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Research Article

Development of AI-enabled multiplex biosensor for simultaneous detection of antimicrobial resistance, biofilm biomarkers, and oncology-associated microbial signatures

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ABSTRACT

Antimicrobial resistance (AMR), biofilm-mediated infections, and microbiota-mediated oncogenesis convergence exists as a meaningful but neglected area for personalized healthcare. Antimicrobial resistance happens when germs change and learn to beat the medicines we use to kill them. It means that everyday infections become impossible to treat, making surgeries much riskier, and sadly, it leads to millions of deaths each year because our standard medical treatments stop working. On top of that, we're not discovering many new drugs, and with everyone traveling globally, this really threatens modern surgeries. It makes us worry about a future where even a common cold or cut could become untreatable. We're also learning that sometimes, when the balance of tiny organisms in our body gets out of whack, it can kick off cancer. This happens because these out-of-balance microbes can mess with our DNA, cause long-term inflammation, and even weaken our body's defences. It's a growing concern because these cancer-linked microbe groups can sometimes fight off chemotherapy and seem to be part of the reason why more younger people are developing cancer worldwide. Many researchers believe that current ways of checking for sickness look at these three things as separate items. An AI-compatible multiplex electrochemical biosensor is created to close this distance by finding genetic and protein signals. The testing tool uses a functionalized three-electrode array containing DNA probes and aptamers for antimicrobial resistance (AMR) genes including mecA, blaKPC, and vanA. The AI-compatible multiplex electrochemical biosensor also identifies biofilm markers like icaA, pslA, and PNAG, along with cancer-related bacterial signs like FadA of *F. nucleatum* and colibactin-producing *E. coli*. Tests established that the detection limits were 0.5-5 nM for nucleic acids and 0.2 ng/mL for protein biomarkers. Experts claim that the AI-based model reached a clarity of more than 94% in the complex signals. In 31% of colorectal cancer cases, previously unknown groups of bacteria were identified through matching signals of antimicrobial resistance (AMR), Biofilm-mediated infections, and microbiota-mediated oncogenesis. Integrating infection details and resistance facts into one test allows the AI-compatible multiplex electrochemical biosensor to serve as a new diagnostic tool. Sensitive signal transduction was done using electrochemical impedance spectroscopy and chronoamperometry. This combined system is a groundbreaking diagnostic system that combines infection profiling, resistance detection, and cancer related microbial analysis in one assay. Integrating biosensing with artificial intelligence, it allows fast, decentralized,

predictive diagnostics, and has potential uses in the management of sepsis, oncology surveillance, and personalized antimicrobial therapy.

Key words: AMR, Biofilm mediated infections, Microbiota-mediated oncogenesis, Biosensor, AI-Integrated diagnostics, DNA Probes, FadA, EIS.

I. INTRODUCTION

Antimicrobial resistance (AMR), biofilm-mediated infections and microbiome linked oncogenesis represent converging global health crises. There is yet no platform which can detect them simultaneously. Due to fragmented testing paradigms, clinical intersections where resistant pathogens form biofilms in oncological patients remain largely undiagnosed.

1.1 The AMR burden

Antimicrobial resistance is a problem that causes around 1.27 million people death every year because of resistance. The World Health Organization says that few bacteria like carbapenem- Enterobacterales, methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus are very dangerous [1]. A study in Brazil found that these types of bacteria cause a lot of infections in hospitals. The common type of bacteria was carbapenem-resistant Enterobacterales, followed by carbapenem-resistant Acinetobacter baumannii and methicillin-resistant Staphylococcus aureus. Another study found that some bacteria have genes that make them resistant to antibiotics. These genes are found in the environment even after the water has been treated. This is a concern because it means that these genes can spread to other bacteria [8].

1.2 Problems with Biofilm

Biofilms are like communities of bacteria that stick together. They are found on devices and on the surfaces of our bodies [9]. Biofilms are very hard to kill with antibiotics because they are protected by a layer. 80% Of chronic infections are caused by biofilms. These infections can be very persistent and hard to treat [10].

Biofilms are like cities for bacteria. They have their systems and ways of working together. They can even share genes with each other which makes them stronger. Some genes are important for biofilms to form and grow. For example, the icaADBC operon is important for some bacteria to stick together [13].

1.3 Microbial oncogenesis

There is a growing amount of evidence that some microbes can cause cancer [3,14]. One type of bacteria called Fusobacterium nucleatum has been found in people with cancer. These bacteria can stick to cancer cells. Help them grow. It can also suppress the system, which makes it harder for the body to fight cancer [16].

Another type of bacteria called Escherichia coli can produce a toxin that damages DNA. This toxin can cause mutations that lead to cancer. Some people with cancer have been found to have this type of bacteria in their tumours. This suggests that the bacteria may play a role in the development of the cancer. Antimicrobial resistance and biofilms are problems that need to be addressed. The link between microbes and cancer is also an area of research. By studying these topics, we can learn more about how to prevent and treat diseases. Antimicrobial resistance is a challenge that we need to overcome [5,17].

1.4 Diagnostic gap

No existing platform simultaneously addresses all three clinical domains. Current limitations include:

- Turnaround: Sequential, siloed assays requiring 24–72 hours for results
- Multiplexing: Inability to multiplex heterogeneous biomarker classes in a single run [20]
- Analytics: Absence of predictive or risk-stratifying analytical output
- Accessibility: High infrastructure requirements precluding point-of-care deployment [21]

II. EMERGING TECHNOLOGICAL SOLUTIONS

Recent advances in technology are helping us make tools. We can use particles like gold and carbon to make things that can detect really small amounts of antibiotics and other things [23]. These tools can even see things that're too small to see with our eyes. We are also using lights to make it easier to see what is going on.

Machine learning is a help with this. It is like a computer program that can learn and get better at things. We are using it to make tools that can tell us what kind of bacteria are making us sick. These tools can even tell us if the bacteria are resistant to the medicines we use to treat them [6,25]. We are also using computers to help doctors make good

decisions about how to treat people who are sick.

This is especially helpful with things like liver disease.
We are using something called nanotechnology and artificial intelligence and machine learning to make all of this happen. Nanostructured interfaces and machine learning algorithms are important for this. We are also using things like surface-enhanced Raman scattering and quantum dot labelling to help us see what is going on. All of this is helping us to make tools to detect antibiotics and nucleic acids and to diagnose diseases, like liver disease [22,23].

III. RESEARCH OBJECTIVES

This project is structured around five primary objectives:

- **Objective 1:** Design and fabricate a nanostructured multiplex biosensor incorporating electrochemical, optical, and piezoelectric transduction zones on a single integrated chip.
- **Objective 2:** Functionalise sensing zones with biomarker-specific bioreceptors — aptamers, CRISPR-Cas12/Cas13 reporters, and DNAzyme probes — capable of simultaneous capture and signal generation.
- **Objective 3:** Develop and validate an AI/ML diagnostic pipeline comprising deep learning classifiers, temporal models, and ensemble decision fusion for integrated clinical interpretation [6].
- **Objective 4:** Validate the platform against clinical isolates representing AMR, biofilm-forming, and oncology-associated bacterial strains, benchmarking sensitivity, specificity, and limit of detection.
- **Objective 5:** Demonstrate proof-of-concept in patient-derived clinical samples (sputum, wound swabs, urine, biopsy), assessing concordance with reference laboratory methods.

IV. TARGETTED BIOMARKERS

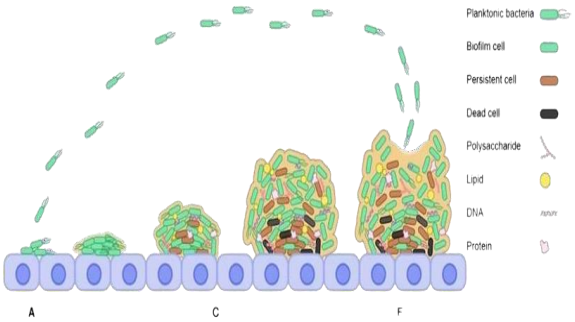
The integration of these 15 biomarkers creates a comprehensive diagnostic profile that spans immediate drug resistance, the physical state of the infection (biofilm), and long-term disease associations (oncology).

4.1 AMR gene targets

The platform monitors critical resistance drivers: blaNDM and KPC, which facilitate broad carbapenem resistance; mecA, which enables methicillin resistance in S. aureus via the PBP2a protein; and vanA, which triggers high-level vancomycin resistance [7]. These markers are essential for identifying pan-drug-resistant pathogens and informing effective clinical interventions.

4.2 Biofilm biomarker targets

The system detects vital resistance genes: blaNDM and KPC for carbapenem immunity, mecA for methicillin-resistant S. aureus via PBP2a, and vanA for vancomycin resistance through cell-wall modification [13]. Targeting these markers allows for the rapid identification of pan-drug-resistant superbugs, enabling more precise and timely clinical treatment strategies.



4.3 Oncology associated microbial targets

The platform targets specific microbial oncoproteins and genotoxins linked to cancer progression. FadA from F. nucleatum acts as a surface adhesin that triggers Wnt/β-catenin signaling, directly accelerating tumor growth in the colon [4,15]. CagA, an effector from H. pylori, is delivered into host cells via a type IV secretion system, where it destabilizes epithelial polarity and cellular growth regulation [5,17]. Additionally, the pks genomic island in
E. coli produces colibactin, a potent genotoxin that induces DNA double-strand breaks. These biomarkers are critical for identifying microbial signatures highly prevalent in colorectal and gastric malignancies.

V. BIOSENSOR PLATFORM DESIGN

5.1 Nanocore architecture

The sensor is constructed on an ITO glass or carbon electrode, supported by a tri-component nanomaterial scaffold that harmonizes optical and

electrical data collection:

• Gold Nanoparticles (Au NPs): These 15–40 nm particles provide a massive surface area for probe attachment. They drive LSPR detection and accelerate electron transfer for sharper electrochemical readings [23].

• Carbon Nanotubes (SWCNTs): Acting as molecular "wires," these nanotubes link probes to the electrode, boosting the sensitivity of DPV and EIS by several magnitudes.

• Molybdenum Disulphide (MoS2): A 2D semiconductor used for "turn-on" optical sensing. It quenches fluorescence in the resting state and releases signals upon target binding to facilitate SERS detection.

5.2 Bioreceptor probe strategy

The platform employs three distinct recognition chemistries to handle varied target types:

1. Aptamer Probes: Synthetic, stable "nucleic acid antibodies" that target quorum-sensing molecules (AHLs) and protein factors (CagA). They are resilient and require no refrigerated transport.

2. CRISPR-Cas Reporters: Cas12a identifies AMR DNA, while Cas13a tracks biofilm RNA. Their "collateral cleavage" mechanism provides massive signal amplification, reaching attomolar sensitivity without PCR.

3. DNAzyme Catalytic Probes: Metal-dependent DNA structures that generate a colorimetric readout to quantify cyclic-di-GMP, helping determine the exact maturation stage of a biofilm.

5.3 Transduction Modalities

Three independent sensing modes operate simultaneously:

• **Electrochemical:** Uses DPV and EIS for ultra-sensitive (attomolar) detection of proteins and genes via electron movement.

• **Optical (SERS/LSPR):** Uses gold arrays for label-free, multi-target detection without the need for complex sample preparation [24].

• **Piezoelectric (QCM-D):** Monitors mass changes at the nanoscale, allowing real-time tracking of biofilm thickness and density.

VI. AI/ML DIAGNOSTIC PIPELINE

6.1 Automated signal preprocessing

Raw data is cleaned in under 200 ms on an edge AI chip using Savitzky-Golay smoothing for noise, least-squares for drift, and z-score normalization to standardize signals across all channels.

6.2 Strategic feature engineering

The AI extracts specific "fingerprints" from the clean data, such as:

• **Electrochemical:** Peak latency and Nyquist plot phase angles.

• **Optical:** SERS spectral ratios at specific wavenumbers [24].

• **Physical:** Viscosity markers from QCM-D and correlation patterns between different analytes.

6.3 Deep learning models

An ensemble of specialized architecture interprets the data [6,25]:

• **1D-CNN:** Classifies AMR genetic signatures in impedance data.

• **LSTM-Transformer:** Models the time-based growth and communication kinetics of biofilms.

• **GNN:** Maps how different virulence factors interact and co-occur.

• **XGBoost + VAE:** Generates oncology risk scores and identifies biological outliers.

• **Bayesian Stacking & SHAP:** Fuses all model data into one result while providing "Explainable AI" to show clinicians exactly why a result was reached.

6.4 Ensemble fusion and clinical output

Final predictions are integrated into a comprehensive report:

• **Resistance Profile:** Probability-weighted AMR detection and antibiotic efficacy predictions.

• **Biofilm Severity (0–100):** A composite score of infection maturity.

• **Oncology Risk (0–1):** The likelihood of a cancer-microbe association [3].

• **Clinical Guidance:** Actionable advice on treatment, monitoring, and specialist referrals, backed by transparent SHAP visualizations for regulatory and clinical trust.

VII. EXPERIMENTAL METHODOLOGY

7.1 Phase I: Biosensor Fabrication

First, we will make the tiny parts needed for the biosensor. These include Gold Nanoparticles, Single-Walled Carbon Nanotubes and MoS_2 nanosheets [22,23]. We will use machines like TEM, AFM and Raman to check if they are made correctly. The biosensor needs these tiny parts to work properly. The biosensor is an important device that will help us detect biomarkers.

Next, we will put these parts together on a surface to make the electrode. We will use a layer-by-layer method to do this. Then we will check the surface with SEM-EDX to make sure it is done right. This is a step for the biosensor. We must make sure that the biosensor is made correctly because the biosensor needs to be able to detect biomarkers.

After that we will find molecules called aptamers that can attach to biomarkers like FadA, CagA, AHLs and AI-2. The biosensor will use these aptamers to detect biomarkers. We will use the SELEX process to find these aptamers and check how strong they attach using MST and SPR. The biosensor needs to be able to detect biomarkers, so we have to find the aptamers for the biosensor.

Then we will work with CRISPR to design special guide RNAs that can target resistance genes. The biosensor will use these guide RNAs to detect resistance genes. We will use DNA to make sure it is accurate. This is a part of the biosensor because the biosensor needs to be able to detect resistance genes.

Finally, we will make DNAzyme probes for the biosensor. We will check how well they work with amounts of c-di-GMP to see how sensitive the biosensor is. This will help us understand how well the biosensor works. The biosensor has to be very sensitive to detect biomarkers, so we must make sure the DNAzyme probes are working correctly.

7.2 Phase II: Analytical Validation

We will test the biosensor to see how accurate it is. We will check the detection limits and the range for all 15 biomarkers the biosensor is targeting. This is a step for the biosensor. We have to make sure that the biosensor is accurate because the biosensor needs to be able to detect biomarkers.

We will also check if the biosensor can tell the difference between biomarkers without getting confused or giving results. The biosensor needs to be able to do this to be useful. The biosensor has to be able to detect biomarkers so we have to make sure it can tell the difference between them [6].

Then we will test the biosensor in fluids like sputum, urine and wound fluid to see if it works well in samples. This will help us understand how the biosensor works in real-world situations. The biosensor has to work in situations, so we have to test it in different fluids [21].

We will compare the results from the biosensor to laboratory methods like qPCR, ELISA and LC-MS/MS to see if it is clinically useful. The biosensor needs to be able to provide results to be useful in healthcare.

The biosensor has to provide results that're accurate and reliable.

7.3 Phase III: AI Training and Validation

We will collect a lot of data from the biosensor using over 2,000 tests and 50 reference strains and 200 clinical samples. This data will help the AI learn to recognize patterns. The AI is a part of the biosensor [6]. The AI will help the biosensor detect biomarkers, so we must make sure it has data to learn.

We will train learning models using this data and find the settings to get results. The AI needs to be able to provide results to be useful [25]. The AI has to be able to detect biomarkers, so we must make sure it is trained correctly.

We will use SHAP analysis to understand how the AI makes its decisions and make sure the results are trustworthy for professionals. This is a step for the biosensor.

We have to make sure that the AI is trustworthy because the AI is a part of the biosensor.

Finally, we will check the performance of the system to see if it is ready for world use. The biosensor needs to be able to provide results to be useful in healthcare. The biosensor must be ready for use, so we have to make sure it is working correctly.

7.4 Phase IV: Clinical Proof-of-Concept

We will test the biosensor using samples like sputum, urine and biopsies to see if it works in infections and conditions. This will help us understand how the biosensor works in real-world situations. The biosensor must work in situations, so

we have to test it in different samples.

We will compare the results from the biosensor to laboratory methods to see if it is accurate. The biosensor needs to be able to provide results to be useful in healthcare. The biosensor must provide results that're accurate and reliable [21].

We will also check how easy it is to use the system and how fast it gives results to see if it is practical for healthcare staff. The biosensor needs to be easy to use and provide results to be useful. The biosensor must be easy to use so we must make sure it is user-friendly.

We will prepare the documents like ISO 13485 to get the device certified for use. This is a step for the biosensor.

We must make sure that the biosensor is certified so we can use it in healthcare.

VIII. EXPECTED OUTCOMES AND IMPACT

8.1 Technical deliverables

• We expect to have a working biosensor that can detect amounts of material and proteins. The biosensor will be able to detect biomarkers. The biosensor will be very useful because it can detect biomarkers.

• We also expect to have a pipeline that meets standards across all three areas we are testing. The biosensor will be able to provide results in situations.

The biosensor will be very helpful because it can provide results in situations.

• We will share our data and AI models with the research community to help accelerate research. This will help other researchers understand how the biosensor works and how to improve it. The biosensor will help researchers because we will share our data and AI models.

• We plan to publish at five peer-reviewed articles to share our findings and methods with the community. This will help other researchers understand the biosensor and its potential applications.

• The biosensor will be very useful because we will share our findings and methods.

8.2 Public health impact

•The biosensor will help doctors diagnose infections faster. Prescribe the antibiotics sooner. This will help patients get the treatment they need quickly [1,2]. The biosensor will help patients because it can detect biomarkers and help doctors diagnose infections faster.

• It will also help detect cancer by integrating cancer screening into infection testing. The biosensor will be able to detect cancer biomarkers and provide warning signs.

The biosensor will help detect cancer because it can detect cancer biomarkers [3,19].

• The biosensor will be made at a cost that makes it accessible to remote or underfunded areas and will help with long-term hospital safety and antibiotic stewardship. The biosensor will be able to provide results in situations and help improve healthcare outcomes [21].

• The biosensor will be very helpful because it will be accessible to remote or underfunded areas.

IX. NOVELY AND INNOVATION

9.1 Biomarker scope

The biosensor is a platform that can detect resistance, biofilm markers and cancer-linked microbial signatures at once. The biosensor is able to detect biomarkers. The biosensor is very advanced because it can detect biomarkers. It bridges the gap between infection diagnostics and cancer screening allowing both to be done in one test. The biosensor is able to provide an understanding of a patient's health. The biosensor is very useful because it can bridge the gap between infection diagnostics and cancer screening [20].

The biosensor can detect over 15 targets across three classes in one integrated device. The biosensor can provide results and detect biomarkers. The biosensor is very powerful because it can detect targets.

9.2 Sensing technology

The biosensor uses a nanocomposite made of Gold, Carbon and MoS_2 to boost detection and sensitivity. The biosensor can detect amounts of biomarkers. The biosensor is very sensitive because it uses a nanocomposite [22,23]. It uses CRISPR-based amplification of lab methods like PCR allowing results. The biosensor is able to provide results. The biosensor is very fast because it uses CRISPR-based amplification.

The biosensor has "encoded" zones that act like lanes for each target preventing data overlaps and allowing for testing. The biosensor can provide

results and detect biomarkers. The biosensor is very advanced because it has "encoded" zones.

9.3 AI/ Clinical intelligence

The biosensor uses an AI system that combines architectures like CNNs and Transformers to analyze sensor data, with diagnostic-grade precision. The AI can provide results and detect biomarkers [6,25]. The AI is very powerful because it can analyze sensor data. The AI system is transparent and explainable showing how it reaches its conclusions, which are important for regulatory standards and building trust with clinicians. AI can provide results and explain its decisions. The AI is very trustworthy because it is transparent and explainable.

The biosensor enables health monitoring by providing a cancer risk score during infection tests identifying issues before symptoms appear. The biosensor is able to provide warning signs and help improve healthcare outcomes. The biosensor is very helpful because it can provide a cancer risk score.

X. DISCUSSION

Antibiotic resistance, infections caused by biofilms, and gut bacteria linked to cancer are three big topics in microbiology. Antibiotic resistance keeps making our current antibiotics less effective, which means more sickness, more deaths, and a bigger burden on healthcare systems everywhere. At the same time, when bacteria form biofilms. which essentially act like a shield, making it easier for bacteria to share genes that help them resist drugs and making antibiotics far less potent. On top of that, we're seeing more evidence that an imbalance in our microbes can play a role in cancer. For instance, specific bacteria like Fusobacterium nucleatum or E. coli that produce certain toxins seem to help start and grow tumors by causing ongoing inflammation, messing with our immune system, and even damaging our DNA. This really highlights why we urgently need a more combined approach to diagnosing these issues.

The system combines different ways of sensing things – like using electricity, light, and pressure – with smart computer learning, which includes AI. This helps it gather a lot of varied information from samples that are often tricky to analyze. When we can quickly link germ resistance, how bad biofilms are, and any cancer-related microbes, it means we can move closer to treatments that are just right for each person and get a deeper understanding of infections.

To address this diagnostic gap, the present study proposes a multiplex biosensing platform that combines nanostructured materials, diverse biorecognition elements, and multi-modal signal transduction. The use of gold nanoparticles, single-walled carbon nanotubes, and MoS_2 provides enhanced surface area, electrical conductivity, and optical responsiveness, thereby improving detection sensitivity across electrochemical, optical, and piezoelectric modalities. The incorporation of aptamers, CRISPR-Cas systems, and DNAzyme probes enable simultaneous targeting of nucleic acids, proteins, and small signaling molecules, allowing comprehensive biomarker profiling within a single assay. Furthermore, the integration of an AI/ML pipeline—including convolutional neural networks, temporal models, and ensemble learning—facilitates high-dimensional data interpretation, feature extraction, and predictive analysis. This methodological synergy enables rapid, multiplexed, and clinically interpretable outputs, overcoming the limitations of conventional single-analyte and time-intensive diagnostic approaches.

To make it all work, we use tiny materials, like gold bits, carbon nanotubes, and MoS_2, which help make the signals much stronger and clearer. This makes the system better at picking up different kinds of things. We also use different biological 'searchers' – like aptamers, CRISPR tools, and DNAzyme probes – to help it find things. These searchers help us find all sorts of biological clues at once, whether they're DNA, proteins, or even small signaling molecules. And the AI part is key: it helps to pick important details and guess what's going on, which makes the diagnosis more accurate and gives doctors practical information they can use. But it's not finished or perfect just yet. We still must `test it on many more patients, figure out how to make the device consistent across the board, and tackle the tricky part of putting all the different data types into one easy-to-understand result.

For doctors and for public health in general, this whole system could really speed up how fast they can diagnose problems. It might also help them make smarter decisions about handling infections,

using antibiotics carefully, and looking for early signs of cancer. If we could use this tool in places that don't have big hospitals or many resources, it could make advanced testing much more accessible, especially in areas where antibiotic resistance and cancers linked to infections are a major issue. Next up, we'll need to make the device smaller, confirm it works reliably for all sorts of patients, and ensure it passes all the necessary rules so it can be used by doctors. Overall, this research shows that it is certainly possible – and looks really promising – to put smart sensing technology together with AI. This could create new ways to diagnose tough diseases that involve many different things.

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