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Detection of *Burkholderia cepacia* by various rapid diagnosis methods

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ABSTRACT

Burkholderia cepacia is an opportunistic pathogen associated with severe infections in immunocompromised and cystic fibrosis patients. Its intrinsic resistance to multiple antibiotics and ability to survive in diverse environments make accurate and rapid detection essential. It also highlights the organism's virulence factors, pathogenesis, and the wide range of available diagnostic techniques, including molecular assays, biosensors, and genomic approaches. Emerging technologies such as CRISPR-based diagnostics, microfluidics, and whole-genome sequencing offer promising prospects for rapid and precise identification. Strengthening diagnostic capacity through these innovative methods can significantly improve infection management and control measures for B. cepacia in clinical and environmental settings.

INTRODUCTION:

Burkholderia cepacia is a Gram-negative, aerobic, motile, non-fermenting bacterium belonging to the Burkholderia cepacia complex (BCC), a group of genetically related species that have emerged as significant opportunistic pathogens [1]. These organisms are ubiquitous in soil, water, and hospital environments and are known for their ability to survive under nutrient-limited and antiseptic conditions [2]. *B. cepacia* is particularly associated with life-threatening respiratory infections in immunocompromised individuals and patients with cystic fibrosis, where it can cause chronic colonization, necrotizing pneumonia, and bacteremia [3,4]. The organism exhibits a remarkable capacity for intrinsic resistance to multiple antimicrobial agents, including β-lactams, aminoglycosides, and polymyxins, due to its impermeable outer membrane, efflux pumps, and chromosomally encoded β-lactamases [5,6]. Its ability to form biofilms on medical devices and moist surfaces further enhances persistence and transmission in hospital settings [7]. Consequently, infections caused by B. cepacia present serious therapeutic challenges and contribute to outbreaks in healthcare environments. Accurate identification and early diagnosis are essential to prevent nosocomial spread and to initiate effective therapy. Conventional culture and biochemical tests, although considered gold standards, are often time-consuming and may fail to differentiate B. cepacia from other non-fermenting Gram-negative bacilli [8]. In contrast, molecular methods provide higher specificity and sensitivity. Among these, Loop-Mediated Isothermal Amplification (LAMP) has emerged as a promising diagnostic tool, offering

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rapid nucleic acid amplification under isothermal conditions without the need for advanced thermocyclers [9]. However, despite its advantages, standard LAMP assays may occasionally suffer from non-specific amplification and lower sensitivity. To address these limitations, modified LAMP methods incorporating fluorescent, colorimetric, and real-time detection systems have been developed, improving diagnostic performance and field applicability [10,11].

Methodology

For the review article publications were identified through extensive searches on PubMed, Scopus, Google Scholar databases using key terms such as "Burkholderia cepacia complex," "LAMP," "modified LAMP," "molecular detection," "PCR," "diagnostic accuracy," "biosensors," and "whole genome sequencing." The literature search covered the period from 1996 to 2025. By initial Peer-review of the articles from the various search engines mentioned above gave a result of 150 articles were initially retrieved. Following a preliminary screening of titles and abstract 92 articles were included and 73 records were excluded for the following reasons. After exclusion, 36 full-text articles were included in the final synthesis. These articles comprised both original research papers and review articles, focusing on the pathogenesis, virulence factors, antimicrobial resistance, and diagnostic technologies applied to Burkholderia cepacia complex detection.

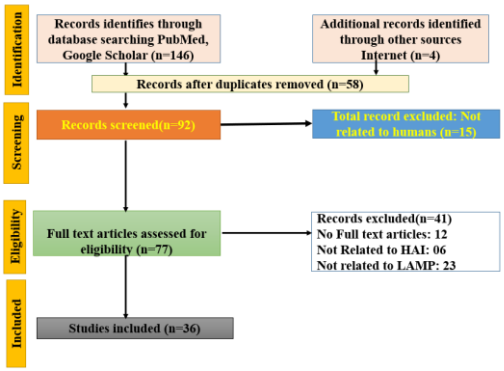

Figure No 1: flowchart showing the article selection process.

Virulence Factors and Pathogenesis of Burkholderia cepacia

The pathogenicity of Burkholderia cepacia arises from its large, flexible genome and an array of virulence determinants that facilitate colonization, invasion, and persistence within the host [12]. The severity of infection depends on both bacterial virulence and host immune competence. In cystic fibrosis and immunocompromised patients, B. cepacia can colonize the respiratory tract and cause chronic infections that are often difficult to eradicate [13]. Adherence to host tissues represents the initial step in infection. B. cepacia expresses Cable pili (CblA) and adhesin BCAM2418A, which mediate strong attachment to epithelial cells and mucins [14]. Following adherence, the bacterium produces exopolysaccharides (EPS) such as cepacian, which facilitate biofilm formation and protect cells from phagocytosis and antibiotic penetration [15].

Virulence is further enhanced by the secretion of extracellular enzymes, including lipases, proteases, and zinc metalloproteases (ZmpA and ZmpB) that degrade host tissues and immune components [16]. B. cepacia also synthesizes siderophores like ornibactin and pyochelin for iron acquisition, crucial for survival in nutrient-limited host environments [17]. The quorum sensing (QS) systems CepIR and CciIR regulate the coordinated expression of multiple virulence factors, including biofilm components, motility, and secretion systems [18]. Additionally, B. cepacia employs Type III and Type VI secretion systems (T3SS and T6SS) to inject effector proteins directly into host cells, promoting intracellular survival, cytotoxicity, and immune evasion [19,20]. Another hallmark of B. cepacia pathogenesis is its intrinsic and acquired antibiotic resistance, mediated through efflux pumps (RND family), β-lactamase production, and restricted outer membrane permeability [21]. These mechanisms collectively contribute to its persistence in clinical environments and resistance to multiple antimicrobial agents [22]. The various virulence determinants and their biological roles in the pathogenesis of *Burkholderia cepacia* are summarized in Table 1.

Table No 1: Major virulence factors of Burkholderia cepacia and their functional roles

Virulence Factor	Functional Role
Cable pili (CblA)	Mediates strong adhesion to respiratory epithelial cells and mucins; promotes colonization of the airways in cystic fibrosis patients.
BCAM2418A adhesin	Facilitates attachment to cytokeratin 13 and epithelial cells surfaces; enhances persistence and invasion.
Exopolysaccharide (Cepacian)	Promotes biofilm formation; protects bacterial cells from phagocytosis, desiccation, and antimicrobial penetration.
Lipases and proteases	Degrade host lipids and proteins, facilitating tissue invasion and immune evasion.
Metalloproteases (ZmpA, ZmpB)	Disrupt epithelial integrity and degrade host immune components such as complement proteins.

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Siderophores (Ornibactin, Pyochelin)	Aid in iron acquisition under host-limited conditions; essential for bacterial growth and virulence.
Quorum sensing systems (CepIr, CcilR)	Regulate biofilm formation, motility, secretion systems, and production of virulence enzymes in a population density-dependent manner.
Type III secretion System (T3SS)	Delivers effector proteins directly into host cells; induces cytotoxicity and interferes with host immune signaling.
Type VI secretion system (T6SS)	Involved in interbacterial competition and host cell interaction; promotes intracellular survival.
Efflux pumps	Confer multidrug resistance by exporting a wide range of antibiotics and toxic compounds.
B-lactamases	Hydrolyze -lactam antibiotics, contributing to intrinsic and acquired antibiotic resistance.
Biofilm formation ability	Enhances survival under environmental stress and confers resistance to antibiotics and host defenses.
Outer membrane low permeability	Limits antibiotic entry; contributes to intrinsic drug resistance.

Various Diagnostic Methods for Detection of Burkholderia cepacia

The detection of Burkholderia cepacia involves both conventional and advanced molecular approaches. Traditional culture methods on selective media, followed by biochemical identification systems such as VITEK-2 and API 20NE, are commonly used but may sometimes misidentify closely related species [23]. PCR and qPCR enable rapid and specific genetic detection, while Loop-Mediated Isothermal Amplification (LAMP) and its modified variants offer simple, field-applicable alternatives [24]. Emerging CRISPR-based assays and multiplex platforms provide highly sensitive and specific detection with rapid turnaround [25,26]. Electrochemical biosensors, microfluidic devices, and DNA hybridization techniques further enhance portability and automation [27,28]. Advanced methods such as MALDI-TOF MS allow precise species identification through protein profiling [29], whereas whole-genome sequencing (WGS) remains the gold standard for comprehensive genetic characterization, outbreak tracing, and antimicrobial resistance profiling of B. cepacia [30].

Table 2: Various diagnostic methods for detection of Burkholderia cepacia

Sr. No	Methods	Mechanism	Advantages	Challenges
1	CRISPR-Based Platforms	Gene-editing enzymes (e.g., Cas12a) detect BCC DNA	Single-base specificity; potential for multiplexing	Limited clinical validation
2	Multiplex Assays	Simultaneous detection of BCC and co-infecting pathogens	Reduces test redundancy; useful in ICU settings	Complex assay design; high manufacturing cost
3	Electrochemical Biosensors	Detect biomarkers via redox reactions	Low-cost; scalable; rapid results (<30 minutes)	Reduced sensitivity in complex biological samples
4	Culture	Growth of BCC on selective media	Gold standard; viable organism recovery	Time-consuming (2–7 days); labour-intensive
5	Biochemical ID (VITEK/API)	Automated or manual profiling of metabolic/enzymatic reactions	Widely used; semi-automated	May misidentify closely related BCC species
6	PCR/qPCR	Amplifies BCC-specific DNA sequences	Sensitive and specific; quantification possible	Requires thermal cycler; contamination risk
7	LAMP	Isothermal amplification of target DNA	Rapid (<1 hour); no need for thermal cycler	Limited commercial availability
8	MALDI-TOF MS	Protein fingerprinting of bacterial isolates	Rapid identification; low per-sample cost	Requires culture; limited resolution for BCC
9	WGS (Whole Genome Sequencing)	Comprehensive sequencing of the entire genome	High-resolution typing; resistance/virulence profiling	Expensive; data analysis requires expertise
10	DNA Hybridization	Probes bind to complementary BCC DNA sequences	High specificity; applicable to multiple targets	Technically complex; slower than PCR-based methods
11	Biosensor technologies	Detects specific targets (e.g., DNA, proteins) using biorecognition and signal transduction.	Fast, portable, sensitive, and suitable for point-of-care diagnostics.	Stability, reproducibility, and interference from complex samples
12	Microfluidics	Miniaturizes lab processes (e.g., PCR, mixing) on a chip with microchannels.	Requires small volumes, offers fast, integrated, and automated analysis.	Complex fabrication and risk of fluidic errors or clogging.

Future Perspectives and Research Directions

Future research on Burkholderia cepacia should focus on improving the accuracy, sensitivity, and speed of diagnostic tools to facilitate early detection and control of infections, particularly in immunocompromised and cystic fibrosis patients [31]. The integration of isothermal amplification methods such as modified LAMP with microfluidic chips and portable biosensors offers promise for point-of-care diagnostics [32]. Moreover, the use of CRISPR-based nucleic acid detection and AI-driven data interpretation could revolutionize rapid

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pathogen identification and antimicrobial resistance prediction [33,34]. Continued efforts in whole-genome sequencing (WGS) and metagenomic surveillance are essential for understanding genetic diversity, virulence evolution, and transmission pathways [35]. Additionally, research into novel therapeutic targets, bacteriophage therapy, and anti-virulence strategies may provide alternative approaches to combat multidrug-resistant B. cepacia infections [36]. Collaborative, multidisciplinary research integrating microbiology, genomics, and bioengineering will be key to addressing the growing clinical and public health challenges posed by this opportunistic pathogen.

CONCLUSION:

In conclusion, Burkholderia cepacia remains a significant opportunistic pathogen, particularly in immunocompromised and cystic fibrosis patients. Accurate and rapid detection is crucial for effective infection control and treatment. While conventional culture and biochemical methods remain important, advanced molecular and biosensor-based techniques such as modified LAMP, CRISPR diagnostics, and whole-genome sequencing offer superior sensitivity and specificity. Continued research into novel diagnostic platforms and targeted therapeutic strategies will be essential to curb the spread of multidrug-resistant B. cepacia and improve clinical outcomes.

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