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REVIEW ARTICLE

Burkholderia Cepacia: Understanding Pathogenicity, Virulence Factors, and Therapeutic Strategies

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ABSTRACT

Burkholderia cepacia formerly described as Pseudomonas cepacia, belongs to Burkholderia cepacia complex (BCC), a collection of closely related gram-negative bacteria is considered as a leading organism in causing pulmonary infections in humans. In healthcare settings, Burkholderia species have the ability to colonize medical equipments and surfaces which then posing threat of nosocomial infections. Moreover, they exhibit inherently resistance to various classes of antibiotics, thus nominated as multi-drug resistant pathogens (MDR), that is why, treatment of infections caused by these organisms is challenging. Enzymes such as metalloproteases, serine proteases, metalloproteases, and other extracellular lipase play a major role in its pathogenesis. In addition to this, different components of such as pili, flagella and lipopolysaccharide (LPS), are have a role in both motility of the bacterial cell as well as its adherence to host cells. Identification is usually done with different biochemical reactions such as late oxidase activity, oxidation of glucose, variable lysine decarboxylation, maltose, mannitol, and lactose, and hydrogen sulphide gas production. Quick and accurate identification is done by advanced techniques such as semi-nested PCR, real-time PCR, or MALDI-TOF mass spectrometry.

Key words: Multi-drug resistant, Polymerase chain reaction, Burkholderia cepacia complex, matrix assisted laser desorption ionization-time of flight mass spectrometry

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Introduction

Burkholderia genus consists of various species, which includes non-pathogenic and extremely pathogenic strains that leads to severe infections and complications in both animals and humans. Major organisms of this genus include Burkholderia cepacia complex, Burkholderia cepacia, Burkholderia mallei and Burkholderia pseudomallie, each have major contribution in causing infectious diseases which challenges the public health strategies and infection control plans.[1] In animals, mainly in horses, B. mallei is the main reason of causing.[2] While in humans, Whitmore's disease or melioidosis is a serious health threat which is caused by Burkholderia

pseudomallei. Another specie of Burkholderia genus, B cepacia is considered as a leading organism in causing pulmonary infections in humans and the most susceptible population for this infection are cystic fibrosis (CF) patients. Burkholderia cepacia complex (BCC) members are Gram-negative rods and are non-fermenters as well as characterized by a series of virulent traits.[3] Burkholderia cepacia formerly described as Pseudomonas cepacia, was recognized in 1950s as the cause of onion sour skin disease. Molecular taxonomic analyses successively reclassified these organisms, categorize them under the genus Burkholderia. At present, the Burkholderia genus encompasses over 50 species, comprising those with pathogenic potential of causing illness in animals, plants, and humans.[4] Advancements in genomic sequencing have provided comprehensions into the virulence mechanisms of Burkholderia species and genetic diversity. Markedly, with enduring efforts to sequence their genomes, it was reported the BCC comprises of at least 17 closely related species. These organisms characterized by the genomic islands and presence of mobile genetic elements, show genomic plasticity, contributing to their pathogenicity and adaptability.[5] In clinical settings, predominantly among CF patients, Burkholderia infections pose major challenges. Although all BCC species are capable of causing infection among CF patients, regional variations in prevalence of different species is reported. For instance, Burkholderia multivorans predominates in Europe, while B. cenocepacia is more common in north America.[6]

Taxonomy

There are at least 22 families of non-fermenters among gram-negative bacilli, with Burkholderiaceae being a remarkable one, categorized as medically significant non-fermenters motile rods conferred by polar flagella. Within the Burkholderiaceae family, genera such as Burkholderia, Lautropia, Ralstonia, Pandoraea and Cupria-Vidas, are members. The Burkholderia cepacia complex (BCC) consist of a group of closely related species within the Burkholderia genus, encompassing at least nine genetic species that are difficult to distinguish phenotypically.[7] In 1992, Burkholderia cepacia and six other species were reassigned to the genus Burkholderia that was initially classified under the genus Pseudomonas. Contrasting to Pseudomonas, Burkholderia falls within the β-subdivision of the Proteobacteria phylum. Since the start of the taxonomy of the Burkholderia genus, it has undergone substantial revisions, with 22 officially documented species up till now, including Burkholderia cepacia, Burkholderia mallei, Burkholderia caryophylli, Burkholderia pseudomallei, and others.[8] These species, characterized based on phenotypic properties, comprised further of the pseudomallei group (also known as rRNA group II) and the Burkholderia cepacia group. Distinctions among these species depend on various features, involving molecular and biochemical characteristics.

Habitat and Dispersion

The Burkholderia cepacia complex (BCC), a collection of closely related gram-negative bacteria, is frequently found in various environmental niches, showcasing copious beneficial traits. Burkholderia species are widespread in nature, booming in diverse habitats such as soils, plants, water bodies, animal species, particularly in healthcare settings.[9] In soils, they contribute to organic matter degradation, nutrient recycling and plant growth promotion.[10] In aquatic ecosystems, Burkholderia species helps in nutrient cycling and biofilm formation, and can also act as an opportunistic pathogen. Some species form symbiotic associations with plants, aiding in pathogen survival and nitrogen fixation. In animal species, Burkholderia may exist as pathogens or commensals causing diseases such as melioidosis and landers. In healthcare settings,

Burkholderia species have the ability to colonize medical equipments and surfaces which then posing threat of nosocomial infections.[11] To evaluate the ecological role of these species in environment and their potential impact on environment and human health, it is important to understand their specie distribution in diverse and unfavorable environmental conditions.

Prevalence and Transmission

The Burkholderia cepacia complex (BCC) can cause serious infections in immunocompromised individuals, often leading to fatal diseases. These microbes are widespread in nature, demonstrating their remarkable ability to survive in environments even after exposure to disinfectants and antiseptics. Moreover, they exhibit inherently resistance to various classes of antibiotics, thus nominated as multi-drug resistant pathogens (MDR), that is why, treatment of infections caused by these organisms is challenging.[12] BCC species can contaminate clinically useful suspensions and fluids, such as intravenous fluids and irrigation solutions, which eventually leads them to cause serious hospital-acquired infections.[13] BCC is frequently associated with nosocomial infections outbreaks, with recent cases of BCC bacteremia in immunocompromised patients reported in India, the United States and Germany.[14] Moreover, there have been reported cases of septic arthritis caused by BCC infection, where hematogenous spread of infection was suspected due to concurrent bacteremia. Within the context of causing infections in the cystic fibrosis (CF) patients, several discussions and studies revolved around the epidemiology of BCC infections, including debates on whether specific genotypes or strains demonstrate higher virulence ability than others. Transmission and assessment risk factors of BCC in cystic fibrosis patients have not been fully documented yet. Specific genetic factors, such as the existence of the cblA gene responsible for cable pilus formation protein in genomovar III strains, and the B. cepacia epidemic strain marker (BCESM), have been linked to epidemic transmission in CF treatment centers, notably in the UK and Canada.[15] Despite efforts to understand BCC epidemiology, there is still ambiguity regarding the varying infection risks associated with different genomovars which can lead to health-care associated concerns among caregivers, CF patients and their families. For better understanding of epidemiology of BCC infections, it is mandatory to perform further studies in this regard. So, the effective control plans and management strategies for the susceptible population could be formulated and implemented.

Virulence Mechanisms

Burkholderia cepacia complex (BCC) depicted itself as a difficult to treat pathogen because of its potentially deadly role in causing severe infections in cystic fibrosis (CF) patients. The genus Burkholderia genus comprises the pathogenic Burkholderia cepacia complex (BCC), which poses serious threat to human health and recurrently results in respiratory failure in patients who are already affected with cystic fibrosis (CF). Remarkably, these bacteria display significant metabolic diversity, enhancing their capability to survive in different environments. Originally recognized as a concern for CF patients, it was subsequently found that BCC also presented a threat to hospitalized individuals with variable underlying disorders, notably in cancer patients because of their weak immune response.[16] The precise mechanism of pathogenesis of BCC in cystic fibrosis (CF) patients depends upon the interaction of various virulence factors within the host which is remain not clear. Enzymes produce by BCC play a major role in causing infections in cystic fibrosis (CF) patient such as metalloproteases, serine proteases, metalloproteases, and other extracellular lipase are important in interacting with epithelial cells. Metalloproteases and serine proteases are also

important in facilitating the breakdown of proteins present in the extracellular matrix, while in invasion of the microorganism in the cell, lipase production played an important role.[17] In addition to this, different components of such as pili, flagella and lipopolysaccharide (LPS), are have a role in both motility of the bacterial cell as well as its adherence to host cells.[18] Additionally, BCC group of microorganisms also have the ability of producing biofilm and have quorum-sensing system. Other virulence mechanisms include intrinsic antimicrobial resistance property and iron-chelating siderophore. These mechanisms including the enzymes enhances the ability of organism to initiate the infections and evade host immune defense mechanisms.[19] The property of BCC of forming biofilm enhances its pathogenicity as it allows the bacteria to protect itself from antibiotic treatment and can withstand challenging and unfavorable environmental conditions. Factors such aa exopolysaccharides and cepacian EPS production enhances the biofilm producing ability and virulence of the bacteria.[21] The genes that are responsible for the biosynthesis of cepacian exopolysaccharides are clustered in discrete locations on the bacterial chromosome, highlighting their significance in resilience and evolution of BCC microorganisms.

Understanding of the molecular mechanisms behind the pathogenicity of this bacteria is important for the development of targeted and effective therapeutic regimes. In spite of the gradual progress in taxonomy, understanding of the mechanisms of pathogenicity and development of novel therapies is not yet fully documented. Advance research regarding the determinants and virulence factors of the Burkholderia cepacia complex (BCC) is important to combat effectively the infections caused by this group of microorganisms.[22]

Burkholderia Cepacia Complex (BCC) and its Disease Association

Although B. cepacia bacteria usually causes mild infections in individuals who have active immune system, however, it can cause serious and deadly infections with underlying chronic lung diseases or cystic fibrosis because of their weekend immune systems. Hospitalized individuals are also more susceptible to B. cepacia infections. Ther are multiple ways by which BCC can cause infections, such as pseudobacteremias resulted from tainted disinfectants, association of urinary tract infections with catheterization or cystoscopy because of contaminated irrigation solutions, pneumonia and pneumonitis from use of contaminated aesthetics, septicemia following heart surgery, and central nervous system (CNS) infections associated with contaminated ventricular-atrial shunts, peritonitis after peritoneal dialysis with contaminated povidone-iodine and secondary brain infections in the form of abscesses after chronic suppurative otitis media (CSOM).[22] These findings highlight the wide spectrum of clinical manifestations associated with these bacteria and the ability of Bcc to cause serious complications in susceptible individuals.

Microbial Detection and Characterization

As the Burkholderia species have the ability to withstand unfavorable conditions, it can be collected by standard protocols of sample collection and transportation used in clinical settings. Different techniques, both phenotypic, which includes culture-based methods and molecular procedures can be used for the isolation and identification of these microorganisms.[23] The BCC comprises non-spore-forming, aerobic, gram-negative bacilli, with the majority of species demonstrating motility attributed to presence of polar flagella. They produce non-lactose fermenting colonies on MacConkey agar. The growth of organisms is facilitated by using selective media, such as oxidation fermentation medium with lactose (OFPBL), bacitracin, and polymyxin B and PC (Pseudomonas cepacia) agar.[24] Nevertheless, identification of BCC through conventional

methods presents challenges due to its intricate composition consisting of 18 distinct genetic species with almost similar morphological features. Typical biochemical characteristics of Bcc include motility, late oxidase activity, varying yellow pigment formation, oxidation (but not fermentation) of glucose, variable lysine decarboxylation (but not arginine), maltose, mannitol, and lactose, and hydrogen sulphide gas production and lack of pyoverdine production.[24]

Although all species may appear similar on gram staining reaction, B. pseudomallei might display a distinctive "safety-pin" appearance because of superior staining of its polar ends, whereas, B.mallei may give the impression of being evenly stained coccobacilli.[25] Ashdown's medium with gentamicin and crystal violet is frequently used to isolate B. pseudomallei.[26] Distinctive colonies with a purple hue, dry texture, and wrinkled appearance typically develop after at least 96 hours of incubation. Unfortunately, B. pseudomallei-induced melioidosis can be challenging to identify in the lab. As a result, sensitive and focused diagnostic techniques such PCR-based approaches and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) are required.[27] For DNA analysis, PCR-based methods are commonly used, especially when focusing on genes like recA, hisA, and fur.[28] With the advancement in PCR techniques such as semi-nested PCR (SN-PCR) and real-time PCR (RT-PCR), the sensitivity of PCR-based techniques for the detection of BCC has been improved remarkably. Next-generation sequencing, an advanced molecular technique, is also a valuable metagenomic techniques in clinical microbiology labs.[29]

Antimicrobial Resistance and Therapeutic Alternatives

Because of the intrinsic resistance ability of the Burkholderia cepacia complex to polymyxin and amino glycosides along with the potential resistance pattern against beta-lactam antibiotics, challenges the treatment plans for such group of bacteria.[30] Another specie of Burkholderia genus, B. pseudomallei, is also intrinsically resistant to penicillin, amino glycosides and macrolides. Treatment options which can be used to treat infections caused by Bcc including carbapenems, ceftazidime and trimethoprim-sulfamethoxazole. Emergence of antibiotic resistance poses the difficulty in treating infections caused by Burkholderia species. Factors which play important role in contributing resistance against different classes of antibiotics are following: Burkholderis's outer membrane serves as a barrier which controls the entry of antimicrobial agents within the cell. Burkholderia has intrinsic polymyxin resistance due to altered lipopolysaccharide, whereas drug penetration is reduced by restrictive porin proteins.[31] Resistance is also significantly influenced by role of efflux pumps. Furthermore, modified targets, such as DNA gyrase and dihydrofolate reductase, are the cause of resistance to trimethoprim and fluoroquinolones, respectively. The determination of breakpoints of antibiotics for the treatment of Bcc for the European Committee on Antimicrobial Susceptibility Testing (EUCAST) is challenging because of problems with reproducibility, accuracy and poor correlation with clinical outcomes. According to the most recent Clinical Laboratory Standard Institute (CLSI) guidelines, Bcc is naturally resistant to a number of antibiotics, including fosfomycin, polymyxin B, amoxicillin, sulbactam, ticarcillin,ampicillin, ertapenem, piper acillin, colistin, ampicillin and amoxicillin-clavulanic acid.[32] It's challenging to definitively confirm intrinsic resistance of this organism to several antimicrobial drugs, such as piperacillin-tazobactam, cefotaxime, ceftriaxone, cefepime, aztreonam, imipenem, aminoglycosides, and trimethoprim, due to discrepancies in vitro and clinical results (in vivo), as well as differences in minimum inhibitory concentration (MIC) between

wild and clinical strains.[33] The complexity in determining effective treatment options for Bcc infections emphasizes the importance of further research and development of advance antimicrobial strategies.

Conclusion

Due to its opportunistic nature, BCC can be found in both community environment and in hospital settings, where they can cause serious infections in susceptible and immunocompromised individuals. In addition to their ability to cause disease outbreaks in hospital environment and transmit potentially fatal illnesses, several genomovars have the potential to spread like an epidemic illness. Perhaps most remarkably, Bcc consistently associates with people who already have cystic fibrosis. BCC's pathogenicity is highlighted by its possession of multiple virulence factors, such as extracellular lipase, quorum sensing systems, 22kDa adhesin, serine proteases, lipopolysaccharide, flagella, iron-chelating siderophores, metalloproteases, pili, and its capability to form biofilms. The combination of these factors strengthens its capability to induce illness and establish infection, categorizing BCC as a true pathogen. Its pathogenicity is further increased by intrinsic resistance to many exorbitant antibiotics and routinely used empirical medications. Bcc's propensity to contaminate widely used disinfectants makes circumstances worse which increases the possibility of infection transmission. Precise and prompt identification of BCC to the species level is pivotal for efficient management and treatment. Conventional approaches or specialized molecular strategies like semi-nested PCR, real-time PCR, or MALDI-TOF mass spectrometry can be used to identify this. These techniques allow for quick and accurate identification, which makes it easier to implement the effective treatment interventions and infection prevention strategies.

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