

## Burkholderia Cepacia ST 767 Causing a Three Years Nosocomial Outbreak in a Hemodialysis Unit

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**Abstract :** Kidney failure causes decreased diuresis and accumulation of nitrogenous substances in the body. To increase patient survival, hemodialysis is used as a partial substitute for renal function. However, contamination of the water used in this treatment, causing bacteremia in patients, is a worldwide concern. The *Burkholderia cepacia* complex (Bcc), a group of bacteria with more than 20 species, is frequently isolated from hemodialysis water samples and comprises opportunistic bacteria, affecting immunosuppressed patients, due to its wide variety of virulence factors, in addition to innate resistance to several antimicrobial agents, contributing to the permanence in the hospital environment and to the pathogenesis in the host. The objective of the present work was to characterize molecularly and phenotypically Bcc isolates collected from the water and dialysate of the Hemodialysis Unit and from the blood of patients at a Public Hospital in Botucatu, São Paulo, Brazil, between 2019 and 2021. We used 33 Bcc isolates, previously obtained from blood cultures from patients with bacteremia undergoing hemodialysis treatment (2019-2021) and 24 isolates obtained from water and dialysate samples in a Hemodialysis Unit (same period). The *recA* gene was sequenced to identify the specific species among the Bcc group. All isolates were tested for the presence of some genes that encode virulence factors such as *cblA*, *esmR*, *zmpA* and *zmpB*. Considering the epidemiology of the outbreak, the Bcc isolates were molecularly characterized by Multi Locus Sequence Type (MLST) and by pulsed-field gel electrophoresis (PFGE). The verification and quantification of biofilm in a polystyrene microplate were performed by submitting the isolates to different incubation temperatures (20°C, average water temperature and 35°C, optimal temperature for group growth). The antibiogram was performed with disc diffusion tests on agar, using discs impregnated with cefepime (30µg), ceftazidime (30µg), ciprofloxacin (5µg), gentamicin (10µg), imipenem (10µg), amikacin 30µg), sulfametazol/trimethoprim (23.75/1.25µg) and ampicillin/sulbactam (10/10µg). The presence of *ZmpB* was identified in all isolates, while *ZmpA* was observed in 96.5% of the isolates, while none of them presented the *cblA* and *esmR* genes. The antibiogram of the 33 human isolates indicated that all were resistant to gentamicin, colistin, ampicillin/sulbactam and imipenem. 16 (48.5%) isolates were resistant to amikacin and lower rates of resistance were observed for meropenem, ceftazidime, cefepime, ciprofloxacin and piperacycline/tazobactam (6.1%). All isolates were sensitive to sulfametazol/trimethoprim, levofloxacin and tigecycline. As for the water isolates, resistance was observed only to gentamicin (34.8%) and imipenem (17.4%). According to PFGE results, all isolates obtained from humans and water belonged to the same pulsotype (1), which was identified by *recA* sequencing as *B. cepacia*, belonging to sequence type ST-767. By observing a single pulse type over three years, one can observe the persistence of this isolate in the pipeline, contaminating patients undergoing hemodialysis, despite the routine disinfection of water with peracetic acid. This persistence is probably due to the production of biofilm, which protects bacteria from disinfectants and, making this scenario more critical, several isolates proved to be multidrug-resistant (resistance to at least three groups of antimicrobials), turning the patient care even more difficult.

**Keywords :** hemodialysis, burkholderia cepacia, PFGE, MLST, multi drug resistance

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