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REVIEW

Burkholderia cepacia Complex Bacteria: a Feared Contamination Risk in Water-Based Pharmaceutical Products

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SUMMARY *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) was once thought to be a single bacterial species but has expanded to the *Burkholderia cepacia* complex (Bcc), comprising 24 closely related opportunistic pathogenic species. These bacteria have a widespread environmental distribution, an extraordinary metabolic versatility, a complex genome with three chromosomes, and a high capacity for rapid mutation and adaptation. Additionally, they present an inherent resistance to antibiotics and antiseptics, as well as the abilities to survive under nutrient-limited conditions and to metabolize the organic matter present in oligotrophic aquatic environments, even using certain antimicrobials as carbon sources. These traits constitute the reason that Bcc bacteria are considered feared contaminants of aqueous pharmaceutical and personal care products and the frequent reason behind nonsterile product recalls. Contamination with Bcc has caused numerous nosocomial outbreaks in health care facilities, presenting a health threat, particularly for patients with cystic fibrosis and chronic granulomatous disease and for immunocompromised individuals. This review addresses the role of Bcc bacteria as a potential public health problem, the mechanisms behind their success as contaminants of pharma-

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ceutical products, particularly in the presence of biocides, the difficulties encountered in their detection, and the preventive measures applied during manufacturing processes to control contamination with these objectionable microorganisms. A summary of Bcc-related outbreaks in different clinical settings, due to contamination of diverse types of pharmaceutical products, is provided.

KEYWORDS *Burkholderia cepacia* complex, pharmaceutical contamination

INTRODUCTION

The *Burkholderia cepacia* complex (Bcc) is a group of Gram-negative nonfermenting betaproteobacteria (1) that are broadly distributed in the environment, colonizing diverse niches, whether natural or human-made (2–6). This feature is attributed to their genotypic and phenotypic plasticity and consequent capacity for rapid mutation and adaptation to challenging environments (1, 7, 8). This group of bacteria has emerged as a worrying opportunistic pathogen, with high potential to cause serious respiratory infections in patients with underlying illnesses, namely, cystic fibrosis (CF) (9–11) and chronic granulomatous disease (CGD) (12, 13). Bcc infection also has been reported in immunocompromised patients (debilitated elderly people, HIV-positive individuals, cancer patients undergoing chemotherapy, etc.) (14–16). Bcc bacteria can adapt to the stressful conditions that characterize the CF lung environment, which makes them virtually impossible to eradicate, leading to unpredictable and variable outcomes, ranging from asymptomatic carriage to a quick and sometimes unexpected deterioration of the patient's condition, culminating in a fatal necrotizing pneumonia (termed "cepacia syndrome") (3, 17).

Remarkably, Bcc bacteria also possess the capacity to survive and proliferate in water-based environments, such as water bodies, lakes, rivers, drinking water, and liquids containing small amounts of nutrients (18–21). In addition to this capacity, these bacteria are described as major contaminants of sterile (e.g., intravenous drugs and solutions) and nonsterile pharmaceuticals (e.g., nasal sprays, water-based products, mouthwash, preoperative skin solutions, and hand sanitizers), being the cause of numerous nosocomial outbreaks registered in the last 2 decades (22–26). Many of these contamination episodes have been associated with the ability of Bcc bacteria to thrive in the presence of antimicrobials and disinfectants, particularly the biocides used in pharmaceutical products' formulations (24, 27–31). Benzalkonium chloride (BZK) solutions have been described as one of the most frequent sources of Bcc contamination (32). Bcc bacteria have developed an array of strategies to cope with the presence of biocides, including their active extrusion from the bacterial cell through the action of efflux pumps or the inactivation by catabolic enzymes and subsequent use as carbon sources for bacterial growth (33–36).

Since Bcc bacteria can survive under nutrient-deprived conditions, water constitutes the most common environment in which they can cause contamination, raising a particular concern when the water supplies of pharmaceutical companies are affected (37, 38). The clinical relevance of Bcc bacteria and their status as one of the most prevalent contaminants in pharmaceutical industries has led authorities, namely, the United States Food and Drug Administration (FDA), to propose the inclusion of these bacteria in the "Objectionable Microorganisms" category (39, 40). In addition to the fact that Bcc bacteria are inherently difficult to detect and identify, due to their specific metabolic requirements and low growth rate, the majority of phenotypic and genotypic methods also demonstrate significant limitations in their capacity to detect Bcc organisms. Therefore, it is important to understand and control the presence of Bcc in pharmaceutical settings, including raw materials and finished products, to avoid their propagation into health care settings, which greatly compromises the treatment options and general quality of life of susceptible patients. Figure 1 provides an overview of the main topics that will be addressed in this review, with special focus on pharmaceutical products' contamination and the associated implications in terms of public health.

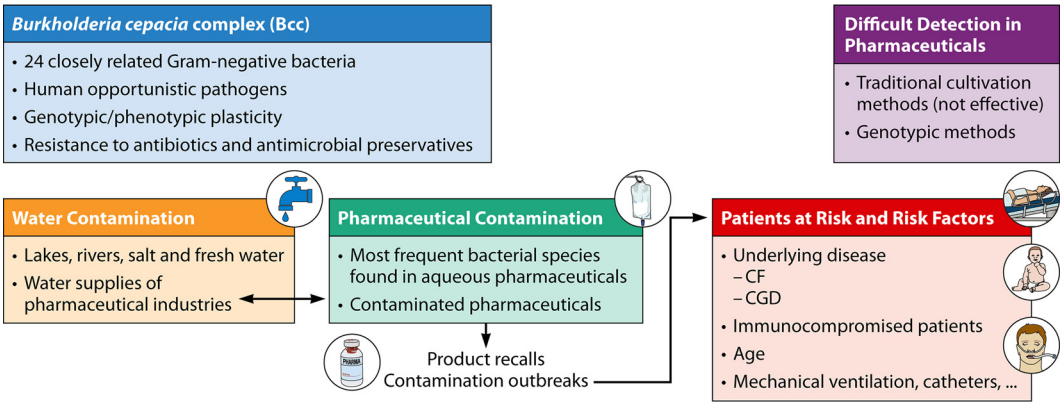

FIG 1 Overview of the main topics addressed in this review, from the description and characterization of *Burkholderia cepacia* complex (Bcc) bacteria and their survival and proliferation in diverse environments, including water supplies and pharmaceutical products, to the related nosocomial outbreaks, especially involving susceptible patients, as well as the difficulty and complexity associated with Bcc detection and identification based on phenotypic and genotypic methods.

Bcc—DIVERSITY AND ECOLOGY

The *Burkholderia cepacia* complex (Bcc) is a group of 24 phylogenetically related Gram-negative betaproteobacteria that are widely distributed in the environment (41–45). They carry complex genomes (with sizes ranging from 6 to 9 Mb and typically 3 chromosomes) and have the ability to rapidly adapt through mutation, which translates into a remarkable genotypic and phenotypic plasticity (1, 3, 7). Consequently, Bcc bacteria are known for their metabolic diversity and capacity to endure environmental stresses (33, 46).

Bcc bacteria are frequently found in natural environments, occupying diverse niches, such as soil, water (including seawater), plant rhizosphere, and agricultural products (2–6). However, the distribution of Bcc species is not homogeneous among these habitats. For instance, *B. cepacia*, *B. cenocepacia*, *B. vietnamiensis*, and *B. ambifaria* are the most representative Bcc species inhabiting the rhizosphere of plants (3–6, 8), while *B. cepacia*, *B. cenocepacia*, *B. vietnamiensis*, *B. anthina*, and *B. seminalis* are commonly found in water-based environments worldwide (6, 19–21). Nevertheless, the precise environmental origin of some Bcc species, namely, *B. multivorans*, is currently unclear, and the reports concerning its presence in different environmental samples are rather contradictory; some describe water as the most common source of this bacterium (19, 21), while others indicate that soil is the most common environmental niche (47). These discrepancies might be due to the limitations associated with the isolation methods (48).

Due to their metabolic diversity and ability to establish mutualistic and symbiotic interactions with plants, the use of *Burkholderia* species for biological control, plant growth promotion, and bioremediation has been attempted, but their pathogenic potential raises concerns (5). Since Bcc species are phylogenetically related, the exchange of genetic material between different strains/species might occur, creating the possibility for apparently innocuous strains to acquire pathogenic characteristics (6). This hypothesis has not yet been described in natural settings but has been demonstrated *in vitro* (6). Field tests have shown that Bcc bacteria can proliferate within the rhizosphere of various economically relevant crops, including rice (where *B. vietnamiensis* has been found in high numbers), pea roots (colonized by *B. ambifaria*), and wheat (with *B. cepacia* and *B. cenocepacia* inhabiting its rhizosphere) (4). Some of these species are also capable of N_2 fixation, thereby contributing to plant growth (8). Another important capacity is the production of biopesticides that protect crops against other bacteria, protozoa, nematodes, and fungal diseases, such as root rot or seed-damaging infections (2, 5). Due to their ability to use complex and diverse carbon sources, some *Burkholderia* isolates potentially can degrade different xenobiotic com-

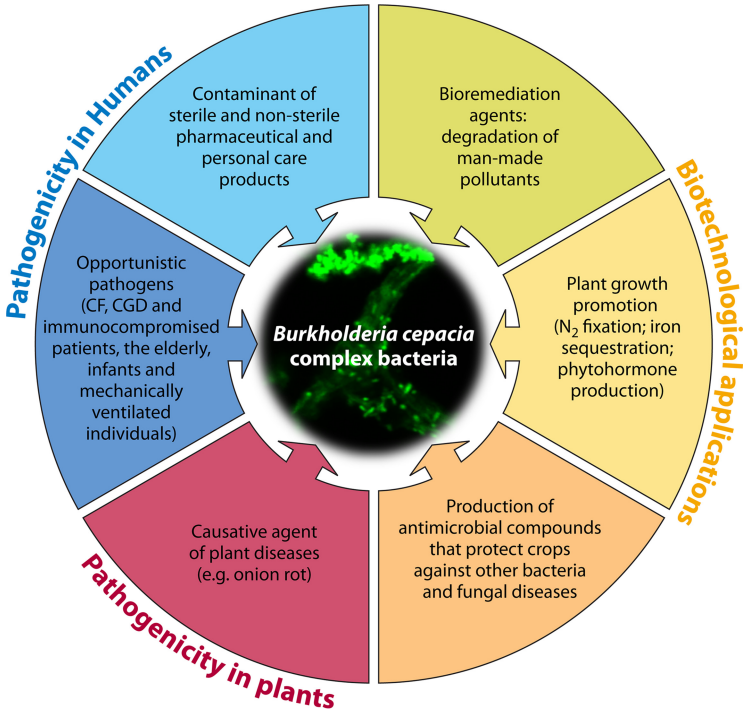

FIG 2 Strains of Bcc bacteria have potentially beneficial ecological and biotechnological applications, including their role in biocontrol, bioremediation, and plant growth promotion. However, their application in environmental settings raises concern, since members of the Bcc are human opportunistic pathogens and have been associated with infection outbreaks due to contaminated pharmaceutical products. These bacteria also can cause disease in plants.

pounds and organic pollutants, including constituents of crude oils, pesticides, phthalates, and solvents, like trichloroethylene (TCE) (3, 8).

Unlike the majority of opportunistic pathogens, members of the Bcc are not prone to commensal carriage, and infections typically are acquired in hospital settings or directly from the environment. In the case of chronically infected CF patients, person-to-person transmission might also occur outside the context of health care facilities (3). In 2002, a soil isolate was proven to be genetically indistinguishable from an epidemic *B. cenocepacia* clone (at the time referred to as *B. cepacia* genomovar III) responsible for widespread infection in CF patients, proving that strains that are pathogenic to humans do not necessarily differ from those found in the environment (49). A number of studies have confirmed the presence of Bcc bacteria in outdoor environments with which people may have regular contact. For example, PCR analysis of DNA extracts from soil and rhizosphere samples, collected from 91 sites located in 3 large U.S. cities (playgrounds, athletic fields, parks, hiking trails, residential yards, and gardens), revealed that around 90% of the samples examined were positive for Bcc (50). Moreover, *B. cenocepacia*, *B. anthina*, *B. multivorans*, and *B. pyrrocinia* strains were isolated from soil and water samples collected in the house and gardens of four CF patients, suggesting that these bacteria are also common in the home environment, possibly acting as a reservoir for infection (51). Therefore, further understanding of the role played by the natural environment in Bcc infection acquisition is of the utmost importance. The positive and negative impacts of the presence of Bcc bacteria in natural and human-made environments are summarized in Fig. 2.

Bcc BACTERIA AS HUMAN OPPORTUNISTIC PATHOGENS

Despite their potential application in bioremediation, the large-scale use of Bcc bacteria deserves attention from the scientific community, especially concerning their release in the environment. This is important, since the multiple bacterial species that

constitute the Bcc may lead to serious respiratory infections among CF (9–11) and CGD patients (12, 13). Immunocompromised individuals, infants, and the elderly also are susceptible to Bcc infection (14–16). Bcc strains rarely cause respiratory infection in healthy (immunocompetent) individuals, since they are cleared by normal airway mucociliary activity (8). In the case of hospitalized non-CF patients, the most commonly reported risk factors comprise the use of venous and urinary catheters, endotracheal tubes (in mechanically ventilated patients), hemodialysis, and long hospitalization periods in intensive care units (17). Patient-to-patient transmission is thought to occur through spreading of aerosol particles, direct physical interaction with infected people, or upon contact with contaminated surfaces (3).

The accumulation of mucous secretions is recurrent in CF patients' airways due to a mutation in the CF transmembrane conductance regulator (CFTR) gene (52). The resulting lung environment is prone to colonization by Bcc bacteria and other pathogens (52), often presenting selective pressures that induce phenotypic and genotypic variation, conferring evolutionary and adaptive advantage (53). Such adaptation mechanisms might include increased antimicrobial resistance, the development of alternative metabolic pathways, and marked genomic expression reprogramming (53–55).

Although a smaller proportion of CF patients are infected with Bcc than with *Pseudomonas aeruginosa*, they represent a major concern, since the clinical outcome is very unpredictable, even if patients are infected with clonal strains (3, 17). Although the Bcc comprises 24 bacterial species, the two most prevalent among the CF community are *B. cenocepacia* and *B. multivorans* (4, 10, 17). However, the less represented species, in particular, *B. dolosa*, *B. stabilis*, *B. contaminans*, and *B. cepacia*, also may result in poor clinical outcomes (11, 56, 57).

B. gladioli, although not a member of the Bcc, is a very closely related bacterial species and accounts for a significant percentage of opportunistic respiratory CF infections (56). This pathogen also has been identified in CGD (58) and other non-CF patients (59, 60). However, to our knowledge, there are no reports on *B. gladioli*-related nosocomial outbreaks, and contamination of pharmaceutical products with this species has not been registered. Contamination episodes involving the presence of *B. gladioli* have been registered only in the water supply system of a laboratory mouse housing facility (61) and within endotracheal tubes used for mechanical ventilation (59). Unlike Bcc species, *B. gladioli* is highly susceptible to certain antibiotics, such as piperacillin-tazobactam, aminoglycosides, quinolones, carbapenem, imipenem, and ticarcillin-clavulanic acid (59, 62, 63), which might, to some extent, explain the lack of contamination outbreaks related to this species.

Bcc BACTERIAL SURVIVAL IN WATER AND UNDER NUTRIENT-DEPLETED CONDITIONS

Besides being widely distributed in the soil, Bcc bacteria also can survive and proliferate in water-based environments, such as water bodies, lakes, rivers, drinking water, and liquids containing small amounts of nutrients. This characteristic is mainly attributed to the genetic and nutritional diversity of these species, which might be responsible for the ability of certain Bcc bacteria to metabolize the organic matter present in oligotrophic aquatic environments (18).

Examples of Bcc Survival in Water Environments

In a study carried out in the province of Bologna (Italy), *B. cepacia* was found to be present in 3.5% of the eighty-five samples of drinking water that were collected from public and private buildings, growing at a temperature of approximately 24°C (20). Additionally, it was demonstrated that the organic matter from the biofilm that is normally formed around the inner walls of water pipes was probably metabolized by the bacterial cells (20). When twenty-eight water samples, collected from the European rivers Schelde and Leie, were analyzed, ten of them were positive for the presence of Bcc bacteria, namely, *B. cepacia*, *B. multivorans*, *B. cenocepacia*, *B. vietnamiensis*, and *B. anthina* (19). More recently, the presence of Bcc bacteria in water bodies of the West

Lake in China was confirmed, where 40% of the 670 bacterial isolates analyzed belonged to five representative Bcc species: *B. multivorans*, *B. cenocepacia*, *B. stabilis*, *B. vietnamiensis*, and *B. seminalis* (21).

Besides being able to survive and proliferate in water-based environments, Bcc bacteria also have been reported to tolerate substantial temperature variations. Early studies performed on three different strains of *P. cepacia* (now *B. cepacia* or another Bcc species), incubated in distilled water, revealed optimum growth rates at 37°C (64). Nonetheless, high population yields were also observed during long-term incubation at temperatures ranging from 18°C to 42°C (64). Moreover, two other *B. cepacia* strains were able to grow in distilled water at temperatures as low as 10°C and as high as 50°C, even surviving under those conditions for 21 days and 48 h, respectively (64). Another study involving six *B. cenocepacia* strains revealed that all of them were able to proliferate or survive in distilled water for at least 40 days at 18°C and 23°C (18).

Mechanisms of Adaptation to Water and Nutrient Scarcity

Several factors can influence bacterial survival in water, namely, their physiological state, intrinsic tolerance to nutrient scarcity, interaction with other bacteria, and temperature variations (18). However, in the case of Bcc bacteria, the reasons behind their remarkable ability to persist in water environments for long periods of time still have not been thoroughly assessed, and to our knowledge, there are no published studies pertaining to the molecular mechanisms behind Bcc bacterial survival in water. Relevant clues about this topic can be extrapolated only from other bacterial species within the *Burkholderia* genus, namely, *B. pseudomallei*, a pathogen endemic to tropical and subtropical regions and the causative agent of melioidosis disease in humans and animals (65). Despite not being a member of the Bcc, it is the best-studied *Burkholderia* species in terms of survival in water environments. Results suggest that the ability to maintain an intact outer membrane architecture is relevant for *B. pseudomallei*'s prolonged survival in water environments (66). A switch of morphology from typical Gram-negative rods to cocci/coccobacilli and the transition into a viable but nonculturable state were also considered to contribute to *B. pseudomallei*'s survival in distilled water at 25°C for 16 years (67). A recent study also suggested that *P. aeruginosa* is capable of long-term survival in a nutrient-deprived environment by existing in a dormant state (68). During long-term incubation in water, *P. aeruginosa*'s cells were found to exhibit a decreased metabolic activity to convert from a rod to a coccoid shape and to change the membrane lipid composition, leading to decreased outer membrane permeability and consequent increased tolerance to polymyxin B (68). A global expression analysis indicated that the majority of genes were repressed, including those required for DNA replication (68). However, a number of genes had increased expression, in particular, amino acid, fatty acid, and phospholipid metabolism genes, suggesting that these compounds can be used as alternative carbon and energy sources under nutrient starvation (68). Water constitutes a hostile environment, presenting several challenges to bacterial growth, including a lack of nutrients and low osmolarity (66). The fact that Bcc bacteria can persist under such harsh conditions is indicative of their remarkable metabolic capacity and deserves special attention, as they can pose serious threats to public safety and health.

Bcc BACTERIA AS CONTAMINANTS OF PHARMACEUTICAL PRODUCTS

Product Recalls Associated with the Presence of Bcc Bacteria

One of the most commonly reported contaminants of nonsterile pharmaceutical products is *Burkholderia cepacia* (69), whether it was definitively identified as *B. cepacia* (the type species) or was just a member of the *Burkholderia cepacia* complex, since most of the literature available on the topic dates back to the pre-Bcc (or even the pre-*Burkholderia*) era. This is a particular concern for recently described members of the Bcc, which may have been involved in contamination episodes in the past, but their role cannot be recognized due to the deficient taxonomic knowledge at the time. Those contaminants can be transmitted through raw materials, water, and machine surfaces

used in pharmaceutical manufacturing. According to United States Food and Drug Administration (FDA) recall data, from 1998 to 2006, *B. cepacia* was identified as the cause of 22% of nonsterile product recalls (22). This tendency has grown over recent years, with this species being involved in 34% of the nonsterile product recalls between 2004 and 2011 (22). *B. cepacia* was considered the most common microbial contaminant found in nonsterile products between January and July 2012, corresponding to 39% of the bacterial species isolated from contaminated samples, while other bacterial genera, including *Pseudomonas*, *Staphylococcus*, and *Enterobacter*, were less prevalent within the collected samples (69). Bcc bacteria have been isolated from diverse types of pharmaceutical and personal care products, including nasal sprays (70, 71), multiple lotions and oils (72), water-based products (73), mouthwash (74, 75), cleansing washcloths and baby wipes, preoperative skin solutions, hand sanitizers (76), and gas relief liquid drops (22, 69).

In an epidemiological survey performed by our group between 1995 and 2002, which included patients receiving treatment at the major Portuguese CF center within a central Lisbon hospital, an unusually high prevalence of *B. cepacia* and *B. contaminans* in sputum cultures was detected (57, 71). Later, a market surveillance conducted by INFARMED, the National Authority of Medicines and Health Products, in 2003 and in 2006, detected Bcc bacteria contamination in several batches of nonsterile saline solutions for nasal application, which are often administered to CF patients for inhalant therapy. Further analysis confirmed that the clinical clones and the strains isolated from contaminated saline solutions were indiscernible based on molecular typing methods (57, 77).

Looking at the FDA's list of recalls, market withdrawals, and safety alerts (78) in recent years, several warnings have been launched concerning the presence of Bcc bacteria in a wide variety of products. In October 2016, the FDA, in collaboration with the Centers for Disease Control and Prevention (CDC), detected the presence of *B. cepacia* in the water system used for oral liquid docusate sodium manufacture. This led to a national alert, and the product was voluntarily recalled from the market by its producing pharmaceutical company (79). In 2017, the FDA detected a potential *B. cepacia*-related contamination of decongestant relief syrups for the treatment of cough, cold, and allergies and advised the voluntary recall of the products from the market (80). More recently, in 2018, the FDA detected the presence of several microbial contaminants in homeopathic drug products, including *B. multivorans*, and the recall of all water-based products from the company in question was recommended (81).

Ability of Bcc Bacteria To Survive and Proliferate in Pharmaceutical Products

Sterile pharmaceutical products typically are intended for intravenous administration (e.g., injection or infusion) or to be applied directly in the eyes. Due to the high infection risk associated with administration through these routes, such products must be manufactured in an environment that is free from bacteria, viruses, and other potentially infectious microorganisms (82). Nonsterile products (e.g., docusate, mouthwash, and body wash) are usually administered orally or topically and, theoretically, have a lower potential to cause infection. For that reason, those compounds do not require sterilization before use (83). Nevertheless, microbiological control of nonsterile products is particularly important, since they are more prone to microbial contamination, which can reduce product quality and, above all, cause drug-related infections (84).

Given their ability to use numerous organic compounds as carbon and energy sources, several of them xenobiotics that are very difficult to catabolize (4, 75), Bcc bacteria can grow and proliferate in a wide variety of medicinal drugs (24). One example of such metabolic diversity is their ability to grow on nitroaromatic and aromatic compounds through the oxidation of aromatic structures and breakdown of halogenated compounds by monooxygenases and dioxygenases (76, 77). Since nitroaromatic compounds make up a vast array of pharmaceutical drugs, Bcc bacteria can lead to the degradation of active ingredients and excipients, compromising drug

stability and purity, as well as their potency and effectiveness (24, 78). Apart from altering the chemical, physical, and organoleptic properties, drug degradation might also lead to the formation of toxic substances (84). This constitutes a serious risk for the consumers of such products, who typically suffer from other pathological conditions. Bcc-contaminated products constitute a vehicle for transmission of these opportunistic pathogens to susceptible individuals, favoring the development of life-threatening chronic infections.

Reasons Underlying Bcc Contamination in Pharmaceutical Settings and Preventive Measures

The persistence of Bcc bacteria in pharmaceutical products is, in part, attributed to the lack of good manufacturing practices. Pharmaceutical companies are responsible for ensuring quality control of their products as well as for monitoring the processing steps and components that are used (26). However, the lack of adequate cleaning procedures; the use of an unsuitable grade of water, associated with poor water control and design systems; the use of the same disinfectants for long periods of time; insufficient microbiological controls; and inadequate testing, specification, and validation guidelines constitute some of the causes of bacterial contamination in pharmaceutical settings (69).

According to the Parenteral Drug Association (PDA) technical report no. 67, *Exclusion of Objectionable Microorganisms from Nonsterile Pharmaceutical and Over-the-Counter (OTC) Drug Products*, *Medical Devices and Cosmetics*, an objectionable organism is defined as a microorganism that can proliferate in a certain pharmaceutical product, causing adverse effects on its chemical, physical, functional, and therapeutic properties (39). This definition also comprises microorganisms with a pathogenic character that, when present in a certain pharmaceutical product in large numbers, can cause infection after administration (22, 39, 40). The high prevalence of product recalls due to contamination with Bcc bacteria and the numerous nosocomial outbreaks verified throughout the years constitute the main reasons why members of the Bcc are considered objectionable organisms (22–24, 62). Moreover, their role as human opportunistic pathogens (affecting both CF and non-CF patients) (3, 17), as well as their resistance to antibiotics and antimicrobial preservatives, also contribute to their inclusion in that category (30, 33, 38, 77).

The U.S. Pharmacopeia (USP) provides several guidelines and methodologies to detect the presence of specific indicator organisms in nonsterile products, namely, the USP guidelines 61, *Microbial Enumeration Tests* (85), and 62, *Tests for Specified Microorganisms* (86). However, despite the fact that Bcc bacteria are widely recognized as objectionable organisms, the USP does not provide any specific test to determine their presence in nonsterile drug products (38, 69). Due to the lack of information and adequate detection methods, Bcc contamination is often disregarded, since these microorganisms are not considered a priority for pharmaceutical manufacturers, who are often unaware of their impact and the consequences that might result from releasing Bcc-contaminated products into the market (38). The concern raised by the undetected presence of Bcc in pharmaceutical products is not a new topic and has been the object of discussion by the FDA since 1981, when the regulatory agency detected irregularities in the validation and control processes of a water deionization system, which resulted in the contamination of a drug product with *B. cepacia* (38, 69). Another example dates back to 1993 and concerns the recall of a metaproterenol sulfate inhalation solution contaminated with *B. cepacia*, which, according to the USP, required no microbial testing before being released onto the market (38, 69). More recently, a chemically preserved oral fexofenadine antihistaminic suspension was found to be contaminated with *B. cepacia* 6 months after the manufacturing process (87). The objectionable organism had not been detected earlier because it was not included in the routine testing described by USP guideline 62 and was not considered a threat, even when its presence had been detected once in the manufacturing company's water system (87). The microbiological counts obtained during product stability testing were

acceptable for 6 months, after which a considerable increase of CFU numbers was registered. *B. cepacia* was subsequently identified through Gram staining and biochemical assays (87). These case reports highlight the urgent need for pharmaceutical companies and regulatory agencies to work together in order to reformulate the current protocols and legislation regarding the presence of Bcc bacteria in pharmaceutical products.

Presently, the FDA continues to warn pharmaceutical companies about the contamination hazard posed by Bcc bacteria when present in sterile and nonsterile pharmaceutical products, reinforcing the fact that the number of product recalls due to Bcc contamination remains high (88). On May 2017, the regulatory agency provided new regulatory guidance for manufacturers of nonsterile water-based drug products, stating the importance of establishing guidelines to prevent contamination with such objectionable organisms, including correct process design and quality assurance of incoming materials, as well as the monitoring of storage conditions and cleaning procedures (88).

In the specific case of Bcc bacteria, the number of product recalls reported in the literature confirms that antimicrobial preservatives are not effective as a way of preventing contamination, since several strains can proliferate in preserved solutions (38). Therefore, manufacturers cannot rely on the addition of antimicrobial preservatives for contamination control and must instead apply stringent in-process control and guarantee sterile conditions during every step of the manufacturing process instead of testing only the finished products (26, 37, 38). Water is the most commonly used raw material in the pharmaceutical industry and is considered a frequent source of Bcc contamination. In fact, several Bcc outbreaks that occurred throughout the years have identified pharmaceutical-grade water as the root cause of product contamination, whereas contamination of raw materials and inadequate quality control procedures were less implicated in product recalls (37, 38). Therefore, in industrial settings, every source of water should be considered a potential reservoir (38), and every piece of equipment used for processing (tanks, pumps, and filling lines) should be properly cleaned, disinfected, and dried, especially the product contact surfaces (37).

Both the FDA and the USP have provided technical guidelines concerning the water used for pharmaceutical manufacturing. The FDA advises manufacturers inspect the source of water used for wet granulations or aqueous liquid preparations, which should be chemically and microbiologically purified USP water (89). Moreover, potable water might only be used for bulk drug manufacturing and not to prepare USP-compliant dosage-form products or laboratory reagents. Nonpotable water systems, such as cooling water for air conditioning or fire sprinklers, should be correctly identified, and cross-connections with potable water must be avoided (89). Regular collection of water samples from piping systems should be a common practice, since these constitute a major source of water contamination. Maintaining the water circulation systems at constantly high temperatures (typically 80°C) is also advised to prevent significant microbial growth (89). Additionally, during the formulation of aqueous oral and topical dosage-form products, reduced water activity (a_w) is important to prevent microbial contamination and guarantee the product's self-preservation capacity (90). Pharmaceutical products with an a_w below 0.75 are significantly less prone to contamination, while nasal inhalants, hair shampoos, and antacids, which have higher water activities (a_w of 0.99), are very susceptible to contamination by Gram-negative bacteria, namely, Bcc (90).

UNIQUE ABILITY OF Bcc TO OVERCOME BIOCIDE ACTION

Bacterial Resistance to Biocides and Outbreaks Associated with Contaminated Solutions

The term biocide is generally employed to describe a chemical agent that destroys or inhibits the growth or activity of living organisms. While antiseptics are substances with antimicrobial activity that are applied to the skin/living tissue in order to reduce the microbial flora, disinfectants are applied to nonliving objects to destroy harmful

microorganisms. Preservatives are added to a wide variety of products, some of them not required to be sterile (e.g., medicines, food, and cosmetics), to prevent microbial growth (27, 32). These chemical agents are also employed at different concentrations, depending on whether the purpose is disinfection or preservation. For example, the biocide biguanide chlorhexidine is used for surface disinfection at concentrations in the range of 0.5% to 4% (vol/vol), for antisepsis at 0.02% to 4% (vol/vol), and for preservation at 0.0025% to 0.01% (vol/vol) (91).

Antiseptics and disinfectants (biocides) are widely used in hospital settings as a way of controlling infections. In general, biocides have a broader spectrum of activity and tend to act upon multiple targets, whereas antibiotics usually have specific intracellular targets (27). The most commonly used biocides include alcohol, iodine, iodophores, triclosan, chloroxylenol, chlorohexidine gluconate (CHX), and quaternary ammonium compounds, such as benzalkonium chloride (BZK) (32). One of the most worrying features of Bcc populations is that they can remain viable and/or proliferate within the currently commercialized biocide formulations, displaying low susceptibility to a variety of compounds, including CHX, cetylpyridinium chloride, triclosan, BZK, and povidone (30). Several studies also have demonstrated that the biocide concentration in many commercial products is insufficient to kill Bcc bacteria, especially when they form biofilms. This is the case for CHX, hydrogen peroxide, and BZK (92–94). For example, the typical BZK and CHX concentrations applied in commercial products range from 0.02% (200 μg/ml) to 5% (50,000 μg/ml), which might not be sufficient to eradicate resistant strains (85). Based on susceptibility assays, proper elimination of Bcc bacteria might require the use of biocide concentrations 25 times higher than those currently applied (24).

Most outbreaks have been associated with improper product utilization, namely, the use of contaminated water during manufacturing processes, overdilution of antiseptic solutions, use of outdated products, prolonged storage periods after opening, and improper storage conditions (32, 93). The use of biocides beyond the expiration date is risky, since changes in the chemical concentration or composition are likely to occur, leading to a decreased bactericidal/bacteriostatic potency (93). Contaminated solutions may appear clear and, therefore, are difficult to distinguish from truly sterile solutions (88). The ability of Bcc bacteria to survive and thrive for long periods of time in biocide-containing solutions raises an alarming concern in terms of public health, since they can be transmitted to patients through the use of contaminated products and cause subsequent problems.

The mechanisms of biocide resistance can be intrinsic or acquired through mutation events or horizontal gene transfer (27, 28). Gram-negative bacteria generally have a higher inherent resistance to biocides than Gram-positive bacteria due to the structure of their outer membrane, which acts as a barrier against the entry of several antimicrobial agents. The presence of an intact and highly charged lipopolysaccharide layer is also an intrinsic Bcc characteristic that helps prevent the diffusion of hydrophobic antimicrobials (27, 28). The presence and activation of efflux pump systems is among the major mechanisms of intrinsic biocide resistance. The most relevant family of efflux pumps comprises the resistance-nodulation-cell division (RND) transporters (95). These are usually chromosomally encoded and composed of a periplasmic membrane fusion protein and an outer membrane factor, allowing efflux of solutes across the inner and outer membranes, thereby reducing accumulation in the periplasm (95). The role of these efflux systems has been described for some Bcc species, conferring resistance to BZK and CHX in *B. cenocepacia* (33, 96) and to methylisothiazolinone-chloromethylisothiazolinone (M-CMIT) and fluoroquinolones in *B. lata* (97). Genomic expression analysis of two sequential *B. cenocepacia* isolates recovered from a CF patient undergoing antibiotic therapy revealed an increased transcription of several genes encoding efflux pumps in the highly resistant clonal isolate, namely, those encoding RND transporters, drug efflux pumps of the major facilitator superfamily (MFS), and ABC transporters (54). The same highly antibiotic-resistant isolate displayed

reduced outer membrane permeability, which was associated with the downregulation of genes encoding porins that may function as channels for the entry of antibiotics (54).

Phenotypic adaptations, such as biofilm growth, also can be considered intrinsic resistance mechanisms. Biofilm formation in Bcc bacteria has been linked to an increased ability to cause persistent infections, especially in CF patients (98, 99). The composition of the biofilm matrix, rich in polymeric substances, might contribute to the development of resistant bacteria by restricting the diffusion of antimicrobial agents (98). However, some compounds, including antibiotics, still can penetrate through biofilms, depending on the nature of both the compound and the biofilm (98, 100). The antimicrobial peptide nisin was able to penetrate through the biofilm structure, causing bacterial death, even though the viability loss was more pronounced in the case of planktonic cells than in biofilm-grown bacterial populations (101). Within the biofilm, the development of a microenvironment characterized by lower nutrient/oxygen availability and reduced bacterial growth is likely to occur together with the production of enzymes that can be involved in the degradation/neutralization of chemicals (27). Overall, biocides like CHX and hydrogen peroxide fail to significantly reduce the viability of sessile cells compared to the effects on planktonic cells (92). The exposure of *B. cenocepacia* biofilms to high levels of oxidizing agents, namely, hydrogen peroxide and sodium hypochlorite, led to the upregulation of several genes, some of which are involved in oxidative stress and general stress responses, as well as others that encode proteins required for the repair of reactive oxygen species (ROS)-induced cellular damage (102). The treatment of *B. cenocepacia* biofilms with CHX also led to the transcriptional upregulation of genes encoding membrane-related and regulatory proteins as well as drug resistance determinants, including sessile-specific RND efflux pumps (96). Bacteria within the biofilms might also resist the action of antimicrobial agents by existing in a slow-growing state, typically characterized by lower antimicrobial susceptibility (98).

Acquired resistance can occur through mutations in genes involved in the biosynthesis of the cell wall, membrane lipids, porins, or outer membrane proteins (OMPs) (28). Horizontal gene transfer may contribute to the acquisition of mobile genetic elements, such as plasmids or genes encoding proteins responsible for modification/degradation of the biocide (e.g., *o*-phosphotransferases, *o*-adenyltransferases, and *n*-acetyltransferases) (28). Another mechanism by which microorganisms can gain biocide resistance is through molecular target alteration. For instance, in *Escherichia coli*, a missense mutation in the FabI protein is sufficient for altering the target site for triclosan, conferring resistance to that compound (28).

Biocides will continue to play an important role in preventing the spread of infection in the health care environment, but it is critical to reevaluate the type of products and the concentrations at which they should be added. For successful biocidal formulations and regulation policies, knowledge about the mechanisms of action and possible development of bacterial resistance is of the utmost importance.

Mechanisms of Benzalkonium and Chlorhexidine Resistance in Bcc Bacteria

BZK and CHX are two of the most common biocides used in health care settings and personal care products, being classified as low-level antiseptics with a broad spectrum of activity against Bcc bacteria (28, 32). BZK is commonly used as an antimicrobial preservative for pharmaceutical products, being the biocide of choice for the majority of multidose aqueous nasal, ophthalmic, and optic products (103). It is a quaternary ammonium compound, composed of a positively charged nitrogen covalently bonded to three alkyl group substituents and a benzyl substituent (34, 104). Bcc-contaminated BZK solutions have been pointed out as the major cause of outbreaks, unlike any other biocide available in the market (32). BZK's effects occur mainly at the level of membrane permeability, starting with its adsorption onto and penetration of the bacterial cell surface, followed by membrane destabilization/disorganization and consequent leakage of cellular constituents, culminating in cell death (27, 104, 105).

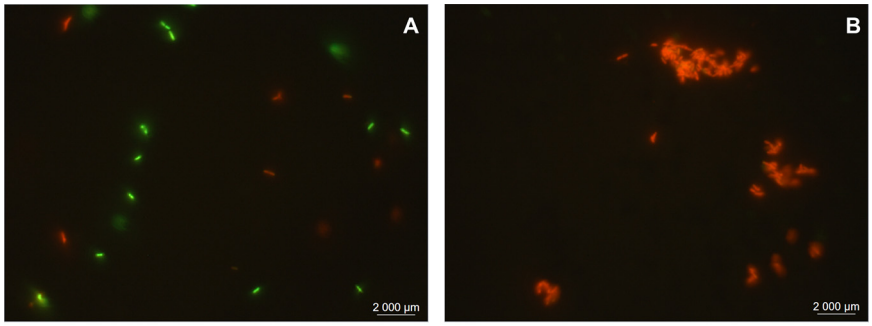

FIG 3 Microscopic observation of cells costained with SYTO9 (green) and propidium iodide (red), showing the effect that the presence of the biocide benzalkonium chloride (BZK) has on *B. cepacia* bacterial viability and aggregation into large clumps that settle on the bottom of the incubation flasks when incubated for several months under conditions mimicking pharmaceutical storage in control saline solution (0.9% [wt/vol] NaCl) (A) or saline solution with 0.05% (wt/vol) BZK (B).

CHX is typically used for hand hygiene and as a component of mouthwash solutions (27, 32, 93). It is a biguanide disinfectant that exerts its effects at the cell membrane level, compromising its structure through the breakdown of associated divalent cations and disruption of lipopolysaccharides, which results in the loss of cytoplasmic components and periplasmic enzymes (93, 96). Several Bcc-related nosocomial outbreaks also have been attributed to the use of contaminated CHX solutions, especially in recent years (106–109).

Early studies identified a *B. cepacia* strain that remained viable for 14 years under nutrient limitation in a saline solution supplemented with 0.05% (wt/vol) BZK (110). More recently, the BZK and CHX susceptibility of 36 different Bcc strains that had been incubated in distilled water for 40 days was assessed through MIC assays and compared to the values at initial inoculation (day zero) (94). Although the 40-day incubation period in distilled water, prior to the addition of the biocides, resulted in a higher susceptibility to both BZK and CHX, six *B. cenocepacia* strains still retained a high level of resistance (94). The same study also reported that clinical strains of *B. contaminans*, *B. multivorans*, *B. vietnamiensis*, and *B. ambifaria* were better recovered after 14 days in the presence of BZK rather than during the first 24 h (94). These observations suggest that BZK was inactivated by the bacteria and was even used as carbon and energy sources for bacterial growth and metabolism (94). Long-term incubation (18 months) with BZK (at 53 μg/ml and 500 μg/ml) also induced structural and organizational alterations in *B. cepacia* cell suspensions incubated in aqueous saline solutions (0.9% [wt/vol] NaCl), including the formation of cellular aggregates, which could be visualized both macro- and microscopically, as evidenced in Fig. 3 (our unpublished results). When the antimicrobial effects of sublethal concentrations of BZK (10 to 50 μg/ml) and CHX (2 to 10 μg/ml) were compared between six *B. cenocepacia* strains, all of them remained viable, exhibiting low susceptibility toward the antiseptics upon incubation at 23°C for 28 days (93). In another study, short-term exposure of a *B. lata* strain to 50 μg/ml of both CHX and BZK resulted in reduced susceptibilities to ceftazidime, ciprofloxacin, imipenem, and, to a lesser extent, meropenem (35). The upregulation of transporter and efflux pump genes, namely, of an outer membrane protein and an ABC transporter, was registered, suggesting that drug efflux plays an important role in reducing the intracellular concentration of these particular antimicrobial peptides (35). Repeated exposure of a *B. cepacia* strain to sublethal concentrations of CHX during 10 passages in the presence of the biocide was reported to induce a 7.3-fold decrease in bacterial susceptibility (111).

An extensive study on the resistance of Bcc bacteria to BZK examined the potential involvement of efflux pumps, the presence of a putative BZK degradation pathway, and the proteome changes induced in response to that biocide. All 20 of the Bcc strains tested were able to partially degrade (4.7 to 42.6% degradation) BZK in a period of

7 days (33). Quantitative proteomic analysis revealed that treatment with BZK resulted in a rapid alteration in the expression patterns of several proteins at a genome-wide level. Proteins belonging to the major facilitator superfamily and to the RND family, presumably involved in multidrug efflux, were upregulated (33). This suggests an intrinsic ability of *B. cenocepacia* to resist BZK's action, which may involve extrusion of the biocide, catalyzed by efflux pumps, and synthesis of metabolic enzymes involved in its degradation. These two mechanisms appear to work synergistically, accelerating the development of resistance (33). The cleavage of the C-N bond in the early steps of the degradation pathway also resulted in reduced BZK toxicity. Finally, the ability of Bcc bacteria to generate acetyl-coenzyme A from BZK, which can be used in central carbon metabolism, allows its utilization as energy and carbon sources, besides being an effective way of degrading BZK without the accumulation of intermediates or toxic metabolites (33).

DETECTION AND IDENTIFICATION OF Bcc BACTERIA IN PHARMACEUTICAL PRODUCTS OR COMPONENTS

Complexity of the Problem

The correct detection of Bcc bacteria is of extreme importance in pharmaceutical industries, allowing the identification of contamination sources and subsequent implementation of corrective measures. The ideal detection method should be rapid and easy to perform due to the clinical relevance of Bcc organisms and the need to ensure the quality and safety of pharmaceutical products. Since Bcc bacteria are adapted to survive and proliferate in water-based environments, as well as under nutrient limitation, they may not grow as well (or at all) if transferred to nutrient-rich culture media (18, 64). In pharmaceutical industries, microorganisms are subjected to multiple stresses, associated not only with the underlying manufacturing processes but also with the lack of nutrients that characterize the raw materials, air, and water, as well as the temperatures applied for manufacturing and storage procedures, which might be below or above room temperature (26). Bcc bacteria have developed several strategies to cope with environmental stresses, among which is the induction of a viable but nonculturable (VBNC) state, a reversible process in which metabolic activity drops to very low levels and no replication occurs (112, 113). Under these conditions, cells might be viable and maintain their integrity and pathogenic potential but cannot be cultured (112, 113). In addition, bacteria adapted to stressful environments, such as nutrient limitation or the presence of antimicrobial agents, often undergo metabolic and morphologic alterations that might interfere with the processes used to eradicate/control their presence. One example is the development of bacterial subpopulations, designated small-colony variants (SCVs), which are characterized for being approximately one-tenth the size of colonies originating from wild-type bacteria and for growing at a rate nine times lower than that of their progenitor cells (114–116). These SCVs have been identified in the respiratory tract of cystic fibrosis patients after lung transplantation, including representative strains of *B. multivorans*, *B. cepacia*, *B. stabilis*, and *B. vietnamiensis* (116). When grown in petri dishes, the reduced size of the colonies may render their visualization with the naked eye difficult, and sometimes even impossible, if a standardized incubation time is used (Fig. 4) (our unpublished results). Moreover, many Gram-negative pathogens alter their characteristic rod-shaped forms to smaller coccoid-like forms after incubation for days or weeks in nutrient-poor environments (117), as observed during long-term colonization of the CF lungs with *B. cenocepacia* (118). Therefore, during membrane filtration processes, those smaller bacterial forms might be able to pass through the 0.2-μm-pore-size filters, which theoretically retain all bacterial species (26). As a result of the referenced limitations, the presence of Bcc bacteria is frequently overlooked during pharmaceutical manufacturing and in the final products, frequently leading to false-negative results (38). Difficulties in detecting the presence of Bcc bacteria within CF patients' respiratory secretions also arise due to their growth rate being lower than that of other coinfecting microorganisms, which might result in the overgrowth of other bacterial populations,

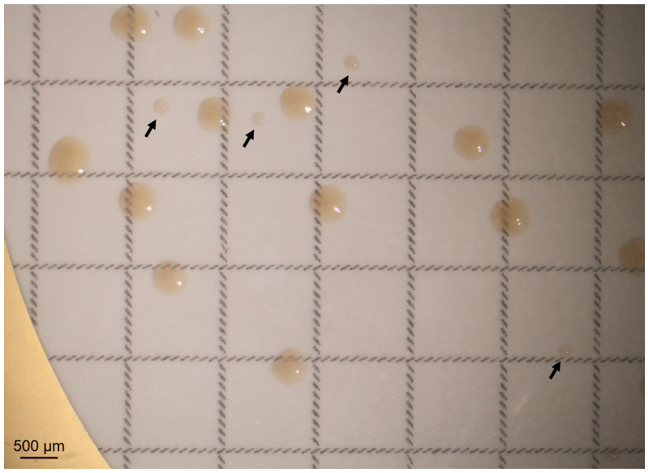

FIG 4 Section of a filter disk corresponding to a *B. cepacia* cell population incubated for several months in a saline solution (0.9% [wt/vol] NaCl) containing benzalkonium chloride (BZK) at 0.05% (wt/vol) under conditions mimicking pharmaceutical storage. The presence of small-colony variants (SCVs), indicated by black arrows, that were absent from the cell population at time zero can be observed on the filter disk following incubation in agar plates (our unpublished results).

masking the presence of Bcc (119). However, the correct detection and identification of Bcc bacteria is of paramount importance for the application of adequate infection control policies as well as to evaluate the most suitable treatment options (120, 121).

Detection and Identification by Phenotypic Methods

Reference laboratories responsible for the quality control of pharmaceutical products typically perform standard phenotypic methods for bacterial isolation and identification (122). In general, these methods rely on enrichment, cultivation, and isolation of microorganisms, requiring long incubation times and extensive manipulation (24). Additionally, traditional cultivation methods and phenotypic assays do not provide enough sensitivity and often underestimate microbial communities, given that some bacteria are not able to grow in certain substrates or do it at such low rates that the incubation periods are not sufficient to allow proper detection (24, 26, 122). Conventional biochemical methods based on catalase, gluconate, malate, phenylacetate, and leucine arylamidase activity are not useful for identification, since Bcc and non-Bcc bacteria cannot be distinguished (123). Many identification errors arise from the phenotypic similarities between *Burkholderia* and other bacterial genera, such as *Cupriavidus*, *Ralstonia*, *Achromobacter*, *Brevundimonas*, *Comamonas*, *Pandoraea*, and *Delfia*, which are frequently misidentified as Bcc species (123, 124). Automated identification systems have also been developed, such as the Vitek MS and the Bruker Biotyper, based on matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (123). This technique consists of the spectral analysis of bacterial ribosomal proteins, which are ionized by laser irradiation of bacterial cells (123). It has a high initial investment cost but provides results in a matter of minutes, as opposed to days, when traditional methods are used (37). Despite the advances registered in this field, further developments still are required for the successful identification of Bcc members, including the development of a database of mass-charge fingerprints, which requires continuous updates to comprise more microorganisms, including Bcc species, which are currently underrepresented (37).

Apart from the correct identification of Bcc bacteria, it is also relevant to know if these contaminants are viable or not. Flow cytometry is a widely used technique that can be useful to characterize bacterial viability by assessing cell membrane integrity (125). Usually, the fluorescent probes SYBR green I (for total cell staining) and propidium iodide (for staining dead cells) are used for nucleic acid costaining. It is a cultivation-independent approach that is particularly useful to detect Bcc bacteria

residing in a viable but nonculturable state (125). Fluorescence species-specific identification could be achieved by labeling with antibodies that bind specifically to Bcc bacterial cell surface molecules. The combination of flow cytometry with fluorescence species-specific detection, although time-consuming, potentially is useful in the pharmaceutical industry (113).

Detection and Identification by Genotypic Methods

Due to the challenges associated with Bcc bacterial identification using traditional phenotypic methods, the application of molecular methods, based on DNA or RNA analysis, is of great importance for quality control in pharmaceutical industries, providing higher resolution, easier analysis, and faster results (123, 126). PCR-based methods are the most widely used for DNA analysis, and their implementation in quality control laboratories has grown over the years. The first reports of the use of PCR-based assays for bacterial detection in artificially contaminated pharmaceutical products date back to 1998, when Jimenez et al. developed the BAX system, which allowed the detection of *Salmonella enterica* serovar Typhimurium within a 30-h period, instead of the 5- to 6-day period associated with traditional cultivation methods (127). Two years later, the same researchers reported a simplified method with PCR beads, which allowed the detection of bacteria in artificially contaminated pharmaceutical products in 27 h (128). The majority of the genotypic methods are based on single genes, such as *recA* (129), *fur* (130), and *hisA* (131), with *recA* being the most widely used. Bcc bacteria exhibit 94 to 95% similarity in terms of *recA* gene sequences among different species and 98 to 99% between sequences of the same species, making it a good candidate for species identification (129). Despite being commonly used for bacterial identification, the 16S rRNA gene sequence has a high similarity level (98 to 100%) among Bcc species, providing only limited resolution for differentiation at the species level (129). A method for the rapid identification of Bcc species based on differences in the *recA* gene sequence, detected by rapid-cycle PCR and through the use of specific fluorescence resonance energy transfer (FRET) probes, was proposed in 2006 that provided results within an hour (132). An expanded multilocus sequence typing (MLST) for the identification of Bcc species, with PCR primers targeting different housekeeping genes (*atpD*, *gltB*, *gyrB*, *lepA*, *phaC*, *recA*, and *trpB*), was proposed later (133). MLST analysis has proven very useful for epidemiological studies, being able to correctly identify Bcc at the genus level and presenting very good results in terms of species identification (134).

To improve the sensitivity of PCR-based methods for Bcc identification, other approaches have been developed, including seminested PCR (SN-PCR) and real-time PCR (RT-PCR). SN-PCR consists of two PCR rounds with two sets of primers, in which the second round uses one of the primers applied in the first round (135). One hundred randomly collected commercial syrup preparations were analyzed by both SN-PCR and conventional methods. The PCR assay detected the presence of *B. cepacia* in two of the samples that had not been detected by standard approaches, highlighting the advantages of using PCR-based methods (136). RT-PCR also has been used for the identification of *B. cepacia* in pharmaceutical products (137). This technical approach allowed bacterial identification in all of the artificially contaminated samples within 30 h (137). The main advantages of RT-PCR include high sensitivity and accuracy, reproducible data, low contamination risk, time efficiency, low labor intensity requirements, and a lack of the need for post-PCR analysis (138).

In recent years, metagenomic approaches, based on next-generation sequencing, have proven very useful for pathogen detection in clinical laboratories by allowing the identification of virtually all pathogens present in a clinical sample without requiring prior cultivation (139). There are at least two strategies for performing metagenomic studies, including deep amplicon sequencing, which involves PCR-based amplification of a highly conserved taxonomic marker, typically the 16S rRNA gene in the case of bacteria, or shotgun metagenomics, where total metagenomic DNA first is extracted and sequenced by a next-generation platform, followed by its assembly and annotation

(139, 140). This approach does not require prior knowledge of microbial identity (139). The main advantages of metagenomics in these settings include quick pathogen identification and the ability to detect less abundant pathogens (139). The generation of complete genome assemblies also allows thorough microorganism characterization, contributing to the development of novel diagnostic methods and the prevention of future contamination outbreaks (139). To our knowledge, there are no current reports on the use of metagenomics for the detection of Bcc bacteria in pharmaceutical or clinical settings, but this approach has successfully detected the presence of bacteria from the *Burkholderia* genus in stream water samples of a polluted urbanized area in São Pedro, Brazil (141).

CONTAMINATION OUTBREAKS IN HEALTH CARE SETTINGS AND CONTROL MEASURES

Throughout the years, a growing body of evidence has confirmed the impact of Bcc bacterial outbreaks, with multiple case studies being reported in order to identify the sources of contamination and their implications in clinical settings. The majority of the cases in which Bcc bacteria have been detected in clinical samples have an environmental origin, typically from contaminated pharmaceutical/personal care products. Contamination of medical products can be classified as extrinsic, when it is introduced during product use, or intrinsic, meaning that the product was already contaminated before use. The easy cross-transmission of Bcc bacteria is particularly worrying, since it facilitates the rapid spreading of infections between different wards within the same facility or even between hospitals situated in different regions of a country (142). The types of products identified as sources of Bcc contamination are diverse, including anesthetic eye drops (143), mouthwash solution (75), fentanyl infusion (144), ultrasound gel (145), antiemetic drugs (15), chlorhexidine solutions (106, 108, 109), liquid soap (76), caffeine citrate (146), distilled water (73), dextrose solutions (147), catheters (16), liquid docusate (148, 149), filters and water oxygen humidifiers (150), washing gloves (151), ventilators (152), and saline flush syringes (153) (Table 1).

Outbreaks and sporadic failures associated with biocides also may be due to user error rather than microbial contamination itself. Common errors include the use of overdiluted solutions and outdated products, the use of tap water to prepare biocide solutions, refilling of small-volume dispensers from large-volume stock containers, and improper product selection (38). Nonsterile products, especially multidose containers, should be handled with extra care, since most of them lose physical integrity after being opened for the first time, becoming more prone to microbial contamination (87). Training of end-users and hospital personnel health care settings should be indispensable (38). Concerning infection control and prevention, there are certain measures that should be followed to minimize the risk of cross-contamination, especially among CF patients. Although associated with anxiety and psychosocial consequences, the segregation of Bcc-positive patients has contributed to a reduction of *B. cepacia* complex-related infections. Additionally, regular microbiological surveillance in clinical settings should include examination for Bcc organisms. Bcc-infected patients should attend different clinics according to the strains they are infected with to avoid superinfections (154). Good hygiene practices are mandatory, including handwashing, disinfection with alcohol rubs, and the use of disposable gloves. Extra care should be taken when handling patient samples, such as sputum specimens and throat swabs. Patients should have well-ventilated, single rooms, and any medical procedure involving respiratory function tests, nebulizations, and airway clearance should be carried out in a separate room (154).

CONCLUSIONS AND PERSPECTIVES

Pharmaceutical products contaminated with Bcc bacteria constitute a serious risk for susceptible patients, particularly those suffering from CF and CGD. Bcc infection also has been reported in immunocompromised individuals (e.g., cancer patients submitted to chemotherapy, HIV/AIDS patients, mechanically ventilated patients, and infants/the

TABLE 1 *Burkholderia cepacia* complex-related outbreaks[a]

Species	Product	Location	Time period	Patient information	No. of patients affected	Reference
Bcc bacteria	Anesthetic eye drops	Madurai, India (Aravind Eye Hospital)	December 2011–February 2012	Patients who underwent cataract surgery and developed acute postoperative endophthalmitis	13	143
B. cepacia	Intrinsically contaminated alcohol-free mouthwash solution	General medical intensive care unit, Vozandes Hospital, Quito, Ecuador	March 2011–May 2012	Ventilated; 3 developed ventilator associated-pneumonia, non-CF patients	13	75
B. contaminans	Continuous fentanyl infusion prepared by institutional compounding pharmacy	Duke University Hospital, Durham, NC	31 August–6 September 2012	1 Child, 6 adults, anemia, cirrhosis, hepatic failure, trauma, HIV, infectious endocarditis, peritonitis	7	144
B. cepacia	Ultrasonic couplant	Hospital in Zhongshan, China	May 2013	Post-C-section patients	4	155
Bcc bacteria	Antiemetic drug granisetron	Mumbai, India (daycare unit of private hospital)	End of 2009	Cancer patients with tunneled catheters	13	15
Bcc bacteria	0.5% Chlorhexidine solution	Seoul, Republic of Korea (Boramae Medical Centre)	10 October–16 December 2013	NR	40	106
B. stabilis, *B. contaminans*, and *B. ambifaria*	Intrinsically contaminated ultrasound gel	Neonatal and adult intensive care units, Argentina	April–July 2013	7 Preterm neonates with respiratory distress, 3 ICU patients who had undergone recent cardiovascular surgery, 1 general ward patient	11	145
B. stabilis	NR[b]	500-Bed tertiary-care hospital in Beijing, China, ophthalmology and otolaryngology	19 March–20 September 2013	Chronic bacterial or fungal sinusitis	53	156
B. cepacia	NR	Hemodialysis center, La Linea de la Concepción, Cadiz, Spain	November 2013–February 2014	NR	7	157
B. cepacia and *B. contaminans*	Nonsterile saline solutions for nasal application	CF care unit of a Lisbon central hospital	2003–2005	CF patients		57, 77
Bcc bacteria	Liquid soap	Near East University Hospital, Nicosia, Cyprus	November 2013	Sickle cell anemia	1	76
B. cepacia	Caffeine citrate	Neonatal intensive care unit, India	September–October 2015	Preterm infants	7	146
B. cepacia	Intravenous solutions of 5% dextrose, normal saline (opened bottles), and continuous-positive-airway-pressure humidifier water	Neonatal intensive care units of 2 hospitals, India	January–March 2014	Neonates, 10 preterm, 11 showed signs of sepsis	12	147
Bcc bacteria	Distilled water	Pediatric intensive care unit, Father Muller Medical College, India	NR	Pediatric patients, 1 with subaortic ventricular septal defect, 1 with rhabdomyosarcoma, on chemotherapy, 1 with pneumonia and pneumothorax	3	73
Bcc bacteria	Central venous catheter, pneumonia	U.S. Veterans Health Administration medical centers	1 January 1999–31 December 2015	Non-CF, advanced age, chronically ill, severe disease	248	16
Bcc bacteria	Liquid docusate	629-Bed, tertiary-care, pediatric hospital in Houston, TX	February 8–July 4, 2016	Pediatric, non-CF	24	148
Bcc bacteria	NR	Urology clinics, Jordan	NR	3 Uncomplicated infections + 1 recurrent infection	4	158
B. cepacia	Flow filter, hemodialysis water, water-oxygen humidifier	Hemodialysis unit, Regional Hospital Coronel Oviedo, Paraguay	November 2013–February 2014	NR	NR	150

(Continued on next page)

TABLE 1 (Continued)

Species	Product	Location	Time period	Patient information	No. of patients affected	Reference
B. cenocepacia	Gel packaged in sachets for use within the sterile ultrasound probe cover	4 Hospitals across Australia	26 March 2017–7 April 2017	Patients with significant preexisting morbidities	11	159
B. stabilis	Commercially available washing gloves	9 Institutions in Switzerland	May 2015–August 2016	Bloodstream and non-bloodstream infections	46	151
B. cepacia	Ventilators	General 1,600-bed hospital, Beijing, China	1–14 June 2015	Ventilator-associated pneumonia	4	152
Bcc bacteria	Rubber stopper of sealed multidose amikacin injection vials	Pediatric ICU and pediatric ward of a tertiary-care hospital, India	June 2012–January 2013	Children with peripheral interavenous catheters	76	160
B. cepacia	Ultrasound probe gel	Referral hospital in Riyadh, Saudi Arabia	8 January–15 June 2016	Non-CF patients	15	161
Bcc bacteria	Oral liquid docusate sodium	Pediatric intensive care unit, The Johns Hopkins Hospital, Baltimore, MD	19 May 2017–30 July 2017	Infants with multiple chronic medical conditions	8	149
Bcc bacteria	Octenidine mouthwash solution	Cardiothoracic intensive care unit in Germany	August–September 2018	Critically ill, postcardiac surgery patients	3	74
Bcc bacteria	Saline flush syringes	59 Nursing facilities in 5 U.S. states	September 2016–January 2017	NR	162	153
B. lata	Chlorhexidine mouthwash	2 Tertiary-care hospitals in Australia	May–June 2016	Intensive care patients	8	107
Bcc bacteria	Prefilled syringe containing liquid docusate	16 Facilities in USA	February 2016	Infants, children, adults and older adults 80 mechanically ventilated and 41 with feeding tubes	108 (63 confirmed and 45 suspect cases)	162
B. cepacia	Intrinsically contaminated commercial 0.5% chlorhexidine solution	600-Bed university-affiliated teaching hospital in South Korea	November 2014–January 2015	Infants, mostly preterm, with respiratory distress syndrome	21	108
B. cepacia	4% Chlorhexidine aqueous body wash	Peritoneal Dialysis Unit, Middlemore Hospital, Auckland, New Zealand	NR	Dialysis patients with peritoneal catheters	9	109
B. cepacia	NR	Tertiary-care hospital, Turkey	2013–2018	NR	46	163
B. cepacia	NR	Neurotrauma critical care unit of a level 1 trauma care center in India	August–November 2014	Mostly patients with head and spinal trauma	48 (15 of which with central line-associated bloodstream infections)	164

[a]*Burkholderia cepacia* complex-related outbreaks that have occurred in different settings and the types of products identified as sources of Bcc contamination, as described in the literature published during the past 5 years. The majority of the contaminations are attributed, in the corresponding publications, to Bcc in general, with no information at the species level, or to the type species *Burkholderia cepacia*. However, since a significant number of reports predate the year in which each Bcc species has been described, the impact of the more recently described species in the contamination outbreaks is currently unknown. The 24 species that currently comprise the *Burkholderia cepacia* complex (and the year of each species description in the literature [41–45, 153, 154, 165–171]) are the following: *B. alpina* (2017), *B. ambifaria* (2001), *B. anthina* (2002), *B. arboris* (2008), *B. catarinensis* (2017), *B. cenocepacia* (2003), *B. cepacia* (1950), *B. contaminans* (2009), *B. diffusa* (2008), *B. dolosa* (2004), *B. lata* (2009), *B. latens* (2008), *B. metallica* (2008), *B. multivorans* (1997), *B. pseudomultivorans* (2013), *B. paludis* (2016), *B. puraquae* (2018), *B. pyrrocinia* (2002), *B. seminalis* (2008), *B. stabilis* (2000), *B. stagnalis* (2015), *B. territorii* (2015), *B. ubonensis* (2000), and *B. vietnamiensis* (1995).
[b]NR, no result.

elderly). Over recent years, both sterile and nonsterile pharmaceutical products have been recalled from the market due to Bcc contamination and subsequent nosocomial outbreaks. The difficulties in terms of detection and correct identification of Bcc bacteria by traditional cultivation techniques or even by genotypic methods, their capacity to grow under conditions of low nutrient availability, and an inherent resistance to chemical preservatives (that can even be used as carbon and energy sources) reinforces their potential to cause disease, hampering the application of adequate infection control policies and therapeutic approaches. Since water is the most common source of this contaminant, aqueous pharmaceutical products represent a particular risk for spreading infection. The negative impact of Bcc bacterial contamination in clinical settings and their prevalence as the number one contaminant in pharmaceutical industries has led authorities, namely, the United States Food and Drug Administration (FDA), to propose the inclusion of these bacteria in the "Objectionable Microorganisms" category. Therefore, it is essential and urgent to prevent Bcc contamination in pharmaceutical manufacturing settings, including the raw materials and finished products, and to develop efficient methods to detect and correctly identify members of this complex when their presence is suspected.

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REFERENCES

1. Depoorter E, Bull MJ, Peeters C, Coenye T, Vandamme P, Mahenthiralingam E. 2016. *Burkholderia*: an update on taxonomy and biotechnological potential as antibiotic producers. Appl Microbiol Biotechnol 100:5215–5229. https://doi.org/10.1007/s00253-016-7520-x.
2. Eberl L, Vandamme P, Eberl L, Vandamme P. 2016. Members of the genus Burkholderia: good and bad guys. F1000Research 5:1007. https://doi.org/10.12688/f1000research.8221.1.
3. Mahenthiralingam E, Urban TA, Goldberg JB. 2005. The multifarious, multireplicon *Burkholderia cepacia* complex. Nat Rev Microbiol 3:144–156. https://doi.org/10.1038/nrmicro1085.
4. Mahenthiralingam E, Baldwin A, Dowson CG. 2008. *Burkholderia cepacia* complex bacteria: opportunistic pathogens with important natural biology. J Appl Microbiol 104:1539–1551. https://doi.org/10.1111/j.1365-2672.2007.03706.x.
5. Compant S, Nowak J, Coenye T, Clément C, Ait Barka E. 2008. Diversity and occurrence of *Burkholderia* spp. in the natural environment. FEMS Microbiol Rev 32:607–626. https://doi.org/10.1111/j.1574-6976.2008.00113.x.
6. Chiarini L, Bevivino A, Dalmastri C, Tabacchioni S, Visca P. 2006. *Burkholderia cepacia* complex species: health hazards and biotechnological potential. Trends Microbiol 14:277–286. https://doi.org/10.1016/j.tim.2006.04.006.
7. Lessie TG, Hendrickson W, Manning BD, Devereux R. 1996. Genomic complexity and plasticity of *Burkholderia cepacia*. FEMS Microbiol Lett 144:117–128. https://doi.org/10.1111/j.1574-6968.1996.tb08517.x.
8. Parke JL, Gurian-Sherman D. 2001. Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. Annu Rev Phytopathol 39:225–258. https://doi.org/10.1146/annurev.phyto.39.1.225.
9. Coutinho CP, Dos Santos SC, Madeira A, Mira NP, Moreira AS, Sá-Correia I. 2011. Long-term colonization of the cystic fibrosis lung by *Burkholderia cepacia* complex bacteria: epidemiology, clonal variation, and genome-wide expression alterations. Front Cell Infect Microbiol 1:12. https://doi.org/10.3389/fcimb.2011.00012.
10. Reik R, Spilker T, LiPuma JJ. 2005. Distribution of *Burkholderia cepacia* complex species among isolates recovered from persons with or without cystic fibrosis. J Clin Microbiol 43:2926–2928. https://doi.org/10.1128/JCM.43.6.2926-2928.2005.
11. Kalish LA, Waltz DA, Dovey M, Potter-Bynoe G, McAdam AJ, LiPuma JJ, Gerard C, Goldmann D. 2006. Impact of *Burkholderia dolosa* on lung function and survival in cystic fibrosis. Am J Respir Crit Care Med 173:421–425. https://doi.org/10.1164/rccm.200503-344OC.
12. Johnston RB, Jr. 2001. Clinical aspects of chronic granulomatous disease. Curr Opin Hematol 8:17–22. https://doi.org/10.1097/00062752-200101000-00004.
13. Greenberg DE, Goldberg JB, Stock F, Murray PR, Holland SM, LiPuma JJ. 2009. Recurrent *Burkholderia* infection in patients with chronic granulomatous disease: 11-year experience at a large referral center. Clin Infect Dis 48:1577–1579. https://doi.org/10.1086/598937.
14. Mann T, Ben-David D, Zlotkin A, Shachar D, Keller N, Toren A, Nagler A, Smollan G, Barzilai A, Rahav G. 2010. An outbreak of *Burkholderia cenocepacia* bacteremia in immunocompromised oncology patients. Infection 38:187–194. https://doi.org/10.1007/s15010-010-0017-0.
15. Singhal T, Shah S, Naik R. 2015. Outbreak of *Burkholderia cepacia* complex bacteremia in a chemotherapy day care unit due to intrinsic contamination of an antiemetic drug. Indian J Med Microbiol 33:117–119. https://doi.org/10.4103/0255-0857.148405.
16. El Chakhtoura NG, Saade E, Wilson BM, Perez F, Papp-Wallace KM, Bonomo RA. 2017. A 17-year nationwide study of *Burkholderia cepacia* complex bloodstream infections among patients in the United States Veterans Health Administration. Clin Infect Dis 65:1253–1259. https://doi.org/10.1093/cid/cix559.
17. Sousa SA, Ramos CG, Leitão JH, Sousa SA, Ramos CG, Leitão JH. 2011. *Burkholderia cepacia* complex: emerging multihost pathogens

equipped with a wide range of virulence factors and determinants. Int J Microbiol 2011:1–9. https://doi.org/10.1155/2011/607575.
18. Ahn Y, Kim JM, Ahn H, Lee YJ, LiPuma JJ, Hussong D, Cerniglia CE. 2014. Evaluation of liquid and solid culture media for the recovery and enrichment of *Burkholderia cenocepacia* from distilled water. J Ind Microbiol Biotechnol 41:1109–1118. https://doi.org/10.1007/s10295-014-1442-3.
19. Vermis K, Brachkova M, Vandamme P, Nelis H. 2003. Isolation of *Burkholderia cepacia* complex genomovars from waters. Syst Appl Microbiol 26:595–600. https://doi.org/10.1078/072320203770865909.
20. Zanetti F, De Luca G, Stampi S. 2000. Recovery of *Burkholderia pseudomallei* and *B. cepacia* from drinking water. Int J Food Microbiol 59:67–72. https://doi.org/10.1016/s0168-1605(00)00255-5.
21. Fang Y, Xie GL, Lou MM, Li B, Muhammad I. 2011. Diversity analysis of *Burkholderia cepacia* complex in the water bodies of West Lake, Hangzhou, China. J Microbiol 49:309–314. https://doi.org/10.1007/s12275-011-0267-2.
22. Sutton S, Jimenez L. 2012. A review of reported recalls involving microbiological control 2004–2011 with emphasis on FDA considerations of "objectionable organisms." Am Pharm Rev 15:42–57.
23. Jimenez L. 2007. Microbial diversity in pharmaceutical product recalls and environments. PDA J Pharm Sci Technol 61:383–399.
24. Jimenez L, Kulko E, Barron E, Flannery T. 2015. *Burkholderia cepacia*: a problem that does not go away! EC Microbiol 2:205–210.
25. Eissa ME. 2016. Distribution of bacterial contamination in non-sterile pharmaceutical materials and assessment of its risk to the health of the final consumers quantitatively. Beni-Suef Univ J Basic Appl Sci 5:217–230. https://doi.org/10.1016/j.bjbas.2016.08.005.
26. Jimenez L. 2004. Microbial contamination control in the pharmaceutical industry, 1st ed. Marcel Dekker, New York, NY.
27. McDonnell G, Russell AD. 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 12:147–179. https://doi.org/10.1128/CMR.12.1.147.
28. Gnanadhas DP, Marathe SA, Chakravortty D. 2013. Biocides: resistance, cross-resistance mechanisms and assessment. Expert Opin Investig Drugs 22:191–206. https://doi.org/10.1517/13543784.2013.748035.
29. Peeters E, Nelis HJ, Coenye T. 2009. *In vitro* activity of ceftazidime, ciprofloxacin, meropenem, minocycline, tobramycin and trimethoprim/sulfamethoxazole against planktonic and sessile *Burkholderia cepacia* complex bacteria. J Antimicrob Chemother 64:801–809. https://doi.org/10.1093/jac/dkp253.
30. Rose H, Baldwin A, Dowson CG, Mahenthiralingam E. 2009. Biocide susceptibility of the *Burkholderia cepacia* complex. J Antimicrob Chemother 63:502–510. https://doi.org/10.1093/jac/dkn540.
31. Rhodes KA, Schweizer HP. 2016. Antibiotic resistance in *Burkholderia* species. Drug Resist Updat 28:82–90. https://doi.org/10.1016/j.drup.2016.07.003.
32. Weber DJ, Rutala WA, Sickbert-Bennett EE. 2007. Outbreaks associated with contaminated antiseptics and disinfectants. Antimicrob Agents Chemother 51:4217–4224. https://doi.org/10.1128/AAC.00138-07.
33. Ahn Y, Kim JM, Kweon O, Kim SJ, Jones RC, Woodling K, da Costa GG, LiPuma JJ, Hussong D, Marasa BS, Cerniglia CE. 2016. Intrinsic resistance of *Burkholderia cepacia* complex to benzalkonium chloride. mBio 7:e01716-16. https://doi.org/10.1128/mBio.01716-16.
34. Kampf G. 2018. Adaptive microbial response to low level benzalkonium chloride exposure. J Hosp Infect 100:e1–e22. https://doi.org/10.1016/j.jhin.2018.05.019.
35. Knapp L, Rushton L, Stapleton H, Sass A, Stewart S, Amezquita A, McClure P, Mahenthiralingam E, Maillard JY. 2013. The effect of cationic microbicide exposure against *Burkholderia cepacia* complex (Bcc); the use of *Burkholderia lata* strain 383 as a model bacterium. J Appl Microbiol 115:1117–1126. https://doi.org/10.1111/jam.12320.
36. Guglierame P, Pasca MR, De Rossi E, Buroni S, Arrigo P, Manina G, Riccardi G. 2006. Efflux pump genes of the resistance-nodulation-division family in *Burkholderia cenocepacia* genome. BMC Microbiol 6:66. https://doi.org/10.1186/1471-2180-6-66.
37. Cundell T. 2019. Excluding Burkholderia cepacia complex from aqueous, non-sterile drug products. Am Pharm Rev https://www.americanpharmaceuticalreview.com/Featured-Articles/358427-Excluding-i-Burkholderia-cepacia-i-complex-from-Aqueous-Non-Sterile-Drug-Products/. Accessed 25 May 2019.
38. Torbeck L, Raccasi D, Guilfoyle DE, Friedman RL, Hussong D. 2011. *Burkholderia cepacia*: this decision is overdue. PDA J Pharm Sci Technol 65:535–543. https://doi.org/10.5731/pdajpst.2011.00793.
39. Parenteral Drug Association, Exclusion of objectionable microorganisms from Nonsterile Pharmaceuticals, Medical Devices, and Cosmetics Technical Report Team. 2014. Technical report no. 67. Exclusion of objectionable microorganisms from nonsterile pharmaceuticals, medical devices, and cosmetics. Parenteral Drug Association, Bethesda, MD. https://store.pda.org/TableOfContents/TR67_TOC.pdf.
40. Sutton S. 2012. What is an "objectionable organism." Am Pharm Rev 15:36–48.
41. De Smet B, Mayo M, Peeters C, Zlosnik JE, Spilker T, Hird TJ, LiPuma JJ, Kidd TJ, Kaestli M, Ginther JL, Wagner DM, Keim P, Bell SC, Jacobs JA, Currie BJ, Vandamme P. 2015. *Burkholderia stagnalis* sp. nov. and *Burkholderia territorii* sp. nov., two novel *Burkholderia cepacia* complex species from environmental and human sources. Int J Syst Evol Microbiol 65:2265–2271. https://doi.org/10.1099/ijs.0.000251.
42. Martina P, Leguizamon M, Prieto CI, Sousa SA, Montanaro P, Draghi WO, Stämmler M, Bettiol M, de Carvalho C, Palau J, Figoli C, Alvarez F, Benetti S, Lejona S, Vescina C, Ferreras J, Lasch P, Lagares A, Zorreguieta A, Leitão JH, Yantorno OM, Bosch A. 2018. *Burkholderia puraquae* sp. nov., a novel species of the *Burkholderia cepacia* complex isolated from hospital settings and agricultural soils. Int J Syst Evol Microbiol 68:14–20. https://doi.org/10.1099/ijsem.0.002293.
43. Ong KS, Aw YK, Lee LH, Yule CM, Cheow YL, Lee SM. 2016. *Burkholderia paludis* sp. nov., an antibiotic-siderophore producing novel *Burkholderia cepacia* complex species, isolated from Malaysian tropical peat swamp soil. Front Microbiol 7:2046. https://doi.org/10.3389/fmicb.2016.02046.
44. Bach E, Sant'Anna FH, Magrich dos Passos JF, Balsanelli E, de Baura VA, Pedrosa FO, de Souza EM, Passaglia L. 2017. Detection of misidentifications of species from the *Burkholderia cepacia* complex and description of a new member, the soil bacterium *Burkholderia catarinensis* sp. Nov Pathog Dis 75:ftx076. https://doi.org/10.1093/femspd/ftx076.
45. Weber CF, King GM. 2017. Volcanic soils as sources of novel CO-oxidizing *Paraburkholderia* and *Burkholderia*: *Paraburkholderia hiiakae* sp. nov., *Paraburkholderia metrosideri* sp. nov., *Paraburkholderia paradisi* sp. nov., *Paraburkholderia peleae*. Front Microbiol 8:207. https://doi.org/10.3389/fmicb.2017.00207.
46. Drevinek P, Holden MT, Ge Z, Jones AM, Ketchell I, Gill RT, Mahenthiralingam E. 2008. Gene expression changes linked to antimicrobial resistance, oxidative stress, iron depletion and retained motility are observed when *Burkholderia cenocepacia* grows in cystic fibrosis sputum. BMC Infect Dis 8:121. https://doi.org/10.1186/1471-2334-8-121.
47. Peeters C, Daenekindt S, Vandamme P. 2016. PCR detection of *Burkholderia multivorans* in water and soil samples. BMC Microbiol 16:184. https://doi.org/10.1186/s12866-016-0801-9.
48. Peeters C, Depoorter E, Praet J, Vandamme P. 2016. Extensive cultivation of soil and water samples yields various pathogens in patients with cystic fibrosis but not *Burkholderia multivorans*. J Cyst Fibros 15:769–775. https://doi.org/10.1016/j.jcf.2016.02.014.
49. LiPuma JJ, Spilker T, Coenye T, Gonzalez CF. 2002. An epidemic *Burkholderia cepacia* complex strain identified in soil. Lancet 359:2002–2003. https://doi.org/10.1016/S0140-6736(02)08836-0.
50. Miller SC, LiPuma JJ, Parke JL. 2002. Culture-based and non-growth-dependent detection of the *Burkholderia cepacia* complex in soil environments. Appl Environ Microbiol 68:3750–3758. https://doi.org/10.1128/aem.68.8.3750-3758.2002.
51. Vanlaere E, Coenye T, Samyn E, Van den Plas C, Govan J, De Baets F, De Boeck K, Knoop C, Vandamme P. 2005. A novel strategy for the isolation and identification of environmental *Burkholderia cepacia* complex bacteria. FEMS Microbiol Lett 249:303–307. https://doi.org/10.1016/j.femsle.2005.06.026.
52. McClean S, Callaghan M. 2009. *Burkholderia cepacia* complex: epithelial cell–pathogen confrontations and potential for therapeutic intervention. J Med Microbiol 58:1–12. https://doi.org/10.1099/jmm.0.47788-0.
53. Coutinho CP, de Carvalho CC, Madeira A, Pinto-de-Oliveira A, Sá-Correia I. 2011. *Burkholderia cenocepacia* phenotypic clonal variation during a 3.5-year colonization in the lungs of a cystic fibrosis patient. Infect Immun 79:2950–2960. https://doi.org/10.1128/IAI.01366-10.
54. Mira NP, Madeira A, Moreira AS, Coutinho CP, Sá-Correia I. 2011. Genomic expression analysis reveals strategies of *Burkholderia cenocepacia* to adapt to cystic fibrosis patients' airways and antimicrobial therapy. PLoS One 6:e28831. https://doi.org/10.1371/journal.pone.0028831.
55. Madeira A, Santos PM, Coutinho CP, Pinto-de-Oliveira A, Sá-Correia I. 2011. Quantitative proteomics (2-D DIGE) reveals molecular strategies

employed by *Burkholderia cenocepacia* to adapt to the airways of cystic fibrosis patients under antimicrobial therapy. Proteomics 11:1313–1328. https://doi.org/10.1002/pmic.201000457.
56. LiPuma JJ. 2010. The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev 23:299–323. https://doi.org/10.1128/CMR.00068-09.
57. Coutinho CP, Barreto C, Pereira L, Lito L, Melo Cristino J, Sá-Correia I. 2015. Incidence of *Burkholderia contaminans* at a cystic fibrosis centre with an unusually high representation of *Burkholderia cepacia* during 15 years of epidemiological surveillance. J Med Microbiol 64:927–935. https://doi.org/10.1099/jmm.0.000094.
58. Boyanton BL, Jr, Noroski LM, Reddy H, Dishop MK, Hicks MJ, Versalovic J, Moylett EH. 2005. *Burkholderia gladioli* osteomyelitis in association with chronic granulomatous disease: case report and review. Pediatr Infect Dis J 24:837–839. https://doi.org/10.1097/01.inf.0000177285.44374.dc.
59. Segonds C, Clavel-Batut P, Thouverez M, Grenet D, Le Coustumier A, Plésiat P, Chabanon G. 2009. Microbiological and epidemiological features of clinical respiratory isolates of *Burkholderia gladioli*. J Clin Microbiol 47:1510–1516. https://doi.org/10.1128/JCM.02489-08.
60. Tong Y, Dou L, Wang C. 2013. Peritonitis due to *Burkholderia gladioli*. Diagn Microbiol Infect Dis 77:174–175. https://doi.org/10.1016/j.diagmicrobio.2013.06.010.
61. Collymore C, Giuliano F, Banks KE. 2019. Head tilt in immunodeficient mice due to contamination of drinking water by *Burkholderia gladioli*. J Am Assoc Lab Anim Sci 58:246–250. https://doi.org/10.30802/AALAS-JAALAS-18-000106.
62. Massip C, Mathieu C, Gaudru C, Miaut V, Floch P, Oswald E, Segonds C, Guet-Revillet H. 2019. *In vitro* activity of seven β-lactams including ceftolozane/tazobactam and ceftazidime/avibactam against *Burkholderia cepacia* complex, *Burkholderia gladioli* and other non-fermentative Gram-negative bacilli isolated from cystic fibrosis patients. J Antimicrob Chemother 74:525–528. https://doi.org/10.1093/jac/dky423.
63. Mazer DM, Young C, Kalikin LM, Spilker T, LiPuma JJ. 2017. *In vitro* activity of ceftolozane-tazobactam and other antimicrobial agents against *Burkholderia cepacia* complex and *Burkholderia gladioli*. Antimicrob Agents Chemother 61:e00766-17. https://doi.org/10.1128/AAC.00766-17.
64. Carson LA, Favero MS, Bond WW, Petersen NJ. 1973. Morphological, biochemical, and growth characteristics of *Pseudomonas cepacia* from distilled water. Appl Microbiol 25:476–483. https://doi.org/10.1128/AEM.25.3.476-483.1973.
65. Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, Rolim DB, Bertherat E, Day NP, Peacock SJ, Hay SI. 2016. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol 1:15008. https://doi.org/10.1038/nmicrobiol.2015.8.
66. Moore RA, Tuanyok A, Woods DE. 2008. Survival of *Burkholderia pseudomallei* in water. BMC Res Notes 1:11. https://doi.org/10.1186/1756-0500-1-11.
67. Pumpuang A, Chantratita N, Wikraiphat C, Saiprom N, Day NP, Peacock SJ, Wuthiekanun V. 2011. Survival of *Burkholderia pseudomallei* in distilled water for 16 years. Trans R Soc Trop Med Hyg 105:598–600. https://doi.org/10.1016/j.trstmh.2011.06.004.
68. Lewenza S, Abboud J, Poon K, Kobryn M, Humplik I, Bell JR, Mardan L, Reckseidler-Zenteno S. 2018. *Pseudomonas aeruginosa* displays a dormancy phenotype during long-term survival in water. PLoS One 13:e0198384. https://doi.org/10.1371/journal.pone.0198384.
69. Ali M. 2016. *Burkholderia cepacia* in pharmaceutical industries. Int J Vaccines Vaccin 3:00064. https://doi.org/10.15406/ijvv.2016.03.00064.
70. Dolan SA, Dowell E, LiPuma JJ, Valdez S, Chan K, James JF. 2011. An outbreak of *Burkholderia cepacia* complex associated with intrinsically contaminated nasal spray. Infect Control Hosp Epidemiol 32:804–810. https://doi.org/10.1086/660876.
71. Cunha MV, Leitao JH, Mahenthiralingam E, Vandamme P, Lito L, Barreto C, Salgado MJ, Sá-Correia I. 2003. Molecular analysis of *Burkholderia cepacia* complex isolates from a Portuguese cystic fibrosis center: a 7-year study. J Clin Microbiol 41:4113–4120. https://doi.org/10.1128/jcm.41.9.4113-4120.2003.
72. Álvarez-Lerma F, Maull E, Terradas R, Segura C, Planells I, Coll P, Knobel H, Vázquez A. 2008. Moisturizing body milk as a reservoir of *Burkholderia cepacia*: outbreak of nosocomial infection in a multidisciplinary intensive care unit. Crit Care 12:R10. https://doi.org/10.1186/cc6778.
73. Antony B, Cherian EV, Boloor R, Shenoy KV. 2016. A sporadic outbreak of *Burkholderia cepacia* complex bacteremia in pediatric intensive care unit of a tertiary care hospital in coastal Karnataka, South India. Indian J Pathol Microbiol 59:197–199. https://doi.org/10.4103/0377-4929.182010.
74. Becker SL, Berger FK, Feldner SK, Karliova I, Haber M, Mellmann A, Schäfers H-J, Gärtner B. 2018. Outbreak of *Burkholderia cepacia* complex infections associated with contaminated octenidine mouthwash solution. Euro Surveill 23:1800540. https://doi.org/10.2807/1560-7917.ES.2018.23.42.1800540.
75. Zurita J, Mejia L, Zapata S, Trueba G, Vargas AC, Aguirre S, Falconi G. 2014. Healthcare-associated respiratory tract infection and colonization in an intensive care unit caused by *Burkholderia cepacia* isolated in mouthwash. Int J Infect Dis 29:96–99. https://doi.org/10.1016/j.ijid.2014.07.016.
76. Süer K, Güvenir M, Otlu B, Tunç E. 2016. Outbreak of *Burkholderia cepacia* complex associated with contaminated liquid soap for hospital use: a case study. African J Microbiol Res 10:791–795. https://doi.org/10.5897/AJMR2015.7661.
77. Cunha MV, Pinto-de-Oliveira A, Meirinhos-Soares L, Salgado MJ, Melo-Cristino J, Correia S, Barreto C, Sá-Correia I. 2007. Exceptionally high representation of *Burkholderia cepacia* among *B. cepacia* complex isolates recovered from the major Portuguese cystic fibrosis center. J Clin Microbiol 45:1628–1633. https://doi.org/10.1128/JCM.00234-07.
78. United States Food and Drug Administration. 2019. Recalls, market withdrawals, & safety alerts. U.S. Food and Drug Administration, Silver Spring, MD. https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts. Accessed 25 May 2019.
79. United States Food and Drug Administration. 2017. FDA updates on multistate outbreak of Burkholderia cepacia infections. U.S. Food and Drug Administration, Silver Spring, MD. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-multistate-outbreak-burkholderia-cepacia-infections. Accessed 25 May 2019.
80. United States Food and Drug Administration. 2017. FDA updates on 2017 *Burkholderia cepacia* contamination. U.S. Food and Drug Administration, Silver Spring, MD. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-2017-burkholderia-cepacia-contamination. Accessed 25 May 2019.
81. United States Food and Drug Administration. 2018. FDA alerts consumers, pet owners not to use products manufactured by King Bio, including Dr. King's label, homeopathic drug and pet products. U.S. Food and Drug Administration, Silver Spring, MD. https://www.fda.gov/news-events/press-announcements/fda-alerts-consumers-pet-owners-not-use-products-manufactured-king-bio-including-dr-kings-label. Accessed 25 May 2019.
82. United States Pharmacopeial Convention. 2008. USP <797> Pharmaceutical compounding–sterile preparations. https://www.sefh.es/fichadjuntos/USP797GC.pdf. Accessed 24 September 2019.
83. United States Pharmacopeial Convention. 2014. USP <795> Pharmaceutical compounding–nonsterile preparations. https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/revisions/gc795.pdf. Accessed 24 September 2019.
84. Ratajczak M, Kubicka MM, Kamińska D, Sawicka P, Długaszewska J. 2015. Microbiological quality of non-sterile pharmaceutical products. Saudi Pharm J 23:303–307. https://doi.org/10.1016/j.jsps.2014.11.015.
85. United States Pharmacopeial Convention. 2008. USP <61> Microbiological examination of non-sterile products: microbial enumeration tests. U.S. Pharmacopeia, Rockville, MD. https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q05b_pf_ira_34_6_2008.pdf. Accessed 6 April 2020.
86. United States Pharmacopeial Convention. 2008. USP <62> Microbiological examination of non-sterile products: tests for specified microorganisms. U.S. Pharmacopeia, Rockville, MD. https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q05a_pf_ira_34_6_2008.pdf. Accessed 6 April 2020.
87. Eissa ME. 2016. *Burkholderia cepacia* growth in preserved pharmaceutical antihistaminic oral suspension. Int J Pharm Pharm Sci 8:464–466.
88. United States Food and Drug Administration. 2017. FDA advises drug manufacturers that Burkholderia cepacia complex poses a contamination risk in non-sterile, water-based drug products. U.S. Food and Drug Administration, Silver Spring, MD. https://www.fda.gov/drugs/drug-safety-and-availability/fda-advises-drug-manufacturers-burkholderia-cepacia-complex-poses-contamination-risk-non-sterile. Accessed 25 May 2019.
89. United States Food and Drug Administration. 2014. Water for pharmaceutical use. U.S. Food and Drug Administration, Silver Spring, MD.

https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/water-pharmacuetical-use. Accessed 24 September 2019.
90. United States Pharmacopeial Convention. 2012. USP <1112> Application of water activity determination to nonsterile pharmaceutical products. U.S. Pharmacopeia, Rockville, MD. http://www.triphasepharmasolutions.com/Private/USP%201112%20APPLICATION%20OF%20WATER%20ACTIVITY%20DETERMINATION%20TO%20NONSTERILE%20PHARMACEUTICAL%20PRODUCTS.pdf. Accessed 24 September 2019.
91. Maillard JY. 2005. Antimicrobial biocides in the healthcare environment: efficacy, usage, policies, and perceived problems. Ther Clin Risk Manag 1:307–320.
92. Peeters E, Nelis HJ, Coenye T. 2008. Evaluation of the efficacy of disinfection procedures against *Burkholderia cenocepacia* biofilms. J Hosp Infect 70:361–368. https://doi.org/10.1016/j.jhin.2008.08.015.
93. Ahn Y, Kim JM, Lee YJ, LiPuma J, Hussong D, Marasa B, Cerniglia C. 2017. Effects of extended storage of chlorhexidine gluconate and benzalkonium chloride solutions on the viability of *Burkholderia cenocepacia*. J Microbiol Biotechnol 27:2211–2220. https://doi.org/10.4014/jmb.1706.06034.
94. Kim JM, Ahn Y, LiPuma JJ, Hussong D, Cerniglia CE. 2015. Survival and susceptibility of *Burkholderia cepacia* complex in chlorhexidine gluconate and benzalkonium chloride. J Ind Microbiol Biotechnol 42:905–913. https://doi.org/10.1007/s10295-015-1605-x.
95. Wiggins P. 2004. Efflux pumps: an answer to Gram-negative bacterial resistance? Expert Opin Investig Drugs 13:899–902. https://doi.org/10.1517/13543784.13.8.899.
96. Coenye T, Van Acker H, Peeters E, Sass A, Buroni S, Riccardi G, Mahenthiralingam E. 2011. Molecular mechanisms of chlorhexidine tolerance in *Burkholderia cenocepacia* biofilms. Antimicrob Agents Chemother 55:1912–1919. https://doi.org/10.1128/AAC.01571-10.
97. Rushton L, Sass A, Baldwin A, Dowson CG, Donoghue D, Mahenthiralingam E. 2013. Key role for efflux in the preservative susceptibility and adaptive resistance of *Burkholderia cepacia* complex bacteria. Antimicrob Agents Chemother 57:2972–2980. https://doi.org/10.1128/AAC.00140-13.
98. Costerton JW, Stewart PS, Greenberg EP. 1999. Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322. https://doi.org/10.1126/science.284.5418.1318.
99. Traverse CC, Mayo-Smith LM, Poltak SR, Cooper VS. 2013. Tangled bank of experimentally evolved *Burkholderia* biofilms reflects selection during chronic infections. Proc Natl Acad Sci U S A 110:E250–E259. https://doi.org/10.1073/pnas.1207025110.
100. Lewis K. 2010. Persister cells. Annu Rev Microbiol 64:357–372. https://doi.org/10.1146/annurev.micro.112408.134306.
101. Santos FG, Pitts B, Stewart PS, Mantovani HC. 2018. Nisin penetrates Staphylococcus aureus biofilms but shows differences in killing effects against sessile and planktonic cells. bioRxiv https://doi.org/10.1101/303636.
102. Peeters E, Sass A, Mahenthiralingam E, Nelis H, Coenye T. 2010. Transcriptional response of *Burkholderia cenocepacia* J2315 sessile cells to treatments with high doses of hydrogen peroxide and sodium hypochlorite. BMC Genomics 11:90. https://doi.org/10.1186/1471-2164-11-90.
103. European Medicines Agency (EMA). 2017. Committee for Human Medicinal Products (CHMP). Benzalkonium chloride used as an excipient. European Medicines Agency, Amsterdam, Netherlands. https://www.ema.europa.eu/en/documents/report/benzalkonium-chloride-used-excipient-report-published-support-questions-answers-benzalkonium_en.pdf. Accessed 25 May 2019.
104. Nagai K, Murata T, Ohta S, Zenda H, Ohnishi M, Hayashi T. 2003. Two different mechanisms are involved in the extremely high-level benzalkonium chloride resistance of a *Pseudomonas fluorescens* strain. Microbiol Immunol 47:709–715. https://doi.org/10.1111/j.1348-0421.2003.tb03440.x.
105. Fazlara A, Ekhtelat M. 2012. The disinfectant effects of benzalkonium chloride on some important foodborne pathogens. Am-Eurasian J Agric Environ Sci 12:23–29.
106. Ko S, An HS, Bang JH, Park SW. 2015. An outbreak of *Burkholderia cepacia* complex pseudobacteremia associated with intrinsically contaminated commercial 0.5% chlorhexidine solution. Am J Infect Control 43:266–268. https://doi.org/10.1016/j.ajic.2014.11.010.
107. Leong LEX, Lagana D, Carter GP, Wang Q, Smith K, Stinear TP, Shaw D, Sintchenko V, Wesselingh SL, Bastian I, Rogers GB. 2018. *Burkholderia lata* infections from intrinsically contaminated chlorhexidine mouthwash, Australia, 2016. Emerg Infect Dis 24:2109–2111. https://doi.org/10.3201/eid2411.171929.
108. Song JE, Kwak YG, Um TH, Cho CR, Kim S, Park IS, Hwang JH, Kim N, Oh GB. 2018. Outbreak of *Burkholderia cepacia* pseudobacteraemia caused by intrinsically contaminated commercial 0.5% chlorhexidine solution in neonatal intensive care units. J Hosp Infect 98:295–299. https://doi.org/10.1016/j.jhin.2017.09.012.
109. Gleeson S, Mulroy E, Bryce E, Fox S, Taylor SL, Talreja H. 2019. *Burkholderia cepacia*: an outbreak in the peritoneal dialysis unit. Perit Dial Int 39:92–95. https://doi.org/10.3747/pdi.2018.00095.
110. Geftic SG, Heymann H, Adair FW. 1979. Fourteen-year survival of *Pseudomonas cepacia* in a salts solution preserved with benzalkonium chloride. Appl Environ Microbiol 37:505–510. https://doi.org/10.1128/AEM.37.3.505-510.1979.
111. Forbes S, Dobson CB, Humphreys GJ, McBain AJ. 2014. Transient and sustained bacterial adaptation following repeated sublethal exposure to microbicides and a novel human antimicrobial peptide. Antimicrob Agents Chemother 58:5809–5817. https://doi.org/10.1128/AAC.03364-14.
112. Gilbert SE, Rose LJ. 2012. Survival and persistence of nonspore-forming biothreat agents in water. Lett Appl Microbiol 55:189–194. https://doi.org/10.1111/j.1472-765X.2012.03277.x.
113. Bergkessel M, Basta DW, Newman DK. 2016. The physiology of growth arrest: uniting molecular and environmental microbiology. Nat Rev Microbiol 14:549–562. https://doi.org/10.1038/nrmicro.2016.107.
114. Johns BE, Purdy KJ, Tucker NP, Maddocks SE. 2015. Phenotypic and genotypic characteristics of small colony variants and their role in chronic infection. Microbiol Insights 8:15–23. https://doi.org/10.4137/MBI.S25800.
115. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, Peters G. 2006. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4:295–305. https://doi.org/10.1038/nrmicro1384.
116. Häussler S, Lehmann C, Breselge C, Rohde M, Classen M, Tümmler B, Vandamme P, Steinmetz I. 2003. Fatal outcome of lung transplantation in cystic fibrosis patients due to small-colony variants of the *Burkholderia cepacia* complex. Eur J Clin Microbiol Infect Dis 22:249–253. https://doi.org/10.1007/s10096-003-0901-y.
117. Yang DC, Blair KM, Salama NR. 2016. Staying in shape: the impact of cell shape on bacterial survival in diverse environments. Microbiol Mol Biol Rev 80:187–203. https://doi.org/10.1128/MMBR.00031-15.
118. Hassan AA, Vitorino MV, Robalo T, Rodrigues MS, Sá-Correia I. 2019. Variation of *Burkholderia cenocepacia* cell wall morphology and mechanical properties during cystic fibrosis lung infection, assessed by atomic force microscopy. Sci Rep 9:112. https://doi.org/10.1038/s41598-019-52604-9.
119. Henry D, Campbell M, McGimpsey C, Clarke A, Louden L, Burns JL, Roe MH, Vandamme P, Speert D. 1999. Comparison of isolation media for recovery of *Burkholderia cepacia* complex from respiratory secretions of patients with cystic fibrosis. J Clin Microbiol 37:1004–1007. https://doi.org/10.1128/JCM.37.4.1004-1007.1999.
120. Miller MB, Gilligan PH. 2003. Laboratory aspects of management of chronic pulmonary infections in patients with cystic fibrosis. J Clin Microbiol 41:4009–4015. https://doi.org/10.1128/jcm.41.9.4009-4015.2003.
121. Kreider M, Kotloff RM. 2009. Selection of candidates for lung transplantation. Proc Am Thorac Soc 6:20–27. https://doi.org/10.1513/pats.200808-097GO.
122. Sandle T. 2017. Microbial identification strategy for pharmaceutical microbiology. Institute of Validation Technology, Burlington, MA. http://www.ivtnetwork.com/article/microbial-identification-strategy-pharmaceutical-microbiology. Accessed 20 May 2019.
123. Devanga Ragupathi NK, Veeraraghavan B. 2019. Accurate identification and epidemiological characterization of *Burkholderia cepacia complex*: an update. Ann Clin Microbiol Antimicrob 18:7. https://doi.org/10.1186/s12941-019-0306-0.
124. Henry DA, Mahenthiralingam E, Vandamme P, Coenye T, Speert DP. 2001. Phenotypic methods for determining genomovar status of the *Burkholderia cepacia* complex. J Clin Microbiol 39:1073–1078. https://doi.org/10.1128/JCM.39.3.1073-1078.2001.
125. Rüger M, Ackermann M, Reichl U. 2014. Species-specific viability analysis of *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Staphylococ-*

cus aureus in mixed culture by flow cytometry. BMC Microbiol 14:56. https://doi.org/10.1186/1471-2180-14-56.
126. Denoya CD. 2009. Nucleic acid amplification-based rapid microbiological methods: are these technologies ready for deployment in the pharmaceutical industry? Am Pharm Rev 12:12.
127. Jimenez L, Smalls S, Scalici C, Bosko Y, Ignar R, English D. 1998. Detection of *Salmonella* spp. contamination in raw materials and cosmetics/pharmaceutical products using the BAX system, a PCR-based assay. J Rapid Methods Autom Microbiol 6:67–76. https://doi.org/10.1111/j.1745-4581.1998.tb00185.x.
128. Jimenez L, Smalls S, Ignar R. 2000. Use of PCR analysis for detecting low levels of bacteria and mold contamination in pharmaceutical samples. J Microbiol Methods 41:259–265. https://doi.org/10.1016/S0167-7012(00)00164-0.
129. Mahenthiralingam E, Bischof J, Byrne SK, Radomski C, Davies JE, Av-Gay Y, Vandamme P. 2000. DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. J Clin Microbiol 38:3165–3173. https://doi.org/10.1128/JCM.38.9.3165-3173.2000.
130. Lynch KH, Dennis JJ. 2008. Development of a species-specific fur gene-based method for identification of the *Burkholderia cepacia* complex. J Clin Microbiol 46:447–455. https://doi.org/10.1128/JCM.01460-07.
131. Papaleo MC, Perrin E, Maida I, Fondi M, Fani R, Vandamme P. 2010. Identification of species of the *Burkholderia cepacia* complex by sequence analysis of the *hisA* gene. J Med Microbiol 59:1163–1170. https://doi.org/10.1099/jmm.0.019844-0.
132. Vonberg RP, Häussler S, Vandamme P, Steinmetz I. 2006. Identification of *Burkholderia cepacia* complex pathogens by rapid-cycle PCR with fluorescent hybridization probes. J Med Microbiol 55:721–727. https://doi.org/10.1099/jmm.0.46457-0.
133. Spilker T, Baldwin A, Bumford A, Dowson CG, Mahenthiralingam E, Lipuma JJ. 2009. Expanded multilocus sequence typing for *Burkholderia* species. J Clin Microbiol 47:2607–2610. https://doi.org/10.1128/JCM.00770-09.
134. Furlan JPR, Pitondo-Silva A, Braz VS, Gallo IFL, Stehling EG. 2019. Evaluation of different molecular and phenotypic methods for identification of environmental *Burkholderia cepacia* complex. World J Microbiol Biotechnol 35:39. https://doi.org/10.1007/s11274-019-2614-0.
135. Moore JE, Xu J, Millar BC, Crowe M, Elborn JS. 2002. Improved molecular detection of *Burkholderia cepacia* genomovar III and *Burkholderia multivorans* directly from sputum of patients with cystic fibrosis. J Microbiol Methods 49:183–191. https://doi.org/10.1016/s0167-7012(01)00367-0.
136. Attia MA, Ali AE, Essam TM, Amin MA. 2016. Direct detection of *Burkholderia cepacia* in susceptible pharmaceutical products using semi-nested PCR. PDA J Pharm Sci Technol 70:99–108. https://doi.org/10.5731/pdajpst.2015.006049.
137. Jimenez L, Jashari T, Vasquez J, Zapata S, Bochis J, Kulko M, Ellman V, Gardner M, Choe T. 2018. Real-time PCR detection of *Burkholderia cepacia* in pharmaceutical products contaminated with low levels of bacterial contamination. PDA J Pharm Sci Technol 72:73–80. https://doi.org/10.5731/pdajpst.2017.007971.
138. Navarro E, Serrano-Heras G, Castaño M, Solera J. 2015. Real-time PCR detection chemistry. Clin Chim Acta 439:231–250. https://doi.org/10.1016/j.cca.2014.10.017.
139. Miller RR, Montoya V, Gardy JL, Patrick DM, Tang P. 2013. Metagenomics for pathogen detection in public health. Genome Med 5:81. https://doi.org/10.1186/gm485.
140. Coughlan LM, Cotter PD, Hill C, Alvarez-Ordóñez A. 2015. Biotechnological applications of functional metagenomics in the food and pharmaceutical industries. Front Microbiol 6:672. https://doi.org/10.3389/fmicb.2015.00672.
141. Medeiros JD, Cantão ME, Cesar DE, Nicolás MF, Diniz CG, Silva VL, Vasconcelos AT, Coelho CM. 2016. Comparative metagenome of a stream impacted by the urbanization phenomenon. Braz J Microbiol 47:835–845. https://doi.org/10.1016/j.bjm.2016.06.011.
142. Abdallah M, Abdallah HA, Memish ZA. 2018. *Burkholderia cepacia* complex outbreaks among non-cystic fibrosis patients in the intensive care units: a review of adult and pediatric literature. Infez Med 26:299–307.
143. Lalitha P, Das M, Purva PS, Karpagam R, Geetha M, Lakshmi Priya J, Naresh Babu K. 2014. Postoperative endophthalmitis due to *Burkholderia cepacia* complex from contaminated anaesthetic eye drops. Br J Ophthalmol 98:1498–1502. https://doi.org/10.1136/bjophthalmol-2013-304129.
144. Moehring RW, Lewis SS, Isaacs PJ, Schell WA, Thomann WR, Althaus MM, Hazen KC, Dicks KV, LiPuma JJ, Chen LF, Sexton DJ. 2014. Outbreak of bacteremia due to *Burkholderia contaminans* linked to intravenous fentanyl from an institutional compounding pharmacy. JAMA Intern Med 174:606–612. https://doi.org/10.1001/jamainternmed.2013.13768.
145. Nannini EC, Ponessa A, Muratori R, Marchiaro P, Ballerini V, Flynn L, Limansky AS. 2015. Polyclonal outbreak of bacteremia caused by *Burkholderia cepacia* complex and the presumptive role of ultrasound gel. Braz J Infect Dis 19:543–545. https://doi.org/10.1016/j.bjid.2015.06.009.
146. Shrivastava B, Sriram A, Shetty S, Doshi R, Varior R. 2016. An unusual source of *Burkholderia cepacia* outbreak in a neonatal intensive care unit. J Hosp Infect 94:358–360. https://doi.org/10.1016/j.jhin.2016.07.008.
147. Paul LM, Hegde A, Pai T, Shetty S, Baliga S, Shenoy S. 2016. An outbreak of *Burkholderia cepacia* bacteremia in a neonatal intensive care unit. Indian J Pediatr 83:285–288. https://doi.org/10.1007/s12098-015-1855-7.
148. Marquez L, Jones KN, Whaley EM, Koy TH, Revell PA, Taylor RS, Bernhardt MB, Wagner JL, Dunn JJ, LiPuma JJ, Campbell JR. 2017. An outbreak of *Burkholderia cepacia* complex infections associated with contaminated liquid docusate. Infect Control Hosp Epidemiol 38:567–573. https://doi.org/10.1017/ice.2017.11.
149. Akinboyo IC, Sick-Samuels AC, Singeltary E, Fackler J, Ascenzi J, Carroll KC, Maldonado Y, Brooks RB, Benowitz I, Wilson LE, LiPuma JJ, Milstone AM. 2018. Multistate outbreak of an emerging *Burkholderia cepacia* complex strain associated with contaminated oral liquid docusate sodium. Infect Control Hosp Epidemiol 39:237–239. https://doi.org/10.1017/ice.2017.265.
150. Rolón Ortiz A, Rios-González CM. 2017. Outbreak of *Burkholderia cepacia* in a hemodialysis unit of Paraguay, 2014. J Infect Public Health 10:688–689. https://doi.org/10.1016/j.jiph.2016.08.011.
151. Sommerstein R, Führer U, Lo Priore E, Casanova C, Meinel DM, Seth-Smith HM, Kronenberg A , Koch D, Senn L, Widmer AF, Egli A, Marschall J. 2017. Burkholderia stabilis outbreak associated with contaminated commercially-available washing gloves, Switzerland, May 2015 to August 2016. Euro Surveill 22:17-00213. https://doi.org/10.2807/1560-7917.ES.2017.22.49.17-00213.
152. Guo L, Li G, Wang J, Zhao X, Wang S, Zhai L, Jia H, Cao B. 2017. Suspicious outbreak of ventilator-associated pneumonia caused by *Burkholderia cepacia* in a surgical intensive care unit. Am J Infect Control 45:660–666. https://doi.org/10.1016/j.ajic.2017.01.024.
153. Brooks RB, *Burkholderia cepacia* Workgroup, Mitchell PK, Miller JR, Vasquez AM, Havlicek J, Lee H, Quinn M, Adams E, Baker D, Greeley R, Ross K, Daskalaki I, Walrath J, Moulton-Meissner H, Crist MB. 2019. Multistate outbreak of *Burkholderia cepacia* complex bloodstream infections after exposure to contaminated saline flush syringes: United States, 2016–2017. Clin Infect Dis 69:445–449. https://doi.org/10.1093/cid/ciy910.
154. The UK Cystic Fibrosis Trust Infection Control Group. 2004. The *Burkholderia cepacia* complex. Suggestions for prevention and infection control. Cystic Fibrosis Trust, London, United Kingdom. https://www.cysticfibrosis.org.uk/the-work-we-do/resources-for-cf-professionals/consensus-documents. Accessed 28 December 2018.
155. Wang M, Zhang L, Xia S, Wu H, Zhang R, Fan M, Wang T. 2014. An investigation on surgical-site infection among post cesarean section patients with *Burkholderia cepacia* contaminated ultrasonic couplant. Zhonghua Liu Xing Bing Xue Za Zhi 35:566–568.
156. Wang L, Wang M, Zhang J, Wu W, Lu Y, Fan Y. 2015. An outbreak of *Burkholderia stabilis* colonization in a nasal ward. Int J Infect Dis 33:71–74. https://doi.org/10.1016/j.ijid.2014.12.046.
157. Montano-Remacha C, Márquez-Cruz MD, Hidalgo-Guzmán P, Sánchez-Porto A, Téllez-Pérez PF. 2015. An outbreak of *Burkholderia cepacia* bacteremia in a hemodialysis unit, Cadiz, 2014. Enferm Infecc Microbiol Clin 33:646–650. https://doi.org/10.1016/j.eimc.2015.02.013.
158. Nimri L, Sulaiman M, Hani OB. 2017. Community-acquired urinary tract infections caused by *Burkholderia cepacia* complex in patients with no underlying risk factor. JMM Case Rep 4:e005081. https://doi.org/10.1099/jmmcr.0.005081.
159. Shaban RZ, Maloney S, Gerrard J, Collignon P, Macbeth D, Cruickshank M, Hume A, Jennison AV, Graham RMA, Bergh H, Wilson HL, Derrington P. 2017. Outbreak of health care-associated *Burkholderia cenocepacia* bacteremia and infection attributed to contaminated sterile gel used

for central line insertion under ultrasound guidance and other procedures. Am J Infect Control 45:954–958. https://doi.org/10.1016/j.ajic.2017.06.025.
160. Mali S, Dash L, Gautam V, Shastri J, Kumar S. 2017. An outbreak of *Burkholderia cepacia* complex in the paediatric unit of a tertiary care hospital. Indian J Med Microbiol 35:216–220. https://doi.org/10.4103/ijmm.IJMM_16_258.
161. Abdelfattah R, Al-Jumaah S, Al-Qahtani A, Al-Thawadi S, Barron I, Al-Mofada S. 2018. Outbreak of *Burkholderia cepacia* bacteraemia in a tertiary care centre due to contaminated ultrasound probe gel. J Hosp Infect 98:289–294. https://doi.org/10.1016/j.jhin.2017.09.010.
162. Glowicz J, *B. cepacia* Investigation Workgroup, Crist M, Gould C, Moulton-Meissner H, Noble-Wang J, de Man TJB, Perry KA, Miller Z, Yang WC, Langille S, Ross J, Garcia B, Kim J, Epson E, Black S, Pacilli M, LiPuma JJ, Fagan R. 2018. A multistate investigation of health care-associated *Burkholderia cepacia* complex infections related to liquid docusate sodium contamination, January-October 2016. Am J Infect Control 46:649–655. https://doi.org/10.1016/j.ajic.2017.11.018.
163. Nazik S, Topal B, Şahin AR, Ateş S. 2018. Nosocomial *Burkholderia cepacia* infection in a tertiary hospital; five-year surveillance: a retrospective cross-sectional study. Urology 6:10–13.
164. Rastogi N, Khurana S, Veeraraghavan B, Yesurajan Inbanathan F, Rajamani Sekar SK, Gupta D, Goyal K, Bindra A, Sokhal N, Panda A, Malhotra R, Mathur P. 2019. Epidemiological investigation and successful management of a *Burkholderia cepacia* outbreak in a neurotrauma intensive care unit. Int J Infect Dis 79:4–11. https://doi.org/10.1016/j.ijid.2018.10.008.
165. Vanlaere E, LiPuma JJ, Baldwin A, Henry D, De Brandt E, Mahenthiralingam E, Speert D, Dowson C, Vandamme P. 2008. *Burkholderia latens* sp. nov., *Burkholderia diffusa* sp. nov., *Burkholderia arboris* sp. nov., *Burkholderia seminalis* sp. nov. and *Burkholderia metallica* sp. nov., novel species within the *Burkholderia cepacia* complex. Int J Syst Evol Microbiol 58:1580–1590. https://doi.org/10.1099/ijs.0.65634-0.
166. Vandamme P, Holmes B, Coenye T, Goris J, Mahenthiralingam E, LiPuma JJ, Govan JR. 2003. *Burkholderia cenocepacia* sp. nov.–a new twist to an old story. Res Microbiol 154:91–96. https://doi.org/10.1016/S0923-2508(03)00026-3.
167. Vermis K, Coenye T, LiPuma JJ, Mahenthiralingam E, Nelis HJ, Vandamme P. 2004. Proposal to accommodate *Burkholderia cepacia* genomovar VI as *Burkholderia dolosa* sp. nov. Int J Syst Evol Microbiol 54:689–691. https://doi.org/10.1099/ijs.0.02888-0.
168. Yabuuchi E, Kawamura Y, Ezaki T, Ikedo M, Dejsirilert S, Fujiwara N, Naka T, Kobayashi K. 2000. *Burkholderia uboniae* sp. nov., L-arabinose-assimilating but different from *Burkholderia thailandensis* and *Burkholderia vietnamiensis*. Microbiol Immunol 44:307–317. https://doi.org/10.1111/j.1348-0421.2000.tb02500.x.
169. Gillis M, Van Van T, Bardin R, Goor M, Hebbar P, Willems A, Segers P, Kersters K, Heulin T, Fernandez MP. 1995. Polyphasic taxonomy in the genus *Burkholderia* leading to an emended description of the genus and proposition of *Burkholderia vietnamiensis* sp. nov. for N2-fixing isolates from rice in Vietnam. Int J Syst Evol Microbiol 45:274–289. https://doi.org/10.1099/00207713-45-2-274.
170. Peeters C, Zlosnik JEA, Spilker T, Hird TJ, LiPuma JJ, Vandamme P. 2013. *Burkholderia pseudomultivorans* sp. nov., a novel *Burkholderia cepacia* complex species from human respiratory samples and the rhizosphere. Syst Appl Microbiol 36:483–489. https://doi.org/10.1016/j.syapm.2013.06.003.
171. Coenye T, Mahenthiralingam E, Henry D, LiPuma JJ, Laevens S, Gillis M, Speert DP, Vandamme P. 2001. *Burkholderia ambifaria* sp. nov., a novel member of the *Burkholderia cepacia* complex including biocontrol and cystic fibrosis-related isolates. Int J Syst Evol Microbiol 51:1481–1490. https://doi.org/10.1099/00207713-51-4-1481.

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