CURRENT APPLICATIONS AND FUTURE PERSPECTIVES IN PHARMACEUTICAL MICROBIOLOGY

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Pharmaceutical microbiology is one of the most critical intersections of biomedical and industrial sciences, directly protecting public health by ensuring the safety, efficacy, and quality of drugs and biological products. Microbiological controls carried out at every stage of the drug production process, from raw materials to the final product, form the indispensable foundation of patient safety.

For centuries, traditional culture methods, sterility tests, and biochemical identification techniques have served as the industry's gold standard. However, in today's pharmaceutical industry, cell therapies, personalized medicines, complex biotechnological drugs, and rapidly changing global health needs require higher analytical speed, sensitivity, and accuracy than ever before in microbiological analyses. The long incubation periods required by traditional methods, the limitations in detecting live but non-culturable microorganisms, and human-factor risks associated with manual evaluations have led the pharmaceutical microbiology industry to adopt more innovative and data-driven approaches.

This work has been written to address this major transformation in pharmaceutical microbiology from a holistic perspective. Throughout the book, starting with the historical and methodological foundations of traditional microbiology practices, the integration of Artificial Intelligence (AI), Machine Learning

(ML), and Deep Learning (DL) algorithms into microbiological data analysis is examined in detail. Furthermore, the role of next-generation analytical approaches such as MALDI-TOF Mass Spectrometry, Next-Generation Sequencing (NGS), Microfluidic Systems, Flow Cytometry, and Biosensor-Based Continuous Monitoring Technologies in sterility testing, environmental monitoring, contamination source tracking, and antimicrobial resistance (AMR) prediction is presented in light of scientific data.

The main goal of this book is not only to introduce existing analytical technologies and algorithms but also to shed light on validation, data reliability, and regulatory processes involved in integrating these technologies into Good Manufacturing Practices and quality assurance systems.

This book will serve as a reliable guide and source of inspiration for pharmaceutical researchers, quality control specialists, clinical researchers, academics, and students in this field. We express our gratitude to all the researchers who push the boundaries of science and technology to contribute to a safer future, and to everyone who helped make this work a reality.

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CHAPTER 1:
INTRODUCTION

Pharmaceutical microbiology is a branch of science that studies microorganisms and the production of drugs. It controls the safety and efficacy of drugs by examining microorganisms such as bacteria, fungi, viruses, and parasites in the drug production process (Haider, 2024). In recent years, with the increase in biological products, cellular therapies, and personalized medicines, the accuracy, speed, and sensitivity of microbiological analyses have become increasingly important (Rawson, 2021).

Traditional microbiological methods have been used for many years as a reliable and standard method for species identification and antimicrobial susceptibility testing (AST). The lengthy nature of traditional microbiological methods presents a disadvantage for emergency situations (Weis, 2020).

Traditional drug development methods are generally slow due to extensive screening processes and repetitive optimization steps. This often results in negative outcomes due to high costs, time loss, toxicity, and insufficient clinical efficacy. To prevent these negative outcomes, data-driven methods are targeted in the pharmaceutical industry to ensure research efficiency and the accuracy of therapeutic methods (Tiwari, 2023). Artificial

intelligence (AI) is a broad scientific discipline, rooted in mathematics, philosophy, and computer science, that aims to understand and improve algorithms and systems that exhibit intelligence (Panch, 2018). AI helps us reduce error rates, lower costs, and save time (Alowais, 2023). The use of AI in microbiology accelerates the classification and analysis of microorganisms, enabling drug discovery, pathogen detection, and antimicrobial resistance (AMR) detection (Microbiome, 2019). The role of AI in pharmaceutical microbiology increases the use of machinery in drug production, enabling the rapid resolution of microbial contamination and quality problems (Reddy, 2023).

AI provides significant advantages in processes such as drug targeting and vaccine prediction, identification of disease-causing microorganisms, antimicrobial resistance classification, outbreak prediction, analysis of microorganism interactions, quality assurance, bacterial identification, and hygiene control (Reddy, 2023; Pena-Guerrero, 2021).

Compared to traditional methods, artificial intelligence aims to rapidly develop vaccines in cases of antimicrobial resistance (AMR) and global pandemics (El Arab, 2025).

In the field of microbiology, machine learning (ML) plays a significant role in research on microbial behavior and diseases by learning from and analyzing data, offering different approaches to microbial analysis (Kavvas, 2018; Baldi, 2018). ML aims to solve computational problems such as supporting vaccine development processes, developing new drugs, identifying microorganisms that cause infectious diseases and predicting outbreaks, and classifying antibiotic resistance (Goodswen, 2021; Camacho, 2018). A

disadvantage of machine learning (ML) is that, if not done correctly, it can pose challenges for data quality (Davis, 2016).

Innovations in artificial intelligence and ML address the goals of exploring the behavior and survival mechanisms of bacterial persistent cells in microbiology and effectively eliminating them (Belghiti, 2025; Zavaleta – Monestal, 2025).

Deep learning (DL) has more comprehensive data processing capabilities than machine learning (ML) (Arango-Argoty, 2018). DL aims to support the prediction of antibiotic resistance genes (ARGs) and environmental monitoring in microbiology (el-Argoty, 2018; Baldi, 2018).

Antimicrobial resistance (AMR), which poses a serious threat to human health, is a global health problem. ML and DL techniques are being developed as diagnostic tools for Antimicrobial Susceptibility Testing (AST), enabling rapid prediction of antibiotic resistance profiles and appropriate antibiotic selection (Liao, 2025).

ML facilitates the classification of microorganisms using genomic data. This enables rapid prototyping and evaluation of developed biological systems (Blanco-Miguez, 2023). It allows for comprehensive examination of microflora-host interaction and the discovery of the functional capabilities of microorganisms (Dhaarani, 2025).

In bioprocesses, ML is used to increase efficiency, reliability, and the detection of various metabolites (Mowbray, 2021). ML saves resources and time by preventing unnecessary downstream monitoring operations (Patel-Shah, 2022).

Clinical microbiology is the specialty where microbiological tests are performed to determine the most appropriate treatment for

a patient. The aim of microbiological tests is to identify the causative agents of infection and determine antibiotic susceptibilities (Wang, 2022). Artificial intelligence is improving accurate diagnosis and efficiency in clinical microbiology (Antonios, 2022).

The safety and quality of pharmaceutical products are directly dependent on maintaining effective microbiological control throughout the manufacturing process. Raw materials, personnel, manufacturing environments, equipment, pharmaceutical water systems, and packaging materials all represent potential sources of microbial contamination. Microorganisms introduced from these sources during production may compromise product stability, efficacy, and patient safety. Consequently, microbiological testing is a fundamental component of product development, manufacturing, batch release, and stability monitoring throughout the product lifecycle. Establishing a robust microbiological control program is particularly critical for sterile products, biological products, and pharmaceutical formulations that are highly susceptible to microbial contamination (Gravante et al., 2025; Tyski et al., 2025).

Conventional methods used in pharmaceutical microbiology are primarily based on the cultivation of microorganisms on appropriate culture media. Sterility testing, microbial limit testing, membrane filtration, plate count methods, and biochemical identification techniques have been widely used for decades and are comprehensively described in pharmacopeial standards. Although these methods provide reliable detection of viable and culturable microorganisms, the need to wait for microbial growth significantly prolongs analytical turnaround times and limits the detection of slow-growing or non-culturable microorganisms. Furthermore, extended incubation periods may delay batch release, hinder timely

responses to manufacturing deviations, and postpone the identification of contamination sources (Marsit et al., 2025).

These limitations have driven the development of analytical technologies that are faster, more sensitive, more specific, and better suited for automation. Molecular diagnostics, next-generation sequencing, mass spectrometry, spectroscopy, biosensors, microfluidic systems, flow cytometry, and advanced imaging techniques enable the detection and characterization of microorganisms based on their nucleic acids, proteins, metabolic activity, or cellular properties without requiring microbial growth (Ferone et al., 2020; Song et al., 2024; Venhuizen et al., 2024). Beyond substantially reducing analytical turnaround times, these technologies provide deeper insights into the physiological states, biochemical characteristics, and potential sources of microbial contamination. As a result, microbiological risks can be identified earlier in the manufacturing process, enabling more effective management of pharmaceutical quality assurance systems (Ferone et al., 2020; Song et al., 2024)

Next-generation technologies offer significant advantages for the detection, enumeration, identification, viability assessment, and source tracking of microorganisms. However, because each technique targets distinct biological characteristics, the resulting data must be interpreted within the context of the method's underlying principle and intended application. Moreover, before these technologies can be implemented in routine quality control laboratories, their performance characteristics—including accuracy, specificity, sensitivity, reproducibility, and comparability with conventional methods—must be rigorously established. Equally important is the scientific validation of their reliable performance

using authentic pharmaceutical samples (Ferone et al., 2020; Song et al., 2024; Venhuizen et al., 2024).

This book discusses the fundamental principles, applications, advantages, and limitations of next-generation diagnostic and analytical technologies used in pharmaceutical microbiology. It also examines the potential of these technologies for sterility testing, microbial enumeration, environmental monitoring, pharmaceutical water system monitoring, and contamination control. In addition, the book provides an overview of the contributions these advanced technologies can make to pharmaceutical quality control while highlighting the key challenges associated with their routine implementation.

CHAPTER 2:
ARTIFICIAL INTELLIGENCE IN PHARMACEUTICAL MICROBILOGY

Definition, Scope, and Importance of Pharmaceutical Microbiology

Pharmaceutical microbiology is a discipline that applies microbiology to drug production and healthcare; it systematically examines the effects of microorganisms on pharmaceutical products, production processes, and production environments (Ahmet, 2024). The laboratory scope of this discipline is quite broad, encompassing many critical processes ranging from susceptibility testing to bioload analysis for determining the efficacy and resistance of pathogenic microorganisms to antimicrobial agents in in-vitro conditions, to sterility assessments and environmental monitoring programs conducted in production facilities (Demirpek, 2012).

Considering the importance of pharmaceutical microbiology to industry and public health, even the lowest level of microbial contamination can have serious consequences, especially in high-risk products administered directly into the circulatory system, such as injectable preparations. Therefore, preventing microbial contamination at all stages of the production process is considered a vital necessity (Sandle, 2016). Finally, in order to overcome the time-

consuming and manual nature of traditional microbiological analysis methods, reduce human-induced errors in laboratory practices, and predict potential contamination risks through predictive models, the integration of artificial intelligence and deep learning-based technologies into the field of pharmaceutical microbiology is considered a strategic transformation process in the modern industry (Attah, 2025).

The Role of Pharmaceutical Microbiology in Drug Development and Manufacturing Processes

Pharmaceutical microbiology, which holds a significant position in the pharmaceutical industry, analyzes the interactions between microorganisms and pharmaceutical products, meticulously evaluating the consequences of these interactions on product stability and patient safety (Dimitrovska, 2025; Demain, 2009). This discipline is not limited to sterility control of final products; it encompasses a broad process, starting from raw material selection and formulation development, to the planning of production facilities and the molecular-level production of biotechnological products (Gunjal 2025).

One of the most critical stages of the drug development process is ensuring the microbiological stability and safety of the final product during the design phase. At this point, microbiologists meticulously assess the suitability of the developed formulations for microbial growth by examining their chemical structure, water activity, and pH. Based on these analyses, the most suitable and effective protective systems for the formulation are selected and integrated, thereby maintaining the product's microbiological safety and sterility throughout its shelf life, from production to consumption (Lolas, 2014).

However, microbiological assurance is not limited to formulation design; it continues to play a vital role in the transition from the laboratory to mass production. When the production process begins, Good Manufacturing Practices (GMP) guidelines, an international standard, come into play to ensure the complete preservation of the product's therapeutic efficacy and chemical integrity. In accordance with GMP protocols, every parameter, from raw material quality and personnel hygiene standards to air filtration in the production area (HEPA filter systems, etc.) and equipment sterilization processes, is considered a potential source of contamination. Controlling these risks proactively through continuous monitoring and validation studies is an indispensable necessity for both protecting patient health and ensuring the therapeutic success of medications (Singer, 2025; Cengiz, 2020).

Traditional Microbiological Methods

Traditional methods commonly used in pharmaceutical microbiological analyses are based on culturing and multiplying microorganisms from samples on suitable media and on ensuring the formation of visible colonies (Memati, 2016). The Gram staining method, commonly used at the outset of these analyses, divides bacteria into two main groups based on differences in peptidoglycan and lipid structures of the cell wall (Paray, 2023). This staining process is based on chemical mechanisms such as alcohol dissolving the lipid structure and removing the crystal violet stain from the cell or the retention of the stain in the dehydrated cell wall (Tripathi, 2025). Following morphological examinations, biochemical tests that reveal the phenotypic characteristics of microorganisms are used to identify them at the species level (Roy-Bhattacharyya, 2023). In the final stage, sterile or non-sterile product samples are directly inoculated onto appropriate culture media, and the total microbial

load is determined by counting the resulting viable colonies (Marsit, 2025).

Advantages and Limitations of Traditional Methods

In industrial production, microbiological quality control processes constitute the most fundamental building block in protecting product safety and public health. However, given the rapid production and logistics dynamics brought about by modern industry, some major limitations inherent in these classical techniques seriously hinder operational efficiency.

Chief among these limitations are the long incubation times required to definitively verify the microbiological safety of products, and especially their sterility. These waiting periods, which can sometimes extend up to four days (or 14 days in sterility tests) depending on the nature of the sample, for microorganisms to form visible colonies, delay the market launch of products and increase warehousing costs, creating bottlenecks in the supply chain (Meamati, 2016). In addition to the time barrier, the reliability of classical methods is also heavily dependent on the human factor. The manual, visual counting and evaluation of microbial colonies developing on culture plates by analysts; Factors directly related to human error, such as fatigue, differences in personal perception, and inattention, bring with them a serious risk element that undermines data integrity and leads to bias in quality control processes (Sandle, 2020). These methodological bottlenecks are currently forcing the industry to turn to more automated and real-time alternative technologies such as Rapid Microbiological Methods.

Pathogens that encounter stress factors such as nutrient deficiencies, temperature fluctuations, or exposure to preservatives during the production process, although they maintain their viability,

may enter a dormant state called ‘live but not culturable’ (VBNC) under standard laboratory conditions (Siegrist, 2024). These VBNC forms, which cannot be detected by traditional culturing methods, pose a significant risk to public health due to their capacity to reactivate under suitable host or storage conditions (Zhao, 2017).

The Concept of Artificial Intelligence and its Role in Pharmaceutical Microbiology

Artificial intelligence is a broad scientific discipline that aims to model cognitive processes specific to human intelligence, such as learning, reasoning, and problem-solving, through computer systems (Uyar, 2025; Li, 2024). This technological discipline is divided into two main classes: systems that currently perform specific functions and models that aim for general competence at the human level but remain theoretical (Sandle, 2026). The digital revolution in microbiology necessitates the use of advanced computational tools, especially for analyzing and transforming the extensive datasets generated by high-throughput sequencing into meaningful information (Gangurde, 2025; Guleria, 2025). Machine learning algorithms are used to detect complex patterns in microbial genome data and, accordingly, to model antibiotic resistance profiles (Makhaole, 2025; Li, 2024).

In drug development, these systems, which utilize virtual screening techniques, accelerate the discovery of new antibiotic compounds, reducing the time constraints of traditional methods (Boudza, 2026; Barron, 2025). Automating routine laboratory workflows, such as colony counting and microscopic image analysis, optimizes operational time and significantly reduces human error rates (Sandle, 2026; Guleria, 2025). Artificial intelligence applications enable the detection of new antibiotic types (e.g.,

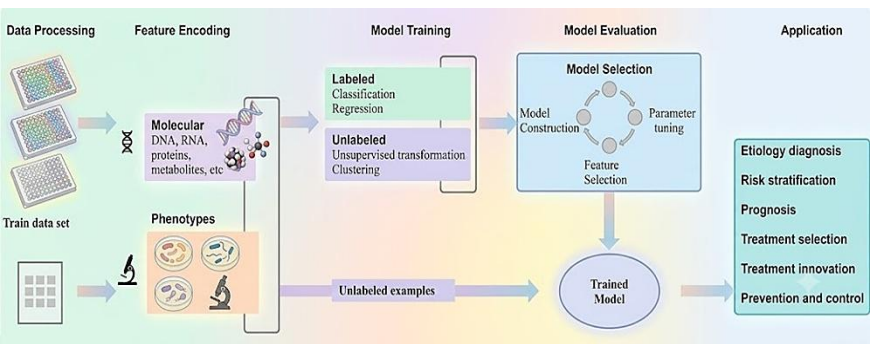
halicin) and also play an effective role in predicting and monitoring contamination hazards in cleanroom conditions (Makhoahle, 2025).

AI-powered digital twins and robotic systems play a fundamental role in creating the scientific infrastructure necessary for the development of personalized treatment methods (Bhalsing, 2025; Debnath, 2025).

Machine learning (ML)

Machine learning (ML) is a subfield of artificial intelligence that enables people to improve and increase their success in specific tasks through data-driven learning processes, without directly programming computer systems with explicit instructions (Figure 1) (Gurajala, 2024). In pharmaceutical microbiology laboratories, machine learning is used as a technological tool that enables faster results compared to traditional analysis methods by digitizing biological data (Wajiej & Aburagaegah, 2025). The field of machine learning utilizes statistical modeling methods to analyze the complexity of microbial structures and generate data-driven predictions in drug development studies (Sanapalli, 2026).

Figure 1. Development and implementation workflow of machine learning models in clinical microbiology and infectious diseases (Adapted from Xu, 2025).



This field, which combines computer science and statistics, particularly analyzes "Big Data" sets generated in microbiological studies to identify hidden structures that are imperceptible to the human eye (Mondal, 2025). In modern research, scientists use these systems as a strategic tool to analyze large volumes of digital data and make laboratory work more efficient (Attah, 2025). This method is based on the principle that the system develops its own rules based on statistical patterns in the data and can make predictions about future situations (Debnath, 2025). The analysis of microbial genomic and proteomic data using machine learning methods is becoming an integral part of current biomedical research (Saleem, 2025). As the amount and quality of data included in the system increase, the error margin of the algorithms decreases, and their predictive capacity increases over time (Vamathevan, 2019).

In the recent past, machine learning has played a significant role in monitoring infectious diseases and determining resistance characteristics (Xu, 2025). The grouping of bacterial species and the analysis of complex ecosystems are undergoing a scientific transformation thanks to the computational power of machine learning (Jiang, 2022). In this context, machine learning is not limited to merely identifying patterns in existing data but is gaining the ability to make logical, data-driven predictions about possible biological outcomes (Kim, 2022).

Definition and Fundamental Principles of Machine Learning

Machine learning enables computers to learn from data and make predictions by analyzing existing data rather than following predetermined commands (Gurajala, 2024). As a general rule, machine learning systems create mathematical structures using "training data" during data processing and produce results through these models (Xu, 2025). The techniques used in this field are

fundamentally divided into two groups: supervised and unsupervised learning, depending on the nature of the available data and the desired outcome (Wajiej and Aburagaegah, 2025). Supervised learning aims to complete a specific task by utilizing "labeled" datasets where inputs and outputs are predefined (Debnath, 2025). On the other hand, unsupervised learning focuses on discovering hidden patterns and clusters in unlabeled data without external guidance (Saleem, 2025). The fundamental criterion for a successful model is not only its ability to memorize the information given to it, but also its capacity to make accurate predictions on new datasets it has not encountered before (Vamathevan, 2019).

The model's perception of unnecessary details in the data as crucial information during the learning phase is considered the most fundamental problem in this field, known as "overfitting" (Mondal, 2025). Validation and test groups are used to objectively evaluate the error rate and real-world performance of the prepared model (Jiang, 2022). The inability of the model to recognize even basic patterns in overly simple datasets during the learning phase is defined as "underfitting" (Kim, 2022).

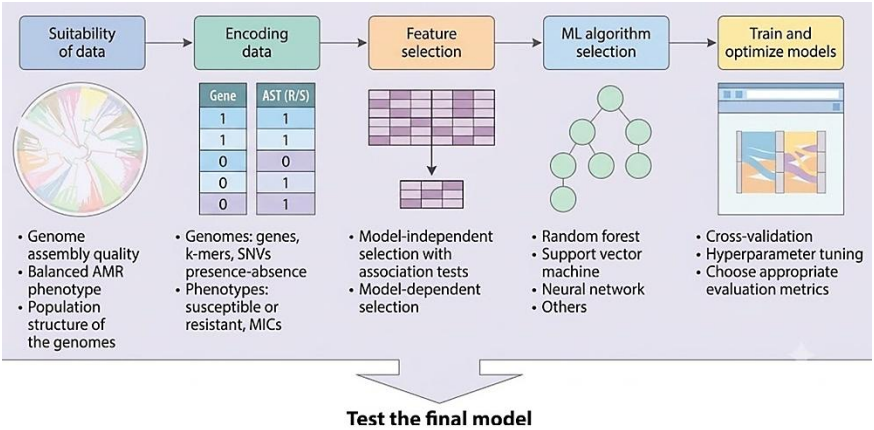
Deep learning (DL) is considered a more advanced field of machine learning that can self-extract features from raw data using artificial neural networks consisting of numerous layers (Attah, 2025). Determining the correct algorithm is carried out meticulously according to the complexity of the scientific problem under consideration and the characteristics of the available data (Sanapalli, 2026). In short, machine learning provides a technical framework that organizes data using mathematical methods and assists in decision-making processes in the field of microbiology (Giacobbe, 2026; Boudza, 2026).

The Role and Applications of Machine Learning in Microbiological Data Analysis

In the field of pharmaceutical microbiology, machine learning offers significant technological advancements by enabling the analysis of large-scale biological datasets quickly and with high accuracy (Sanapalli, 2026). Machine learning algorithms achieve higher success rates than manual techniques, particularly for identifying and species-level classifying bacteria in genomic data analysis (Xu, 2025).

In antibiotic resistance (AMR) prediction, predicting resistance status by analyzing the gene sequences or k-mer profiles of microorganisms accelerates clinical decision-making processes (Figure 2) (Kim, 2022). Processing MALDI-TOF mass spectrometry data with machine learning models, in particular, allows for the direct derivation of resistance profiles from raw data (López-Cortés, 2024). In image analysis, machine learning minimizes human-induced errors in the automated counting of cells in digital microscopy images and of colonies on agar plates (Gurajala, 2024).

Figure 2. Workflow for predicting antimicrobial resistance (AMR) using machine learning algorithms (Adapted from Kim, 2022).



In the process of discovering new antimicrobial agents, machine learning models play a significant role in identifying candidates that may be effective against bacteria by scanning large molecular domains (Saleem, 2025). Metagenomic research and disease outbreak prediction are increasingly highlighting the importance of this technology in understanding the microbial world (Jiang, 2022). For example, software such as DeepMicrobes can significantly speed up diagnostic processes by analyzing short sequences in metagenomic samples (Mondal, 2025).

Machine learning-supported sensors used in pharmaceutical production processes enable real-time monitoring and prevention of microbial contamination risks (Attah, 2025). Furthermore, in molecular design processes, these models enable the design of unique antibiotic candidates with targeted characteristics (Boudza, 2026).

Risk assessment of infections caused by resistant bacteria in clinical settings is becoming increasingly reliable with machine-

learning-based predictive tools (Zhao, 2025). These new data-driven approaches accelerate drug development processes, reducing costs and increasing the probability of success (Vamathevan, 2019; Debnath, 2025). Digital microbiology applications are ushering in a modern era in terms of protecting public health and increasing laboratory efficiency (Wajiej and Aburagaegah, 2025; EMA, 2025).

Advantages and Limitations of Machine Learning

The integration of machine learning technologies into pharmaceutical microbiology offers significant advantages while also presenting technical challenges (Boudza, 2026). The biggest advantages of this technology are its ability to process datasets beyond human capacity, its high analysis speed, and its ability to discover hidden patterns in the data (Attah, 2025). Machine learning systems automate quality control processes in production areas, increasing the consistency and verifiability of the results obtained (Gurajala, 2024). However, the accuracy of these models is directly determined by the quality and unbiasedness of the data used (Kim, 2022). If there are deficiencies or biases in the datasets, the model can produce erroneous results, negatively impacting healthcare processes (Mondal, 2025). The ‘black box’ nature of deep learning methods can lead to trust issues among experts because they cannot fully explain the system's prediction process (Wajiej and Aburagaegah, 2025). Overfitting of models to training data alone can lead to decreased prediction performance on new or different data (López-Cortés, 2024).

In the pharmaceutical sector, legal regulations present various obstacles that make it difficult to formally validate these constantly updated models (EMA, 2025; Sanapalli, 2026). Furthermore, the need for explainable AI tools to enable physicians to interpret the results in a clinical setting is of great importance

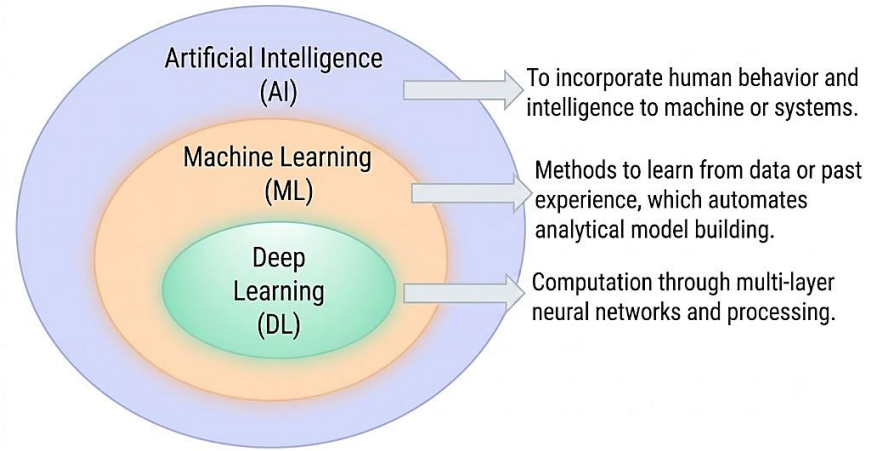
(Zhao, 2025). Rules for data protection, ethical principles, and algorithmic accountability are considered necessary steps for the approval of this technology across all areas (Debnath, 2025). The expensive technical equipment required to prepare and update complex AI systems can sometimes hinder scientific research (Giacobbe, 2026).

Consequently, the gap between microbiological expertise and data analysis expertise poses a practical challenge to the full adaptation of these models to laboratory settings (Saleem, 2025; Xu, 2025). Balancing these advantages and disadvantages is considered necessary for machine learning technology to demonstrate its full potential in healthcare (Vamathevan, 2019).

Deep learning (DL)

Deep learning (DL), considered a subfield of AI and ML, is based on artificial neural network structures that model the information processing processes of the human brain (Figure 3) (Sarker, 2021).

Figure 3. The comprehensive relationship between artificial intelligence, machine learning, and deep learning (Adapted from Sarker, 2021).



This advanced technology makes it possible to use a structure consisting of numerous hidden layers and arranged sequentially for the purpose of analyzing and interpreting data (Przymus, 2025). Today, the exponential increase in data volume due to the impact of digitalization has placed big data systems at the center, and unlike traditional methods, deep learning techniques have enabled much higher performance on these large datasets (Yang, 2019). In large datasets where classical analytical methods are insufficient, deep learning programs can learn the fundamental, complex structure of the data on their own (Shen, 2022).

In fields such as pharmaceutical microbiology, the increasing volume of biological data processing directly increases the need for this advanced analytical technology (Wajiej - Aburagaegah, 2023). These highly distributed artificial neural networks offer significant contributions to researchers, particularly in areas such as the degradation processes of new drugs, the distribution of

microorganisms, and the explanation of changes in diseases (Koutroumpa, 2023). This makes analyses in pharmaceutical microbiology more accessible, transforms them into actionable results, and creates a more efficient structure (Li, 2024).

Definition of Deep Learning

Deep learning is an approach based on multilayer artificial neural networks that enables the analysis of data through highly abstract features, and is considered one of the most advanced subfields of machine learning (Chen, 2018). When trained on large, complex datasets, these algorithms can learn hidden relationships in the data with minimal human intervention and produce high-accuracy results (Shen, 2022). Thanks to this unique capacity, deep learning is not limited to processing simple numerical data but also stands out as an effective approach for analyzing heterogeneous data with diverse characteristics (Przymus, 2025).

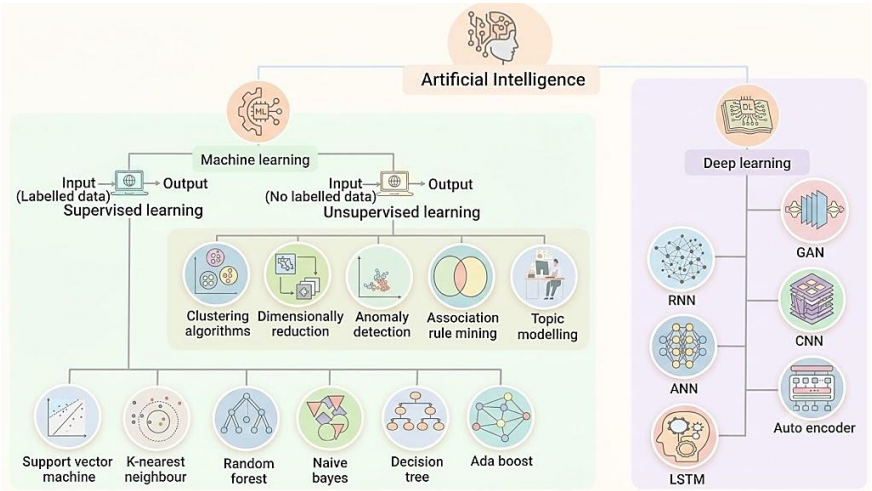
In the analysis of high-resolution microscopic images, complex genome sequences, and multidimensional molecular data structures, which are critical in pharmaceutical sciences, these approaches can yield more advantageous results than traditional statistical methods (Roy, 2024). At a basic level, this technology can be described as an independent system that accepts raw data as input, processes the data incrementally within a multi-layered structure to recognize features, and generates predictive results based on the learning rules it obtains (Wu-Gadsden, 2023). In line with these qualities, deep learning helps in the automated analysis of data in microbiological studies and makes it possible to obtain results with high accuracy and reliability (Asnicar, 2023).

Differences Between Machine Learning and Deep Learning

One of the fundamental differences between traditional machine learning and deep learning is deep learning's ability to automatically identify and directly extract meaningful features from data (Wu-Gadsden, 2023). While machine learning methods require experts to manually determine the features to be analyzed and the data to be prepared in advance, deep learning, thanks to its multi-layered structure, can learn these features directly from the data without the need for human intervention (Greenspan, 2016). Furthermore, while machine learning models generally reach a saturation point and stabilize after a certain amount of data, deep learning models continue to improve their performance as they are trained with larger datasets (Roy, 2024). Despite significant advantages such as performance enhancement and automated feature extraction, deep learning models require high computational power, particularly advanced graphics processing units (GPUs) (Koutroumpa, 2023).

While machine learning methods generally offer more transparent and interpretable results, deep learning models are often described as ‘black boxes’ due to their complex structures, making direct interpretation of the results more difficult (Sarker, 2021). In pharmaceutical research, these two approaches are considered complementary methods, taking into account the available data volume and the computational complexity of the problem (Przymus, 2025).

Figure 4. Classification of machine learning and deep learning algorithms (Adapted from Debnath, 2025).



The Importance of Deep Learning in Genomic and Metagenomic Analyses

Genomic and metagenomic data obtained in the field of pharmaceutical microbiology are, by their nature, high-dimensional, sparsely feature-rich, and exhibit a complex data structure (Przymus, 2025). Uncovering nonlinear relationships within these large and heterogeneous genetic datasets presents a challenging process for classical sequence alignment tools and classical statistical methods (Pei, 2024).

Deep learning algorithms enable the reliable and highly accurate differentiation of different microorganism species in clinical or environmental metagenomic samples (Roy, 2024). Furthermore, these advanced architectures contribute to the general classification of pathogens and the understanding of disease

distribution by analyzing complex relationships in DNA sequences (Li, 2024).

Deep learning models, which can overcome the limitations of traditional reference databases, enable highly accurate prediction of novel antibiotic resistance genes using features directly derived from sequences (Pei, 2024). In conclusion, deep learning helps transform genomic data from simple sequence data into meaningful, usable information for pharmaceutical research and resistance management (Koutroumpa, 2023).

AI-Based Antimicrobial Susceptibility Testing and Diagnostic Approaches

Traditional microbiological diagnostic methods used in the fight against antimicrobial resistance involve time-consuming application processes (Askar, 2026). Culture-based analyses performed on clinical samples require considerable time, and obtaining definitive results of standard antimicrobial susceptibility tests (ASTs) can often take 24–48 hours, and in some cases up to 72 hours (Behera, 2019). This long waiting period forces physicians to apply broad-spectrum empirical treatment to patients in emergency situations and increases the need for rapid diagnostic methods in clinical practice (Eubank, 2020). To overcome these limitations of classical methods and to accelerate diagnostic processes, the use of artificial intelligence applications in laboratory environments is becoming increasingly widespread (Atria, 2025).

AI-supported systems can evaluate characteristics such as colony morphology of microorganisms found in culture plates within seconds through advanced image analysis techniques (Askar, 2026). At the same time, the genetic structures of microorganisms are being examined through machine learning-based genomic data analysis

methods, and antibiotic resistance that may develop can be predicted with high accuracy (Liao, 2025). AI-powered solutions are providing physicians with faster results by shortening laboratory investigations that can take days to just a few hours (Guerro-Lopez, 2023; Wang, 2022). This speed advantage enables the application of the appropriate targeted treatment at the earliest stage for patients, thereby accelerating clinical recovery (Pennisi, 2025). In addition, systematic automation applications used in laboratory workflows minimize errors caused by manual evaluation and increase the laboratory efficiency of healthcare personnel (Magimai Rufus, 2025).

Clinical Microbiology and Artificial Intelligence Applications

Clinical microbiology is a fundamental medical discipline that studies the distribution and spread of pathogenic microorganisms causing disease in economically developed regions (Gurajala, 2024). Standard laboratory procedures in this field include sample preparation, isolation of microorganisms by culture, and antibiotic testing (Ding, 2025). The data obtained provide critical clinical decision support for physicians in making appropriate treatment decisions (Xu, 2025).

In traditional culture methods, the multiplication of pathogens and the acquisition of biological data generally require 24 to 72 hours (Ghedia, 2026). Furthermore, manual test evaluation can introduce human error and discrepancies in assessment (Gurajala, 2024). These time-consuming characteristics of classical methods are emerging as a key factor necessitating the transition to artificial intelligence applications in medical laboratories (Askar, 2026). Today, artificial intelligence technologies enable the efficient application of various diagnostic processes in microbiology to laboratory studies (Alsulimani, 2024). Digital image analysis allows

for the automated evaluation of microscopic images and the classification of bacterial colonies in culture plates (Ghedia, 2026). Furthermore, machine learning-based programs enable the rapid and reliable analysis of large genomic data. This allows for the highly accurate prediction of potential antibiotic resistance profiles of pathogens (Cesaro, 2025).

The integration of artificial intelligence into laboratory processes significantly contributes to reducing error rates associated with data transfer processes and manual examination (Askar, 2026). The rapid diagnostic capability provided by AI-based software ensures the timely and uninterrupted initiation of antimicrobial treatment in clinics (Alsulimani, 2024). Consequently, the rapid diagnosis and accurate antibiotic selection capabilities achieved through artificial intelligence are among the most effective strategies in combating antimicrobial resistance (AMR) today, making significant contributions to this field (Ding, 2025).

AI-Supported Approaches in Microbiological Quality Control Processes in Pharmaceutical Production

In pharmaceutical production, microbiological quality control plays a critical role in ensuring product safety and assessing contamination risks (Tyski, 2025). Within the scope of Good Manufacturing Practices (GMP), comprehensive monitoring strategies are implemented to manage microbiological hazards originating from raw materials, personnel, and equipment, and to control microbial load (Marsit, 2025).

Traditional culture methods, microbial counting, and sterility tests form the basis of these processes, but they have some limitations due to their time-consuming nature and the need for manual evaluation (Nemati, 2016). This makes it difficult to make

quick decisions in potential contamination cases and increases the need for new technologies in quality assurance processes (Marsit, 2025). In this context, artificial intelligence systems developed enable big data analysis by monitoring production processes and contribute to the prediction of contamination risks (Vora, 2023).

Automation systems; It enables the acquisition of laboratory data by allowing colony counting through image analysis, evaluation of microbial growth patterns, and automatic analysis of production data (Bard, 2025). As a result, artificial intelligence supports the proactive improvement of production safety standards by enabling rapid data analysis, reduction of error rates, and early detection of potential contaminations (Vora, 2023). This approach, which can contribute more efficiently without completely transforming production processes, contributes to strengthening the relationship between drug safety and patient safety (Tyski, 2025).

CHAPTER 3:
NEXT GENERATION DIAGNOSTIC AND ANALYSIS TECHNOLOGIES IN PHARMACEUTICAL MICROBIOLOGY

Molecular Diagnostics and Sequencing Technologies

Molecular diagnostic technologies are based on the detection of microbial genetic material without requiring prior cultivation in culture media. These methods are used to detect microbial contamination, identify microorganisms, investigate antimicrobial resistance and virulence genes, trace contamination sources, and characterize microbial communities. Unlike culture-based methods, molecular approaches are less affected by the growth rate of the target microorganism or its ability to proliferate on culture media. They can therefore offer substantial advantages in the investigation of microorganisms present at low abundance, slow-growing organisms, or those requiring specialized culture conditions. Nevertheless, the success of a molecular method depends on the selection of an appropriate target region, the extraction of nucleic acids of sufficient quality from the sample, and the effective control of analytical interference (Gao et al., 2021; Song et al., 2024; Totten et al., 2023).

Polymerase chain reaction is one of the fundamental molecular techniques used to amplify specific DNA regions derived from microorganisms. In real-time PCR, amplification is monitored concurrently through fluorescent signals, enabling the rapid detection and quantification of the target nucleic acid. Target-specific primers and probes facilitate the differentiation of closely related species, whereas multiplex systems allow multiple microorganisms or gene regions to be investigated within a single reaction. The cycle number at which the fluorescent signal generated during amplification exceeds a defined threshold provides quantitative information on the initial amount of the target. This feature enables not only qualitative presence–absence assessment but also estimation of the microbial load when appropriate calibration procedures and controls are used. Although multiplex PCR systems can reduce analytical time and sample consumption, they require more extensive optimization because of potential interactions among primers and the need to balance the amplification efficiencies of different targets (Ndraha et al., 2023; Totten et al., 2023).

Reverse transcription PCR is used to analyze RNA targets. In this method, RNA is first converted into complementary DNA and subsequently amplified by PCR. Because RNA generally persists in the environment for a shorter period than DNA, it may offer an advantage in assessing metabolically active cells. However, its inherently unstable nature requires careful handling during sample collection, storage, and extraction. Messenger RNA, in particular, is associated with cellular activity and may therefore provide more functionally relevant information for viability assessment than DNA-based analyses. Nevertheless, because different RNA species may persist for varying periods after cell death, the presence of RNA should not invariably be interpreted as direct evidence of viability,

and the findings should be supported by culture-based methods or measurements of metabolic activity (Gao et al., 2021).

Real-time PCR can be used for the microbiological control of raw materials, manufacturing environments, pharmaceutical water systems, biological products, and short-shelf-life cell therapy products. Its principal applications include the rapid detection of specified pharmacopeial microorganisms, mycoplasmas, and known environmental contaminants. Interest in molecular methods has increased particularly for cell therapy products, for which rapid results are essential, because the prolonged incubation period required for conventional sterility testing may prevent completion of the test before product release. Target-specific assays can facilitate the rapid monitoring of previously identified high-risk microorganisms during manufacturing, thereby supporting the early assessment of process deviations. However, preservatives, proteins, salts, and other excipients present in pharmaceutical products may inhibit amplification. Accordingly, sample preparation, nucleic acid extraction, and the use of internal controls are critical to method reliability. An internal amplification control helps determine whether a negative result reflects the true absence of the target microorganism or inhibition of the reaction. Extraction controls, positive controls, and negative controls designed to detect contamination should also be used in combination (Gebo & Lau, 2020; Ndraha et al., 2023; Totten et al., 2023).

One of the main limitations of PCR-based methods is their inability to directly distinguish between viable and nonviable microorganisms. Residual DNA from dead cells may produce positive results. To mitigate this limitation, membrane integrity–dependent dyes such as propidium monoazide, RNA-based analyses, or short pre-enrichment steps may be used. Propidium monoazide is

intended to bind to the DNA of cells with compromised membrane integrity, thereby limiting amplification of that DNA. Pre-enrichment, in turn, allows viable microorganisms to proliferate while reducing the relative contribution of free DNA and may improve sensitivity in cases of low-level contamination. However, these approaches must be optimized separately for each product and microorganism. Because dye penetration into cells, the sample matrix, the physiological state of the microorganism, and the applied light conditions can all influence the results, viability PCR should not be regarded as a direct and universally applicable measure of microbial viability (Gao et al., 2021).

In digital PCR, the reaction mixture is partitioned into a large number of small compartments, each of which is classified as positive or negative. The absolute copy number can then be calculated from the proportion of positive compartments without the need for a standard curve. Target molecules are assumed to be randomly distributed among the compartments, and the results are calculated using Poisson statistics. Partitioning the reaction into numerous independent microreactions facilitates the discrimination of low-abundance targets from background signals and the detection of rare genetic variants. This method offers advantages for detecting targets at low concentrations, resolving small differences in concentration, and obtaining more precise quantitative results from complex samples. Its potentially greater tolerance to PCR inhibitors than real-time PCR may represent an important advantage when analyzing pharmaceutical product matrices. Furthermore, the absence of a requirement for a standard curve may improve the interlaboratory comparability of quantitative results. However, instrument cost, sample throughput, and standardization requirements limit its routine use. Because the number of partitions, sample volume, and saturation of positive compartments affect the

measurement range, samples must be diluted appropriately according to the target concentration (Lei et al., 2021).

Isothermal amplification methods enable nucleic acid amplification at a constant temperature. Eliminating the need for a thermal cycler facilitates the development of simpler and more portable systems. Loop-mediated isothermal amplification, recombinase polymerase amplification, helicase-dependent amplification, and nucleic acid sequence–based amplification are the principal methods in this group. Loop-mediated isothermal amplification can generate large quantities of amplification products, and the results can be assessed through fluorescence, turbidity, or colorimetric changes. Recombinase polymerase amplification is performed rapidly at relatively low temperatures, whereas nucleic acid sequence–based amplification is used primarily for RNA targets. The ability to interpret assay results visually through color changes may facilitate their use in settings with limited laboratory infrastructure and in near-manufacturing applications. When integrated with microfluidic platforms and lateral flow systems, sample preparation, amplification, and result interpretation may be combined within a single device. Although these methods are well suited for near-site testing, they are limited by factors such as complex primer design, nonspecific amplification, and the risk of cross-contamination. The generation of large quantities of amplification products may increase the risk of amplicon contamination in the laboratory when closed-tube systems are not used. Therefore, results should be evaluated without opening the reaction tubes, and work areas should be physically separated (Ndraha et al., 2023).

CRISPR-based diagnostic systems use guide RNA and Cas enzymes to recognize target nucleic acid sequences. Upon detection

of the target sequence, the activated enzyme cleaves labeled reporter molecules, generating a measurable fluorescent or colorimetric signal. Cas12 systems are primarily used for DNA targets, whereas Cas13 systems are generally applied to RNA targets. Because guide RNA can be designed to be complementary to a specific target sequence, these systems may distinguish single-nucleotide differences and strain-specific genetic regions. The collateral cleavage activity triggered after Cas enzymes bind to their targets is used to convert low-abundance targets into measurable signals. CRISPR-based systems offer high specificity, short analytical turnaround times, and the potential for adaptation to portable devices. They can be used to detect microorganisms, antimicrobial resistance genes, and strain-specific regions. Their integration with isothermal amplification and lateral flow strips may enable result interpretation without the need for advanced laboratory instrumentation. However, the frequent requirement for a pre-amplification step to improve sensitivity complicates the workflow and increases the risk of contamination. Standardization and validation studies are required before these systems can be implemented routinely for pharmaceutical samples. In addition, guide RNA design, variations within the target genome, and the effects of pharmaceutical matrix components on enzyme activity may influence method performance (Kaminski et al., 2021).

Next-generation sequencing technologies enable the parallel analysis of millions of DNA fragments, allowing the genomic characteristics of microorganisms to be examined in detail. Whole-genome sequencing can identify microorganisms at the species and strain levels, detect antimicrobial resistance and virulence genes, and compare isolates recovered from different sources. This approach is an important tool for investigating recurrent contamination events and tracing contamination sources within manufacturing facilities.

Comparing the genomic similarity of microorganisms isolated from manufacturing areas at different time points can help determine whether contamination originates from a persistent source, repeated introduction, or independent events. Whole-genome sequencing enhances epidemiological resolution by revealing differences among isolates identified as the same species by conventional phenotypic methods (Song et al., 2024). Short-read sequencing platforms provide high accuracy and throughput, whereas long-read technologies enable more comprehensive analysis of complex genomic regions, plasmids, and genetic rearrangements. Hybrid approaches combining short and long reads can integrate high sequence accuracy with improved genome completeness. Long reads are particularly useful for determining the genomic locations of repetitive sequences, mobile genetic elements, and plasmids carrying antimicrobial resistance genes. In contrast, short-read data can reduce base-level error rates and thereby improve the accuracy of hybrid genome assemblies (Di Marco et al., 2023; Song et al., 2024).

In metagenomic analyses, the total genetic material present in a sample is examined directly without prior cultivation of microorganisms. Amplicon sequencing typically targets the 16S rRNA gene in bacteria and the internal transcribed spacer (ITS) regions in fungi. Although these methods are relatively inexpensive and can be applied to large numbers of samples, they may introduce primer bias and often lack sufficient resolution for species- or strain-level discrimination. Preferential amplification of certain microbial groups by the selected primers can cause the apparent community structure to deviate from its actual composition. In addition, insufficient sequence variability within the targeted gene region among different species may limit taxonomic resolution (Durazzi et al., 2021; Fierer et al., 2025).

In shotgun metagenomics, the total DNA in a sample is sequenced without prior target selection. This approach enables bacteria, fungi, viruses, and archaea to be evaluated within a single analysis and allows the investigation of genes associated with antimicrobial resistance, virulence, and metabolic functions. Unlike amplicon sequencing, shotgun metagenomics is not restricted to a predefined taxonomic target and may therefore detect unexpected or difficult-to-culture microorganisms. The resulting data can be used to evaluate not only the taxonomic composition of the microbial community but also its potential functional characteristics. However, high costs, extensive bioinformatics requirements, and the masking of microbial signals by product- or host-derived DNA remain major limitations. Detecting low-abundance microorganisms may require substantial sequencing depth, further increasing analytical costs and computational demands (Durazzi et al., 2021).

Pharmaceutical water systems, cleanrooms, and raw materials are among the principal application areas of metagenomic methods for characterizing microbial communities. This approach can help identify community members that remain undetected by culture-based analyses, monitor dominant microorganisms within a system, and evaluate changes in community composition over time. In pharmaceutical water systems in particular, routine monitoring of the microbial community may provide early indications of biofilm development or changes in system performance (Shikama et al., 2025; Song et al., 2024). However, in low-biomass samples, contamination from reagent-derived and environmental DNA can substantially affect the results. Extraction kits, laboratory environments, consumables, and analytical reagents may contain trace amounts of microbial DNA. When the amount of authentic microbial DNA in a sample is low, this background contamination may account for a substantial proportion of the detected signal.

Therefore, extraction blanks, reagent controls, and process controls should be included in the analysis (Fierer et al., 2025; Gao et al., 2021). Furthermore, the detection of DNA does not directly demonstrate that the microorganism is viable or poses a risk to the product (Fierer et al., 2025).

Although molecular diagnostic and sequencing technologies provide rapid and detailed information, they do not always directly indicate microbial viability or the capacity for proliferation. Furthermore, determining whether genetic signals detected at very low levels represent clinically or pharmaceutically meaningful contamination is not always straightforward. The results must therefore be interpreted in the context of the product type, manufacturing stage, level of microbial risk, and the method's limit of detection. While molecular methods are powerful tools for rapid screening and source investigation, culture-based methods remain essential for recovering viable microorganisms, examining their phenotypic characteristics, and performing further identification. Accordingly, combining these technologies with culture-based analyses enhances the reliability of pharmaceutical microbiological assessments (Deutschmann et al., 2023; Gao et al., 2021; Song et al., 2024).

Rapid Microbial Identification and Spectroscopic Methods

In pharmaceutical manufacturing, it is important not only to detect a contaminant but also to identify it accurately and rapidly. The identity of the microorganism provides critical guidance for investigating the source of contamination, assessing risk, and planning corrective actions. Identification results support the evaluation of the risk posed by the detected microorganism to product quality and patient safety, help determine potential routes of dissemination within the manufacturing environment, and facilitate

the linkage of recurrent contamination events. In particular, repeated detection of the same or closely related species at different stages of production may warrant investigation of a persistent environmental source or deficiencies in cleaning and disinfection practices. Although conventional morphological and biochemical identification methods are reliable, obtaining results may require several days (Guimarães et al., 2023; Song et al., 2024).

Methods based on mass spectrometry and vibrational spectroscopy use characteristic profiles derived from the protein, lipid, carbohydrate, and nucleic acid composition of microorganisms. By comparing these profiles with reference data, microorganisms can be identified at the genus, species, or, in some cases, strain level. Because these methods evaluate multiple molecular components simultaneously rather than relying on a single metabolic or biochemical characteristic, they may offer a significant advantage in distinguishing isolates with similar phenotypic properties. However, because profile accuracy may be influenced by the physiological state of the microorganism and by preanalytical procedures, standardization remains a fundamental requirement (Kassem et al., 2023; McGalliard et al., 2024; Rebrosova et al., 2022).

MALDI-TOF MS is one of the most widely used technologies for rapid microbial identification. The microbial sample is mixed with an appropriate matrix and ionized by laser irradiation, and a characteristic mass spectrum is generated from the time of flight of the resulting ions. The spectrum is composed predominantly of ribosomal and other small proteins. The profile obtained from an unknown isolate is compared with a reference database, enabling rapid identification (Chudzik et al., 2024; Guimarães et al., 2023; Song et al., 2024). The resulting spectrum is

matched against reference profiles in the database using a similarity score, and the confidence level of the identification is assessed accordingly. When a spectrum of sufficient quality is obtained, identification can be completed within minutes, providing a substantial time advantage over conventional biochemical tests. Bacterial and yeast colonies can generally be transferred directly onto the target plate, whereas protein extraction may be required for microorganisms with thick cell walls, molds, and certain yeasts. Extraction with formic acid and organic solvents facilitates cell wall disruption and more efficient release of cellular proteins. In filamentous fungi in particular, growth morphology and sporulation can influence the spectral profile, necessitating more extensive sample preparation. Because the sample preparation technique, culture medium, colony age, and incubation conditions may affect spectral quality, standardization of analytical conditions is essential (Chudzik et al., 2024; Guimarães et al., 2023).

In pharmaceutical microbiology, MALDI-TOF MS is used to identify environmental isolates, microorganisms recovered from water systems, and product contaminants. Rapid identification facilitates the assessment of whether the same microorganism recurs at different time points or sampling locations. Prompt identification of isolates obtained through environmental monitoring programs can support the development of a facility-specific microbial flora profile and enable the tracking of microbial groups that become predominant in particular areas. These data are useful for evaluating microbial trends within manufacturing environments and for the early detection of unusual isolates. However, because the method generally requires a pure culture, it does not eliminate the time required for microbial growth; rather, it accelerates the post-culture identification step (Guimarães et al., 2023; Song et al., 2024). In spectra obtained from mixed cultures, signals from multiple

microorganisms may overlap, preventing reliable identification. Therefore, colony isolation and purification remain necessary in most applications. Identification performance depends on the scope of the reference database. Although clinical isolates are extensively represented, environmental microorganisms encountered in pharmaceutical manufacturing facilities may be underrepresented. This limitation can be mitigated by establishing in-house spectral libraries using facility isolates confirmed by molecular methods. Regularly updating these in-house databases may facilitate the identification of rare environmental species and enable more consistent evaluation of microorganisms repeatedly isolated from the same facility (Chudzik et al., 2024; Mazhari et al., 2025; Song et al., 2024).

Raman spectroscopy is based on measuring energy shifts that occur when monochromatic light interacts with molecules. Signals arising from proteins, nucleic acids, lipids, carbohydrates, and pigments collectively form the biochemical profile of the cell. The ability to analyze samples with minimal preparation and the weak Raman signal of water are important advantages (Pezzotti, 2021; Rebrosova et al., 2022). Raman spectroscopy can enable the examination of cells without staining or labeling and, when combined with appropriate optical systems, can provide measurements at the single-cell level. This capability is particularly valuable for evaluating cells in different physiological states within the same microbial population and investigating cellular heterogeneity. Moreover, because the sample remains largely intact during analysis, the same cell may subsequently be subjected to further examination using other methods. However, the inherently weak Raman signal can hinder the detection of microorganisms present at low abundance. Long acquisition times and fluorescence background may also reduce spectral quality, particularly in

pigmented microorganisms or complex product matrices. The laser wavelength and power level used can influence not only signal intensity but also the extent of photothermal damage to cells (Pezzotti, 2021; Rebrosova et al., 2022; Zhang et al., 2024).

Surface-enhanced Raman spectroscopy increases signal intensity through the use of gold or silver nanostructures. SERS systems may operate on the basis of whole-cell profiles or be rendered target-specific through the incorporation of antibodies, aptamers, or nucleic acid probes. The electromagnetic field generated near the metal surface markedly enhances the Raman signal originating from the microorganism or target biomolecule. This enhancement enables the detection of low-abundance targets that are difficult to identify using conventional Raman spectroscopy. The use of target-specific recognition elements can facilitate the selective binding of microbial cells to the surface and improve specificity in complex samples. Although SERS provides high sensitivity, variability in metal surface fabrication, nanoparticle properties, and sample distribution across the substrate may affect reproducibility. Because pharmaceutical product components may also cause spectral interference, surface characteristics and sample preparation procedures must be standardized. Variations in nanoparticle size, shape, degree of aggregation, and mode of interaction with target cells can alter signal intensity, potentially leading to performance differences among manufacturing batches. Therefore, both substrate fabrication and signal interpretation criteria must be validated before routine implementation (Hassan et al., 2024; Usman et al., 2023).

FTIR spectroscopy is based on the absorption of infrared light by molecules at specific frequencies. Amide bonds in proteins, membrane lipids, cell wall carbohydrates, and phosphate groups in

nucleic acids generate signals in different spectral regions. The resulting profile serves as a fingerprint reflecting the overall chemical composition of the cell (Kassem et al., 2023). The intensity and position of bands within specific spectral regions may vary among microorganisms depending on characteristics such as cell wall structure, membrane composition, and protein content. FTIR can therefore be used not only for taxonomic identification but also to evaluate phenotypic differences within the same species and cellular changes induced by environmental conditions. Its short analytical time and low consumable requirements may offer advantages for microbial identification and typing. The ability to discriminate certain microorganisms at the strain level is particularly useful for comparing recurrent environmental isolates. Assessing the spectral similarity of isolates recovered from the same manufacturing area at different time points may provide preliminary insight into potential persistent contamination sources. However, strain-level discriminatory power varies depending on the microbial species and the classification model used. Culture medium, incubation time, growth phase, and drying procedures may also alter the spectral profile. In particular, changes in cellular protein, lipid, and carbohydrate proportions across different growth phases may cause spectra from the same strain to appear dissimilar. Therefore, isolates intended for comparison should be analyzed under comparable culture and sample preparation conditions (Kassem et al., 2023; McGalliard et al., 2024).

Because Raman and FTIR spectra consist of numerous measurement points, multivariate statistical methods are used to evaluate the resulting data. Principal component analysis, clustering, and discriminant analysis enable the grouping of similar microorganisms and the classification of unknown samples. Principal component analysis helps visualize the main sources of

variation within the data, whereas hierarchical clustering groups isolates with similar spectral profiles. Linear discriminant analysis, support vector machines, and other supervised classification methods can be used to assign unknown samples to predefined groups. Spectral preprocessing may include baseline correction, normalization, and derivative transformations, all of which can substantially influence model performance. Reliable models require a sufficient number of validated reference spectra. Validation using independent samples, rather than only the data set used for model development, is essential to prevent overfitting and demonstrate real-world performance. Variability among spectra acquired using different instruments, in different laboratories, or on different analytical days may also limit model transferability (Ma et al., 2023; McGalliard et al., 2024; Usman et al., 2023; Yang et al., 2023; Zhang et al., 2024).

In addition to microbial identification, spectroscopic methods can be used to investigate biofilm formation, antimicrobial effects, and cellular stress responses. Polysaccharides, proteins, and nucleic acids within the biofilm matrix may produce characteristic changes in Raman and FTIR spectra. Similarly, alterations in signals associated with membrane lipids, protein structures, and nucleic acids in cells exposed to antimicrobial agents can be used to assess cellular damage or adaptive responses. Single-cell Raman analysis may provide information that facilitates the discrimination of susceptible and tolerant cells within the same population. However, the biological significance of spectral changes should be confirmed using culture-based, molecular, or phenotypic assays. Because a change in the intensity of a single spectral band does not, by itself, establish a specific biological mechanism, interpreting the results alongside complementary measurements of cell viability, metabolic activity, membrane integrity, or gene expression provides a more

reliable approach (Jelli et al., 2023; Kassem et al., 2023; Pezzotti, 2021; Rebrosova et al., 2022).

MALDI-TOF MS, Raman spectroscopy, SERS, and FTIR spectroscopy shorten microbial identification times by using characteristic molecular profiles of microorganisms. MALDI-TOF MS is particularly well suited for the rapid routine identification of pure isolates recovered from culture, whereas Raman and FTIR spectroscopy provide more comprehensive profiles of cellular biochemical composition and enable phenotypic changes to be monitored in research applications. SERS is particularly promising for investigating low microbial loads because of its high sensitivity and its potential for integration with target-specific detection systems. However, the reliability of these methods depends on sample preparation, the scope of the reference database, and the standardization of analytical conditions. For routine implementation, reference data sets must adequately represent pharmaceutical environmental isolates, instrument performance must be monitored regularly, and result acceptance criteria must be predefined. Accordingly, rather than completely replacing conventional methods, these technologies are regarded as complementary tools that strengthen post-culture identification and contamination source investigation processes (Guimarães et al., 2023; Kassem et al., 2023; McGalliard et al., 2024; Rebrosova et al., 2022; Song et al., 2024).

Biosensors, Microfluidic Systems, and Lab-On-A-Chip Technologies

Biosensor and microfluidic systems enable rapid, portable analyses using small sample volumes. Biosensors rely on the detection of microorganisms or microbial components through biological recognition elements, whereas microfluidic platforms integrate sample preparation, separation, reaction, and measurement

steps within a miniaturized system (Figure 5). These technologies can streamline processes that are performed as separate stages in conventional laboratory workflows, reducing analysis time and minimizing user intervention. In pharmaceutical manufacturing, rapid results may be particularly valuable for assessing potential microbial contamination before it progresses to subsequent stages of production (Abdelhamied et al., 2023; Dönmez et al., 2022; Jóskowiak et al., 2023).

A biosensor consists of a biological recognition element, a transducer, and a measurement unit. Antibodies, aptamers, nucleic acid probes, enzymes, and bacteriophages may be used for target recognition. The interaction with the target is converted into an electrical, optical, or mechanical signal. Sensor specificity depends largely on the recognition element, whereas sensitivity is determined by how efficiently the target–sensor interaction is converted into a measurable signal. Accordingly, the manner in which the recognition element is immobilized on the surface, its stability, and its interactions with nontarget components directly influence sensor performance (Abdelhamied et al., 2023; Beyazit et al., 2024; Liu et al., 2024; Ning et al., 2023; Zhang et al., 2025).

Antibody-based sensors provide high specificity but are sensitive to temperature and storage conditions. Their production costs, potential loss of activity during long-term storage, and batch-to-batch variability in performance may limit routine implementation. Aptamers represent an important alternative because they can be chemically synthesized and exhibit high stability. Their amenability to chemical modification also facilitates adaptation to different sensor surfaces. However, complex product matrices may alter the three-dimensional structure of an aptamer and its target-binding capacity (Beyazit et al., 2024; Ning et al., 2023).

Bacteriophages, in contrast, can be used to capture specific viable bacteria because of their natural host specificity. Because phage replication requires a viable and susceptible host cell, phage-based systems offer an important advantage for detecting viable microorganisms. In addition to intact phages, phage-derived proteins that recognize bacterial surfaces may also serve as biological recognition elements. However, a narrow host range may require the use of multiple phages to detect different strains. Changes in bacterial surface receptors or the development of phage resistance may also hinder the detection of target cells (Abdelhamied et al., 2023; Liu et al., 2024).

Electrochemical biosensors measure changes in current, potential, conductivity, or impedance resulting from target binding. Their compatibility with miniaturized devices, low power requirements, and limited susceptibility to sample color are important advantages. Because these sensors can be readily integrated with portable electronic systems, they have considerable potential for near-manufacturing applications. In impedimetric systems in particular, electrical changes caused by the attachment or proliferation of microorganisms on the electrode surface can be monitored without the use of labels (Abdelhamied et al., 2023; Liu et al., 2024). However, binding of product components to the electrode surface, surface fouling, and matrix-induced changes in conductivity may produce erroneous results. Therefore, appropriate electrode surface preparation and the prevention of nonspecific binding are essential. In pharmaceutical products containing proteins, lipids, surfactants, and preservatives, matrix components may coat the electrode surface and reduce target accessibility. Although surface modification and suitable blocking procedures can limit these interactions, they must not adversely affect the electrical properties of the sensor (Abdelhamied et al., 2023).

Optical biosensors operate through fluorescence, absorbance, chemiluminescence, colorimetric changes, or surface plasmon resonance. Fluorescent probes provide high sensitivity, whereas colorimetric systems allow results to be interpreted visually. The use of different fluorescent labels may enable multiple microbial targets to be investigated within a single assay. However, intrinsic product fluorescence, turbidity, or light scattering may interfere with optical measurements. Although colorimetric systems are simple and inexpensive, visual assessment of color intensity may result in only semiquantitative outcomes (Zhao et al., 2023). Surface plasmon resonance can provide real-time, label-free measurements; however, instrument cost and nonspecific binding may limit its use. Real-time monitoring of association and dissociation processes enables evaluation not only of the presence of an interaction between the target and the recognition element but also of its binding behavior (Ning et al., 2023).

Piezoelectric and mass-sensitive sensors measure changes in mass resulting from target binding to the sensor surface. These label-free systems can provide real-time analysis. However, temperature, sample viscosity, and the distribution of cells across the surface may influence the results. Measurement conditions must therefore be maintained consistently, and control systems appropriate to the sample matrix should be used (Beyazit et al., 2024).

Nanomaterials are used to increase the sensor surface area, enhance signal intensity, and separate target microorganisms from the sample. Gold and silver nanoparticles, graphene derivatives, carbon nanotubes, quantum dots, and magnetic particles are among the principal examples. Their large surface area allows a greater number of biological recognition elements to be immobilized on the sensor surface, thereby contributing to stronger signals at low target

concentrations. Gold nanoparticles can enhance sensor performance through their optical and electrochemical properties, whereas graphene and carbon nanotubes can improve performance through their high electrical conductivity (Abdelhamied et al., 2023; Beyazit et al., 2024). In particular, magnetic particles coated with target-specific molecules can facilitate the separation and concentration of microorganisms from complex product matrices. The application of a magnetic field enables target cells to be separated from other sample components, inhibitory substances to be removed, and microorganisms to be concentrated into a smaller volume. This approach can improve the limit of detection of subsequent biosensor or molecular analyses (Beyazit et al., 2024; Garlan et al., 2024; Jóskowiak et al., 2023).

Variations in nanomaterial size, shape, surface charge, and manufacturing method can affect sensor performance. Particle aggregation and batch-to-batch variability are major challenges for standardization and reproducibility. During functionalization of nanomaterial surfaces with biological recognition elements, control over immobilization density and orientation may also influence sensor specificity and signal intensity. Excessively dense immobilization of recognition elements may restrict target accessibility, whereas insufficient surface coverage may result in inadequate signal generation. In addition, particle aggregation during storage can alter target-capture capacity as well as optical or electrical properties (Abdelhamied et al., 2023; Beyazit et al., 2024).

In microfluidic systems, microliter- or nanoliter-scale fluid volumes are precisely controlled within miniature channels. Short diffusion distances reduce reaction times and reagent consumption while enabling sample preparation and measurement steps to be performed within the same device. The high surface-area-to-volume

ratio of microchannels can promote more rapid contact between target cells or molecules and sensor surfaces. Control of flow rate and channel geometry directly affects reaction efficiency and microbial recovery. The parallel use of multiple channels also enables the simultaneous analysis of different targets or samples (Dönmez et al., 2022; Garlan et al., 2024; Jóskowiak et al., 2023).

Microorganisms can be separated and concentrated using filtration, size-based separation, magnetic fields, dielectrophoresis, and specific surface binding. Filtration and size-based separation methods exploit the physical properties of cells, whereas dielectrophoresis directs cells according to differences in their polarization behavior under an electric field. Surfaces coated with antibodies or aptamers can selectively capture target cells within microchannels. In pharmaceutical samples with low microbial loads, concentrating target cells into a small volume can improve the limit of detection. Microfluidic systems integrated with magnetic nanoparticles have been shown to enable the separation and concentration of bacteria from complex samples. However, adhesion of cells to microchannel surfaces or cell loss within the channels may lead to falsely low results. Device performance should therefore be evaluated not only on the basis of the limit of detection but also by determining the proportion of microorganisms added to the sample that can be recovered at the end of the analysis (Garlan et al., 2024; Jóskowiak et al., 2023).

Lab-on-a-chip systems integrate sample preparation, lysis, amplification, identification, and signal detection on a single chip. These platforms can be combined with PCR, isothermal amplification, electrochemical sensors, or cellular analysis. The use of a closed system reduces the number of manual handling steps and the risk of contamination. Eliminating sample transfer between

different instruments can limit operator-related errors and cross-contamination. Preloaded reagent cartridges and automated result-reading systems may also contribute to greater consistency among users. However, to ensure that processes such as lysis, amplification, and signal detection function compatibly on the same chip, temperature, buffer composition, and flow conditions must be optimized collectively (Garlan et al., 2024; Wu et al., 2024).

In pharmaceutical microbiology, these systems can be applied to sterility testing, environmental monitoring, pharmaceutical water system control, and the rapid detection of specified contaminants. Performing analyses near the manufacturing area can reduce sample transportation time and delays in obtaining results. This may enable earlier recognition of potential contamination during in-process controls and allow corrective actions to be initiated without delay. However, devices intended for use in manufacturing areas must be suitable with respect to cleaning, disinfection, data integrity, and operator safety. The integration of microfluidic systems with portable imaging or magnetic detection devices offers substantial potential for applications outside conventional laboratory settings (Dönmez et al., 2022; Garlan et al., 2024; Wu et al., 2024).

Paper-based microfluidic devices operate through capillary forces and do not require external pumps. Their low cost and portability are important advantages. The porous structure of paper enables spontaneous fluid flow, while hydrophobic barriers can be used to create multiple reaction zones. Reagents can also be dried and stored on the paper substrate, allowing the devices to be prepared in a ready-to-use format. Integrating paper-based microfluidic platforms with isothermal nucleic acid amplification and lateral flow strips may enable the rapid visual detection of multiple microbial

targets. However, these systems generally provide qualitative or semiquantitative results and may be affected by colored pharmaceutical products. Although measuring color intensity with smartphone cameras can support more objective result interpretation, ambient lighting conditions and camera characteristics may introduce measurement variability. The migration of product color or turbidity into the test zone may also lead to false-positive or false-negative results (Wu et al., 2024).

In droplet-based microfluidic systems, the sample is partitioned into numerous small droplets, each of which serves as an independent reaction compartment. This approach has potential applications in single-cell analysis, rapid growth monitoring, and antimicrobial susceptibility testing. Because each droplet functions as an individual microreactor, single cells can be examined independently, allowing growth heterogeneity within the same microbial population to be assessed. Simultaneous analysis of large numbers of droplets may enable different antimicrobial concentrations to be compared within a short period. The small droplet volume facilitates the rapid accumulation of cellular metabolites, thereby supporting the early detection of microbial growth. It has been demonstrated that minimum inhibitory concentrations can be determined within a few hours by encapsulating bacteria together with different antimicrobial concentrations in droplets (Kim et al., 2024).

Air bubbles, particle accumulation, and channel blockage may occur within microchannels. Because product viscosity and particulate content can affect fluid flow, devices must be optimized for different pharmaceutical matrices. Variations in channel dimensions and surface properties introduced during microfluidic device manufacturing may also influence flow behavior and cell

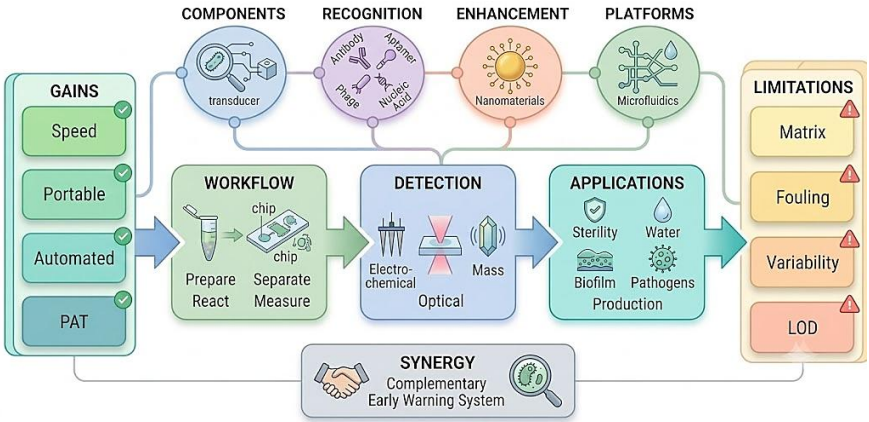
recovery rates. Air bubbles can disrupt fluid flow and cause irregular distribution of cells within the channels. In addition, some materials used to fabricate microchannels may adsorb proteins or small molecules onto their surfaces, thereby altering sample composition. Therefore, microchannel integrity, flow stability, leak tightness, and cell recovery should be included in device performance assessments (Dönmez et al., 2022; Garlan et al., 2024; Jóskowiak et al., 2023).

Because most biosensors are specific to predefined targets, they may fail to detect unexpected contaminants. Target-specific systems can therefore be used to monitor known high-risk microorganisms, whereas systems that measure overall cell growth or microbial activity may be more appropriate for broader screening. Target-specific sensors may be suitable for critical microorganisms frequently encountered in a particular product or manufacturing area. In contrast, systems that measure total cell counts or metabolic activity provide broader microbial screening but offer limited information regarding microbial identity. The analytical approach should therefore be selected according to the intended outcome of the analysis and the microbial risk being monitored. The choice of biological recognition element, such as a phage, aptamer, or antibody, directly influences the target range of the sensor and its ability to detect viable microorganisms. Antibodies and aptamers may also bind to preserved structures on nonviable cells, whereas phage-based systems generally depend on the presence of viable and susceptible bacteria. This distinction is important when interpreting sensor results in terms of microbiological risk. Biosensor, microfluidic, and lab-on-a-chip technologies enable rapid, low-volume analyses that are well suited for automation. Their combined use may allow target cells to be separated, concentrated, recognized, and measured within an integrated workflow. This can reduce analytical time and operator intervention while increasing the

likelihood of detecting low microbial loads. Nevertheless, a prototype that performs well under laboratory conditions should not be assumed to demonstrate comparable performance in complex pharmaceutical products without appropriate evaluation (Abdelhamied et al., 2023; Beyazit et al., 2024; Liu et al., 2024; Ning et al., 2023; Zhang et al., 2025).

For routine implementation, the limit of detection, matrix effects, sensor stability, instrument calibration, and comparability of results with conventional methods must be evaluated in detail. Selectivity, accuracy, precision, microbial recovery, false-positive and false-negative rates, and batch-to-batch variability in performance should also be determined. The shelf life of biological recognition elements, storage conditions for disposable cartridges, and the reliability of the software used for result interpretation are additional factors that influence the sustainability of routine use. Rather than completely replacing existing culture-based methods, these technologies can be regarded as complementary systems that enable the early detection of microbial contamination and accelerate targeted analyses. When used for rapid screening, they may facilitate the early identification of suspect samples and help prioritize culture-based confirmatory testing (Abdelhamied et al., 2023; Beyazit et al., 2024; Dönmez et al., 2022; Jóskowiak et al., 2023).

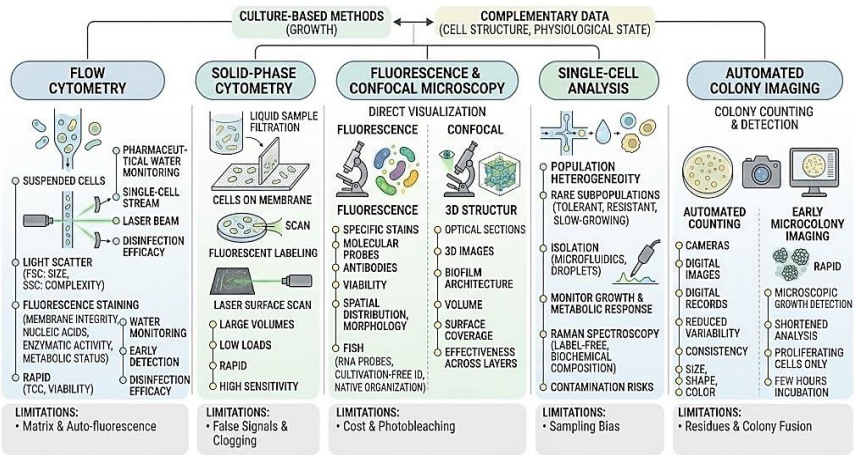
Figure 5. Schematic presentation of Biosensors and Lab-on-a-Chip Platforms in Pharmaceutical Microbiological Quality Control: Workflows, Enhancements, and Challenges



Advanced Imaging and Cellular Analysis Technologies

Advanced imaging and cellular analysis technologies can provide information on microbial abundance, viability, morphology, and metabolic status more rapidly than culture-based methods. Flow cytometry, solid-phase cytometry, fluorescence and confocal microscopy, single-cell analysis, and automated colony imaging systems are among the principal approaches in this field (Figure 6). These methods complement the information on microbial growth obtained from culture-based analyses with data on cellular structure and physiological state. They are particularly valuable for assessing stressed or damaged cells and microorganisms that proliferate slowly under culture conditions (Mhade & Kaushik, 2023; Silberreiss et al., 2026; Zand et al., 2021).

Figure 6. Overview of Advanced Microbial Imaging and Cellular Analysis Technologies



In flow cytometry, cells suspended in a liquid are passed individually through a laser beam, and their light-scattering and fluorescence characteristics are measured. Forward scatter provides information on cell size, whereas side scatter reflects cellular complexity. However, these signals alone are insufficient for microbial identification and may overlap with signals from extracellular particles of similar size. Scatter data are therefore generally interpreted in conjunction with fluorescence staining results. Fluorescent dyes enable the assessment of properties such as membrane integrity, nucleic acid content, enzymatic activity, and membrane potential (Daddy Gaoh et al., 2022; Zand et al., 2021). The combined use of membrane-permeable dyes and dyes that enter only damaged cells allows viable, injured, and dead cells to be distinguished. The use of multiple fluorescent markers also enables cells in different physiological states to be examined as distinct subpopulations within the same sample. Nevertheless, membrane integrity does not always directly reflect a cell's capacity to

proliferate. Some cells may retain an intact membrane yet fail to grow in culture, whereas others may recover and resume growth after limited membrane damage. Flow cytometry results should therefore be interpreted together with culture-based data (Lindivat et al., 2021; Zand et al., 2021).

In pharmaceutical water systems, monitoring total cell counts and cells with intact membranes can facilitate the early detection of microbial changes within the system. Sudden shifts in cell numbers or viability profiles may indicate a potential decline in system performance before such changes are reflected in culture results. Flow cytometry is also used to evaluate the effectiveness of disinfection and the effects of antimicrobial treatments on microbial cells. Comparing cell populations before and after treatment enables the monitoring of both changes in total cell counts and the extent of cellular damage (Daddy Gaoh et al., 2022; Lindivat et al., 2021; Zand et al., 2021). Particles, protein aggregates, and oil droplets present in pharmaceutical products may generate signals similar to those of microorganisms. Autofluorescent components may also interfere with the results. These effects can make it particularly difficult to distinguish microbial signals in suspensions, emulsions, and high-protein products. Appropriate controls, cell concentration procedures, and matrix removal steps may therefore be required (Daddy Gaoh et al., 2022; Zand et al., 2021).

In solid-phase cytometry, the sample is passed through a membrane filter, the cells retained on the filter are labeled with fluorescent dyes, and the surface is scanned with a laser. A major advantage of this approach is its ability to concentrate cells from large-volume samples with low microbial loads. By collecting a small number of cells on a limited filter surface, the method can improve analytical sensitivity. Furthermore, direct detection of

labeled cells without waiting for conventional colony formation can shorten the analysis time. However, product components and fluorescent particles retained on the filter may generate false signals. Filter clogging or uneven distribution of cells across the surface may also affect cell detection. Therefore, filter characteristics and sample preparation conditions should be optimized according to the product being analyzed (Silberreiss et al., 2026).

Fluorescence microscopy enables the direct visualization of microorganisms using nucleic acid stains, antibodies, molecular probes, and viability markers. Pharmaceutical water samples, surface samples, and biofilms can be examined using this method. In addition to cell number and morphology, the spatial distribution of cells within a sample or across a surface can be assessed directly. The combined use of probes emitting signals at different wavelengths may enable multiple microbial groups or cellular characteristics to be examined within the same image (Barbosa et al., 2023; Mhade & Kaushik, 2023). Fluorescence in situ hybridization uses labeled probes that bind to ribosomal RNA regions. This approach enables specific microbial groups to be identified without cultivation and their locations within the sample to be visualized. Target-specific probes can help distinguish morphologically similar cells on the basis of their molecular characteristics. Moreover, the spatial distribution of microorganisms within biofilms or complex microbial communities can be examined while preserving their native organization. However, low ribosomal content, limited cell permeability, and autofluorescence may reduce sensitivity. Probe selection, permeabilization, and hybridization conditions must therefore be optimized for the target microorganism (Barbosa et al., 2023).

Confocal laser scanning microscopy generates three-dimensional images by acquiring optical sections at different depths. It is particularly useful for examining biofilm thickness, cellular distribution, and the structure of the extracellular matrix. By reconstructing sequential optical sections, biofilm volume, surface coverage, and structural heterogeneity can be evaluated. This capability facilitates the separation of overlapping structures that are difficult to distinguish using conventional microscopy. When combined with viability stains, confocal microscopy can be used to assess the effects of disinfectants across different biofilm layers. This allows determination of whether cells located at the surface and in deeper layers respond differently to the applied treatment (Barbosa et al., 2023; Jelli et al., 2023; Mhade & Kaushik, 2023). Confocal imaging is limited by high cost, complex sample preparation, and time-consuming image analysis. Uneven penetration of fluorescent dyes into the biofilm, photobleaching, and operator-dependent image analysis may affect the results. In thick biofilms, insufficient penetration of fluorescent dyes or laser light into deeper layers may lead to underestimation of cell abundance. Standardization of image acquisition, segmentation, and threshold settings is essential for the reliability of quantitative results. Parameters such as laser power, image resolution, and optical section spacing should be kept constant across experimental groups (Jelli et al., 2023; Mhade & Kaushik, 2023).

Single-cell analyses are based on the recognition that cells within a microbial population do not necessarily share the same physiological state. Cells within the same culture may differ in growth rate, metabolic activity, and antimicrobial susceptibility. Population-averaged analyses may obscure the characteristics of small but biologically important subpopulations. Single-cell technologies enable the detection of rare tolerant, resistant, or slow-

growing cells. In particular, identifying cells that remain viable after antimicrobial treatment is important for assessing the risk of contamination recurrence (Jelli et al., 2023; Zhang et al., 2024). Microfluidic compartments, optical systems, droplet-based platforms, and single-cell Raman spectroscopy can be used for this purpose. Isolating cells in separate compartments allows their growth and metabolic responses to be monitored independently of other cells. Exposing individual cells to different antimicrobial concentrations in separate compartments enables rapid monitoring of growth and metabolic responses. Single-cell Raman spectroscopy, in turn, can provide information on cellular biochemical composition without the need for labeling. However, the limited number of cells examined may reduce the extent to which the findings represent the entire population. Cell selection and cell loss during analysis may also introduce sampling bias. Therefore, a sufficient number of cells and independent experimental replicates should be evaluated (Jelli et al., 2023; Zhang et al., 2024).

Automated colony counting and imaging systems detect and enumerate colonies by analyzing digital images of agar surfaces. These systems reduce operator-dependent variability in counting and enable small or closely spaced colonies to be evaluated more consistently. Recording digital images also allows results to be reviewed retrospectively and colony characteristics such as size, shape, and color to be compared. However, system performance may vary depending on colony density, microbial morphology, and the culture medium used. Colonies in contact with one another may be recognized as a single colony, whereas precipitates or air bubbles in the medium may be incorrectly classified as colonies (Heuser et al., 2023; Ma et al., 2023).

Early microcolony imaging detects microscopic growth before colonies become visible to the naked eye. This approach preserves the culture-based principle while shortening the analysis time. Automated detection of small cell clusters formed after a brief incubation period can substantially reduce the prolonged waiting times associated with conventional counting methods. Because only proliferating cells are detected, the risk of positive results caused by DNA from dead cells is reduced. However, cells that have sustained sublethal injury or require an extended adaptation period may fail to form microcolonies within the specified detection window. Optical imaging combined with automated analysis has been shown to enable the detection of microorganisms at the microcolony stage after only a few hours of incubation (Ma et al., 2023).

Product residues, irregular colony morphologies, mold growth, and colonies in contact with one another may complicate image analysis. Residues left by colored or particulate-containing products on filters or culture media can reduce image contrast. In addition, small, transparent, or spreading colonies may not be adequately distinguished using standard imaging parameters. These systems should therefore be validated using different culture media, microorganisms, and product matrices. Validation should assess counting accuracy, precision, limit of detection, microbial recovery, and agreement with conventional methods (Heuser et al., 2023; Ma et al., 2023).

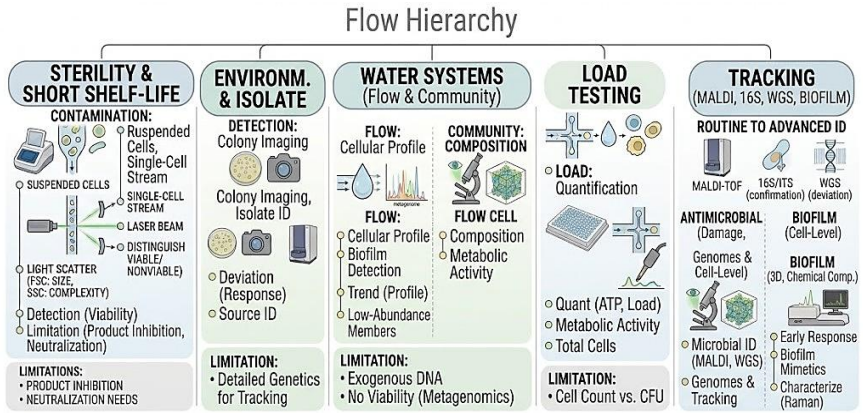
Advanced imaging and cellular analysis technologies enable detailed evaluation of the physiological characteristics of microbial cells and the structural organization of biofilms. Flow cytometry and solid-phase cytometry allow rapid assessment of cell counts and viability status, whereas fluorescence and confocal microscopy enable visualization of cellular distribution within samples and

characterization of biofilm architecture. Single-cell analyses reveal heterogeneity within microbial populations, while automated imaging systems may accelerate the evaluation of culture-based results. However, because viability indicators do not always directly reflect proliferative capacity, the findings should be interpreted in conjunction with culture-based methods. Sample matrix effects, fluorescent markers, instrument settings, and image analysis parameters may also influence result reliability; therefore, these methods should be validated according to their intended use (Lindivat et al., 2021; Mhade & Kaushik, 2023; Silberreiss et al., 2026; Zand et al., 2021).

Applications in Pharmaceutical Microbiology

Next-generation technologies can be applied to sterility testing, microbial load testing, environmental monitoring, pharmaceutical water systems, microbial identification, source tracking, antimicrobial susceptibility testing, and biofilm analysis. However, because each technology measures a different biological characteristic, method selection should be based on the analytical objective and the properties of the product. Some methods assess microbial proliferation, whereas others evaluate cellular viability, metabolic activity, the presence of nucleic acids, or structural characteristics of microorganisms. Results obtained using different methods should therefore not be regarded as directly equivalent but should be interpreted in the context of the specific biological property being measured (Figure 7). (Gebo & Lau, 2020; van Hoogstraten et al., 2024; Venhuizen et al., 2024).

Figure 7. Technological Flow Hierarchy for Microbial Identification and Risk-Based Control in Pharmaceutical Systems.



Sterility testing is one of the most critical quality control analyses for sterile products. Because conventional methods require prolonged monitoring of microbial growth, rapid methods are particularly needed for products with short shelf lives, such as cell and gene therapy products. Delayed availability of test results may pose a significant challenge with respect to product usability and patient safety. Rapid methods can facilitate the earlier detection of contamination during manufacturing and support more timely quality decisions. Real-time PCR, isothermal amplification, solid-phase cytometry, bioluminescence, and early microcolony imaging can be used for rapid sterility testing. Molecular methods provide high sensitivity within a short period but are limited in their ability to distinguish viable from nonviable microorganisms. Microcolony imaging detects only microorganisms capable of proliferation, although it still requires a brief incubation period. Therefore, the speed advantage of the method, its capacity to detect viable microorganisms, and its compatibility with the product matrix

should be evaluated collectively (Deutschmann et al., 2023; Gebo & Lau, 2020; Silberreiss et al., 2026).

The antimicrobial properties of a product may interfere with sterility testing. Preservatives, antibiotics, and other product components may suppress microbial growth and may also disrupt PCR, fluorescence, or sensor-based signals. Whereas inhibition in culture-based methods can lead to false-negative results, interference in molecular and optical systems may either reduce or enhance the detected signal. Accordingly, it is necessary to evaluate not only the effect of the product on microbial growth but also its potential impact on the detection system itself. Product-specific procedures such as neutralization, dilution, filtration, or cell concentration should therefore be developed. It is essential that sample preparation steps do not reduce microbial recovery or adversely affect the method's limit of detection (Deutschmann et al., 2023; Gebo & Lau, 2020; Venhuizen et al., 2024).

Flow cytometry, ATP bioluminescence, impedance-based systems, and automated microcolony imaging can be used for microbial load testing. Flow cytometry enables the detection of total cells or cells with intact membranes, ATP bioluminescence reflects metabolic activity, and microcolony imaging allows the early detection of proliferating cells. However, total cell counts, numbers of metabolically active cells, and colony-forming unit values do not represent the same biological measurement. Acceptance criteria should therefore be established according to the specific parameter measured by the method. The fact that a new method does not produce results identical to those obtained by conventional colony counting does not, by itself, indicate inadequate performance. The key requirement is to relate the measured parameter to meaningful

limits in terms of product quality and microbial risk (Lindivat et al., 2021; Venhuizen et al., 2024; Zand et al., 2021).

In environmental monitoring programs, automated colony imaging, MALDI-TOF MS, and molecular methods can shorten analytical turnaround times. Early detection of colonies and rapid identification of isolates enable a more timely response to microbiological deviations. This may facilitate assessment of potential contamination within the manufacturing environment before it spreads and allow corrective actions to be initiated more rapidly. Automated imaging systems reduce operator-dependent variability in colony counting, whereas rapid identification methods support early assessment of the potential risk associated with an isolate. Comparing environmental isolates across different time points and sampling locations can help identify persistent sources of contamination. Repeated recovery of the same microorganism may indicate a problem associated with the water system, equipment, personnel, or cleaning practices. However, isolates identified as the same species should not necessarily be assumed to originate from the same contamination source. More detailed genetic comparisons can determine whether recurrent findings are linked to a common source or represent independent contamination events. MALDI-TOF MS can be used for routine identification, whereas whole-genome sequencing is suitable for detailed source tracking (Guimarães et al., 2023; Heuser et al., 2023; Ma et al., 2023; Mazhari et al., 2025; Song et al., 2024).

In pharmaceutical water systems, flow cytometry, molecular methods, and metagenomic analyses can reveal cell populations that remain undetected by culture-based methods. Sudden changes in total cell counts may serve as early indicators of problems such as biofilm formation, inadequate sanitization, or system stagnation.

These technologies can also support the monitoring of changes caused by microorganisms that require prolonged incubation or fail to proliferate on the selected culture media. Evaluating data obtained from routine measurements over time may provide more meaningful information than interpreting a single result in isolation (Daddy Gaoh et al., 2024; Venhuizen et al., 2024).

Flow cytometry results should not be compared directly with pharmacopeial colony count limits. Instead, it is more meaningful to establish the normal cellular profile of the water system and monitor deviations over time through trend analysis. Because each water system differs in design, usage intensity, temperature, and sanitization program, normal cellular ranges should be defined on a system-specific basis. Alert and action levels can be established by evaluating historical data in conjunction with conventional microbiological results (Venhuizen et al., 2024).

Metagenomic analyses can characterize the microbial community structure of water systems independently of culture. This approach enables the examination not only of dominant microbial groups but also of low-abundance community members or microorganisms that cannot be detected under routine culture conditions. Changes in microbial community composition can be compared across different points within the system or before and after sanitization. However, exogenous DNA contamination and the inability to obtain direct information on microbial viability are important limitations in low-biomass samples. Even trace amounts of DNA originating from reagents or the laboratory environment may affect the results. Metagenomic data should therefore be supported by culture-based and cellular analyses (Daddy Gaoh et al., 2024; Fierer et al., 2025; Shikama et al., 2025).

Accurate identification of microorganisms is essential for assessing product-related risk. Different microorganisms present at the same concentration may pose distinct risks in terms of pathogenicity, antimicrobial resistance, toxin production, or the ability to degrade product components. The capacity of a microorganism to proliferate within the product, withstand preservative systems, or form biofilms in the manufacturing environment should also be considered in the risk assessment. Microbial load results should therefore be interpreted not only quantitatively but also in conjunction with the characteristics of the identified microorganism (Guimarães et al., 2023; Mazhari et al., 2025; Song et al., 2024).

MALDI-TOF MS can be used for routine identification, 16S rRNA gene or ITS sequencing for confirmation, and whole-genome sequencing for advanced source tracking. This tiered approach provides a balance between cost and analytical depth. MALDI-TOF MS is suitable for identifying large numbers of isolates within a short period, whereas sequencing methods can be used to confirm isolates with low-confidence identifications or those that are insufficiently represented in reference databases. Whole-genome sequencing, in contrast, provides the high discriminatory power required primarily for deviation and contamination investigations rather than routine identification. By comparing the genetic relatedness of isolates recovered from products, the environment, personnel, and water systems, whole-genome sequencing enables investigation of potential contamination routes. It can also determine more reliably whether isolates with similar colony morphologies originate from the same or different sources (Mazhari et al., 2025; McGalliard et al., 2024).

Next-generation technologies can also accelerate antimicrobial susceptibility testing. Flow cytometry can rapidly assess membrane damage and metabolic activity, microfluidic systems can monitor single-cell growth, and Raman and FTIR spectroscopy can detect cellular biochemical changes. These approaches may reveal early cellular responses to antimicrobial agents without requiring visible microbial growth. Single-cell analyses, in particular, can help identify subpopulations with differing susceptibility profiles within the same microbial population. However, these findings must be correlated with standard minimum inhibitory concentration values. Because the detection of cellular damage or metabolic alterations does not necessarily indicate complete loss of proliferative capacity, these methods should be validated through comparison with standard susceptibility testing procedures (Kassem et al., 2023; Rebrosova et al., 2022; Reszetnik et al., 2024).

Biofilms can serve as persistent sources of contamination in pharmaceutical water systems, pipelines, and manufacturing equipment. The biofilm matrix can protect microorganisms from disinfectants and environmental stresses, enabling their long-term persistence within the system and the intermittent release of planktonic cells into the surrounding environment. Therefore, when recurrent microbial findings are investigated, biofilm formation should be assessed in addition to free-living cells. Confocal microscopy can visualize biofilm architecture in three dimensions, whereas molecular methods can characterize community composition and identify antimicrobial resistance genes. Raman and FTIR spectroscopy can provide information on the chemical composition of the biofilm matrix (Barbosa et al., 2023; Mhade & Kaushik, 2023; van Hoogstraten et al., 2024).

No single method is sufficient for biofilm investigations. More reliable results can be obtained by combining the identification of recurrent isolates, monitoring of changes in cell counts, imaging, and molecular source tracking. Imaging methods reveal the structural characteristics of biofilms, whereas culture-based and cellular analyses provide information on viable or proliferating cells. Molecular methods, in turn, enable assessment of community composition and potential antimicrobial resistance characteristics. Integrated interpretation of these data supports a more accurate determination of biofilm presence and its role in contamination (Daddy Gaoh et al., 2024; Jelli et al., 2023; Mazhari et al., 2025; van Hoogstraten et al., 2024).

Next-generation technologies not only accelerate microbiological quality control but also provide more detailed information on manufacturing systems. These methods can support the early detection of microbial changes, investigation of contamination sources, and evaluation of microbiological trends within the manufacturing system over time. This enables the development of a more comprehensive control system in which data obtained from different stages of the manufacturing process are evaluated collectively, rather than relying solely on end-product testing. However, it should be recognized that target-specific methods may fail to detect unexpected microorganisms, molecular analyses do not provide direct information on viability, and cellular methods may be affected by the product matrix. These methods should therefore be used in combination within a risk-based control strategy. The strengths and limitations of each method should be considered, and results obtained using rapid methods should be supported, when necessary, by culture-based, identification, or confirmatory analyses (Deutschmann et al., 2023; Gebo & Lau, 2020; van Hoogstraten et al., 2024; Venhuizen et al., 2024).

Validation, Limitations, and Future Perspectives

For next-generation methods to be implemented in routine pharmaceutical quality control, their suitability for the intended use must be scientifically demonstrated. Because the performance requirements for sterility testing, microbial load testing, identification, and environmental monitoring differ, the validation approach should be defined according to the quality decision the method is intended to support. When the same technology is used for a different analytical purpose, the performance characteristics requiring evaluation may also change. Therefore, the validation plan should be developed not on the basis of the instrument or technology itself, but according to the intended role of the method's results within the quality control process (Deutschmann et al., 2023; Marsit et al., 2025; Nonaka et al., 2025; Venhuizen et al., 2024).

Validation evaluates accuracy, precision, specificity, sensitivity, limit of detection, limit of quantification, linearity, analytical range, robustness, and repeatability. For qualitative methods, the primary consideration is the correct classification of positive and negative results; for quantitative methods, it is the extent to which the measurement reflects the true microbial load; and for identification systems, it is the accurate discrimination of species. Not every performance characteristic is expected to be equally important for all methods. For example, reliable detection of low numbers of microorganisms is a priority in sterility testing, whereas counting accuracy and analytical range may be more critical in microbial load testing (Deutschmann et al., 2023; Marsit et al., 2025).

Specificity studies should demonstrate whether the target microorganism can be distinguished from closely related species, product components, and environmental particles. Cross-

amplification in molecular methods, nonspecific binding in biosensors, similar profiles in spectroscopic systems, and particulate interference in cellular analyses may produce erroneous results. Such interferences may lead not only to false-positive results but also to false-negative results by suppressing the true microbial signal. Therefore, in addition to the target microorganism, closely related species, product blanks, negative controls, and potentially interfering components should be included in the validation study (Deutschmann et al., 2023; Marsit et al., 2025; Silberreiss et al., 2026).

Validation should be performed not only with reference strains but also with environmental isolates encountered in pharmaceutical manufacturing environments and with actual product matrices. Otherwise, a method that performs successfully under laboratory conditions may not demonstrate the same performance in routine application. Environmental isolates may differ from reference strains in terms of stress tolerance, growth rate, surface properties, and metabolic activity. Similarly, the physical and chemical characteristics of the product may affect microbial recovery and signal generation. Validation samples should therefore represent the method's actual conditions of use as closely as possible (Deutschmann et al., 2023; Nonaka et al., 2025).

The limit of detection is particularly critical in sterility testing and low-level microbial load analyses. Cell concentration and short-term enrichment can improve sensitivity; however, they may prolong the analysis time and result in cell loss. Although procedures such as filtration, centrifugation, or microfluidic concentration can facilitate the detection of low numbers of cells, cell adhesion to surfaces or damage during processing may reduce recovery rates. Short-term enrichment allows viable microorganisms to proliferate but may

partially diminish the speed advantage of the analysis. An appropriate balance must therefore be established between speed and sensitivity (Deutschmann et al., 2023; Nonaka et al., 2025; Silberreiss et al., 2026).

Comparison of an alternative method with the pharmacopeial method is important. However, when different methods measure distinct biological characteristics, their results should not be expected to be identical. Plate counting detects proliferating cells, flow cytometry assesses membrane integrity, bioluminescence measures metabolic activity, and PCR detects the presence of nucleic acids. Differences among methods should therefore not be interpreted solely as numerical discrepancies. Instead, it should be determined whether the alternative method identifies microbiological risk with a level of reliability equivalent or superior to that of the pharmacopeial method. Comparative studies should consider the microbial species, inoculum level, product matrix, and physiological state of the cells. Equivalence should be evaluated in light of these differences (Deutschmann et al., 2023; Lindivat et al., 2021; Venhuizen et al., 2024; Zand et al., 2021).

Pharmaceutical product matrices can substantially affect the performance of next-generation methods. Preservatives and proteins may interfere with amplification, colorants with optical measurements, surfactants with biosensors, and particulates with microfluidic systems. High viscosity, low or high pH, salt concentration, and intrinsic product fluorescence may also alter sample transfer or signal detection. Consequently, the same method cannot always be applied directly across different pharmaceutical products. Sample preparation procedures should therefore be developed specifically for each product type. The effects of dilution, neutralization, filtration, or extraction procedures on method

sensitivity and microbial recovery must also be demonstrated (Marsit et al., 2025; Nonaka et al., 2025; Silberreiss et al., 2026).

Distinguishing viable from nonviable cells remains a major challenge. DNA-based methods may detect dead cells, whereas membrane integrity or metabolic activity does not necessarily provide definitive evidence of proliferative capacity. Residual nucleic acids from dead cells may continue to generate signals in molecular assays for extended periods. Conversely, stressed but viable cells may exhibit low metabolic activity or temporarily lose the ability to proliferate. This issue complicates the interpretation of results, particularly in assessments of disinfection efficacy and low-level microbial load analyses. The biological meaning of the viability indicator used should therefore be clearly defined (Lindivat et al., 2021; Marsit et al., 2025; Venhuizen et al., 2024; Zand et al., 2021).

Sample preparation protocols, instrument settings, signal thresholds, data processing steps, and reference databases may vary among laboratories. Standardized protocols, interlaboratory comparisons, and appropriate reference materials are therefore required. Differences in signal threshold settings may cause the same sample to be classified as positive in one laboratory and negative in another. Similarly, software algorithms and data preprocessing methods may affect quantitative results. Standardization should encompass the entire analytical process, from sample preparation to result reporting, rather than being limited to instrument operation alone (Deutschmann et al., 2023; Marsit et al., 2025; Nonaka et al., 2025; Yang et al., 2023).

In MALDI-TOF MS, genomic analyses, and spectroscopic methods, the scope of the reference database directly affects identification performance. The limited representation of

microorganisms specific to pharmaceutical manufacturing environments in commercial databases may necessitate the development of in-house reference libraries. However, when establishing such databases, reference identifications must be verified, spectra must be acquired under standardized conditions, and the database must be updated regularly. Inclusion of an incorrectly or incompletely identified reference entry may lead to systematic errors in subsequent analyses. Therefore, isolates added to the database should be confirmed using reliable molecular methods, and routine performance checks should be conducted with quality control strains. Compatibility of data generated across different instruments and laboratories should also be taken into account (Chudzik et al., 2024; Guimarães et al., 2023; Song et al., 2024).

The cost of new systems should not be evaluated solely in terms of the initial instrument investment. Indirect benefits, such as shorter product hold times, reduced labor requirements, faster deviation investigations, and prevention of production losses, should also be considered. Conversely, the costs associated with instrumentation, consumables, validation, personnel training, and regulatory submissions may affect method feasibility. Testing frequency, annual sample volume, instrument utilization capacity, and maintenance requirements should also be included in the economic assessment. A system with a high initial cost may provide long-term advantages when sample throughput is high and product release times are substantially reduced. However, the same investment may not be economically justified in laboratories with low sample volumes (Deutschmann et al., 2023; Marsit et al., 2025).

Data integrity becomes increasingly important with the implementation of automation. Raw data must be retained, user

activities monitored, changes to results documented, and software validated. Access to raw data should be ensured in addition to the final report generated by the instrument. Although automated data processing can reduce operator-dependent errors, deficiencies in software algorithms or user authorization controls may compromise result reliability. Electronic records must be secured with respect to timestamps, audit trails, backup procedures, and access controls. Automated reading systems can reduce operator-dependent variability in result interpretation and minimize the need for manual data transfer (Deutschmann et al., 2023).

In the future, systems that integrate sample preparation, cell concentration, amplification, and signal detection within closed cartridges are expected to become increasingly widespread. The integration of microfluidic platforms with biosensors, isothermal amplification, and cellular analysis will facilitate the development of rapid tests for near-manufacturing applications. Closed systems can reduce sample transfer steps, thereby limiting the risk of external contamination and operator-dependent variability. The incorporation of standardized reagents into disposable cartridges may also contribute to greater comparability of results across laboratories. However, factors such as cell recovery within the cartridge, reagent stability, and compatibility with different product matrices will require careful evaluation (Jóskowiak et al., 2023; Marsit et al., 2025; Nonaka et al., 2025).

Continuous microbiological monitoring systems will also become increasingly important. Current sampling strategies based on measurements taken at predefined intervals may fail to capture short-term changes occurring between sampling points. Continuously operating sensors, flow cytometry, and automated imaging systems can provide early warning signals and shift quality

control from a reactive to a preventive approach. Monitoring time-dependent changes in cell counts and analytical signals may help establish the normal microbiological profile of a manufacturing system and enable minor deviations to be detected at an early stage. This approach is particularly important for identifying contamination trends in pharmaceutical water systems and continuous manufacturing lines. However, appropriate alert thresholds and data analysis models must be developed to interpret the large volumes of data generated (Lindivat et al., 2021; Venhuizen et al., 2024; Zand et al., 2021).

Portable devices may offer particular advantages, particularly for short-shelf-life products, aseptic manufacturing areas, and cell therapy centers. Performing the analysis at the manufacturing or point-of-use site, without sending samples to a centralized laboratory, can reduce transportation time and minimize changes that may occur during sample handling and transfer. These systems may also enable rapid preliminary assessment when contamination is suspected. However, portable devices must meet the same performance requirements as laboratory-based systems and should remain unaffected by environmental conditions. The effects of variables such as temperature, humidity, vibration, and operator experience on the accuracy of portable systems should be demonstrated during validation (Deutschmann et al., 2023; Nonaka et al., 2025; Wu et al., 2024).

In the future, microbiological requirements are more likely to be addressed through the combined use of different methods than by a single technology. Biosensors may provide early warning, MALDI-TOF MS may enable rapid identification, genomic analyses may support source tracking, and culture-based methods may be used to isolate viable microorganisms. Within this complementary

approach, rapid methods can detect potential microbial changes at an early stage, while culture-based and advanced identification methods can confirm the finding and support investigation of the contamination source. In this way, the limitations of one method can be balanced by the information provided by another (Deutschmann et al., 2023; Song et al., 2024; Venhuizen et al., 2024).

The widespread adoption of next-generation technologies will depend on the concurrent advancement of validation, standardization, economic feasibility, and regulatory acceptance. Pharmaceutical microbiological quality control is expected to evolve from culture-based end-point testing toward faster, continuous, and risk-based monitoring systems. However, rather than being completely replaced, culture-based methods are more likely to remain in use alongside rapid methods for confirmatory testing and microbial isolation. In the future, integrating analytical results with manufacturing process data, environmental monitoring findings, and water system data will support earlier and more comprehensive assessment of microbiological risks (Deutschmann et al., 2023; Marsit et al., 2025; Nonaka et al., 2025; Venhuizen et al., 2024).

CHAPTER 4:
CONCLUSUION

The development and ensuring the safety of natural and synthetic pharmaceutical products are of critical importance for the protection of human health. In this context, AI, ML, and DL technologies are increasingly gaining importance in the field of pharmaceutical microbiology thanks to their high analytical capacity and data processing speeds. Traditional microbiological methods have been used as standard techniques for many years and form the basis of quality and safety processes. However, the long incubation times required for analyses, particularly sterility tests, limit these methods in urgent clinical and industrial settings. Furthermore, manual evaluation of colonies in culture plates can lead to human error and data bias.

Data-driven artificial intelligence applications developed to overcome these limitations offer significant opportunities in pharmaceutical microbiology. Image analysis techniques allow for the rapid evaluation of microscopic images and colony formations on agar plates, thus speeding up analysis processes and optimizing workflows. In addition, the analysis of genomic data using artificial intelligence algorithms enables the highly accurate prediction of antimicrobial resistance profiles of microorganisms. Among the

main contributions of artificial intelligence technologies to pharmaceutical microbiology are the efficient analysis of large datasets and the automation of quality control processes. In particular, the real-time monitoring and prediction of contamination risks in production environments contributes to strengthening the relationship between product safety and patient safety.

However, artificial intelligence applications also have some limitations. The "black box" nature of deep learning models makes interpreting the results difficult and raises various concerns regarding reliability. Furthermore, the performance of the models is directly dependent on the quality of the datasets used; working with incomplete or erroneous data can lead to incorrect results. In this context, several suggestions are presented to enable the more effective use of artificial intelligence applications in pharmaceutical microbiology. Firstly, it is necessary to strengthen the digital microbiology infrastructure and increase research and development investments in advanced technologies. Secondly, developing explainable artificial intelligence approaches is crucial to make the results more reliable and understandable. Finally, existing legal regulations need to be updated to include AI-based systems.

The need for rapid and reliable analyses in pharmaceutical microbiology has made it necessary to complement conventional culture-based methods with next-generation technologies. Molecular diagnostics, sequencing, mass spectrometry, spectroscopy, biosensors, microfluidic systems, and cellular analysis technologies offer valuable capabilities for the detection, identification, enumeration, and source tracking of microorganisms.

The contribution of these methods extends beyond reducing analytical turnaround times. Culture-independent characterization of microbial communities, assessment of cellular physiological states,

visualization of biofilm architecture, and strain-level source tracking have enabled a more comprehensive approach to microbiological quality control. Nevertheless, no single next-generation method can meet all analytical requirements. The limitations of nucleic acid–based methods in distinguishing viable from nonviable cells, the target specificity of biosensors, the susceptibility of spectroscopic methods to matrix effects, and the extensive data analysis required for sequencing technologies necessitate careful interpretation of the results. Culture-based methods remain essential for isolating viable microorganisms and evaluating their phenotypic characteristics. Therefore, conventional and next-generation methods should be regarded not as alternatives, but as complementary approaches. Method selection should be based on product characteristics, the analytical objective, the required turnaround time, and regulatory requirements, and appropriate validation and standardization should be established under actual sample conditions.

In conclusion, artificial intelligence technologies are considered not as replacements for traditional methods in pharmaceutical microbiology, but rather as complementary tools that support and enhance the effectiveness of these methods. It is anticipated that, with appropriate data infrastructure and regulatory frameworks, these technologies will make significant contributions to the development of faster, more reliable, and more efficient microbiological analysis processes.

In the future, portable biosensors, microfluidic platforms, automated imaging, and continuous monitoring systems are expected to become increasingly integrated. These advances will contribute to the transformation of pharmaceutical microbiological quality control into a faster, more preventive, and risk-based framework. This transformation will not only enable the early

detection of biological risks, but will also significantly improve operational cost effectiveness by minimizing downtime and product losses in production processes.

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