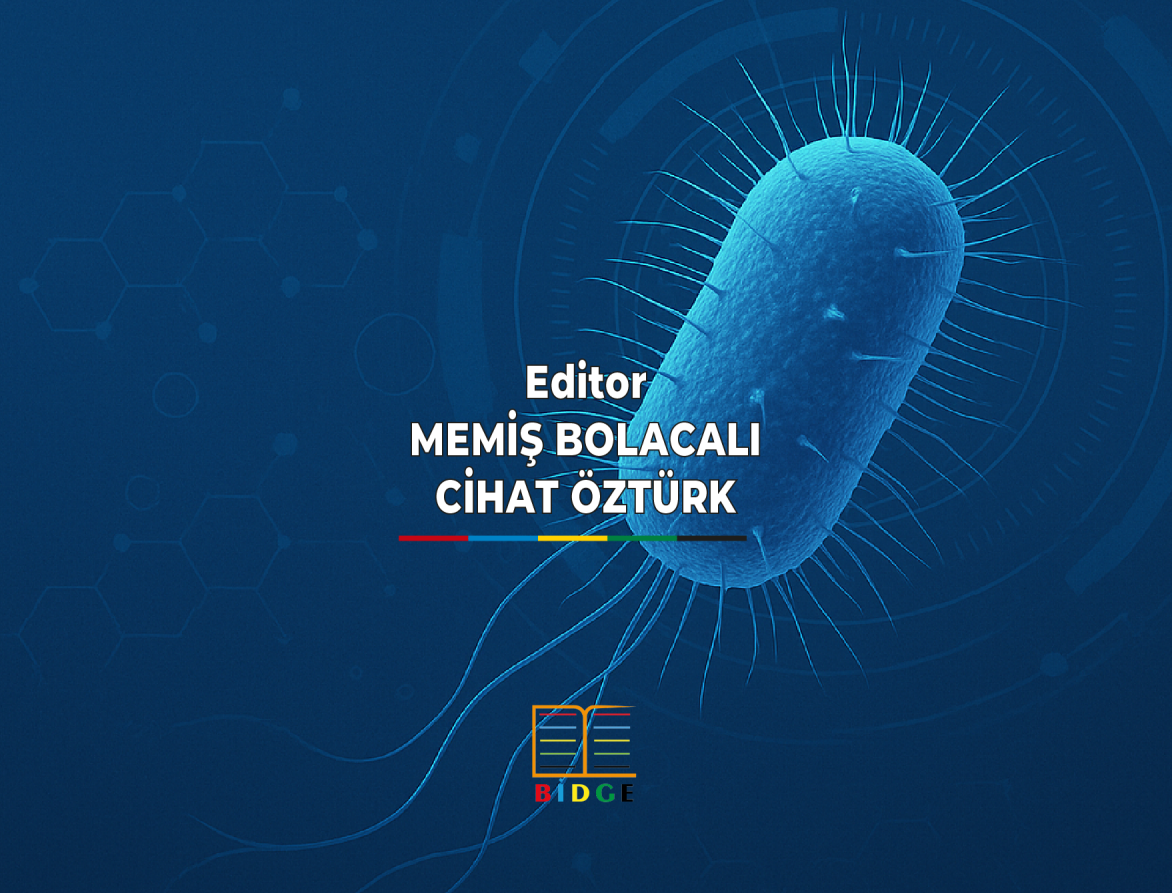
CURRENT TRENDS IN MICROBIOLOGY

Integrating Clinical Insight with Molecular and Applied Methodologies



Editor
MEMİŞ BOLACALI
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BİDGE

BİDGE Yayınları

CURRENT TRENDS IN MICROBIOLOGY: INTEGRATING CLINICAL INSIGHT WITH MOLECULAR AND APPLIED METHODOLOGIES

Editor: MEMİŞ BOLACALI & CİHAT ÖZTÜRK

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PREFACE

Microbiology has evolved from being a discipline solely focused on the identification of microorganisms into a multifaceted and dynamic science that directly influences human health, the environment, and modern therapeutic approaches. Today, the rise of antibiotic resistance, complex microbial interactions, advanced diagnostic technologies, and biotherapeutic applications are reshaping microbiology as an interdisciplinary research field.

The primary aim of this book is to comprehensively address recent advancements in microbiology from clinical, molecular, and applied perspectives. It covers a broad range of topics, from infectious diseases and microbial regulation of the immune system to antimicrobial resistance genes and the role of microbial biofilms in pathogenesis. Furthermore, the book explores innovative approaches such as the therapeutic use of viruses in cancer treatment, as well as the diagnosis and treatment of localized microbial infections like ocular infections, and up-to-date diagnostic strategies for zoonotic diseases—each of critical clinical importance.

Additionally, the book presents current knowledge on microbial–immune system interactions, including the immunomodulatory effects of probiotics and the management of systemic infections caused by microbes. Thanks to advanced techniques in molecular microbiology, resistance mechanisms and microbial responses to treatment can now be analyzed in greater detail, facilitating the development of targeted therapeutic strategies.

This work is intended to serve as both a refresher of foundational knowledge and a scientific guide to current developments in the field for researchers, clinicians, academics, and students working in various branches of microbiology. Prepared with contributions from leading experts, this volume stands as a reliable

and comprehensive resource for anyone interested in the science of microbiology.

With the hope that it contributes to a healthier future…

Asst. Prof. CİHAT ÖZTÜRK

Prof. Dr. MEMİŞ BOLACALI

KIRŞEHİR AHİ EVRAN UNIVERSITY

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CHAPTER 1

ONCOLYTIC VIRUSES IN CANCER TREATMENT

Murat ŞEVİK[1]

Introduction

Cancer is the most common cause of death worldwide, with almost 10 million deaths caused by cancer in 2020 (Aydın et al., 2018: 426; Bray et al., 2024: 229). The incidence and mortality rates continue to increase despite significant progress having been made in cancer prevention and diagnosis (Bray et al., 2018: 394; Segovia-Siapco & Sabaté, 2019: 60; Yalçın et al., 2020: 124). Conventional therapies like surgery, chemotherapy, radiation therapy, or hormonal therapies only provide limited durable responses for most cancer patients (Cerullo et al., 2018: 124; Hemminki et al., 2018: 41).

Oncolytic viruses (OVs), monoclonal antibodies, and adoptive cell therapies are currently the most prominent advances in cancer treatment because they provide effective and durable clinical responses in cancer patients (Lizée et al., 2013: 71). Viruses have been reported to have therapeutic benefits in cancer, resulting in multiple reports of leukaemia patients becoming

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disease-free after viral infections in the last century. During the 1950s and 1960s, hundreds of cancer-treatment cases were conducted using various wild-type viruses, like West Nile, Epstein-Barr, and yellow fever (Kelly & Russell, 2007: 651).

Clinical trials are being conducted to investigate the potential use of OVs, which consist of DNA and RNA viruses, as a cancer treatment (Fu et al., 2019: 270). OVs are able to infect and lyse different tumour cells in order to stabilise and decrease tumour progression. Their natural affinity for cancer cells or genetic orientation can be used to identify specific targets (Russell, Peng & Bell, 2012: 658). Furthermore, the OVs are capable of influencing the immune system's activation against tumour cells, which influences the development of an antitumour response (Desjardins, Vlahovic & Friedman, 2016: 211). OVs have become a viable way to modify the tumour environment by enabling the immune system to eliminate abnormal cells when there is an immune deficiency state (Rosewell Shaw & Suzuki, 2018: 2103). Additionally, viruses have distinct mechanisms that cause infected cells to lyse, which consequently leads to tumour cell death and enhanced immunotherapy effectiveness (Lawler et al., 2017: 841).

In the beginning, research on oncolytic effects was generally centred on wild-type or naturally occurring viruses, and the mechanism was merely assumed to be their lytic characteristics (Kelly & Russell, 2007: 651). Genetic engineering has made it technically feasible to perform a variety of modifications on wild-type viruses since 2000. Therapeutic genes can be added to modify OVs to enhance their antitumour effects through various mechanisms. Initially, the primary objective of reconstruction was to enhance target specificity, selective replication, and oncolysis. A tumour immunoreactive response was detected during tumour lysis, which is another advantage of OVs as immunotherapy (Lizée et al., 2013: 71; Li et al., 2020b: 2943). Therefore, in recent years,

strategies have shifted towards developing viral vectors to enhance immune responses within tumours (Lin, Shen & Liang, 2023: 156).

Mechanism of OVs

A race between virus replication, immune activation, and tumour growth determines the outcomes of OVs (Wu, Kirn & Wein, 2004: 605). OVs cause cancer cells to be disrupted by interacting with particular cellular receptors or by designing virus vectors with specific gene knockouts or using tumour suppressor gene defects to downregulate the antiviral pathway in tumour cells (Lin, Shen & Liang, 2023: 156). OVs are capable of infecting abnormal cells by targeting specific targets, such as cyclooxygenase-2, endothelial growth factor, CD20, and Her2/neu, which are produced by tumour cells (Russell, Peng & Bell, 2012: 658). Furthermore, pathogenic viral gene deletion can increase selectivity for tumour cells and decrease OVs' aggressiveness towards normal tissues (Thorne et al., 2007: 3350). The benefits of cell death vary depending on the characteristics of tumour cell type and virus vectors, and most of them have the ability to induce immunogenic cell death, release antigens related to tumours, and initiate antitumour immune responses. It is important to remember that antiviral immunity can be activated simultaneously with the initiation of infection (Lin, Shen & Liang, 2023: 156).

The route of administering OVs is based on the type of tumour being treated. Because the effectiveness of treatment depends on the viral route, which is caused by the virus being present in situ and the organism's natural resistance to antigens. Delivery can be subcutaneous, intravenous, intrathecal, intraperitoneal, and intratumoral, providing better control of viral abundance in the tumour environment and preventing side effects (Li et al., 2020a: 475).

When viruses are present in the organism, the virus's structure, including DNA, RNA, viral proteins, and the pathogen-associated molecular patterns, are recognised by the immune system, resulting in immunogenic cell death (Chiocca & Rabkin, 2014: 295). Immunogenic cell death is a key factor in OVs' ability to induce antitumor immunity, which involves a variety of cell deaths such as immunogenic apoptosis, autophagy, pyroptosis, and necrosis in cancer cells (Guo, Liu & Bartlett, 2014: 74; Inoue & Tani, 2014: 39).

When pathogen-associated molecular patterns are recognised through toll-like receptors (TLRs), dendritic cells produce inflammatory molecules (such as type 1 interferons, cytokines and TNF-alpha) with antiviral properties (Marchini et al., 2019: 1848). The response to viral infection is influenced by TNF-alpha and has a positive impact on the action of caspase enzymes and cell apoptosis in certain tumours (Conlon, Miljkovic, & Waldmann, 2019: 6). Through the mechanisms of necrosis and apoptosis, TNF-alpha is able to stimulate cancer cell death and cause thrombosis due to its antiangiogenic effects (Shen et al., 2018: 12441; Aydın, 2023: 1). The response of type 1 (Th1) helper cells is stimulated by TNF-alpha (Conlon, Miljkovic, & Waldmann, 2019: 6; Parlar et al., 2021: 48). The Th1 inflammatory profile is characterised by elevated levels of CD4+ T and CD8+ T effector cells, activation and differentiation of T lymphocytes, and maturation of dendritic cells, which contribute to reversing the immunosuppressive state of the tumour and promoting an inflammatory response (Zamarin et al., 2014: 226).

IL-2, a cytokine created after antigen activation, has the ability to regulate homeostasis and T regulatory cell action, which can help fight the tumour by creating an inflammatory environment (Kim, Lee & Lee, 2021: 21). The lysis and death of aberrant cells are heavily influenced by the viral action inside the cell, as well as

the damage caused by the inflammatory response. Disruptions of organelles like mitochondria, lysosomes, or endoplasmic reticulum could be caused by the presence of OVs, which could compromise normal cellular function. Additionally, the virus can trigger oxidative stress, resulting in the stabilisation and decrease of the tumour (Marchini et al., 2019: 1848).

Combining OVs and cell checkpoint blockers is an essential method to improve viral survival rates in humans. By negatively regulating PD-L1, the tumour can circumvent the immune system and avoid T cell maturation. Th1-positive response can be triggered by PD-L1 inhibition, leading to the appearance of CD8+ T cells against the tumours and the activation of natural killer cells (Rajani et al., 2016: 166). Furthermore, studies have proven that immunotherapy's effectiveness was enhanced by using monoclonal antibodies and OVs that inhibited the action of cytotoxic T lymphocyte-associated antigen 4 (CTLA4) (Zamarin et al., 2014: 226).

OVs

OVs can be categorised into natural and genetically modified viruses, with the majority of oncolytic viruses being genetically modified for oncolytic activity. The OVs have evolved from the original natural viruses to gene-edited viruses and now include over ten commonly used viruses, including adenovirus, coxsackie virus, Newcastle disease virus (NDV), measles virus (MeV), herpes simplex virus, poliovirus, reovirus, and vesicular stomatitis virus. Natural forms were used for most naturally occurring viruses, including NDV, enteroviruses, reovirus, and MeV, while genetic modification has taken place on herpes simplex virus and adenovirus (Abd-Aziz & Poh, 2021: 98).

Adenoviruses

Adenoviruses lack an envelope and have an icosahedral capsid and double-stranded linear DNA (Lasswitz et al., 2018: 1863). Several receptors, such as CD46, CD80, CD86, and the human coxsackie-adenovirus receptor, can be used by these viruses because various tissues of the organism, including lymphoid tissues, respiratory, enteric, renal, and ocular tissues, are highly susceptible to their affinity (Uusi-Kerttula et al., 2015: 6009). Therefore, various immune therapies have been developed using adenoviruses due to their ability to serve as viral vectors (Hendrickx et al., 2014: 265).

Viral replication begins in the cell nucleus and initiates the expression of certain proteins in the cytoplasm, such as E1a and E1b that are connected to the autophagy process. Autophagosomes are produced by this mechanism, which can bind with lysosomes and cause organelles or even the cell to die (Tazawa et al., 2017: 1479). Furthermore, previous studies reported that E1a and E1b contribute to tumour stabilisation and reduction (Rodriguez-Rocha et al., 2011: 9). After adenovirus infection, inflammatory cytokines, such as IL-12 and TNF-alpha, are produced to stimulate cytotoxic cells such as natural killer cells and CD8+ T cells, which also contribute to the maturation of immune cells and anti-tumour activity. Oncolytic therapy is frequently carried out with adenovirus type-5 (Ad5) due to its ability to trigger TLRs on either the inside of the cell (TLR-9) or the cellular membrane (TLR-2) (Khare et al., 2011: 1254).

Adenoviruses have the ability to activate other pathways of the immune system, including increasing the migration rates of inflammatory cells and production of inflammatory cytokines, which contributes to destroying infected cells (Khare et al., 2011: 1254). Cellular stress caused by viral infection and inflammation results in tumour cell death through apoptosis, necrosis, or autophagy. In addition to killing tumour cells directly, adenoviruses

can also create an antitumour immune response that helps in the fight in metastatic sites (Gürel et al., 2002: 6; Uusi-Kerttula et al., 2015: 6009). Strong humoral immune responses are caused by oncolytic adenoviruses. Neutralising antibodies are often viewed as an unwanted adverse event in oncolytic therapy that significantly reduces virus applicability and efficacy. However, it has been proven that completely neutralised oncolytic viruses can still have antitumoural effects when delivered to monocytes. Furthermore, it has been recently demonstrated that pre-existing immune responses can even enhance the immune response of oncolytic viruses, suggesting that neutralising antibodies are part of an unrealised immune potential (Ricca et al., 2018: 1008).

Herpes simplex virus type I (HSV-1)

HSV-1, a significant human pathogen, can both latently infect neurones and produce lytic infection in other tissue cells. HSV-1 does not cause insertional mutagenesis, even though it replicates in the nucleus (Duan, Sun & Li, 2023: 97). The fact that it has a large genome makes it easy to modify to enhance its oncolytic properties and patient safety (Ma, He & Wang, 2018: 40). The oncolytic herpes simplex virus has been found to cause a dual mechanism in tumour cells. These mechanisms involve activating antiviral pathways via cell death signalling cascades and inducing host antitumour immune responses through mobilising and activating surrounding immune cells, resulting in tumour killing (Yin, Markert & Leavenworth, 2017: 136).

There are many oncolytic HSV-1 drugs that have been developed, and Talimogene Laherparepvec (T-VEC) is one of the products that has been approved by the FDA. T-VEC is an oncolytic HSV-1 virus that has been extensively studied. It is created by deleting ICP47 and γ34.5 and adding GM-CSF to inactivate neurovirulence factors and increase virus replication and

immunogenicity (Liu et al., 2003: 292). It was discovered that HSV-1 can be linked to the Ras signalling pathway to facilitate viral replication (Pan et al., 2009: 6514). These viruses, particularly T-VEC, have a dual mechanism of action. The primary objective is to kill tumour cells directly, allowing viruses to enter the tumour environment and then proliferate and lyse the infected tumour cell, causing the expression of tumour antigens and a local immune response (Bommareddy et al., 2017: 1). Furthermore, GM-CSF expression permits dendritic cells to migrate and mature appropriately and presents more antigens to CD8+ T and CD4+ T cells that are capable of reaching distant metastases. Interferon response has been shown to cause an increase in PD-L1 expression, which results in T cell infiltration in the tumour environment (Atherton & Lichty, 2013: 1191; Thomas et al., 2019: 214).

Measles virus (MeV)

MeV is an RNA virus that has negative strands and is classified as a member of the genus *Morbillivirus* within the family *Paramyxoviridae* (Cox & Plemper, 2017: 105). Wild-type MeV primarily infects cells via CD150/SLAM, whereas MeV vaccine strains infect cells through CD46 as receptors. Mutations in the receptor attachment protein hemagglutinin (H) in vaccine strain MeV resulted in CD46's high affinity (Tahara et al., 2007: 2564). MeV has many benefits, including its excellent safety profile and the absence of genotoxicity in oncolytic vaccine strains, its immunogenicity and, specifically, the numerous engineering opportunities provided by the MeV reverse genetics system (Engeland & Ungerechts, 2021: 544).

Case reports linking measles infection to tumour remission led to the idea of treating cancer patients with MeV. Haematological malignancies were the target entities in many early studies. The natural lymphotropism of MeV provided support for

this (Grote, Cattaneo & Fielding, 2003: 6463; Kelly & Russell, 2007: 651). MeV oncolysis was found to be sensitive to other malignancies, such as ovarian cancer and glioblastoma, but not normal cells (Peng et al., 2001: 2002; Peng et al., 2002: 4656). Preclinical research has demonstrated the oncolytic properties of Edmonston B derivatives, rMV-Hu191, Edmonston-Zagreb, Moraten-Schwarz, AIK-C, and Leningrad-16 vaccine strains (Engeland & Ungerechts, 2021: 544). Furthermore, clinical evidence also indicates that oncolytic measles virotherapy enhances the immune system to prevent tumours. After treatment, lymphoma lesions in cutaneous T cell lymphoma showed a shift toward a Th1-biased T cell population (Heinzerling et al., 2005: 2287). Also, the majority of patients with multiple myeloma who received an oncolytic measles virus encoding sodium iodide symporter (MV-NIS) had increases in IFN-γ levels for cancer testis antigens (Packiriswamy et al., 2020: 3310).

MeV-induced cell death produces an immunogenic effect, generating a distinct immunopeptidome and promoting antitumour T cell responses by plasmacytoid and conventional dendritic cells (Guillerme et al., 2013: 1147; Rajaraman et al., 2018: 147). MeV oncolysis has been found to augment the cytotoxicity induced by ligand-mediated apoptosis in myeloid and plasmacytoid DCs, and it also modulates macrophages towards an antitumor phenotype additionally directing macrophages towards an antitumor phenotype (Achard et al., 2016: 1261240). Furthermore, activation of neutrophils results in the release of monocyte chemoattractant protein, interleukin-8 (IL-8), and tumour necrosis factor (TNF)-α, which can be beneficial or harmful depending on the tumour model (Zhang et al., 2012: 1002). Immunotherapeutic effects can be increased by inserting immunomodulatory transgenes into the MeV genome. MeV has the potential to act as a delivery system for immunomodulators to the tumour site, which can cause significant

harm when administered systemically (Grote, Cattaneo & Fielding, 2003: 6463).

Granulocyte macrophage colony-stimulating factor (GM-CSF) was the initial immunomodulatory transgene identified for MeV (Grote, Cattaneo & Fielding, 2003: 6463). Neutrophic infiltration was increased by MV GM-CSF in a lymphoma xenograft model, which was correlated with tumour regression. A MeV vector that encodes for IFN-β can cause immune infiltration and remodelling of the tumour microenvironment in mesothelioma xenografts (Li et al., 2010: 550). In both breast cancer xenograft pleural effusion and lung colonisation models, a MeV that encodes the immunomodulatory neutrophil-activating protein of Helicobacter pylori had a beneficial cytokine response and improved survival (Iankov et al., 2012: 1139).

Newcastle disease virus (NDV)

NDV is an enveloped avian paramyxovirus that has the ability to attack human cancers through its oncolytic properties. Membrane fusion or endocytosis can be used to infect cells by NDV. NDV has the capability to use different pathways: macropinocytosis and phagocytosis in mixed membrane domains, clathrin-mediated endocytosis in non-lipid raft membrane domains, and RhoA-dependent endocytosis in membrane domains that contain lipid rafts (El-Sayed & Harashima, 2013: 1118; Song et al., 2019: 45057). NDV has a tendency to replicate more efficiently in tumour cells than in normal cells (Krishnamurthy et al., 2006: 5145).

A significant inflammatory response is triggered by the intracellular insertion of viral hemagglutinin-neuraminidase and fusion protein antigens, which leads to cytokines, chemokines, and type I interferons being secreted (Washburn & Schirrmacher, 2002: 85; Krishnamurthy et al., 2006: 5145). Modification of tumour cell

surface markers by these proteins leads to downstream apoptosis. Especially, oncolysis can be achieved by NDV through the activation of either the intrinsic or extrinsic caspase-dependent pathways of apoptosis. Other mechanisms include secreting tumour necrosis factor- α from infected tumour cells and activating the caspase-12 and endoplasmic eIF2a kinase PERK (Washburn & Schirrmacher, 2002: 85; Elankumaran, Rockemann & Samal, 2006: 7522). NDV pathogenic classification and oncolytic properties are closely linked. The findings indicate that lentogenic NDV strains are non-lytic, while velogenic and mesogenic NDV strains are lytic. The replication of non-lytic virus is only single-loop, while lytic NDV is multi-loop. Velogenic NDV destroys the cytoplasmic membrane of infected cells, leading to the rapid destruction of cancer cells (Fournier et al., 2012: 177).

NDV replication occurs in the cytoplasm. The virus is unable to integrate with the host genome due to this replication mode. Being non-pathogenic to humans and having a relatively low risk of side effects are significant advantages of NDV as an oncolytic agent (Ganar et al., 2014: 71). NDV oncolysis transforms cold tumours into hot tumours by altering the tumour microenvironment. This situation allows immune cells to infiltrate tumours. Furthermore, danger-associated molecular patterns, pathogen-associated molecular patterns, and tumour-associated antigens are released in response to NDV, which is a strong antitumour immunity. The activation of innate immune cells (NK cells), tumour-specific T cells (CD8+ T and CD4+ T cells), and recruitment of antigen presenting cells (APCs) into the tumour is initiated by these key risk-related molecular models (Ricca et al., 2018: 1008). The immune system can clear tumours through the inflammatory response to NDV infection, but it can also cause immune cells to clear NDV, which diminishes its anti-tumour effects (Buijs et al., 2014: 24).

NDV has been found to have a positive effect on multiple cancers, including fibrosarcoma, neuroblastoma, colorectal, breast, gastric, hepatocellular, lung, and prostate carcinoma, and glioblastoma in preclinical animal studies (Matveeva et al., 2015: 15017). The safety and non-neurotropic profile of its selective replication in cancer cells is higher than that of other oncolytic viruses (Washburn & Schirrmacher, 2002: 85; Krishnamurthy et al., 2006: 5145). Oncolytic activity has been demonstrated by multiple NDV strains (e.g., AF2240, HUJ, LaSota, MTH-68/H, Ulster, and 73-T) in glioblastoma, and Ulster, HUJ, and MTH68/H strains have been mainly studied in early phase studies in patients with glioblastoma (Cuoco, Rogers & Mittal, 2021: 8). The development of the reverse genetic system of NDV has resulted in the identification of more transgenic NDVs, making the use of NDV a new phase in cancer therapy. NDV-F3aa, an NDV strain that has been genetically modified, is effective in treating a gastric tumour in peritoneal models without significant side effects, and it has the potential to completely cure gastric tumours in certain cases (Song et al., 2010: 589).

Protoparvoviruses

Protoparvoviruses are viruses that have single-stranded DNA, are not enveloped, and belong to the *Parvoviridae* family. Fixation factors such as the glycosidic substances or transferrin receptor are used by protoparvoviruses to infect human cells (Ros et al., 2017: 313). VP1, the major capsid protein, is responsible for coordinating the penetration of protoparvoviruses into the host cell. Furthermore, VP1 possesses nuclear localisation signals that aid in the movement of viral proteins to the cell nucleus. Blocking cell genome replication and integrating viral material with the host genetic material is possible from this point to ensure viral survival (Marchini et al., 2015: 6). During the lytic phase, lysosome membrane permeability is increased by viral action, which allows

cathepsin enzymes to pass into the cytoplasm and decreases the impact of inhibitory agents on these proteases. The accumulation of cathepsins in the cell cytoplasm is influenced by both factors, which in turn causes their effects and contributes to apoptosis pathways and tumour cell death (Di Piazza et al., 2007: 4186).

Apoptosis and DNA damage are caused by oxidative stress caused by a rat parvovirus H-1PV infection. The NS1 protein acts as a mediating agent for these events and alone is sufficient to trigger the complete cell death cascade caused by the complete virus (Hristov et al., 2010: 5909). Furthermore, viral DNA replication, gene expression and activation of apoptosis pathways are regulated by the NS1 protein. Viruses can cause tumour cells to undergo cellular necrosis by stimulating the expression of proteases from the lysosome to the cytoplasm. The stimulation of T lymphocytes can also be achieved by H-1PV and the formation of immune memory against tumours (Nüesch et al., 2012: 3516). In addition, an inflammatory response with antitumorigenic properties can be induced by protoparvoviruses, which leads to the production of Th1-related cytokines including IL-2 and TNF-alpha (Geletneky et al., 2015: 23).

Reoviruses

The respiratory and gastrointestinal tracts of humans are the primary targets of reoviruses (Tyler et al., 2001: 560). The fundamental link for reoviruses having an oncolytic role was found in 1977; a study reported their oncolytic role by demonstrating their cytotoxic effect on “transformed cells”, while normal cells were immune to the virus (Hashiro, Loh & Yau, 1977: 307). Reovirus has three distinct serotypes: Lang type I (TIL), Jones type II (T2J), and Abney and Dearing type III (T3D). The most extensively studied potential therapeutic for cancer treatment is T3D, which is also referred to as Reolysin (Gong et al., 2016: 25). Additionally,

reoviruses depend on a mutation in the ras gene to replicate properly in tumour cells, a fact that limits their use because only about 30% of human tumours have this mutation. The Ras pathway is crucial in the mechanism of reovirus proliferation in tumour cells by inhibiting protein kinase R and enabling viral protein synthesis (Norman et al., 2004: 11099).

Certain elements can activate the Ras pathway, resulting in the possibility of viral oncolytic therapy for more cancer types (up to 80%) (Hirasawa et al., 2002: 1696; Norman et al., 2004: 11099). The immunomodulatory properties of the virus and virus-induced apoptosis are the main causes of tumour lysis by reoviruses. The activation of an apoptotic pathway in tumour cells can be accomplished by the viral capsid proteins, which release smac/DIABLO and cytochrome c from the mitochondria into the cytosol. Once reoviruses initiate protein synthesis, proinflammatory cytokines and chemokines are secreted via damage-associated molecular patterns and pathogen-associated molecular patterns, facilitating the generation of an adaptive antitumor immune response. Then, reovirus antigens are recognised by cytotoxic CD8+ T cells and lysed cells, which leads to dendritic cell maturation, activation of natural killer cells, and more cytotoxicity (Ferlazzo et al., 2004: 16606; Steele et al., 2011: 20).

Vaccinia virus (VACV)

VACV is a complex and enveloped poxvirus that has a large DNA genome and is only replicated in the cytoplasm. It has been in use as a smallpox vaccine for many years and has not caused any significant adverse reactions (Smith et al., 2013: 2367).

VACVs offer several advantages in oncolytic virotherapy when compared to other virus vectors. These advantages are:

1. VACV replication is restricted to the cytoplasm, and viral DNA is not integrated into the host genome.

2. The large genome (~190 kb) of VACVs enables them to insert and express exogenous therapeutic genes of at least 25 kb in a single vector with high stability.
3. VACVs possess their own tumour tropism, which has the potential to be administered systemically.
4. VACVs have a replication cycle that is both rapid and lytic.
5. VACVs are capable of replicating under hypoxic conditions.
6. VACVs are able to exhibit high infectivity in multiple host species and tissues due to the lack of receptor restrictions during entry (Yu et al., 2004: 313; Hiley et al., 2010: 281; Mercer et al., 2010: 9346).

The three main mechanisms employed by oncolytic VACVs for tumour tissue destruction include direct oncolysis of tumour cells, disruption of tumour vasculature, and activation of anti-tumour immunity (Xu et al., 2024: 1324744).

Using DNA recombinant technology, engineered oncolytic VACVs can be produced due to the increasing knowledge in cancer cell biology. Preclinical and clinical evidence suggests that engineered oncolytic VACVs (e.g., Pexa-Vec (pexastimogene devacirepvec, JX-594)) have the potential to be used for intravenous infusion and tumour therapy by expressing numerous therapeutic genes that are highly and stably expressed (Chon et al., 2019: 1612). Additionally, Pexa-Vec has tropism towards endothelial cells that play a role in tumour growth through the expression of vascular endothelial growth factor or fibroblast growth factor. It leads to destruction of the vascular structure that feeds the tumour, resulting in tissue necrosis and a decrease in tumour spread (Breitbach et al., 2013: 1265). Administration of

VACVs was found to be associated with the induction and expression of IL-24 and GM-CSF, factors that together may contribute to the stabilisation and death of tumour cells. GM-CSF has an impact on the maturation and differentiation of immune system cells, including dendritic cells and neutrophils, creating an inflammatory environment that facilitates the fight against tumours (Gujar et al., 2018: 209).

Tumour angiogenesis is suppressed by IL-24, which positively affects the apoptosis pathways and antitumour response while also avoiding tumour metastases (Gujar et al., 2018: 209). Different cell death pathways, like necrosis and apoptosis, are triggered by the viral activity of certain VACV strains, which results in the release of damage- and danger-associated substances that provide an immunogenic environment. Afterwards, the damage-associated molecular patterns help to cross-present themselves with tumour antigens, which stimulate CD8+ T cell activity and contribute to the antitumor response (Gujar et al., 2018: 209; Xu et al., 2024: 1324744).

Conclusion

There are still very few approved OVs and applicable cancer types in the clinic. Intratumour administration is one of the most important limiting factors. Antiviral neutralising antibodies that appear during viral therapy cause repeated systemic treatments of OVs to be ineffective and limit their application in certain types of cancer that have metastases or cannot be treated in situ. Therefore, it is urgently required to develop novel and more potent oncolytic viruses.

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CHAPTER 2

BACTERIAL INFECTIONS OF THE EYE

Funda ÇİMEN AÇIKGÜL[1]
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Introduction

The eye is a highly specialized sensory organ responsible for the sense of vision. Anatomically, it is composed of three primary layers: the outer, middle, and inner layers (Li, Chen, & Fu, 2023). The outermost layer consists of the cornea and sclera. The cornea, with its transparent and dome-shaped structure, enables the refraction of light into the eye and protects the anterior segment. The sclera, on the other hand, is an opaque, fibrous tissue providing mechanical support to the globe (Nishida, Saika, & Morishige, 2021). The middle layer, known as the uveal tract, is a vascular and pigmented structure comprising the choroid, ciliary body, and iris (Kumar, Kumar, & Attuluri, 2024). The choroid, with its dense vascular network, is primarily responsible for nourishing the retina, while the ciliary body contains the ciliary muscles essential for

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accommodation and epithelial cells that produce the aqueous humor (Nishida et al., 2021). The iris, rich in pigmented cells determining eye color, regulates the amount of light entering the eye through its central aperture, the pupil (Valmaggia, Inglin, Kaiser, Scholl, & Maloca, 2022).

The innermost layer, the retina, contains photoreceptor cells (rods and cones) that convert light stimuli into neural impulses, transmitted them to the brain via the optic nerve (Kiptenko, Hryntsova, & Tymakova, 2021). Additionally, auxiliary structures such as the anterior and posterior chambers, lens, vitreous humor, and optic disc collectively maintain the integrity of the optical system. This multi-layered and highly organized anatomical design is crucial for precise image formation and healthy visual perception (Kiptenko et al., 2021).

As a sensory organ continuously exposed to intrinsic factors and environmental risks, the eye is susceptible to various infections. These infections may involve individual ocular layers or affect multiple structures simultaneously.

Bacterial Infections of the Eye

Bacterial Infections of the Eyelids

The eyelids, with their delicate skin and glandular structures, offer a suitable environment for microbial colonization and infection. The Meibomian, Zeis, and Moll glands, located at the base of the eyelashes, secrete oils and sweat to maintain ocular surface hydration while potentially providing a niche for microorganisms (Sadig et al., 2022). The most frequently isolated pathogens in these areas are *Staphylococcus aureus* and coagulase-negative staphylococci, notably *Staphylococcus epidermidis* (Adeghate, 2024).

Staphylococcal blepharitis is characterized by keratinized crusts at the lash bases, erythema, and mild edema along the eyelid margins. Changes in lash color, misalignment, and loss (madarosis)

may also occur (Yeu et al., 2022). Abscesses of the deeper Zeis and Moll glands manifest as external hordeolum (styes), whereas inflammation of the Meibomian glands presents as internal hordeola (Kim, Afshin, & Elahi, 2023). Oral tetracyclines are typically effective in treating internal hordeolum (Alsoudi et al., 2022). Chronic abscesses may evolve into chalazia, requiring surgical intervention (Barshak & Durand, 2022). In an era marked by increasing antibiotic resistance, hygiene-centered therapeutic approaches have gained prominence. These include daily cleansing of the eyelid margins using warm compresses and baby shampoo, as well as antiseptic treatments with 0,5-1% povidone-iodine solution (Zhang, Wang, & Gao, 2023). Topical erythromycin or bacitracin ointments are preferred for mild to moderate cases, while oral tetracyclines or macrolides may be necessary for resistant or internal hordeolum cases (Kreft & Wohlrab, 2022).

Bacterial Conjunctivitis

The conjunctiva, a mucous membrane covering the sclera and inner eyelids, serves as a robust immunological barrier (Diebold & García-Posadas, 2021). However, when these defenses are compromised, or the infecting bacteria surpass host resistance, infectious conjunctivitis may develop. The most common pathogens in acute bacterial conjunctivitis include *Staphylococcus aureus*, *Haemophilus aegyptius*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* (Alrawyah et al., 2024). Clinically, the disease manifests with conjunctival hyperemia, excessive tearing, mucopurulent discharge, and eyelash crusting, especially in the mornings. Infections caused by *H. aegyptius* can progress to serious systemic complications such as preseptal cellulitis, sepsis, and meningitis in children (Cantor, 1990).

Hyperacute bacterial conjunctivitis is primarily associated with *Neisseria gonorrhoeae* and *Neisseria meningitidis* (Bhat & Jhanji, 2020). This severe form is characterized by pronounced eyelid edema, copious purulent discharge, lymphadenopathy, and potential corneal perforation (Lindquist & Lindquist, 2021).

Immediate and aggressive management with both systemic and topical antibiotics is vital.

Chronic bacterial conjunctivitis is typically caused by *Staphylococcus aureus* and *Moraxella lacunata* (Bhat & Jhanji, 2020). This condition usually presents with mild hyperemia and a small amount of mucous discharge, particularly in the mornings. Management involves selecting appropriate antibiotics based on culture and susceptibility results, alongside eyelid hygiene, warm compresses, and regular cleansing (Høvding, 2008).

Bacterial Keratitis

The cornea, as an avascular and transparent structure, has limited natural defenses against infection. Bacterial keratitis often develops following epithelial damage due to contact lens wear, trauma, or topical corticosteroid or antibiotic use (Wilhelmus, 2002). Common causative agents include *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Moraxella lacunata* (Teweldemedhin, Gebreyesus, Atsbaha, Asgedom, & Saravanan, 2017). Notably, *Pseudomonas aeruginosa* is the most common cause of rapidly progressive keratitis that can lead to perforation, particularly in contact lens wearers (Hilliam, Kaye, & Winstanley, 2020).

Clinically, bacterial keratitis presents with pain, photophobia, decreased visual acuity, corneal ulceration, stromal infiltrates, anterior chamber reaction, and hypopyon formation (Dumitrache, 2024). Epithelial defects with surrounding edema and stromal infiltration are frequently observed (Cabrera-Aguas, Khoo, & Watson, 2022). For definitive diagnosis, corneal scrapings should be obtained for microbiological culture. Gram and Giemsa stains offer rapid diagnostic insights, while multiplex PCR techniques have recently gained importance for early and specific pathogen detection (Zemba et al., 2022).

Empirical treatment typically involves intensive topical administration of broad-spectrum antibiotics such as cefazolin

combined with tobramycin or fluoroquinolones (Zhou, Wang, Yuan, Zhou, & Xue, 2024). Therapy is adjusted according to culture results and antibiotic susceptibility patterns. Severe cases may require subconjunctival antibiotic injections, cycloplegic agents, or surgical interventions including collagen shields, tissue adhesives, or corneal transplantation (Mohan, Natarajan, Kaur, & Gurnani, 2023).

Bacterial Endophthalmitis

Endophthalmitis is a severe bacterial infection involving the internal structures of the eye and represents one of the most serious ocular infectious diseases (Simakurthy & Tripathy, 2023). Causative agents may enter the eye via endogenous hematogenous dissemination or through exogenous routes such as surgery or trauma (Cunningham, Flynn, Relhan, & Zierhut, 2018). Postoperative endophthalmitis is most frequently associated with *Staphylococcus epidermidis*, though more virulent pathogens such as *Staphylococcus aureus*, *Proteus* species, and *Pseudomonas aeruginosa* can also be responsible (Benz, Scott, Flynn Jr, Unonius, & Miller, 2004).

Clinically, endophthalmitis presents with severe ocular pain, eyelid edema, conjunctival hyperemia, corneal edema, anterior chamber reaction, hypopyon, and vitritis (Simakurthy & Tripathy, 2023). Diagnosis is established through culture of vitreous samples (Kehrmann et al., 2018). Management involves intravitreal injection of antibiotics such as gentamicin and vancomycin, supported by topical and systemic antimicrobial therapy. In advanced cases, pars plana vitrectomy may be necessary (Blom et al., 2023).

Retinal Infections

The retina is susceptible to bacterial, viral, and fungal infections. Among bacterial causes, ocular complications secondary to systemic diseases such as syphilis and tuberculosis are particularly notable (Hu et al., 2022). Endophthalmitis can progress to involve both the retina and vitreous. Although bacterial retinitis is rare, it

carries a high risk of irreversible vision loss (Haseeb, Elhusseiny, Siddiqui, Ahmad, & Sallam, 2021).

Differential diagnosis of retinal infections, particularly in immunocompromised patients such as those with AIDS, is essential (Kuo et al., 2025). Opportunistic infections like ocular histoplasmosis, cryptococcosis, and aspergillosis can lead to retinal scarring and macular damage (Stiles, 2021). In select cases, argon laser photocoagulation may be used to manage subretinal neovascularization (Huang, Zheng, Li, Sun, & Lin, 2023).

Conclusion

The eye's intricate anatomical architecture and limited vascularization necessitate unique defense mechanisms against infections. Nevertheless, environmental factors and systemic diseases can disrupt these protective barriers, facilitating bacterial invasion of ocular tissues. Bacterial infections present with diverse clinical manifestations, ranging from superficial involvement to life- and vision-threatening intraocular complications.

Early diagnosis, targeted antimicrobial therapy, and the rational selection of antibiotics based on regional resistance profiles are vital in the management of bacterial ocular infections. Preventive strategies, including patient education on ocular hygiene, adherence to safe contact lens use, and the cautious application of topical corticosteroids, are equally critical in minimizing infection risk. The rising trend of antibiotic resistance highlights the importance of updating treatment protocols in accordance with current guidelines and local epidemiological data. Integrating multidisciplinary approaches and novel diagnostic technologies into routine clinical practice will further enhance therapeutic outcomes and preserve visual function in affected patients.

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CHAPTER 3

BRUCELLOSIS AND DIAGNOSIS

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Introduction

Brucellosis is a zoonotic infectious disease caused by bacteria of the genus *Brucella*, leading to serious health problems in both humans and animals (Berhanu and Pal, 2020). The disease is a significant source of infection in terms of public health, particularly affecting occupational risk groups. While brucellosis is primarily transmitted through direct contact with animals, consumption of contaminated animal products, and inhalation, mother-to-child transmission is also clinically significant (Huy, 2024).

Brucella Pathogen and Its Characteristics

Brucellosis is a disease caused by bacteria of the genus *Brucella*, leading to systemic infections in humans and animals. *Brucella* bacteria are Gram-negative, non-motile, non-capsulated,

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and non-spore-forming coccobacilli, classified as obligate aerobic organisms. These bacteria are catalase-, oxidase-, and urease-positive and possess the ability to survive intracellularly within phagocytic cells (Aksoy, 2021). This intracellular localization contributes to the chronic course of brucellosis and its ability to evade host defense mechanisms. The most common pathogenic species of *Brucella* in humans are: *Brucella melitensis* (from sheep and goats, the most virulent species for humans), *Brucella abortus* (from cattle), *Brucella suis* (from pigs), and *Brucella canis* (from dogs). *Brucella melitensis* is the most frequently isolated species in human brucellosis cases and accounts for the majority of infections (Shoukat et al., 2017).

Epidemiology

Brucellosis in the world and Turkey Brucellosis is a widespread zoonotic disease globally, being more prevalent in endemic regions such as the Mediterranean countries, the Middle East, Central Asia, Africa, and Central and South America (Bagheri Nejad et al., 2020). Approximately 500,000 cases are reported annually; however, seroprevalence studies suggest that the actual number may be much higher (Khoshnood et al., 2022). Turkey is among the countries where brucellosis is endemic, with an annual incidence rate ranging between 29 and 200 cases per 100,000 people (Liu et al., 2024). The infection is more frequently observed in regions where sheep and goat farming is common and among individuals involved in animal husbandry.

Clinical Symptoms

Brucellosis can present as acute, subacute, or chronic and exhibits highly variable clinical manifestations (Tekin et al., 2024). Common symptoms include fever, night sweats, fatigue, headache, anorexia, weight loss, and myalgia (Ulu Kilic et al., 2013). In addition, organ involvement may occur, including osteoarticular (arthritis, spondylitis, sacroiliitis), genitourinary (epididymo-

orchitis, testicular infections), gastrointestinal (hepatosplenomegaly, liver dysfunction), and cardiovascular (endocarditis, though rare, has a high mortality rate) complications (Jin et al., 2023).

Brucella bacteria can be transmitted to humans through various routes. The most common mode of transmission is direct contact with infected animals. Occupational risk groups, including veterinarians, farmers, butchers, and laboratory workers, are at higher risk when exposed to infected animal blood, urine, milk, placenta, and other body fluids (Mia et al., 2022). Another major route of transmission is the consumption of unpasteurized milk and dairy products. Raw consumption of products such as cheese, butter, and cream derived from infected animals can contribute to the spread of the disease. Airborne transmission poses a risk particularly for laboratory workers or individuals present in animal shelters, where aerosols generated during animal birthing or from contaminated materials can be inhaled (Chiang and Palmore, 2021). Furthermore, transmission through skin and mucosal contact is possible, especially through open wounds that come into contact with infected animals or contamination of the eyes, mouth, or nasal mucosa (Tryland et al., 2018). In pregnant women, *Brucella* can be transmitted to the fetus via transplacental transmission, during childbirth, or through breast milk (Chiang and Palmore, 2021). Rarely, transmission through blood transfusion and organ transplantation has also been reported.

Diagnostic Methods

The definitive diagnosis of brucellosis is based on the evaluation of clinical symptoms along with laboratory tests. The primary diagnostic methods include:

Culture Tests

Brucella species can be isolated from blood, bone marrow, cerebrospinal fluid, synovial fluid, or other sterile body fluids

(Bwisa, 2014). However, due to the slow growth of the bacteria, obtaining results can take time. Culture media such as blood agar, chocolate agar, *Brucella* broth, and *Brucella* agar can be used (Di Bonaventura et al., 2021). Specimens with low bacterial loads, such as blood and cerebrospinal fluid, should be inoculated into large volumes of liquid media. Incubation should occur in a 5-10% CO_2 environment, with duplicate cultures recommended when possible (Di Bonaventura et al., 2021). Contaminated specimens, such as pus, should be plated directly onto solid media. The incubation period ranges from 7 to 21 days, but given the slow growth of initial isolates, cultures should be incubated for up to 28 days before being considered negative (Bwisa, 2014). Pure colonies on agar are used for identification, revealing Gram-negative coccobacilli under Gram staining. Sand-like clumps of bacteria appear in the Giemsa stain (Qiangsheng et al., 2023). *Brucella* species exhibit variable hydrogen sulfide (H_2S) production times: *B. suis* produces H_2S in over three days, *B. abortus* within two days, and *B. melitensis* in small amounts within one day (Occhialini et al., 2022). On Christensen's urea agar, *B. suis* exhibits urease activity within 15-20 minutes,
B. abortus after two hours, and *B. melitensis* shows delayed or negative results (Nayak, 2023).

Serological Tests

Serological tests are the most commonly used methods for diagnosing brucellosis. Tests such as the Rose Bengal Test, Agglutination Test, Coombs Tube Agglutination Test, and ELISA (IgG and IgM) can indicate the presence of the disease.

Slide Agglutination Tests
Rose Bengal Test

A standardized suspension of *Brucella abortus* bacteria stained with Rose Bengal dye in saline is used as an antigen (Anbazhagan, 2024). Approximately 300 µL of patient serum is

placed on plastic plates with wells or a clean white tile. An equal volume of antigen, which has been kept at room temperature for 10 minutes, is added to the serum. The mixture is stirred using a wooden stick and spread in a circle of 1.5 cm diameter. Afterward, it is mixed in a circular motion for 4 minutes. If large granular precipitates form, the result is positive; if fine granules appear, it is suspicious; and a homogeneous appearance indicates a negative result (Díaz et al., 2011).

Spot Test

As an antigen, prepared from *Brucella* bacteria stained with a special method, is placed on a clean tile or plate. The patient's finger prick is pricked, and after wiping away the first drop, a loopful of blood from the second drop is taken and placed on the antigen (Tschopp et al., 2021). The reaction is observed for 4 minutes by tilting the plate back and forth. In positive cases, agglutinated particles form a blue ring at the edges, while a red-green color appears in the center (Saavedra et al., 2019).

Slide agglutination tests are preliminary screening methods. If the results are positive, serum samples should be re-evaluated with tube agglutination tests.

Wright Test

Suspensions of *Brucella* bacteria killed by heat and preserved in phenol, obtained from S colonies, are used as antigens. Antibodies appear in the blood about two weeks after infection begins and remain for a long time. The serum is inactivated at 56°C for 30 minutes, and tests are conducted using both active and inactive sera for comparison (Yagupsky et al., 2019).

In the acute phase of brucellosis, IgM antibodies first appear after the first week, reaching their highest levels within three months before gradually decreasing (Jindan et al., 2019). However, in many cases, they may persist in the blood at low or sometimes high levels

for extended periods. IgG antibodies become detectable approximately three weeks after the onset of the disease, reaching their highest levels in 6-8 weeks (Jindan et al., 2019). They remain in the blood at significant levels during chronic infection. To detect only IgG antibodies in chronic cases, the 2-Mercaptoethanol test should be performed (Zhou et al., 2021).

Due to the predominance of IgG antibodies in brucellosis and antigen-antibody interactions, the agglutination mechanism may be impaired. Therefore, if clinical symptoms suggest brucellosis but agglutination results are negative, the Coombs test should be performed to detect blocking antibodies (Turan and Karslıgil). If agglutination inhibition occurs due to an excess of antibodies, further dilutions should be made.

Agglutination Test

At least ten test tubes are arranged in a rack. The first tube is filled with 0.9 mL of physiological saline, while the others receive 0.5 mL each (Nayak, 2023). Then, 0.1 mL of patient serum, inactivated at 56°C for 30 minutes, is added to the first tube. After pipetting, 0.5 mL of the suspension is transferred from the first tube to the second tube, continuing this process until the ninth tube, from which 0.5 mL is discarded. This completes the serial dilutions. The tenth tube serves as the antigen control tube and does not receive any serum. Each tube contains 0.5 mL of suspension. The serum is diluted in a series (1:10, 1:20, 1:40, etc.) (Nigudgi, 2008).

Antigen (0.5 mL) is added to all tubes, including the last one, further diluting the serum by one fold. The tubes are shaken and incubated at 37°C for 48 hours (Caswell, 2019). At the end of the incubation period, the antigen control tube is checked to ensure no agglutination occurs. The other tubes are evaluated based on the clarity of the upper fluid and the degree of agglutination, graded as 4+, 3+, 2+, 1+, or negative (-), and titers are recorded accordingly.

The results are written for each tube according to the dilution factor, e.g., 1:80 4+, 1:160 2+, etc. The agglutination titer is defined as the highest dilution that still gives a result of at least 2+. A titer of ≥1:160 supports an active infection (Barkay et al., 2024). A brucellosis diagnosis requires a titer above 1:100 with a significant increase in titer after two weeks.

2-Mercaptoethanol Test

In the acute phase of brucellosis, IgM antibodies appear first, followed by IgG. IgM levels decrease and disappear more rapidly, while IgG production continues in chronic cases. However, in many cases, IgM antibodies may persist at low or significant levels. In such cases, it is necessary to determine whether a positive agglutination result is due to IgM antibodies or an ongoing chronic infection. The presence of IgG antibodies, which indicate active infection, must be identified (Zhou et al., 2021).

To distinguish between the two, agglutination tests are performed after destroying IgM antibodies in the patient's serum. For this purpose, 2-Mercaptoethanol or rivanol is used. These substances break the disulfide bonds in IgM and depolymerize the antibodies, eliminating their agglutination activity. If agglutination tests remain positive after treatment with these substances, this indicates the presence of IgG antibodies and suggests a chronic infection. If agglutination becomes negative, it means the initial agglutination was due to IgM antibodies (Ullah et al. 2025).

In this test, 0.05M 2-Mercaptoethanol saline solution is used instead of physiological saline for serial dilution. The evaluation is conducted in the same manner as described above.

Coombs Tube Test

In some cases, clinical symptoms of brucellosis are present, but agglutination tests yield negative results. This occurs due to mechanisms that prevent agglutination reactions despite antigen-

antibody binding. To overcome this agglutination blockage, the Coombs test is performed using human antiglobulin serum (Oktari et al. 2021).

The patient's serum is diluted and antigen is added, as described in the agglutination test. The mixture is incubated at 37°C for 24 hours, and tubes with positive agglutination results are removed. All tubes showing no agglutination are centrifuged at 3000 rpm for 10 minutes to precipitate the bacteria. The supernatant is discarded, and each tube is washed with saline, followed by another round of centrifugation. This process is repeated three times (Ullah et al. 2024).

After the final wash, 0.45 mL of saline is added to each tube, and the contents are resuspended. The human antiglobulin (Coombs) serum is diluted tenfold with saline, and 0.05 mL of this dilution is added to each tube, resulting in a 1:10 final dilution. The tubes are incubated at 37°C for 24 hours and evaluated for agglutination.

Other Diagnostic Methods

In addition to these tests, Enzyme Immunoassay (EIA), Polymerase Chain Reaction (PCR), and imaging methods such as MRI, CT, or ultrasound can assist in diagnosing brucellosis (Liang et al., 2022).

Conclusion

Brucellosis is a zoonotic infectious disease caused by *Brucella* species, posing significant health risks to both humans and animals. The transmission routes of the disease directly impact occupational risk groups and public health. Clinically, it can present in acute, subacute, or chronic forms, and its nonspecific symptoms make diagnosis challenging.

Culture, serological, and molecular methods play crucial roles in the diagnosis of brucellosis. Although culture remains the gold standard, the slow growth of *Brucella* species makes it time-

consuming. Serological tests such as RBT, SAT, and Coombs tests are widely used for diagnosis. In cases of agglutination blocking, the Coombs test can confirm the presence of antibodies, while the 2-Mercaptoethanol test helps determine the presence of IgG antibodies, indicating chronic infection. PCR is a critical diagnostic tool due to its rapid and high sensitivity.

Preventive measures, including vaccination of animals, adherence to hygiene protocols, and avoiding the consumption of unpasteurized dairy products, are essential for the control and prevention of brucellosis. Given its public health implications, early diagnosis and treatment are crucial to preventing complications and controlling the spread of the disease.

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CHAPTER 4
THE *ermTR* GENE: AN UNIQUE PLAYER IN ANTIBIOTIC RESISTANCE

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1.Introduction

The *ermTR* gene, initially identified in *Streptococcus pyogenes* by Seppälä et al. (1998), is a member of the erm gene family associated with antibiotic resistance. This gene is key for bacteria, especially *S. pyogenes*, to adapt to antibiotics such as erythromycin and clindamycin. The importance of *ermTR* lies in its capacity to provide low-level resistance to erythromycin while allowing for high-level resistance to clindamycin (Oryaşın et al., 2020).

Lincosamides such as clindamycin are especially valuable for treating infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and other Gram-positive bacteria. The increasing prevalence of bacteria exhibiting resistance to both macrolides and lincosamides, often driven by *erm* genes such as *ermTR*, poses a significant clinical challenge particularly when treating streptococcal infections.

Macrolides, lincosamides, and streptogramins (MLS) are a group of antibiotics that inhibit bacterial growth by binding to the ribosome, demonstrating strong efficacy against Gram-positive bacteria (Oryaşın et al., 2020). The growing problem of bacteria developing resistance to antibiotics, especially those affecting streptococci, has become a major clinical issue (Cattoir & Leclercq, 2017).

2. The molecular mechanism behind *ermTR*-mediated resistance

The structure of a gene and its resulting protein product

The *ermTR* gene consists of 732 nucleotides, displaying 82.5% similarity with *ermA*, and is expected to encode a polypeptide of 243 amino acids (Seppälä et al., 1998). Its expression and induction can be influenced by the presence or absence of the gene's regulatory region, as noted by Oryaşın et al. in 2020.

Methylation of 23S rRNA

The resistance profile conferred by *ermTR* is primarily due to the methylation of adenine residues in the 23S rRNA, which alters antibiotic binding sites (Cattoir & Leclercq, 2017). Specifically, methylases encoded by *erm* genes methylate the adenine residue at position 2058 of the ribosomal RNA 23S subunit. The modification enables bacteria to endure antibiotic treatment.

The Erm family of methyltransferases (erythromycin resistance methyltransferase) facilitates the methylation of the N6 position of nucleotide A2058 in the 23S rRNA. This methylation process prevents macrolides from interacting with the ribosome, effectively conferring high resistance to macrolide antibiotics (Golkar et al., 2018). Methylation also impacts the binding of lincosamides, which is why cross-resistance is often seen between macrolides and lincosamides. ErmTR's distinct resistance profile, characterised by both low- and high-level resistance to clindamycin, offers a potentially unique mechanism of action in comparison to other *erm* genes. The differing impacts of macrolides and lincosamides on antibiotic resistance underscore the multifaceted nature of resistance mechanisms and their practical effects on treatment.

So far, six mobile linezolid resistance classes, encompassing *lnu, cfr, erm, vga, lsa,* and *sal,* have been recognised. Lincosamide resistance genes are commonly encountered on mobile genetic elements, including plasmids, transposons, integrative and conjugative elements, genomic islands, and prophages (Yang et al., 2024). The Erms enzymes promote the addition of either one or two methyl groups to the A2058 residue, this process being influenced by the *erm* gene (Svetlov et al., 2021).

Svetlov et al. (2021) offered significant understanding of how the *ermTR* gene is responsible for macrolide resistance, primarily through the dimethylation of the A2058 residue in 23S rRNA. The high-resolution crystal structures show that this modification happens during ribosome assembly (30S and 70S), with Erm methyltransferases targeting the A2058 site, remaining accessible in the process. These results highlight the crucial phase of ribosomal maturation. This study questioned established beliefs about significant changes to ribosomal structure after post-dimethylation, finding that the modification does not cause major changes to ribosomal architecture or operation.

Svetlov et al. (2021) found that the dynamics of ribosomal interactions are precise and the desosamine moiety plays a vital role in macrolide binding. These insights lay the groundwork for developing rational drug design strategies to tackle the pressing issue of antibiotic resistance. These discoveries hold significant importance in medical settings, where the widespread presence of erm genes in disease-causing bacteria hinders treatment choices.

Comparison with Other *erm* Genes

To date, at least 55 *erm* genes have been identified, with *erm*(A), *erm*(B) and *erm*(C) being the most frequently encountered (Yang et al., 2024). Each class shows distinct resistance profiles, induction methods, and patterns of occurrence among bacterial species. Widely distributed among various Gram-positive and Gram-negative bacteria, *ermB* is found in contrast to *ermTR*, which is predominantly located in *Streptococci*.

In contrast to *ermA* and *ermB*, which encode methyltransferases that methylate adenine at position 2058 of 23S rRNA and impart high-level resistance to both macrolides and clindamycin, ermTR displays a distinct resistance profile (Oryaşın et al., 2020). Constitutive expression of *ermTR* results in low-level erythromycin resistance at 8 mg/L and high-level resistance to clindamycin at 128 mg/L (Oryaşın et al., 2020).

3. Regulatory Mechanisms and Induction

The expression of *erm* genes can be either constitutive or inducible, depending on specific stimuli. When the leader peptides are being translated before the genes that encode methyltransferases, such as *erm*(B), in the presence of erythromycin, ribosome stalling occurs, which in turn leads to the induction of downstream methyltransferase expression (Arenz et al., 2014). The *erm* genes can be expressed constitutively either with a mutated leader peptide or in the absence of the leader peptide (Yang X et al., 2024).

The Regulatory Region of ermTR

The regulatory region of *ermTR* is vital for its adaptive response to antibiotics. This area encompasses three key hairpin formations, with the third featuring a higher delta energy that conceals the ribosome's binding site and the *ermTR* gene's start codon on the mRNA (Oryaşın et al., 2020).

Induction Mechanism

Compounds such as erythromycin are hypothesized to induce conformational changes in the mRNA structure., enabling the ribosome to bind and start the synthesis of the ErmTR enzyme (Oryaşın et al., 2020). The induction mechanism enables a quick response to the presence of antibiotics in the environment.

The *ermTR* induction mechanism is particularly relevant to lincosamide resistance. Constitutive expression provides a high degree of resistance to clindamycin, and erythromycin induction can substantially boost this resistance. Inducible clindamycin resistance has significant clinical implications because it may result in treatment failure if clindamycin is administered to treat infections caused by bacteria that appear to be resistant to erythromycin but susceptible to clindamycin.

The induction mechanism of erm genes, encompassing *ermTR*, involves an intricate process including ribosome stalling and translational attenuation. Binding of an inducer antibiotic to the ribosome causes the ribosomal process to halt at a particular location within the leader peptide region. This stalling event initiates a conformational shift in the mRNA structure, revealing the Shine-Dalgarno sequence and the erm gene start codon, which then enables its translation to proceed.

Differential Induction Effects

A noticeable impact of the regulatory region was evident in *S. pyogenes* NZ131, where the clindamycin MIC rose by more than 16-fold following induction with erythromycin (Oryaşın et al., 2020). The presence of the regulatory region (*ermTR*+rr) results in erythromycin and clindamycin MICs that are nearly identical, however, clindamycin MICs rise by more than 32-fold (from 4 to over 128 mg/L) following erythromycin induction as reported by Oryaşın et al. (2020).

This differential induction effect is not exclusive to *ermTR* and can also be seen in other *erm* genes. The induction profile for macrolide resistance can differ based on the *erm* gene and the specific bacterial species involved, which adds to the complexity of macrolide resistance in clinical environments.

Translational Attenuation and Inducible Expression of *ermTR*

The inducible expression of erm genes, such as *ermTR*, is primarily regulated by translational attenuation. This mechanism allows bacteria to detect the presence of macrolide antibiotics and activate resistance genes on demand, thereby reducing the metabolic strain of continuous expression.

The process of translational attenuation incorporates a leader peptide sequence positioned upstream of the erm gene coding region. A specific sequence of nucleotides is essential for controlling the initiation of translation by causing ribosomes to pause and altering the structure of messenger RNA (Bozdogan and Appelbaum, 2004).

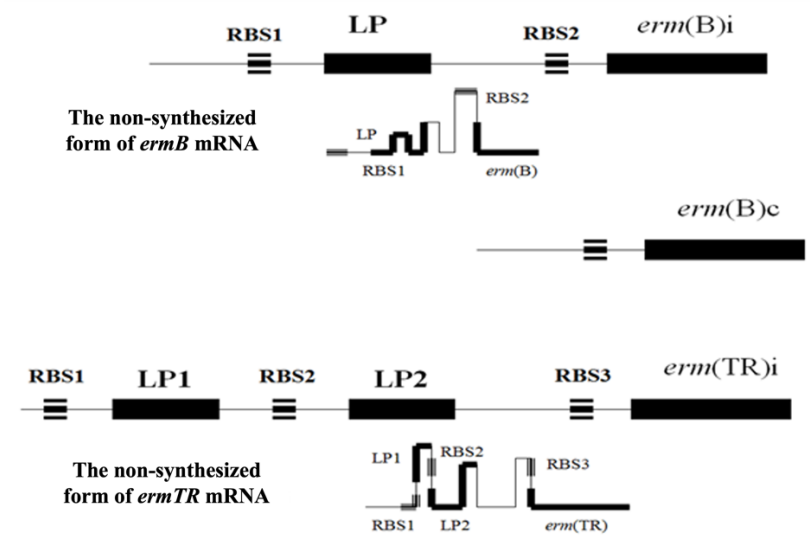

Figure 1: The inducible and constitutive regulatory regions of the *ermB* gene and the inducible regulatory region of *ermTR* have been shown (Bozdogan and Appelbaum, 2004).

The Mechanism of Ribosomal Stalling Induced by Macrolide Antibiotics

1. In the absence of a macrolide antibiotic, ribosomes are able to efficiently translate the leader peptide, which in turn prevents the downstream expression of *ermTR*. However, when a macrolide antibiotic, such as erythromycin, binds to the ribosome, it results in ribosome stalling at a specific codon within the leader peptide region.

2. The stalling of the ribosome causes a change in the mRNA structure, resulting in the unfolding of an inhibitory hairpin structure that normally blocks the Shine-Dalgarno sequence and the *ermTR* start codon. This structural change makes the Shine-Dalgarno sequence accessible, enabling ribosome binding and the initiation of *ermTR* translation.

3. Once the SD sequence is accessible, the full *ermTR* gene is transcribed and translated into an Erm methyltransferase enzyme, which then methylates the 23S rRNA at the A2058 position, preventing macrolide binding to the ribosome and thereby conferring resistance.

The distinctions between ermTR and other erm genes.

Unlike *ermA* or *ermB*, which display robust inducibility, *ermTR* has been documented to have a weaker ribosome stalling effect, resulting in partial constitutive expression even in the absence of macrolides. This distinctive regulatory feature may underlie its distinct resistance profile, which involves low-level erythromycin resistance and high-level clindamycin resistance.

The *ermA* and *ermC* genes are most commonly found in staphylococci, the *ermB* gene in *enterococci* and *streptococci*, while the *ermTR* gene was first identified in *Streptococcus pyogenes* strains and constitutes the most prevalent methylase group in this species. The synthesis of Erm methylase can be induced by the presence of macrolides in the environment. Typically, 14- and 15-membered macrolides induce resistance to a greater extent than 16-membered macrolides. In *staphylococci*, lincosamides do not induce resistance. The induction of resistance occurs during the protein synthesis phase, specifically at the translational stage. Upon erythromycin binding, conformational changes in the mRNA occur, and an RBS (ribosome binding site) previously inaccessible to the ribosome in the secondary RNA structure becomes accessible for ribosome binding (Bozdogan and Appelbaum, 2004) (Fig 1).

Translational attenuation has significant clinical implications.

- The presence of a working mechanism for slowing down translation means bacteria initially seem to be susceptible to clindamycin in standard tests but can develop resistance when exposed to macrolides. The correct interpretation of this phenomenon is essential for making informed decisions about antibiotic treatment, since misinterpretation could result in unsuccessful treatments.

- Developing novel antibiotics could be achieved by targeting the mechanism that causes attenuation, which would prevent ribosome stalling and block the activation of ermTR.

4. Experimental Findings and Clinical Implications

Isogenic Conditions Study

Oryaşın et al. (2020) investigated the impact of the *ermTR* gene on macrolides and lincosamides under isogenic conditions to clarify why ErmTR varies from other methylases. The researchers isolated *ermTR* from *Streptococcus pyogenes* C1 and then moved it to *S. pyogenes* NZ131, both instances occurring with and without its regulatory region intact.

MIC Determination

The results showed that ermTR, which is always expressed, provided low-level resistance to erythromycin at a concentration of 8 mg/L, but high-level resistance to clindamycin at a concentration of 128 mg/L (Oryaşın et al., 2020). The regulatory region's presence led to a substantial rise in clindamycin resistance under erythromycin induction, with resistance increasing from 4 mg/L to greater than 128 mg/L.

When interpreting the results of antibiotic susceptibility tests, it is essential to take into account both the inherent and acquired resistance that may be present. In healthcare environments, untreatable bacterial resistance can arise if it is not identified and managed correctly.

The distinct resistance characteristics of *ermTR*, most notably its influence on lincosamide resistance, have substantial clinical repercussions. The coexistence of low-level erythromycin resistance and high-level clindamycin resistance complicates traditional diagnostic approaches. There is a requirement for more advanced diagnostic methods, like molecular detection of *erm* genes or inducible clindamycin resistance testing, to inform the selection of suitable antibiotic treatments.

Double Disk Testing

The double disk test, also referred to as the D-test, is a significant diagnostic tool for identifying clindamycin resistance that can be induced in clinical isolates. The procedure entails positioning erythromycin and clindamycin disks in close proximity on an agar plate that has been inoculated with the test organism. A reduction or diminishment in the size of the clindamycin inhibition zone adjacent to the erythromycin disk (D-shaped) is indicative of inducible resistance.

Induction with erythromycin and azithromycin led to a decrease in the clindamycin inhibition zone for *S. pyogenes* transformed with *ermTR* and regulatory regions, but had no effect on telithromycin inhibition, according to Oryaşın et al. (2020). This provides additional evidence for the distinct induction profile of *ermTR*.

5. Environmental Factors and Resistance Spread

Antibiotic Pressure

Environmental factors have a substantial impact on *ermTR* gene expression in *Streptococcus pyogenes.* Antibiotics at levels below the minimum needed to inhibit bacterial growth can stimulate stress responses, causing an increase in ermTR and allowing bacteria to adapt rapidly (Motallebirad et al., 2021).

Recent studies indicate that sub-MIC antibiotic concentrations can selectively promote the growth of resistant bacteria. The "selective window" refers to a phenomenon that has significant implications for the dissemination of antibiotic resistance in environmental contexts (Wang et al., 2025).

Genetic Mobility

The spread of *ermTR* is accelerated by mobile genetic elements, such as plasmids and transposons, which speed up the widespread distribution of resistance characteristics (Varaldo et al., 2009).

Furthermore, MGEs carry genes that not only provide resistance to antimicrobial agents from other classes, but also to metals and biocides (Yang et al., 2024).

The horizontal transfer of erm genes, which include *ermTR*, can occur through processes like conjugation, transformation, and transduction. Genetic mobility enables the spread of resistance traits not only within a species but also between various bacterial species, thus facilitating the swift evolution and widespread dissemination of antibiotic resistance.

6.The unique characteristics of *ermTR*

Differential Resistance Levels

Two key differences set *ermTR* apart from other methylases: (1) the level of erythromycin resistance provided by constitutively expressed *ermTR* is relatively low at 8 mg/L in comparison to >128 mg/L for other methylases, and (2) erythromycin induction does not enhance resistance to erythromycin but does increase resistance to clindamycin (Oryaşın et al., 2020).

Methylation Mechanism

The level of macrolide resistance caused by *ermTR* is lower than the resistance profiles associated with alterations at position 2058 of 23S rRNA (Oryaşın et al., 2020). The distinction between the methylation mechanism or target site of *ermTR* and those of other extensively researched methylases may be significant.

Recent research into the structural composition has shed light on the erm-mediated resistance process. According to a 2021 study by Svetlov et al., dimethylating A2058 does not bring about substantial structural modifications to the ribosome, but rather impacts the binding of macrolides via interactions facilitated by water molecules.

7.Clinical Implications

The unique resistance characteristics of *ermTR* have substantial clinical implications, particularly in the treatment of infections resulting from *S. pyogenes* and other *streptococci*. The dual resistance phenomenon complicates treatment because first-line treatments such as erythromycin and alternatives including clindamycin may not be effective against strains positive for *ermTR* (Pinheiro et al., 2009).

The presence of *ermTR* in *Streptococcus agalactiae* (Group B Streptococcus) can result in clindamycin resistance that can be triggered, thereby increasing the risk of treatment failure in conditions like neonatal sepsis (DiPersio & DiPersio, 2007). The significance of precise detection and identification of *ermTR* in clinical samples is underscored by this point.

The existence of *ermTR* and other *erm* genes in clinical isolates requires prudent antibiotic management and the creation of innovative treatment approaches. Newer macrolides and ketolides, like telithromycin, have been developed in order to overcome several types of *erm*-mediated resistance.

8.Geographical Distribution and Prevalence

Research has demonstrated that the frequency and spread of *ermTR* can differ across different geographical areas. Zhou et al. (2014) found evidence of erythromycin-resistant genes such as *ermTR* in group A beta-hemolytic Streptococci in Chengdu, Southwestern China. Lo et al. (2015) examined the antibiotic resistance patterns and the mechanisms behind erythromycin resistance in erythromycin-resistant group G *Streptococcus dysgalactiae* subspecies equisimilis isolates from central Taiwan.

Regional variations underscore the necessity for localized surveillance and tailored antibiotic stewardship programs (Zakerifar et al., 2023). The spread of *ermTR* and other erm genes is influenced by factors like antibiotic usage patterns, healthcare practices, and environmental conditions.

9.Future Directions

Identification of Methylation Sites

Further investigation is required to precisely determine the methylation site of the methylase encoded by *ermTR* (Oryaşın et al., 2020). This could offer vital information about its distinct mode of operation and might lead to the creation of novel methods to counteract *ermTR*-mediated resistance.

Sophisticated techniques including cryo-electron microscopy and high-resolution X-ray crystallography can be utilised to elucidate the precise structural changes resulting from ErmTR methylation. These studies could expand upon the research by Svetlov et al. (2021) and lead to a more comprehensive understanding of the molecular basis of *ermTR*-mediated resistance.

Development of New Strategies

Comprehending the complex processes behind *ermTR*-mediated resistance is vital for creating novel antibiotics and implementing successful treatment plans to counteract the escalating problem of antibiotic resistance. The options may involve designing new medications that can counteract resistance or creating diagnostic equipment for the quick identification of *ermTR*-positive strains (Gygax et al., 2006; Gygax et al., 2007).

Advances in CRISPR-Cas9 and other gene-editing technologies offer promising strategies for combating antibiotic resistance. These approaches could be employed to target and inactivate erm genes or to alter the ribosomal binding sites of antibiotics in order to overcome resistance.

Combination therapies and the development of antibiotic adjuvants which can either inhibit Erm methyltransferases or boost the effectiveness of existing antibiotics against resistant bacterial strains are current research focuses.

10.Conclusion

The *ermTR* gene illustrates the intricacies of antibiotic resistance through a combination of genetic adaptation and environmental responsiveness. Macrolide resistance mechanisms are complicated by the antibiotic's unique properties, specifically its low-level resistance to erythromycin paired with high-level resistance to clindamycin (Oryaşın et al., 2020).

Svetlov et al. (2021) has greatly enhanced our comprehension of the molecular processes driving *erm*-mediated resistance, laying the groundwork for forthcoming drug development initiatives. Research on the part water plays in macrolide binding and the fact that dimethylated ribosomes show no substantial structural changes provides the basis for creating more targeted and logical drug treatments.

Antibiotic resistance remains a major health concern worldwide, underscoring the need for in-depth research into singular resistance mechanisms such as that mediated by *ermTR*. Comprehending the distinct properties of *ermTR* and its influence on both macrolide and lincosamide resistance is essential for creating innovative therapeutic approaches. Future studies should concentrate on explaining the exact molecular process of *ermTR*-mediated resistance, especially its varying impacts on macrolides and lincosamides. This discovery could lay the groundwork for the creation of new antibiotics that can counteract this resistance mechanism, possibly addressing both macrolide- and lincosamide-resistant strains at the same time.

The purpose of discovering and summarizing bacterial resistance is to prevent, control, and combat resistance effectively. This review highlights four promising strategies, including chemical modification of antibiotics, the development of antimicrobial peptides, the initiation of bacterial self-destruct programs, and antimicrobial stewardship, to fight against resistance and safeguard global health. By unraveling the intricacies of these adaptive bacterial responses, we can hope to stay one step ahead of the ongoing evolutionary arms race between bacteria and antibiotics.

The fight against antibiotic resistance requires a multifaceted approach, including continued basic research into resistance mechanisms, development of new antibiotics and alternative therapies, improved diagnostic techniques, and responsible antibiotic stewardship. The *ermTR* gene and its counterparts within the *erm* family represent crucial targets in antibiotic resistance research, with ongoing studies expected to provide valuable insights.

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CHAPTER 5

MİKROBİYAL BİYOFİLMLER: YAPISI, OLUŞUMU VE KLİNİK ÖNEMİ

ZELİHA SEYFİ ŞANDA[1]

Giriş

Biyofilmin ilk keşfi, 17. yüzyılda Antonie van Leeuwenhoek'ın kendi geliştirdiği mikroskobuyla, kendi dişinden aldığı örneklerde plaklar içinde yaşayan mikroorganizmaları tanımlamasıyla başlamıştır. Biyofilm kavramı zaman içinde farklı çalışmalarla şekillenmiş ve çeşitli biçimlerde tanımlanmıştır (Köremezli, Kariptaş & Erdem, 2022: 153). Günümüzde biyofilmler, mikroorganizmaların kendi ürettikleri hücre dışı polimerik madde (extracellular polymeric substance, EPS) içerisinde gömülü olarak yaşayan organize topluluklar olarak tanımlanmaktadır (Solano, Echeverz & Lasa, 2014: 96). Bu yapılar, endüstriyel su sistemleri, ekolojik su kaynakları, yerleşik tıbbi cihazlar ve canlı dokular gibi canlı veya cansız birçok yüzeyde oluşabilmektedir (Donlan, 2002: 881). Okyanus derinlikleri ve derin yeraltı suları dışında kalan tüm ekosistemlerde biyofilmin varlığı kabul edilmektedir (Donlan &

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Costerton, 2002: 167). Nemli yüzeylerde sıkça karşılaşılan biyofilm tabakası; endüstri, sağlık hizmetleri ve gıda sanayinde olmak üzere mikroorganizmaların çoğalabileceği her alanda önemli bir tehdit oluşturmaktadır. Özellikle sağlık sektöründe biyofilmler, antimikrobiyallere ve dezenfektanlara karşı gelişen direnç nedeniyle tedavisi güç klinik tabloların ortaya çıkmasına neden olmaktadır. Bu direnç tedavi sürecini zorlaştırmakta ve biyofilmle ilişkili enfeksiyonların kronikleşmesine zemin hazırlamaktadır (Kostakioti, Hadjifrangiskou & Hultgren, 2013: 2).

Biyofilm Yapısı

Biyofilm yapısı, saf bir mikroorganizma türünün oluşturduğu homojen biyofilmler şeklinde veya birden fazla türün bir araya gelmesiyle oluşan heterojen biyofilmler şeklinde gelişebilir. Heterojen yapı gösteren biyofilmlerde aynı türden olan bakteriler kendi aralarında bir araya gelerek, her tür kendi mikrokolonilerini oluşturur. Bu farklı mikrokoloniler arasında su kanalları bulunmaktadır. Bu sayede biyofilmin iç kısımlarına oksijen ve temel besin maddelerinin ulaşımı sağlanır. Doğada biyofilmler çoğunlukla heterojen yapıda bulunmaktadır. Bu organizasyon, biyofilmin yapısal stabilitesini korurken aynı zamanda antimikrobiyal ajanlara ve çevresel stres faktörlerine karşı daha dayanıklı hale gelmesini sağlamaktadır (Flemming & ark., 2016: 569-570; Gün & Ekinci, 2009: 166-167).

Biyofilm yapısının, %97 gibi büyük bir kısmını su oluşturmaktadır ve matriks yüksek oranda su içeren, iyonlarla yüklü bir mikroçevre olarak tanımlanmaktadır. Diğer bileşenleri ise, %2-5 mikroorganizma, %1-2 protein, %1-2 ekzopolisakkarit, %1-2 DNA ve iyonlar oluşturmaktadır (Yüksekdağ & Baltacı, 2013: 78).

Ekzopolisakkaritler

Biyofilmin ana ekstraselüler bileşimini oluşturan ekzopolisakkaritler, yapının fiziksel bütünlüğü ve stabilitesiyle

ilişkilendirilmiştir. Jelimsi ya da viskoelastik özellikler sergilemekle birlikte doğrusal veya dallanmış şeritler halinde hücrenin çevresinde karmaşık bir ağ yapısı göstermektedir. Bu yapı proteinler, kalsiyum iyonları ve çeşitli polisakkaritlerle daha stabil ve dayanıklı bir hal almaktadır. Biyofilmin bu mekanik dayanıklılığı, çevresel stres faktörlerine karşı koruyucu bir bariyer işlevi görmektedir (Gün & Ekinci, 2009: 166). Biyofilmler içerisinde en fazla bulunan ve incelenen ekzopolisakkaritlerden biri olan poli-N-asetil glukozamin (PNAG), özellikle gram pozitif patojenlerin biyofilm oluşumuyla yakından ilişkilendirilmiştir. PNAG, *Staphylococcus aureus* ve *Staphylococcus epidermidis* gibi mikroorganizmalarda hücreler arası yapışmayı kolaylaştırarak biyofilmin matriks bütünlüğünü sürdürmesinde kritik bir rol oynamaktadır (Cerca & ark., 2006: 4849). Benzer şekilde *Pseudomonas aeruginosa* da biyofilm oluşumunda aljinat adı verilen viskoz bir polisakkarit üretir. Özellikle kistik fibrozisli hastalarda olmak üzere kronik enfeksiyonlara yol açan aljinat, önemli bir virülans faktörü olarak rol oynamaktadır. Her iki türün oluşturduğu biyofilmler, hem konak bağışıklık yanıtını baskılayarak immün kaçışa katkıda bulunmakta hem de antibiyotiklere karşı direnç kazandırmaktadır (Rabin & ark., 2015: 495).

Proteinler

Biyofilm matriksinde yer alan proteinler, biyofilm oluşumunda ve çözünmesinde çeşitli roller oynamaktadır. Bu proteinler; hücrelerin yüzeylere bağlanmasını kolaylaştırmak, yapının mekanik stabilitesini sağlamak, üç boyutlu biyofilm mimarisinin gelişimini desteklemek ve hücreler arası etkileşimi düzenlemek gibi kritik süreçlerde görev alır. Ayrıca bazı matriks proteinleri, biyofilm matriksini parçalayarak biyofilm hücrelerinin serbest kalmasını ve böylece yeni bir biyofilm yaşam döngüsünün oluşturulmasını sağlar. Proteinler, bu çok yönlü işlevleriyle biyofilm

oluşumu, olgunlaşması ve dağılması süreçlerinin temel düzenleyici bileşenleri arasında yer almaktadır (Fong & Yildiz, 2015: 201-202).

Pseudomonas spp. biyofilmlerinde bulunan Fap (functional amyloid protein) amiloidleri, aşırı ekspresyon durumunda hücre agregasyonunu artırarak biyofilm oluşumunu destekleyen yapısal proteinlerdendir. Aynı şekilde TasA amiloid proteini, *Bacillus subtilis* biyofilmlerinin ana bileşenlerinden biridir. Bu protein, biyofilm hücrelerini bir arada tutan sağlam lifli yapılar oluşturur. *S. aureus'ta* tanımlanan Bap (biofilm-associated protein) ise hem yapısal hem de adezyon proteini olarak biyofilm oluşumu ve enfeksiyon süreçlerinde rol alır (Rabin & ark., 2015: 496).

Diğer adezyon proteinleri arasında yer alan FnBPA ve FnBPB, *S. aureus* kaynaklı fibronectin bağlayıcı proteinlerdir. Bu proteinler, bakterinin konak hücrelere tutunmasını kolaylaştırarak biyofilm oluşumunun başlangıcında kritik rol oynamaktadır (O'Neill & ark., 2008: 3841-3842). Benzer şekilde *P. aeruginosa*'da bulunan LecA ve LecB adlı lektin türü adezyon proteinleri, hücre-hücre etkileşimini destekleyerek matriksin yapısal organizasyonunun sürdürülmesine katkıda bulunur (Passos da Silva & ark., 2019: 2).

Biyofilm gelişiminde yapısal bileşenler kadar, çevresel yanıtların ve hücreler arası iletişimin koordine edilmesini sağlayan sinyal iletim ve düzenleyici proteinler de önemli yer almaktadır. Bu proteinler genellikle çoğunluğu algılama (Quarum sensing) sistemleri ve iki bileşenli düzenleyici mekanizmalar aracılığıyla çalışmaktadır. Bu mekanizmalara örnek olarak *S. aureus'*ta bulunan AgrA ve AgrC proteinleri gösterilebilir. Bu proteinler çevrede biriken sinyal moleküllerini algılayarak biyofilme özgü genlerin düzenlenmesinde görev almaktadır (Yarwood & Schlievert, 2003: 1620-1621).

Bazı biyofilm matriks proteinleri ise yapının çözülmesinde görev alarak hücrelerin serbest kalmasını ve yeni yüzeylere kolonize

olmasını mümkün kılar. Bu süreç biyofilmin yaşam döngüsünün son ancak aktif bir aşamasını oluşturur. *P. aeruginosa*'da ekspresyonu artan nükleazlar ve aljinat liyaz gibi enzimatik proteinler, ekstraselüler DNA'yı ve aljinatı parçalayarak biyofilmin dağılmasına katkıda bulunur (Flemming & ark., 2016: 568).

Hücre Dışı DNA (eDNA)

Hücre dışı DNA (Ekstraselüler DNA, eDNA), ekzopolisakkarit yapısında fazlaca bulunan, biyofilm oluşumunda yapısal ve işlevsel roller üstlenen önemli bir matriks elemanıdır. Yüzeye ilk tutunma aşamasında negatif yük taşıması sebebiyle başlangıçta itici bir kuvvet oluştursa da yüzeyle hücre arasındaki mesafe birkaç nanometreye ulaştığında substrat üzerindeki pozitif yüklü reseptörlerle etkileşime girerek adezyonu destekler. Bunun yanı sıra, eDNA'nın biyofilm yapısının genişlemesinde rol aldığı, hücresel hareketliliği koordine ettiği görülmüştür. Negatif yükü sayesinde eDNA, pozitif yüklü bazı antibiyotikleri bağlayarak mikroorganizmaların antimikrobiyal ajanlardan korunmasında rol almaktadır (Rabin & ark., 2015: 496; Das & ark., 2010: 3407).

Biyofilmin Oluşumu

Bakterilerin planktonik formdan biyofilm oluşturan yerleşik forma geçişini içeren karmaşık bir süreçtir. Büyük ölçüde çevresel koşulların elverişliliğine bağlıdır. Ortam sıcaklığı, pH düzeyi ve besin maddelerinin varlığı gibi değişken faktörler biyofilm oluşumunun başlaması ve devamlılığı açısından belirleyicidir. Uygun koşullar altında mikroorganizmalar yüzeylere tutunarak biyofilm gelişimini başlatır. Ancak besin maddeleri tükendiğinde ya da uygun çevresel şartlar sağlanamadığında, yüzeye tutunma zayıflar ve hücreler planktonik yaşam formuna geri döner (Köremezli, Kariptaş & Erdem, 2022: 154-155). Biyofilm oluşumu beş aşamada gerçekleşmektedir.

Dönüşümlü Tutunma

Biyofilm oluşumunun ilk aşaması olan dönüşümlü tutunma mikroorganizmaların bir yüzeye geçici olarak bağlandığı ve bu bağlanmanın geri döndürülebilir olduğu bir süreçtir. Bu aşamada bakteri hücreleri yüzeyle tam anlamıyla temas etmemekle birlikte yüzey ile arasında hidrofobik etkileşimler, elektrostatik ve Van der Walls kuvvetleri gibi hem çekici hem de itici güçler meydana gelmektedir. Bakteriyel hücre yüzeyleri ve çevresel yüzeylerin genellikle negatif yüklü olması sebebiyle elektrostatik etkileşimler daha çok itici güçlerdir. Bunun yanı sıra, özellikle Van der Waals kuvvetleri çekici etkileşim göstermektedir. Hidrofobik etkileşimler ise hücrelerin yüzeye ilk bağlanmasında önemli bir rol oynar ve yüzeye tutunma kapasitesini artırarak biyofilm oluşum sürecini başlatmada etkilidir. Bu aşamada EPS üretimi oldukça sınırlıdır. Biyofilm yapısının tam olarak gelişmediği bu evrede basit yıkama işlemleriyle hücreler yüzeyden kolayca uzaklaştırılabilir (Gün & Ekinci, 2009: 168). Sonuç olarak, dönüşümlü tutunma aşaması mikroorganizmaların yüzeyle ilk etkileşime geçtiği ancak henüz kalıcı bir biyofilm oluşturmadığı bir basamaktır.

Dönüşümsüz Tutunma

Mikroorganizmaların yüzeye kalıcı olarak bağlanmasını sağlayan bir süreçtir. Bu aşamada bakteriler yüzeyle daha kısa mesafeli ve güçlü etkileşimler kurarlar. Tutunmada dipol-dipol, iyon-dipol, iyonik ve kovalent bağların yanında hidrofobik ve hidrojen bağları gibi etkileşimler de rol oynamaktadır. Bakteriler, yüzeye kalıcı olarak bağlanabilmek için koruyucu ve yapıştırıcı bir bariyer olarak EPS üretirler. Aynı zamanda flagella, fimbria ve pili gibi yüzeye daha sıkı tutunmayı sağlayan yapıları da bu aşamada kullanırlar. Dönüşümsüz tutunma aşamasındaki bakterilerin yüzeyden uzaklaştırılması için basit yıkama işlemleri yetersiz kalır. Bu aşamada biyofilmi ortadan kaldırmak için kazıma gibi güç

gerektiren mekanik müdahaleler gerekmektedir (Poulsen, 1999: 322).

Kolonizasyon

Dönüşümsüz olarak bağlanan mikroorganizmalar çoğalmaya başlar ve bir araya gelerek mikrokolonileri oluşturur. Yüzeye bağlanan bakteriler birincil koloniyi oluştururken, aynı yüzey üzerinde farklı bakteri türlerinin de katılımıyla ikincil kolonilerin oluşumu görülür. Bu koloniler, matriks içinde büyümeye ve olgunlaşmaya devam ederek karmaşık bir topluluk yapısı oluşur (Köremezli, Kariptaş & Erdem, 2022: 155).

Mikrokoloni oluşması ile birlikte "Quorum sensing" adı verilen iletişim sisteminden gelen sinyaller ile mikroorganizmalar arası haberleşme başlar. Yüzeye tutunan her mikroorganizma, ortama özel sinyal molekülleri salgılayarak hücreler arası iletişimi sağlar. Yüzeye tutunmuş bakterilerin miktarı arttıkça, bölgedeki sinyal yoğunluğu da artar. Bu sistemle mikroorganizmalar çevrelerindeki popülasyonu algılar (Bayrakal & Baskın, 2018: 9).

Olgun Biyofilm

Tutunma ve mikrokoloni oluşum aşamalarından sonra kule şeklinde hücre tabakaları ve bu yapıların içerisinde de su kanalları oluşur. Bu aşamalardan sonra yapı üç boyutlu şeklini kazanır ve olgun biyofilm oluşur. (Kartal, Ekinci & Poyraz, 2021: 356).

Ayrılma

Biyofilm oluşum sürecinin son aşamasında, yapının belirli bir olgunluğa erişmesiyle birlikte bireysel hücreler veya büyük biyofilm parçaları yapıdan ayrılarak yeni yüzeylere yayılım gösterir. Küçük hücre grupları şeklinde ayrılma erozyon ve aşınma şeklinde gerçekleşebilir. Erozyon, biyofilm yüzeyi ile temas eden sıvının kayma kuvvetiyle meydana gelirken, aşınma ise biyofilm kaplı yüzeyin mekanik etkileşimler veya çarpışmalar sonucu zarar

görmesi sonucu gerçekleşmektedir. Büyük hücre grupları şeklinde ayrılma ise besin kaynaklarının azalması sebebiyle dökülme şeklinde gerçekleşir. Bir diğer ayrılma ise protozoaların bakterileri besin olarak tüketmesi ile oluşmaktadır (Yeşilkaya & Merey, 2024: 85).

Klinik Önemi

Biyofilm ile İlişkili Enfeksiyonlar

Mikrobiyal enfeksiyonların yaklaşık %65'inin biyofilm kaynaklı olduğu düşünülmektedir (Khalil & ark., 2022: 2). Özellikle immün sistemi baskılanmış bireylerde, uzun süreli tıbbi cihaz kullanımı gereken ya da kateteri olan hastalarda biyofilm varlığı klinik olarak ciddi seyreden enfeksiyonların gelişimine yol açabilmektedir (Lindsay & Von Holy, 2006: 318). Koagülaz negatif stafilokoklar, hastane enfeksiyonlarının önde gelen etkenleri arasında olup, yapay kalp kapakçıkları ve kateterler gibi biyomateryallerin yaygın kullanımı sonucunda biyofilm tabakası oluşumuyla karakterize enfeksiyonlarda belirgin bir artışa yol açmıştır (Vogel & ark., 2000: 139-140).

Klinik örneklerden kolonizasyon ve enfeksiyon etkeni olarak izole edilen bakterilerde biyofilm oluşturma yeteneğinin yaygın olduğu görülmüştür (Hortaç İştar, Alışkan & Başustaoğlu, 2020: 232). Endotrakeal entübasyonu takiben gelişen ventilatör ilişkili pnömoni, yüksek mortaliteyle seyreden tıbbi cihaz enfeksiyonlarından biridir. *P. aeruginosa* ve *Acinetobacter baumannii* gibi patojenler, endotrakeal tüplerin yüzeyinde biyofilm oluşturarak, uzun süreli kolonizasyon oluştururlar. Artan kolonizasyon süresi pnömoni gelişimi için risk oluşturmaktadır (Taner & ark., 2024: 4).

Biyofilmlerin klinik önemi yalnızca tıbbi cihazlarla sınırlı değildir. *P. aeruginosa*'nın neden olduğu solunum yolu enfeksiyonları, özellikle kistik fibrozis hastalarında biyofilm

oluşumuyla karakterize kronik enfeksiyonların tipik bir örnektir (Aydemir, 2018: 220).

Antibiyotik Direnci

Biyofilm yapısındaki mikroorganizmalar, serbest halde bulunan planktonik formlarına göre antimikrobiyal ajanlara karşı daha yüksek düzeyde direnç geliştirme eğilimindedir. Biyofilm ortamı horizontal gen transferi için elverişli bir alan sağlayarak direnç genlerinin yayılmasını kolaylaştırır. Bunun yanında biyofilmin yapısal özellikleri de direnç gelişimine katkıda bulunmaktadır. Biyofilm matriksinde bulunan polimerik maddelerin, antimikrobiyal molekülün biyofilm içine taşınma hızını veya antimikrobiyal materyalin matriks materyaliyle reaksiyonunu etkileyerek bir difüzyon bariyeri oluşturmaktadır. Bu sebeple antimikrobiyal ajanlar biyofilm yapısının tüm katmanlarına nüfuz edemez ve hedef bölgelere yeterli konsantrasyonda ulaşamaz. Ayrıca biyofilmin iç kısımlarında bulunan mikroorganizmaların bir kısmı besin maddelerine olan sınırlı erişimleri sebebiyle sıklıkla daha yavaş büyür, bu da onları hızla bölünen hücreler için üretilen antibiyotiklere karşı duyarsız hale getirir (Høiby & ark., 2010: 323-325).

Sonuç

Sonuç olarak; biyofilmler, karmaşık yapılarıyla ve aynı zamanda antimikrobiyal direnç, kronik enfeksiyon oluşumu ve zorlu tedavi süreçleriyle hem klinik hem de mikrobiyolojik açıdan dikkatle ele alınması gereken yapılardır. Bu nedenle biyofilm oluşumunun mekanizmalarının, yapısal bileşenlerinin ve dirence katkı sağlayan faktörlerinin anlaşılması, enfeksiyonların önlenmesi ve etkili tedavi stratejilerinin geliştirilmesi açısından önemlidir.

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CHAPTER 6

PROBİYOTİKLERİN İMMÜNOMODÜLATÖR ETKİLERİ: SEPSİS ÖNLEME VE TEDAVİSİNDEKİ YERİ

Necip Gökhan TAŞ[1]

Giriş

Probiyotikler, canlı mikroorganizmalar olup yeterli miktarda alındığında konak sağlığı üzerinde olumlu etkiler gösteren biyolojik ajanlardır. "Probiyotik" terimi ilk kez Lilly ve Stillwell tarafından 1965 yılında tanımlanmış, günümüzde yaygın olarak kullanılan anlamına ise Fuller'ın 1989'daki tanımıyla kavuşmuştur (Fuller, 1989; Lilly & Stillwell, 1965). Probiyotiklerin terapötik potansiyeli ilk olarak Nobel ödüllü bilim insanı Elie Metchnikoff tarafından 20. yüzyılın başlarında, fermente süt ürünleri tüketiminin insan ömrü üzerindeki olumlu etkilerini ileri sürmesiyle gündeme gelmiştir (Metchnikoff, 1907).

Modern mikrobiyoloji ve immünoloji literatüründe, probiyotiklerin gastrointestinal sistemde epitel bütünlüğünü koruma, konak immün yanıtını modüle etme ve patojenlerle rekabet etme gibi

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çok yönlü etkileri detaylı biçimde tanımlanmıştır (Ouwehand ve ark., 2002:279-289; Hill ve ark., 2014: 506-514). Bu mikroorganizmalar başta Lactobacillus, Bifidobacterium, Saccharomyces boulardii ve bazı Bacillus türleri olmak üzere, çeşitli türleri içermektedir. Son yıllarda yapılan deneysel ve klinik çalışmalar, probiyotiklerin sistemik inflamatuar yanıtların düzenlenmesinde de rol oynayabileceğini ortaya koyarak, bu ajanların sepsis gibi ciddi immün yanıt bozukluklarıyla ilişkili hastalıklarda potansiyel kullanımını gündeme getirmiştir (Hemarajata & Versalovic, 2013:70-73).

Sepsis, enfeksiyona karşı gelişen kontrolsüz immün yanıt sonucunda meydana gelen ve yaşamı tehdit eden çoklu organ disfonksiyonu ile karakterize bir klinik sendromdur (Singer ve ark., 2016: 801-810). Sepsis insidansı dünya genelinde artmakta olup, yüksek morbidite ve mortalite oranları ile sağlık sistemleri üzerinde ciddi bir yük oluşturmaktadır. Modern yoğun bakım uygulamalarına ve erken antibiyotik tedavisine rağmen, sepsis vakalarında klinik başarı sınırlı kalmakta ve özellikle dirençli mikroorganizmaların yaygınlığı bu durumu daha da karmaşık hale getirmektedir (Rudd ve ark., 2020: 200-211).

Bağırsak, hem mikrobiyal translokasyonun başlangıç noktası olması hem de sistemik inflamasyonun tetiklenmesindeki rolü nedeniyle sepsisin immünopatogenezinde merkezi bir konuma sahiptir (Fukuda ve ark., 2011: 543-547). Artmış intestinal permeabilite ve bozulmuş mikrobiyal denge, sepsis sürecini hızlandıran temel faktörler arasında yer alır. Bu doğrultuda, bağırsak mikrobiyotasını modüle eden ve epitel bariyer bütünlüğünü destekleyen probiyotiklerin, sepsis gelişimini önleme ya da hastalığın ilerleyişini yavaşlatma potansiyeli taşımaktadır (Shimizu, 2014: 1-5; Ewaschuk & Dieleman, 2006: 5941-5950).

Bu bölümde, probiyotiklerin immünomodülatör etkileri bağlamında sepsisin önlenmesi ve tedavisindeki potansiyel rolleri detaylı olarak ele alınacaktır. Deneysel modellerden elde edilen güncel veriler ışığında, probiyotiklerin immün sistem etkileşimleri, inflamatuar süreçlerdeki düzenleyici rolleri ve klinik kullanıma dair avantajları ve sınırlılıkları değerlendirilecektir. Ayrıca, gelecekteki araştırmalar açısından probiyotik temelli terapötik stratejilere yönelik önerilere de yer verilecektir.

Probiyotiklerin İmmünomodülatör Etkileri

Probiyotiklerin immünomodülatör etkileri, onların konak bağışıklık sistemiyle olan karmaşık ve çok yönlü etkileşimlerinden kaynaklanır. Bu mikroorganizmalar, bağırsak epiteli, mukozal immün hücreler ve mikrobiyal ürünlerle olan ilişkileri yoluyla hem doğuştan gelen (innate) hem de adaptif (kazanılmış) bağışıklık yanıtlarını etkileyebilir (Bron ve ark., 2011: 1714-1728).

Probiyotikler, öncelikle toll-like reseptörler (TLR'ler) ve nükleotid-bağlayıcı oligomerizasyon domain (NOD)-benzeri reseptörler gibi pattern recognition receptor (PRR) mekanizmaları aracılığıyla bağışıklık hücreleri tarafından tanınır (Lebeer ve ark., 2010:171-184). Bu tanıma süreci, dendritik hücreler ve makrofajlar başta olmak üzere çeşitli antijen sunucu hücrelerde sitokin üretimini tetikleyerek immün yanıtın şekillenmesinde önemli rol oynar.

Örneğin, bazı Lactobacillus rhamnosus suşlarının TLR2 üzerinden sinyal iletimiyle interlökin-10 (IL-10) gibi antienflamatuar sitokinlerin salımını artırdığı ve bu yolla immün toleransı desteklediği gösterilmiştir (Ng ve ark., 2009:300-310). Aynı şekilde, Bifidobacterium bifidum gibi türlerin, pro-inflamatuar TNF-α ve IL-6 düzeylerini azaltarak sistemik inflamasyonu modüle ettiği bildirilmiştir (Mohamadzadeh ve ark., 2005: 5132-5137).

Mukozal bağışıklık sisteminde, probiyotiklerin etkisi özellikle IgA üretimi, T regülatuvar hücre aktivasyonu ve Th1/Th2

dengesinin düzenlenmesi yoluyla ortaya çıkar. Probiyotikler, bağırsak lenfoid doku (GALT) üzerinden adaptif immün yanıtın şekillenmesine katkı sağlar. Bu etkileşim, yalnızca gastrointestinal hastalıklarda değil, sistemik hastalıklarda da bağışıklık dengesinin sağlanmasına yardımcı olabilir (O'Mahony ve ark., 2005: 541-551).

Ek olarak, bazı probiyotik suşları bağırsak epitel hücreleriyle doğrudan etkileşime girerek epitel bariyer bütünlüğünü güçlendiren zonulin, okludin ve klaudin proteinlerinin ekspresyonunu artırabilir. Bu etki, sepsis gibi durumlarda mikrobiyal translokasyonu ve sistemik inflamasyonu önlemeye katkı sağlayabilir (Anderson ve ark., 2010).

Deneysel sepsis modellerinde probiyotiklerin uygulaması sonucunda, sitokin profillerinde düzenlenme, bakteriyel yükte azalma ve organ hasarında gerileme gibi sonuçlar elde edilmiştir. Özellikle Lactobacillus plantarum ve Saccharomyces boulardii gibi türler, immün sistemin inflamatuar bileşenlerini baskılayarak çoklu organ disfonksiyonunun şiddetini azaltabilmektedir (Oliveira ve ark., 2016: 104-112).

Bu mekanizmalar bütüncül olarak değerlendirildiğinde, probiyotiklerin sepsiste yalnızca bağırsak mikrobiyotasını düzenlemekle kalmayıp, sistemik immün yanıtı da yönlendirebilecek potansiyele sahip olduğu görülmektedir. Ancak bu etkinin suş-spesifik olduğu ve her probiyotiğin aynı etkiyi göstermediği unutulmamalıdır. Bu nedenle, klinik uygulamalarda kullanılacak suşların moleküler profillerinin ve immünolojik etkilerinin ayrıntılı olarak karakterize edilmesi büyük önem taşımaktadır (Hill ve ark., 2014: 506-514).

Sepsisin İmmünopatogenezi ve Organ Hasarı

Sepsis, patojenlerle karşılaşıldığında aktivite kazanan doğuştan gelen bağışıklık sisteminin, dengeleyici mekanizmaların yetersizliği sonucu hiperinflamatuar bir tabloya sürüklenmesiyle

ortaya çıkan karmaşık bir immün yanıt bozukluğudur. Bu süreç, enfeksiyona yanıt olarak başlatılan normal immün yanıtın, hem pro-inflamatuar hem de anti-inflamatuar medyatörlerin dengesiz üretimi nedeniyle patolojik bir karakter kazanmasıyla sonuçlanır (Hotchkiss ve ark., 2016: 862-874).

Sepsis patogenezinde, lipopolisakkarit (LPS) gibi mikrobiyal yapılar, konak hücrelerinde yer alan Toll-like reseptör 4 (TLR4) aracılığıyla tanınır ve bu tanıma süreci sonucunda NF-κB, MAPK gibi sinyal yolakları aktive edilir. Bu aktivasyon, sitokin fırtınası olarak bilinen yoğun IL-1β, TNF-α, IL-6 üretimiyle sonuçlanır ve sistemik inflamatuar yanıt sendromuna (SIRS) yol açar (Takeuchi & Akira, 2010: 805-820). Hiperinflamatuar evreyi takiben, bağışıklık sisteminde immün paralizi olarak bilinen derin bir immünosupresyon dönemi gelişebilir. Bu evrede, lenfosit apoptozu, monosit disfonksiyonu ve antijen sunum yetersizliği gibi değişiklikler dikkat çeker (Delano & Ward, 2016: 23-31).

Organ disfonksiyonu, sepsisin en dramatik ve mortal yönlerinden biridir. Mikrovasküler düzeyde oluşan endotel disfonksiyonu, kılcal sızıntı, koagülopati ve doku perfüzyonunun bozulması, hipoksiye ve organ iskemisine zemin hazırlar. Karaciğer, böbrek, akciğer ve kalp en sık etkilenen organlar arasındadır. Örneğin, akut akciğer hasarı (ARDS), alveolo-kapiller membran bütünlüğünün bozulmasıyla gelişir ve yüksek mortalite oranlarına sahiptir (Bosmann & Ward, 2013: 129-136). Akut böbrek hasarı (AKI) ise renal peritübüler kapillerlerde azalmış perfüzyon ve inflamatuar hücre infiltrasyonunun bir sonucudur (Gómez ve ark., 2014: 3-11).

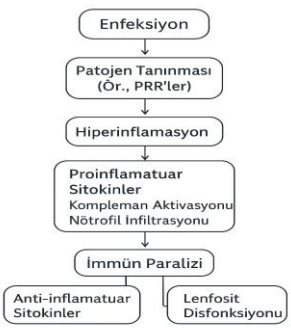
Bağırsak epitel bariyerinin bütünlüğünün bozulması da sepsisin progresyonunda kritik bir rol oynamaktadır. İnflamasyon, hipoksi ve apoptoz sonucu gelişen mukozal bütünlük kaybı, bağırsak kaynaklı mikrobiyal translokasyonu tetikler ve bu durum, sistemik inflamasyonu daha da körükleyen bir geri besleme döngüsüne neden

olur (Fukuda ve ark., 2011: 543-547). Bu noktada, bağırsak bariyer fonksiyonunu koruyan ve inflamasyonu sınırlayan biyoterapötik ajanların (örneğin probiyotikler) sepsis tedavisinde potansiyel katkı sağlayabileceği öne sürülmektedir.

Hayvan modellerinde yapılan çalışmalar, sepsisin immün yanıtı hem sistemik hem de doku düzeyinde derinlemesine etkilediğini ortaya koymuştur. LPS, CLP (cecal ligation and puncture) gibi modellerde pro-inflamatuar medyatörlerin ve oksidatif stres göstergelerinin artışı ile hücresel hasar belirginleşmektedir (Rittirsch ve ark., 2009: 31-36). İnsan verileri de bu bulguları desteklemekte; özellikle yoğun bakım hastalarında dolaşımdaki inflamatuar belirteç düzeyleri, hastalığın şiddeti ve mortalite ile doğrudan ilişkilidir (Singer ve ark., 2016: 801-810).

Sepsis, yalnızca enfeksiyonun neden olduğu akut bir sendrom değil; aynı zamanda immün disregülasyonun, endotel disfonksiyonunun ve organ düzeyinde inflamatuar yıkımın birleşimiyle oluşan kompleks bir tablodur. Bu nedenle sepsise yönelik terapötik müdahalelerin, yalnızca enfeksiyona değil; aynı zamanda inflamatuar dengenin yeniden sağlanmasına, endotel bütünlüğünün korunmasına ve organ disfonksiyonunun engellenmesine yönelik olması gerekmektedir.

Şekil 1 Sepsisin immünopatogenezi



Kaynak: (Hotchkiss ve ark., 2013; Takeuchi & Akira, 2010; Bosmann & Ward, 2013; Delano & Ward, 2016; Singer ve ark., 2016).

Deneysel ve Klinik Veriler Işığında Probiyotiklerin Sepsiste Kullanımı

Son yıllarda yapılan deneysel ve klinik çalışmalar, probiyotiklerin sepsis patogenezinde yer alan çeşitli immünolojik ve fizyopatolojik süreçleri modüle edebileceğine dair giderek artan kanıtlar sunmaktadır. Hayvan modellerinde ve sınırlı sayıda klinik çalışmada, belirli probiyotik suşlarının sistemik inflamasyonun şiddetini azalttığı, intestinal bariyer bütünlüğünü koruduğu ve mortalite oranlarını düşürdüğü gösterilmiştir (Zhang ve ark., 2022; Liu ve ark., 2023:56).

Deneysel Bulgular

Deneysel sepsis modellerinde (özellikle cecal ligation and puncture – CLP ve LPS indüksiyon modelleri) yapılan çalışmalar, Lactobacillus plantarum, Lactobacillus rhamnosus GG, Bifidobacterium longum ve Saccharomyces boulardii gibi suşların immün yanıt üzerinde düzenleyici etkiler oluşturduğunu göstermektedir. Bu suşlar, proinflamatuar sitokin düzeylerini azaltmakta (TNF-α, IL-6, IL-1β) ve antiinflamatuar sitokinleri (IL-10) artırarak konak savunma dengesini yeniden sağlamaktadır (Chen ve ark., 2022).

Ayrıca, probiyotik uygulaması ile intestinal mikrobiyota kompozisyonunda olumlu değişiklikler gözlemlenmiş; patojenik türlerin yerini faydalı anaerobik bakterilere bıraktığı, bu durumun da mikrobiyal translokasyonu azalttığı bildirilmiştir (Wang ve ark., 2021: 5692-5706). Aynı çalışmalarda, oksidatif stres belirteçlerinde azalma, doku düzeyinde apoptotik hücre sayısında gerileme ve çoklu organ hasarında düzelme gibi bulgular elde edilmiştir (Kong ve ark., 2023).

Klinik Bulgular

Klinik açıdan değerlendirildiğinde, probiyotiklerin sepsisli veya sepsise yatkın hasta gruplarında (örneğin yoğun bakım hastaları, neonatal sepsis riski taşıyan yenidoğanlar) kullanımı hâlâ tartışmalıdır ancak bazı sonuçlar umut vericidir. 2020 sonrası yapılan randomize kontrollü çalışmalarda, probiyotik kullanımının ventilatör ilişkili pnömoni insidansını azalttığı, gastrointestinal enfeksiyon oranlarını düşürdüğü ve bazı çalışmalarda hastanede kalış süresini kısalttığı rapor edilmiştir (Manzanares ve ark., 2021; Sharma ve ark., 2022: 1-5).

Bununla birlikte, klinik çalışmalarda dikkat çeken temel sınırlılıklar arasında çalışma tasarımlarındaki heterojenlik, kullanılan suşların çeşitliliği, doz ve uygulama süresinin standardize olmaması yer almaktadır. Ayrıca, immün baskılanmış hastalarda probiyotik kullanımına bağlı nadir de olsa bakteriyemi ve fungemi bildirimleri, bu tedavi yaklaşımının dikkatli ve hasta seçimine dayalı olarak ele alınması gerektiğini göstermektedir (Brooks ve ark., 2020).

Genel Değerlendirme

Genel olarak değerlendirildiğinde, probiyotiklerin sepsis sürecinde immün dengeyi yeniden sağlamaya yönelik etkili biyolojik ajanlar olabileceği öne sürülmektedir. Bununla birlikte, mevcut kanıtlar henüz büyük ölçekli klinik uygulamalar için yeterli değildir ve özellikle yüksek riskli hasta gruplarında daha güvenli ve standardize edilmiş protokollere ihtiyaç duyulmaktadır. Gelecekte yapılacak çok merkezli, iyi tasarlanmış klinik çalışmalar, probiyotiklerin sepsisteki rolünü daha net ortaya koyacaktır. Bu alandaki seçilmiş deneysel ve klinik çalışmalardan elde edilen temel bulgular Tablo 1'de özetlenmiştir.

Tablo 1 Sepsiste Probiyotik Kullanımına Ait Seçilmiş Deneysel ve Klinik Bulgular

Çalışma	Model/Hasta Grubu	Kullanılan Suş(lar)	Temel Bulgular	Yıl
Chen ve ark. (2022)	LPS ile indüklenen sepsisli fare modeli	*Lactobacillus rhamnosus* GG	IL-6 ve TNF-α azaldı, IL-10 arttı, çoklu organ hasarı hafifledi	2022
Kong ve ark. (2023)	CLP ile sepsis modeli (fare)	*Saccharomyces boulardii*	Bağırsak bariyeri korundu, epitel sıkı bağlantı proteinleri arttı	2023
Zhang ve ark. (2022)	LPS ile sepsis (fare)	*Lactobacillus plantarum*, *B. longum*	Sistemik inflamasyon azaldı, oksidatif stres göstergelerinde iyileşme	2022
Sharma ve ark. (2022)	Yoğun bakım hastaları (meta-analiz)	Çeşitli (çoklu suş kombinasyonu)	Sepsis riski ve hastane kalış süresi azaldı	2022
Manzanares ve ark. (2021)	Yoğun bakım hastaları (randomize çalışma)	Probiotik + prebiyotik (synbiotik)	VİP insidansı azaldı, inflamasyon düzeyleri düştü	2021

Kısıtlılıklar, Güvenlik ve Klinik Uygulamaya Entegrasyon

Probiyotiklerin sepsis gibi sistemik inflamatuar hastalıklarda potansiyel faydaları bulunsa da bu yaklaşımın rutin klinik uygulamaya geçebilmesi için dikkate alınması gereken çok sayıda sınırlılık ve güvenlik meselesi mevcuttur. Özellikle ağır hastalık durumlarında uygulanan probiyotik tedaviler hem etkinlik hem de güvenlik açısından dikkatli bir şekilde değerlendirilmelidir (Brooks ve ark., 2020; Liu ve ark., 2023: 56).

Suş-Spesifik Etki ve Doz Standartizasyonunun Eksikliği

Probiyotiklerin immünomodülatör etkileri büyük oranda suş-spesifiktir. Örneğin, Lactobacillus rhamnosus GG ile elde edilen olumlu etkiler, başka bir Lactobacillus türüyle replike

edilemeyebilir. Bu durum, hem klinik çalışmalardaki heterojenliğe neden olmakta hem de tedavi protokollerinin standardizasyonunu zorlaştırmaktadır (Hill ve ark., 2014: 506-514). Ayrıca optimal doz, uygulama süresi ve kombinasyonların belirlenmesine yönelik kanıtlar halen sınırlıdır.

Güvenlik Riskleri ve Bakteriyemi/Oportunistik Enfeksiyon Riski

Yoğun bakım hastaları, prematüre bebekler, kemoterapi veya immünsüpresif tedavi alan bireylerde probiyotiklerin nadiren de olsa fungemi (S. boulardii) veya bakteriyemi (Lactobacillus spp.) gibi komplikasyonlara yol açabildiği rapor edilmiştir (Venugopalan ve ark., 2010: 1661-1665). Bu nedenle, probiyotik uygulaması yapılmadan önce hastanın immün durumu ve bağırsak bariyer bütünlüğü mutlaka değerlendirilmelidir.

Klinik Çalışmalarda Yöntemsel Zorluklar

Sepsis gibi heterojen yapıya sahip hastalıklarda, klinik çalışma tasarısında homojen hasta gruplarının belirlenmesi ve klinik sonuçların objektif kriterlerle ölçülmesi önemli zorluklar yaratmaktadır. Ayrıca, plasebo kontrollü çalışmaların azlığı ve randomizasyon yöntemlerinin farklılığı, elde edilen verilerin karşılaştırılabilirliğini sınırlamaktadır (Sharma ve ark., 2022: 1-5).

Klinik Uygulamaya Geçiş İçin Gereken Koşullar

Probiyotiklerin sepsis tedavi rehberlerine entegre edilebilmesi için aşağıdaki kriterler önem arz etmektedir:

Klinik çalışmalarda yüksek kalitede randomizasyon ve körleme, spesifik suşlara ait etkinlik ve güvenlik profillerinin net olarak belirlenmesi, endikasyona özel risk grupları ve kontrendikasyonların tanımlanması, farmasötik ürün olarak kullanılan suşların genomik karakterizasyonunun yapılmış olması

Avrupa Gıda Güvenliği Otoritesi (EFSA) ve ABD Gıda ve İlaç Dairesi (FDA), probiyotiklerin "GRAS (Generally Recognized As Safe)" statüsünde olmasını güvenlik açısından zorunlu kılmakta; ancak sepsis gibi kritik hastalık tablolarında bu statü tek başına yeterli görülmemektedir (Sanders ve ark., 2019: 605-616).

Gelecek Perspektifi

Gelecekte, mikrobiyota temelli bireyselleştirilmiş tedavi yaklaşımlarının gelişimiyle birlikte, probiyotiklerin genetik profili ve hasta mikrobiyotasına göre seçilmesi mümkün olacaktır. Ayrıca, postbiyotikler ve paraprobiotikler gibi inaktif veya yapılandırılmış bakteriyel ürünlerin daha güvenli alternatifler olarak öne çıkması beklenmektedir (Zorzela ve ark., 2022)

Sonuç ve Genel Değerlendirme

Probiyotikler, bağışıklık sistemini modüle edebilme kapasiteleri ve bağırsak mikrobiyotasını dengeleyici etkileri nedeniyle, sepsis gibi sistemik inflamatuar durumların önlenmesi ve yönetiminde dikkat çeken biyoterapötik ajanlar haline gelmiştir. Gerek hayvan modellerinde gerekse erken dönem klinik çalışmalarda, belirli probiyotik suşlarının inflamatuar medyatörlerin düzenlenmesinde, bağırsak bariyer bütünlüğünün korunmasında ve sistemik immün yanıtın dengelenmesinde anlamlı etkiler sağladığı gösterilmiştir.

Bununla birlikte, mevcut veriler ışığında probiyotiklerin sepsis tedavisinde rutin klinik kullanımı için henüz yeterli düzeyde kanıt bulunmamaktadır. Suş-spesifik etkilerin varlığı, klinik protokollerde standardizasyon eksikliği, güvenlik ile ilgili endişeler ve çalışma tasarılarındaki heterojenlik, bu alandaki başlıca sınırlamaları oluşturmaktadır. Özellikle bağışıklık sistemi baskılanmış bireylerde nadir görülen fakat ciddi olabilen advers olaylar, hasta seçiminin önemini bir kez daha vurgulamaktadır.

Gelecek araştırmalarda, genetik ve metagenomik teknolojilerin kullanımıyla bireyselleştirilmiş probiyotik tedavi yaklaşımları geliştirilebilir. Ayrıca postbiyotikler ve paraprobiotikler gibi alternatif ajanlar, hem daha güvenli hem de daha kontrollü bir etki profiline sahip olmaları nedeniyle öne çıkmaktadır. Klinik karar vericilerin, bu ajanları sadece destekleyici değil, aynı zamanda hedefe yönelik immünomodülatör araçlar olarak değerlendirmesi, sepsis tedavisinde paradigma değişikliklerine yol açabilir.

Sonuç olarak, probiyotikler sepsise yönelik tedavi stratejilerinde henüz deneysel aşamada olmakla birlikte, potansiyelleri göz ardı edilemeyecek kadar önemlidir. Bu potansiyelin bilimsel temellere dayalı ve kontrollü bir şekilde değerlendirilmesi hem hasta güvenliği hem de klinik etkinlik açısından zorunludur.

Klinik Pratikte Probiyotik Kullanımına Yönelik Öneriler

Sepsis gelişme riski yüksek hasta gruplarında probiyotiklerin kullanımı dikkatli hasta seçimi, suş spesifikliği ve güvenlik önlemleri dikkate alınarak yapılmalıdır. Klinik kullanımı destekleyen veriler çoğunlukla önleyici (profilaktik) amaçlıdır; özellikle yoğun bakım hastalarında ve prematüre yenidoğanlarda gastrointestinal enfeksiyonların azaltılmasına yönelik çalışmalardan elde edilmiştir.

Öneri 1: Hasta Seçimi

Yararlanabilecek gruplar:

- Mekanik ventilasyonda olan erişkin yoğun bakım hastaları
- Prematüre veya düşük doğum ağırlıklı bebekler
- Yoğun antibiyotik tedavisi alan hastalar
- Gastrointestinal disbiyozis bulgusu olanlar

Kaçınılması gerekenler:

- Ağır immünsüpresyon (kemoterapi, neutropeni)
- Ciddi mukozal hasar (mukozit, kısa bağırsak sendromu)
- Şüpheli bakteriyemi/fungemi öyküsü

Öneri 2: Suş ve Dozaj Spesifikliği

- Klinik çalışmalarda en çok kullanılan suşlar:
- *Lactobacillus rhamnosus GG* (1–10 × 10^9 CFU/gün)
- *Bifidobacterium longum* (1–5 × 10^9 CFU/gün)
- *Saccharomyces boulardii* (250–500 mg/gün)

Öneri 3: Uygulama Süresi ve İzlem

- Uygulama süresi genellikle 7–14 gün arasında değişmektedir.
- Tedavi süresince ateş, enfeksiyon belirteçleri ve dışkı kültürleri ile izlem önerilir. (Liu ve ark., 2023: 56)

Tablo 2 Klinik Kullanım İçin Probiyotik Rehberi (Sepsis Riski Taşıyan Hastalar İçin)

Kriter	Öneri
Hasta Profili	VİP riski taşıyan, antibiyotik alan, bağırsak disbiyozisli ICU hastası
Kullanım Zamanı	İlk 24–48 saatte profilaktik başlama
Uygulama Yolu	Oral veya nazogastrik sonda
Süre	7–14 gün (klinik yanıta göre uzatılabilir)
Sık Kullanılan Suşlar	*L. rhamnosus GG*, *B. longum*, *S. boulardii*
Ortalama Doz Aralığı	10^9–10^{10} CFU/gün
Güvenlik İzlemi	Ateş, lökositoz, kültür takibi, transaminazlar
Kontrendikasyonlar	Ağır nötropeni, invaziv kateter enfeksiyonu şüphesi, akut fungemi

Kaynak: (Liu ve ark., 2023)

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CHAPTER 7

MATERNAL MICROBIOTA AND NEWBORN MICROBIOTA

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Intraductıon

Human and microbial genomes have evolved together over time, and their metabolisms and survival features are inseparably intertwined. The gut microbiota is formed by the collection of microorganisms consisting of bacteria, viruses and some single-celled eukaryotes. The microbiome is the sum of all microorganisms found in humans, including the genome, gene products and metabolites of microorganisms (Gomaa, 2020). Humans are a superorganism formed by the combination of 10%

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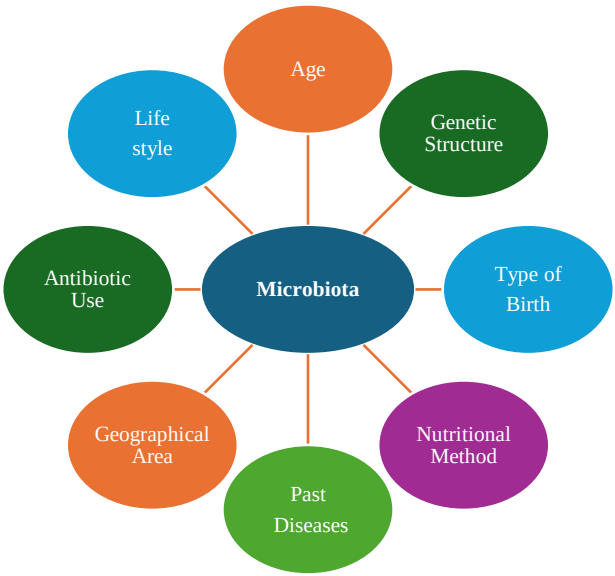
Produced from Gülsüm Kaya's doctoral thesis.

human and 90% microbial cells (Pascale et al., 2018; Yılmaz and Altındiş, 2017).

Intestinal microbiota, which is like a fingerprint, has characteristics specific to the individual as well as a common composition and distribution with other individuals. Microbiota changes depending on various endogenous and exogenous factors that change throughout the individual's life, such as geography, genetic structure, birth type, age, lifestyle, diet, antibiotic use and past diseases (Figure 1).

Figure 1. Factors affecting microbiota



For example, starting from infancy to old age, *Firmicutes* bacteria increase, while *Bacteriodetes* decrease. While high protein, red meat and animal fat consumption affects the Bacteriodes genus; carbohydrate-rich or vegetarian diets affect the *Prevotella* genus, and studies have shown that a diet rich in resistant starch affects the

Ruminococcus family. Antibiotic use causes temporary or permanent unhealthy microbiota changes in the microbiota, depending on the antibiotic group and the age period used (Gomaa, 2020; Pascale et al., 2018; Kalip & Atak, 2018).

The microbiota is formed by bacteria coming from the mother and the environment during birth (Kalip & Atak, 2018). Although it was accepted that the newborn flora was sterile until recently, some studies have shown that meconium microbiota is present. The bacteria in meconium are transmitted from the mother to the newborn through the mother's microbiota. This contributes to the formation of the newborn microbiota in the prenatal period (Blandino et al., 2016). The newborn encounters many microorganisms during birth, thus forming the gastrointestinal system microbiota. This microbiota, which is specific to the newborn, is affected by many internal and external factors. Factors such as the mother's diet, use of antibiotics and probiotics during pregnancy, the baby being born by elective cesarean section (CS), physiological stress and the absence of some hormones affect the microbiota (Chang et al., 2020; Turnbaugh et al. 2007). In newborns born via normal vaginal delivery (VD), the microbiota consists of the mother's genitourinary system microorganisms, while in newborns born via CS, the microbiota has been reported to be similar to skin flora microorganisms (Wei et al., 2019). In their study, Jakobsson et al. reported that CS birth reduced the diversity of the intestinal microbiome and resulted in lower Bacteroidetes abundance (Hollister et al., 2014).

The knowledge about the effects of infant gut microbiota colonization on health and disease in later life is rapidly increasing (Jandhyala et al., 2015). A blueprint for the final shape of the microbiota composition is established in early infancy. During this critical window in early life, commensal microorganisms interact

with the mucosal surface and are responsible for programming the immune system (Sekirov et al., 2010). While postnatal events are thought to have the greatest impact on microbiome formation, recent evidence has emerged that prenatal factors may also play a role in infant microbiome development (Kuzu, 2017). The intestinal colonization of the newborn is influenced by many perinatal factors, such as mode of delivery, type of feeding, gestational age, and newborn medication use (especially antibiotics) (Rajilić-Stojanović M de Vos, 2014). It is also thought that maternal antibiotic use affects this neonatal colonization process (Lagier et al., 2015).

Maternal Microbiota and Neonatal Microbiota

Indications that environmental influences may have an effect on immune regulation, host susceptibility to disease, and microbiota in early life are beginning to appear in epidemiological studies. Researchers have reported a positive correlation between higher and more hygienic living conditions in industrialized countries and increased rates of autoimmune and allergic diseases due to the creation of a less microbially rich environment (Bach, 2002). For example, it has been shown that allergic asthma is prevented in children growing up on farms with a microbially rich environment, and this effect is long-lasting (Ege et al., 2011; Eriksson et al., 2010). However, optimal nutrition is very important for both mother and fetus throughout pregnancy. Disruptions in the neonatal window of opportunity in early life put the fetus at risk of developing many chronic diseases such as metabolic syndrome, type 2 diabetes, coronary heart disease, adiposity and osteoporosis (Gluckman et al., 2008; Fleming et al., 2018). The microbiota is the main key factor in the window of opportunity period. Early colonization by a microbial consortium during this period is crucial for the healthy development of the immune system (Cahenzli et al., 2013; Hornef & Torow 2020). Studies in mice have shown that the

window of opportunity closes around the time of weaning. It is known that a stable microbiota composition is formed by the age of 2-3 in humans. However, there is no published data yet to evaluate when the window of opportunity closes in humans. While the immune system in children matures predominantly in the first few years after birth, it continues to strengthen and shape in later childhood. Observational studies based on epidemiological correlation have turned into experimental controlled studies. This evidence-based research has yielded mechanistic insights into how events during this critical neonatal window impact the long-term health of the host. The window of opportunity opens not only after birth but also during pregnancy, with a variety of effects on the developing fetus.

Maternal Microbiota

Maternal microbiota affects the development of the baby in the prenatal and postnatal periods (Mesa et al., 2020). In a study investigating the effect of the maternal microbiota on the baby's immunity during pregnancy, it was reported that maternal intestinal strains were more persistent in the baby's intestine compared to other strains and their adaptation was better (Nyangahu & Jaspan 2019). It is known that many physiological changes occur in the mother's body during pregnancy. However, there is evidence that the microbiota content changes during pregnancy (Mesa et al., 2020). In a study examining the reshaping of the intestinal microbiome and metabolic changes during pregnancy, it was stated that there was a difference between the first trimester and third trimester microbiotas of 91 pregnant women, an increase in Proteobacteria and Actinobacteria and a loss of richness, but gene diversity did not change (Nyangahu & Jaspan 2019). In a study investigating the temporal and spatial changes in human microbiota during pregnancy, it was determined that microbiota diversity remained relatively similar during pregnancy (Koren et al., 2012).

In a systematic analysis of the intestinal microbiota of pregnant women and their correlations with individual heterogeneity, conducted with the participation of 1479 pregnant women, it was shown that individual heterogeneity was the main factor shaping the intestinal microbiota during pregnancy (Yang et al., 2020).

Since gut microbiota plays an important role in physiological processes during pregnancy, recent studies have focused on the relationship between maternal gut microbiota and pregnancy complications. Recent studies have shown that maternal gut microbiota is associated with gestational diabetes mellitus (GDM), hypertensive disorders in pregnancy (HDP), and preterm birth. In addition, recent evidence has shown that gut microbial dysbiosis affects Th1/Th17 cytokine levels, thereby leading to recurrent miscarriage (Liu et al., 2021; Lu et al., 2024).

Factors affecting the microbiota during pregnancy can be listed as maternal diet, gestational age, pre-pregnancy body mass index of the mother, body weight gain during pregnancy, use of antibiotics and probiotics, diseases of the mother before or during pregnancy (gestational diabetes mellitus, liver diseases, etc.), mood of the mother and other factors (Edwards et al., 2017; Grech et al., 2021). Factors such as gestational diabetes mellitus, maternal obesity, Western-style diet, maternal disease in the mother cause maternal and neonatal dysbiosis (Stanislawski et al., 2017). In the postnatal period, the mother's microbiota continues to form the baby's microbiota through breast milk (Nyangahu & Jaspan 2019). It has been reported that Lactobacillus and Bifidobacterium species found in breast milk are useful probiotics and some subspecies of these microorganisms are also effective against pathogens such as Escherichia coli 0157 H7, Staphylococcus epidermidis and Listeria monocytogenes (Lyons et al., 2020).

Placental microbiome

The adaptations of the maternal body during pregnancy affecting all organs and systems, and the development of the placenta, a highly specialized organ that ensures anatomical separation of the fetus and the mother to prevent maternal immunogenicity towards the fetus, are particularly noteworthy. This highly complex organ ensures maternofetal exchange of molecules, including those originating from the maternal microbiota. Recently, the possible existence of a placental microbiome has become a topic that has attracted the attention of researchers (Ganal-Vonarburg et al., 2020).

The development of the microbiota begins long before the baby is born. Contrary to previous belief, amniotic fluid is not sterile (DiGiulio, 2012; DiGiulio et al., 2008). Studies on the microbial community of the placenta have shown the presence of bacteria during term and preterm labor (Rautava et al., 2012; Stout et al., 2013). Bacteria have also been isolated from umbilical cord blood, meconium, and amniotic fluid. In some cases, the presence of bacteria in amniotic fluid is associated with disease status. Mycoplasma and Ureaplasma in amniotic fluid are common health problems associated with diseases such as chorioamnionitis, preterm labor, and necrotizing enterocolitis (NEC) (Kwak et al., 2014; Goldenberg & Culhane 2003). However, women with vaginal infections have a much higher risk of premature birth (Menon et al., 2011). Bacteria are also frequently detected in the amniotic fluid and placentas of healthy full-term infants (Koenig et al., 2011; Hill et al., 2011). Other phyla detected in the amniotic fluid and placenta overlap with the phyla Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Fusobacteria, which are commonly found in the oral microbiota (DiGiulio, 2012; Aagaard et al., 2014). In addition, in mouse experimental studies, genetically labeled E. faecium was administered orally to pregnant mice and

then isolated from amniotic fluid and meconium cultures (Jiménez et al., 2005; Jiménez et al., 2008). Subsequently, many studies have been conducted indicating the existence of a placental microbiome.

Along with these studies, in 2014, Aagaard and colleagues performed 16S sequencing on human placenta samples and this issue became a topic of discussion again with the detection of a microbial community (Aagaard et al., 2014). Harvey J Kliman pointed out in his study that the detection of DNA alone does not provide evidence for the presence of living microbes (Kliman, 2014). Over time, it became clear that contamination problems and problems with the test kit's own microbiome (pseudo-chitome) pose great difficulties in the search for a living microbiota in the placenta (Salter et al. 2014; Olomu et al., 2020). To address these issues, researchers have made more careful evaluations in the processes of controlled progress at every step of the process, including tissue samples from CS birth to prevent contamination at birth, combining high-throughput DNA sequencing with qPCR and bacterial culture, comparing bacterial taxa found in the immediate vicinity (e.g., the processing room where the samples were studied), and removing taxa overlapping with the kitome. Even with these precautions, researchers could not detect the placental microbiome (Olomu et al., 2020; Theis et al., 2019). Along with these studies and problems, a recently published article reported the detection of bacterial DNA and live bacteria in the fetal intestine using 16S rRNA gene sequencing, qPCR, electron microscopy, and bacterial culture (Rackaityte et al., 2021). In this context, the presence of microbiome in the placenta is still a controversial issue.

Metabolites from commensal bacteria are transported via the placenta. The intestinal maternal microbiota plays an important role in this maternofetal molecular transfer and modulates fetal development (Macpherson et al., 2017). An important noteworthy point is the observation that a healthy pregnancy causes changes in

the microbiota composition that resemble a dysbiotic association. However, these stimulations are physiological during the pregnancy process, which has unique needs and requirements (Martino et al., 2022; Sato et al., 2019). For example, the SCFA producer Faecalibacterium decreases in abundance during the last trimester of pregnancy. This decrease in Faecalibacterium has also been observed in populations with metabolic syndrome (Haro et al., 2016). In general, pregnancy has been shown to be associated with a decrease in microbial diversity and richness, an overall increase in Proteobacteria and Actinobacteria, and an increase in bacterial load (Koren et al., 2012). This change in the microbiota of pregnant women is attributed to adjustments in dietary habits accompanied by changes in the bacterial metabolite pool to fully support the development of the fetal immune system. A fiber-rich diet during pregnancy protected the offspring against the onset of asthma, probably through acetate-mediated inhibition of histone deacetylase 9 (HDAC9), resulting in higher gene transcription of Foxp3 in Tregs. They further reduced the frequency of eosinophils and macrophages in blood and bronchoalveolar lavage fluid and serum IgE levels of the infants (Thorburn et al., 2015).

Neonatal Microbiota

Although it is generally thought that the development of microbiota begins at birth, there are also studies showing the presence of microorganisms in structures such as the placenta (Sarkar et al., 2021; Liang et al., 2018). It has been reported that bacteria grow in meconium samples collected from newborns born via CS or VD within the first 2 hours after birth, and thus the theory that the fetus may not be sterile has been proposed (Jiménez et al., 2008). In another study, amniotic fluid, placenta, colostrum, meconium and mother-baby stool samples were collected from mothers and newborns born at term via CS delivery, and microbiota analysis was performed using culture, PCR (polymerase chain

reaction) and rRNA sequencing methods. As a result of the analyses, similarities were observed between the microbiota detected in the placenta, amniotic fluid and neonatal meconium, and it was suggested that the intestinal microbiota colonization process begins before birth (Collado et al., 2016). It was thought that microorganisms could be transferred vertically from the mother through the circulatory system, via the vagina and/or urine, through intercellular permeability and/or dendritic cell transport (Milani et al., 2017).

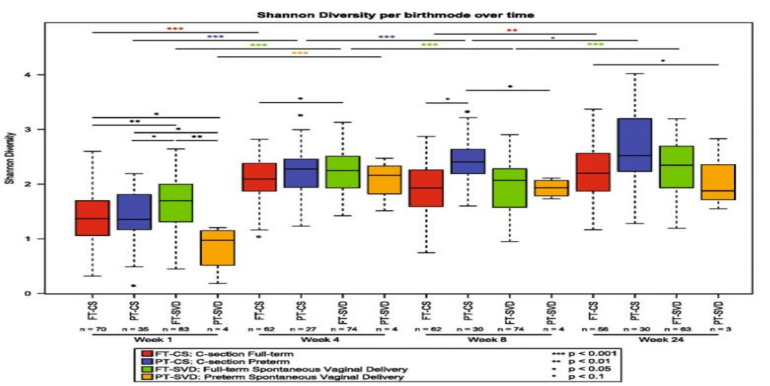
Although the newborn's intestinal microbiome completes its development in the first 1000 days of life, it undergoes changes due to various factors during this process, and it is known that there are many factors that affect the microbiota. Factors such as delivery method, exposure to antibiotics during pregnancy or infancy, maternal diet, breastfeeding or formula feeding, and transition to solid food are known to affect the microbiota in the first days of life, while the host genetic background is estimated to constitute only approximately 9% of the intestinal microbiota (Bäckhed et al., 2015; Dierikx et al., 2020; García-Mantrana et al., 2020; Ma et al., 2020).

Bifidobacteria, which are found in high amounts in the neonatal microbiota, are the main members of the microbiota, but the bacterial diversity of the microbiota is low, unstable and dynamic (Arrieta et al., 2014; Bergström et al., 2014). 80 subspecies of *Bifidobacteria* belonging to the *Actinobacteria* phylum have been identified. The subspecies of Bifidobacterium specifically identified in human intestinal microbiota profiles are *Bifidobacterium bifidum*, *Bifidobacterium longum* and *Bifidobacterium breve*, and they are predominantly present in the intestinal microbiota of breast-fed newborns (Duranti et al., 2017; Duranti et al., 2019). However, other species that are frequently seen besides *Bifidobacteria* are *Streptococcus, Veillonella,*

Escherichia, Citrobacter, Bacteroides and *Clostridium* (Turroni et al., 2020; Vallès et al., 2014).

The diversity of intestinal microbiota increases in direct proportion to the growth week of the baby. The microbiome of the baby up to the age of three can be clearly distinguished from an adult microbiome by a lower diversity index reflected in operational taxonomic units (OTUs) and higher interindividual variability, which is half that of adults (Yatsunenko et al., 2012; Koenig et al., 2011). The type of birth (CS/VD) of the newborn and whether it is born term or preterm also affect the increase and content of microbiota diversity. The diversity of the community in a sample is defined as Alpha diversity and is measured by the Shannon index. In a cohort study investigating the development of intestinal microbiota from birth to 24 weeks, the results of the change in Alpha diversity of the infant microbiota between 1-24 weeks and the Shannon index according to the type of birth and the term/preterm status of the baby are shown in Figure 2 (Hill et al., 2017).

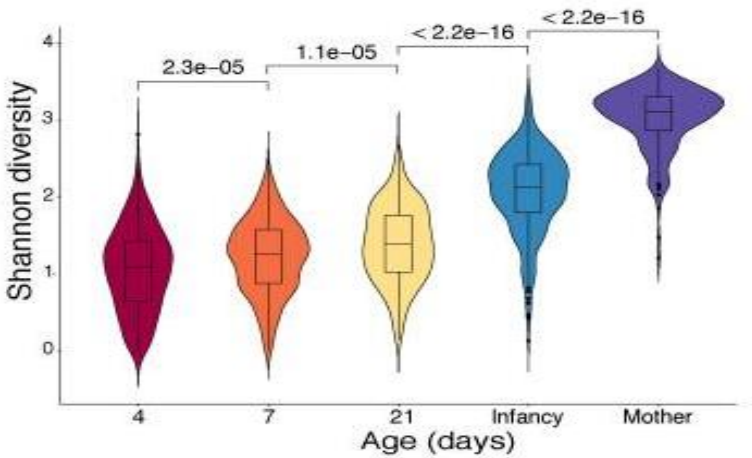
Figure 2. *Changes in Alpha diversity of infant gut microbiota between 1 and 24 weeks after birth according to age and delivery type.*



Hill et al., 2017

In the study conducted by Shao and his colleagues examining 771 term newborns, stool samples were taken from the babies on the 4th, 7th and 21st days and 4-12 months after birth, and stool samples were collected from the mothers just before or immediately after birth to investigate the changes in their microbiota with the Shannon index. As a result of the study, the researchers reported that the Alpha diversity of the microbiota increased throughout the developmental process of the baby (Figure 3) (Shao et al., 2019).

Figure 3. *Comparison of infant and maternal microbiota with Shannon index on postpartum days 4-7-21*

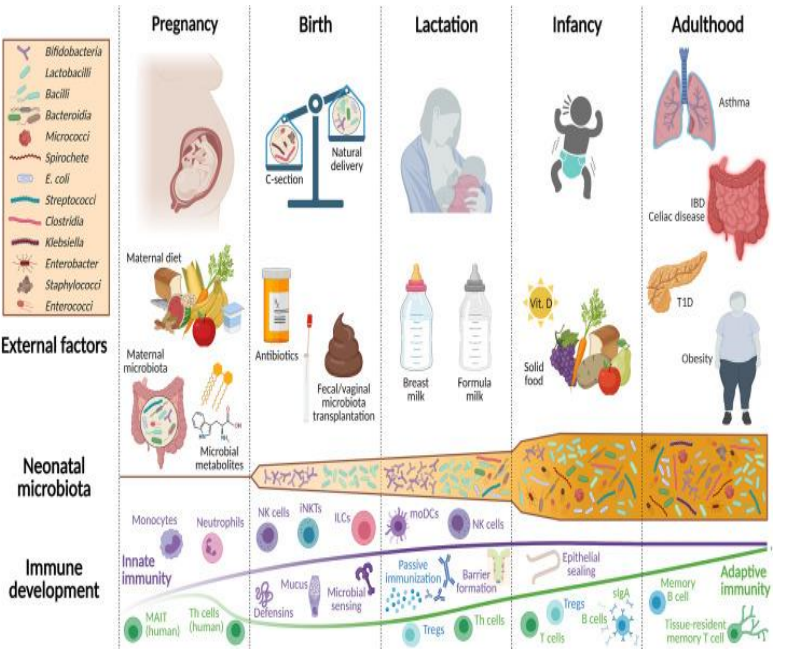


Shao et al., 2019

The dominant bacterial taxa in the microbiota of a newborn infant in the first weeks of life are *Enterococcacae, Clostridiaceae, Lactobacillaceae, Bifidobacteriaceae,* and *Streptococcaceae. Bifidobacteriaceae* develop in the newborn microbiota because they are fed with oligosaccharides abundant in breast milk, which is the main energy source of newborn infants in the first months of life. During

weaning, when solid foods are introduced, the abundance of *Bifidobacteriaceae* in the microbiota decreases, while *Bacteroides*, *Ruminococcus*, and *Clostridium* become more prevalent (Milani et al., 2017) (Figure 4).

Figure 4. *Environmental factors shaping the development of the neonatal microbiota and mucosal immune system*



Kalbermatter et al., 2021

The infant gut undergoes significant developmental stages that depend entirely on microbial colonization from birth onwards. The direct feeding from the mother's skin, the constant putting of hands, feet and other objects in the mouth, and especially the crawling and walking stages with hands touching surfaces at an early age during the first 3 years of life promote significant exposure to microorganisms. Furthermore, children are more likely to contract infectious diseases than adults. Not surprisingly, the

microbiota in children under the age of 3 years fluctuates greatly and is more susceptible to environmental factors than the adult microbiota (Koenig et al., 2011). Modern lifestyle changes, including improved environments, CS birth, antibiotic use and immunization, are among some of the factors that may alter the microbiota and are being studied as potential drivers of the sudden increase in immune-related diseases in the developed world. It has been postulated that there is a "critical window" in early life during which the microbiota may be disrupted in a way that may favor disease development later in life (Penders et al., 2007). Antibiotic treatment only in the perinatal period has been shown to cause a more severe disease phenotype in an animal model of asthma (Russell et al., 2012)

Meconium Microbiota

Meconium is not sterile, supporting the idea that microbes in amniotic fluid have access to the unborn fetus. Ardisson et al compared the meconium microbiota of preterm infants with separate datasets of amniotic, vaginal, and oral cavity microbiota and found that most of the overlap between meconium was due to the amniotic datasets (Hu et al., 2013; Ardissone et al., 2014).

Bacterial taxa isolated in meconium using culture-dependent and culture-independent approaches overlap with the adult gut microbiota. *Enterobacteriaceae* (including *Escherichia coli* and *Shigella spp.), Enterococci, Streptococci, Staphylococci* (including *Staphylococcus epidermidis*) and *Bifidobacteria* have been detected in healthy term infants (Ardissone et al., 2014; Tuddenham & Sears 2015). Jimenez et al. administered *Enterococcus faecium* to pregnant rats and isolated the same bacteria as CS in the meconium of term pups shortly after birth (Jiménez et al., 2008). Thus, while exposure to pathogenic vaginal microbes can be considered infectious events, prenatal exposure to fecal microbes is likely a

natural part of in utero development. How these microbes gain access to the maternal uterus is unknown, although bacterial translocation from the gut to the bloodstream and from there to the maternal uterus is a theory that has been proposed but has not yet been tested experimentally (Funkhouser & Bordenstein, 2013).

Conclusion

A healthy maternal microbiota is essential for infant health and development. The composition of the maternal gut microbiota is relatively stable during the progression of pregnancy and lactation. Individual heterogeneity is a major factor shaping the maternal gut microbiota. During pregnancy, maternal microbial metabolites may be transported across the placenta to the fetus to regulate immunity and recognition.

Microbial colonization of newborns begins at birth. The transfer of microbes from mother to newborn is influenced by many factors, including the route of delivery and breastfeeding. Vaginal delivery, breastfeeding, and skin-to-skin contact are considered to be the dominant factors in establishing the close relationship between the mother and infant microbiome. Maternal microbes originating from the maternal gut but not the vagina mainly contribute to the early colonization of the microbiome. Disruption of microbiome transfer from mother to child may lead to short-term and/or long-term adverse health outcomes.

Consequently, further studies are needed to determine the important roles that the maternal gut microbiota plays in the health of mothers and offspring, as well as other important microbial factors that play a role in regulating fetal immunity and infant development.

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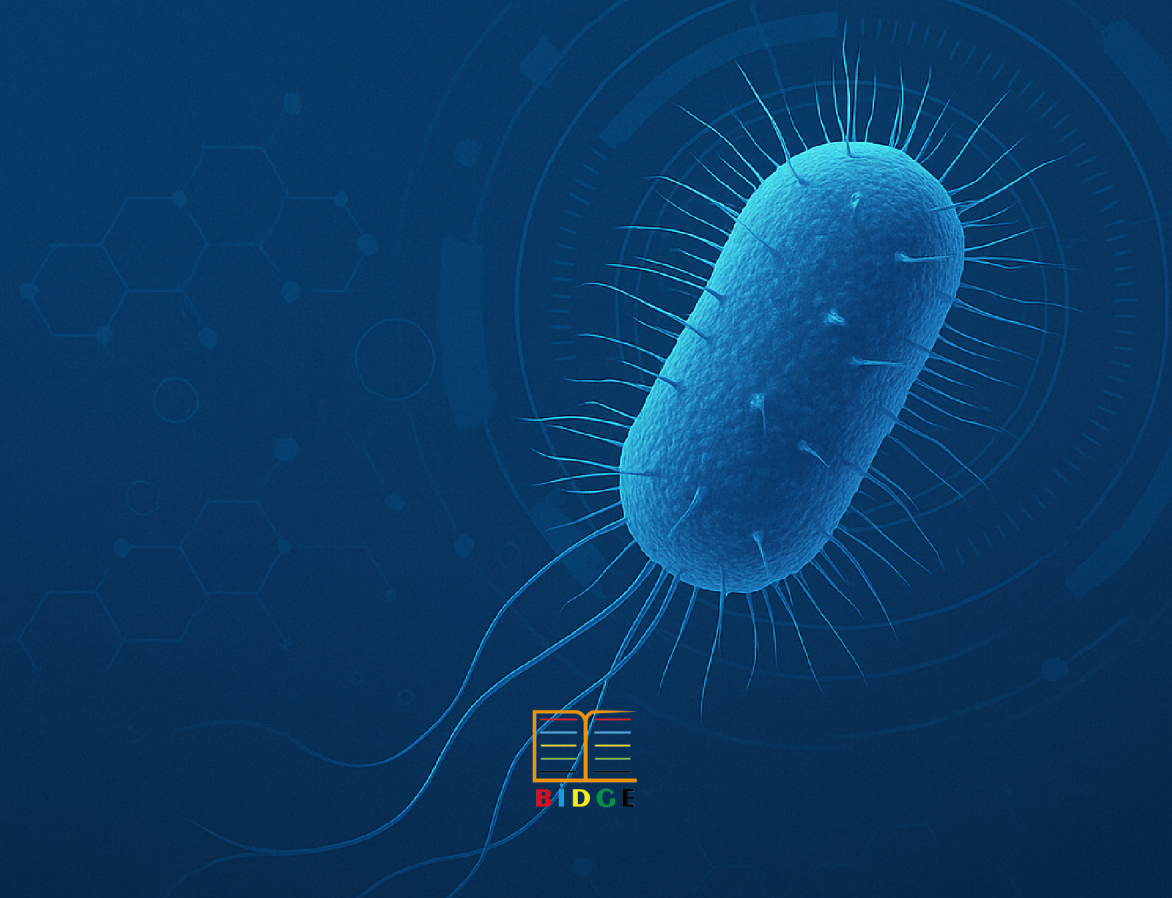
Integrating Clinical Insight with Molecular and Applied Methodologies



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