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Original Article

Burkholderia cepacia ST 767 causing a three-year nosocomial outbreak in a hemodialysis unit

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ABSTRACT

Background: The risk of bloodstream infections increases in patients undergoing chronic hemodialysis, primarily due to water contamination or improper handling during dialyzer reprocessing. This study aimed to identify virulence factors and gather epidemiological data on the Burkholderia cepacia complex (Bcc), isolated from water and blood cultures during an outbreak in a hemodialysis unit.
Methods: We analyzed 33 Bcc isolates from blood cultures of patients with bacteremia undergoing hemodialysis and 24 isolates from water samples collected between 2019 and 2022. All isolates were tested for virulence factor-encoding genes, including cblA, esmR, zmpA, and zmpB, using polymerase chain reaction (PCR) assays. The isolates were further characterized using Multi-Locus Sequence Typing (MLST) and Pulsed-Field Gel Electrophoresis (PFGE). Biofilm production was assessed on polystyrene microplates at 20°C and 35°C.
Results: The genes zmpB and zmpA were present in 100 % and 96.5 % of the isolates, respectively. However, none of the isolates carried the cblA or esmR genes. Within the Bcc, the species B. cepacia (genomovar I) was identified through recA gene sequencing. PFGE analysis revealed that all human- and water-derived isolates shared the same pulsotype, classified as ST-767.
Conclusions: Despite routine water disinfection with peracetic acid, the detection of a single pulsotype of this species over three years indicates the persistent of Burkholderia cepacia ST 767 in the plumbing system, leading to the contamination of patients undergoing hemodialysis. This persistence is likely facilitated by biofilm formation, as all water-derived isolates demonstrated biofilm production at 20°C. To exacerbate the situation, all isolates exhibited multidrug resistance, further complicating patient treatment and management.

Introduction

Burkholderia cepacia is classified within the *Burkholderia cepacia* Complex (Bcc) due to their genotypic and phenotypic similarities to another 25 Gram-negative, non-fermenting bacilli *Burkholderia* species, also named as genomovars [1].

These complexes of bacteria exhibit extensive metabolic versatility, enabling adaptation to various ecological niches, survival under nutrient-limited conditions, and metabolism of organic matter present in oligotrophic aquatic environments [2].

Resistance to antimicrobials, disinfectants, and antiseptic solutions are the major concerns about Bcc [3–5], often being associated with hospital-acquired infections. There is evidence that Bcc species also produce biofilms, mainly composed of exopolysaccharides (EPS), external DNA (eDNA), and proteins, protecting bacterial cells against antibiotics, disinfectants, and host defense mediators [6]. These polysaccharides also interact with antimicrobial peptides and interfere with neutrophil chemotaxis, compromising this important

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host defense mechanism [7]. Moreover, most species exhibit innate resistance to various antimicrobials, such as aminoglycosides, cephalosporins, polymyxins, and beta-lactams [8].

Evidence suggests that Bcc is frequently detected in hemodialysis units, including in water supplies, filter membranes, and antiseptic solutions, often leading to nosocomial outbreaks [9,10]. A significant concern is the extensive array of virulence factors contributing to its persistence in the environment, invasion, and intracellular survival in human respiratory epithelial cells and macrophages [11]. Among them, in addition to biofilm production, Bcc species utilize protein and enzyme secretion system (e.g., T3SS and T6SS), adhesin attachment mechanisms, efflux pumps, iron acquisition systems, and motility. For cellular adhesion and invasion, Bcc species require cable pili and the 22 kDa adhesin. In association with this adhesin, the former binds to the intermediate filament protein cytokeratin 13 (CK13), acting as a receptor on the host cell [12]. The *cblA* gene encodes the principal pilin subunit of the giant cable pili, a distinctive surface structure expressed by an epidemic strain of *Burkholderia cepacia* with high transmissibility among individuals with cystic fibrosis. These unusually elongated and intertwined pili exhibit high-affinity binding to mucin, a property that may underlie the enhanced capacity of these isolates for host colonization and inter-patient transmission [13]. Besides, the *cblA* gene is a marker associated with transmissible of Bcc isolates [14]. ZmpA and ZmpB are zinc metalloproteases, both with a broad-spectrum of action causing evasion of host defense mechanisms (cleaves antimicrobial peptides), and degrading substrates involved in tissue integrity and host defense, such as type IV collagen, fibronectin [15,16]. Still, both cleave elafin and secretory leukocyte inhibitor, and neutrophil elastase inhibitors [17]. Specifically, ZmpA cleaves cathelicidin LL-37, interferon gamma, neutrophil a-1 proteinase inhibitor, a2-macroglobulin, gamma interferon, type IV collagen, and fibronectin, causing tissue damage as well as it modulates the host immune system [18] while ZmpB degrades other substrates such as immunoglobulins (IgA, IgG, and IgM), lactoferrin, transferrin, and b-defensin-1 [17]. The specificity of each enzyme differs markedly: ZmpA typically cleaves its substrates into two to three peptides, while ZmpB cleaves substrates into a greater number of smaller peptides [16,17]. The epidemic strain marker of the epidemic strain known as *Burkholderia cepacia* (BCESM) is identified by the *esmR* gene, that is considered a virulence factor marker and is part of a genomic island (cci), that encodes genes related to virulence and metabolism, but there was no evidence that *esmR* gene itself contributes to pathogenicity [12,19].

Epidemic strain marker of the epidemic strain known as Burkholderia cepacia (BCESM) is identified by the esmR gene …."

Their multidrug resistance profile correlates with high mortality rates, considering that the affected patients already have compromised immunity, making Bcc a significant agent in nosocomial infections [20]. The study aimed to describe the molecular and epidemiological characterization of Bcc isolates obtained from water and blood cultures of patients with bacteremia undergoing hemodialysis treatment between 2019 and 2021. The specific objectives were to evaluate the ability of Bcc isolates to form biofilms at different temperatures (20°C and 35°C), and to detect the presence of markers and virulence factor-encoding genes using polymerase chain reaction (PCR). The genetic relatedness of the isolates was assessed through Pulsed-Field Gel Electrophoresis (PFGE)."

Materials and methods

Burkholderia cepacia complex isolates and other groups of bacteria

Bcc isolates originating from water at a Public University Hospital Hemodialysis Unit, were identified through a surveillance program that monitored the microbiological quality of water monthly. The outbreak was detected in November 2019, marked by a sudden increase in Bcc isolations in water samples, despite prior occurrences of Bcc presence. Consequently, results from water and blood cultures from preceding months were reviewed, and Bcc isolates from both sample types were recovered starting from May 2019, with the investigation extending until April 2022.

Water samples were analyzed at the Department of Genetics, Microbiology, and Immunology, São Paulo State University (UNESP), Botucatu, SP, Brazil. Four aliquots of 100 mL each were filtered through 0.22 µm membrane filters (Millipore), which were subsequently placed onto the surfaces of MacConkey agar, Mannitol Salt Agar, Cetrimide agar, and Plate Count Agar (PCA). The plates were incubated at 35°C for 48 hours. Heterotrophic bacteria detected on PCA were only considered in the absence of growth on selective media for pathogenic organisms. For pathogen-specific media, the colonies of the Bcc and other morphologically distinct groups were identified according to the methodology described by Procop et al. [21]. Colonies with morphological characteristics consistent with Bcc, isolated from MacConkey and Cetrimide agars, were subjected to biochemical characterization using Triple Sugar Iron (TSI) agar (Difco), followed by identification with the API 20 NE system (bioMérieux).

The Bcc isolates from patients' blood cultures with bacteremia undergoing hemodialysis were kindly provided by the Clinical Laboratory of the Hospital. Blood samples were inoculated into aerobic and anaerobic bottles of the Bact/Alert system (Virtuo) and incubated at 35°C for up to seven days. Aliquots from bottles flagged as positive by the system were subcultured onto 5 % sheep blood agar and MacConkey agar, followed by incubation at 35°C for 48 hours. Then, the colonies were identified using BD Phoenix m50 (Beckton Dickinson, NJ, EUA) (Ethics Committee in Research Involving Human Subjects No. 5411). Additionally, the drug susceptibility test results accompanied these samples. The BD Phoenix M50 system uses a standard panel for Gram-negative bacteria, regardless of species, testing for cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, amikacin, meropenem, colistin, and piperacillin/tazobactam.

From March 2020 to June 2021, the COVID-19 pandemic interrupted the sample collections and analysis, leading to a data gap, but the isolates from both sources were stored at −70°C (Thermo Scientific, Forma 900 Series). The Bcc isolates were frozen in brain heart infusion broth (BHI, Difco) supplemented with 15 % glycerol.

The surveillance program performed PFGE on some isolates before the COVID-19 pandemic. However, four human isolates could not be recovered from freezing at −70°C. Consequently, only the PFGE test includes 35 blood culture isolates. All subsequent tests conducted after the pandemic, including biofilm production, antibiogram, and PCR were performed on 33 isolates. For the same reason, although there was a total of 40 water isolates, only 25 were subjected to PFGE and 24 to the other tests. MLST was performed for only one isolate

Molecular characterization of Bcc isolates

For DNA extraction, a single colony of each isolate was transferred to 200 µL of sterile Milli-Q water from an overnight MacConkey agar, boiled for 10 minutes, and then centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant was frozen for future PCR reactions [22].

We employed the *recA* gene (Table 1) to identify the species within the Bcc, and the resulting amplicons were sequenced by Sanger. The PCR amplicons were purified using SpeedBeads Magnetic Carboxylate-modified Particles (Cytiva), following the methodology described by Oberacker et al. [23]. Subsequent Sanger sequencing was performed using the same primer employed in the amplification reaction, at a final concentration of 5 pmol/µL.

Table 1
Primers and references used in PCR reactions for genes involved in the pathogenicity and identification of bacteria from the *Burkholderia cepacia* complex.

Gene	Primer Sequence (5´´3´)	bp	Cycle	Reference
cblA	CCAAAGGACTAACCCA ACGCGATGTCCATCACA	664	ID:94°C/5 min −30 cycles: 94°C/1 min 55°C/1 min 72°C/1 min FE: 72°C/10 min	[24]
esmR	CCACGGACGTGACTAACA CGTCCATCCGAACACGAT	1400	ID:94°C/5 min −30 cycles: 94°C/1 min 63°C/1 min 72°C/2 min FE: 72°C/10 min	[14]
recA	TGACCGCCGAGAAGAGCAA GACCGAGTCGATGACGAT	597	ID:94°C/5 min −30 cycles: 94°C/30 sec 61°C/45 sec 72°C/1 min FE: 72°C/10 min	[25]
zmpA	CCAACCTGAACTATTCGG TGCGGATCGTGGCGC	228	ID:96°C/5 min −30 cycles: 96°C/30 sec 56°C/30 sec 72°C/1 min FE: 72°C/10 min	[26]
zmpB	GCAGACATCACGCAAAGCATTG GCAGCTCTTGTTGTACGCGTTG	1700	ID:95°C/7 min −30 cycles: 95°C/1 min 56°C/30 sec 72°C/2 min FE: 72°C/20 min	[16]

BP: base pairs; ID: initial denaturation; FE: final extension; Min: minute; Sec: second.

After identification, we investigated the presence of some genes *(cblA, esmR, zmpA*, and *zmpB*). The oligonucleotides are presented in Table I. Positive control (*B. cepacia* ATCC 25416) and negative control (*E. coli* ATCC 25922) were included in all reactions. The PCR products underwent electrophoresis using an Electrophoresis Power Supply Model EPD 600 (Amersham Pharmacia Biotech Inc.) using 400 amp and 80 volts for 30 minutes, in a 1.5 % agarose gel (Sigma-Aldrich, prepared in Tris-borate-EDTA buffer). The resulting bands were stained with SYBR Safe (Invitrogen). Image capture was performed using the SmartView Pro Imager System 1200 (Major Science).

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) assay was conducted according to Gautom [27], using *Spe*I (Thermo Scientific) (50 U for 2 h at 37°C) as the restriction endonuclease to digest *B. cepacia* DNA. The CHEF (Clamped Homogeneous Electric Fields) Mapper (Biorad, Hercules, California) was used, with an initial switch time of 2.2 s, final switch time of 54.2 s, voltage 6 V/cm, and 120° angle for 19 h. The gel was stained with ethidium bromide and photographed by Alphaimager—AlphaEase FC software (Alpha Innotech Corporation). The images were analyzed using Bionumerics software (version 7.6) (Applied Maths, Sint-Martens-Latem, Belgium), and clustering was carried out by the unweighted pair group method with arithmetic mean (UPGMA), using the Dice coefficient, and a similarity index of 80 %. The DNA fragments of ATCC BAA-664 (*Salmonella* ser. Braenderup H9812) were used as molecular weight markers for the *Xba*I digestion standard.

Two *B. cepacia* isolates were included as an outgroup, indicating that the technique is discriminatory. These isolates were obtained from blood cultures of hospitalized patients who were not undergoing hemodialysis treatment.

Multilocus sequence typing

The PCR amplicons of housekeeping genes (*atpD, gltB, gyrB, recA, lepA, phaC, trpB*) were purified using AMPure XP (Beckman Coulter) according to manufacturer recommendations and then sequenced using Sanger. The sequence types were determined in pubMLST platform (https://pubmlst.org/organisms/burkholderia-cepacia-complex).

In vitro biofilm production

Each isolate was inoculated into BHI incubated with agitation at 35°C for 48 hours and diluted to 1.5 × 108 CFU/mL (0.5 McFarland scale). Aliquots of 200 µl were distributed in a 96well-plate in quadruplicate and incubated at 35°C and 20°C for 48 hours without agitation. After incubation, the plate was washed three times with phosphate-buffered saline (PBS, pH 7.4), air-dried at room temperature, and stained with 1 % crystal violet for 15 minutes. After removing the dye, the plate was washed three times with PBS. The biofilm was resuspended in 200 µl of 33 % glacial acetic acid and read using an ELISA reader (Biotek, EPOCH2C 200–999 nm) at 570 nm. Uninoculated BHI was used as a blank to correct the absorbance value. Biofilm production was classified as non-producers or weak, moderate and strong producers [28]. *S. epidermidis* ATCC 14990 was used as a negative control.

Statistical analysis

The results of the biofilm tests were subjected to the Chi-square or Fisher Exact tests (PROC FREQ; SAS Institute, Cary, NC, USA). The analysis was performed at a level of significance of 0.05.

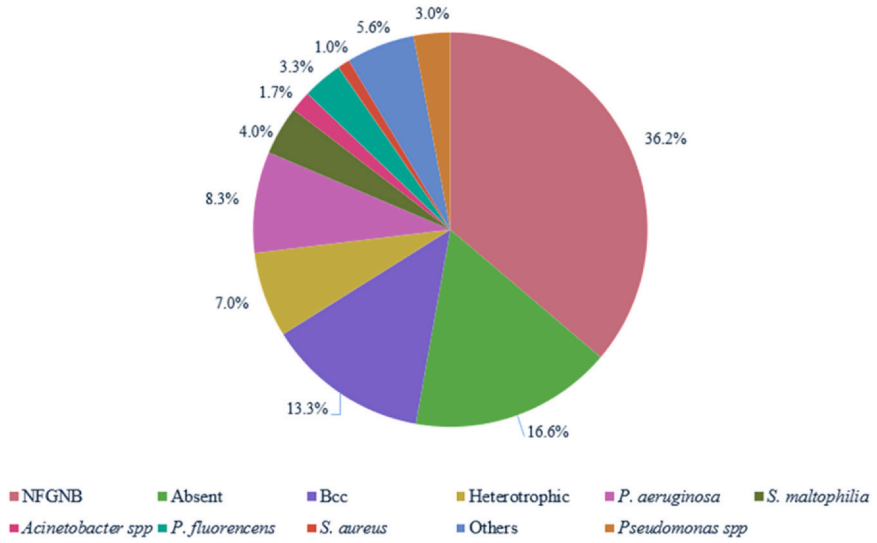


Fig. 1. Bacterial species identified from water, in a Hemodialysis Unit (2019-2022). NFGNB: Non-fermenting Gram-negative bacilli.

Results

Monitoring the microbiological quality of water, the Bcc isolates were the second most frequent microorganism, representing 13.3 % (40) out of the 300 samples collected from 2019 to 2022 (Fig. 1). We considered hard-to-identify (probably environmental) species that could not be identified as Bcc, *Pseudomonas* spp, *Stenotrophomonas maltophilia* or *Acinetobacter* spp as NFGB. Heterotrophs bacteria in PCA were only considered in the absence of growth in culture media specific for pathogens, such as MacConkey agar, Salt Mannitol agar and Cetrimide agar.

Regarding the presence of genes encoding the researched virulence factors, all 57 (100 %) isolates exhibited the *zmpB* gene, while 55 (96.5 %) possessed the *zmpA* gene (absent in one isolate from each source). None of the isolates harbored *cblA* or the virulent strain marker *esmR* gene.

In the biofilm production assay performed on polystyrene microplate, the same inoculum from each isolate was incubated at 35°C, and 20°C, the average annual temperature in Botucatu City/São Paulo State/Brazil [29]. Table 2 shows the results of the assay at these temperatures, revealing that 26 isolates (45.6 %) were classified as non-producers at 35°C. However, this number decreased significantly to three (5.3 %) at 20°C ($p < 0.0001$). The same chi-squared value was observed for the behavior of strong producer isolates at both temperatures. Notably, at 35°C, a significantly higher number of human isolates ($p = 0.001187$) were classified as strong producers compared to isolates obtained from water, at the same temperature.

The 33 isolates recovered from freezing, provided by the Clinical Laboratory of the Hospital, were accompanied by the results of antimicrobial susceptibility testing, since the BD Phoenix M50 system uses a standard panel for Gram-negative bacteria. Supplementary Table 1 presents the resistance profile of these isolates and all of them were resistant to gentamicin, colistin, ampicillin/sulbactam, and imipenem, with 16 (48.5 %) isolates showing resistance also to amikacin. Lower rates of resistance were observed for meropenem, ceftazidime, cefepime, ciprofloxacin, and piperacillin/tazobactam (2/33; 6.1 %). All isolates were sensitive to sulfamethoxazole/trimethoprim, levofloxacin, and tigecycline.

PFGE analysis demonstrated that all isolates obtained from both hemodialysis patients' blood cultures and water samples belonged to the same pulsotype (0001) with similarity > 80 % (Fig. 2). The two isolates from pulsotype 0002 (H65 and H66) are of human origin but from patients with another source of infection who were not undergoing hemodialysis. These isolates are considered "outgroup," indicating that the technique is discriminatory.

Since all isolates were classified as the same pulsotype, only two water isolates and two blood culture isolates were subjected to Sanger sequencing of the *recA* gene for species identification. The analysis revealed identical sequences across all four isolates, which were then compared using Blast/GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This comparison yielded accession number GU951989.1, with a 100 % compatible identity, confirming these isolates as belonging to *B. cepacia* species (genomovar I) within the Bcc complex. Likewise, due to the uniformity in pulsotype, only one isolate was selected for MLST, and it was identified as sequence type ST-767.

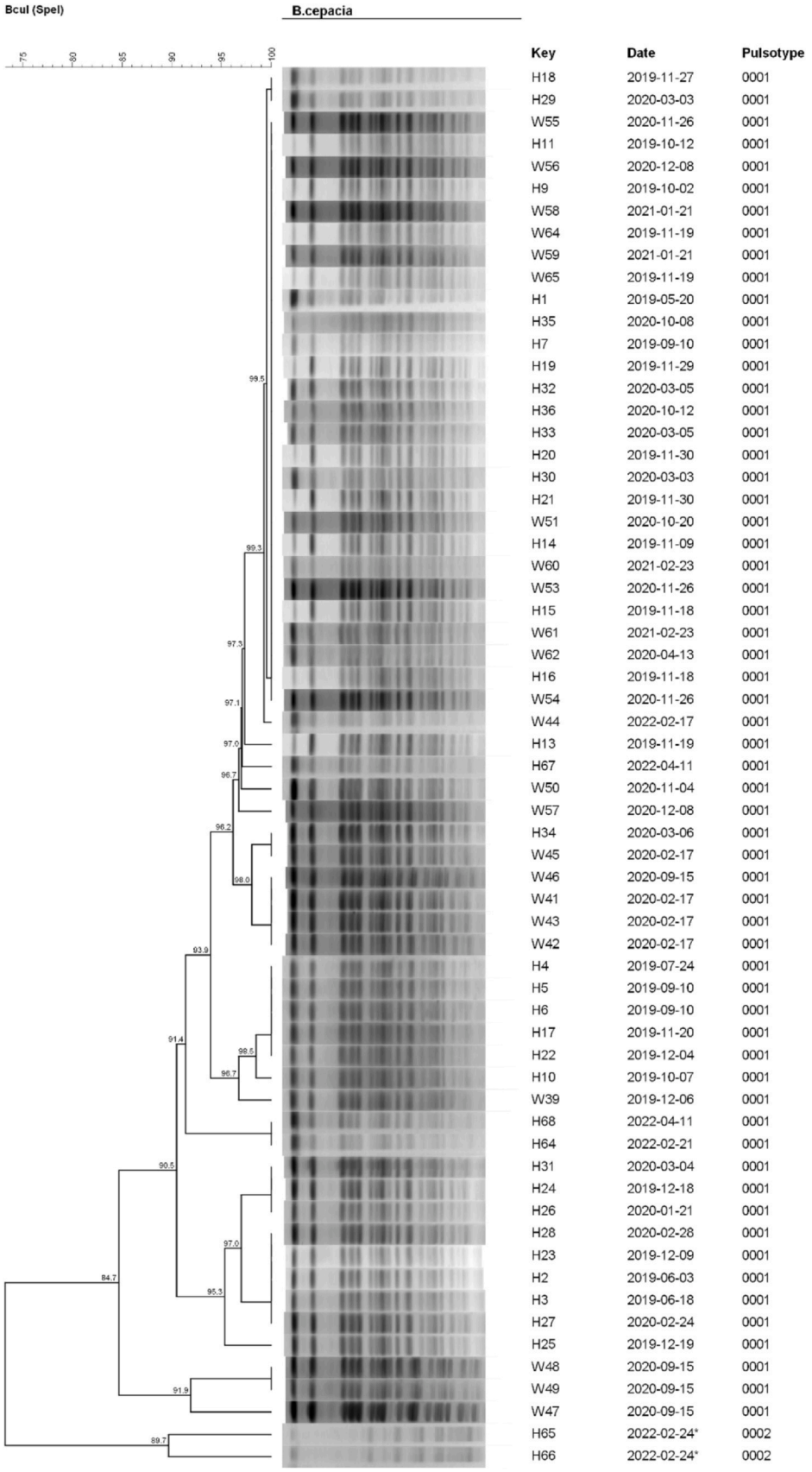
Discussion

Bcc isolates from water were consistently detected throughout the collection period, despite the implementation of reverse osmosis (RO) processing, a purification method that removes ions and

Table 2
Biofilm production capacity, at 20°C and 35°C, by isolates of *B. cepacia*, obtained from water samples from a Hemodialysis Unit and from blood cultures of patients undergoing hemodialysis treatment.

Classification	20°C Blood culture	Water	Overall	35°C Blood culture	Water	Overall
Strong producers	29 (50.9 %)	20 (35.1 %)	49 (86 %)*	12 (21 %)	1 (1.75 %)	13 (22.8 %)
Moderate producers	1 (1.75 %)	3 (5.26 %)	4 (7 %)	6 (10.5 %)	1 (1.75 %)	7 (12.3 %)
Weak producers	0	1 (1,75 %)	1 (1.8 %)	5 (8,8 %)	6 (10,5 %)	11 (19.3 %)
Total of producers			54 (94.7 %)			31 (54.5 %)
Non producers	3	0	3 (5.3 %)*	10 (17.5 %)	16 (28.1 %)	26 (45.6 %)
Total	57			57		

* $p < 0.0001$, Pearson Chi-Square test



Key	Date	Pulsotype
H18	2019-11-27	0001
H29	2020-03-03	0001
W55	2020-11-26	0001
H11	2019-10-12	0001
W56	2020-12-08	0001
H9	2019-10-02	0001
W58	2021-01-21	0001
W64	2019-11-19	0001
W59	2021-01-21	0001
W65	2019-11-19	0001
H1	2019-05-20	0001
H35	2020-10-08	0001
H7	2019-09-10	0001
H19	2019-11-29	0001
H32	2020-03-05	0001
H36	2020-10-12	0001
H33	2020-03-05	0001
H20	2019-11-30	0001
H30	2020-03-03	0001
H21	2019-11-30	0001
W51	2020-10-20	0001
H14	2019-11-09	0001
W60	2021-02-23	0001
W53	2020-11-26	0001
H15	2019-11-18	0001
W61	2021-02-23	0001
W62	2020-04-13	0001
H16	2019-11-18	0001
W54	2020-11-26	0001
W44	2022-02-17	0001
H13	2019-11-19	0001
H67	2022-04-11	0001
W50	2020-11-04	0001
W57	2020-12-08	0001
H34	2020-03-06	0001
W45	2020-02-17	0001
W46	2020-09-15	0001
W41	2020-02-17	0001
W43	2020-02-17	0001
W42	2020-02-17	0001
H4	2019-07-24	0001
H5	2019-09-10	0001
H6	2019-09-10	0001
H17	2019-11-20	0001
H22	2019-12-04	0001
H10	2019-10-07	0001
W39	2019-12-06	0001
H68	2022-04-11	0001
H64	2022-02-21	0001
H31	2020-03-04	0001
H24	2019-12-18	0001
H26	2020-01-21	0001
H28	2020-02-28	0001
H23	2019-12-09	0001
H2	2019-06-03	0001
H3	2019-06-18	0001
H27	2020-02-24	0001
H25	2019-12-19	0001
W48	2020-09-15	0001
W49	2020-09-15	0001
W47	2020-09-15	0001
H65	2022-02-24*	0002
H66	2022-02-24*	0002

Fig. 2. Dendrogram of PFGE patterns, using the Dice coefficient, and a similarity index of 80 % for *B. cepacia* isolates obtained from patients undergoing haemodialysis blood cultures and water samples. * These isolates are considered "outgroup," indicating that the technique is discriminatory.

dissolved chemical compounds from water. However, this process also eliminates chlorine, thereby removing a key antibacterial agent [30]. While the RO membrane system is highly effective at removing bacteria, viruses, and fungi (up to 99 %) [31], it is not without challenges. Biofouling and flow reduction, caused by certain microorganisms capable of forming biofilms on the membranes, are well-documented issues. These issues can result in system malfunctions [32] and facilitate the transfer of microorganisms into the treated water. The situation becomes particularly concerning as previous studies have reported significant microbial growth in RO-treated water, even under oligotrophic conditions, posing a potential health risk to patients undergoing hemodialysis [33].

Bcc isolates can survive and proliferate in nutrient-deprived conditions [2], causing outbreaks such as the one described in this study, as well as others previously reported in Brazil [34,35] and other countries such as Morocco [36], Spain [37], and Paraguay [38]. Although the mechanisms involved in the survival of these bacteria in water have not yet been fully elucidated, they are related to the genetic and nutritional diversity, particularly in their ability to metabolize organic matter in oligotrophic aquatic environments [39].

In addition to nutrient-poor water, the biofilm production was higher at 20°C. The tolerance of Bcc to a large range of temperature had already been reported [2,39], as well as biofilm formation [40]. Carson et al. [41] pointed out the temperature of 25°C as the maximum growth point for *B. cepacia*, when compared to the other temperatures evaluated. In a study on the same genus, Ahn et al. [2] demonstrated that *B. cenocepacia* could persist in distilled water for 40 days at temperatures of 18°C and 23°C. This higher multiplication rate at lower temperatures may explain the higher biofilm production, since its production is controlled by quorum sensing [42]. Biofilm production ensures greater resistance to antimicrobials and disinfectants such as sodium hypochlorite and peracetic acid [43]. In an outbreak in a hemodialysis unit caused by *B. cepacia* and *S. maltophilia* in Salvador, Brazil, these bacteria were not eliminated even after the water system disinfection protocol was intensified and dialyzers were replaced. Eradication occurred only after the hemodialysis unit's plumbing system was replaced, probably due to biofilm formation [35]. Anversa et al. [44] reported the presence of strong biofilm-producing Bcc isolates when they analyzed the stage of post-reverse osmosis, reuse and dialysate in three hemodialysis centres in Brazil.

Sequencing of the *recA* gene for species identification confirmed that PFGE pulsotype 0001 belongs to a clone of *Burkholderia cepacia* (genomovar I), a species frequently associated with infections in hospitalized patients [45]. The isolates were assigned to sequence type ST-767, which has been previously linked to an outbreak caused by contamination of gloves used for bathing patients in an intensive care unit in Belgium [46].

None of the isolates from this clone exhibited BCESM, aligning with findings by Cunha et al. [47], who reported the *esmR* gene exclusively in genomovar III isolates, with all genomovar I isolates testing negative for this gene. The prevalence of BCESM appears to be higher in isolates from cystic fibrosis cases, which are predominantly caused by *B. cenocepacia* (genomovar III) [19,48,49]. This may explain the absence of the gene in the isolates obtained from hemodialysis water and blood cultures in this study.

Similarly to the *esmR* gene, *cblA* was not detected in any of the isolates, consistent with the findings of Martins et al. [49]. This absence aligns with the strong association of *cblA* with *Burkholderia cenocepacia*, particularly the epidemic strain ET12 [50,51], although Sajjan et al. [50] found *cblA* in a small number of isolates of genomovar I.

The *zmpA* (96.5 %) and *zmpB* (100 %) genes showed higher prevalence than the 69 %-88 % range reported by Corbett et al. [52]. This likely reflects the study's focus on *B. cepacia* isolates from genomovar I, as these protease genes are predominantly found in genomovars I and III [53]. Their presence increases the pathogenic potential of the isolates, as mutants lacking *zmpA* and *zmpB* have been linked to less persistent infections and reduced tissue damage [16,52].

A significant concern regarding Bcc pathogens is their intrinsic resistance to many commonly used antibiotics. As known, this group is inherently resistant to several classes of antibiotics, such as aminoglycosides, polymyxins, and beta-lactams [54,55] and we included our results to support the existing literature. Indeed, our findings revealed that all blood culture isolates were resistant to colistin, gentamicin, imipenem, and ampicillin/sulbactam. Consistent with our results, previous studies by El Chakhtoura et al. [54], Saran et al. [55] and Uwumiro et al. [56] have reported Bcc sensitivity to ceftazidime, meropenem, levofloxacin, and sulfamethoxazole/trimethoprim. This pattern was observed in our study as well, except for one isolate resistant to ceftazidime and two isolates resistant to meropenem.

A major limitation of this study is the interruption of sample collection and analysis due to the COVID-19 pandemic. This led to a gap in longitudinal data, which may have compromised the ability to comprehensively track the outbreak dynamics and bacterial spread during that critical period.

Conclusions

The study identified a single *B. cepacia* pulsotype persisting in the hospital water supply over time, continuously affecting hemodialysis patients despite regular disinfection with peracetic acid. This persistence is likely attributed to biofilm production, which protects the bacteria from disinfectants. Compounding the issue, all isolates were multi-drug resistant, limiting treatment options. Additionally, the presence of virulence genes such as *zmpA* and *zmpB* suggests an enhanced capacity to evade host defenses, increasing the risk of bacteremia in immunocompromised patients, and highlight the need for improved infection control and treatment strategies

CRediT authorship contribution statement

Stéfani T. A. Dantas: Formal analysis, Methodology, Writing – original draft; **Gousilin R. Silva**: Investigation, Methodology, Writing; **Iranildo A. Fernandes**: Investigation, Methodology; **Carlos H. Camargo**: Investigation, Methodology; **Ary Fernandes Júnior**: Formal analysis, Resources, Writing – review and editing; **José C. F. Pantoja**: Formal analysis, Software, Writing – review and editing; **Juliano L. Gonçalves**: Conceptualization, Writing – review and editing; **Ivana G. Castilho**: Formal analysis, Project administration; **Nathália C. C. Silva**: Project administration, Writing – review and editing; **Vera L. M. Rall**: Conceptualization; Data curation, Resources, Project administration, Supervision, Writing – original draft, Writing – review and editing

Ethics statement

The study was approved by the Ethics Committee in Research Involving Human Subjects (No. 5411) at the Clinical Hospital/São Paulo State University, Botucatu, SP/Brazil.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2025.102843.

References

[1] Velez LS, Aburjaile FF, Farias ARG, Baia ADB, Oliveira WJ, Silva AMF, Benko-Iseppon AM, Azevedo V, Brenig B, Ham JHG, Souza EB, Gama MAS. *Burkholderia semiarida* sp. nov. and *Burkholderia sola* sp. nov., two novel *B. cepacia* complex species causing onion sour skin. Syst Appl Microbiol 2023;46:e126415. https://doi.org/10.1016/j.syapm.2023.126415

[2] Ahn Y, Kim JM, Ahn H, Lee YJ, LiPuma JJ, Hussong D, Cerniglia CE. Evaluation of liquid and solid culture media for the recovery and enrichment of *Burkholderia cenocepacia* from distilled water. J Ind Microbiol Biotechnol 2014;41:1109–18. https://doi.org/10.1007/s10295-014-1442-3

[3] Lo Cascio G, Bonora MG, Zorzi A, Mortani E, Tessitore N, Loschiavo C, Lupo A, Solbiati M, Fontana R. A napkin-associated outbreak of Burkholderia cenocepacia bacteremia in hemodialysis patients. 56e62 J Hosp Infect 2006;64. https://doi.org/10.1016/j.jhin.2006.04.010

[4] Al Zunitan M, Aldawood F, El-Saed A, Azzam M, Yassine KA, Alshammari L, Alshamrani MM. Two consecutive outbreaks caused by chlorhexidine mouthwash contaminated with Burkholderia contaminans in a two-hospital tertiary care system. 96e104 J Hosp Infect 2023;142. https://doi.org/10.1016/j.jhin.2023.10.005

[5] Abdulqawi R, Saleh RA, Alameer RM, Aldakhil H, AlKattan KM, Almaghrabi RS, Althawadi S, Hashim M, Saleh W, Yamani AH, Al-Mutairy EA. Donor respiratory multidrug-resistant bacteria and lung transplantation outcomes. J Infect 2024;8:139–48. https://doi.org/10.1016/j.jinf.2023.12.013

[6] Rabin N, Zheng Y, Opoku-Temeng C, Du Y, Bonsu E, Sintim HO. Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Med Chem 2015;7:493–512. https://doi.org/10.4155/fmc.15.6

[7] Zlosnik JE, Hird TJ, Fraenkel MC, Moreira LM, Henry DA, Speert DP. Differential mucoid exopolysaccharide production by members of the *Burkholderia cepacia* complex. J Clin Microbiol 2008;46:1470–3. https://doi.org/10.1128/JCM.02273-07

[8] Bazzini S, Udine C, Riccardi G. Molecular approaches to pathogenesis study of *Burkholderia cenocepacia*, an important cystic fibrosis opportunistic bacterium. Appl Microbiol Biotechnol 2011;92:887–95. https://doi.org/10.1007/s00253-011-3616-5

[9] Agrawal V, Valson AT, Mohapatra A, David VG, Alexander S, Jacob S, Bakthavatchalam YD, Prakash JAA, Balaji V, Varughese S. Fast and furious: a retrospective study of catheter-associated bloodstream infections with internal jugular nontunneled hemodialysis catheters at a tropical center. Clin Kidney J 2019;12:737–44. https://doi.org/10.1093/ckj/sfy138

[10] Backman L, Dumigan DG, Oleksiw M, Carusillo E, Patel PR, Nguyen DB, Moulton-Meissner H, Boyce JM. A cluster of gram-negative bloodstream infections in Connecticut hemodialysis patients associated with contaminated wall boxes and prime buckets. A J Infect Control 2023;51:638–43. https://doi.org/10.1016/j.ajic.2022.08.007

[11] Ganesh PS, Vishnupriya S, Vadivelu J, Mariappan V, Vellasamy KM, Shankar EM. Intracellular survival and innate immune evasion of *Burkholderia cepacia*: Improved understanding of quorum sensing-controlled virulence factors, biofilm, and inhibitors. Microbiol Immunol 2020;64:87–98. https://doi.org/10.1111/1348-0421.12762

[12] Mahenthiralingam E, Urban TA, Goldberg JB. The multifarious, multireplicon *Burkholderia cepacia* complex. Nat Rev Microbiol 2005;3:144–56. https://doi.org/10.1038/nrmicro1085

[13] Richardson J, Stead DE, Coutts RHA. Incidence of the *cblA* major subunit pilin gene amongst *Burkholderia* species. FEMS Microbiol Lett 2001;196:61–6. https://doi.org/10.1111/j.1574-6968.2001.tb10541.x

[14] Mahenthiralingam E, Simpson DA, Speert PD. Identification and characterization of a novel DNA marker associated with epidemic *Burkholderia cepacia* strains recovered from patients with cystic fibrosis. J Clin Microbiol 1997;35:808–16. https://doi.org/10.1128/jcm.35.4.808-816.1997

[15] Rosales-Reyes R, Aubert DF, Tolman JS, Amer AO, Valvano MA. *Burkholderia cenocepacia* type VI secretion system mediates escape of type II secreted proteins into the cytoplasm of infected macrophages. PLoS One 2012;7:e41726. https://doi.org/10.1371/journal.pone.0041726

[16] Kooi C, Subsin B, Chen R, Pohorelic B, Sokol PA. *Burkholderia cenocepacia* ZmpB is a broad-specificity zinc metalloprotease involved in virulence. Infect Immun 2006;74:4083–93. https://doi.org/10.1128/IAI.00297-06

[17] Kooi C, Sokol PA. *Burkholderia cenocepacia* zinc metalloproteases influence resistance to antimicrobial peptides. Microbiology 2009;155:2818–25. https://doi.org/10.1099/mic.0.028969-0

[18] Kooi C, Corbett CR, Sokol PA. Functional analysis of the *Burkholderia cenocepacia* ZmpA metalloprotease. J Bacteriol 2005;187:4421–9. https://doi.org/10.1128/JB.187.13.4421-4429.2005

[19] Baldwin A, Sokol PA, Parkhill J, Mahenthiralingam E. The *Burkholderia cepacia* epidemic strain marker is part of a novel genomic island encoding both virulence and metabolism-associated genes in *Burkholderia cenocepacia*. Infect Immun 2004;72:1537–47. https://doi.org/10.1128/IAI.72.3.1537-1547.2004

[20] Lo Priore E, Bernasconi S, Schlotterbeck H, Seth-Smith HM, Egli A, Martinetti G, Balmelli C. *Burkholderia cepacia* complex outbreak originating from contaminated wash gloves. Infect Prev Pract 2020;2:100039. https://doi.org/10.1016/j.infpip.2020.100039

[21] Procop GW, Church DL, Hall GS, Janda WM, Koneman EW, Schreckenberger PC, Woods GL, editors. Koneman's Color Atlas and Textbook of Diagnostic Microbiology 7th. Burlington: Jones & Bartlett Learning; 2018. p. 326–98.

[22] Motta RG, Motta LSAM, Bertolini AB, Prado AM, Thyssen PJ, Hernandes RT, Orsi H, Rall VLM, Gouvea FLR, Guerra ST, Guimarães FP, Joaquim SF, Pantoja JC, Langoni H, Ribeiro MG. Identification of *Escherichia coli* isolated from flies (*Insecta: Diptera*) that inhabit the environment of dairy farms harboring extra-intestinal virulence markers. J Appl Microbiol 2023;34:1–9. https://doi.org/10.1093/jambio/lxad301

[23] Oberacker P, Stepper P, Bond DM, Hohn S, Focken J, Meyer V, Schelle L, Sugrue VJ, Jeunen GJ, Moser T, Hore SR, von Meyenn F, Hipp K, Hore TA, Jurkowski TP. Bio-OnMagnetic-Beads (BOMB): open platform for high-throughput nucleic acid extraction and manipulation. PLoS Biol 2019;17:e3000107. https://doi.org/10.1371/journal.pbio.3000107

[24] Urban TA, Goldberg JB, Forstner JF, Sajjan US. Cable pili and the 22-kilodalton adhesin are required for *Burkholderia cenocepacia* binding to and transmigration across the squamous epithelium. Infect Immun 2005;73:5426–37. https://doi.org/10.1128/IAI.73.9.5426–5437.2005

[25] Mahenthiralingam E, Bischof J, Byrne SK, Radomski C, Davies JE, Av-Gay Y, Vandamme P. 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 2000;38:3165–73. https://doi.org/10.1128/JCM.38.9.3165-3173.2000

[26] Gingues S, Kooi C, Visser MB, Subsin B, Sokol PA. Distribution and expression of the ZmpA metalloprotease in the *Burkholderia cepacia* complex. J Bacteriol 2005;1876:8247–55. https://doi.org/10.1128/JB.187.24.8247–8255.2005

[27] Gautom R. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. J Clin Microbiol 1997;35:2977–80. https://doi.org/10.1128/jcm.35.11.2977-2980.1997

[28] Stepanović S, Vuković D, Hola V, Bonaventura GC, Djukić S, Cirković I, Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Acta Pathol Microbiol Scand Suppl 2007;115:891–999. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x

[29] CLIMATE DATA. ⟨https://pt.climate-data.org/america-do-sul/brasil/sao-paulo/botucatu-4227/⟩. [Accessed 15 February 2023].

[30] Li Y, Yang Z, Yang K, Wei J, Li Z, Ma C, Yang X, Wang T, Zeng G, Yu G, Yu Z, Zhang C. Removal of chloride from water and wastewater: removal mechanisms and recent trends. Sci Total Environ 2022;821:153174. https://doi.org/10.1016/j.scitotenv.2022.153174

[31] Popova A, Boivin S, Shintani T, Fujioka T. Development of high-integrity reverse osmosis membranes for enhanced removal of microorganisms. Desalination 2024;572:117155. https://doi.org/10.1016/j.desal.2023.117155

[32] Khan MM, Sewart PS, Moll DJ, Mickols WE, Nelson SE, Camper AK. Characterization and effect of biofouling on polyamide reverse osmosis and nanofiltration membrane surfaces. Biofouling 2011;27:173–83. https://doi.org/10.1080/08927014.2010.551766

[33] Park SK, Hu JY. Assessment of the extent of bacterial growth in reverse osmosis system for improving drinking water quality. J Environ Sci Health Part A 2010;45:968–77. https://doi.org/10.1080/10934521003772386

[34] Magalhães M, Doherty C, Govan JR, Vandamme P. Polyclonal outbreak of *Burkholderia cepacia* complex bacteremia in hemodialysis patients. J Hosp Infect 2003;54:120–3. https://doi.org/10.1016/S0195-6701(03)00118-X

[35] Rocha VFD, Cavalcanti TP, Azevedo J, Leal HF, Silva GEO, Malheiros ARX, Ataide LA, Lima Filho JA, Almeida ARP, Khouri NA, Reis MG, Reis JN. Outbreak of *Stenotrophomonas maltophilia* and *Burkholderia cepacia* bloodstream infections at a hemodialysis center. Am J Trop Med Hyg 2021;104:848–53. https://doi.org/10.4155/fmc.15.6

[36] Oumokhtar B, Ael OL, Mahmoud M, Berrada S, Arrayhani M, Houssaini TS. Prevent infection linked to the dialysis water in a hemodialysis center in Fez city (Morocco). Pan Afr Med J 2013;16:e122. https://doi.org/0.11604/pamj.2013.16.122.2877.

[37] Montaño-Remacha C, Márquez-Cruz MD, Hidalgo-Guzmán P, Sánchez-Porto A, Téllez-Pérez FP. Brote de bacteremia por *Burkholderia cepacia* en una unidad de hemodiálisis de Cádiz. Enferm Infecc Microbiol ClíN 2015;33:646–50. https://doi.org/10.1016/j.eimc.2015.02.013

[38] Ortiz AR, Rios-González CM. Outbreak of *Burkholderia cepacia* in a hemodialysis unit of Paraguay, 2014. J Infect Publ Health 2017;10:688–9. https://doi.org/10.1016/j.jiph.2016.08.011

[39] Tavares M, Kozak M, Balola A, Sá-Correia I. *Burkholderia cepacia* complex bacteria: a feared contamination risk in water-based pharmaceutical products. e00139-19 Clin Microbiol Rev 2020;33. https://doi.org/10.1128/cmr.00139-19

[40] Nörnberg MF, Mentges ML, Silveira ST, Tondo EC, Brandelli A. A psychrotrophic *Burkholderia cepacia* strain isolated from refrigerated raw milk showing proteolytic activity and adhesion to stainless steel. J Dairy Res 2011;78:257–62. https://doi.org/10.1017/S002202991100015X

[41] Carson LA, Favero MS, Bond WW, Petersen NJ. Morphological, biochemical, and growth characteristics of *Pseudomonas cepacia* from distilled water. Appl Microbiol 1973;25:476–83. https://doi.org/10.1128/am.25.3.476-483.1973
[42] Malott RJ, Baldwin A, Mahenthiralingam E, Sokol PA. Characterization of the cciIR *quorum-sensing* system in *Burkholderia cenocepacia*.. Infect Immun 2005;73:4982–92. https://doi.org/10.1128/IAI.73.8.4982-4992.2005
[43] Tomičić R, Tomičić Z, Nićetin M, Knežević V, Kocić-Tanackov S, Raspor P. Food grade disinfectants based on hydrogen peroxide/peracetic acid and sodium hypochlorite interfere with the adhesion of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Listeria monocytogenes* to stainless steel of differing surface roughness. Biofouling 2023;39:990–1003. https://doi.org/10.1080/08927014.2023.2288886
[44] Anversa L, Romani CD, Caria ES, Saeki EK, Nascentes GAN, Garbelotti M, Stancari RCA, Dantas STA, Rall VLM, Ruiz LS, Camargo CH, Richini VBP. Quality of dialysis water and dialysate in hemodialysis centers: highlight for occurrence of non-fermenting gram negative bacilli. J Appl Microbiol 2022;132:3416–29. https://doi.org/10.1111/jam.15470
[45] Kenna DTD, Lilley D, Coward A, Martin K, Perry C, Pike R, Hill R, Turton JF. Prevalence of *Burkholderia* species, including members of *Burkholderia cepacia* complex, among UK cystic and non-cystic fibrosis patients. J Med Microbiol 2017;66:490–501. https://doi.org/10.1099/jmm.0.000458
[46] Peeters C., Echahidi F., De Bel A., Boudewijns M., Bruyneel H., De Canck E., Wybo I., Piérard D., Vandamme P. Polyclonal Burkholderia cepacia outbreak originating from contaminated wash gloves. ⟨https://www.sciensano.be/sites/default/files/polyclonal_burkholderia_cepacia_outbreak_originating_from_contaminated_wash_gloves_0.pdf⟩. [Accessed 16 January 2023].
[47] Cunha MV, Leitão JH, Mahenthiralingam E, Vandamme P, Lito L, Barreto C, Salgado MJ. Sá-Correia I. Molecular analysis of *Burkholderia cepacia* complex isolates from a Portuguese cystic fibrosis center: a 7-year study. J Clin Microbiol 2003;41:4113–20. https://doi.org/10.1128/JCM.41.9.4113-4120.2003
[48] Bevivino A, Dalmastri C, Tabacchioni S, Chiarini L, Belli ML, Piana S, Materazzo A, Vandamme P, Manno G. *Burkholderia cepacia* complex bacteria from clinical and environmental sources in Italy: genomovar status and distribution of traits related to virulence and transmissibility. J Clin Microbiol 2002;40:846–51. https://doi.org/10.1128/JCM.40.3.846-851.2002
[49] Martins KM, Fongaro GF, Rodrigues ABD, Tateno AF, Azzuz-Chernishev AC, Oliveira-Garcia D, Rodrigues, Silva Filho LVF. Genomovar status, virulence markers and genotyping of *Burkholderia cepacia* complex strains isolated from Brazilian cystic fibrosis patients. J Cyst Fibros 2008;7:336–9. https://doi.org/10.1016/j.jcf.2007.12.001
[50] Sajjan U, Liu L, Lu A, Spilker T, Forstner J, LiPuma JJ. Lack of cable pili expression by cblA-containing *Burkholderia cepacia* complex. Microbiology 2002;148:3477–84. https://doi.org/10.1099/00221287-148-11-3477
[51] Turton JF, Arif N, Hennessy D, Kaufmann ME, Pitt TL. Revised approach for identification of isolates within the *Burkholderia cepacia* complex and description of clinical isolates not assigned to any of the known genomovars. J Clin Microbiol 2007;45:3105–8. https://doi.org/10.1128/JCM.00976-07
[52] Corbett CR, Burtnick MN, Kooi C, Woods DE, Soko PA. An extracellular zinc metalloprotease gene of *Burkholderia cepacia*. Microbiology 2003;149:2263–71. https://doi.org/10.1099/mic.0.26243-0
[53] Gotschlich A, Huber B, Geisenberger O, Tögl A, Steidle A, Riedel K, Hill P, Tümmler B, Vandamme P, Middleton B, Camara M, Williams P, Hardman A, Eberl L. Synthesis of multiple N-acylhomoserine lactones is widespread among the members of the Burkholderia cepacia complex. Syst Appl Microbiol 2001;24:1–14. https://doi.org/10.1078/0723-2020-00013
[54] El Chakhtoura NG, Saade E, Wilson BM, Perez F, Papp-Wallace KM, Bonomo RA. A 17-year nationwide study of *Burkholderia cepacia* complex bloodstream infections among patients in the United States Veterans Health Administration. Clin Infect Dis 2017;65:1253–9. https://doi.org/10.1093/cid/cix559
[55] Saran S, Azim A, Gurjar M. Multidrug-resistant *Burkholderia cepacia* bacteremia in an immunocompetent adult diagnosed with dengue and scrub coinfection: a rare case report. Int J Crit Illn Inj 2018;8:173–5. https://doi.org/10.4103/IJCIIS.IJCIIS_31_18
[56] Uwumiro F, Edigin E, Okpujie V. Atypical *Burkholderia cepacia* resistance to ceftazidime/avibactam and co-trimoxazole. Cureus 2021;13:e15836. https://doi.org/10.7759/cureus.15836