapten-protein complexes formed by oxazolone, triggering T-cell-mediated immune responses. This model replicates IBD-like pathology, with symptoms such as diarrhea and weight loss emerging within 7–14 days post-administration.[26,27]

Indomethacin-induced colitis model

An animal model of inflammatory bowel disease (IBD) called indomethacin-induced colitis is used to investigate the involvement of prostaglandins and the inhibition of cyclooxygenase (COX) in colonic inflammation. A nonsteroidal anti-inflammatory medication (NSAID), indomethacin works mainly by blocking COX enzymes, especially COX-1 and COX-2, which are involved in prostaglandin formation. This model is helpful for examining how NSAIDs contribute to the pathophysiology of IBD and how intestinal injury and inflammation result from the disruption of prostaglandin synthesis, which disrupts prostaglandin synthesis, causing epithelial damage and inflammation. Effects are observable within 24–48 hours. [28,29]

Acetic acid-induced colitis model

One popular animal model for researching inflammatory bowel disease (IBD), especially ulcerative colitis, is acetic acid-induced colitis. This model is particularly useful for examining the mechanisms behind intestinal inflammation, assessing the efficacy of proposed treatment options, and comprehending how different immune pathways contribute to colitis.[30]

By injecting acetic acid, commonly referred to as vinegar, into the colon of rodents, colitis is induced, causing localized inflammation and damage akin to that seen in IBD in humans. While Acetic Acid Is being Administered: Acetic acid is usually injected intrarectally into rodents (mice or rats). Applying the acid solution directly to the colon, often at a concentration of 3–5%, damages the colonic mucosa. The acid's corrosive properties cause damage by rupturing the epithelial lining's integrity, which subsequently results in inflammation and ulceration." Acetic acid mostly damages the colon's epithelial lining. The acidic environment damages the mucosal barrier, which normally protects the underlying tissue from impurities and pathogens. It also weakens the close bonds that hold epithelial cells together. [30,31]The gut lining becomes more permeable as a result of this injury, which permits immunological activation and bacterial migration.

***Peptidoglycan-polysaccharide (PG-PS) model*:**

An experimental model called peptidoglycan-polysaccharide (PG-PS) induced colitis is used to investigate the pathophysiology of Crohn's disease and ulcerative colitis, two types of inflammatory bowel disease (IBD). By inducing a specialized immune response to bacterial components, specifically peptidoglycan and polysaccharides, which are parts of bacterial cell walls, this model is unique in that it attempts to reproduce the immunologically mediated inflammation of the colon. The relationship between the gut microbiota, the innate immune system, and the inflammatory pathways that lead to IBD is frequently investigated using PG-PS colitis. Both Gram-positive and Gram-negative bacteria contain peptidoglycan (PG), a structural

element of the bacterial cell wall. Particularly in Gram-positive bacteria, polysaccharides (PS) are carbohydrate polymers that make up the outer layer of the bacterial cell wall. Peptidoglycan and polysaccharides derived from bacterial sources (such as Staphylococcus aureus or Enterococcus faecalis) are combined to cause colitis in the PG-PS colitis model. It is well known that these bacterial components cause a powerful immunological reaction. [32,33]

Spontaneous animal model

In the spontaneous induced colitis model, C3H/HeJBir and SAMP1/Yit mice play a major role as examples of spontaneous models of colitis that develop the illness naturally without the need for outside induction. Beginning at 3–6 weeks, C3H/HeJBir mice experience acute colitis in the right colon and cecum; by 12 weeks, the symptoms have subsided. Granulomas, intestinal wall thickening, ulcers, crypt abscesses, and elevated IFN-γ and IL-2 expression—all of which signify a Th1 immune response—are caused by the CsCs1 gene on chromosome 3. SAMP1/Yit mice, which are descended from AKR/J mice, are beneficial for researching the pathophysiology of Crohn's disease because they exhibit traits similar to those of Crohn's disease, including granulomas, fistulas, transmural inflammation, and elevated production of TNF-α and IFN-γ.[34,35]

Adoptive transfer models

Adoptive transfer models involve transferring immune cells from donor animals with IBD into immune-compromised recipient animals, such as SCID or RAG knockout mice, to induce and study the disease. The CD45RB model, developed by Dr. Powrie, demonstrates that transferring naïve CD4+ T cells into immune-deficient mice results in transmural inflammation within 5–10 weeks. This approach highlights the roles of immune and genetic factors in IBD pathophysiology and the function of T cells in mucosal immunity. The procedure involves selecting donor cells, often genetically modified or treated to mimic IBD, isolating CD4+ T cells from tissues like the spleen or lymph nodes, and transferring them into preconditioned recipient animals via intravenous injection or other methods. Disease progression is monitored through clinical symptoms (e.g., diarrhea, weight loss), histopathology (e.g., tissue inflammation, fibrosis, goblet cell loss), and molecular analyses (e.g., cytokine levels, gene expression). This model provides critical insights into IBD mechanisms and aids in the development of therapeutic strategies. [36,37]

Genetic Animal model:

For the research on (IBD), which encompasses ulcerative colitis and Crohn's disease, genetic animal models are crucial. These models help test new therapies, explore disease mechanisms, and understand the role of cytokines in IBD.

Notable models include knockout (KO) models, where specific genes are inactivated, and transgenic models, where additional copies of genes are expressed. In IL-10 KO mice, intestinal inflammation develops at 12–15 weeks, characterized by lesions, intestinal wall thickening, and a reduction in goblet cells. However, these mice do not develop colitis if raised in a germ-free environment. In IL-2 KO mice, the loss of IL-2 causes systemic diseases, including anemia, pancreatitis, and colitis, with pathology showing epithelial damage, crypt deformation, and neutrophil infiltration. [38]These mice typically develop colitis between 6 and 15 weeks of age. In transgenic models, such as those over expressing IL-7, colitis develops within 1 to 3 months, with features like neutrophil infiltration, goblet cell depletion, crypt damage, and rectal bleeding, resembling chronic colitis. These genetic models provide critical insights into the pathophysiology of IBD and the immune mechanisms involved.[38,39]

Future Directions

Future directions in IBD research should focus on refining experimental models to more accurately reflect patient heterogeneity, encompassing variations in sex, age, genetic background, and immune responses. Incorporating advanced *multi-omics* technologies—such as genomics, transcriptomics, proteomics, and metabolomics—will be essential for capturing the complex biological networks involved in disease progression. Additionally, integrating humanized models and organoid systems may offer more translational relevance, bridging the gap between preclinical findings and clinical applications. Collectively, these advancements will enhance our ability to dissect IBD pathophysiology and accelerate the development of targeted, patient-specific therapies.

Discussion:

The multifactorial origin of IBD necessitates diverse experimental models to dissect its underlying mechanisms. Animal models—especially those involving rodents—are fundamental in replicating aspects of IBD pathology and testing candidate therapies. Chemically induced models such as DSS and TNBS are among the most commonly employed. The DSS model is particularly suited to studying ulcerative colitis, as it leads to epithelial barrier disruption and immune activation, mimicking mucosal damage and inflammatory cytokine release (TNF-α, IL-6, IL-1β). In contrast, TNBS induces transmural inflammation reflective of Crohn's disease, engaging Th1/Th17 responses and severe colonic injury. The oxazolone model selectively induces Th2-driven colitis, paralleling some features of UC, while indomethacin and acetic acid models are valuable for examining NSAID-induced mucosal injury and barrier compromise. These models are dose- and duration-dependent and allow for the assessment of acute versus chronic inflammatory responses. Beyond chemical induction, microbial and immune-based

models provide mechanistic insights. The peptidoglycan-polysaccharide (PG-PS) model recreates bacterial antigen-driven colitis and helps link microbial components with innate immune activation. Adoptive T-cell transfer models, such as the CD45RBhi transfer into immunodeficient hosts, establish the pivotal role of T-cell dysregulation in colitis pathogenesis. Meanwhile, genetically modified models like IL-10 or IL-2 knockout mice demonstrate spontaneous colitis and highlight cytokine imbalances central to disease maintenance. Spontaneous models such as C3H/HeJBir and SAMP1/Yit mice, which develop IBD-like features without experimental manipulation, underscore the importance of genetic and microbiota interactions.

Each model has inherent limitations but offers distinct advantages depending on the specific hypothesis or therapeutic target under investigation. For instance, chemically induced models allow rapid and reproducible inflammation induction, whereas genetic and adoptive models offer sustained disease and immunological relevance. Thus, the appropriate selection or combination of models is essential for replicating human IBD complexity.

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