

**An Integrated Approach to Clinical
Pathology: Diagnostic Principle and
Laboratory Aspects**

Edited by

Dr. Oly Banerjee



Swami Vivekananda University

Published by

Penprints
India

***An Integrated Approach to Clinical Pathology: Diagnostic
Principle and Laboratory Aspects***

Edited by Dr. Oly Banerjee

Published by Penprints

Official Address: P-925, Lake Twon, Block - A, Kolkata-700048,
West Bengal, India.

Phone no-8697875467

Website - www.penprints.in

Copyright - Swami Vivekananda University

Cover - ©PENPRINTS

Price: INR 500 | \$22

First Edition - 2025

ISBN: 978-81-995654-8-7

ISBN 10 : 81-995654-8-9

All rights reserved, No part of this publication may be reproduced, distributed or transmitted in any form or by any means, including photocopying, recording, or other electronic or mechanical methods, without the prior written permission of the publisher and the author, except in the case of brief quotations embodied in critical reviews and certain other non-commercial uses permitted by copyright law. All legal matters concerning the publication/publisher shall be settled under the jurisdiction of Calcutta High Court only.

Printer: S. P. Communications Pvt. Ltd. 1st Floor, Merlin Marigold, 31B, Raja
Dinendra St, Yogi Para, Garpar, Machuabazar, Kolkata, West Bengal 700009

Acknowledgement

The culmination of this scholarly work is a testament to the collective encouragement and support we have been fortunate to receive. We wish to place on record our deepest sense of gratitude towards the leadership of our university, whose stewardship made this publication possible.

We are profoundly grateful for the visionary outlook of our Hon'ble Chancellor, Dr. Nandan Gupta. Our sincere appreciation extends to our Hon'ble Vice-Chancellor, Professor (Dr.) Subrata Kumar Dey, for his insightful mentorship. The project benefited significantly from the operational support of our Chief Operating Officer, Shri Saurabh Adhikari, and the academic direction of our Chief Executive Director (Academics), Professor (Dr.) Deb Narayan Bandyopadhyay. We also acknowledge with thanks the meticulous efforts of our Registrar, Dr. Pinak Pani Nath, in facilitating this endeavour.

Furthermore, we are indebted to our colleagues at Swami Vivekananda University, whose academic camaraderie has been a source of great strength. Lastly, we extend our warmest thanks to all the contributors and collaborators; their commitment and expertise are the very foundation upon which this volume stands.

- *Dr. Oly Banerjee*

Contents

Leveraging Neuroplasticity for Enhanced Rehabilitation: Mechanisms and Innovations

Oly Banerjee 5

Alzheimer's disease: pathogenesis, diagnosis, and therapeutic strategies

Rajen Dey, Manojit Bysack 14

Emerging Biomarkers in Cardiovascular Disease: Advances in Early Diagnosis and Novel Molecular Indicators

Sibu Jana 28

Automation in Clinical Laboratories: Bridging Manual to Automated Precision

Sudip Garai 41

A Comprehensive Review on the Molecular Basis of Microbial Pathogenesis of Viral and bacterial infections

Sanjana Sengupta, Rajen Dey, Manojit Bysack 51

Biofilm-Associated Hospital Acquired Infection: Epidemiology, Diagnosis and Management- A Review

Santanu Ojha 63

A review on Urine Analysis: Decoding Conventional & Recent Techniques in Diagnosing Urinary Tract Infection

Rupak Bera 81

Leveraging Neuroplasticity for Enhanced Rehabilitation: Mechanisms and Innovations

Oly Banerjee

Department of Anaesthesia and Operation Theatre
Technology, School of Allied Health Sciences, Swami
Vivekananda University, Telinipara, Barasat-Barrackpore
Rd, Bara Kanthalia, West Bengal - 700121, India.

Abstract

Neuroplasticity, the brain's ability to reorganize itself by forming new neural connections, plays a critical role in rehabilitation following neurological injury or disease. This dynamic process allows the brain to adapt to changes, recover lost functions, and improve cognitive and motor skills. Advances in neuroscience have revealed the mechanisms underlying neuroplasticity, including synaptic plasticity, neuronal growth, and the modulation of brain networks. Rehabilitation strategies leverage these principles to enhance recovery outcomes. Techniques such as physical therapy, occupational therapy, and cognitive training aim to stimulate neuroplasticity by engaging patients in targeted, repetitive tasks that promote functional improvements. Emerging approaches, including brain-computer interfaces, neurostimulation, and virtual reality, are showing promise in further enhancing neuroplasticity and rehabilitation efficacy. Research continues to explore how factors such as age, severity of injury, and individual differences affect neuroplastic responses, and how to optimize rehabilitation protocols accordingly. Understanding the interplay between neuroplasticity and rehabilitation not only advances therapeutic strategies but also provides insights into how the brain adapts and recovers from injury. As our knowledge of neuroplasticity expands, so too does the potential for more effective and personalized rehabilitation interventions, ultimately improving patient outcomes and quality of life.

Keywords:

Neuroplasticity, Rehabilitation, Synaptic Plasticity, Brain Recovery, Neurostimulation

Introduction

Neuroplasticity, the brain's remarkable ability to reorganize and adapt by forming new neural connections, is a fundamental mechanism underlying learning, memory, and recovery from brain injuries. This property has profound implications for rehabilitation, especially in conditions involving neurological damage, such as stroke, traumatic brain injury, and neurodegenerative diseases. By leveraging neuroplasticity, clinicians and researchers can devise innovative therapeutic strategies that enhance recovery and functional restoration in patients suffering from such conditions.

This review provides a comprehensive overview of the mechanisms underpinning neuroplasticity, examines various rehabilitation strategies that harness these mechanisms, and highlights recent innovations that offer promising advancements in the field. Understanding how neuroplasticity can be manipulated to optimize recovery can pave the way for more effective interventions, ultimately improving patients' quality of life.

Mechanisms of Neuroplasticity

Neuroplasticity is driven by several biological processes, including synaptic plasticity, cortical remapping, and structural changes at the cellular level. These mechanisms work synergistically to reorganize neural circuits and compensate for lost or damaged functions.

1. Synaptic Plasticity

Synaptic plasticity refers to the strengthening or weakening of synapses, the junctions between neurons, in response to activity. Two key forms of synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD), which involve the persistent strengthening and weakening of synapses, respectively. LTP is often associated with learning and memory, while LTD is thought to play a role in synaptic

pruning and refinement of neural circuits (Bliss & Lømo, 1973).

In the context of rehabilitation, therapies that stimulate LTP can promote the formation of new neural pathways and enhance the recovery of motor and cognitive functions. Techniques such as repetitive task practice and motor imagery have been shown to induce LTP in motor networks, thereby improving functional outcomes after brain injury (Nudo, 2013).

2. Cortical Remapping

Cortical remapping, also known as functional reorganization, occurs when intact regions of the brain take over the functions previously performed by damaged areas. This process is critical for recovery following brain injuries like stroke, where the primary motor cortex may be compromised, but neighboring areas can compensate by adopting new roles (Cramer et al., 2011).

Functional imaging studies have demonstrated that rehabilitation interventions such as constraint-induced movement therapy (CIMT) and mirror therapy can promote cortical remapping. These interventions enhance the brain's ability to reorganize its functional map, allowing patients to regain lost abilities (Taub et al., 2006).

3. Structural Changes

Neuroplasticity also involves structural changes in the brain, including dendritic branching, axonal sprouting, and the formation of new synapses (synaptogenesis). These structural adaptations are essential for long-term recovery and are often accompanied by changes in brain volume and connectivity, as observed through neuroimaging techniques (Johansen-Berg et al., 2010).

Rehabilitation strategies that promote neurogenesis and synaptogenesis, such as aerobic exercise and cognitive training, have been found to enhance these structural changes. These strategies not only aid in recovery but also help to maintain brain health and prevent further decline in neurodegenerative conditions (Kempermann et al., 2008).

Rehabilitation Strategies Leveraging Neuroplasticity

Several rehabilitation techniques have been developed to exploit neuroplasticity and facilitate functional recovery in patients with neurological impairments. These strategies include task-specific training, non-invasive brain stimulation, and pharmacological interventions.

1. Task-Specific Training

Task-specific training involves repetitive practice of tasks that are directly relevant to the patient's functional goals. This approach is based on the principle of "use-dependent" plasticity, where repeated use of neural circuits strengthens them over time (Kleim & Jones, 2008). Task-specific training has been particularly effective in stroke rehabilitation, where patients engage in activities such as grasping, reaching, and walking to improve motor function.

Studies have shown that task-specific training induces LTP-like changes in the motor cortex, leading to improved coordination and muscle control (Buch et al., 2017). Moreover, this approach is often combined with techniques such as robotic-assisted therapy and virtual reality (VR) to enhance the intensity and variability of training, further promoting neuroplasticity.

2. Non-Invasive Brain Stimulation

Non-invasive brain stimulation techniques, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), have gained significant attention in rehabilitation for their ability to modulate neural activity and promote plasticity (Bolognini et al., 2009). TMS uses magnetic fields to induce electric currents in specific brain regions, while tDCS applies low-intensity electrical currents to enhance or inhibit neural activity.

Both TMS and tDCS have been used to enhance motor recovery in stroke patients by stimulating the motor cortex and facilitating the reorganization of neural circuits. These techniques can be combined with physical therapy to optimize outcomes, as the stimulation primes the brain for plastic changes, making rehabilitation exercises more effective (Hummel & Cohen, 2006).

3. Pharmacological Interventions

Pharmacological agents that enhance neuroplasticity are being explored as adjuncts to rehabilitation therapies. Drugs such as selective serotonin reuptake inhibitors (SSRIs), dopamine agonists, and N-methyl-D-aspartate (NMDA) receptor modulators have been shown to facilitate plasticity by modulating neurotransmitter systems involved in synaptic transmission and learning (Berthier et al., 2011).

For example, SSRIs like fluoxetine have been found to enhance motor recovery in stroke patients by promoting neurogenesis and synaptic plasticity in the motor cortex (Chollet et al., 2011). Similarly, dopamine agonists have been used to improve cognitive function and attention in patients with traumatic brain injury by enhancing dopaminergic transmission in the prefrontal cortex (Whyte et al., 2008).

Innovations in Rehabilitation

Recent technological advancements and emerging therapies are pushing the boundaries of neuroplasticity-based rehabilitation, offering new hope for patients with neurological impairments.

1. Virtual Reality and Gaming

Virtual reality (VR) has emerged as a powerful tool for rehabilitation, providing immersive and engaging environments for patients to practice motor and cognitive tasks. VR-based therapies leverage the brain's natural ability to learn through experience, creating a motivating and interactive platform for promoting neuroplasticity (Laver et al., 2017).

Studies have shown that VR-based rehabilitation can improve balance, coordination, and motor function in patients with stroke, Parkinson's disease, and cerebral palsy (Massetti et al., 2018). By providing real-time feedback and allowing for individualized task progression, VR enhances the intensity and effectiveness of rehabilitation, driving greater neuroplastic changes.

2. Brain-Computer Interfaces (BCIs)

Brain-computer interfaces (BCIs) are cutting-edge technologies that enable direct communication between the brain and external devices, bypassing damaged neural pathways (Lebedev & Nicolelis, 2006). BCIs can be used in rehabilitation to restore motor function in patients with paralysis or severe motor impairments.

By detecting neural signals related to movement and translating them into actions (e.g., controlling a robotic arm or computer cursor), BCIs provide patients with real-time feedback, promoting neuroplasticity and motor learning. Recent studies have demonstrated that BCI-based interventions can enhance motor recovery in stroke patients by facilitating the reorganization of motor networks (Ramos-Murguialday et al., 2013).

3. Stem Cell Therapy

Stem cell therapy holds great promise for enhancing neuroplasticity and promoting recovery in neurological disorders. Stem cells have the potential to differentiate into various cell types, including neurons and glial cells, and can contribute to the repair of damaged brain tissue (Lindvall & Kokaia, 2006).

Preclinical studies have shown that stem cell transplantation can promote neurogenesis, synaptogenesis, and axonal sprouting in animal models of stroke and spinal cord injury (Li et al., 2010). While clinical applications are still in the early stages, ongoing research is exploring the potential of stem cell therapy to enhance neuroplasticity and improve outcomes in patients with neurological injuries.

Challenges and Future Directions

While significant progress has been made in leveraging neuroplasticity for rehabilitation, several challenges remain. One of the main challenges is individual variability in the capacity for neuroplasticity. Factors such as age, genetics, and the severity of brain injury can influence the brain's ability to reorganize and recover (Johansson, 2000).

Additionally, the timing and intensity of rehabilitation interventions are critical factors that affect outcomes. Research suggests that early and intensive rehabilitation is more effective in promoting neuroplasticity, but the optimal dosage and timing of interventions remain unclear (Kwakkel et al., 2004).

Future research should focus on developing personalized rehabilitation strategies that take into account individual differences in neuroplasticity and recovery potential. Advances in neuroimaging and neurophysiological techniques will also enable more precise monitoring of plasticity changes, allowing for more targeted and effective interventions.

Conclusion

Neuroplasticity offers a powerful framework for enhancing rehabilitation and promoting recovery in patients with neurological impairments. By understanding the mechanisms underlying neuroplasticity, clinicians and researchers can develop innovative therapies that optimize functional recovery and improve quality of life. Emerging technologies such as virtual reality, brain-computer interfaces, and stem cell therapy hold great promise for the future of neuroplasticity-based rehabilitation. However, further research is needed to refine these approaches and overcome the challenges associated with individual variability and intervention timing. With continued advancements in the field, neuroplasticity will remain a cornerstone of rehabilitation, offering hope for patients with debilitating neurological conditions.

References

- Berthier, M. L., Pulvermüller, F., Dávila, G., & Green, C. (2011). Drug therapy of post-stroke aphasia: a review of current evidence. *Neuropsychology Review*, 21(3), 302-319.
- Bliss, T. V. P., & Lømo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *Journal of Physiology*, 232(2), 331-356.

Bolognini, N., Pascual-Leone, A., &Fregni, F. (2009). Noninvasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *The Lancet Neurology*, 8(8), 702-712.

Buch, E. R., et al. (2017). Enhancing motor skill learning through neuroplasticity induction in chronic stroke patients. *Journal of Neuroscience*, 37(3), 671-684.

Chollet, F., et al. (2011). Fluoxetine for motor recovery after acute ischemic stroke (FLAME): a randomized placebo-controlled trial. *The Lancet Neurology*, 10(2), 123-130.

Cramer, S. C., et al. (2011). Understanding recovery following stroke: leveraging the brain's neuroplastic potential. *Journal of Clinical Investigation*, 121(4), 1102-1109.

Hummel, F. C., & Cohen, L. G. (2006). Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *The Lancet Neurology*, 5(8), 708-712.

Johansson, B. B. (2000). Brain plasticity and stroke rehabilitation: the Willis lecture. *Stroke*, 31(1), 223-230.

Johansen-Berg, H., et al. (2010). Structural plasticity in the human brain: can training change the structure of the brain? *Nature Reviews Neuroscience*, 11(9), 672-682.

Kempermann, G., et al. (2008). The neuroplasticity of the hippocampus in adult mammals: how much and how far? *Annual Review of Neuroscience*, 31, 563-588.

Kleim, J. A., & Jones, T. A. (2008). Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. *Journal of Speech, Language, and Hearing Research*, 51(1), S225-S239.

Kwakkel, G., et al. (2004). Intensity of leg and arm training after primary middle-cerebral-artery stroke: a randomised trial. *The Lancet*, 364(9431), 191-196.

Laver, K. E., et al. (2017). Virtual reality for stroke rehabilitation. *Cochrane Database of Systematic Reviews*, 11, CD008349.

Lebedev, M. A., & Nicolelis, M. A. L. (2006). Brain-machine interfaces: past, present and future. *Trends in Neurosciences*, 29(9), 536-546.

Li, Y., et al. (2010). Stem cell therapy for ischemic stroke: progress and challenges. *Translational Stroke Research*, 1(3), 151-160.

Lindvall, O., & Kokaia, Z. (2006). Stem cells for the treatment of neurological disorders. *Nature*, 441(7097), 1094-1096.

Massetti, T., et al. (2018). Virtual reality in pediatric rehabilitation: a systematic review of randomized controlled trials. *Pediatric Physical Therapy*, 30(1), 84-92.

Nudo, R. J. (2013). Recovery after brain injury: mechanisms and principles. *Frontiers in Human Neuroscience*, 7, 887.

Ramos-Murguialday, A., et al. (2013). Brain-machine interface in chronic stroke rehabilitation: a controlled study. *Annals of Neurology*, 74(1), 100-108.

Taub, E., et al. (2006). Constraint-induced movement therapy: a new family of techniques with broad application to physical rehabilitation—a clinical review. *Journal of Rehabilitation Research and Development*, 43(3), 293-304.

Whyte, J., et al. (2008). Dopamine agonists and neurorehabilitation after traumatic brain injury: a randomized, double-blind, placebo-controlled study. *Journal of Head Trauma Rehabilitation*, 23(5), 288-296.

Alzheimer's disease: pathogenesis, diagnosis, and therapeutic strategies

Rajen Dey*, Manojit Bysack

Department of Medical Laboratory Technology, School of Allied Health Sciences, Swami Vivekananda University, Telinipara, Barasat-Barrackpore Rd, Bara Kanthalia, West Bengal - 700121, India.

Abstract:

Presence of Alzheimer's disease (AD) is one of the main causes of progressive dementia. The pathophysiology of AD, a neurodegenerative disease, has been linked to intracellular neurofibrillary tangles composed of hyperphosphorylated τ -protein and extracellular aggregation of amyloid β ($A\beta$) plaques in the cortical and limbic regions of the human brain. Memory loss and increasing neurocognitive impairment are its hallmarks. Senile plaques are formed when $A\beta_{40}$ and $A\beta_{42}$ monomers are produced as a result of the aberrant processing of APP by β - and γ -secretases. These monomers then oligomerize and aggregate. Additionally, infectious infections such as HIV exacerbate the condition. Furthermore, microglial infiltration is triggered during disease pathogenesis by high quantities of $A\beta$ peptides in the central nervous system. When microglia come into contact with $A\beta$, they become activated, endocytose $A\beta$, and help to remove them through TREM2 surface receptors, all the while inducing an innate immune response to the aggregation. The current review covers the state of the art in AD therapies and diagnostics, including labeling and imaging techniques used as contrast agents for enhanced vision and sensing of the plaques. It also provides a full report on the causative elements leading to AD. The analysis highlights the critical need for nanotechnology as a successful therapeutic approach to boost medication bioavailability in the central nervous system.

Keywords: amyloid beta, amyloidogenesis, amyloid precursor proteins, β -secretases, tau phosphorylation

Introduction

The primary etiology of Alzheimer's disease (AD), a major protein conformational disease (PCD) with neurodegenerative symptoms, is abnormal protein processing and polymerization of usually soluble proteins [1, 2]. Soluble neuronal proteins can get misfolded as a result of aging, genetic mutation, or environmental influences. This can lead to aberrant conformations that accumulate and cause atypical neuronal functioning as well as loss [4]. Alois Alzheimer, a German neurologist, is credited with identifying AD as a neurodegenerative illness after studying Auguste Deter, a 51-year-old patient who had memory loss, language impairment, disorientation, and hallucinations. Plaques and tangles in the cerebral cortex were discovered during her autopsy, [5] which persuaded him that this was more than just ordinary dementia. Subsequent investigations of his findings demonstrated that dementia patients have neuritic amyloid β ($A\beta$) plaques [6]. Predisposition to the rare but powerful PS1 genetic mutation is thought to be the cause of the disease's early start [7]. Parkinson's disease, Creutzfeldt-Jakob disease, Huntington's disease, and Machado-Joseph disease are additional neurodegenerative diseases linked to aberrant protein conformations. These diseases stem from abnormalities in the α -synuclein, Cellular Prion protein (PrPc), Scrapie prion protein (PrPSc), Htt, and Ataxin3 proteins, respectively. It becomes crucial to address areas like AD mechanisms, pathogenesis, and diagnostics after learning the disease's causative factors and pathogenesis mechanism. Finally, it becomes important to figure out how to develop novel therapies to combat AD. Nanoparticle (NP)-based sensitive early-phase detection of AD biomarkers such as $A\beta$ and τ in patient cerebrospinal fluid (CSF) samples is one of the diagnostic and imaging approaches used. Additionally, aggregated $A\beta$ plaques can be seen using nanomaterials as contrast agents. It is critical to comprehend how NPs contribute to the drug's increased effectiveness and bioavailability through the blood-brain barrier (BBB) and into the central

nervous system (CNS). This study highlights the possible application of nanotechnology in therapies to prevent the advancement of AD, covers the present state of diagnostics and treatments, and provides a thorough understanding of the pathogenic route leading to full-blown AD.

AD pathogenesis

There is a lot of research being done to better understand AD pathophysiology and develop effective therapeutics. AD is a progressive neurodegenerative disease that is extremely complex [8]. It ranks among the main causes of dementia cases worldwide. Approximately 5.3 million Americans, 500,000 of whom have younger-onset AD and 5.1 million of whom are 65 years of age or older, suffer from AD in the US alone [9]. Extracellular aggregates of A β plaques and intracellular aggregations of neurofibrillary tangles (NFTs), which are made of hyperphosphorylated microtubule-associated τ , are described as histological features of AD. A β plaques begin to form in the brain's orbitofrontal, basal, and temporal neocortex areas before spreading to the hippocampus, diencephalon, amygdala, and basal ganglia. A β can also be detected in the cerebellar cortex, lower brain stem, and mesencephalon in severe situations. The locus coeruleus, transentorhinal, and entorhinal regions of the brain are the sites where τ -tangle development is initiated by this concentration of A β . It expands to the neocortex and hippocampal regions during the crucial stage [10]. The cause, pathophysiology, and factors linked to the progression of AD are the main topics of this review. A β and NFTs are thought to be the main players in the disease's evolution. Amyloid precursor protein (APP), an essential protein on the plasma membrane, is first alteredly cleaved by β -secretases (BACE1) and γ -secretases to form insoluble A β fibrils, which is the beginning of amyloid pathology. Subsequently, A β oligomerizes, diffuses into clefts in synapses, and tampers with synaptic transmission [11,12]. It then polymerizes into insoluble amyloid fibrils, which collect to form plaques. As a result of this polymerization, kinases are activated, which causes the microtubule-associated τ protein to become hyperphosphorylated and polymerize into insoluble NFTs. Microglia surrounding plaques are

recruited subsequent to the formation of plaques and tangles. This increases local inflammatory response and microglial activation while also increasing neurotoxicity.

Structure and function of APP

APP is a member of the same protein family as the amyloid precursor-like proteins (APLP1 and APLP2) found in mammals and the amyloid precursor protein-like (APPL) found in *Drosophila*. It is a transmembrane protein that is essential and has extracellular domains. When APP is sick, different enzymes cleave it differently, producing amyloidogenic fragments. There is still much to learn about APP's physiological roles. Research utilizing transiently transfected cell lines demonstrates that APP regulates neurite outgrowth and functioning, as well as cell survival, proliferation, and motility. These effects are linked to the release of soluble ectodomains that occur during normal APP cleavage [13, 14]. Studies showing neural defects in mice injected with APP RNAi [15] and increased cognitive performance and synaptic density following intracerebral injections of APP-ectodomain have demonstrated the relevance of APP [16]. Type 1 transmembrane glycoprotein, which is encoded by APP, can be cleaved through an amyloidogenic mechanism in a diseased condition or through a nonamyloidogenic pathway in a normal state [17]. Different polypeptides that may result from complicated proteolysis, glycosylation, phosphorylation, or alternative splicing are released by APP [18, 19]. Out of the 770 amino acids in APP, A β consists of 28 residues and 14 more residues from the transmembrane domain. The large soluble ectodomain APP α is released into the medium by α -secretase at the cleavage site, while the C-terminal fragment C83 remains in the membrane. γ -secretase then cleaves C83 at position 711, releasing the soluble P3 peptide. As an alternative, aberrant cleavage in a sick condition is caused by β -secretase, which releases shortened APP β , and C-terminal fragment C99, which is kept in the membrane and cleaves further to release insoluble A β peptides by γ -secretase. The APP intracellular domain is released into the cytoplasm upon γ -secretase's cleavage of both C83 and C99. This soluble domain then translocates to nuclei to facilitate further gene expression functions.

Amyloidogenic pathway

In the sick state, APP is cleaved differentially. A β is liberated from APP via consecutive cleavages by BACE-1, an intramembrane aspartyl protease composed of four proteins: presenilin, nicastrin, anterior pharynx-defective 1 (Aph1), and Psen2 complexed together [20]. BACE-1 is a membrane-spanning aspartyl protease with its active site located in lumen. This complex aids in the production of neurotoxic and insoluble A β fragments by γ -secretase. The first and rate-limiting step is β -secretase cleavage, which results in a cut at the N-terminus of A β . The protein's extracellular region is mostly removed, leaving only the C-terminal of APP [21], which is then further cleaved at the C-terminus of A β to create A β oligomers that subsequently polymerize to form aggregated plaques.

A β 40 and A β 42 are the two primary subtypes of A β polymers that directly contribute to the development of plaque and produce neurotoxicity. Compared to A β 42, which functions as a toxic building block of A β assembly and is less abundant, highly insoluble, severely neurotoxic, and more aggregation-prone, A β 40 is more plentiful and less neurotoxic. A β 40/A β 42 aggregation leads to ion channel blockage, disrupted calcium homeostasis, increased oxidative stress in the mitochondria, and impaired glucose regulation and energy metabolism, all of which worsen neuronal health and ultimately cause neuronal cell death.

Epigenetics of AD

The study of gene-gene interactions, genotype expression, and different biological processes that translate genotype expression into corresponding phenotypes is known as epigenetics [22]. Neuroepigenetics, the branch of epigenetics that studies neurological diseases, has progressed quite well and has been extensively researched in CNS-associated diseases, including pathologies and disorders related to learning, movement, behavior, and cognition [23, 24]. Understanding epigenetics is crucial to comprehending the extent to which environmental factors, paternal genes, dietary practices, traumatic experiences, stress, learning

difficulties, chemical or drug addiction exposure, and the ensuing structural disruptions, mutations, or alterations in DNA are influenced [25, 26]. Recent research has investigated the role of epigenetics in AD, one of the most complicated aging-related neurological disorders [27]. Aging, genetic abnormalities, nutritional and metabolic problems, exposure to environmental variables, and—most importantly—the involvement of social factors all play intricate roles in the onset and progression of AD [28]. It's likely that variables including obesity, diabetes, hypertension, and inflammatory illnesses, in addition to aging, affect AD and cause epigenetic changes or perhaps cause AD-like pathogenesis in children. It is plausible [29] and has been documented in several brain regions [30] that there are correlations between DNA-methylation patterns in the brain and aging. It is hypothesized that an understanding of DNA epigenetic mechanisms is crucial to comprehending aging and the complications associated with Alzheimer's disease patients, as these mechanisms play a part in memory formation and maintenance, much as a decrease in DNA methylation deteriorates neuronal plasticity and causes memory loss [31]. Histone alterations might be just as significant as DNA methylation. Histone acetylation in APP-PSEN1 double-mutant transgenic mice has been studied; associative learning deficit was linked to a decrease in H4K14 histone acetylation [32]. Histone deacetylase (HDAC) inhibitors also impact the aggregation and synthesis of A β in AD animals. Promising research has been done with their inhibitors, including vorinostat, valproic acid, and trichostatin A. Thus, in order to target the mechanisms and complexities associated with AD, it becomes crucial to comprehend the epigenetic mechanisms involved in aging [33].

Diagnostics for AD: labeling and imaging

Neuropsychological testing is the main basis for the current diagnosis of AD. Neuroimaging and the monitoring of established biomarkers, such as A β peptide concentrations (A β 1-42:A β 1-40 ratio) and total and hyperphosphorylated τ (Thr181 and Thr231) proteins in the CSF, are necessary for a clinical diagnosis of AD. With 18F-florbetapir (or alternatively 11C Pittsburgh compound B) positron emission tomography

(PET), amyloid oligomers and plaque accumulation can also be visualized; nevertheless, nonlinear connection between A β concentration in CSF and PET scans is still a cause for worry. But for many older patients, CSF sample is not always practical or well tolerated due to its rather intrusive nature. Clinically useful noninvasive imaging techniques include fludeoxyglucose PET, which provides information on brain metabolism. Indeed, different stages of Alzheimer's disease have been linked to altered brain metabolism, including hyper- and hypometabolism. Another useful noninvasive method for identifying functional problems is magnetic resonance imaging (MRI) at increasing field strength and resolution. Iron oxide nanoparticles (NPs) are used as contrast agents in magnetic resonance imaging (MRI) to detect and identify amyloid plaques. To enhance detection efficiency, the NPs can be tagged with fluorescent probes [34]. It is said that these iron oxide nanoparticles bond to the N terminal of A β , facilitating their imaging. Furthermore, it has been observed that using an external magnetic field, nonfluorescent or fluorescent rhodamine-tagged γ Fe₂O₃ NPs may selectively identify A β fibrils and extract them from solubilized A β [35, 36]. Polystyrene-block-poly (n-butyl cyanoacrylate) NPs containing thioflavin T to target A β have also been reported, in addition to iron NPs [37]. Using fluorescent semiconductor nanocrystals (quantum dots) for labeling and gold NPs as contrasting agents to explore structural stages in A β self-assembly, MRI has been employed [38, 39]. An ultrasensitive NP-based biobarcode system that uses oligonucleotide (DNA barcode)-modified AuNPs and magnetic microparticles functionalized with monoclonal/polyclonal antibodies has been used to detect soluble forms of A β from CSF [40]. Additionally, electrochemical sensing using click chemistry, which uses AuNPs and assembled monolayers to interact with A β peptide [41], and ultrasensitive electrical detection for A β 1-42 using scanning tunneling microscopy [42] have also been employed. The ability to diagnose AD has significantly increased thanks to recent conceptual and technological advancements. Following an AD diagnosis, the available therapeutic options are limited to disease-modifying therapies with comparatively low efficacy.

AD therapeutics

Misfolding of proteins in AD causes cellular processes to become distorted and results in neuronal death. A protein that has misfolded can lose its function or gain it in a harmful way. This could happen as a result of aberrant protein aggregation, which renders the protein unable to carry out its intended function and prevents it from being eliminated by the cellular environment, which can have detrimental biological effects. In order to reduce the toxicity brought on by aberrant proteins, there are ongoing AD investigations focused on blocking the synthesis of misfolding proteins as well as their aggregation and dissemination [43]. Since a wide range of neurodegenerative mechanisms underlie both early- and late-stage AD, most AD treatment strategies concentrate on lowering toxic forms of $A\beta$ and τ . After analysis, a number of medications have advanced to Phase I, II, and III clinical trials.

None of the current treatment targets (such as galantamine, donepezil, and rivastigmine) are believed to be causally linked to the development of AD; instead, they all appear secondary. Unfavorable medication pharmacokinetics and pharmacodynamics frequently lead to therapy failure. Inadequate drug physical chemistry (hydrophobicity, for example), unfavorable biological membrane absorption, unfavorable pharmacokinetic parameters (intense and plasma metabolism), unstable drug chemistry (oxidation, hydrolysis, or photolysis), and tissue toxicity (hepatotoxicity, neurotoxicity, or kidney toxicity) are all causes of pharmacotherapy failure. A number of therapy approaches have been put out and tried in an effort to remove $A\beta$. There are a number of medications used to treat $A\beta$ breakdown, but most of them that performed well in in vivo experiments failed in human clinical trials, thus new approaches are desperately needed. Many of the medications on the market have low brain bioavailability and lose effectiveness as they cross the blood-brain barrier. This calls for fresh research that delves into effective CNS-specific neuroprotective tactics. Because of their capacity for multifunctionalization, which allows them to imitate the physiological mechanisms of transport across

the blood-brain barrier, NPs are attractive candidates for this use. This crucial cell-based physical barrier shields the brain from potentially dangerous compounds in the bloodstream, but it also blocks the passage of 98% of neuropharmaceuticals and diagnostics currently on the market.

Conclusion

Alzheimer's disease (AD) remains one of the most complex and devastating neurodegenerative disorders, characterized by progressive cognitive decline and neuronal loss. The disease is primarily driven by the aberrant processing of amyloid precursor protein leading to amyloid- β plaque formation, along with tau hyperphosphorylation and neurofibrillary tangle development. These pathological events initiate synaptic dysfunction, neuroinflammation, mitochondrial impairment, and ultimately neuronal death. In addition to classical genetic and molecular mechanisms, emerging evidence highlights the significant role of epigenetic modifications and environmental factors in disease onset and progression. Current diagnostic strategies rely on neuropsychological evaluation, cerebrospinal fluid biomarkers, and advanced imaging techniques; however, early and noninvasive detection remains a major clinical challenge. Therapeutic options available today are largely symptomatic and fail to halt disease progression, mainly due to poor drug bioavailability and limited penetration across the blood-brain barrier. In this context, nanotechnology-based approaches offer promising solutions for improved diagnosis and targeted drug delivery to the central nervous system. Overall, a deeper understanding of AD pathogenesis combined with innovative diagnostic tools and advanced therapeutic strategies is essential for developing effective disease-modifying interventions and improving patient outcomes in the future.

References:

Adav, S. S., & Sze, S. K. (2016). Insight of brain degenerative protein modifications in the pathology of neurodegeneration and dementia by proteomic profiling. *Molecular brain*, 9(1), 92.

Leandro, P., & Gomes, C. M. (2008). Protein misfolding in conformational disorders: rescue of folding defects and chemical chaperoning. *Mini reviews in medicinal chemistry*, 8(9), 901–911.

Tran, L., & Ha-Duong, T. (2015). Exploring the Alzheimer amyloid- β peptide conformational ensemble: A review of molecular dynamics approaches. *Peptides*, 69, 86–91.

Horwich A. (2002). Protein aggregation in disease: a role for folding intermediates forming specific multimeric interactions. *The Journal of clinical investigation*, 110(9), 1221–1232.

Selkoe D. J. (2004). Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. *Nature cell biology*, 6(11), 1054–1061.

Blessed, G., Tomlinson, B. E., & Roth, M. (1968). The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *The British journal of psychiatry : the journal of mental science*, 114(512), 797–811.

O'Brien, R. J., & Wong, P. C. (2011). Amyloid precursor protein processing and Alzheimer's disease. *Annual review of neuroscience*, 34, 185–204.

Querfurth, H. W., & LaFerla, F. M. (2010). Alzheimer's disease. *The New England journal of medicine*, 362(4), 329–344.

Alzheimer's Association (2015). 2015 Alzheimer's disease facts and figures. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 11(3), 332–384.

Goedert M. (2015). NEURODEGENERATION. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled A β , tau, and α -synuclein. *Science (New York, N.Y.)*, 349(6248), 1255–1255.

Chen, J. X., & Yan, S. S. (2010). Role of mitochondrial amyloid-beta in Alzheimer's disease. *Journal of Alzheimer's disease : JAD*, 20 Suppl 2, S569–S578.

Crews, L., & Masliah, E. (2010). Molecular mechanisms of neurodegeneration in Alzheimer's disease. *Human molecular genetics*, 19(R1), R12–R20.

Oh, E. S., Savonenko, A. V., King, J. F., Fangmark Tucker, S. M., Rudow, G. L., Xu, G., Borchelt, D. R., & Troncoso, J. C. (2009). Amyloid precursor protein increases cortical neuron size in transgenic mice. *Neurobiology of aging*, 30(8), 1238–1244.

Thinakaran, G., & Koo, E. H. (2008). Amyloid precursor protein trafficking, processing, and function. *The Journal of biological chemistry*, 283(44), 29615–29619.

Young-Pearse, T. L., Bai, J., Chang, R., Zheng, J. B., LoTurco, J. J., & Selkoe, D. J. (2007). A critical function for beta-amyloid precursor protein in neuronal migration revealed by in utero RNA interference. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(52), 14459–14469.

Meziane, H., Dodart, J. C., Mathis, C., Little, S., Clemens, J., Paul, S. M., & Ungerer, A. (1998). Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnesic mice. *Proceedings of the National Academy of Sciences of the United States of America*, 95(21), 12683–12688.

Selkoe D. J. (1994). Cell biology of the amyloid beta-protein precursor and the mechanism of Alzheimer's disease. *Annual review of cell biology*, 10, 373–403.

Hefter, D., Kaiser, M., Weyer, S. W., Papageorgiou, I. E., Both, M., Kann, O., Müller, U. C., & Draguhn, A. (2016). Amyloid Precursor Protein Protects Neuronal Network Function after Hypoxia via Control of Voltage-Gated Calcium Channels. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 36(32), 8356–8371.

Shoji, M., Golde, T. E., Ghiso, J., Cheung, T. T., Estus, S., Shaffer, L. M., Cai, X. D., McKay, D. M., Tintner, R., & Frangione, B. (1992). Production of the Alzheimer amyloid beta protein by normal proteolytic processing. *Science (New York, N.Y.)*, 258(5079), 126–129.

Bergmans, B. A., & De Strooper, B. (2010). gamma-secretases: from cell biology to therapeutic strategies. *The Lancet. Neurology*, 9(2), 215–226.

Tu, S., Okamoto, S., Lipton, S. A., & Xu, H. (2014). Oligomeric A β -induced synaptic dysfunction in Alzheimer's disease. *Molecular neurodegeneration*, 9, 48.

Waddington C. H. (2012). The epigenotype. 1942. *International journal of epidemiology*, 41(1), 10–13.

Lardenoije, R., Iatrou, A., Kenis, G., Kompotis, K., Steinbusch, H. W., Mastroeni, D., Coleman, P., Lemere, C. A., Hof, P. R., van den Hove, D. L., & Rutten, B. P. (2015). The epigenetics of aging and neurodegeneration. *Progress in neurobiology*, 131, 21–64.

Sweatt J. D. (2013). The emerging field of neuroepigenetics. *Neuron*, 80(3), 624–632.

Tsankova, N., Renthal, W., Kumar, A., & Nestler, E. J. (2007). Epigenetic regulation in psychiatric disorders. *Nature reviews. Neuroscience*, 8(5), 355–367.

Day, J. J., & Sweatt, J. D. (2010). DNA methylation and memory formation. *Nature neuroscience*, 13(11), 1319–1323.

Landgrave-Gómez, J., Mercado-Gómez, O., & Guevara-Guzmán, R. (2015). Epigenetic mechanisms in neurological and neurodegenerative diseases. *Frontiers in cellular neuroscience*, 9, 58.

Sezgin, Z., & Dincer, Y. (2014). Alzheimer's disease and epigenetic diet. *Neurochemistry international*, 78, 105–116.

Balazs R. (2014). Epigenetic mechanisms in Alzheimer's disease. *Degenerative neurological and neuromuscular disease*, 4, 85–102.

Hernandez, D. G., Nalls, M. A., Gibbs, J. R., Arepalli, S., van der Brug, M., Chong, S., Moore, M., Longo, D. L., Cookson, M. R., Traynor, B. J., & Singleton, A. B. (2011). Distinct DNA methylation changes highly correlated with chronological age in the human brain. *Human molecular genetics*, 20(6), 1164–1172.

Mastroeni, D., Grover, A., Delvaux, E., Whiteside, C., Coleman, P. D., & Rogers, J. (2011). Epigenetic mechanisms in Alzheimer's disease. *Neurobiology of aging*, 32(7), 1161–1180.

Francis, Y. I., Fà, M., Ashraf, H., Zhang, H., Staniszewski, A., Latchman, D. S., & Arancio, O. (2009). Dysregulation of histone acetylation in the APP/PS1 mouse model of Alzheimer's disease. *Journal of Alzheimer's disease : JAD*, 18(1), 131–139.

Delgado-Morales, R., Agís-Balboa, R. C., Esteller, M., & Berdasco, M. (2017). Epigenetic mechanisms during ageing and neurogenesis as novel therapeutic avenues in human brain disorders. *Clinical epigenetics*, 9, 67.

Wadghiri, Y. Z., Sigurdsson, E. M., Sadowski, M., Elliott, J. I., Li, Y., Scholtzova, H., Tang, C. Y., Aguinaldo, G., Pappolla, M., Duff, K., Wisniewski, T., & Turnbull, D. H. (2003). Detection of Alzheimer's amyloid in transgenic mice using magnetic resonance microimaging. *Magnetic resonance in medicine*, 50(2), 293–302.

Skaat, H., & Margel, S. (2009). Synthesis of fluorescent-maghemite nanoparticles as multimodal imaging agents for amyloid-beta fibrils detection and removal by a magnetic field. *Biochemical and biophysical research communications*, 386(4), 645–649.

Skaat, H., Sorci, M., Belfort, G., & Margel, S. (2009). Effect of maghemite nanoparticles on insulin amyloid fibril formation: selective labeling, kinetics, and fibril removal by a magnetic field. *Journal of biomedical materials research. Part A*, 91(2), 342–351.

Siegemund, T., Paulke, B. R., Schmiedel, H., Bordag, N., Hoffmann, A., Harkany, T., Tanila, H., Kacza, J., & Härtig, W. (2006). Thioflavins released from nanoparticles target fibrillar amyloid beta in the hippocampus of APP/PS1 transgenic mice. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience*, 24(2-3), 195–201.

Choi, J. S., Choi, H. J., Jung, D. C., Lee, J. H., & Cheon, J. (2008). Nanoparticle assisted magnetic resonance imaging of the early reversible stages of amyloid beta self-assembly. *Chemical communications (Cambridge, England)*, (19), 2197–2199.

Dubertret, B., Skourides, P., Norris, D. J., Noireaux, V., Brivanlou, A. H., & Libchaber, A. (2002). In vivo imaging of quantum dots encapsulated in phospholipid micelles. *Science (New York, N.Y.)*, 298(5599), 1759–1762.

Georganopoulou, D. G., Chang, L., Nam, J. M., Thaxton, C. S., Mufson, E. J., Klein, W. L., & Mirkin, C. A. (2005).

Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 102(7), 2273–2276.

Kolb, H. C., Finn, M. G., & Sharpless, K. B. (2001). Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angewandte Chemie (International ed. in English)*, 40(11), 2004–2021.

Kang, D. Y., Lee, J. H., Oh, B. K., & Choi, J. W. (2009). Ultra-sensitive immunosensor for beta-amyloid (1-42) using scanning tunneling microscopy-based electrical detection. *Biosensors & bioelectronics*, 24(5), 1431–1436.

Scannevin R. H. (2018). Therapeutic strategies for targeting neurodegenerative protein misfolding disorders. *Current opinion in chemical biology*, 44, 66–74.

Emerging Biomarkers in Cardiovascular Disease: Advances in Early Diagnosis and Novel Molecular Indicators

Sibu Jana*

Department of Medical Laboratory Technology, School of
Allied Health Sciences, Swami Vivekananda University,
Telinipara, Barasat-Barrackpore Rd, Bara Kanthalia, West
Bengal - 700121, India.

Email: rupakbera164@gmail.com

Abstract

The global burden of cardiovascular disease (CVD) continues to rise, posing a major challenge to public health systems, particularly in low- and middle-income countries. Early diagnosis remains crucial for effective management and prevention of CVD-related morbidity and mortality. In this context, emerging biomarkers offer promising avenues for improving diagnostic precision and risk assessment. This review synthesizes recent findings from diverse medical and scientific sources to identify and evaluate novel biomarkers with diagnostic and prognostic potential in CVD. Biomolecules such as high-sensitivity cardiac troponins, natriuretic peptides, C-reactive protein, microRNAs, and novel omics-based markers have demonstrated significant utility in predicting disease onset and progression. These biomarkers provide molecular insights into underlying pathophysiological mechanisms, enabling earlier and more targeted intervention strategies. Additionally, integrating biomarker profiling with advanced bioinformatics and molecular diagnostics may further enhance personalized cardiovascular care. Overall, emerging biomarkers represent a transformative step toward redefining diagnostic frameworks and improving clinical outcomes in CVD management.

Keywords: Emerging biomarkers, Cardiovascular disease, Diagnostic markers, Cardiac troponins, MicroRNAs, Molecular diagnostics, Predictive medicine.

Introduction

Cardiovascular diseases (CVDs), including coronary artery disease (CAD), cerebrovascular disease (CD), and rheumatic heart disease (RHD), remain the leading non-communicable causes of morbidity and mortality worldwide. According to the World Health Organization, CVDs account for approximately 17.9 million deaths each year, representing about 31% of all global deaths, with coronary heart disease and stroke responsible for nearly 7.3 and 6.2 million fatalities, respectively. The mortality burden is projected to rise to 23.6 million deaths annually by 2030, primarily due to ischemic heart disease and cerebrovascular disorders. [1]

To counter this growing threat, the identification and utilization of reliable biomarkers have emerged as vital strategies for early diagnosis, prognosis, and disease monitoring. Cardiac biomarkers—biochemical molecules released into the bloodstream following myocardial injury or stress—provide critical insights into cardiac function and pathology. These molecular indicators, typically obtained through venous blood sampling, assist clinicians in evaluating cardiovascular risk, diagnosing acute coronary syndromes (ACS), and monitoring therapeutic outcomes.[2]

Despite significant advances, the availability of consistent and specific biomarkers for early-stage CVD detection remains limited. Therefore, exploring emerging biomarkers that exhibit strong diagnostic and prognostic value is essential for improving patient management and reducing global cardiovascular mortality [3].

Carbohydrate-Based Biomarkers

Glycogen Phosphorylase BB (GPBB)

The heart is particularly vulnerable to hypoxia caused by carbon monoxide (CO) exposure because of its high oxygen requirements. However, CO-related cardiac damage often goes unnoticed, as patients typically exhibit no distinct

symptoms or specific electrocardiogram (ECG) alterations. Therefore, there is an increasing need for early and specific biomarkers capable of detecting myocardial hypoxia.[4]

One promising candidate is the glycogen phosphorylase BB isoenzyme (GPBB), a relatively new cardiac biomarker that may enable the early identification of myocardial ischemia. Under conditions of low oxygen or glucose availability, GPBB plays a key role as the enzyme responsible for the initial, rate-limiting step in glycogen breakdown, converting it to glucose-1-phosphate.[5]

In humans, three isoforms of glycogen phosphorylase have been characterized: GPMM (found in skeletal muscle), GPLI (in the liver), and GPBB (in the brain and heart). Although markers such as myoglobin and ischemia-modified albumin (IMA) exhibit good sensitivity for ischemic events, they lack cardiac tissue specificity. In contrast, GPBB has demonstrated superior sensitivity and specificity for diagnosing acute coronary syndrome (ACS) within the first four hours following the onset of chest pain – reported at 81% and 93%, respectively.[6]

Studies indicate that GPBB is a valuable clinical biomarker for myocardial ischemia and can assist in diagnosing ACS. Despite the fact that GPBB levels may rise during pregnancy and are also detectable in the brain, making it not entirely cardiac-specific, plasma GPBB remains a useful surrogate marker for acute coronary syndromes in numerous investigations. Consequently, GPBB can serve as a secondary biomarker for the detection of acute myocardial infarction. [7,8]

Protein-Based Biomarkers

Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST), also known as a liver transaminase, is an intracellular enzyme that catalyzes transamination reactions between amino acids and keto acids. Although AST is predominantly located in hepatocytes and commonly used as a serum marker for liver injury, it is also found in the heart, skeletal muscles, brain, and kidneys. [9,10]

Historically, AST was the first enzyme identified as a potential cardiac biomarker in 1954. However, due to its lack of cardiac tissue specificity, it is not considered reliable for diagnosing acute myocardial infarction (AMI). Later developments introduced other enzymes such as creatine kinase (CK), which became a recognized indicator of muscle injury in 1959, and lactate dehydrogenase (LDH), which was applied to AMI diagnosis in 1960. By 1979, the World Health Organization (WHO) recommended a diagnostic panel combining CK, AST, and LDH for confirming AMI. [11]

LDH, or lactate dehydrogenase

Lactate dehydrogenase (LDH), an intracellular enzyme, which is readily available, catalyzes the transformation of pyruvic acid, the last byproduct of the glycolytic process, into lactate. As a sign of heart failure, LDH has been used to detect acute myocardial infarction since the 1960s. Five isozymes are produced by the two self-contained subunits that make up LDH. Every isozyme is expressed in a different organ:

Hepatocytes express LDH5, lung tissue contains LDH3, and cardiomyocytes express LDH1 [12, 13]. LDH is expressed by numerous organs, such as the kidney, liver, heart, lung, skeletal muscle, and erythrocytes. One of the five isoenzymes that make up LDH is present in nature.

It is currently not used to diagnose AMI, despite reports indicating an LDH1-to-LDH2 ratio greater than 1 is specific for AMI [14]. Nowadays, LDH is only utilized to distinguish between acute and subacute myocardial infarction (MI) in patients who have positive troponins and whose CK and CK-MB readings have returned to normal by the time they get to the hospital [15].

Carbonic anhydrase III (CA-III)

Carbonic anhydrases (CAs), the main protein components of red blood cells, are metalloproteins that include zinc, agents that quicken carbon dioxide's hydration and dehydration [16]. Numerous physiological processes, such as gluconeogenesis, acid-base homeostasis, adipogenesis, and

calcification, are carried out by CAs, which are present in a variety of tissues [17]. A distinct member of the CAs family, the CA-III protein is involved in a number of conditions, such as skeletal muscle damage, MI, and type-2 diabetes. The cytosolic enzyme CA-III protein is less active than carbon dioxide hydratase [18].

Creatine Kinase-MB (CK-MB)

CK-MB, an isoenzyme of creatine kinase, is predominantly expressed in cardiac muscle and released into circulation following myocardial cell necrosis. It has long been used as a biochemical marker for acute myocardial infarction (AMI). CK-MB typically rises 4–6 hours after myocardial injury, peaks within 24 hours, and normalizes in 48–72 hours. Despite its diagnostic value, CK-MB can also elevate in skeletal muscle injury and certain non-cardiac conditions, limiting its specificity. It remains useful when troponin assays are unavailable or when reinfarction is suspected. [19]

Cardiac Troponins (cTnI and cTnT)

Cardiac troponins are regulatory proteins that control cardiac muscle contraction and are highly specific to myocardial tissue. They are currently considered the gold standard for diagnosing AMI. Elevated levels of troponins indicate myocardial cell damage and correlate with infarct size and prognosis. High-sensitivity cardiac troponin (hs-cTn) assays allow detection of minute increases in circulating troponin concentrations, enabling diagnosis of myocardial injury even in the early stages or in chronic cardiac conditions such as heart failure. [20]

Hydroxybutyrate Dehydrogenase (HBDH)

HBDH, an isoenzyme of lactate dehydrogenase (LDH), was historically used in MI diagnosis before troponin testing became standard. Its elevation reflects tissue necrosis, including cardiac muscle injury. Although less specific than troponins, HBDH measurement can still support differential diagnosis of myocardial injury when used alongside other markers. [21]

Biomarkers of Neurohormonal Activation

Brain Natriuretic Peptide (BNP) and N-terminal pro-BNP (NT-proBNP)

BNP and its inactive fragment NT-proBNP are released from cardiac ventricles in response to increased wall stretch and volume overload. They are established markers for diagnosing and prognosticating heart failure (HF). Elevated BNP and NT-proBNP levels correlate with ventricular dysfunction, increased filling pressures, and mortality risk. Beyond HF, these peptides may also predict adverse outcomes in acute coronary syndromes and hypertensive heart disease. Their rapid measurement and high negative predictive value make them valuable in distinguishing cardiac from non-cardiac causes of dyspnea. [22]

Biomarkers of Inflammation and Immune Activation

C-Reactive Protein (CRP)

CRP is an acute-phase protein synthesized in the liver under the regulation of interleukin-6 (IL-6). High-sensitivity CRP (hs-CRP) assays can detect subclinical inflammation and have been shown to independently predict CVD risk. Elevated hs-CRP concentrations are associated with atherosclerotic plaque instability and increased risk of myocardial infarction, stroke, and cardiovascular mortality. CRP is thus a marker of systemic inflammation rather than direct myocardial injury. [23]

Interleukin-6 (IL-6)

IL-6 is a pleiotropic proinflammatory cytokine involved in endothelial activation, vascular inflammation, and atherogenesis. Elevated IL-6 levels correlate with CVD severity, plaque instability, and adverse cardiac remodeling after MI. IL-6 acts upstream of CRP synthesis, suggesting that IL-6 blockade could have therapeutic potential in attenuating inflammatory-driven cardiovascular events. [24]

Tumor Necrosis Factor-Alpha (TNF- α)

TNF- α contributes to the inflammatory cascade, promoting endothelial dysfunction, myocardial apoptosis, and

remodeling. Elevated TNF- α levels are observed in heart failure and coronary artery disease. While it is not a diagnostic marker, it provides mechanistic insight into inflammation-driven cardiac injury and may serve as a prognostic biomarker in chronic CVD and heart failure. [25]

Biomarkers of Oxidative Stress, Fibrosis, and Remodeling

Galectin-3

Galectin-3 is a β -galactoside-binding lectin secreted by activated macrophages that plays a key role in inflammation, fibrosis, and myocardial remodeling. Elevated plasma galectin-3 levels are associated with worsening heart failure and higher mortality. It has prognostic value independent of natriuretic peptides and can help identify patients at risk for progression of cardiac dysfunction. [26]

Pregnancy-Associated Plasma Protein-A (PAPP-A)

PAPP-A is a zinc-binding metalloproteinase that regulates insulin-like growth factor availability and is released by unstable atherosclerotic plaques. Elevated circulating PAPP-A levels have been linked to plaque rupture and acute coronary syndromes (ACS). It is considered a promising biomarker for early detection of plaque instability and subclinical atherosclerosis. [27]

Fibrinogen

Fibrinogen, a glycoprotein synthesized by the liver, plays a central role in coagulation and is an acute-phase reactant. Elevated plasma fibrinogen levels are associated with increased blood viscosity, platelet aggregation, and thrombus formation. High fibrinogen concentrations correlate with increased risk of myocardial infarction, stroke, and peripheral artery disease, making it a predictive biomarker of cardiovascular risk and inflammation. [28]

Biomarkers of Vasopressin Pathways and Stress Response

Copeptin

Copeptin is the C-terminal fragment of the vasopressin prohormone and is released in equimolar amounts with

arginine vasopressin (AVP). As AVP is unstable and difficult to measure, copeptin serves as a reliable surrogate marker. It rises rapidly after acute myocardial infarction and reflects endogenous stress responses, complementing cardiac troponin testing for early rule-out of AMI. Combined measurement of copeptin and troponin improves diagnostic accuracy in the emergency setting. [29]

Procalcitonin (PCT)

Procalcitonin, a precursor of calcitonin, is primarily a biomarker of bacterial infection and systemic inflammation but has recently been associated with adverse cardiovascular outcomes. Elevated PCT levels have been reported in heart failure, myocarditis, and post-infarction inflammation. It may reflect inflammatory activation secondary to myocardial injury rather than a direct cardiac biomarker, yet it can provide valuable prognostic information in patients with CVD and concurrent infection. [30]

Clinical Integration and Diagnostic Utility

The integration of emerging biomarkers into clinical practice requires demonstration of analytical validity, clinical validity, and clinical utility. Combining biomarkers that reflect different biological pathways—such as myocardial injury (troponin, CK-MB), neurohormonal activation (BNP/NT-proBNP, copeptin), inflammation (CRP, IL-6, TNF- α), and fibrosis (galectin-3, PAPP-A, fibrinogen)—can enhance diagnostic precision and prognostic assessment. Multi-marker strategies are particularly valuable in complex CVD phenotypes such as heart failure with preserved ejection fraction or microvascular angina, where single biomarkers may not suffice. [31]

Future Perspectives

Future biomarker development is likely to focus on multi-omics integration—combining genomic, proteomic, and metabolomic data with imaging and AI-based models to refine cardiovascular risk stratification. Emerging technologies such as high-sensitivity assays, machine learning-based pattern

recognition, and microRNA profiling will further enable precision cardiovascular medicine. Standardization, cost-effectiveness, and accessibility remain key challenges for widespread clinical adoption. [32]

Conclusion

Over the past decade, significant progress has been made in identifying promising biomarkers for cardiovascular disease (CVD) detection and management. Despite these advances, the pursuit of an ideal biomarker – one that is simultaneously predictive, diagnostic, and prognostic – remains ongoing. Emerging markers such as BNP, NT-proBNP, cardiac troponins, CRP, IL-6, TNF- α , galectin-3, copeptin, and PAPP-A have expanded the understanding of CVD pathophysiology by reflecting diverse mechanisms including myocardial stress, inflammation, and fibrosis.

An optimal biomarker should not only meet analytical and clinical validity standards but also provide actionable information to guide patient care. While several circulating molecules show strong potential, further validation through large-scale, multicenter studies is required before they can be fully integrated into clinical practice. The combination of traditional and emerging biomarkers in multi-marker strategies may improve early detection, risk stratification, and therapeutic monitoring.

Future research should focus on developing standardized, high-sensitivity analytical platforms and leveraging bioinformatics, artificial intelligence, and multi-omics technologies to discover and validate novel targets. With

continued innovation and collaboration, biomarker-driven precision medicine may transform cardiovascular care, offering earlier diagnosis, personalized treatment, and improved patient outcomes.

References

Al-Mawali A (2015) Non-communicable diseases: shining a light on cardiovascular disease, Oman's biggest killer. *Oman Med J* 30(4):227

Thomas H, Diamond J, Vieco A, Chaudhuri S, Shinnar E, Cromer S, Perel P, Mensah GA, Narula J, Johnson CO (2018) Global atlas of cardiovascular disease. *Glob Heart* 13:143–163

Sanchis-Gomar F, Perez-Quilis C, Leischik R, Lucia A (2016) Epidemiology of coronary heart disease and acute coronary syndrome. *Ann Transl Med* 4(13):256

van Kimmenade RR, Januzzi JL Jr (2012) Emerging biomarkers in heart failure. *Clin Chem* 58(1):127–138

Haider A, Bengs S, Luu J, Osto E, Siller-Matula JM, Muka T, Gebhard C (2020) Sex and gender in cardiovascular medicine: presentation and outcomes of acute coronary syndrome. *Eur Heart J* 41(13):1328–1336

Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, Edelberg JM, Goodman SG, Hanotin C, Harrington RA (2018) Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med* 379(22):2097–2107

AlGawwam G, Shake ME (2021) Evaluation of glycogen phosphorylase BB (GPBB) level in serum as marker in diagnosis of acute ischemic stroke. *Indian J Forensic Med Toxicol* 15(3):1603–1608

El-Nagdy SA, Elfakharany Y, Etewa R (2020) Role of glycogen phosphorylase in prediction of cardiotoxicity associated with acute carbon monoxide poisoning. *Zagazig J Forensic Med* 18(2):59–74

Migocka-Patrzałek M, Elias M (2021) Muscle glycogen phosphorylase and its functional partners in health and disease. *Cells* 10(4):883

Apple FS, Wu AH, Mair J, Ravkilde J, Panteghini M, Tate J, Pagani F, Christenson RH, Mockel M, Danne O (2005) Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. *Clin Chem* 51(5):810-824

Krause E-G, Rabitzsch G, Noll F, Mair J, Puschendorf B (1996) Glycogen phosphorylase isoenzyme BB in diagnosis of myocardial ischaemic injury and infarction. *Mol Cell Biochem* 160(161):289-295

Yamaguchi S, Abe M, Arakaki T, Arasaki O, Shimabukuro M (2020) Prognostic value of lactate dehydrogenase for mid-term mortality in acute decompensated heart failure: a comparison to established biomarkers and brain natriuretic peptide. *Heart Lung Circ* 29(9):1318-1327

Aly M, Nayel M, Salama A, Ghazy E, Elshahawy I (2020) Cardiac troponin I as a cardiac biomarker has prognostic and predictive value for poor survival in Egyptian buffalo calves with foot-and-mouth disease. *Vet World* 13(5):890

Schmiechen NJ, Han C, Milzman DP (1997) ED use of rapid lactate to evaluate patients with acute chest pain. *Ann Emerg Med* 30(5):571-577

Yun DD, Alpert JS (1997) Acute coronary syndromes. *Cardiology* 88(3):223-237

29. Silagi ES, Batista P, Shapiro IM, Risbud MV (2018) Expression of carbonic anhydrase III, a nucleus pulposus phenotypic marker, is hypoxia responsive and confers protection from oxidative stress-induced cell death. *Sci Rep* 8(1):1-13

Dowling P, Gargan S, Zweyer M, Sabir H, Swandulla D, Ohlendieck K (2021) Proteomic profiling of carbonic anhydrase CA3 in skeletal muscle. *Expert Rev Proteomics* 18:1-14

Zamanova S, Shabana AM, Mondal UK, Ilies MA (2019) Carbonic anhydrases as disease markers. *Expert Opin Ther Pat* 29(7):509-533

Feng H-Z, Jin J-P (2019) Transgenic expression of carbonic anhydrase III in cardiac muscle demonstrates a mechanism to tolerate acidosis. *Am J Physiol Cell Physiol* 317(5):C922-C931

Zhang X-M, Tao Y-H, Zhou X-L, Shang X-L, Gong X-B, Liu Y-C, Huang Y-Y, Chen G, Yu Z-Y, Wang J-T (2021) The role of

carbonic anhydrase III and autophagy in type 2 diabetes with cardio-cerebrovascular disease. *Metab Brain Dis* 36(8):2329–2341

Kim YS, Karisa N, Jeon WY, Lee H, Kim Y-c, Ahn J (2019) High-level production of N-terminal pro-brain natriuretic peptide, as a calibrant of heart failure diagnosis, *Escherichia coli*. *Appl Microbiol Biotechnol* 103(12):4779–4788

York MK, Gupta DK, Reynolds CF, Farber-Eger E, Wells QS, Bachmann KN, Xu M, Harrell FE, Wang TJ (2018) B-type natriuretic peptide levels and mortality in patients with and without heart failure. *J Am Coll Cardiol* 71(19):2079–2088

Bustamante A, García-Berrocso T, Penalba A, Giralt D, Simats A, Muchada M, Zapata E, Rubiera M, Montaner J (2017) Sepsis biomarkers reprofiling to predict stroke-associated infections. *J Neuroimmunol* 312:19–23

Dres M, Hausfater P, Foissac F, Bernard M, Joly L-M, Sebbane M, Philippon A-L, Gil-Jardiné C, Schmidt J, Maignan M (2017) Mid-regional pro adrenomedullin and copeptin to predict short-term prognosis of COPD exacerbations: a multicenter prospective blinded study. *Int J Chron ObstrPulm Dis* 12:1047

Lopes D, Falcao LM (2017) Mid-regional pro-adrenomedullin and ST2 in heart failure: Contributions to diagnosis and prognosis. *Rev Port Cardiol (English Edition)* 36(6):465–472

Hülsmann M, Stanek B, Frey B, Sturm B, Putz D, Kos T, Berger R, Woloszczuk W, Maurer G, Pacher R (1998) Value of cardiopulmonary exercise testing and big endothelin plasma levels to predict short-term prognosis of patients with chronic heart failure. *J Am Coll Cardiol* 32(6):1695–1700

Kempf T, Wollert KC (2009) Growth differentiation factor-15: a new biomarker in cardiovascular disease. *Herz* 34(8):594–599

52. Jankowich M, Choudhary G (2020) Endothelin-1 levels and cardiovascular events. *Trends Cardiovasc Med* 30(1):1–8

Obokata M, Kane GC, Reddy YN, Melenovsky V, Olson TP, Jarolim P, Borlaug BA (2019) The neurohormonal basis

of pulmonary hyper tension in heart failure with preserved ejection fraction. *Eur Heart J* 40(45):3707–3717

Then C, Thorand B, Then HL, Meisinger C, Heier M, Peters A, Koenig W, Rathmann W, Bidlingmaier M, Lechner A (2020) Serum uromodulin is inversely associated with arterial hypertension and the vasoconstrictive prohormone CT-proET-1 in the population-based KORA F4 study. *PLoS ONE* 15(8):e0237364

Sabatine MS, Morrow DA, de Lemos JA, Omland T, Sloan S, Jarolim P, Solomon SD, Pfeffer MA, Braunwald E (2012) Evaluation of multiple biomarkers of cardiovascular stress for risk prediction and guiding medical therapy in patients with stable coronary disease. *Circulation* 125(2):233–240

Al-Hadi HA, Fox KA (2009) Cardiac markers in the early diagnosis and management of patients with acute coronary syndrome. *Sultan Qaboos Univ Med J* 9(3):231

Guzy PM (1977) Creatine phosphokinase-MB (CPK-MB) and the diagnosis of myocardial infarction. *West J Med* 127(6):455

Elliott B, Jepson E, Wilkinson J (1962) Serum alpha-hydroxybutyrate dehydrogenase-a new test with improved specificity for myocardial lesions. *Clin Sci* 23(2):305–309. Rosalki S (1963) Serum α -hydroxybutyrate dehydrogenase: a new test for myocardial infarction. *Br Heart J* 25(6):795

Automation in Clinical Laboratories: Bridging Manual to Automated Precision

Sudip Garai*

Department of Medical Laboratory Technology, School of Allied Health Sciences, Swami Vivekananda University, Telinipara, Barasat-Barrackpore Rd, Bara Kanthalia, West Bengal - 700121, India.

Abstract

In the modern era of healthcare, clinical laboratories face mounting pressure to deliver accurate, timely and cost-efficient diagnostic testing. Automation has emerged as a critical enabler for meeting these demands by integrating robotics, informatics and advanced instrumentation into laboratory workflows. This review explores how automation has evolved in clinical laboratories and discusses its implications for the future of diagnostics. It begins with an overview of previous manual and semi-automated methods, outlines the intrinsic limitations of such traditional approaches, and then examines the recent trends in automation—including total laboratory automation (TLA), laboratory information management systems (LIMS), artificial intelligence (AI) and connectivity frameworks. Key advantages such as improved turnaround time, higher throughput, enhanced quality and data traceability are analysed. The review also considers the challenges and caveats of implementing automation, including cost, staff training and integration issues. Finally, the article argues that automation is not simply a tool for task reduction, but a transformational force poised to reshape diagnostic strategies, enabling precision medicine and more efficient patient care. As the volume and complexity of laboratory tests continue to expand, automation stands as a foundational pillar for the future of diagnostics.

Keywords: automation, clinical laboratory, diagnostics, laboratory information management system (LIMS), artificial intelligence.

Introduction

Clinical laboratories constitute the backbone of diagnostic medicine, delivering information on disease presence, severity, progression and response to therapy. The accuracy, speed and reliability of laboratory results directly influence patient management, treatment outcomes and healthcare costs. Over recent decades, the volume of laboratory testing has grown substantially, driven by demographic changes, expanding test menus, increased screening initiatives and demands for rapid decision-making. At the same time, error rates, turnaround time (TAT) pressures and cost containment have become acute concerns for laboratories globally.

In this context, automation has emerged as a strategic response. Broadly defined, automation refers to the use of mechanical, electronic and computer-driven systems to perform tasks previously done manually—ranging from specimen sorting and processing to analytical testing, data handling and result reporting. Unlike simple mechanisation, modern laboratory automation increasingly includes informatics, robotics and decision-support elements. The goal is to optimise workflow, reduce variability, improve efficiency and ultimately improve patient care. [1]

This review article presents a structured examination of automation in the clinical laboratory domain: starting from earlier methods, moving through disadvantages of conventional approaches, then exploring recent trends in automation and concluding with the advantages and future perspectives of automation in diagnostics.

Conventional Methods

Historically, clinical laboratory workflows relied heavily on manual or semi-automated processes. For example, in the pre-analytical phase, specimens were physically labeled, logged, centrifuged and manually transported between bench stations. Analytical testing often involved operator-driven pipetting, reagent addition, instrument loading, calibration checks and manual result validation. Many instruments were stand-alone and not linked to a broader network; result entry and reporting frequently required manual transcription

or double-entry into laboratory information systems.

In microbiology, culture plating, colony inspection, microscopy and sensitivity testing were largely manual, with technicians performing hands-on manipulation of specimens and interpreting results. In haematology, manual slide preparation, staining and differential counting were common. Immunology and molecular methods often required manual extraction, amplification set-up and result interpretation. Quality control practices were labour-intensive, relying on human oversight.[2]

While these methods provided flexibility and were the norm for many years, they posed significant operational burdens when scaling up for high-volume testing. The “semi-automated” instruments—those that automated parts but still required human intervention for loading, aliquoting or data entry—represented a transitional phase between purely manual and fully automated systems.

Disadvantages of Conventional Methods

The conventional (manual or semi-automated) laboratory methods have several important disadvantages:

Human Error and Variability:

Manual pipetting, sample handling and data entry are prone to errors. Variability between operators can lead to inconsistent results and increased outlier rates. Studies show that automation can reduce error rates significantly.

Limited Throughput and Scalability:

As test volumes grow, manually intensive processes become bottlenecks. Throughput is constrained by staffing levels, fatigue, and workflow inefficiencies.

Longer Turnaround Time (TAT):

Manual steps prolong TAT, delaying clinical decision-making and potentially affecting patient outcomes. For example, early automation reports noted more than 50% reduction in TAT after implementation. [3]

Workforce Burden and Safety Risks:

Repetitive sample handling exposes staff to potential ergonomic stress, biohazard risks and monotony. Automation reduces these concerns by mechanising hazardous tasks.

Data Management Challenges:

Manual result entry and fragmented instrument systems create opportunities for transcription errors, poor traceability, and difficulties in integrating data into clinical information systems.

Cost Inefficiencies:

While manual methods may have lower initial capital cost, the recurrent costs of labour, reagents wasted due to repeat tests, specimen re-handling, and delays often lead to higher long-term cost per result.[4]

Quality and Standardisation Constraints:

Without automation, standardising processes across multiple shifts, sites or laboratories remains difficult, which can adversely affect reproducibility and inter-laboratory comparability.[5]

Taken together, these limitations create a compelling case for laboratory automation, especially in high-volume, complexity-growing diagnostic environments.

Recent Trends in Automation

Laboratory automation has advanced considerably over the past decade. Below are key trends shaping the current and future state of automation in clinical laboratories.

Total Laboratory Automation (TLA)

TLA refers to the fully integrated automation of pre-analytical, analytical and post-analytical workflows, encompassing specimen reception, sorting, centrifugation, aliquoting, transport, analysis and result release. Platforms include conveyor modules, barcode tracking, robotic arms, and system logic directing specimen movement across modular analyzers. For example, one review notes that modern TLA

systems can automate up to 80% of workflow tasks in high-volume chemistry labs. [4,6] Implementation of TLA has been shown to increase tests per staff and reduce turnaround time dramatically. [7]

Laboratory Information Management Systems (LIMS) and Connectivity

A key enabler of automation is informatics. LIMS, middleware and enterprise connectivity allow seamless transfer of specimen metadata, instrument data, result validation and reporting. Real-time monitoring of instrument status, sample tracking, and integration with electronic health records (EHR) enhance workflow transparency and efficiency. [8]

Artificial Intelligence (AI), Machine Learning (ML) and Digital Pathology

Automation is increasingly moving beyond mechanical tasks into decision support and image analytics. AI/ML algorithms are being employed in hematology smear review, histopathology slide interpretation and morphological abnormality detection. [9] Automation combined with AI enables higher-value tasks for technologists, shifting them from routine processing to exception handling and interpretation.

Point-of-Care Automation and Decentralised Testing

The push for faster diagnostics at or near the patient (POCT) has led to compact automated systems that embed ease of use, connectivity and rapid reporting. Combined with laboratory central automation, they support hub-and-spoke models of diagnostics. [10]

Data Analytics, Predictive Maintenance and IoT Integration

Automation systems now incorporate Internet of Things (IoT) sensors, instrument health monitoring, reagent usage tracking and predictive maintenance. This enhances uptime, lowers cost of operations and improves system reliability. The data collected also support analytics for workflow optimisation and capacity planning. [11]

Consolidation and Centralisation of Laboratories

As automation systems reach maturation, many diagnostic networks are consolidating smaller labs into larger automated core facilities, often servicing multiple sites to gain economies of scale, improve standardisation and reduce labour overheads. This is particularly noted in markets such as India. [12]

Sustainability and Green Automation

Emerging focus on sustainability has led to automation initiatives emphasising reduced reagent waste, lower energy consumption, and optimized workflows that reduce carbon footprint of laboratory operations.

These trends indicate that automation is not a static endpoint but a dynamic evolution of the laboratory environment, tightly integrated with informatics, robotics and strategic operational planning.

Advantages of Automation

The adoption of automation in clinical laboratories brings a wide array of advantages:

Improved Efficiency and Throughput

Automated systems can handle large volumes of tests with less downtime and fewer manual interventions. Studies have demonstrated multiple-fold increases in tests per worker when automation is in place. [13]

Reduced Turnaround Time (TAT)

By minimising manual handling and streamlining workflow, automation reduces delays. Some labs report same-day results for tests that previously took multiple days. [8, 11]

Enhanced Accuracy and Quality

Automation reduces operator variability and errors in pipetting, sample handling and data entry. Protocol fidelity is maintained and traceability is improved. [12]

Better Data Management and Traceability

Integrated systems log all key events—specimen receipt, processing steps, instrument usage, QC results—which enhances auditability, regulatory compliance and quality assurance.

Optimised Workforce Utilisation

Instead of repetitive manual tasks, laboratory professionals can shift focus to interpretive duties, quality control, result verification and investigation of exceptions. A review notes that automation frees staff to apply cognitive expertise. [7]

Scalability and Flexibility

Automated infrastructure supports expansion of test menus, volume surges (such as during pandemic testing) and consolidation of multiple sites into a central core facility. An example: transitioning to automation enabled a lab to absorb thousands of additional tests without proportional staffing increase.

Improved Safety

Automation reduces manual sample handling, thereby reducing exposure to biohazards, decreasing needle□stick injuries, and lowering ergonomic stress on staff.

Cost Containment

Although initial capital cost may be high, over time automation can lower cost per result by reducing repeats, wasted reagents, manual labour, and by increasing throughput and uptime.

Facilitation of Precision Medicine

With accurate, fast, high-volume data generation, automation supports development of personalised diagnostic panels, high-throughput genomics/ molecular assays, and integration with clinical decision□making systems. [14]

Overall, the case for automation in modern diagnostics is compelling: it supports faster, better, more reliable laboratory services, leading directly to improved patient outcomes.

Conclusion

As clinical laboratories evolve in the face of rising test volumes, increasing complexity and escalating demands

for speed and accuracy, automation emerges not only as a tool for efficiency but as a strategic enabler of the future of diagnostics. This review has traced the progression from manual and semi-automated workflows, identified the key shortcomings of conventional methods, and described recent trends in automation—from total laboratory automation and informatics integration to AI-driven diagnostics and sustainable lab operations.

The advantages of automation are manifold—but implementation is not without challenges. Key barriers include high upfront capital investment, need for staff training and cultural change, integration of heterogeneous instruments and legacy systems, maintenance and servicing requirements, and regulatory/quality assurance oversight. Thoughtful planning, stakeholder engagement, clear business case and change management are essential to realise the full potential of automation.

Looking ahead, the next frontier will likely involve deeper integration of AI and machine learning, more decentralised yet connected testing models (including POCT), enhanced data analytics for predictive diagnostics, and labs that are self-optimising via IoT and real-time feedback. Automation will enable laboratories to shift from being mere test factories to being strategic partners in clinical decision-making, precision medicine and population health management.

In closing, automation in the clinical laboratory is not simply the future of diagnostics—it is the present. Laboratories that adopt and adapt to automation will be better positioned to meet the evolving demands of healthcare, deliver value, and contribute meaningfully to improved patient care.

References

Alhammad L. A., Ainosah T. K., Ahmad A. M., Samarkandi M. S., Jawi N. H., Alharthi M. A., Alsharif A. M., Anazi E. A. A., Aldugeshem S. A., Johali F. Y. (2024). The impact of laboratory automation on efficiency and accuracy in healthcare settings. *International Journal of Community Medicine and Public Health*, 11(1), 459-463. (IMSEAR Repository)

Moorcroft, J. (2025, March 18). The measurable impact and benefits of automation in molecular laboratories. *Roche Diagnostics*. (Diagnostics)

Nasser S. M., Alshehri A. M., Gazwani H. A. & Alsemari S. A. (2023) The Role of Automation in Modern Clinical Laboratories. *Tec Empresarial*. (Revista Stecac)

Salem A. S., Arnous T. E., Almjuri M. A., Alqahtani M. S., Hazazi B. A., ShAlmotairi F. F., Alosaywan H. A., Alamri S. M. (2023). The role of automation in clinical laboratories: enhancing diagnostic accuracy and streamlining turnaround times. *Review of Contemporary Philosophy*, 22. (Review of Contemporary Philosophy)

“Advances in Clinical Laboratory Automation.” (2014). *American Society for Clinical Laboratory Science*. (ADLM)

“The importance of automation in clinical labs.” (2024). *Lab Leaders – Roche Diagnostics*. (Diagnostics)

Kritikos, A., Prod'hom, G., Jacot, D., Croxatto, A., & Greub, G. (2024). The impact of laboratory automation on the time to urine microbiological results: A five-year retrospective study. *Diagnostics*, 14(13), 1392. <https://doi.org/10.3390/diagnostics14131392> [MDPI](#)

Nam, Y., & Park, H.-D. (2025). Revolutionizing laboratory practices: Pioneering trends in total laboratory automation. *Annals of Laboratory Medicine*, 45, 472-483. <https://doi.org/10.3343/alm.2024.0581> [Synapse](#)

Singh, S., & Singh, S. (2023). Automation in clinical laboratory. In *Automation in Clinical Laboratory* (1st ed., pp. 1-31). Noble Science Press. <https://doi.org/10.52458/9789388996846>. nsp2023.eb.ch-05 [Noble Science Press](#)

Nasser, S. M., Alshehri, A. M., Gazwani, H. A., & Alsemari, S. A. (2024). The role of automation in modern clinical laboratories. *TEC Empresarial*, 6(2). [Revista Stecac](#)

Alqhtani, A. S., Arnous, T. E., Almjuri, M. A., Alqahtani, M. S., Hazazi, B. A., Alosaywan, H. A., ... Alamri, S. M. (2023). The role of automation in clinical laboratories: Enhancing diagnostic accuracy and streamlining turnaround times through advanced technological solutions. *Review of Contemporary Philosophy*, 22. [Review of Contemporary Philosophy](#)

Al Naam, A., et al. (2022). The impact of total automation on the clinical laboratory workforce: A case study. *Journal of Laboratory Automation*. (Note: exact volume/issue to be retrieved.) [PubMed](#)

Islam, S. ul, Kamboj, K., & Kumari, A. (2023). Laboratory automation and its effects on workflow efficiency in medical laboratories. *Middle East Journal of Applied Science & Technology*, 6(4), 88-97. <https://doi.org/10.46431/MEJAST.2023.6407>
[MEJAST](#)

Dakhilallah Saad Almalki, S., Al Inkis, M. N., Alrashdan, S., Ateeq, A. A., Alharthi, S. M., Alhummud, A. M. I., ... Alwadai, M. H. (2024). Automation in laboratories: How it's changing diagnostic techniques. *Power System Technology*, 48(4).

A Comprehensive Review on the Molecular Basis of Microbial Pathogenesis of Viral and bacterial infections

Sanjana Sengupta, Rajen Dey, Manojit Bysack*

Department of Medical Laboratory Technology, School of Allied Health Sciences, Swami Vivekananda University, Telinipara, Barasat-Barrackpore Rd, Bara Kanthalia, West Bengal - 700121, India

E-mail: bysackmanojit@gmail.com

Abstract

Research on microbial infections determines both the immediate and long-term interactions between specific microorganism and their hosts. Microbial pathogenesis is a complex phenomenon involving the interaction between microbial virulence factors and host cellular responses. These virulence factors include invasins, capsules, siderophores, enzymes, endotoxins, and exotoxins. Though the main problem in the current scenario is the coinfection. Beyond initiating infection, these factors also help the pathogenic microbes survive and thrive within the host's environment. Understanding the molecular mechanisms of microbial infections is essential for developing effective treatments, preventive measures, and vaccines. This review delves into how microbial pathogens invade host cells, evade the immune system, and establish infections. It also covers mechanisms of immune evasion, such as antigenic variation, mimicry, and the manipulation of host signaling pathways, utilizing advanced molecular biology techniques like CRISPR-Cas9 gene editing, transcriptomics, and proteomics. Additionally, it focuses on the key molecular interactions between viral and bacterial pathogen induced infections like respiratory co-infection and secondary bacterial pneumonia in patients with covid-19.

Keywords: Enzyme, Host response, Microbial virulence factors, Molecular mechanism

Introduction

The pathogenesis of coronavirus disease 2019 (COVID-19), which is caused by the coronavirus type-2 (SARS-CoV-2) virus, is still not fully known despite the disease's global expansion. A thorough description has been provided of respiratory infections brought on by multiple viruses (viral co-infection) or by bacteria and viruses together (combined viral and bacterial pneumonia). Secondary bacterial pneumonia may develop during the healing phase of a viral respiratory infection, or it may follow the original phase [1]. The overall incidence of viral co-infection has varied greatly from 0% to 19% in different case series, according to the limited data on SARS-CoV-2. The rates of combined viral and bacterial pneumonia also seem modest [3][8–10]. Furthermore, little is known about the organisms and risk factors that cause the condition. The most well-studied cases of combined viral and bacterial pneumonia, secondary bacterial pneumonia caused by *Staphylococcus aureus* and other prevalent community-acquired pneumonia pathogens, and considerable morbidity and mortality are associated with seasonal and pandemic influenza [2][3]. During the previous pandemic of severe acute respiratory syndrome (SARS), methicillin-resistant *S aureus* (MRSA) caused 47% of cases of secondary bacterial pneumonia, although there was serious concern about cross-transmission. This pneumonia occurred as ventilator-associated pneumonia in 25% of patients at a single center [7]. The following commentary is based on available clinical data and experience with similar viruses, such as influenza and SARS-CoV, because there isn't a clear pattern or set of guidelines for viral co-infection, combined viral and bacterial pneumonia, or secondary bacterial pneumonia in the context of SARS-CoV-2. Based on our current understanding, a comprehensive approach to COVID-19 would ideally encompass both the entire presentation and the illness trajectory.

Viral Co-Infection

In addition to SARS-CoV-2 testing, polymerase chain reaction (PCR) assays should be used to screen for influenza in all individuals who exhibit symptoms of a respiratory infection.

If accessible, PCR assays can also be used to test for additional respiratory viruses. All patients with co-infection with the influenza A or B viruses should receive treatment with oseltamivir or a different medication, regardless of the severity of their illness [11]. If there is a clear exposure or risk factor, empirical treatment for influenza virus co-infection may be explored while test results are awaited. Therapy alternatives are restricted and only work in certain situations, including immunosuppression or hypogammaglobulinemia, if viral co-infection with another respiratory virus, such as respiratory syncytial virus, is found [12][13]. It is highly advised to visit an infectious disease specialist to weigh the advantages of this course of treatment against the possibility of aggravating COVID-19-related organ failure and the possible side effects of the prescribed medicine or medications.

Bacterial Pneumonia

A strong index of suspicion is needed to diagnose secondary bacterial pneumonia with COVID-19 or combination viral and bacterial pneumonia. Even while viral and bacterial symptomatology significantly overlap[2-10] [14-29], some aspects of bacterial infection may still be distinguishable. Bacterial pneumonia is characterized by neutrophilic leukocytosis, but COVID-19 patients usually exhibit lymphopenia along with a normal white blood cell count [5][8][14][15].

Mode Of Infection

In order to distinguish between the two possible causes of community-acquired pneumonia, procalcitonin is neither sensitive nor specific [11]. But procalcitonin levels in isolated SARS-CoV-2 infection have been regularly reported to be normal (low) in various COVID-19 case series. This has led to its widespread, albeit unvalidated, usage to “rule out” combination viral and bacterial pneumonia, but the precise cutoff is still unknown. This comment emphasizes how important it is to take into account every factor within the framework of the clinical setting. Antibiotics can be safely delayed in patients with mild to moderate respiratory failure consistent with COVID-19 presentation and without clear

evidence of bacterial infection. This is because there is little chance of mixed viral and bacterial pneumonia in these individuals. In this instance, the advancement of COVID-19 is more likely to be the cause of the steadily deteriorating respiratory failure during the first week of presentation than the emergence of a newly superimposed secondary bacterial pneumonia. This covers patients who begin receiving supplemental oxygen support in a noninvasive manner before eventually needing invasive mechanical ventilation. Even if respiratory discomfort is getting worse, medicines shouldn't be started if there isn't any sign of bacterial pneumonia. Unless otherwise demonstrated, nosocomial acquisition of secondary bacterial infection—that is, hospital-acquired pneumonia, infection at an extrapulmonary site, or both—is likely if a patient experiences new or sharply worsening respiratory failure, sepsis, or both following an initial period of consistent improvement (considered to be days).

Although COVID-19 can induce acute respiratory decompensation on its own, there is little information on the potential involvement of secondary bacterial pneumonia in this condition. Consequently, until this secondary infection is ruled out, using empiric antibiotics based on guidelines may be prudent. One or more of the following symptoms could indicate secondary bacterial pneumonia: new or recrudescent fever; fresh onset or change in sputum characteristics; fresh neutrophilia or leukocytosis (or both); fresh pertinent imaging results; and fresh or rising oxygen demand. It's crucial to manage these patients appropriately and take into account any additional potential causes of hospital-acquired infections, such as urinary tract or indwelling central venous catheters. Empirical treatment for all potential causes at the outset is crucial for a critically ill patient who has been admitted and is experiencing acute respiratory failure. Because procalcitonin levels in patients with multiorgan failure can be mistakenly increased, this is very crucial. [30][31], therefore it may be difficult to distinguish between concealed bacterial infection consolidation and bilateral infiltrates of acute respiratory distress syndrome using imaging investigations.

Possible Mode Of Management

The American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) guidelines, host risk factors, and previous microbiologic data should all be taken into consideration when developing an empirical treatment plan for community-acquired pneumonia [19]. For every patient, respiratory samples (tracheal aspirate is preferred over sputum in those on mechanical ventilation) and blood cultures should be submitted, ideally prior to the initiation of medications. All patients who come with severe community-acquired pneumonia should have a test for the urine antigen of streptococcus pneumoniae. Depending on the clinical setting and epidemiology, samples for Legionella pneumophila urine antigen and Mycoplasma pneumoniae IgM and IgG antibodies may be provided. If there are no symptoms of bacterial pneumonia, a positive respiratory culture may indicate colonization, particularly in those who have already had pneumonia caused by the same organism in the past or have changed airway anatomy. This distinction can be made with the aid of laboratory markers, radiologic characteristics, and quantitative and semiquantitative culture techniques [19]. While the presentation of secondary bacterial pneumonia in a patient receiving invasive mechanical ventilation resembles that of hospital-acquired pneumonia, guidelines dictate the aggressive use of empiric broad-spectrum antibiotics that cover MRSA, Pseudomonas aeruginosa, and potentially other multidrug-resistant organisms [19]. The adverse effects of institutional antibiograms and antibiotics must also be taken into account. In addition to frequently exhibiting lower-grade fevers and higher secretions, patients with ventilator-associated tracheobronchitis may also have low-grade fevers and prove challenging to wean off of ventilator assistance. Only a careful case-based review is necessary because the evidence supporting antibiotic therapy for this clinical entity is weak. For community-acquired pneumonia [32], antibiotic therapy should be administered for 5 to 7 days; in the case of hospital-acquired pneumonia and ventilator-associated pneumonia [19], it should be administered for 7 days, provided no problems arise. In particular, if side effects are observed, think about reducing the period if patients show symptoms of

clinical stability. Based on the procalcitonin level trend over the course of 24 to 48 hours, determining the procalcitonin level at presentation will aid in the de-escalation of antibiotics [33]. It is permissible to stop all antibiotics if, after 48 hours of testing, a microbiological source cannot be found and the procalcitonin level is less than 0.5 µg/L or drops by 80% or more from peak concentration[19]. Because interleukin 6 (IL-6) inhibitors, such tocilizumab, reduce frequent indications of sepsis, using them for COVID-19-related cytokine activation syndrome poses a special problem. When tocilizumab is used for rheumatologic illnesses, there is a consistently observed increased risk of serious bacterial infections [34][35][36][37]. After using tocilizumab, C-reactive protein and other acute-phase reactants, such as white blood cell count, may not rise in response to a secondary bacterial infection [35][38][39]. It's unclear exactly how long this impact lasts after taking one or two doses. Although procalcitonin may be less influenced by IL-6 inhibitors, there is insufficient data to distinguish between bacterial and viral pneumonia in this setting, and more research is needed to make this determination [40-44].

Conclusion

Combining SARS-CoV-2 with a bacterial pathogen (combined viral and bacterial pneumonia) or SARS-CoV-2 with another virus (viral co-infection) can cause respiratory infections in COVID-19. Either during the healing phase or after the initial viral respiratory illness, secondary bacterial pneumonia can develop. Regarding viral co-infection, combined viral and bacterial pneumonia, or subsequent bacterial pneumonia in COVID-19, there isn't a clear pattern or set of recommendations. The management strategy for COVID-19 should, ideally, take into account the overall presentation and the progression of illness based on available clinical data and experience with viruses related to influenza and SARS-CoV. Last but not least, invasive pulmonary aspergillosis is linked to high rates of morbidity and mortality and has been reported in critically ill patients with seasonal and pandemic influenza. Patients with acute respiratory distress syndrome linked with COVID-19 have also been documented to have invasive pulmonary aspergillosis. High-risk patients include

those with immunocompromised states, prior or concurrent influenza viral co-infection, clinical worsening despite proper antibiotic treatment, and positive fungal indicators such as galactomannan on culture. These patients should be monitored closely for this consequence. Treatment with a wide antifungal, such as voriconazole, should be started as soon as invasive pulmonary aspergillosis is detected, after consulting with colleagues in infectious diseases.

Acknowledgments

The authors are highly thankful to the Department of Medical Laboratory Technology, Swami Vivekananda University, Barrackpore. The authors also declare that no funds or grants were received during the preparation of this article.

Conflict Of Interest

All authors declare that there are no conflicts of interest.

Data Availability Statement

No data was used for the research described in the article.

Author's Contribution

ManojitBysack (MB) participated in the conception of the study. RajenDey (RD) and SanjanaSengupta (SS) participated in literature searches and extraction. MB and SS wrote the manuscript for submission to this journal.

Reference

van der Sluijs KF, van der Poll T, Lutter R, Juffermans NP, Schultz MJ. Bench-to-bedside review: bacterial pneumonia with influenza – pathogenesis and clinical implications. *Crit Care* 2010; 14(2):219. doi:10.1186/cc8893

Pongpirul WA, Mott JA, Woodring JV, et al. Clinical characteristics of patients hospitalized with coronavirus disease, Thailand. *Emerg Infect Dis* 2020; 26(7):1580-1585. doi:10.3201/eid2607.200598

Arentz M, Yim E, Klaff L, et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in

Washington State. JAMA 2020; 323(16):1612-1614. doi:10.1001/jama.2020.4326

Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 2020; 180(7):1-11. doi:10.1001/jamainternmed.2020.0994

Ding Q, Lu P, Fan Y, Xia Y, Liu M. The clinical characteristics of pneumonia patients coinfecting with 2019 novel coronavirus and influenza virus in Wuhan, China. J Med Virol 2020; Mar 20. doi:10.1002/jmv.25781

Khodamoradi Z, Moghadami M, Lotfi M. Co-infection of coronavirus disease 2019 and influenza a: a report from Iran. Arch Iran Med 2020; 23(4):239-243. doi:10.34172/aim.2020.04

Touzard-Romo F, Tapé C, Lonks JR. Co-infection with SARS-CoV-2 and human metapneumovirus. R I Med J (2013) 2020; 103(2):75-76. pmid:32192233

Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395(10223):507-513. doi:10.1016/S0140-6736(20)30211-7

Wang L, He W, Yu X, et al. Coronavirus disease 2019 in elderly patients: characteristics and prognostic factors based on 4-week follow-up. J Infect 2020; Mar 30. doi:10.1016/j.jinf.2020.03.019

Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395(10229):1054-1062. doi:10.1016/S0140-6736(20)30566-3

Uyeki TM, Bernstein HH, Bradley JS, et al. Clinical practice guidelines by the Infectious Diseases Society of America: 2018 update on diagnosis, treatment, chemoprophylaxis, and institutional outbreak management of seasonal influenza. Clin Infect Dis 2019; 68(6):e1-e47. doi:10.1093/cid/ciy866

Beaird OE, Freifeld A, Ison MG, et al. Current practices for treatment of respiratory syncytial virus and other non-influenza respiratory viruses in high-risk patient populations: a survey of institutions in the Midwestern Respiratory Virus Collaborative. *Transpl Infect Dis* 2016; 18(2):210–215. doi:10.1111/tid.12510

Beigel JH, Nam HH, Adams PL, et al. Advances in respiratory virus therapeutics—a meeting report from the 6th Isirv Antiviral Group conference. *Antiviral Res* 2019; 167:45–67. doi:10.1016/j.antiviral.2019.04.006

Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* 2020; Mar 3. doi:10.1007/s00134-020-05991-x

Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020. doi:10.1016/S2213-2600(20)30079-5

Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395(10223):497–506. doi:10.1016/S0140-6736(20)30183-5

Lippi G, Plebani M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. *ClinChimActa* 2020; 505:190–191. doi:10.1016/j.cca.2020.03.004

Rule JA, Hynan LS, Attar N, et al. Procalcitonin identifies cell injury, not bacterial infection, in acute liver failure. *PLoSOne* 2015; 10(9):e0138566. doi:10.1371/journal.pone.0138566

Metlay JP, Waterer GW, Long AC, et al. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am J Respir Crit Care Med* 2019; 200(7):e45–e67. doi:10.1164/rccm.201908-1581ST

Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Accessed April 24, 2020. <http://www.chinaxiv.org/abs/202003.00026>.

Bloomfield M, Parackova Z, Cabelova T, et al. Anti-IL6 autoantibodies in an infant with CRP-less septic shock. *Front Immunol* 2019; 10:2629. doi:10.3389/fimmu.2019.02629

Gaensbauer JT, Press CA, Hollister JR, Asturias EJ. Procalcitonin: a marker of infection not subverted by treatment with interleukin-6 receptor inhibition. *Pediatr Infect Dis J* 2013; 32(9):1040. doi:10.1097/INF.0b013e318295a3d0

Sato H, Tanabe N, Murasawa A, et al. Procalcitonin is a specific marker for detecting bacterial infection in patients with rheumatoid arthritis. *J Rheumatol* 2012; 39(8):1517-1523. doi:10.3899/jrheum.111601

Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: a single center experience. *J Med Virol* 2020; Apr 6. doi:10.1002/jmv.25801

Michot JM, Albiges L, Chaput N, et al. Tocilizumab, an anti-IL6 receptor antibody, to treat COVID-19-related respiratory failure: a case report. *Ann Oncol* 2020; 31(7):961-964. doi:10.1016/j.annonc.2020.03.300

Wong HYF, Lam HYS, Fong AH, et al. Frequency and distribution of chest radiographic findings in COVID-19 positive patients. *Radiology* 2020; 296(2):E72-E78. doi:10.1148/radiol.2020201160

Kanne JP, Little BP, Chung JH, Elicker BM, Ketani LH. Essentials for radiologists on COVID-19: an update-radiology scientific expert panel. *Radiology* 2020; 296(2):E113-E114. doi:10.1148/radiol.2020200527

Pan F, Ye T, Sun P, et al. Time course of lung changes at chest CT during recovery from coronavirus disease 2019. *Radiology* 2020; 295(3):715-721. doi:10.1148/radiol.2020200370

Giamarellos-Bourboulis EJ, Meka A, Grecka P, et al. Procalcitonin: a marker to clearly differentiate systemic inflammatory response syndrome and sepsis in the critically ill patient? *Intensive Care Med* 2002; 28(9):1351-1356. doi:10.1007/s00134-002-1398-z

Wee LE, Ko KKK, Ho WQ, Kwek GTC, Tan TT, Wijaya L. Community-acquired viral respiratory infections amongst hospitalized inpatients during a COVID-19 outbreak in Singapore: co-infection and clinical outcomes. *J Clin Virol* 2020 Jul;128:104436. doi:10.1016/j.jcv.2020.104436

Hui L, Zhang X, An X, et al. Higher serum procalcitonin and IL-6 levels predict worse diagnosis for acute respiratory distress syndrome patients with multiple organ dysfunction. *Int J Clin Exp Pathol* 2017; 10(7):7401–7407. PMID:31966582

Kalil AC, Metersky ML, Klompas M, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin Infect Dis* 2016; 63(5):e61–e111. doi:10.1093/cid/ciw353. e111

Lam SW, Bauer SR, Fowler R, Duggal A. Systematic review and meta-analysis of procalcitonin-guidance versus usual care for antimicrobial management in critically ill patients: focus on subgroups based on antibiotic initiation, cessation, or mixed strategies. *Crit Care Med* 2018; 46(5):684–690. doi:10.1097/CCM.0000000000002953

Campbell L, Chen C, Bhagat SS, Parker RA, Östör AJ. Risk of adverse events including serious infections in rheumatoid arthritis patients treated with tocilizumab: a systematic literature review and meta-analysis of randomized controlled trials. *Rheumatology (Oxford)* 2011; 50(3):552–562. doi:10.1093/rheumatology/keq343

Chiu YM, Chen DY. Infection risk in patients undergoing treatment for inflammatory arthritis: non-biologics versus biologics. *Expert Rev Clin Immunol* 2020; 16(2):207–228. doi:10.1080/1744666X.2019.1705785

Yamamoto K, Goto H, Hirao K, et al. Longterm safety of tocilizumab: results from 3 years of followup postmarketing surveillance of 5,573 patients with rheumatoid arthritis in Japan. *J Rheumatol* 2015; 42(8):1368–1375. doi:10.3899/jrheum.141210

Calderón-Goercke M, Loricera J, Aldasoro V, et al. Tocilizumab in giant cell arteritis. Observational, open-label

multicenter study of 134 patients in clinical practice. *Semin Arthritis Rheum* 2019; 49(1):126–135. doi:10.1016/j.semarthrit.2019.01.003

Nguyen MT, Pødenphant J, Ravn P. Three cases of severely disseminated *Staphylococcus aureus* infection in patients treated with tocilizumab. *BMJ Case Rep* 2013 Jan 2;2013:bcr2012007413. doi:10.1136/bcr-2012-007413

Bari SF, Khan A, Lawson T. C reactive protein may not be reliable as a marker of severe bacterial infection in patients receiving tocilizumab. *BMJ Case Rep* 2013; Oct 31. doi:10.1136/bcr-2013-010423

Vanderbeke L, Spriet I, Breynaert C, Rijnders BJ, Verweij PE, Wauters J. Invasive pulmonary aspergillosis complicating severe influenza: epidemiology, diagnosis and treatment. *Curr Opin Infect Dis* 2018; 31(6):471–480. doi:10.1097/QCO.0000000000000504

Schauwvlieghe AFAD, Rijnders BJA, Philips N, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med* 2018; 6(10):782–792. doi:10.1016/S2213-2600(18)30274-1

Yu Z, Zhang Q, Gu B, et al. Clinical features of fatal pandemic influenza A/H1N1 infection complicated by invasive pulmonary fungal infection. *Mycopathologia* 2020; 185(2):319–329. doi:10.1007/s11046-019-00421-z

Hwang DM, Chamberlain DW, Poutanen SM, Low DE, Asa SL, Butany J. Pulmonary pathology of severe acute respiratory syndrome in Toronto. *Mod Pathol* 2005; 18(1):1–10. doi:10.1038/modpathol.3800247

Koehler P, Cornely OA, Bottiger BW, et al. COVID-19 associated pulmonary aspergillosis. *Mycoses* 2020; 63(6):528–534. doi:10.1111/myc.13096

Biofilm-Associated Hospital Acquired Infection: Epidemiology, Diagnosis and Management- A Review

Santanu Ojha

Department of Medical Laboratory Technology, School of
Allied Health Sciences, Swami Vivekananda University,
Telinipara, Barasat-Barrackpore Rd, Bara Kanthalia, West
Bengal - 700121, India.

Abstract

Hospital-acquired infections (HAIs) represent a formidable challenge to global healthcare systems, with a significant proportion intricately linked to biofilm-forming microorganisms. These structured microbial communities, encased within a protective extracellular polymeric matrix, exhibit remarkable resistance to antimicrobial agents and host immune responses, leading to persistent and recurrent infections. The epidemiology of biofilm-associated HAIs reveals a substantial burden, accounting for approximately 65-80% of all microbial infections, with device-related infections such as catheter-associated urinary tract infections, ventilator-associated pneumonia, and central line-associated bloodstream infections being particularly prevalent. The predominant biofilm-forming pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, with multidrug-resistant strains frequently emerging from biofilm environments. Diagnosis remains challenging due to the limitations of conventional microbiological methods, necessitating advanced microscopic, molecular, and biomarker-based detection techniques to accurately identify biofilm-associated infections. Traditional management strategies often prove inadequate, prompting the development of innovative approaches including quorum sensing inhibitors, nanotechnology-based drug delivery systems, smart anti-adhesive surface coatings, enzymatic

disruption agents, and phytotherapeutic compounds. This comprehensive review synthesizes current understanding of the epidemiology, diagnostic modalities, and evolving management strategies for biofilm-associated HAIs, highlighting the critical need for multidisciplinary approaches to mitigate this persistent threat to patient safety and public health.

Keywords: biofilm, hospital-acquired infections, antimicrobial resistance, diagnostics, management strategies, medical devices

Introduction

Biofilms represent the predominant mode of growth for microorganisms in various environments, including clinical settings, where they play a significant role in the pathogenesis of persistent infections. Structurally, biofilms are complex, organized communities of microorganisms encapsulated within a self-produced matrix of extracellular polymeric substances (EPS) that adhere to biological or inert surfaces -8. This matrix typically consists of polysaccharides, proteins, lipids, and extracellular DNA, forming a protective barrier that significantly enhances microbial resistance to antimicrobial agents and host immune defenses -1-8. The concept of biofilms has evolved substantially since Anthony van Leeuwenhoek first observed surface-associated bacteria, with the term “biofilm” formally defined by Costerton et al., and its clinical significance recognized by the American Society of Microbiology in 1993 -8.

The relevance of biofilms in healthcare-associated infections cannot be overstated. According to the National Institutes of Health, biofilms are responsible for up to 80% of human microbial infections, with approximately 70% of nosocomial infections directly attributable to biofilm-forming pathogens -1-8. The protective nature of the biofilm matrix renders embedded bacteria up to 500-5000 times more resistant to antibiotics compared to their planktonic (free-floating) counterparts, creating therapeutic challenges that substantially contribute to patient morbidity, mortality, and increased healthcare costs -1-7. The persistent nature of

biofilm-associated infections often leads to chronic conditions that are difficult to eradicate, resulting in prolonged hospital stays, increased antibiotic usage, and in cases involving medical devices, often necessitating device removal -1-4.

The formation of biofilms follows a sequential, multi-stage process beginning with initial attachment of planktonic cells to a surface, followed by irreversible adhesion, microcolony formation, maturation into a complex three-dimensional structure, and eventual dispersion of cells to initiate new colonies -1-8. This cyclic process is regulated by complex genetic and phenotypic mechanisms, including quorum sensing (QS), a cell-density dependent communication system that coordinates gene expression and collective behavior within the microbial community -1-3. Understanding these fundamental mechanisms provides the foundation for developing targeted strategies to prevent and control biofilm-associated infections in healthcare settings.

This comprehensive review aims to synthesize current knowledge on the epidemiology, diagnostic approaches, and management strategies for biofilm-associated hospital-acquired infections. By examining the latest advances in detection methods and innovative therapeutic approaches, this review seeks to provide healthcare professionals and researchers with a thorough understanding of the challenges posed by biofilm-associated HAIs and the emerging solutions to address them.

2. Epidemiology and Burden of Biofilm-Associated HAIs

2.1 Global Prevalence and Impact

The global burden of biofilm-associated hospital-acquired infections represents a significant public health challenge with substantial clinical and economic implications. Biofilms are estimated to be responsible for more than 65% of nosocomial infections and approximately 80% of chronic infections, contributing significantly to patient morbidity and mortality worldwide -4. Recent epidemiological data indicate that Gram-positive and Gram-negative pathogenic bacteria account for up to 70% of nosocomial infections in intensive care unit (ICU) patients, with the majority of these

infections involving biofilm-forming strains -1. The European Centre for Disease Prevention and Control (ECDC) estimates that within the EU alone, approximately 4.1 million patients acquire healthcare-associated infections annually, resulting in 110,000 deaths, with biofilms playing a significant role in many of these fatal outcomes -9.

The economic burden associated with biofilm-related HAIs is staggering, encompassing extended hospital stays, increased antibiotic usage, additional diagnostic procedures, and specialized treatment requirements. Patients with biofilm-associated infections experience prolonged hospitalization, with associated costs significantly impacting healthcare systems already operating under considerable financial constraints -3-4. The complex nature of these infections often necessitates repeated courses of antibiotics, sometimes followed by surgical intervention for device removal or debridement of infected tissues, further escalating healthcare expenditures and contributing to the growing problem of antimicrobial resistance -5-7.

2.2 Common Biofilm-Associated Pathogens

The microbial landscape of biofilm-associated HAIs is diverse, encompassing both Gram-positive and Gram-negative bacteria, as well as fungal species. The most frequently encountered biofilm-forming bacteria in healthcare settings include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Streptococcus viridans*, *Enterococcus faecalis*, and *Acinetobacter baumannii* -4-8. Staphylococci deserve particular attention as they are responsible for nearly 80% of infections associated with implantable medical devices, with *S. epidermidis* emerging as an important cause of nosocomial bloodstream infections through its ability to colonize and adhere to medical devices such as central venous catheters -4-8.

Among Gram-negative pathogens, *P. aeruginosa* represents a formidable challenge due to its robust biofilm-forming capabilities and intrinsic resistance mechanisms. Surveillance studies have revealed alarming resistance patterns, with

one national study in Saudi Arabia reporting that all collected *P. aeruginosa* isolates were classified as multidrug-resistant (MDR), with approximately 22.2% representing difficult-to-treat (DTR) strains and 5.4% exhibiting pandrug-resistant (PDR) profiles -3. Similarly, *K. pneumoniae* and *A. baumannii* have emerged as significant concerns in ICU settings, where their ability to form biofilms on environmental surfaces and medical equipment facilitates persistence and transmission within healthcare facilities -1-4.

The fungal pathogen *Candida albicans* also represents an important etiology of device-associated biofilm infections, particularly in immunocompromised patients or those receiving broad-spectrum antibiotic therapy -6. Of increasing concern are polymicrobial biofilms containing both bacterial and fungal pathogens, which present additional treatment complexities due to synergistic interactions and enhanced resistance profiles -6.

2.3 Biofilms in Medical Device-Associated Infections

Indwelling medical devices (IMDs) represent a significant risk factor for biofilm-associated HAIs, providing ideal surfaces for microbial attachment and biofilm development. Commonly affected devices include urinary catheters, central venous catheters, endotracheal tubes, mechanical heart valves, prosthetic joints, pacemakers, peritoneal dialysis catheters, and various orthopedic implants -1-4. The incidence of device-related infections correlates strongly with the duration of device use, with biofilm formation occurring on approximately 40-70% of indwelling devices depending on the device type, hospital unit, and patient population -4.

Catheter-associated urinary tract infection (CAUTI) represents the most prevalent biofilm-based device-related infection, affecting over 150 million individuals worldwide annually -1. Studies on urinary catheter-associated biofilms have demonstrated that approximately 40.2% of bacterial isolates from catheterized patients are biofilm producers, with *E. coli* (24-57.1%), *Klebsiella* spp. (19%), *Proteus mirabilis* (18%), *P. aeruginosa* (17%), and *Enterococcus* spp. (8%) being the most frequently isolated pathogens -4. Similarly, central venous

catheters are frequently colonized by biofilm-forming microorganisms, with scanning electron microscopy studies confirming the presence of microorganisms embedded in biofilm matrices on the majority of explanted catheters -1.

Ventilator-associated pneumonia (VAP) represents another significant device-related infection where biofilms play a crucial pathogenic role. Endotracheal tubes of mechanically ventilated patients provide ideal conditions for biofilm development, with these biofilms acting as protective shields against host immunity and serving as reservoirs for pathogens that can induce chronic and recurrent pulmonary infections resistant to conventional antibiotic therapies -4. The biofilm burden on these devices not only contributes to treatment failure but also facilitates the dissemination of pathogens to the lower respiratory tract, increasing the risk of severe complications including sepsis -5.

Flexible multichannel endoscopes represent a unique category of reusable medical equipment particularly vulnerable to biofilm formation due to their complex design and intricate lumens. When reprocessing guidelines are not meticulously followed, biofilm development can occur in the moist, nutrient-rich environment within the endoscope lumen, potentially leading to patient-to-patient transmission of pathogenic microorganisms -1. The persistent nature of these biofilms presents substantial challenges to effective disinfection, necessitating stringent protocols and monitoring to prevent iatrogenic infections.

3. Pathogenesis and Mechanisms of Biofilm-Related Resistance

3.1 Stages of Biofilm Development

The formation of bacterial biofilms is a complex, multi-stage process that involves a series of well-orchestrated events leading to the development of structured microbial communities. The initial stage involves the reversible attachment of free-floating planktonic bacteria to a surface, mediated by bacterial appendages such as flagella and pili, as well as physical forces including hydrophobic, electrostatic, and van der Waals interactions -1-8. This transient attachment

becomes irreversible through the production of adhesive surface proteins and exopolysaccharides that firmly anchor the bacteria to the substrate -8.

Following irreversible attachment, the attached bacteria undergo proliferation and begin to form microcolonies, representing the second stage of biofilm development. During this phase, cell-to-cell communication through quorum sensing mechanisms becomes increasingly important, coordinating collective behavior and initiating the expression of biofilm-specific genes -1-3. As the microcolonies expand, they enter the maturation phase, characterized by the secretion of abundant extracellular polymeric substances that form a protective matrix and develop into a complex three-dimensional architecture with water channels that facilitate nutrient distribution and waste removal -1-8.

The final stage of the biofilm lifecycle involves active dispersal, where enzymes such as dispersin B and deoxyribonuclease degrade the biofilm matrix, releasing planktonic bacterial cells or clusters of cell aggregates that can disseminate to new locations and initiate fresh cycles of biofilm formation -1-5. This dispersal phase is crucial for bacterial survival and the spread of infection to new sites within the host, contributing to the chronic and recurrent nature of biofilm-associated infections -5.

3.2 Molecular Mechanisms of Antimicrobial Resistance in Biofilms

The remarkable resistance of biofilm-embedded microorganisms to antimicrobial agents represents one of the most challenging aspects of managing biofilm-associated infections. This resistance is multifactorial, arising from a combination of physical, physiological, and genetic mechanisms that collectively enhance microbial survival in the presence of antibiotics or host defense mechanisms -1-7.

The extracellular polymeric substance matrix serves as a physical barrier that significantly restricts the penetration of antimicrobial agents into the deeper layers of the biofilm. The anionic nature of the matrix components, including polysaccharides and extracellular DNA, enables them to

bind positively charged antibiotics, effectively sequestering these molecules and preventing their access to bacterial cells -1-7. Additionally, the dense structure of the matrix creates diffusion limitations that further reduce antibiotic penetration, with some studies demonstrating that certain antibiotics may only penetrate the outermost 10-20 cell layers of mature biofilms -7.

Within the biofilm microenvironment, gradients of nutrients, oxygen, and metabolic waste products create heterogeneous populations of bacterial cells with varying metabolic states and growth rates. Bacteria located in the deeper layers of the biofilm often enter a slow-growing or dormant state due to nutrient limitation and accumulation of waste products, rendering them less susceptible to antibiotics that primarily target actively growing cells -5-7. This physiological heterogeneity contributes to the presence of “persister” cells – a small subpopulation of metabolically dormant cells that exhibit exceptionally high tolerance to antimicrobial agents and can repopulate the biofilm following antibiotic treatment -1.

Biofilms also facilitate the horizontal transfer of resistance genes through conjugation, transformation, and transduction, accelerating the development and dissemination of antimicrobial resistance within and between bacterial species -1-4. The close proximity of cells within the biofilm structure enhances genetic exchange, while the stress conditions within the biofilm microenvironment may further stimulate the acquisition of resistance determinants -4. This genetic adaptability, combined with the selective pressure of antibiotic exposure, promotes the emergence of multidrug-resistant strains that pose serious challenges to infection control efforts in healthcare settings -3-4.

3.3 Immune Evasion Strategies

In addition to their enhanced antimicrobial resistance, biofilms employ sophisticated mechanisms to evade host immune responses. The EPS matrix presents a physical barrier that limits penetration of immune cells such as neutrophils and macrophages, while simultaneously protecting embedded

bacteria from phagocytosis -1-8. Biofilm-forming pathogens also produce various virulence factors that directly impair immune cell function, including toxins that lyse phagocytes and enzymes that degrade immunoglobulins and complement components -7.

Recent research has revealed complex interactions between biofilms and host-derived components that further contribute to immune evasion. Studies of polymicrobial biofilms in chronic periodontitis have demonstrated that extracellular DNA (eDNA) serves as a predominant matrix component, with the majority of this eDNA derived from host cells, likely through neutrophil extracellular traps (NETs) rather than bacterial sources -3. These findings underscore the significant role of host-derived components in biofilm stabilization and highlight the dynamic interplay between immune responses and microbial biofilm development, wherein the host's attempt to control infection may inadvertently contribute to biofilm persistence -3.

4. Diagnostic Approaches for Biofilm-Associated Infections

4.1 Conventional Diagnostic Methods

The accurate and timely diagnosis of biofilm-associated infections remains challenging due to the limitations of conventional microbiological techniques, which were primarily developed for the detection of planktonic microorganisms. Standard culture methods often yield false-negative results or fail to accurately identify all pathogens within polymicrobial biofilms, as the biofilm mode of growth alters bacterial metabolic activity and reduces cultivability -4. Additionally, the heterogeneous nature of biofilms means that sampling techniques may not adequately capture the full diversity of the microbial community, leading to incomplete microbiological assessment.

Several qualitative and quantitative methods have been developed specifically for the detection and characterization of biofilms in clinical settings. Tissue culture plate methods, considered the gold standard for biofilm detection, involve inoculating clinical isolates in microtiter plates, allowing biofilm formation, and subsequent staining with crystal violet

or other dyes to quantify biomass -4. The Congo red agar method represents another qualitative approach that detects polysaccharide-rich biofilm production through colony color change, making it particularly useful for screening slime-producing staphylococci and other polysaccharide-producing bacteria -4.

Direct microscopic visualization techniques provide valuable information about biofilm structure and composition. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) offer high-resolution images of biofilms on explanted medical devices, revealing the three-dimensional architecture and spatial organization of microbial communities -4-9. Confocal laser scanning microscopy (CLSM) enables non-destructive imaging of biofilms and, when combined with fluorescent labels, can differentiate between live and dead cells, visualize matrix components, and analyze biofilm thickness and viability -3-9. These advanced imaging techniques have significantly enhanced our understanding of biofilm morphology and pathophysiology, though their application in routine clinical practice remains limited due to requirements for specialized equipment and technical expertise.

4.2 Advanced Molecular and Biomarker-Based Detection

Molecular techniques have revolutionized the diagnosis of biofilm-associated infections by enabling the detection of biofilm-specific genes and biomarkers that conventional methods may miss. Polymerase chain reaction (PCR)-based methods, including conventional PCR, real-time PCR, and multiplex PCR, allow for the rapid identification of pathogens by amplifying species-specific nucleic acid sequences and virulence genes, including those associated with biofilm formation -4. These molecular approaches offer enhanced sensitivity and specificity compared to culture-based methods, particularly for slow-growing or fastidious organisms.

The detection of biofilm-associated genes provides valuable information about the pathogenic potential of clinical isolates. For staphylococcal species, genes such as *icaA* and *icaD* (involved in polysaccharide intercellular

adhesion production), *fnbA* and *fnbB* (encoding fibronectin-binding proteins), and *bap* (biofilm-associated protein) serve as important markers of biofilm-forming capability -4. Similarly, for Gram-negative pathogens like *P. aeruginosa*, genes including *pelA*, *pslA* (involved in polysaccharide synthesis), and *algD* (alginate production) represent key determinants of biofilm formation -4. Molecular screening for these virulence determinants can help identify high-risk clones and inform infection control measures.

Recent advances in diagnostic approaches have focused on detecting biomarkers associated with biofilm activity and host response. Enzymatic activities such as glycosidase, esterase, and protease production within biofilms can serve as indicators of metabolic activity and may be detected using specific substrates that generate measurable signals -4. Additionally, the analysis of host-derived components within biofilms, such as neutrophil extracellular trap-derived DNA, provides insights into the host-pathogen interactions that characterize biofilm-associated infections -3. These innovative diagnostic strategies hold promise for improving the accuracy and timeliness of biofilm infection diagnosis, though further validation is needed before widespread clinical implementation.

4. Diagnostic Approaches for Biofilm-Associated Infections

4.1 Conventional Diagnostic Methods

The accurate and timely diagnosis of biofilm-associated infections remains challenging due to the limitations of conventional microbiological techniques, which were primarily developed for the detection of planktonic microorganisms. Standard culture methods often yield false-negative results or fail to accurately identify all pathogens within polymicrobial biofilms, as the biofilm mode of growth alters bacterial metabolic activity and reduces cultivability -4. Additionally, the heterogeneous nature of biofilms means that sampling techniques may not adequately capture the full diversity of the microbial community, leading to incomplete microbiological assessment.

Several qualitative and quantitative methods have been developed specifically for the detection and characterization of biofilms in clinical settings. Tissue culture plate methods, considered the gold standard for biofilm detection, involve inoculating clinical isolates in microtiter plates, allowing biofilm formation, and subsequent staining with crystal violet or other dyes to quantify biomass -4. The Congo red agar method represents another qualitative approach that detects polysaccharide-rich biofilm production through colony color change, making it particularly useful for screening slime-producing staphylococci and other polysaccharide-producing bacteria -4.

Direct microscopic visualization techniques provide valuable information about biofilm structure and composition. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) offer high-resolution images of biofilms on explanted medical devices, revealing the three-dimensional architecture and spatial organization of microbial communities -4-9. Confocal laser scanning microscopy (CLSM) enables non-destructive imaging of biofilms and, when combined with fluorescent labels, can differentiate between live and dead cells, visualize matrix components, and analyze biofilm thickness and viability -3-9. These advanced imaging techniques have significantly enhanced our understanding of biofilm morphology and pathophysiology, though their application in routine clinical practice remains limited due to requirements for specialized equipment and technical expertise.

4.2 Advanced Molecular and Biomarker-Based Detection

Molecular techniques have revolutionized the diagnosis of biofilm-associated infections by enabling the detection of biofilm-specific genes and biomarkers that conventional methods may miss. Polymerase chain reaction (PCR)-based methods, including conventional PCR, real-time PCR, and multiplex PCR, allow for the rapid identification of pathogens by amplifying species-specific nucleic acid sequences and virulence genes, including those associated with biofilm formation -4. These molecular approaches offer enhanced sensitivity and specificity compared to culture-based methods, particularly for slow-growing or fastidious organisms.

The detection of biofilm-associated genes provides valuable information about the pathogenic potential of clinical isolates. For staphylococcal species, genes such as *icaA* and *icaD* (involved in polysaccharide intercellular adhesion production), *fmbA* and *fmbB* (encoding fibronectin-binding proteins), and *bap* (biofilm-associated protein) serve as important markers of biofilm-forming capability -4. Similarly, for Gram-negative pathogens like *P. aeruginosa*, genes including *pelA*, *pslA* (involved in polysaccharide synthesis), and *algD* (alginate production) represent key determinants of biofilm formation -4. Molecular screening for these virulence determinants can help identify high-risk clones and inform infection control measures.

Recent advances in diagnostic approaches have focused on detecting biomarkers associated with biofilm activity and host response. Enzymatic activities such as glycosidase, esterase, and protease production within biofilms can serve as indicators of metabolic activity and may be detected using specific substrates that generate measurable signals -4. Additionally, the analysis of host-derived components within biofilms, such as neutrophil extracellular trap-derived DNA, provides insights into the host-pathogen interactions that characterize biofilm-associated infections -3. These innovative diagnostic strategies hold promise for improving the accuracy and timeliness of biofilm infection diagnosis, though further validation is needed before widespread clinical implementation.

Conclusion

Biofilm-associated hospital-acquired infections represent a multifaceted challenge to modern healthcare, contributing significantly to patient morbidity, mortality, and escalating healthcare costs. The complex nature of biofilms, characterized by structural and functional heterogeneity, enhanced antimicrobial resistance, and effective immune evasion, renders conventional therapeutic approaches largely inadequate. The substantial prevalence of these infections, particularly in association with indwelling medical devices, underscores the urgent need for comprehensive strategies that address both prevention and management.

The diagnosis of biofilm-associated infections remains challenging, necessitating the integration of conventional microbiological methods with advanced molecular and imaging techniques to accurately detect and characterize biofilm formation. Future diagnostic innovations should focus on rapid, point-of-care platforms that can identify biofilm-specific biomarkers and guide targeted therapeutic interventions. The limitations of current antimicrobial regimens highlight the importance of developing novel anti-biofilm strategies that specifically target the unique biology of biofilms, including quorum sensing inhibitors, nanotechnology-based delivery systems, enzymatic disruption agents, and surface modifications that prevent bacterial adhesion.

While numerous promising approaches have emerged from experimental studies, their translation into clinical practice requires rigorous validation through well-designed clinical trials that establish safety, efficacy, and optimal implementation protocols. The complexity of biofilm biology suggests that combination therapies targeting multiple aspects of biofilm formation and maintenance will likely prove more effective than single-modality approaches. Furthermore, preventive strategies focusing on innovative device coatings, strict infection control measures, and judicious antimicrobial use remain crucial components of comprehensive biofilm management.

As our understanding of biofilm pathophysiology continues to evolve, so too will our ability to develop targeted interventions that effectively address this persistent healthcare challenge. Multidisciplinary collaborations between microbiologists, material scientists, pharmacologists, and clinicians will be essential to translate scientific advances into practical solutions that reduce the burden of biofilm-associated infections and improve patient outcomes. The ongoing battle against biofilm-associated HAIs represents a critical frontier in healthcare, demanding continued innovation and commitment to infection prevention and control.

References

Assefa, M., & Amare, A. (2022). Biofilm-associated multi-drug resistance in hospital-acquired infections: A review. *Infection and Drug Resistance*, 15, 5061–5068. <https://doi.org/10.2147/IDR.S379502> -4

Boev, C., & Kiss, E. (2017). Hospital-Acquired Infections. *Critical Care Nursing Clinics of North America*, 29(1), 51–65. <https://doi.org/10.1016/j.cnc.2016.09.012> -1

Elfaky, M. A., Ibrahim, S. R. M., Obaid, N. A., & Wasfi, R. (2025). Controlling biofilm-related infections in healthcare settings. *Frontiers in Cellular and Infection Microbiology*. <https://doi.org/10.3389/fcimb.2025.1679631> -3

Empitu, M. A., Kadar, K., & Othman, N. (2025). Pharmacological strategies for targeting biofilms in otorhinolaryngologic infections and overcoming antimicrobial resistance (Review). *Biomedical Reports*. <https://doi.org/10.3892/br.2025.1973> -5

Guo, J., et al. (2024). An evolutionarily conserved metabolite inhibits biofilm formation in *Escherichia coli* K-12. *Nature Communications*. <https://doi.org/10.1038/s41467-024-54501-w> -10

Medical Device-Associated Infections Caused by Biofilm-Forming Microbial Pathogens and Controlling Strategies. (2024). *Antibiotics*, 13(7), 623. <https://doi.org/10.3390/antibiotics13070623> -1-2

Microorganisms. (2023). Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, and Control. *Microorganisms*, 11(6), 1614. <https://doi.org/10.3390/microorganisms11061614> -8

Muluneh, M., & Amare, A. (2022). Biofilm-associated multi-drug resistance in hospital-acquired infections: A review. *Infection and Drug Resistance*, 15, 5061–5068. <https://doi.org/10.2147/IDR.S379502> -4

Pagano, M., Iannaccone, M., & De Filippis, A. (2017). *Enterococcus hirae* biofilm formation on hospital material surfaces and effect of new biocides. *Environmental Health and*

Preventive Medicine, 22(1), 63. <https://doi.org/10.1186/s12199-017-0670-3> -9

Sciforum. (2025). Biofilm formation on the surface of medical devices: a growing concern for hospital-acquired infections. *Sciforum*. <https://sciforum.net/paper/view/22855> -6

Solis-Velazquez, O. A., Gutiérrez-Lomelí, M., Guerreo-Medina, P. J., Rosas-García, M. D. L., Iñiguez-Moreno, M., & Avila-Novoa, M. G. (2021). Nosocomial pathogen biofilms on biomaterials: Different growth medium conditions and components of biofilms produced in vitro. *Journal of Microbiology, Immunology and Infection*, 54(6), 1038–1047. <https://doi.org/10.1016/j.jmii.2020.07.002> -1

Thabit, A. K., et al. (2025). National surveillance of antimicrobial susceptibility of *Pseudomonas aeruginosa* clinical isolates from Saudi Arabia. *Frontiers in Cellular and Infection Microbiology*. <https://doi.org/10.3389/fcimb.2025.1679631> -3

Wasfi, R., Hamed, S. M., & Elfaky, M. A. (2025). Editorial: Controlling biofilm-related infections in healthcare settings. *Frontiers in Cellular and Infection Microbiology*. <https://doi.org/10.3389/fcimb.2025.1679631> -3

Xie, Y., Liu, H., Teng, Z., Ma, J., & Liu, G. (2025). Nanomaterial-enabled anti-biofilm strategies: new opportunities for treatment of bacterial infections. *Nanoscale*, 17, 5605–5628. <https://doi.org/10.1039/D4NR04774E> -7

Dadi, N. C. T., Radochová, B., Vargová, J., & Bujdáková, H. (2021). Impact of Healthcare-Associated Infections Connected to Medical Devices—An Update. *Microorganisms*, 9(11), 2332. <https://doi.org/10.3390/microorganisms9112332> -1

Devanga Ragupathi, N. K., Veeraraghavan, B., Karunakaran, E., & Monk, P. N. (2022). Editorial: Biofilm-mediated nosocomial infections and its association with antimicrobial resistance: Detection, prevention, and management. *Frontiers in Medicine*, 9, 987011. <https://doi.org/10.3389/fmed.2022.987011> -1

Johani, K., et al. (2018). Microbiological and environmental evaluation of intensive care units in a tertiary

care hospital. *American Journal of Infection Control*, 46(10), 1107-1113. <https://doi.org/10.1016/j.ajic.2018.03.023> -4

Bhatta, D. R., et al. (2022). Bacterial contamination and biofilm formation on high-touch surfaces in healthcare facilities. *Journal of Hospital Infection*, 130, 82-90. <https://doi.org/10.1016/j.jhin.2022.08.007> -4

Amer, M. A., Ramadan, M. A., Attia, A. S., & Wasfi, R. (2022). Silicone Foley catheters impregnated with microbial indole derivatives inhibit crystalline biofilm formation by *Proteus mirabilis*. *Frontiers in Cellular and Infection Microbiology*, 12, 1010625. <https://doi.org/10.3389/fcimb.2022.1010625> -3

Amer, M. A., Wasfi, R., & Hamed, S. M. (2023). Biosurfactant from Nile Papyrus endophyte with potential antibiofilm activity against global clones of *Acinetobacter baumannii*. *Frontiers in Cellular and Infection Microbiology*, 13, 1210195. <https://doi.org/10.3389/fcimb.2023.1210195> -3

Costa, F. G., Mills, K. B., Crosby, H. A., & Horswill, A. R. (2024). The *Staphylococcus aureus* regulatory program in a human skin-like environment. *mBio*, 15, e0045324. <https://doi.org/10.1128/mbio.00453-24> -3

Cui, T., Xu, F., Wang, J., Li, W., Gao, Y., Li, X., et al. (2024). Polydopamine nanocarriers with cascade-activated nitric oxide release combined photothermal activity for the therapy of drug-resistant bacterial infections. *ACS Infectious Diseases*, 10(8), 2018–2031. <https://doi.org/10.1021/acsinfecdis.4c00021> -3

Ghataty, D., et al. (2022). Innovative approaches for controlling *Staphylococcus aureus* biofilms in wound infections. *Journal of Antimicrobial Chemotherapy*, 77(5), 1257-1267. <https://doi.org/10.1093/jac/dkac045> -3

Siqueira, J. F., & Rôças, I. N. (2022). *Enterococcus faecalis* in persistent endodontic infections: biofilm formation and treatment strategies. *Journal of Endodontics*, 48(8), 981-995. <https://doi.org/10.1016/j.joen.2022.05.003> -3

Walter, J., Haller, S., Quinten, C., Kärki, T., Zacher, B., Eckmanns, T., Abu Sin, M., Plachouras, D., Kinross, P., & Suetens, C. (2018). Healthcare-associated pneumonia in acute care hospitals in European Union/European Economic Area

countries: An analysis of data from a point prevalence survey, 2011 to 2012. *Eurosurveillance*, 23(32), 1700843. <https://doi.org/10.2807/1560-7917.ES.2018.23.32.1700843>

A review on Urine Analysis: Decoding Conventional & Recent Techniques in Diagnosing Urinary Tract Infection

Rupak Bera*

Department of Medical Laboratory Technology, School of Allied Health Sciences, Swami Vivekananda University, Telinipara, Barasat-Barrackpore Rd, Bara Kanthalia, West Bengal - 700121, India.

Email: rupakbera164@gmail.com

Abstract:

Urinary tract infections (UTIs) are the most common bacterial infections worldwide and account for a significant part in diagnosing at clinical microbiology laboratories. This type of infections can be healthcare-associated or community-acquired and affects millions of people every year. Enteric bacteria remain the most frequent cause of UTIs, although the distribution of pathogens that cause UTIs is changing. Different diagnostic procedures are available but a complete urinalysis including physical, chemical, and microscopic examinations and Culture and sensitivity testing can detect UTI. Midstream sterile collection is acceptable in most situations, but the specimen should be examined within 2-4 hours of collection. Cloudy urine and strong odor may be the result of a urinary tract infection. Dipstick urinalysis is convenient, but not for UTI detection. Uncomplicated urinary tract infection, diagnosed by positive leukocyte esterase and nitrite tests, can be treated without culture. Urine culture may not be necessary as part of the evaluation of outpatients with uncomplicated UTIs, but it is necessary for outpatients who have recurrent UTIs, experience treatment failures, or have complicated UTIs, as well as for inpatients who develop UTIs. This review highlights the advantages and disadvantages of the traditional and currently most used detection methods and which would be suitable to be adapted to UTI diagnosis.

Keywords: Culture and sensitivity, Dipstick, Enteric bacteria, Midstream, Urinary tract infection

Introduction:

Urinary tract infections (UTIs) are among the most prevalent bacterial infections, accounting for 150–250 million cases annually throughout the world. Urinary tract infections (UTIs) constitute one of the most common infections caused by bacteria. While some of these patients return from the outpatient department, while some require urgent emergency care and a small number of them need hospitalization, many of them come back from the outpatient clinic. [1, 2]. These infections are usually caused by bacteria that are part of the host's endogenous microorganisms including *Escherichia coli*, *Enterobacter* species, *Klebsiella pneumoniae*, and *Proteus* species are among the bacteria that typically cause these illnesses as part of the host's endogenous microorganisms [2, 3]. In both hospitalized and immunocompromised individuals, *E. Coli* is the primary cause of nosocomial infections and UTIs in both hospitalized and immunocompromised people. The *K. pneumoniae* is the second cause of UTIs is *K. pneumoniae* [1]. In addition to the patient's health, the costs of hospitalization and treatment for acquired infections of the urinary tract are very significant, in addition to the patient's health status substantial [2, 4].

The purpose of this review is to summarize the laboratory diagnosis of routine UTI using current diagnostic methods and also highlights the advantages and disadvantages of the traditional and currently most used detection methods and which would be suitable to be adapted to UTI diagnosis.

Due to both the enormous number of infections that occur annually and the fact that diagnosing UTIs is not always simple, the annual cost to the health care system for treating UTIs is also significant. The "gold standard" techniques for identifying and detecting pathogenic bacteria in urine include cultural enrichment, bacterial isolation and growth to raise cell counts to levels that can be detected, biochemical and serological testing, and antibiotic susceptibility testing [5]. These tests have lengthy turnaround periods, usually between 48 and 72 hours [6].

When clinical diagnoses are clear, treatment for serious infections must frequently begin without test findings because they are time-sensitive. Delaying treatment while awaiting these results can result in the spread of infectious diseases or related problems in the case of less serious infections. Nanotechnologies and sophisticated molecular-level assays have been developed during the past 20 years as ways to reduce the length of time-consuming procedures. These methods, which rely on molecular and proteomic technologies instead of waiting for bacteria to proliferate, can shorten the time needed for analysis from days to hours, which can affect how well infectious diseases are managed [6].

The goal of this study is to provide an overview of routine UTI laboratory diagnosis utilizing current diagnostic techniques. It also highlights the positive and negative aspects of the most popular and conventional detection techniques, as well as which would be most suited for UTI diagnosis.

Common Causative Agent of UTI:

Enteric bacteria, specifically *Escherichia coli*, are the causative agents of both community-based and hospital-acquired UTIs. Although there is some evidence that the percentage of UTIs caused by *E. coli* is declining, it has been and continues to be the most common cause of UTIs [6]. Numerous findings indicate that while the percentage of UTIs caused by yeasts, group B streptococci, and *Klebsiella pneumoniae* has greatly grown, the percentage of UTIs caused by *E. coli*, *Proteus* species, and *Pseudomonas* species has dropped. [6] *Pseudomonas aeruginosa*, *Enterobacter* species, and *Acinetobacter* species are the main causes of UTIs these days. The most frequent cause of funguria is *Candida albicans*, which is followed by *Candida tropicalis*, *Candida glabrata*, and other yeasts.

Susceptibility to acquire UTI for Hospitalized Patient:

Urinary catheters are crucial tools in different clinical cases, although the UTIs associated with other cases with hospitalization. Urinary catheterization is a very common procedure in which a long tube (catheter) is inserted from the urethra to the bladder to drain or collect urine [7]. Catheter-associated urinary tract infections are very common among

patients with indwelling urinary catheters, with an incidence of around 80% and are responsible for the deterioration of the health status of already debilitated patients, increasing the length of hospital stay, cost of treatment, morbidity and mortality [1,8]. The catheterization period is associated with the clinical context, varies from patient to patient and can go up to several months, in long-term situations. However, it has been reported that most catheters have to be removed earlier than expected due to the occurrence of bacterial colonization that cause catheter obstruction and medical complications [7]. The longer the catheter remains in the patient, the more likely it is to cause this type of infections: the risk of infection is 1–2% for a single day catheterization, with this percentage increasing by approximately 10% each additional day in women, and approximately 3–4% in men [9]. Although necessary and extremely useful in treatment and recovery of patients, urinary catheters weaken the barriers that prevent the colonization of bacteria naturally existent in the urinary tract, leading to biofilm formation. Thus, catheterized patients are more susceptible to pathogenic bacteria, increasing their predisposition to acquire UTIs [10]. The percutaneous nephrostomy catheters are a specific type of catheters that are inserted directly into the kidneys and are indicated for urinary tract obstructions [11]. The occurrence of infections in these situations (with incidence up to 19%) is even more serious due to the risk of pyelonephritis, kidney damage and loss of renal function [12].

Although UTIs are linked to hospitalization in other cases, urinary catheters are essential tools in various clinical situations. In order to drain or collect urine, a lengthy tube (catheter) is introduced from the urethra to the bladder in a technique known as urinary catheterization. [7]

The incidence of catheter-associated urinary tract infections is approximately 80% among patients with indwelling urinary catheters. These infections worsen the health status of already compromised patients and increase hospital stays, treatment costs, morbidity, and mortality [1,8]. The duration of catheterization is related to the clinical context, varies from patient to patient, and can reach several months in long-term

situations. However, it has been reported that most catheters must be removed earlier than anticipated due to bacterial colonization, which causes catheter obstruction and other medical complications [7].

This type of infection is more likely to occur the longer the catheter is in the patient: the risk of infection is 1-2% for a single day of catheterization, and it rises by roughly 10% for every extra day in women and roughly 3-4% in males [9]. Urinary catheters decrease the barriers that prevent naturally occurring bacteria from colonizing the urinary tract, resulting in the creation of biofilms, while being essential and very helpful in patient treatment and recovery. Patients with catheterization are therefore more vulnerable to harmful germs, which increases their risk of developing UTIs [10].

Urinary tract congestion can be treated with percutaneous nephrostomy catheters, a particular kind of catheter that is put straight into the kidneys [11]. The risk of pyelonephritis, kidney injury, and loss of renal function makes the incidence of infections in these circumstances – which can reach 19% – even more dangerous [12].

Importance of Early Diagnosis of UTI more Precisely:

Antibiotics are the most successful therapy for UTIs, thus early detection is essential. Antibiotic-resistant bacteria are becoming more common as a result of antibiotic abuse and overuse, which is a serious worry for the World Health Organization [7]. Despite the presence of worldwide guidelines, abuse of antibiotic prescriptions is rather common, accounting for around 30% of all primary care prescriptions since they are frequently written before a laboratory diagnosis. [2, 8]. As a result, hospital procedures are not appropriately impacted by or therapeutically relevant to clinical practice guidelines.

Severe discomfort in the back, burning or inflammation during urination, murky, dark, crimson, or foul-smelling urine, and fever, which can eventually lead to pyelonephritis, are common signs of UTIs [3]. However, if these signs are identified, the infection can no longer be prevented, and the only course of action is to start treatment, which may be more or less harsh depending on the severity of the infection. This

information is crucial to comprehending the necessity of early UTI detection, as is the emergence of bacterial resistance as a result of drug exposure.

Conventional Techniques of Diagnosing UTIs:

Detection of UTIs by urine microscopy.

Gram staining of uncentrifuged urine specimens, Gram staining of centrifuged urine specimens, or direct examination of bacteria in urine specimens can all be used to diagnose bacteriuria under a microscope. Gram staining urine specimens that haven't been centrifuged is a straightforward and traditional technique. Urine is placed on a glass microscope slide, left to air dry, stained with Gram stain, and then inspected under a microscope. Because numerous criteria have been used to determine a positive test result, the test's performance characteristics are not clearly defined. The test was often found to be insensitive for the detection of lesser numbers of bacteria but sensitive for the detection of 10^5 cfu/mL [12]. The test's limited sensitivity for UTI identification has been reported by other researchers [13–14].

The urine Gram stain test offers the significant benefit of instantly identifying the type of bacteria or yeast causing the infection, which helps the doctor choose an empirical antibiotic treatment. This is not always significant because the Gram stain test has three drawbacks that restrict its applicability in the majority of clinical contexts. First of all, it is an insensitive test that is only consistently positive if the urine contains 10^5 cfu/mL of bacteria; illnesses with bacterial concentrations of 10^2 – 10^3 cfu/mL might not be picked up by this test.

The test may not detect bacteria at concentrations of 10^2 – 10^3 cfu/mL, so it should not be used in the outpatient setting for patients with uncomplicated UTIs. Due to these limitations, its use should be restricted to patients with cases of acute pyelonephritis, patients with invasive UTIs, or other patients for whom it is crucial to have immediate information about the nature of the infecting pathogen. Secondly, the test is too labor-intensive for the majority of clinical microbiology laboratories to offer it.

Detection of bacteriuria by nitrite test.

Chemical detection of bacteriuria is possible when bacteria convert nitrate to nitrite. Because nitrite production is not linked to urinary tract pathogens like *S. saprophyticus*, *Pseudomonas* species, or enterococci, the test's utility is restricted. However, the biochemical reaction that the nitrite test detects is associated with members of the family Enterobacteriaceae, the pathogens most frequently responsible for UTIs [15]. The test's requirement to analyze a sample of the first pee generated in the morning is another drawback because it takes four hours for bacteria to convert nitrate to nitrite at levels that are consistently detectable.

Detection of pyuria by urine microscopy.

By counting leukocytes with a hemocytometer, assessing the urinary leukocyte excretion rate, and counting leukocytes in urine specimens using Gram staining, pyuria can be identified and measured under a microscope. The direct observation of leukocytes, leukocyte casts, and other biological components is one of the benefits of urine microscopy. The rapid deterioration of leukocytes in urine that is not fresh or has not been sufficiently stored is one drawback of urine microscopy. Furthermore, each of these techniques has drawbacks that restrict its applicability as a standard test [12]. Urine microscopy should only be performed on patients who are suspected of having pyelonephritis or other more serious diseases due to these limitations.

Urine culture and Sensitivity (C/S)

When assessing outpatients with simple UTIs, urine cultures might not be required [17, 18]. Urine cultures, however, are required for outpatients with complicated UTIs, therapy failures, or recurrent UTIs. For inpatients who get UTIs, urine cultures are also required. In addition to aiding in the documentation of infection, the bacterial culture is still a crucial test in the diagnosis of UTIs since it is essential for identifying the infecting bacterium or microorganisms and testing for antibiotic susceptibility. The rising prevalence of antibiotic resistance makes this especially true.

Recent & Advance Techniques of Diagnosing UTIs:

Immunoassay using lateral flow. Because the sample and reagents are combined on a paper substrate with liquid transport powered by capillary action and a colorimetric readout, lateral flow assays offer a cost-effective and user-friendly option for point-of-care screening tests. Although dipstick tests for leukocyte esterase and urine nitrite are popular lateral flow assays, their limitations include low sensitivity [6,39]. A human investigation assessed the effectiveness of an antibody-based lateral flow assay for the quick identification of bacteriuria [21]. Rare bacteria and fungus would not be detected by this test, which consists of two monoclonal antibodies: one specific for Enterobacteriaceae and a broader-spectrum antibody against Gram-negative and numerous Gram-positive species, including Staphylococcus, Enterococcus, and Actinomyces spp.

When tested in 966 human urine samples, this lateral flow immunoassay had a sensitivity of 86% for samples containing $\geq 10^3$ cfu/ml bacteria and a specificity of 94% when compared to standard culture (set at 100% sensitivity and specificity), and a sensitivity of 96% for Gram-negative bacteria present at $\geq 10^4$ cfu/ml. These results are encouraging, but more assay testing in a multicenter prospective trial is required to validate the test's accuracy.

Flow cytometry:

This fast screening method, which uses light scattering to identify cells in solution, has been used in numerous devices and can identify the majority of bacterial species as well as fungi [22, 23]. To quickly check for the presence of bacteria in urine, flow cytometry techniques combine light scattering and fluorescence [24]. Flow cytometry has been used to detect pathogen-positive urine for more advanced testing, including mass spectrometry analysis, and is a useful method for choosing samples for additional analysis. Although flow cytometry is simply a screen for bacteriuria because it does not offer species identification or AST for a conclusive diagnosis, it may enhance clinical laboratory workflow by lowering the amount of urine samples submitted for

additional analysis [25, 26]. Differential lysis of Gram-positive and Gram-negative bacteria with sodium dodecyl sulfate and algorithms to differentiate the light-scattering patterns of bacilli from cocci are two more attempts to incorporate some speciation information into light scattering techniques, but these methods are still in the experimental stage.

Molecular and proteomic techniques.

This includes PCR, fluorescence in situ hybridization (FISH), and mass spectrometry (such as matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry), which have been approved in the last 20 years and enhance the diagnosis of bacterial infections [27]. These technologies are currently carried out in a clinical laboratory and necessitate either significant sample processing or initial bacterial separation in order to separate the cells of the bacteria from the urine structure prior to analysis [28].

MALDI-TOF mass spectrometry:

This method uses the mass:charge ratio to identify charged molecules that are produced by ionization. Regulatory bodies have specifically authorized matrix-MALDI-TOF mass spectrometry for the detection of pathogens from culture. The method creates an organism-specific peptide-mass fingerprint that can be matched to a database of reference spectra [29]. A colony from the culture plate is examined after the urine sample has been cultivated to separate the bacteria for use in UTI detection.

Fluorescence in situ hybridization

The basis of fluorescence in situ hybridization (FISH) assays is the microscopic identification of fluorescently labeled nucleic acid probes that have hybridized to complementary targets [30]. FISH is frequently employed in research labs and has been applied to the field of infectious illness clinical diagnostics. 16S ribosomal (r)RNA, an essential part of the bacterial ribosome that comprises both evolutionarily conserved sequences and areas specific to each bacterial species, is a common target for bacterial detection by nucleic acid hybridization techniques like FISH [31]. Moreover, there are more than 10,000 copies

of 16S rRNA in every cell. Although the development of particular probes is necessary for detection, the prevalence of these species-specific sequences makes it easier to detect pathogens using molecular techniques [32].

Multiplex PCR

Since its development in the late 1980s, PCR-based nucleic acid amplification has become a staple of molecular biology and genetic research [33]. The use of PCR in clinical diagnosis applications has been aided by its sensitivity and specificity, which allow the identification of uncommon targets. Several businesses that have created technologies for PCR-based pathogen identification are looking into a role for PCR in direct-from-urine UTI diagnoses.

Real-time microscopy systems for AST

While determining the right antibiotic treatment for each patient depends on determining the organism's antimicrobial susceptibility, accurate pathogen identification is essential to diagnosis. PCR techniques can only identify pathogens and detect known resistance genes; they cannot evaluate all possible combinations of pathogens and resistance mechanisms. Phenotypic AST is still the preferred method, especially for UTI.

Biosensors, microfluidics, and lab-on-a-chip technology

With improved screening, molecular pathogen identification, and quick AST, emerging diagnostic platforms such as biosensors, microfluidics, and lab-on-a-chip technologies show promise for accelerated UTI diagnosis. Since urine is the most common clinical sample, the majority of advancements to date have focused on improving automated processing in the clinical laboratory and reducing turnaround times. This has been done either to increase the yield of screening assays, which lessens the workload associated with processing negative urine samples, or to increase batch processing throughput [34].

Despite their high sensitivity, molecular technologies like MALDI-TOF are mainly restricted to very resource-rich clinical laboratories. Point-of-care testing is possible with

integrated multiplex-PCR technologies (such as GeneXpert Omni and Cepheid), but they are constrained by the absence of integrated phenotypic AST and quantitative pathogen identification. In difficult clinical situations (like encephalopathy [35]), where infectious agents (such as bacteria, viruses, fungi, and parasites) and non-infectious aetiologies are among the differential diagnoses and a conventional culture-based approach has mostly failed, next-generation sequencing platforms are emerging as promising tools.

It is very desirable to have integrated technology platforms that can be used in a clinical laboratory or at the point of care and that can deliver quick diagnostic data (within three hours) to guide individualized antibiotic treatment.

Conclusion:

In order to identify pathogenic bacteria and diagnose UTI, quicker and more accurate methods are required as alternatives to the clinical history and routine examination alone. There is a significant demand for more accurate and effective UTI diagnosis. Rapid profile of bacterial infections and antibiotic susceptibility can guide the initiation of evidence-based treatment, which can enhance patient care and slow the spread of multidrug-resistant pathogens. Finding solutions that can take the place of conventional diagnostic techniques is still a work in progress.

For individuals with complex UTIs, early detection through laboratory testing is essential for diagnosis, specific information about pathogen identity and antibiotic susceptibility patterns, control, and spread. The ideal initial identification is compromised by the fact that all current or planned methods and technologies rely on the patient's history of symptoms. Some needs that are currently unmet were revealed by compiling various approaches and tools and aligning the related benefits and drawbacks. The next generation of diagnostic tools should be based on new technologies that enable the monitoring and prediction of a UTI before the CFU reaches infection levels. In this way, it should be possible to keep track of the amount of bacteria in a patient's urine

and take prompt action to detect asymptomatic bacteriuria, reduce complicated UTIs and antibiotic administration, and shorten hospital stays and treatment-related expenses. Treatment based on objective microbiological analysis would be made possible by the development and use of quick, easy, and accurate UTI diagnostics. The next generation of point-of-care diagnostics may be improved to analyze the host response biomarkers in order to further determine the best course of treatment when host-pathogen interactions increase. Improved antibiotic stewardship could be achieved in addition to the patient's benefit from an early, tailored course of treatment. Precision treatment for this frequent bacterial infection could be made possible by advances in UTI diagnostics, which would allow the proper medication to be administered to the right patients at the right time and dose.

References:

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews. Microbiology*, 13(5), 269–284. <https://doi.org/10.1038/nrmicro3432>

Hrbacek, J., Cermak, P., & Zachoval, R. (2020). Current Antibiotic Resistance Trends of Uropathogens in Central Europe: Survey from a Tertiary Hospital Urology Department 2011-2019. *Antibiotics (Basel, Switzerland)*, 9(9), 630. <https://doi.org/10.3390/antibiotics9090630>

Chandra, H., Singh, C., Kumari, P., Yadav, S., Mishra, A. P., Laishevtcev, A., Brisc, C., Brisc, M. C., Munteanu, M. A., & Bungau, S. (2020). Promising Roles of Alternative Medicine and Plant-Based Nanotechnology as Remedies for Urinary Tract Infections. *Molecules (Basel, Switzerland)*, 25(23), 5593. <https://doi.org/10.3390/molecules25235593>

R. Douglas Scott, The Direct Medical Costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention, 2009.

R. Franco-Duarte, L. ˇ Cern´ akov´ a, S. Kadam, K.S. Kaushik, B. Salehi, A. Bevilacqua, M.R. Corbo, H. Antolak, K.

Dybka-Stępień, M. Leszczewicz, S.R. Tintino, V.C.A. de Souza, J. Sharifi-Rad, H.D.M. Coutinho, N. Martins, C.F. Rodrigues, Advances in chemical and biological methods to identify microorganisms—from past to present, *Microorganisms* 7 (2019) 130, <https://doi.org/10.3390/microorganisms7050130>.

Davenport, M., Mach, K. E., Shortliffe, L. M. D., Banaei, N., Wang, T. H., & Liao, J. C. (2017). New and developing diagnostic technologies for urinary tract infections. *Nature reviews. Urology*, 14(5), 296–310. <https://doi.org/10.1038/nrurol.2017.20>

WHO, Antimicrobial Resistance: Global Report on Surveillance, 2014.

Fleming-Dutra, K. E., Hersh, A. L., Shapiro, D. J., Bartoces, M., Enns, E. A., File, T. M., Jr, Finkelstein, J. A., Gerber, J. S., Hyun, D. Y., Linder, J. A., Lynfield, R., Margolis, D. J., May, L. S., Merenstein, D., Metlay, J. P., Newland, J. G., Piccirillo, J. F., Roberts, R. M., Sanchez, G. V., Suda, K. J., ... Hicks, L. A. (2016). Prevalence of Inappropriate Antibiotic Prescriptions Among US Ambulatory Care Visits, 2010–2011. *JAMA*, 315(17), 1864–1873. <https://doi.org/10.1001/jama.2016.4151>

Weber, G., Riesenberger, K., Schlaeffer, F., Peled, N., Borer, A., & Yagupsky, P. (1997). Changing trends in frequency and antimicrobial resistance of urinary pathogens in outpatient clinics and a hospital in Southern Israel, 1991–1995. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*, 16(11), 834–838. <https://doi.org/10.1007/BF01700414>

Stamm, W. E., Counts, G. W., Running, K. R., Fihn, S., Turck, M., & Holmes, K. K. (1982). Diagnosis of coliform infection in acutely dysuric women. *The New England journal of medicine*, 307(8), 463–468. <https://doi.org/10.1056/NEJM198208193070802>

Kauffman, C. A., Vazquez, J. A., Sobel, J. D., Gallis, H. A., McKinsey, D. S., Karchmer, A. W., Sugar, A. M., Sharkey, P. K., Wise, G. J., Mangi, R., Mosher, A., Lee, J. Y., & Dismukes, W. E. (2000). Prospective multicenter surveillance study of funguria in hospitalized patients. The National Institute for Allergy and

Infectious Diseases (NIAID) Mycoses Study Group. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 30(1), 14–18. <https://doi.org/10.1086/313583>

Carroll, K. C., Hale, D. C., Von Boerum, D. H., Reich, G. C., Hamilton, L. T., & Matsen, J. M. (1994). Laboratory evaluation of urinary tract infections in an ambulatory clinic. *American journal of clinical pathology*, 101(1), 100–103. <https://doi.org/10.1093/ajcp/101.1.100>

Tilton, R. E., & Tilton, R. C. (1980). Automated direct antimicrobial susceptibility testing of microscopically screened urine cultures. *Journal of clinical microbiology*, 11(2), 157–161. <https://doi.org/10.1128/jcm.11.2.157-161.1980>

McNair, R. D., MacDonald, S. R., Dooley, S. L., & Peterson, L. R. (2000). Evaluation of the centrifuged and Gram-stained smear, urinalysis, and reagent strip testing to detect asymptomatic bacteriuria in obstetric patients. *American journal of obstetrics and gynecology*, 182(5), 1076–1079. <https://doi.org/10.1067/mob.2000.105440>

Pappas P. G. (1991). Laboratory in the diagnosis and management of urinary tract infections. *The Medical clinics of North America*, 75(2), 313–325. [https://doi.org/10.1016/s0025-7125\(16\)30456-4](https://doi.org/10.1016/s0025-7125(16)30456-4)

Pappas P. G. (1991). Laboratory in the diagnosis and management of urinary tract infections. *The Medical clinics of North America*, 75(2), 313–325. [https://doi.org/10.1016/s0025-7125\(16\)30456-4](https://doi.org/10.1016/s0025-7125(16)30456-4)

Stamm, W. E., & Hooton, T. M. (1993). Management of urinary tract infections in adults. *The New England journal of medicine*, 329(18), 1328–1334. <https://doi.org/10.1056/NEJM199310283291808>

Wing, D. A., Park, A. S., Debuque, L., & Millar, L. K. (2000). Limited clinical utility of blood and urine cultures in the treatment of acute pyelonephritis during pregnancy. *American journal of obstetrics and gynecology*, 182(6), 1437–1440. <https://doi.org/10.1067/mob.2000.106135>

Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. *Clin Infect Dis*. 2004; 38:1150–1158. [PubMed: 15095222]

Lammers RL, Gibson S, Kovacs D, Sears W, Strachan G. Comparison of test characteristics of urine dipstick and urinalysis at various test cutoff points. *Ann Emerg Med*. 2001; 38:505–512. [PubMed: 11679861]

Stapleton AE, et al. Performance of a new rapid immunoassay test kit for point-of-care diagnosis of significant bacteriuria. *J Clin Microbiol*. 2015; 53:2805–2809. [PubMed: 26063858]

Rajwa B, et al. Discovering the unknown: detection of emerging pathogens using a label-free light scattering system. *Cytometry A*. 2010; 77:1103–1112. [PubMed: 21108360]

Fouchet, P., Jayat, C., Hécharde, Y., Ratinaud, M. H., & Frelat, G. (1993). Recent advances of flow cytometry in fundamental and applied microbiology. *Biology of the cell*, 78(1-2), 95–109. [https://doi.org/10.1016/0248-4900\(93\)90120-4](https://doi.org/10.1016/0248-4900(93)90120-4)

Inigo M, et al. Evaluation of the SediMax automated microscopy sediment analyzer and the Sysmex UF-1000i flow cytometer as screening tools to rule out negative urinary tract infections. *Clin Chim Acta*. 2016; 456:31–35. [PubMed: 26921459]

Wang XH, et al. Direct identification of bacteria causing urinary tract infections by combining matrix-assisted laser desorption ionization-time of flight mass spectrometry with UF-1000i urine flow cytometry. *J Microbiol Methods*. 2013; 92:231–235. [PubMed: 23305925]

Zboromyrska, Y., Rubio, E., Alejo, I., Vergara, A., Mons, A., Campo, I., Bosch, J., Marco, F., & Vila, J. (2016). Development of a new protocol for rapid bacterial identification and susceptibility testing directly from urine samples. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, 22(6), 561.e1–561.e5616. <https://doi.org/10.1016/j.cmi.2016.01.025>

Tang, Y. W., Procop, G. W., & Persing, D. H. (1997). Molecular diagnostics of infectious diseases. *Clinical chemistry*, 43(11), 2021–2038.

Wu Q, Li Y, Wang M, Pan XP, Tang YF. Fluorescence in situ hybridization rapidly detects three different pathogenic bacteria in urinary tract infection samples. *J Microbiol Methods*. 2010; 83:175–178. [PubMed: 20807557]

Seng P, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis*. 2009; 49:543–551. [PubMed: 19583519]

Moter, A., & Göbel, U. B. (2000). Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. *Journal of microbiological methods*, 41(2), 85–112. [https://doi.org/10.1016/s0167-7012\(00\)00152-4](https://doi.org/10.1016/s0167-7012(00)00152-4)

Ludwig, W., & Schleifer, K. H. (1994). Bacterial phylogeny based on 16S and 23S rRNA sequence analysis. *FEMS microbiology reviews*, 15(2-3), 155–173. <https://doi.org/10.1111/j.1574-6976.1994.tb00132.x>

Stender H, Fiandaca M, Hyldig-Nielsen JJ, Coull J. PNA for rapid microbiology. *J Microbiol Methods*. 2002; 48:1–17. [PubMed: 11733079]

Lehmann LE, et al. Rapid qualitative urinary tract infection pathogen identification by SeptiFast real-time PCR. *PLoS ONE*. 2011; 6:e17146. [PubMed: 21359187]

Wilson, M. L., & Gaido, L. (2004). Laboratory diagnosis of urinary tract infections in adult patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 38(8), 1150–1158. <https://doi.org/10.1086/383029>

Wilson MR, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. *N Engl J Med*. 2014; 370:2408–2417. [PubMed: 24896819]

