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Biotechnological Approaches in the Detection, Control, and Prevention of Candida Auris

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Abstract: Background- *Candida auris* is an emerging multidrug-resistant (MDR) fungal pathogen that poses a significant global health threat, particularly in healthcare settings. Its ability to cause invasive infections, coupled with its resistance to multiple antifungal drugs and propensity for hospital outbreaks, necessitates rapid and accurate diagnostic and control strategies.

Aim- This report aims to comprehensively review the biotechnological approaches employed in the rapid detection, effective control, and proactive prevention of *C. auris* infections. A particular focus will be placed on CRISPR-based diagnostic methods and novel biotechnological interventions for managing this formidable pathogen.

Methodology- Conventional diagnostic methods for *C. auris* often suffer from limitations such as prolonged turnaround times and misidentification issues. The advent of molecular diagnostics, including PCR, sequencing, and MALDI-TOF, has significantly improved detection capabilities. Among these, CRISPR technology has emerged as a highly promising tool for rapid, sensitive, and specific *C. auris* detection. Systems like SHERLOCK and DETECTR, utilizing Cas12 and Cas13 enzymes, offer point-of-care potential and the ability to detect resistance genes. Beyond diagnostics, biotechnological strategies for control and prevention involve the development of novel antifungal compounds, nanotechnology-based solutions such as antifungal coatings and targeted nanoparticles, and CRISPR-based gene editing for identifying therapeutic targets. These innovations are crucial for mitigating the spread and impact of *C. auris*.

Conclusion- CRISPR-based diagnostic approaches represent a significant advancement in the rapid and accurate identification of *C. auris*, facilitating timely intervention and outbreak control. Coupled with other biotechnological innovations in prevention and control, these strategies are poised to revolutionize the management of this challenging fungal pathogen, ultimately improving patient outcomes and public health safety.

***Keywords**: Candida auris; CRISPR diagnostics; SHERLOCK; DETECTR; Cas12; Cas13; molecular diagnostics; antifungal resistance; outbreak surveillance; point-of-care testing; nanotechnology; fungal infection control.*

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Introduction

Candida auris is emerging, multidrug-resistant (MDR) yeast that has rapidly become a global public health concern since its initial identification in Japan in 2009 [1]. This opportunistic fungal pathogen is capable of causing a wide range of infections, from superficial skin colonization to severe invasive candidiasis, particularly in immunocompromised patients [2]. The importance of *C. auris* stems from several critical factors. Firstly, its inherent resistance to multiple classes of antifungal drugs, including fluconazole, voriconazole, and echinocandins, significantly complicates treatment and often leads to therapeutic failures [3]. This MDR nature distinguishes it from other *Candida* species and contributes to higher mortality rates in infected individuals [4].

Secondly, *C. auris* has demonstrated a remarkable ability to cause hospital outbreaks, leading to prolonged hospital stays, increased healthcare costs, and significant morbidity and mortality [5]. Its capacity to persist on environmental surfaces and medical equipment, coupled with its efficient transmission between patients, makes infection control challenging in healthcare settings

[6]. The global spread of *C. auris* has been rapid, with cases reported across all continents, highlighting the urgent need for effective diagnostic and control measures [7].

Finally, the need for rapid diagnosis is paramount. Conventional diagnostic methods often lead to misidentification of *C. auris* as other *Candida* species, delaying appropriate treatment and infection control interventions [8]. Early and accurate identification is crucial for implementing timely antifungal therapy, preventing further transmission, and controlling outbreaks. Biotechnological advancements, particularly in molecular diagnostics, offer promising avenues to address these diagnostic challenges and enhance the overall management of *C. auris* infections [9].

Limitations of Conventional Diagnostic Methods

The accurate and timely diagnosis of *Candida auris* is critical for effective patient management and infection control. However, conventional diagnostic methods often present significant limitations that hinder rapid identification and contribute to the pathogen's spread [8].

Culture Methods: Traditional culture-based methods, while considered the gold standard for fungal identification, are time-consuming. *C. auris* typically takes several days to grow and be identified, delaying the initiation of appropriate antifungal therapy [10]. Furthermore, *C. auris* can be misidentified as other *Candida* species, such as *Candida haemulonii* or *Candida famata*, by standard phenotypic methods, leading to inappropriate treatment decisions [11].

Biochemical Methods: Biochemical identification systems, such as API 20C AUX or Vitek 2, rely on metabolic profiles to identify yeast species. These methods frequently misidentify *C. auris* due to its unique biochemical characteristics, often reporting it as other *Candida* species or even other yeasts [12]. This misidentification can lead to the use of ineffective antifungal agents and further contribute to the development of drug resistance.

Delay in Diagnosis: The cumulative effect of these limitations is a significant delay in diagnosis. This delay has profound implications, including delayed initiation of appropriate treatment, increased risk of patient morbidity and mortality, and enhanced opportunities for *C. auris* transmission within healthcare facilities, exacerbating outbreak potential [13]. The urgent need for rapid and accurate diagnostic tools is therefore evident to combat the growing threat of *C. auris.*

Overview of Biotechnology in Fungal Diagnostics

The limitations of conventional diagnostic methods for fungal infections, particularly for emerging pathogens like *Candida auris*, have driven the development and adoption of advanced biotechnological approaches. These molecular diagnostics offer enhanced sensitivity, specificity, and speed, revolutionizing the landscape of clinical microbiology [9].

Molecular Diagnostics: This broad category encompasses techniques that detect specific genetic material (DNA or RNA) of pathogens. Unlike culture-based methods, molecular diagnostics do not rely on organism viability, allowing for earlier detection and identification [14].

Polymerase Chain Reaction (PCR): PCR-based assays are widely used for fungal detection due to their high sensitivity and specificity. Real-time PCR (qPCR) can rapidly amplify and detect *C. auris*-specific DNA sequences directly from clinical samples, significantly reducing turnaround time compared to culture [15]. Multiplex PCR can simultaneously detect multiple fungal pathogens or resistance genes, providing comprehensive diagnostic information.

Sequencing: DNA sequencing, particularly next-generation sequencing (NGS), offers unparalleled precision in fungal identification and characterization. It can accurately identify *C. auris* to the species level, differentiate between clades, and detect antifungal resistance mutations, which is crucial for guiding treatment strategies and epidemiological surveillance [16].

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS): MALDI-TOF MS has transformed fungal identification in clinical laboratories. This proteomic-based method rapidly identifies microorganisms by analyzing their unique protein fingerprints. For *C. auris*, MALDI-TOF MS provides accurate species identification within minutes to hours, directly from culture, significantly faster than traditional biochemical methods [17]. However, it still requires an initial culture step, which can be a limitation.

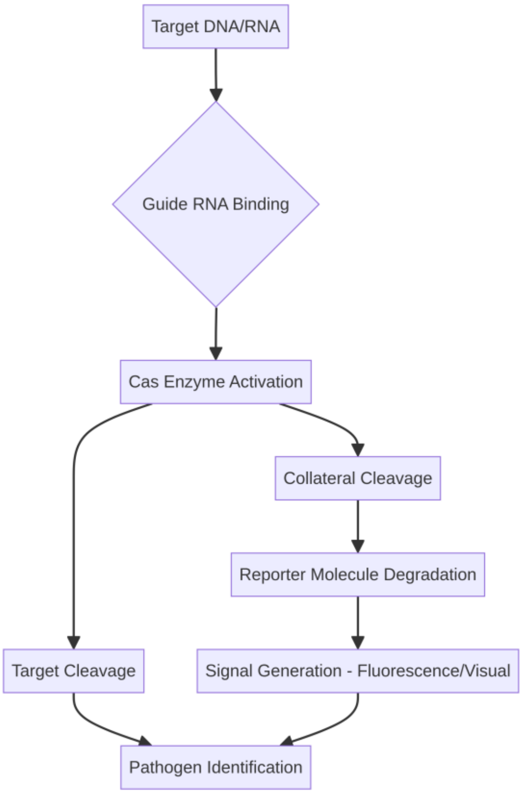
CRISPR Technology: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology, originally known for its gene-editing capabilities, has been repurposed for highly sensitive and specific nucleic acid detection. CRISPR-based diagnostics represent a cutting-edge biotechnological advancement with the potential for rapid, point-of-care detection of *C. auris* and its associated resistance markers [18]. This technology leverages the precision of CRISPR-Cas systems to target and cleave pathogen-specific DNA or RNA, offering a new paradigm in fungal diagnostics.

Principle of CRISPR Technology

CRISPR, an acronym for **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats, represents a revolutionary biotechnological tool initially discovered as an adaptive immune system in bacteria and archaea [19]. This natural defense mechanism allows prokaryotes to detect and destroy foreign genetic material, such as from bacteriophages or plasmids. The core principle of CRISPR technology, when applied to diagnostics, revolves around the precise recognition and cleavage of target nucleic acid sequences.

At the heart of the CRISPR system are two key components: **Cas enzymes** (CRISPR-associated proteins) and **guide RNA** (gRNA) [20]. Cas enzymes are nucleases capable of cutting DNA or RNA. Different Cas enzymes exhibit distinct characteristics and target specific types of nucleic acids. The gRNA is a synthetic RNA molecule designed to be complementary to a specific target sequence within the pathogen's genome. It acts as a 'GPS' for the Cas enzyme, directing it to the precise location on the target nucleic acid.

The diagnostic process typically begins with the gRNA binding to the complementary target DNA or RNA sequence. This binding event activates the Cas enzyme, which then undergoes a conformational change. Upon activation, the Cas enzyme cleaves the target nucleic acid. In many diagnostic applications, this cleavage event is coupled with a reporter system. For instance, some Cas enzymes, once activated by target binding, exhibit a non-specific collateral cleavage activity, meaning they will cut any nearby single-stranded DNA (ssDNA) or RNA molecules [21]. This collateral activity can be harnessed by including a reporter molecule (e.g., a fluorophore-quencher pair) linked by a short ssDNA or RNA sequence. When the activated Cas enzyme cleaves the reporter molecule, a detectable signal (e.g., fluorescence) is released, indicating the presence of the target pathogen [22]. This mechanism allows for highly sensitive and specific detection of even minute quantities of pathogen genetic material.



Types of CRISPR Diagnostic Systems

The versatility of CRISPR-Cas systems has led to the development of various diagnostic platforms, each leveraging different Cas enzymes and their unique properties. The most prominent systems for nucleic acid detection include those based on Cas12 and Cas13, which have been integrated into platforms like SHERLOCK and DETECTR [23].

Cas12

Cas12 (formerly Cpf1) is a Class 2 type V CRISPR-Cas enzyme that targets double-stranded DNA (dsDNA). Upon binding to its specific dsDNA target guided by a crRNA (CRISPR RNA), activated Cas12 exhibits a non-specific collateral single-stranded DNA (ssDNA) cleavage activity [21]. This 'bystander' effect is crucial for diagnostic applications. In a typical Cas12-based diagnostic assay, a reporter molecule, often a fluorophore-quencher labelled ssDNA, is included. When Cas12 detects its target DNA, it becomes activated and indiscriminately cleaves the reporter ssDNA, leading to the release of the fluorophore and a detectable fluorescent signal. This allows for highly sensitive detection of the target DNA [24].

Cas13

Cas13 is a Class 2 type VI CRISPR-Cas enzyme that specifically targets RNA. Similar to Cas12, when Cas13 binds to its target RNA guided by a crRNA, it becomes activated and exhibits a collateral RNA cleavage activity [25]. This means that once activated, Cas13 will cleave any nearby single-stranded RNA molecules, including reporter RNAs. This collateral RNAse activity is utilized in diagnostic assays by incorporating a fluorophore-quencher labeled RNA reporter. Upon target RNA detection and Cas13 activation, the reporter RNA is cleaved, generating a fluorescent signal indicative of the target RNA's presence [26].

SHERLOCK (Specific High-sensitivity Enzymatic Reporter UnLOCKing)

SHERLOCK is a highly sensitive and specific CRISPR-based diagnostic platform that can detect DNA or RNA targets at attomolar concentrations. It typically utilizes Cas13 (for RNA targets) or Cas12 (for DNA targets) in conjunction with isothermal amplification methods, such as Recombinase Polymerase Amplification (RPA) or Loop-mediated Isothermal Amplification (LAMP), to amplify the target nucleic acid before CRISPR detection [27]. The amplified target then activates the Cas enzyme, leading to collateral cleavage of a reporter molecule and signal generation. SHERLOCK can be multiplexed to detect multiple targets simultaneously and can also differentiate between closely related sequences, including single-nucleotide polymorphisms (SNPs), making it ideal for detecting specific pathogen strains or resistance mutations [28].

DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter)

DETECTR is another CRISPR-based diagnostic platform that primarily employs Cas12a for DNA detection. Similar to SHERLOCK, DETECTR combines isothermal amplification (e.g., LAMP) with the collateral activity of Cas12a. After amplification of the target DNA, the activated Cas12a cleaves a fluorescently labeled ssDNA reporter, generating a detectable signal [29]. DETECTR offers high sensitivity and specificity, and its relative simplicity makes it suitable for point-of-care applications. Both SHERLOCK and DETECTR represent significant advancements in rapid and accurate pathogen detection, including for challenging organisms like *Candida auris.*

Application of CRISPR in Detection of *Candida auris*

CRISPR-based diagnostic systems have shown immense promise in addressing the critical need for rapid, accurate, and specific detection of *Candida auris*. Their ability to target specific nucleic acid sequences makes them highly suitable for various applications in the context of this challenging fungal pathogen [18].

Species Identification

One of the primary applications of CRISPR diagnostics is the unambiguous identification of *C. auris* at the species level. Traditional methods often misidentify *C. auris* as other *Candida* species, leading to delayed or incorrect treatment [11]. CRISPR assays can be designed with guide RNAs that specifically target unique genetic sequences of *C. auris*, ensuring accurate differentiation from closely related species. For instance, dSHERLOCK, a digital CRISPR-based diagnostic platform, has demonstrated excellent specificity for *C. auris* across its major clades, while maintaining specificity against other common fungal pathogens [30].

Rapid Diagnosis

The speed of CRISPR-based detection is a significant advantage. By integrating with isothermal amplification techniques (e.g., LAMP or RPA), CRISPR assays can provide results in under an hour, a substantial improvement over culture-based methods that can take several days [31]. This rapid turnaround time is crucial for initiating timely antifungal treatment and implementing infection control measures, thereby reducing patient morbidity and preventing further transmission [13].

Detection from Clinical Samples

CRISPR diagnostics are being developed to directly detect *C. auris* from various clinical samples, including blood, urine, and surveillance swabs (e.g., naris-axilla-groin swabs). This direct detection bypasses the need for lengthy culture steps, further accelerating diagnosis. Studies have shown the clinical applicability of dSHERLOCK to detect *C. auris* from patient and environmental surveillance swabs with high sensitivity, making it a valuable tool for active surveillance and early detection in healthcare settings [30].

Outbreak Surveillance

Given *C. auris*'s propensity for hospital outbreaks, rapid and accurate surveillance is paramount. CRISPR-based tools can facilitate real-time monitoring of *C. auris* presence in healthcare environments and patient populations. Their ability to provide quick results allows for prompt identification of new cases and clusters, enabling swift implementation of isolation protocols and environmental decontamination, thereby curbing the spread of outbreaks [5].

Resistance Gene Detection

Beyond species identification, CRISPR diagnostics can also be engineered to detect specific antifungal resistance genes or mutations within the *C. auris* genome. This capability is particularly important given the MDR nature of *C. auris*. For example, dSHERLOCK has been shown to detect antifungal resistance mutations in *C. auris* from patient samples [31]. Identifying resistance markers early can guide clinicians in selecting appropriate antifungal therapies, avoiding ineffective treatments, and improving patient outcomes [3]. This targeted approach to resistance profiling is a critical step towards personalized medicine in fungal infections.

Workflow of CRISPR-Based Detection

The workflow for CRISPR-based detection of *Candida auris* typically involves several key steps, designed to ensure efficient and accurate identification of the pathogen from various sample types. While specific protocols may vary depending on the chosen CRISPR system (e.g., SHERLOCK, DETECTR) and the nature of the sample, the general sequence of events remains consistent [27].

Sample Collection

The process begins with the collection of appropriate clinical samples. This can include blood, urine, respiratory secretions, or environmental swabs (e.g., from hospital surfaces or patient skin). Proper sample collection techniques are crucial to minimize contamination and ensure sufficient pathogen load for detection [13].

DNA/RNA Extraction

Once collected, nucleic acids (DNA or RNA) are extracted from the sample. This step involves lysing the fungal cells to release their genetic material and then purifying the DNA or RNA from other cellular components and inhibitors that might interfere with downstream reactions. Various commercial kits are available for rapid and efficient nucleic acid extraction [14].

Amplification

For most CRISPR-based diagnostic assays, an amplification step is necessary to increase the copy number of the target nucleic acid to a detectable level. Isothermal amplification methods, such as Recombinase Polymerase Amplification (RPA) or Loop-mediated Isothermal Amplification (LAMP), are commonly employed due to their speed and ability to operate at a constant temperature, eliminating the need for a thermocycler [31]. This step generates millions of copies of the specific *C. auris* target sequence.

CRISPR Targeting

Following amplification, the amplified target nucleic acids are introduced to the CRISPR reaction mixture. This mixture contains the specific Cas enzyme (e.g., Cas12 or Cas13) and the designed guide RNA (gRNA) that is complementary to the *C. auris* target sequence. The gRNA directs the Cas enzyme to bind to the amplified target [20].

Signal Detection

Upon successful binding of the gRNA-Cas complex to the target nucleic acid, the Cas enzyme becomes activated. This activation triggers its collateral cleavage activity, leading to the degradation of a reporter molecule. The reporter molecule is typically a fluorophore-quencher pair linked by a short nucleic acid sequence. Cleavage of this reporter releases the fluorophore, generating a detectable fluorescent signal. This signal can be measured using a fluorimeter, a lateral flow strip, or even visualized by eye, depending on the assay design [22]. The presence of a signal indicates a positive result for *C. auris.*

Comparison with Conventional Methods

The landscape of *Candida auris* diagnostics has evolved significantly with the introduction of molecular and CRISPR-based methods, offering distinct advantages over traditional approaches. A comparative analysis highlights the strengths and weaknesses of each method across key parameters such as time, accuracy, cost, and sensitivity [13], [15], [17], [31].

Method	Time to Result	Accuracy (Specificity/Sensitivity)	Cost	Sensitivity (Detection Limit)
Culture	2-7 days	Good (but prone to misidentification)	Low	Moderate (requires viable cells)
PCR	2-4 hours	High (90-95% specificity/sensitivity)	Moderate	High (1-10 CFU/mL)

MALDI-TOF	1-2 days (after culture)	High (90-99% identification accuracy)	Moderate-High	Moderate (requires sufficient biomass)
CRISPR	<1 hour (with amplification)	Very High (near 100% specificity/sensitivity)	Moderate-High	Very High (attomolar concentrations)

Culture methods are inexpensive and allow for antifungal susceptibility testing, but their prolonged turnaround time and potential for misidentification are significant drawbacks [10]. **PCR-based methods** offer a substantial improvement in speed and sensitivity, making them valuable for rapid screening and detection directly from clinical samples [15]. However, they may require specialized equipment and trained personnel, contributing to moderate costs. **MALDI-TOF MS** provides rapid and accurate identification once a pure culture is obtained, but the initial culture step still introduces a delay [17]. Its cost can be higher due to instrument acquisition and maintenance.

CRISPR-based diagnostics represent the cutting edge, offering the fastest turnaround times, often within an hour, especially when coupled with isothermal amplification. Their specificity and sensitivity are exceptionally high, capable of detecting minute quantities of target nucleic acids [31]. While the initial development and reagent costs can be moderate to high, the potential for point-of-care applications and simplified workflows could reduce overall costs in the long run. The ability of CRISPR to detect specific resistance genes further enhances its utility, providing actionable information for clinical management [3].

Advantages of CRISPR Diagnostics

CRISPR-based diagnostic platforms offer a multitude of advantages that position them as transformative tools in the fight against *Candida auris* and other infectious diseases [18]. These benefits address many of the shortcomings of conventional diagnostic methods, leading to improved patient care and public health outcomes.

Rapid Turnaround Time: One of the most significant advantages of CRISPR diagnostics is their speed. By combining with isothermal amplification techniques, these assays can deliver results in less than an hour, a stark contrast to the several days required for culture-based methods [31]. This rapid diagnosis enables clinicians to initiate appropriate antifungal treatment much sooner, which is critical for infections with high mortality rates like invasive candidiasis.

High Sensitivity: CRISPR systems are renowned for their exceptional sensitivity, capable of detecting target nucleic acids at attomolar concentrations [30]. This high sensitivity allows for the detection of *C. auris* even when present in very low numbers in clinical samples, facilitating early diagnosis before the infection becomes severe or widespread.

High Specificity: The precise targeting mechanism of CRISPR-Cas enzymes, guided by highly specific guide RNAs, ensures that these diagnostics can accurately differentiate *C. auris* from other closely related *Candida* species and other fungal pathogens [11]. This reduces the risk of misidentification, which is a common problem with conventional biochemical methods.

Point-of-Care Potential: The simplicity of CRISPR-based assays, particularly those that produce visual readouts (e.g., lateral flow strips), makes them highly suitable for point-of-care (POC) testing. POC diagnostics can be performed outside of centralized laboratories, such as in clinics or even at the patient's bedside, enabling rapid decision-making and immediate intervention without the need for specialized equipment or extensive training [29].

Early Outbreak Control: The rapid and accurate detection capabilities of CRISPR diagnostics are invaluable for early outbreak control. By quickly identifying new cases and tracking the spread of *C. auris*, healthcare facilities can implement timely infection control measures, such as patient isolation and environmental decontamination, to prevent further transmission and contain outbreaks effectively [5].

Detection of Resistance Genes: Beyond mere identification, CRISPR assays can be designed to detect specific antifungal resistance mutations within the *C. auris* genome [31]. This provides crucial information for guiding therapeutic decisions, allowing clinicians to select effective antifungal agents and avoid those to which the pathogen is resistant, thereby optimizing treatment strategies and combating the rise of multidrug resistance.

Limitations and Challenges

Despite the significant advantages offered by CRISPR-based diagnostics for *Candida auris*, several limitations and challenges need to be addressed for their widespread adoption and implementation in clinical microbiology laboratories [32].

Cost: The initial development and production of CRISPR-based diagnostic kits can be relatively expensive, particularly for specialized reagents like Cas enzymes and custom guide RNAs. While the cost per test may decrease with mass production, the upfront investment for laboratories to adopt these technologies can be a barrier, especially in resource-limited settings [33].

Need for Validation: Before widespread clinical use, CRISPR diagnostic assays require rigorous validation against a diverse range of clinical samples and *C. auris* strains. This includes extensive testing to confirm their sensitivity, specificity, reproducibility, and robustness in real-world scenarios. The validation process can be time-consuming and resource-intensive, necessitating collaborative efforts between academic institutions, industry, and regulatory bodies [34].

Limited Availability: While research and development in CRISPR diagnostics are rapidly advancing, the commercial availability of validated and regulatory-approved kits for *C. auris* detection is still limited. This restricts their accessibility to a broader range of clinical laboratories, particularly those without the capacity for in-house assay development [35].

Standardization Issues: The lack of standardized protocols for sample processing, nucleic acid extraction, assay execution, and result interpretation can hinder the comparability of results across different laboratories and diagnostic platforms. Establishing universal standards and quality control measures is essential to ensure the reliability and consistency of CRISPR-based *C. auris* diagnostics [36].

Complexity of Interpretation: While some CRISPR assays offer simple visual readouts, others may require specialized equipment and expertise for signal detection and interpretation. This can add to the complexity of implementation, particularly in settings where trained personnel are scarce [37].

Regulatory Hurdles: The introduction of novel diagnostic technologies like CRISPR-based assays into clinical practice requires navigating complex regulatory pathways. Obtaining approvals from health authorities necessitates demonstrating clinical utility, safety, and efficacy, which can be a lengthy and challenging process [38].

Addressing these limitations through continued research, technological advancements, cost reduction strategies, and international collaboration will be crucial for realizing the full potential of CRISPR diagnostics in combating *C. auris.*

Future Perspectives

The rapid evolution of biotechnological tools, particularly CRISPR technology, promises a transformative future for the detection, control, and prevention of *Candida auris*. Several key areas are poised for significant advancements, offering innovative solutions to combat this formidable pathogen [39].

Portable Diagnostics: The development of highly portable and user-friendly CRISPR-based diagnostic devices is a major future direction. These devices, often integrated with microfluidics and lateral flow assays, could enable rapid, on-site detection of *C. auris* in diverse settings, including remote clinics, emergency rooms, and even during outbreak investigations in resource-limited areas. This would significantly reduce turnaround times and facilitate immediate public health interventions [40].

AI Integration: The integration of Artificial Intelligence (AI) and machine learning algorithms with CRISPR diagnostics and other biotechnological approaches holds immense potential. AI can enhance the analysis of complex diagnostic data, improve the accuracy of pathogen identification, predict antifungal resistance patterns, and even aid in the design of novel gRNAs for CRISPR systems. Furthermore, AI can be leveraged for real-time surveillance and prediction of *C. auris* outbreaks, optimizing resource allocation and intervention strategies [41].

Biosensors: The development of advanced biosensors incorporating CRISPR technology is another promising avenue. These biosensors could offer continuous or near real-time monitoring of *C. auris* in environmental samples, healthcare surfaces, or even directly from patient fluids. Combining CRISPR with nanotechnology-enabled biosensors could lead to ultra-sensitive and highly specific detection platforms with rapid response times [42].

Nanotechnology: Nanotechnology is expected to play an increasingly crucial role in both diagnostics and therapeutic strategies against *C. auris*. Beyond biosensors, nanoparticles can be engineered to deliver antifungal agents more effectively to infection sites, overcome drug resistance mechanisms, and even act as direct antifungal agents themselves. Nanocoatings with antimicrobial properties could be developed for hospital surfaces and medical devices to prevent *C. auris* colonization and transmission [43], [44].

Routine Fungal Diagnostics: Ultimately, the goal is to integrate these advanced biotechnological approaches into routine fungal diagnostics. This would involve establishing standardized, cost-effective, and highly accessible CRISPR-based assays and other molecular tools as the first line of defense against *C. auris*. Such integration would ensure rapid and accurate identification, timely treatment, and robust infection control, thereby significantly improving patient outcomes and mitigating the global health threat posed by *C. auris* [45].

Conclusion

Candida auris presents a formidable challenge to global public health due to its multidrug resistance, propensity for outbreaks, and diagnostic complexities. The advent of biotechnological approaches, particularly CRISPR-based diagnostic systems, offers a promising paradigm shift in our ability to detect, control, and prevent infections caused by this pathogen. CRISPR technology, with its inherent rapidity, sensitivity, and specificity, stands out as a powerful tool for accurate species identification, early diagnosis from clinical samples, and the crucial detection of antifungal resistance genes. These capabilities are vital for timely clinical intervention and effective outbreak management [30], [31].

Beyond diagnostics, biotechnological innovations in prevention and control, including advanced antifungal compounds, nanotechnology-based solutions, and the potential for AI integration, are continuously evolving. The future landscape of *C. auris* management will likely feature portable, integrated diagnostic platforms and novel therapeutic strategies that leverage these cutting-edge technologies. By embracing and further developing these biotechnological approaches, we can significantly improve our capacity to combat *C. auris*, safeguard patient health, and enhance infection control in healthcare settings, ultimately shaping the future of fungal diagnostics and therapeutics [39], [43].

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