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Biosensor-Based Detection of Klebsiella pneumoniae: Recent Advances and Future Opportunities

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Abstract: *Klebsiella pneumoniae is a Gram-negative opportunistic pathogen responsible for a wide range of community-acquired and healthcare-associated infections, including pneumonia, bloodstream infections, urinary tract infections, and sepsis. The global emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and carbapenem-resistant K. pneumoniae (CRKP) has significantly increased morbidity, mortality, and healthcare costs, emphasizing the urgent need for rapid, accurate, and point-of-care diagnostic technologies. Conventional diagnostic methods, including culture-based techniques, biochemical assays, and antimicrobial susceptibility testing, remain the clinical gold standard but are labor-intensive and require prolonged turnaround times, potentially delaying appropriate antimicrobial therapy. Molecular diagnostic techniques such as polymerase chain reaction (PCR), quantitative PCR (qPCR), multiplex PCR, and next-generation sequencing (NGS) have substantially improved diagnostic sensitivity and specificity; however, their widespread implementation is constrained by high costs, sophisticated instrumentation, and the need for trained personnel. In recent years, biosensor technologies have emerged as promising alternatives, offering rapid, sensitive, selective, and cost-effective detection of K. pneumoniae. Electrochemical, optical, surface plasmon resonance (SPR), based biosensors have demonstrated excellent analytical performance by integrating advanced biorecognition elements such as antibodies, nucleic acids, aptamers, bacteriophages, antimicrobial peptides, and molecularly imprinted polymers.*

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Furthermore, advances in nanotechnology, artificial intelligence, and smartphone-assisted platforms have considerably enhanced biosensor sensitivity, multiplexing capability, and real-time data analysis, facilitating decentralized diagnostics. This review comprehensively summarizes recent developments in biosensor-based detection strategies for K. pneumoniae, highlighting sensing principles, nanomaterial-assisted signal amplification,CRISPR-based biosensors, point-of-care applications, and emerging intelligent diagnostic platforms. Additionally, current challenges, including clinical validation, large-scale manufacturing, regulatory approval, and commercialization, are critically discussed alongside future perspectives for translating biosensor technologies into routine clinical practice to improve infection management and antimicrobial stewardship.

Keywords: Klebsiella pneumoniae, Biosensors, Point-of-care diagnostics, Nanobiosensors diagnostics, Antimicrobial resistance

1. Introduction

2. *Klebsiella pneumoniae* is a Gram-negative, encapsulated, non-motile bacterium belonging to the family *Enterobacteriaceae*. It is one of the leading opportunistic pathogens responsible for both community-acquired and healthcare-associated infections, including pneumonia, urinary tract infections, bloodstream infections, liver abscesses, wound infections, and sepsis. Among these, pneumonia caused by *K. pneumoniae* is associated with high morbidity and mortality, particularly in elderly individuals, immunocompromised patients, and those admitted to intensive care units (ICUs) [1, 2]. The remarkable ability of this pathogen to colonize the respiratory and gastrointestinal tracts, survive under adverse environmental conditions, and rapidly acquire antimicrobial resistance has made it a significant global public health concern.
3. The increasing prevalence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and carbapenem-resistant *K. pneumoniae* (CRKP) has further complicated infection management. Resistance is primarily mediated by carbapenemase genes including KPC, NDM, OXA-48, VIM, and IMP, which are commonly carried on transferable plasmids and other mobile genetic elements, promoting horizontal gene transfer among bacterial populations.In addition, hypervirulent strains possessing enhanced virulence factors, including polysaccharide capsules, siderophores,

fimbriae, biofilm-forming ability, and hypermucoviscosity-associated genes (*rmpA* and *rmpA2*), have emerged worldwide. The convergence of antimicrobial resistance and hypervirulence has created strains that are increasingly difficult to diagnose and treat, highlighting the urgent need for rapid and reliable diagnostic technologies [3,4].

4. Conventional laboratory diagnosis of *K. pneumoniae* relies on bacterial culture, Gram staining, biochemical identification, and antimicrobial susceptibility testing. Although these methods remain the clinical gold standard because of their high specificity, they generally require 24–72 h or longer to generate definitive results, thereby delaying targeted antimicrobial therapy. Furthermore, culture-based techniques may exhibit reduced sensitivity in patients receiving prior antibiotic treatment or in samples containing low bacterial loads [5]). Molecular diagnostic methods, including polymerase chain reaction (PCR), real-time PCR (qPCR), multiplex PCR, loop-mediated isothermal amplification (LAMP), and next-generation sequencing (NGS), have substantially improved the speed and accuracy of pathogen detection while enabling simultaneous identification of antimicrobial resistance genes. However, their widespread implementation remains limited by expensive instrumentation, complex workflows, and the requirement for skilled personnel, particularly in resource-limited settings [6].
5. To address these limitations, biosensor-based diagnostic platforms have emerged as promising alternatives for the rapid detection of bacterial pathogens. A biosensor integrates a biological recognition element, such as antibodies, nucleic acids, aptamers, bacteriophages, antimicrobial peptides, or molecularly imprinted polymers, with a physicochemical transducer that converts the biorecognition event into a measurable electrical, optical, or mechanical signal. Biosensors offer several advantages over conventional diagnostic methods, including rapid analysis, high sensitivity and specificity, portability, minimal sample preparation, low reagent consumption, and compatibility with point-of-care (POC) testing [7].
6. Recent advances in nanotechnology have significantly enhanced biosensor performance. Nanomaterials such as gold nanoparticles, graphene, carbon nanotubes, quantum dots, magnetic nanoparticles, and metal-organic frameworks increase the surface area available for biomolecule immobilization, improve electron transfer efficiency, and amplify detection signals, resulting in lower detection limits and faster response times [8,9]. Simultaneously, the integration of CRISPR/Cas systems with electrochemical, fluorescence, and lateral flow biosensors has enabled highly sensitive and sequence-specific detection of *K. pneumoniae* and clinically important antimicrobial resistance genes within a short time frame [10].

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7. Another emerging trend is the incorporation of artificial intelligence (AI), smartphone-assisted sensing, and microfluidic lab-on-a-chip technologies into biosensing platforms. These innovations facilitate automated signal interpretation, multiplex pathogen detection, wireless data transmission, and decentralized testing, making biosensors increasingly suitable for clinical diagnostics, infection surveillance, and antimicrobial stewardship [11]. Such platforms align well with the World Health Organization's reassured criteria for next-generation point-of-care diagnostics, emphasizing affordability, sensitivity, specificity, rapidity, user-friendliness, connectivity, and accessibility.
8. Despite remarkable progress, several challenges remain before biosensors can be routinely adopted in clinical laboratories. These include limited clinical validation using large patient cohorts, difficulties in assay standardization, long-term stability of biorecognition elements, regulatory approval, and large-scale commercialization. Addressing these challenges will be critical for translating laboratory prototypes into clinically reliable diagnostic devices [7,8].
9. This review summarizes recent advances in biosensor technologies for the detection of *K. pneumoniae*, with particular emphasis on electrochemical, optical, nanomaterial-enabled, CRISPR-based, and microfluidic biosensors. Current challenges, clinical applications, and future opportunities for developing rapid, sensitive, and point-of-care diagnostic platforms are also discussed.

10. 2. Clinical Importance of *Klebsiella pneumoniae*

11. The clinical significance of *Klebsiella pneumoniae* has increased markedly over the past decade owing to its widespread distribution, diverse virulence mechanisms, and rapidly evolving antimicrobial resistance. It is one of the leading causes of both community-acquired and healthcare-associated infections, particularly in hospitalized and immunocompromised patients. *K. pneumoniae* is frequently associated with pneumonia, ventilator-associated pneumonia (VAP), bloodstream infections, urinary tract infections (UTIs), liver abscesses, surgical site infections, and sepsis, contributing substantially to global morbidity, mortality, and healthcare expenditure [1,2].
12. A major factor underlying the clinical success of *K. pneumoniae* is its extensive repertoire of virulence determinants. The polysaccharide capsule is the primary virulence factor, enabling the bacterium to evade phagocytosis and complement-mediated killing. Other important factors include lipopolysaccharide (LPS), type 1 and type 3 fimbriae, siderophores (enterobactin, aerobactin, yersiniabactin, and salmochelin), and the ability to form biofilms on medical devices such as urinary catheters and endotracheal tubes. Biofilm formation enhances bacterial persistence, promotes antibiotic tolerance, and contributes to chronic and recurrent infections [12]. In recent years, hypervirulent *K. pneumoniae*

(hvKP) strains possessing hypermucoviscosity-associated genes (*rmpA* and *rmpA2*) have emerged worldwide, causing severe invasive infections, including pyogenic liver abscesses, meningitis, endophthalmitis, and necrotizing pneumonia, even in healthy individuals [3,4].

13. The rapid emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and carbapenem-resistant *K. pneumoniae* (CRKP) has further intensified the clinical challenge. Resistance is primarily mediated by carbapenemase genes, including KPC, NDM, OXA-48, VIM, and IMP,which are frequently located on transferable plasmids, facilitating rapid horizontal gene transfer. Consequently, infections caused by CRKP are associated with prolonged hospitalization, therapeutic failure, and significantly higher mortality rates [13,14].
14. The convergence of hypervirulence and antimicrobial resistance has created highly pathogenic clones that are increasingly difficult to diagnose and treat. Early identification of *K. pneumoniae* and its resistance determinants is therefore critical for timely antimicrobial therapy, infection control, and antimicrobial stewardship. However, the prolonged turnaround time of conventional microbiological techniques highlights the need for rapid, sensitive, and point-of-care diagnostic platforms. In this context, biosensor technologies have emerged as promising tools for the early detection of *K. pneumoniae*, offering high analytical sensitivity, specificity, portability, and rapid response, thereby facilitating improved clinical decision-making and patient outcomes.

15. **3. Limitations of Conventional Diagnostic Methods**

16. Accurate and timely diagnosis of *K. pneumoniae* infections is essential for appropriate antimicrobial therapy and effective infection control. Conventional diagnostic methods, including bacterial culture, Gram staining, biochemical identification, and antimicrobial susceptibility testing (AST), remain the gold standard in clinical microbiology because of their high specificity and ability to provide phenotypic resistance profiles. However, these techniques are associated with several limitations that restrict their effectiveness in the management of rapidly progressing infections [15].
17. The primary drawback of culture-based methods is their prolonged turnaround time, typically requiring 24–72 h or longer for pathogen identification and antimicrobial susceptibility testing. This delay often results in empirical broad-spectrum antibiotic administration, which may contribute to the emergence of antimicrobial resistance and poorer clinical outcomes. Furthermore, the sensitivity of culture can be reduced in patients receiving prior antibiotic therapy or when bacterial loads are low. Conventional biochemical assays may also fail to accurately differentiate *K. pneumoniae* from closely related *Klebsiella* species, leading to potential misidentification [16].

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18. Although molecular techniques such as PCR and next-generation sequencing have significantly improved diagnostic accuracy, their routine clinical use remains constrained by expensive instrumentation, specialized laboratory infrastructure, and the need for trained personnel. These limitations underscore the need for rapid, sensitive, cost-effective, and point-of-care diagnostic platforms. Biosensor technologies have emerged as promising alternatives, offering shorter detection times, high analytical performance, portability, and the potential for real-time detection directly from clinical samples [17].

19. **4. Principles of Biosensors and Types of Biosensors**

20. Biosensors are analytical devices that combine a biological recognition element (bioreceptor) with a physicochemical transducer to detect specific analytes and convert the biological interaction into a measurable signal. Since their introduction, biosensors have revolutionized clinical diagnostics by enabling rapid, sensitive, and selective detection of pathogens, biomarkers, toxins, and nucleic acids. In the diagnosis of *Klebsiella pneumoniae*, biosensors have emerged as attractive alternatives to conventional culture-based methods because they provide rapid analysis, minimal sample preparation, high analytical sensitivity, and compatibility with point-of-care (POC) applications [18].

21. A typical biosensor consists of three major components: (i) a bioreceptor, (ii) a transducer, and (iii) a signal processing system. The bioreceptor specifically recognizes the target analyte through biological interactions such as antigen–antibody binding, nucleic acid hybridization, enzyme–substrate reactions, or aptamer-target recognition [19]. Common biorecognition elements employed for *K. pneumoniae* detection include antibodies, DNA probes, aptamers, bacteriophages, antimicrobial peptides, and molecularly imprinted polymers (MIPs). Following target recognition, the transducer converts the biological event into an electrical, optical, or mechanical signal, which is subsequently amplified and processed to produce quantitative or qualitative analytical results [20].

22. Based on the signal transduction mechanism, biosensors used for microbial detection are broadly classified into electrochemical, optical, piezoelectric, thermal, and field-effect transistor (FET)-based biosensors. Among these, electrochemical and optical biosensors are the most extensively investigated for the rapid detection of *K. pneumoniae* due to their excellent analytical performance and ease of miniaturization [21].

23. **4.1 Electrochemical Biosensors**

24. Electrochemical biosensors detect changes in electrical properties generated during the interaction between the target analyte and the biorecognition element. Depending on the measured electrical parameter, these

biosensors are categorized as amperometric, potentiometric, conductometric, or impedimetric sensors. Electrochemical biosensors are highly sensitive, inexpensive, portable, and suitable for real-time analysis, making them ideal candidates for point-of-care diagnostics [22]. Recent advances in nanomaterials, including gold nanoparticles, graphene, carbon nanotubes, MXenes, and metal-organic frameworks, have significantly improved electron transfer, signal amplification, and detection sensitivity. Electrochemical biosensors have been successfully developed for detecting *K. pneumoniae* whole cells, virulence-associated genes, carbapenemase genes (e.gKPC, NDM, VIM, and IMP,) and antimicrobial resistance biomarkers, with detection limits reaching only a few colony-forming units (CFU) per milliliter [23,24].

25. **4.2 Optical Biosensors**

26. Optical biosensors detect biological interactions by measuring changes in optical properties such as fluorescence, absorbance, luminescence, colorimetric response, or refractive index. These platforms provide high specificity, rapid response, and multiplex detection without requiring extensive sample processing. Fluorescence-based biosensors, quantum dot sensors, colorimetric assays utilizing gold nanoparticles, and chemiluminescence sensors have demonstrated excellent performance for bacterial pathogen detection. The integration of nanomaterials and CRISPR/Cas systems has further enhanced optical biosensor sensitivity, enabling rapid identification of *K. pneumoniae* directly from clinical specimens. Because optical biosensors often provide visual or digital readouts, they are increasingly incorporated into portable diagnostic devices and smartphone-assisted platforms for decentralized testing [25].

27. **4.3 Surface Plasmon Resonance (SPR) Biosensors**

28. Surface plasmon resonance (SPR) biosensors represent a label-free optical sensing technology that measures changes in the refractive index occurring at a metal surface, typically a thin gold film, during biomolecular interactions. When target molecules bind to immobilized recognition elements on the sensor surface, they alter the resonance angle of incident light, producing a real-time analytical signal without the need for fluorescent or enzymatic labels. SPR biosensors enable rapid determination of binding kinetics, affinity constants, and bacterial concentration with exceptional sensitivity. In *K. pneumoniae* diagnostics, SPR platforms functionalized with antibodies, aptamers, or nucleic acid probes have demonstrated rapid and highly specific detection of bacterial cells and antimicrobial resistance markers [26]. Their label-free operation, high reproducibility, and capability for real-time monitoring make SPR biosensors valuable tools for clinical diagnostics, antimicrobial susceptibility studies, and biomarker discovery. However, the widespread adoption of SPR systems

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remains limited by relatively high instrumentation costs and the requirement for sophisticated optical components [27, 28].

29. Overall, continuous advances in nanotechnology, microfluidics, and molecular recognition strategies are substantially improving the analytical performance of electrochemical and optical biosensors, making them promising candidates for rapid, sensitive, and point-of-care detection of *K. pneumoniae*. Figure 1: showing the conventional and biosensor methods for the detection of *K. pneumoniae.*

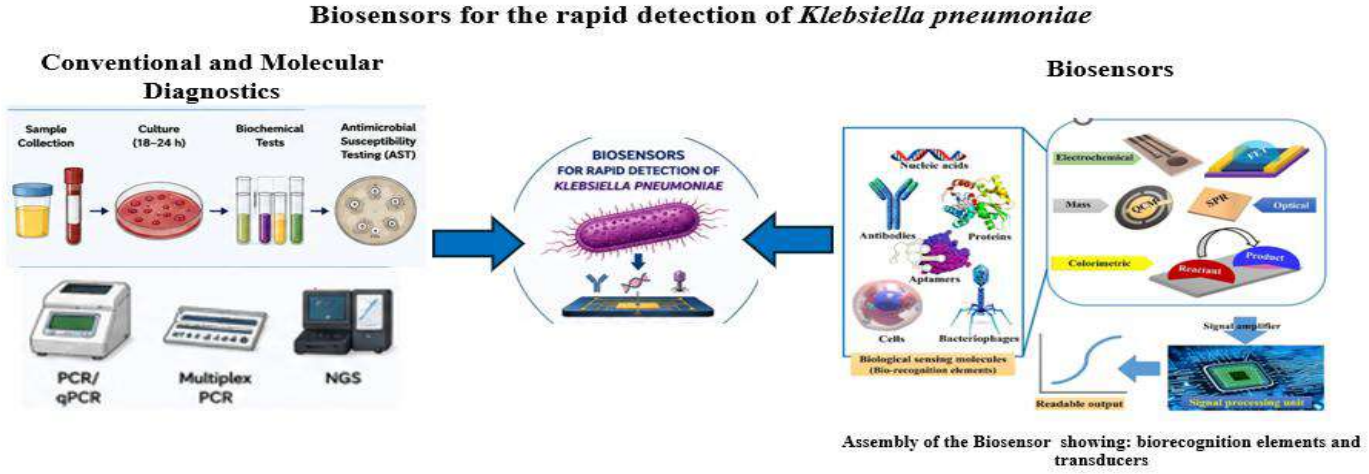

Figure 1: Schematic representation of conventional methods and biosensors for the detection of *K. pneumoniae.*

5. Nanotechnology-Enabled Biosensors for the Detection of *Klebsiella pneumoniae*

Nanotechnology has revolutionized biosensor development by significantly enhancing the sensitivity, specificity, and analytical performance of diagnostic platforms. The incorporation of nanomaterials into biosensors provides unique physicochemical properties, including a high surface-to-volume ratio, superior electrical conductivity, enhanced optical characteristics, excellent biocompatibility, and efficient biomolecule immobilization. These features improve signal amplification, accelerate electron transfer, and enable the detection of *Klebsiella pneumoniae* at extremely low concentrations, making nanotechnology-enabled biosensors promising tools for rapid clinical diagnosis [29, 8].

Among the various nanomaterials, gold nanoparticles (AuNPs) are the most widely used because of their excellent conductivity, chemical stability, and ease of surface functionalization with antibodies, DNA probes, and aptamers. AuNP-based electrochemical and colorimetric

biosensors have demonstrated rapid and highly sensitive detection of *K. pneumoniae* by amplifying electrochemical signals or producing visible color changes through nanoparticle aggregation. Their compatibility with lateral flow assays has further facilitated the development of portable point-of-care diagnostic devices [30]. Carbon-based nanomaterials, including graphene, graphene oxide (GO), reduced graphene oxide (rGO), carbon nanotubes (CNTs), and carbon quantum dots (CQDs), have attracted considerable attention because of their exceptional electrical conductivity, large surface area, and mechanical stability. These nanomaterials improve electron transport between the sensing interface and transducer while providing abundant functional groups for immobilizing nucleic acids, antibodies, or antimicrobial peptides. Graphene- and CNT-modified electrodes have been successfully employed for the electrochemical detection of *K. pneumoniae*, achieving rapid response times and low limits of detection suitable for clinical applications [31].

Recent studies have also explored magnetic nanoparticles (MNPs) as efficient sample preparation and target enrichment tools. Functionalized MNPs selectively capture *K. pneumoniae* cells from complex biological samples such as blood, urine, and sputum, reducing matrix interference and improving assay sensitivity. When integrated with electrochemical or fluorescence biosensors, magnetic separation significantly shortens analysis time and enhances diagnostic accuracy [8].

Another emerging class of nanomaterials includes metal-organic frameworks (MOFs) and MXenes, which possess exceptionally high porosity, tunable surface chemistry, and excellent conductivity. These materials provide abundant active sites for immobilizing biorecognition molecules and facilitate efficient signal transduction. MOF- and MXene-based biosensors have shown considerable potential for multiplex pathogen detection and ultrasensitive identification of antimicrobial resistance genes, including carbapenemase genes associated with *K. pneumoniae* ([32].

The integration of nanotechnology with CRISPR/Cas systems, microfluidics, and smartphone-assisted platforms has further advanced biosensor performance. Nanomaterials enhance CRISPR-based signal amplification, enabling highly specific detection of bacterial DNA or antimicrobial resistance genes within a short time. Coupling nanobiosensors with microfluidic chips reduces sample volume and reagent consumption while supporting automated, high-throughput analysis. In addition, smartphone-based optical and electrochemical readers enable real-time data acquisition and remote monitoring, making these platforms suitable for decentralized and point-of-care diagnostics [11,12].

Despite these remarkable advances, challenges such as nanoparticle synthesis reproducibility, long-term stability, large-scale manufacturing, regulatory approval, and clinical validation remain barriers to commercialization. Future research should focus on developing cost-effective, multiplexed, and clinically validated nanobiosensors capable of simultaneously detecting *K. pneumoniae*, its virulence factors, and antimicrobial resistance determinants directly from clinical specimens. Such next-generation diagnostic platforms have the potential to significantly improve early disease diagnosis, infection control, and antimicrobial stewardship.

6. DNA and Aptamer Biosensors for the Detection of *Klebsiella pneumoniae*

DNA- and aptamer-based biosensors have emerged as highly promising molecular diagnostic platforms for the rapid and specific detection of *Klebsiella pneumoniae*. Unlike conventional immunoassays, these biosensors utilize nucleic acid probes or synthetic aptamers as biorecognition elements to identify bacterial genomic DNA, virulence-associated genes, antimicrobial resistance determinants, or intact bacterial cells. Their high specificity, excellent stability, ease of synthesis, and compatibility with electrochemical, optical, and microfluidic platforms make them attractive alternatives for point-of-care (POC) diagnostics. Recent advances in nanotechnology and CRISPR-based signal amplification have further enhanced the sensitivity and clinical applicability of these biosensors.

6.1 DNA Biosensors

DNA biosensors detect specific nucleotide sequences by hybridization between an immobilized single-stranded DNA probe and complementary target DNA extracted from bacterial cells. The target genes commonly employed for *K. pneumoniae* identification include 16S rRNA, phoE, rpoB, ureD, and clinically important carbapenemase genes such as KPC, NDM, OXA-48, VIM, and IMP. Hybridization events are converted into measurable electrochemical, fluorescent, or colorimetric signals, allowing highly specific pathogen detection even at low bacterial concentrations.

Several studies have demonstrated the effectiveness of DNA biosensors for *K. pneumoniae*. In [33] developed a glycine-functionalized iron oxide nanoparticle(glycine@Fe_3 O_4) electrochemical DNA biosensor capable of specifically detecting *K. pneumoniae* DNA. The functionalized nanoparticles enhanced DNA immobilization and electron transfer, producing high analytical sensitivity, excellent selectivity, and rapid detection, demonstrating its potential for clinical diagnosis.

Similarly, [34]reported a label-free gold nanoparticle (AuNP)-based colorimetric DNA biosensor combined with duplex PCR targeting the K2A and orf10 genes. The assay produced a

visible color change within approximately 60 min and demonstrated high analytical sensitivity while eliminating the need for expensive instrumentation, making it suitable for resource-limited laboratories and point-of-care applications.

DNA biosensors have also been integrated with isothermal amplification techniques such as recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP). More recently, [35] combined dual RPA with CRISPR-Cas12a for the simultaneous detection of *Escherichia coli* and *K. pneumoniae*. The assay demonstrated rapid amplification, excellent specificity, and sensitive detection suitable for clinical specimens, highlighting the growing role of DNA-based molecular biosensors in infectious disease diagnostics.

6.2 Aptamer Biosensors

Aptamers are short single-stranded DNA or RNA oligonucleotides selected through the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process. They bind specifically to bacterial surface proteins, capsular polysaccharides, lipopolysaccharides, or other cellular targets with affinities comparable to monoclonal antibodies. Compared with antibodies, aptamers offer several advantages, including lower production costs, superior thermal stability, chemical synthesis, easy modification, and longer shelf life, making them highly suitable for biosensor development.

Although aptamer biosensors for *K. pneumoniae* remain less numerous than DNA biosensors, significant progress has been made in recent years. Researchers have developed electrochemical and fluorescence aptasensors capable of recognizing intact *K. pneumoniae* cells without requiring DNA extraction. These sensors provide rapid detection with excellent specificity and can distinguish *K. pneumoniae* from closely related Gram-negative bacteria [36]. Integration with nanomaterials such as gold nanoparticles, graphene, and carbon nanotubes has substantially improved signal amplification and analytical sensitivity.

A major advance in functional nucleic acid diagnostics has been the combination of aptamers with nanomaterials and CRISPR-based amplification systems. Recent reviews indicate that aptamer-based electrochemical, optical, and DNAzyme biosensors provide rapid, multiplexed, and highly sensitive pathogen detection while maintaining compatibility with portable and smartphone-assisted diagnostic devices. These platforms are increasingly viewed as promising candidates for next-generation point-of-care diagnostics because they combine high specificity with rapid response times and simplified assay procedures.

Overall, DNA and aptamer biosensors represent an important class of next-generation molecular diagnostics for *K. pneumoniae*. Their integration

with nanotechnology, isothermal amplification, CRISPR/Cas systems, and microfluidic platforms is expected to facilitate rapid, sensitive, and multiplex detection of bacterial pathogens and antimicrobial resistance genes, thereby supporting early clinical intervention and improved antimicrobial stewardship.

7. Point-of-Care (POC) Diagnostic Platforms for *Klebsiella pneumoniae*

Point-of-care (POC) diagnostic platforms have emerged as transformative tools for the rapid detection of *Klebsiella pneumoniae*, enabling timely clinical decision-making and improving patient outcomes. Unlike conventional laboratory-based diagnostics, POC devices provide on-site testing with minimal sample preparation, short turnaround times, and reduced dependence on sophisticated laboratory infrastructure. These characteristics are particularly valuable in emergency departments, intensive care units (ICUs), outpatient clinics, and resource-limited settings, where rapid diagnosis is essential for initiating targeted antimicrobial therapy and preventing the spread of multidrug-resistant (MDR) pathogens [37].

Recent advances in biosensor technology have facilitated the development of portable POC platforms integrating electrochemical, optical, and microfluidic sensing systems. Lab-on-a-chip (LOC) and microfluidic devices can perform automated sample preparation, nucleic acid amplification, and signal detection within a single miniaturized platform, significantly reducing reagent consumption and analysis time. When coupled with nanomaterials and isothermal amplification techniques such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), these systems enable rapid detection of *K. pneumoniae* and antimicrobial resistance genes directly from clinical specimens without requiring complex instrumentation [38].

Lateral flow biosensors and paper-based analytical devices have also gained considerable attention because of their low cost, portability, ease of use, and suitability for field deployment. Integration with CRISPR/Cas-based molecular diagnostics has further enhanced the sensitivity and specificity of these platforms, enabling highly accurate identification of bacterial pathogens within 30–60 min [39]. The World Health Organization's reassured framework emphasizes that next-generation POC diagnostics should be affordable, sensitive, specific, user-friendly, rapid, equipment-free, environmentally sustainable, and capable of real-time connectivity. Biosensor-based POC platforms increasingly fulfill these criteria and hold significant promise for improving antimicrobial stewardship, infection surveillance, and decentralized healthcare.

8. AI and Smartphone-Integrated Biosensors

Artificial intelligence (AI) and smartphone-integrated biosensors are redefining the future of infectious disease diagnostics by combining

rapid biosensing with automated data analysis and real-time communication. AI algorithms, particularly machine learning (ML) and deep learning (DL), improve biosensor performance by analyzing complex datasets, reducing background noise, identifying subtle signal variations, and enhancing diagnostic accuracy. These computational approaches facilitate rapid interpretation of biosensor outputs while minimizing operator-dependent variability, making them highly suitable for detecting *K. pneumoniae* in clinical settings [40].

Smartphone-assisted biosensors integrate portable sensing devices with mobile applications to capture, process, and transmit analytical data. Modern smartphones equipped with high-resolution cameras, optical sensors, wireless communication, and cloud connectivity serve as compact analytical platforms capable of quantifying fluorescence, colorimetric, and electrochemical signals. For *K. pneumoniae* detection, smartphone-based biosensors enable rapid analysis of electrochemical or optical responses, allowing clinicians to receive diagnostic results remotely and in real time. Such systems are particularly advantageous in remote healthcare facilities and low-resource environments where conventional laboratory infrastructure is unavailable [11].

Recent developments combine AI with nanotechnology, CRISPR/Cas diagnostics, and Internet of Medical Things (IoMT) technologies to create intelligent biosensing platforms capable of automated pathogen identification and antimicrobial resistance prediction. AI-assisted algorithms can support outbreak surveillance, monitor antimicrobial resistance trends, and assist clinicians in selecting appropriate antibiotic therapy based on biosensor-derived data. Furthermore, cloud-based connectivity enables secure storage and sharing of diagnostic information, supporting telemedicine and public health monitoring. Although challenges related to data privacy, algorithm validation, regulatory approval, and device standardization remain, AI-enabled smartphone biosensors are expected to play a pivotal role in next-generation point-of-care diagnostics for *K. pneumoniae* and other clinically important bacterial pathogens.

9. Challenges and Future Opportunities

Despite significant advances in biosensor technology, several challenges must be addressed before these platforms can be routinely implemented for the clinical diagnosis of *Klebsiella pneumoniae*. Most biosensors reported to date remain at the proof-of-concept stage and have been evaluated using limited numbers of laboratory strains or spiked samples rather than large-scale clinical specimens. Consequently, extensive multicenter clinical validation is required to establish their diagnostic accuracy, reproducibility, and robustness under real-world conditions.

Another major challenge is the standardization of biosensor fabrication, including the immobilization of biorecognition elements,

signal amplification strategies, and assay protocols. Variations in sensor design and testing conditions can affect analytical performance and hinder comparisons among studies. In addition, maintaining the long-term stability and shelf life of biological recognition elements such as antibodies, enzymes, and aptamers remains a concern for commercial deployment. Manufacturing scalability, quality control, regulatory approval, and cost-effectiveness also represent significant barriers to widespread commercialization.

Future biosensor development should focus on multiplexed platforms capable of simultaneously detecting *K. pneumoniae*, virulence-associated biomarkers, and antimicrobial resistance genes directly from complex clinical samples. The integration of nanotechnology, CRISPR/Cas systems, microfluidics, artificial intelligence (AI), and wireless communication technologies will further enhance sensitivity, automation, and real-time data analysis. Smartphone-enabled biosensors and Internet of Medical Things (IoMT)-based diagnostic systems are expected to facilitate decentralized healthcare and continuous infection surveillance. With continued interdisciplinary collaboration among microbiologists, engineers, clinicians, and industry partners, next-generation biosensors have the potential to become reliable point-of-care diagnostic tools that improve early disease diagnosis, antimicrobial stewardship, and infection control worldwide.

10. Conclusions

Klebsiella pneumoniae continues to pose a major global health threat due to its increasing prevalence, remarkable virulence, and rapid acquisition of multidrug and carbapenem resistance. Conventional diagnostic methods, although reliable, are often time-consuming and insufficient for the timely initiation of targeted antimicrobial therapy. Consequently, there is an urgent need for rapid, sensitive, and cost-effective diagnostic approaches that can support early clinical decision-making and improve patient outcomes.

Biosensor technologies have emerged as promising alternatives to conventional microbiological techniques by providing rapid detection, high analytical sensitivity and specificity, portability, and compatibility with point-of-care testing. Significant progress has been achieved in the development of electrochemical, optical, surface plasmon resonance, DNA, aptamer, nanotechnology-enabled, and CRISPR-based biosensors for detecting *K. pneumoniae* and its antimicrobial resistance determinants. Furthermore, the integration of nanomaterials, artificial intelligence, smartphone-assisted devices, and microfluidic systems has considerably improved biosensor performance while enabling automated, real-time, and decentralized diagnostics.

Despite these advances, further efforts are required to improve clinical validation, assay

standardization, long-term stability, regulatory approval, and large-scale manufacturing before biosensor technologies can be routinely implemented in healthcare settings. Future research should prioritize the development of multiplex, low-cost, and user-friendly biosensors capable of simultaneously detecting bacterial pathogens and resistance biomarkers directly from clinical samples. With continued innovations in nanotechnology, molecular diagnostics, and digital health, biosensor-based platforms are expected to transform the diagnosis of *K. pneumoniae*, supporting precision medicine, antimicrobial stewardship, and effective infection control strategies in both hospital and community settings.

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