

Polyclonal *Burkholderia cepacia* Complex Outbreak in Peritoneal Dialysis Patients Caused by Contaminated Aqueous Chlorhexidine

Sally C.Y. Wong,¹ Shuk-Ching Wong, Jonathan H.K. Chen, Rosana W.S. Poon, Derek L.L. Hung, Kelvin H.Y. Chiu, Simon Y.C. So, Wing Shan Leung, Tak Mao Chan, Desmond Y.H. Yap, Vivien W.M. Chuang, Kwok-Yung Yuen,² Vincent C.C. Cheng²

Whether *Burkholderia cepacia* complex should be an objectionable organism in antiseptic solutions with acceptable total bacterial counts is controversial. By using next-generation sequencing, we documented a polyclonal *B. cepacia* complex outbreak affecting peritoneal dialysis patients in Hong Kong that was caused by contaminated chlorhexidine solutions. Epidemiologic investigations at a manufacturing site identified a semiautomated packaging machine as the probable source of contamination in some of the brands. Use of whole-genome sequencing differentiated the isolates into 3 brand-specific clonal types. Changes in exit site care recommendations, rapid recall of affected products, and tightening of regulatory control for chlorhexidine-containing skin antiseptics could prevent future similar outbreaks. Environmental opportunistic pathogens, including *B. cepacia* complex, might be included in regular surveillance as indicator organisms for monitoring environmental contamination.

Burkholderia cepacia is the type species of the genus *Burkholderia* and is a ubiquitous multidrug-resistant, motile, non-glucose-fermenting, gram-negative organism found in water and soil (1). The *B. cepacia* complex (BCC) contains ≥ 17 closely related species that require molecular methods for accurate differentiation (2). Previous typing methods, such as pulsed-field gel electrophoresis, restriction fragment-length polymorphism, or

multilocus sequence typing (MLST), are ineffective and only enable differentiation into genome variants.

BCC is a major pathogen among patients with cystic fibrosis and an opportunistic pathogen affecting patients with indwelling medical devices and immunosuppression (3). Although >50 BCC-related nosocomial outbreaks associated with contaminated antiseptics or medications have been described, none of the skin disinfectant-related outbreaks were documented by next-generation genome sequencing as the typing method. The exact mode of contamination of commercial antiseptics was often not found (4,5). Implicated disinfectants and medications included intrinsically or extrinsically contaminated chlorhexidine (4–11), povidone-iodine (12,13), benzalkonium chloride (14–16), intravenous fluids or drugs (17–20), sodium docusate (21,22), eye drops (23), alcohol-free mouthwash, and nebulized salbutamol and albuterol (24–26).

There is a lack of consensus on whether *B. cepacia* should be considered an objectionable organism in nonsterile pharmaceuticals according to guidelines for the United States and Europe (27–30). We report an outbreak involving ≥ 2 clusters of BCC strains among peritoneal dialysis patients caused by multiple brands of contaminated, prepackaged, single-use, 0.05% aqueous chlorhexidine (aqCHX) solutions.

Materials and Methods

Outbreak Investigation

On September 6, 2019, we conducted an investigation at the Queen Mary Hospital Dialysis Unit in

Author affiliations: Queen Mary Hospital, Hong Kong, China

(S.C.Y. Wong, S.-C. Wong, J.H.K. Chen, R.W.S. Poon,

D.L.L. Hung, K.H.Y. Chiu, S.Y.C. So, W.S. Leung,

Vincent C.C. Cheng); The University of Hong Kong, Hong Kong

(T.M. Chan, D.Y.H. Yap, K.Y. Yuen); Hospital Authority, Hong Kong

(V.W.M. Chuang)

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¹Current affiliation: Queen Elizabeth Hospital, Hong Kong, China.

²These senior authors contributed equally to this article.

Hong Kong when a cluster of 4 dialysis patients had BCC isolated from their exit site. All 4 patients had recent-onset serous to bloody discharge from their exit site (3 peritoneal dialysis catheter exit sites and 1 hemodialysis catheter exit site). The hospital is a 1,700-bed university-affiliated tertiary referral center serving ≈270 peritoneal dialysis and 110 hemodialysis patients. Noting the unusual number of BCC among renal patients, we performed case finding and established baseline incidence rate of BCC during January 1, 2014–September 9, 2019, by using a laboratory information system (software system that records, manages, and stores data for clinical laboratories).

For the outbreak investigation, we defined a case-patient as a peritoneal dialysis patient who had BCC isolated from clinical specimens during March 13, 2018–October 30, 2019. The medical records of case-patients were reviewed by the infection control team as described (31). Epidemiologic investigation at the renal unit was performed, and nursing staff were interviewed and observed for any changes in their patient care practice; patients and their relatives, if available, were interviewed about their exit site care procedures. Environmental surveillance was conducted as described in the next section. Active surveillance was initiated for all peritoneal dialysis patients; we collected exit site swab specimens to screen for additional BCC cases. Ethics approval was obtained from the institutional review board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

Environmental Surveillance

Air, water, and environmental samples from the peritoneal dialysis unit, together with various antiseptics used for exit site care from our hospital and the community, were collected and microbiologically analyzed as described (31,32) (Appendix, <https://wwwnc.cdc.gov/EID/article/26/9/19-1746-App1.pdf>). In brief, we collected surface specimens by using premoistened, Polywipe sponge swabs (Medical Wire & Equipment, <https://www.mwe.co.uk>). We sampled faucets and drains of sinks by using transport rayon swabs (Copan Diagnostics, <https://www.copanusa.com>). We collected tap water (250 mL) into labeled sterile bottles. We used an air sampler, SAS Super ISO 180 model 86834 (VWR International PBI Srl, <https://it.vwr.com>), to collect 1,000 liters of air onto MacConkey agar (CM 0507; Oxoid, <http://www.oxoid.com>) containing 0.0005% crystal violet (Merck KGaA, <https://www.emdgroup.com>) and 4 µg/mL gentamicin (CG-MAC). We collected in-use and unopened antiseptics in the hospital.

Unopened 0.05% aqCHX was also obtained from other (outside) stores.

Tap water was filtered through a 0.45-µm membrane, which was then inoculated onto CG-MAC. Sponge swabs and transport rayon swabs were incubated in sterile selective brain heart infusion broth (CM1135; Oxoid) containing 4 µg/mL gentamicin, 15 µg/mL vancomycin, and 1 µg/mL amphotericin B (G3632, V2002, and A4888, respectively; Sigma-Aldrich, <https://www.sigmaaldrich.com>) at 37°C overnight before inoculation onto CG-MAC. All disinfectants and antiseptics were subjected to 1:10 dilution with neutralization broth (brain-heart infusion plus 2% Tween 80 [P1754; Sigma-Aldrich], 0.3% sodium thiosulphate pentahydrate [27910.260; VWR Chemicals, <https://us.vwr.com>], 0.4% potassium dihydrogen phosphate [26936.260; VWR Chemicals], and 0.5% lecithin). The suspension was left at room temperature for 5 min, then 100 µL of the suspension was spread onto blood agar (CM0331; Oxoid). Water and air samples were incubated at 37°C for 1 day, followed by room temperature for 5 days. Other specimens were incubated at 37°C for 5 days and examined daily for visible bacterial growth. Any bacterial growth was further speciated, and bacterial CFUs were also counted for air and antiseptic cultures.

Clinical Specimens

We processed all clinical specimens obtained before the outbreak investigation according to standard laboratory operating procedures. We performed active surveillance for BCC collected by swabbing catheter exit sites for all peritoneal dialysis patients. These swab specimens were inoculated onto CG-MAC for incubation at 37°C for 2 days. Patients with clinical symptoms suggestive of invasive catheter-related infection were investigated accordingly (e.g., peritoneal fluid or blood culture).

Field Investigation at Brand B Manufacturing Site

On September 19, 2019, a joint field investigation at brand B manufacturing site was conducted by a team of field epidemiologists, infection control professionals, and clinical microbiologists. The process of reconstitution, dilution, and packaging of 5% chlorhexidine solution into individually packed 25-mL volumes of 0.05% aqCHX was directly observed. Environmental samples and antiseptics were collected for microbiological investigations as described in the previous sections.

Identification by Matrix-Assisted Laser

Desorption/Ionization Time-of-Flight Mass Spectrometry

We picked bacterial colonies from blood agar or CG-MAC for matrix-assisted laser desorption/ionization

ionization time-of-flight (MALDI-TOF) mass spectrometry identification with bacterial colony protein extraction by using a direct transfer method. We measured mass spectra of isolates by using the MBT Smart Mass Spectrometer (Bruker Daltonik, <https://www.bruker.com>) and the Bruker MBT Database 9.0 (8326 spectra). Scores >2.0 were considered as showing high-confidence identification and scores of 1.7–2.0 as showing low-confidence identification.

Whole-Genome Sequencing and Bioinformatic Analysis

We further analyzed environmental and clinical BCC isolates by using the NovaSeq 6000 Sequencing System (Illumina Inc., <https://www.illumina.com>) at The University of Hong Kong Li Ka Shing Faculty of Medicine, Centre for PanorOmic Sciences, Genomics Core (Appendix). Two archived outbreak-unrelated BCC isolates were used as controls. We extracted MLST profiles from whole-genome assemblies by using BIGSdb, which is available on the BCC PubMLST website (33). We performed phylogenetic analysis according to single-nucleotide polymorphisms (SNPs) by using CSIphylogeny version 1.4 with default settings (Appendix) (34). Results from CSIphylogeny were subsequently imported into FigTree version 1.4.4 (<http://tree.bio.ed.ac.uk>) for visualizing the phylogenetic tree.

Statistical Analysis

We used the exact rate ratio test to compare exit site infection (ESI) rates between centers with and without routine chlorhexidine use. A *p* value <0.05 was considered statistically significant. We applied the Holm-Bonferroni correction for multiple comparisons to control the familywise error rate at 0.05. We used the R package *rateratio.test* (<https://www.r-project.org>) to perform calculations. We used an independent *t*-test to compare means of outbreak durations involving nonsterile and sterile sites. We used SPSS Statistics 20 (IBM, <https://www.ibm.com>) to perform this analysis.

Results

Epidemiologic Investigation

On September 6, 2019, we launched an outbreak investigation when BCC was isolated from 3 peritoneal dialysis catheter exit sites and 1 hemodialysis catheter exit site for 4 patients (2 women and 2 men; age range 49–90 years, median age 60.5 years). The exit site swab specimens were used for investigation of suspected ESI on September 4, 2019. Three patients had BCC isolated from previous exit site specimens,

1 from as early as September 24, 2018. The number of days from catheter insertion to first isolation of BCC ranged from 300 to 2,329 days (mean 1,084.5 days, median 854.5 days).

Retrospective case finding of BCC showed an increasing trend over time among nonduplicated dialysis patients since March 2018. During March 13, 2018–September 6, 2019, BCC was isolated from 53 renal patients, including 47 peritoneal dialysis catheter exit sites and 2 peritoneal fluid specimens (Table 1). The incidence rate of BCC isolated from peritoneal dialysis catheters during 2018–2019 was >2 SD from baseline (Figure 1), confirming an outbreak of BCC among peritoneal dialysis patients. Interviews with ward staff and observation of patient care practice found no recent changes or irregularity but showed that peritoneal dialysis patients purchased 0.05% aqCHX from community stores and used this solution for routine exit site care. Brands A and B were the commonest aqCHX bought by peritoneal dialysis patients because they were the most readily available brands in the community.

Environmental Surveillance

We collected 63 environmental and antiseptic specimens used in peritoneal dialysis catheter exit site care from the renal unit (Table 2). Different brands of aqCHX were purchased in the community (brands A–F) and collected in the hospital (brands G and H). All 77 aqCHX collected in the hospital were culture negative, but 103 of the 104 community aqCHX showed bacterial growth (Table 2). Brand A of aqCHX had an average bacterial load of 3.6×10^5 , and brand B had a value of 5.9×10^4 CFU/mL. No BCC was isolated from environmental samples and other antiseptics collected from the renal unit.

Clinical Specimens

We collected peritoneal dialysis catheter exit site swab specimens from 275 patients for BCC surveillance. A total of 62 (22.5%) patients were positive for BCC, 33.9% (21/62) of whom had a genuine infection. A total of 29.0% (18/62) were among the 53 BCC-positive peritoneal dialysis patients identified from retrospective case finding.

Field Investigation at Brand B Manufacturing Site

We observed the entire process from dilution to packaging of aqCHX. In brief, 5% aqCHX was diluted with distilled water in the mixing compartment of a semiautomated packaging machine, which channeled and packed the diluted solution into

SYNOPSIS

Table 1. Specimen types and demographic characteristics for 53 renal dialysis patients from whom *Burkholderia cepacia* complex was isolated, Hong Kong, China, March 13, 2018–September 6, 2019*

Characteristic	2018, 25 patients	2019, 28 patients	Total, 53 patients
Specimen type	23 PD catheter ES; 1 HD catheter ES; 1 ES swab specimen not otherwise specified	23 PD catheter ES; 2 HD catheter ES; 2 peritoneal fluid; 1 blood culture from HD catheter	46 PD catheter ES; 3 HD catheter ES; 2 peritoneal fluid; 1 blood culture from HD catheter; 1 ES swab specimen not otherwise specified
Age, y, mean (median, range)	60.1 (65, 24–81)	65.8 (66, 46–90)	63.1 (66, 24–90)
Sex ratio, F:M	16:9	13:15	29:24
Days from PD/HD catheter insertion until first isolation of <i>B. cepacia</i> complex, mean (median, range)	1,192 (648, 58–2,349)	1,140 (769.5, 70–6,098)	1,163, (713, 58–6,198)
<i>B. cepacia</i> complex peritonitis	1	4	5
Removal of PD catheter	1	3 (2 caused by renal transplant)	4
Previous infections			
ESI caused by other organisms	8	7	15
Peritonitis caused by other organisms	4	6	10
Antimicrobial drug use \leq 1 y before isolation of <i>B. cepacia</i> complex	19	26	45
No. deaths†	2	2	4

*ES, exit site; ESI, exit site infection; HD, hemodialysis; PD, peritoneal dialysis.

†None of the 4 deaths were attributable to infection by *B. cepacia* complex.

25-mL sachets (Figure 2). Samples of antiseptics were taken before and after each step, together with additional environmental samples from the site. BCC was found in 19 of 29 environmental samples and antiseptics collected, and 3 freshly packed antiseptics also yielded *Achromobacter* species (Table 2). BCC was first detected at a low level after chlorhexidine was diluted with distilled water in the semi-automated machine, then at high level in all subsequent packaged aqCHX, implying that the machine was the probable source of contamination. No BCC was found in the distilled water, air samples, or samples taken from measuring beaker, mixing rod, and unused package material.

Identification by MALDI-TOF Mass Spectrometry

All isolates were identified correctly to the genus level and had scores \geq 1.7. Further species identification within the BCC was not possible.

Whole-Genome Sequencing and Bioinformatic Analysis

A total of 80 isolates (52 patient isolates from active surveillance; 26 chlorhexidine-related isolates, including 5 isolates from the manufacturing site; and 2 outbreak-unrelated strains) were subjected to genome sequencing (Appendix Tables 1, 2). MLST analysis identified 2 predominant types. All BCC isolated from brands A, D, and E aqCHX (from the same company) were *B. cenocepacia* genomovar IIIA sequence

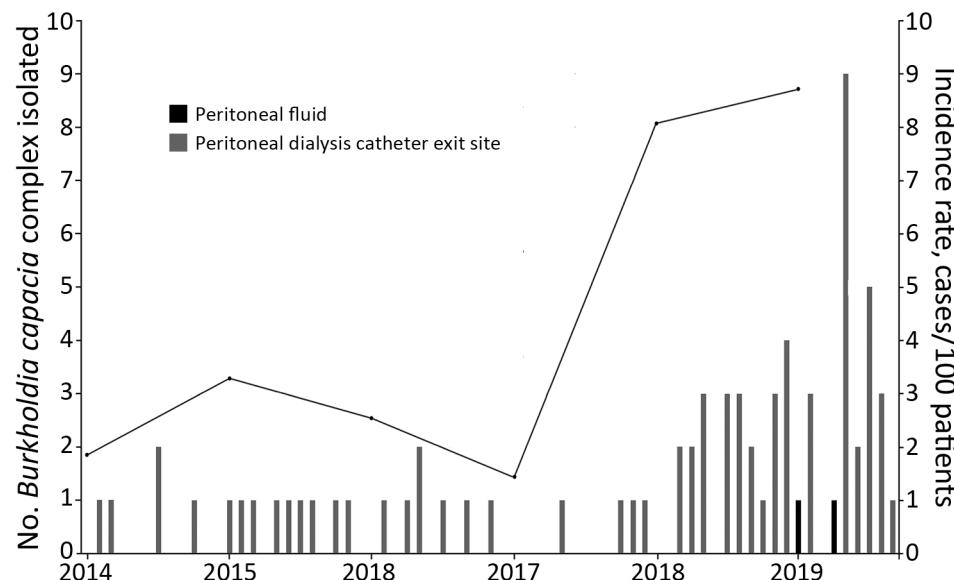


Figure 1. Epidemic curve and incidence rate of *Burkholderia cepacia* complex isolated from peritoneal dialysis patients, Hong Kong, China, January 2014–September 2019.

Table 2. Environmental specimens collected and tested for investigation of *Burkholderia cepacia* complex outbreak in peritoneal dialysis unit, Hong Kong, China, March 13, 2018–September 6, 2019*

Characteristic	No. specimens	Culture result (mean, median, range), CFU/mL
Peritoneal dialysis unit		
Environment		
Air samples	2	Negative for BCC
Swab specimens from sink and faucet	12	
Water samples from sink in ward	10	
Soaps from dispensers next to patient sinks	4	
Swab specimens from wound dressing trolleys	3	
Blood pressure cuffs, gloves, and tissue paper	6 (2 each)	
Connection shield SysIK with povidone–iodine solution†	3	
Exit site care agents		
In-use povidone–iodine	10	Negative for BCC
Single-use prepackaged saline and sterile water	10 (5 each)	
White wine vinegar	3	
Aqueous chlorhexidine		
Brand A (outside hospital)	51	43 with BCC only (3.6×10^3 , 1.9×10^2 , $2.7\text{--}7.6 \times 10^4$); 4 with <i>Ralstonia</i> species only (77, 85, 46–93); 4 with BCC and <i>Ralstonia</i> species (120, 120, 94–130)
Brand B (outside hospital)	45	45 with BCC (5.9×10^4 , 4.6×10^4 , $2.9 \times 10^4\text{--}1.2 \times 10^5$)
Brand C (outside hospital)	4	4 with BCC (8.3×10^3 , 6.8×10^3 , $8 \times 10^2\text{--}1.9 \times 10^4$)
Brand D (outside hospital)	2	2 with BCC (2.8×10^5 , 2.8×10^5 , $2.4\text{--}3.2 \times 10^5$)
Brand E (outside hospital)	1	1 with BCC (1.5×10^5)
Brand F (outside hospital)	1	Negative for BCC
Brand G (from hospital)	47	Negative for BCC
Brand H (from hospital)	30	Negative for BCC
Brand B manufacturing site		
Environment		
Air samples	2	Negative for BCC
Plastic packaging	1	Negative for BCC
Plastic container in preparation room	1	Negative for BCC
Surface of fan in preparation room	1	Negative for BCC
Surface of air conditioner in preparation room	1	Negative for BCC
Specimens collected during dilution and packaging process		
5% chlorhexidine from original bottle	1	Negative for BCC
Chlorhexidine in measuring beaker	1	Negative for BCC
Distilled water	1	Negative for BCC
Diluted chlorhexidine in mixing compartment of semiautomated packaging machine ([I] in Figure 2), before mixing with stirring rod	1	BCC from enriched culture method with overnight incubation in neutralization broth
Stirring rod surface swab specimen, before mixing diluted chlorhexidine solution	1	Negative for BCC
Stirring rod surface swab specimen, after mixing diluted chlorhexidine solution	1	BCC from enriched culture method with overnight incubation in neutralization broth
Diluted chlorhexidine in mixing bowl of packaging machine, after mixing with stirring rod	1	BCC from enriched culture method with overnight incubation in neutralization broth
Newly packed 25 mL 0.05% aqueous chlorhexidine	16	16‡ with BCC 1.2×10^5 , 1.2×10^5 , $3.6 \times 10^4\text{--}2.4 \times 10^5$; 3 with concurrent <i>Achromobacter</i> species

*BCC, *Burkholderia cepacia* complex.†Baxter Healthcare SA, <https://www.baxter.com>.‡Three specimens had concurrent *Achromobacter* species found in culture.

type (ST) 1547, and all BCC isolated from brand B aqCHX and its manufacturing site were *B. cepacia* that had a novel ST (ST1693). The 2 BCC isolates from brand C were *B. cenocepacia* that had another novel sequence (ST1694).

The phylogenetic tree based on core SNPs was consistent with the MLST results showing 2 predominant clusters with highly related strains within

each cluster (Figure 3). Strains from clusters A corresponded to brand A (and D and E) aqCHX and cluster B corresponded to brand B aqCHX, except that 1 brand A isolate (BCAP168) was different from cluster A strains. Both strains in cluster C corresponded to brand C aqCHX produced by a different company. A total of 47/52 patient isolates were indistinguishable or closely related to those in cluster A. Forty of these

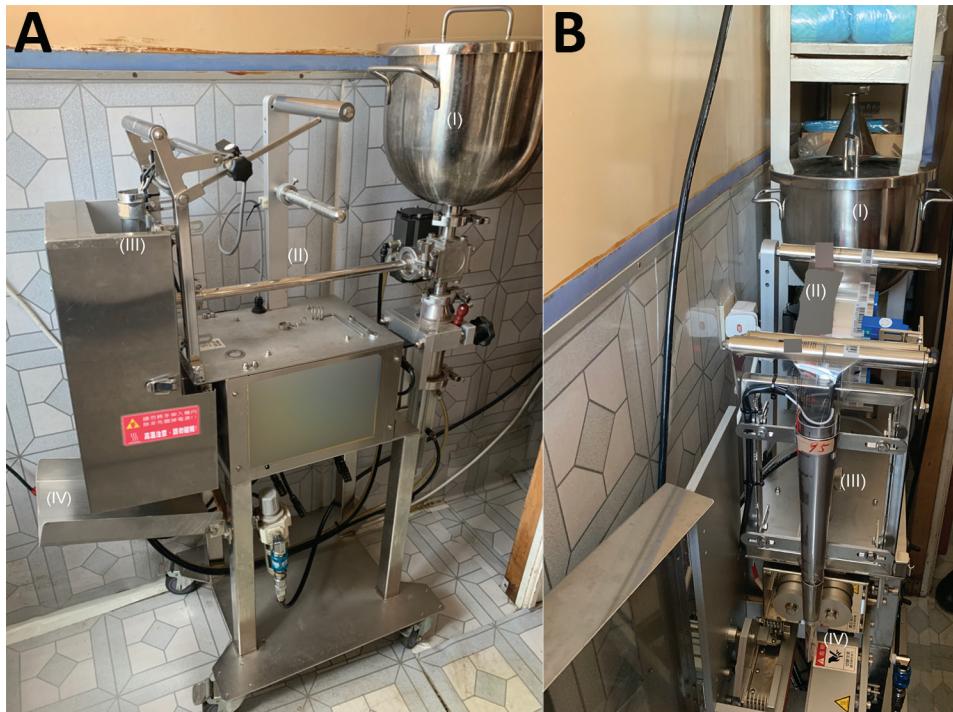


Figure 2. Semiautomated packing machine for aqueous chlorhexidine in brand B manufacturing site, Hong Kong, China. A) Mixing compartment (I), transfer tube from mixing compartment to dispensing end (II), area in which unused plastic packages are threaded (III), collection tray of newly packed 25 mL 0.05% aqueous chlorhexidine (IV). B) mixing compartment (I), unused plastic package (II), unused plastic package funneled to dispensing end (III), heat seal of 0.05% aqueous chlorhexidine into 25-mL packages (IV).

patients recalled using brand A for exit site care, 4 could not recall the brand used, and 3 reported using brand B. Of the 5 patients with isolates closely related to those in cluster B, 2 reported using brand B for exit site care, 2 reported using brand A, and 1 could not recall the brand used. The number of SNP differences in pairwise comparison of environment and patient isolates within cluster A was 0–165 and within cluster B was 0–32.

Outbreak Control

Upon reasonable suspicion of BCC contamination affecting prepacked aqCHX purchased in the community, the renal unit called all patients to stop such a practice and arranged alternative means of exit site disinfection. On September 17, 2019, the Hospital Authority and Centre for Health Protection (CHP), Department of Health, Hong Kong, were notified of the finding of BCC in prepackaged aqCHX. Further investigation by CHP identified 183 affected patients in public and private hospitals in Hong Kong (35). Several additional affected brands of aqCHX were identified and voluntarily recalled by the corresponding companies (36). We performed snapshot ESI surveillance between centers with routine and nonroutine chlorhexidine use by using data provided by the Hospital Authority; no major difference were found between the 2 practices (Table 3). Thus, sterile saline was recommended for routine exit site care in peritoneal dialysis patients instead of aqCHX.

On October 8, 2019, the Guidance Notes on Classification of Products as Pharmaceutical Product under the Pharmacy and Poisons Ordinance (Cap. 138) related to chlorhexidine was revised. Skin antiseptic products containing chlorhexidine are now classified as pharmaceutical products unless otherwise stated or under certain exceptions. This guidance took effect on July 8, 2020 (37).

Discussion

We report a polyclonal outbreak of BCC among peritoneal dialysis patients in our hospital that was caused by several contaminated brands of prepackaged aqCHX, which led to a territory-wide contact tracing that identified additional affected patients in other hospitals. Some observations can be made from this and previous BCC outbreaks. First, BCC outbreaks involving nonsterile sites were usually more prolonged; the mean outbreak duration was 85.4 days (median 66 days) when $\geq 50\%$ of outbreak strains were isolated from sterile sites, compared with a mean of 245.9 days and a median of 199 days when $\geq 50\%$ of BCC were isolated from nonsterile sites ($p = 0.001$) (Appendix Tables 3, 4). This finding might have occurred because BCC isolated from nonsterile sites might go unnoticed or were dismissed as sporadic, especially for patients with known risk factors, such as peritoneal dialysis catheters.

Also, the number of patients involved in an outbreak correlated with geographic distribution of the

contaminated source(s). For example, 2 recent, large BCC outbreaks involving 162 and 138 patients were caused by intrinsically contaminated intravenous saline and liquid docusate (17,21,22); both items were distributed to multiple states in the United States. From these and previous experiences (38), opportunistic environmental pathogens, such as BCC and nonanthrax *Bacillus*, might be used as indicator organisms for environmental contamination and be included as part of routine surveillance.

The use of whole-genome sequencing (WGS) provided high-resolution information for further analysis of this outbreak. First, it enabled accurate identification of BCC to species level and preliminary typing of bacterial strains through MLST. Phenotypic tests and MALDI-TOF mass spectrometry are inaccurate in speciation within BCC, and unlike previous BCC outbreaks, in which identical antibiogram profiles were found among outbreak-related BCC (4,39), the antibiogram profiles among isolates from our patients were variable.

Although WGS is becoming increasingly used for outbreak investigations, the technology is not readily available in usual clinical microbiology laboratories and can be costly. Thus, alternative molecular typing

methods, such as MLST or restriction fragment length polymorphism, remains the first choice for nosocomial outbreak investigations because they often provide sufficient information for evaluation of smaller scale, more focused outbreaks. In addition, these methods are also helpful for preliminary evaluation of larger outbreaks. Nevertheless, we opted for WGS in our investigation because of anticipated large-scale involvement, and the need for high-resolution data for analysis to enable rapid enforcement of corrective measures at a regional level.

Phylogenetic analysis of the WGS data based on SNP differences unambiguously differentiated the outbreak BCC isolates into distinct clusters. Combined with epidemiologic findings and field investigation at brand B manufacturing site, we believe that the contamination of aqCHX most likely occurred at their corresponding manufacturing sites. First, brand A aqCHX was manufactured outside Hong Kong and had no direct geographic linkage with the brand B manufacturing site. Second, the 5% chlorhexidine from the unopened bottle at the brand B manufacturing site did not show any growth of BCC, and presence of BCC was only detected in samples taken from the semiautomated machine, implying that the

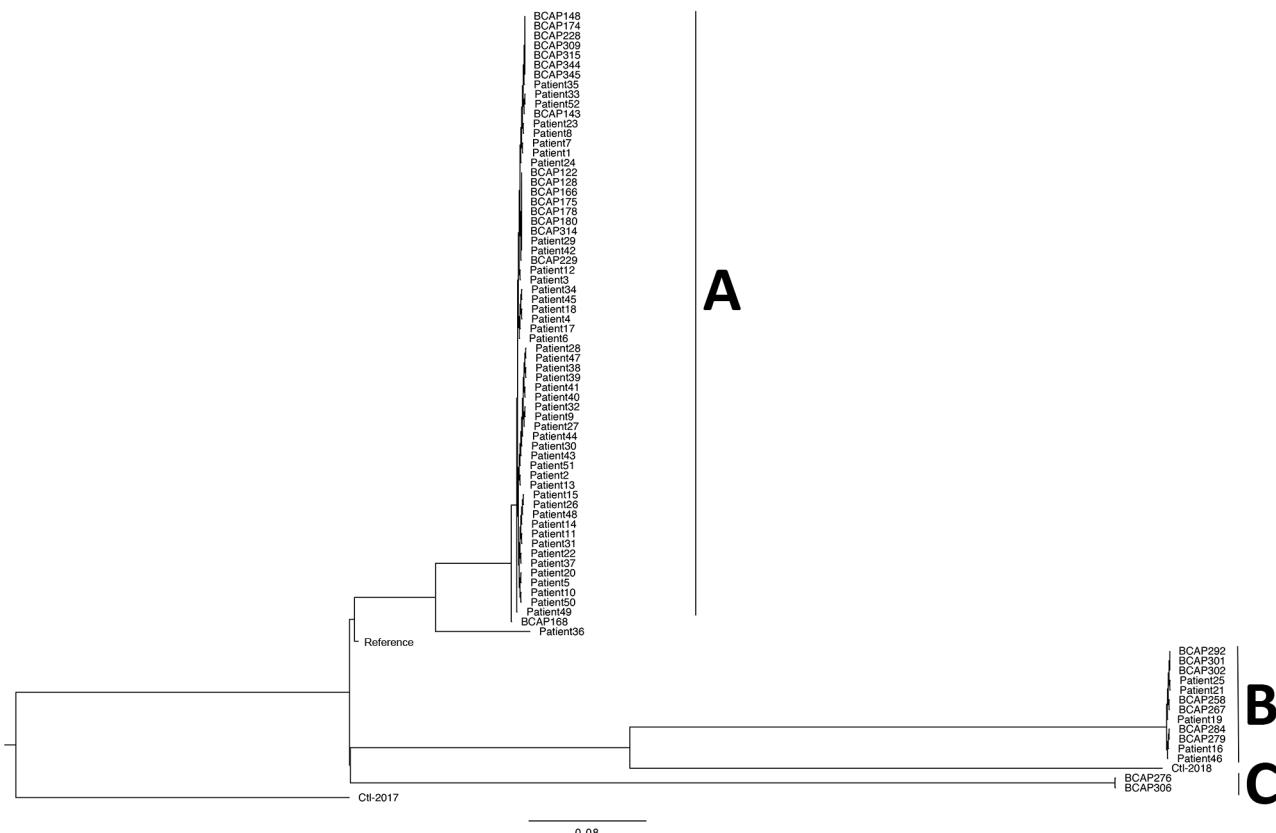


Figure 3. Maximum-likelihood phylogenetic tree of 80 *Burkholderia cepacia* complex isolates based on single-nucleotide polymorphisms, Hong Kong, China. A, B, and C indicate clusters. Scale bar indicates nucleotide substitutions per site.

Table 3. Exit site infection rate of various microorganisms for patients in peritoneal dialysis centers in public hospitals, Hong Kong, China*

Microorganisms causing peritoneal dialysis catheter exit site infections	No. infections/1,000 patient-years		
	Centers with routine CHX use, n = 2,530 patients	Centers without routine CHX use, n = 2,030 patients	p value
Coagulase-negative staphylococci	95.25	72.41	0.0096†
Diphtheroid bacilli	19.37	31.53	0.0128
<i>Streptococcus</i> species	74.70	65.02	0.2424
Methicillin-resistant <i>Staphylococcus aureus</i>	49.41	57.64	0.2570
Methicillin-sensitive <i>S. aureus</i>	62.45	70.44	0.3241
Enterobacteriales‡	95.65	86.70	0.3457
<i>Candida</i> species§	21.34	25.62	0.3990

*Values are pooled data from centers with and without routine CHX use for exit site care (deduplication done per center). CHX use included 0.05% aqueous CHX and 2% and 4% CHX body wash. CHX, chlorhexidine.

†Not statistically significant after Holm-Bonferroni sequential correction (0.05/7 = 0.007).

‡Included *Citrobacter freundii*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella* species, *Morganella* species, *Morganelia* species, *Providencia alcalifaciens*, *P. rettgeri*, *P. stuartii*, *Proteus* species, *Raoultella ornithinolytica*, *R. planticola*, and *R. terrigena*.

§Included *C. holmii*, *C. valida*, *Candida* species, and *Pichia* species.

contamination had occurred during processing at the manufacturing site, rather than in the raw material. Third, the WGS analysis of clusters A and B, corresponding to brands A and B, were genetically distant. Although we cannot be certain of the exact time and duration of contamination, the retrospective case finding of *B. cepacia* isolated among our peritoneal dialysis patients during 2014–2019 showed a substantial increase only since March 2018, suggestive of a relatively recent event. We suspect that lapses in good manufacturing practices (GMPs) at various sites of chlorhexidine dilution led to bacterial contamination from the environment into the production line. BCC are ubiquitous in the environment and strains that have a MIC (>100 mg/L chlorhexidine) have been described, in which the minimum bactericidal concentration can be 3 times higher than the MIC (40).

The relative chlorhexidine resistance of BCC was believed to be caused by chromosomally encoded, resistant-nodulation-division efflux pumps, which up-regulate in the presence of sublethal concentrations of chlorhexidine (41). Thus, chlorhexidine led to the selection of a predominant BCC strain exhibiting high levels of resistance to chlorhexidine specific to each manufacturing site. In comparison, chlorhexidine has better antibacterial activities against staphylococci and Enterobacteriales; thus, contamination of chlorhexidine by these organisms is rare, even at low chlorhexidine concentrations (Appendix Figure) (42).

The peritoneal dialysis catheter exit site care practice was revisited during this outbreak. Our local guideline stated that sterile saline and antiseptics, such as aqCHX, are acceptable (43), and the International Society for Peritoneal Dialysis 2017 guidelines stated that there is no evidence to suggest any antiseptics being superior in lowering the ESI rate (44). Some peritoneal dialysis centers have adopted routine use of chlorhexidine for ES care but a local snapshot audit

on ESI rate supported the use of either sterile saline or aqCHX for exit site care.

Before the described outbreak, prepackaged aqCHX products were not considered to be pharmaceutical products in our locality because they were not labeled for use on broken skin nor had medicinal claims, and as such, these products were not registered with the Pharmacy and Poisons Board. The updated CHP guidance issued in response to this outbreak compels all chlorhexidine-containing skin antiseptic for human and animal use to be classified as pharmaceutical products unless otherwise stated, or except that these products are clearly labeled in English and Chinese for washing hands only (or equivalent); or chlorhexidine is used as a preservative or antimicrobial agent in cosmetic products, and necessitates that GMPs be observed, together with additional regulatory measures (45). Because terminal sterilization might inactivate or compromise the antimicrobial activity of particular antiseptics including, chlorhexidine, GMPs are relied upon to ensure the quality of the chlorhexidine produced, coupled with microbial testing of products to demonstrate their compliance with the limit laid out by the authorities (27,28,46). We believe that antiseptics that are potentially used on wounds, compromised mucosal surfaces, exit sites or in immunocompromised patients should be subjected to regulations as pharmaceutical products to avoid future similar outbreaks.

This study had several limitations. First, the outbreak that we described was restricted to peritoneal dialysis patients. Non-peritoneal dialysis-related infections associated with contaminated aqCHX would not have been readily identified during initial case finding. Subsequent case finding based on exposure to contaminated aqCHX identified other affected groups of patients (e.g., persons with left ventricular-

assisted devices). Also, BCC isolated from peritoneal dialysis patients before September 6, 2019, and older lots of aqCHX were not available. Thus, only BCC strains identified from active patient surveillance and recent lots of prepackaged aqCHX were included for WGS. Therefore, phylogenetic analysis of the environmental and clinical strains might only reflect recent transmissions. Nevertheless, isolates subjected to WGS were from 12 patients who were among the 53 patients identified by the initial retrospective case finding. All of these isolates were highly related to strains within cluster A. Finally, investigation of brand C was not performed because there were no patient isolates within cluster C and none of the peritoneal dialysis patients used this brand. Other affected brands of aqCHX were imported from outside Hong Kong. Therefore, field investigation at the manufacturing sites for these brands was also not possible. Nevertheless, all affected brands were recalled and will be subject to the new regulatory measures.

In conclusion, our investigations identified a polyclonal outbreak of BCC caused by contamination of multiple brands of commercial aqCHX. The findings illustrated that genome sequencing enabled high-resolution and accurate analysis of the outbreak strains, which facilitated identification of the probable cause or point of contamination. Timely actions and coordination between renal units, the Microbiology and Infection Control Services, Hospital Authority, and Department of Health ensured prompt control of the outbreak and amendment of peritoneal dialysis catheter exit site care practice guidelines, voluntary territory-wide recall of the contaminated aqCHX, and tightening of regulatory control of chlorhexidine-containing skin antiseptics to prevent additional cases. Surveillance of environmental opportunistic pathogens, such as BCC, might enable these indicator organisms to be used to monitor environmental contamination for early detection of similar outbreaks.

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About the Author

Dr. Sally C.Y. Wong is an honorary assistant professor in the Department of Microbiology, The University of Hong Kong, Hong Kong, China. Her research interests include *Corynebacterium kroppenstedtii*, multidrug-resistant organisms, and infection control and prevention.

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- Address for correspondence: Vincent C.C. Cheng, Department of Microbiology, Queen Mary Hospital, Hong Kong, China; email: vccheng@hku.hk

EID Podcast TB in Internationally Displaced Children in Texas

Internationally displaced children often face a barrage of conditions—such as poor sanitation, nutrition, and access to healthcare—that increase their risk for disease. Upon the children’s arrival in the United States, medical examinations can help uncover infectious diseases such as tuberculosis, which can remain latent for years before progressing into its more serious, contagious state. As testing methods improve, researchers are learning how tuberculosis rates can be complicated.

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Polyclonal *Burkholderia cepacia* Complex Outbreak in Peritoneal Dialysis Patients Caused by Contaminated Aqueous Chlorhexidine

Appendix

Collection and Laboratory Processing of Environmental, Air, Water, and Antiseptic Samples

Environmental samples were collected by using Polywipe sponge swabs (Medical Wire & Equipment, <https://www.mwe.co.uk>). These swabs are sterile, premoistened, thin, flexible sponges tailor-made for sampling environmental surfaces. The sampled sponge swabs were put into sealed sterile plastic bags individually and were properly labeled before further processing in the laboratory. Samples from the faucets and drains of sinks in renal units were taken by using transport rayon swabs (Copan Diagnostics, <https://www.copanusa.com>) and placed in sterile selective brain heart infusion (BHI) broth (CM1135; Oxoid, <http://www.oxoid.com>) containing 4 µg/mL gentamicin, 15 µg/mL vancomycin, and 1 µg/mL amphotericin B (G3632, V2002, and A4888, respectively; Sigma-Aldrich, <https://www.sigmaaldrich.com>) (CG-BHI) before further processing in the laboratory.

An air sampler, SAS Super ISO 180 model 86834 (VWR International PBI Srl, <https://it.vwr.com>), was used to collect 1,000 liters of air at a rate of 180 liters of air/min for each bacterial air sampling. The air collected was directly pass onto MacConkey agar (CM 0507; Oxoid) containing 0.0005% crystal violet (Merck KGaA, <https://www.emdgroup.com>) and 4 µg/mL gentamicin (CG-MAC) during a 5.5-min process. Because water has been implicated in *Burkholderia cepacia*

complex (BCC) nosocomial outbreaks, 250 mL of water from sinks in renal units were collected into labeled sterile bottles for processing in the laboratory.

Both in-use and unopened antiseptics were collected from the renal unit. Unopened 0.05% aqueous chlorhexidine (aqCHX) were also collected from other units in our hospital. Because many peritoneal dialysis patients obtain their aqCHX from the community, 0.05% aqCHX was also obtained from a medical equipment store in the hospital and outside pharmacies.

Specimen Processing

The air samples on CG-MAC were incubated directly after collection at 37°C in air for 1 day and then at room temperature. Water samples were filtered by using MicroFunnel filter funnels (Pall, <https://www.pall.com>) through a 0.45- μ m membrane. The membrane was then placed onto CG-MAC and incubated at 37°C for 1 day, and then at room temperature. All initial processing of other environmental samples was performed in class II biosafety cabinets. For each sponge swab specimen, 3 mL CG-BHI was added into a plastic bag, in which the medium was absorbed by the sponge swab specimen. The sponge swab specimen was then squeezed repeatedly for proper mixing. Then, 2 mL of suspension was extracted from the bag and incubated at 37°C overnight, then subcultured onto CG-MAC for incubation at 37°C in air. Swabs in CG-BHI broth were incubated at 37°C overnight, then subcultured onto CG-MAC for incubation at 37°C in air.

All antiseptics were processed in class II biosafety cabinets and 70% alcohol was used to disinfect the surface of the container immediately before specimen collection. Sterile needles and syringes were used to aspirate the antiseptics from the container under aseptic condition. One milliliter of the antiseptic was transferred to 9 mL neutralization broth (BHI plus 2% Tween 80) (P1754; Sigma-Aldrich), 0.3% sodium thiosulphate pentahydrate (27910.260; VWR Chemicals, <https://us.vwr.com>), 0.4% potassium dihydrogen phosphate (26936.260; VWR Chemicals), and 0.5% lecithin. The

suspension was left at room temperature for 5 min. Then, 100 µL of suspension was spread onto blood agar (CM0331; Oxoid) for incubation at 37°C in air.

All culture plates were incubated for ≤ 5 days and were examined daily for visible bacterial growth. Any bacterial growth was further speciated. For air samples and antiseptic cultures, bacterial CFUs were also counted.

Peritoneal dialysis catheter exit site swab specimens for BCC surveillance were inoculated onto CG-MAC agar upon arrival at the microbiology laboratory. The inoculated agar was incubated at 37°C in air for 2 days and examined daily for bacterial growth.

Genome Sequencing

The BCC isolates were further analyzed by genome sequencing with the NovaSeq 6000 sequencing system (Illumina Inc., <https://www.illumina.com>) at The University of Hong Kong. A BCC isolate from a peritoneal swab specimen from a patient with acute necrotizing pancreatitis during 2017 and a blood culture isolated during 2018 from a patient with atonic urinary bladder with recurrent urinary tract infection were included as unrelated controls.

Libraries (pair-end sequencing of 151 bp) were prepared on the basis of the protocol for the Nextera XT DNA Sample Prep Kit (Illumina). Enriched libraries were validated by using a Fragment Analyzer (<https://www.agilent.com>) and Qubit (<https://www.thermofisher.com>), and quality control analysis was performed by using a quantitative PCR. The libraries were denatured and diluted to optimal concentration. Illumina NovaSeq 6000 was used for Pair-End 151-bp sequencing.

Using software from Illumina (bcl2fastq), we assigned sequencing reads into individual samples; each sample had an average throughput of 1.7 Gb and a total throughput of 137.9 Gb. In terms of sequence quality, an average of 93% of the bases achieved a quality score of Q30, in which Q30 indicates the accuracy of a base call to be 99.9%.

Sequencing reads were filtered for adaptor sequence and low-quality sequence, followed by retaining only reads with read length ≥ 40 bp by using Cutadapt version 1.8.1 (1) and custom scripts. Low quality is defined as reads with $>5\%$ unknown bases N and reads having $>50\%$ of bases with a quality value ≤ 11 .

De novo genome assembly was performed on samples by using preprocessed reads with SPAdes assembler version 3.13.0 (2). A range of k-mer sizes of 21, 33, 55, and 77 were used. The assembly yielded an average genome size of 8.1 Mb and an average N50 value of 322 Kb, and number of scaffolds ranged from 53 to 134. All assembled sequences were annotated by using Prokka version 1.14.0 (3) and setting genus as *Burkholderia* and species as *cepacia*. Multilocus sequence typing profiles were extracted from whole-genome assemblies by using BIGSdb (4), which is available on the *B. cepacia* complex PubMLST website (<https://pubmlst.org/bcc/>).

Phylogenetic Analysis

Scaffold sequences and reference genome sequence of BCC ST32 were uploaded to the CSIPhylogeny 0v1.4 Web site (5) with default settings. Results from CSIPhylogeny were subsequently imported into FigTree version 1.4.4 (<http://tree.bio.ed.ac.uk>) for visualizing the phylogenetic tree.

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Appendix Table 1. Summary of library preparation for whole-genome sequencing of *Burkholderia cepacia* isolates

Characteristic	Summary
Average input DNA	1 ng
Library preparation protocol	Nextera XT DNA Library Prep Kit Reference Guide (15031942 v02)
Index system	IDT UDI Nextera Primer Pairs
Changes made to library preparation protocol	None
Sequencer model	Novaseq 6000
Run type	Pair end 151 bp

Appendix Table 2. Nature and distribution of throughput of each sample for whole-genome sequencing of *Burkholderia cepacia* isolates

Sample name	Associated brand	No. raw reads (Read1 + Read2)	Total throughput, Gb	% \geq Q30 bases
BCAP122	Brand A	11,950,402	1.8	94
BCAP128	Brand A	11,733,806	1.8	93
BCAP143	Brand A	6,274,224	0.9	84
BCAP148	Brand A	9,639,536	1.5	94
BCAP166	Brand A	10,257,462	1.5	93
BCAP168	Brand A	11,702,132	1.8	93
BCAP174	Brand A	9,678,166	1.5	91
BCAP177	Brand A	8,848,674	1.3	90
BCAP178	Brand A	11,773,222	1.8	94
BCAP180	Brand A	11,652,572	1.8	94
BCAP228	Brand A	11,807,954	1.8	93
BCAP229	Brand A	9,000,840	1.4	89
BCAP258	Brand B	12,118,698	1.8	94
BCAP267	Brand B	11,566,100	1.7	94
BCAP276	Brand C	12,404,438	1.9	94
BCAP279	Brand B	12,791,322	1.9	94
BCAP284	Brand B	10,421,288	1.6	93
BCAP292	Brand B	13,069,666	2.0	93
BCAP301	Brand B	9,275,042	1.4	90
BCAP302	Brand B	11,509,782	1.7	94
BCAP306	Brand C	11,978,500	1.8	93
BCAP309	Brand E	11,226,918	1.7	94
BCAP314	Brand D	13,238,160	2.0	94
BCAP315	Brand D	10,598,990	1.6	94
BCAP344	Brand A	13,624,626	2.1	94
BCAP345	Brand A	14,178,784	2.1	93
Ctl-2017	Outbreak unrelated blood culture isolate from 2017	12,130,950	1.8	94
Ctl-2018	Outbreak unrelated blood culture isolate from 2018	12,620,538	1.9	92
Patient 1	Brand A	12,806,494	1.9	94
Patient 2	Brand A	12,273,168	1.9	93
Patient 3	Patient using brand A aqCHX	10,764,710	1.6	93
Patient 4	Brand A	11,176,822	1.7	94
Patient 5	Brand A	10,950,102	1.7	92
Patient 6	Brand A	11,085,726	1.7	94
Patient 7	Brand A	11,562,168	1.7	93
Patient 8	Brand A	11,588,802	1.8	93
Patient 9	Brand A	14,410,584	2.2	94
Patient 10	Brand A	11,509,524	1.7	94
Patient 11	Brand A	5,409,852	0.8	88
Patient 12	Brand A	10,538,318	1.6	94
Patient 13	Brand B	13,177,162	2.0	94
Patient 14	Brand B	10,687,054	1.6	91
Patient 15	Brand B	12,277,634	1.9	92
Patient 16	Brand B	12,691,674	1.9	94
Patient 17	Unknown	11,599,212	1.8	94

Sample name	Associated brand	No. raw reads (Read1 + Read2)	Total throughput, Gb	% \geq Q30 bases
Patient 18	Brand A	11,848,136	1.8	93
Patient 19	Brand A	12,330,168	1.9	94
Patient 20	Brand A	12,214,730	1.8	93
Patient 21	Brand A and B	10,843,768	1.6	92
Patient 22	Brand A	12,736,614	1.9	93
Patient 23	Brand A	11,019,666	1.7	92
Patient 24	Brand A	11,704,104	1.8	94
Patient 25	Brand A	12,997,772	2.0	93
Patient 26	Brand A	12,828,720	1.9	94
Patient 27	Brand A	12,098,718	1.8	94
Patient 28	Brand A	9,875,416	1.5	95
Patient 29	Brand A	10,455,064	1.6	94
Patient 30	Brand A	12,178,434	1.8	94
Patient 31	Brand A	10,247,674	1.5	94
Patient 32	Brand A	11,413,460	1.7	94
Patient 33	Brand A	9,016,084	1.4	76
Patient 34	Brand A	3,923,344	0.6	86
Patient 35	Brand A	11,181,508	1.7	91
Patient 36	Brand A	8,991,288	1.4	90
Patient 37	Brand A	9,862,958	1.5	94
Patient 38	Brand A	10,110,866	1.5	92
Patient 39	Brand A	13,828,424	2.1	93
Patient 40	Brand A	12,469,812	1.9	94
Patient 41	Brand A	13,330,094	2.0	94
Patient 42	Brand A	10,407,292	1.6	94
Patient 43	Brand A	13,431,490	2.0	94
Patient 44	Brand A	12,053,012	1.8	94
Patient 45	Brand A	11,246,962	1.7	94
Patient 46	Unknown	13,231,332	2.0	94
Patient 47	Unknown	13,667,616	2.1	94
Patient 48	Unknown	12,343,144	1.9	93
Patient 49	Unknown	11,251,576	1.7	92
Patient 50	Unknown	13,368,328	2.0	94
Patient 51	Brand A	13,624,934	2.1	93
Patient 52	Brand A	9,421,366	1.4	89

Appendix Table 3. Summary of antiseptic- and medication-related *Burkholderia cepacia* complex outbreaks involving \geq 50% sterile sites*

Year of outbreak, country	Site(s) of BCC isolation	Duration of outbreak, d†	Type of patients involved	No. affected patients	Implicated source (intrinsic or extrinsic contamination)	Multistate or multiple hospital involvement	Reference
1981, United States	Blood (pseudobacteremia)	210	Various wards	52	Povidone-iodine (intrinsic contamination)	4 hospitals	(6)
1992, United States	Peritoneal fluid (4) and blood (2)	25	ICU and HD center in pediatric facilities	6	Povidone-iodine (intrinsic contamination)	No	(7)
1993, Georgia	Blood	85	Oncology clinic	14	Multiuse IV fluid used for dilution of multiuse vial heparin flush solution (extrinsic contamination)	No	(8)
1998, Belgium	Blood	3	Cardiology ward	8	1 L dextrose used for heparin dilution (extrinsic contamination)	No	(9)
2000, Thailand	Blood (subclavicular line infection)	7	HD	9	1.5% chlorhexidine-cetrimide prepared from in pharmacy department	No	(10)

Year of outbreak, country	Site(s) of BCC isolation	Duration of outbreak, d†	Type of patients involved	No. affected patients	Implicated source (intrinsic or extrinsic contamination)	Multistate or multiple hospital involvement	Reference
2004, France	Blood (IV catheter as source in 75%)	210	NICU, PICU, pediatric gastroenterology	8	Contaminated condensate on the plastic stoppers in lipid emulsion	No	(11)
2006, Saudi Arabia	Blood	21	Tertiary care hospital	5	0.5% salbutamol solution (intrinsic contamination)	No	(12)
2007, United States	Blood/intravenous catheter tips	214	Pediatric hematology and oncology practice, patients with subcutaneous port catheters	10	Multidose medications (extrinsic contamination)	No	(13)
2008, Taiwan	9 blood, 7 central venous catheter tips, 2 urine, 1 HD catheter tip	90	Hospital respiratory care ward and general ward	15	Extrinsic contamination of daily prepared diluted heparin solution in the ward	No	(14)
2008, South Korea	Blood	23	Cancer center	8	0.5% chlorhexidine solution diluted at hospital site	No	(15)
2008, South Korea	Blood (6), urine (1), wound (3), catheter tip (1), unknown (2)	21	Various wards, especially hemato-oncology and endocrine patients	13	Benzalkonium chloride diluted in hospital pharmacy	No	(16)
2008, Spain	Blood	151	HD patients	5	Contaminated deionized water used for dilution of 2.5% chlorhexidine at hospital site	No	(17)
2009, United States	Eye (endophthalmitis)	30	Hospital A (4)	4	Contaminated trypan blue dye from compounding pharmacy (unopened bottles were contaminated)	Yes	(18)
	Eye (endophthalmitis)	60	Hospital B (2)	2	Contaminated trypan blue dye from compounding pharmacy (unopened bottles were contaminated)	Yes	
2010, Brazil	Blood	88	Various wards	25	IV bromopride (antiemetics)	3 hospitals	(19)
2013, Brazil	Blood (4) and urine (3)	59	350-bed private tertiary care hospital	7	3% mannitol (intrinsically contaminated) for bladder irrigation	No	(20)
2014, Brazil	Blood	60	Hematology and BMT outpatient unit	24	Multidose vial of IV drug (extrinsic contamination) and a laminar flow cabinet	No	(21)
2014, India	Vitreous samples	91	Postcataract surgery patients	13	Local anesthetic eye drops	No	(22)

Year of outbreak, country	Site(s) of BCC isolation	Duration of outbreak, d†	Type of patients involved	No. affected patients	Implicated source (intrinsic or extrinsic contamination)	Multistate or multiple hospital involvement	Reference
2014, United States	Blood	7	350-bed private tertiary care hospital	7	Contaminated fentanyl solution (intrinsic contamination)	No	(23)
2015, South Korea	Blood (pseudobacteremia)	66	ICU and general wards	40	Commercial 0.5% chlorhexidine (intrinsic contamination).	No	(24)
2015, Spain	Blood	91	HD center	7	Chlorhexidine.	No	(25)
2016, India	Blood	90	Neonatal unit	12	In-use IV fluid bottles, ventilator humidifier	No	(26)
2017, India	Blood	240	Pediatric unit	76	Amikacin with contaminated rubber stopper.	No	(27)
2018, South Korea	Blood (pseudobacteremia)	42	NICU	21	Commercial 0.5% chlorhexidine (intrinsic contamination)	No	(28)
2019, United States	Blood	150	Skilled nursing facilities	162	IV saline (intrinsic contamination)	5 states, 59 facilities	(29)

*An outbreak in Lebanon was excluded because the prolonged outbreak duration was attributed to the political instability at the time of outbreak. Only reports where outbreak duration were described are included). BCC, *Burkholderia cepacia* complex; BMT, bone marrow transplant; HD, hemodialysis; ICU, intensive care unit; IV, intravenous; NICU, neonatal intensive care unit; PICU, pediatric intensive care unit.
†If exact dates are not specified in the report, the whole month will be counted toward duration of outbreak.

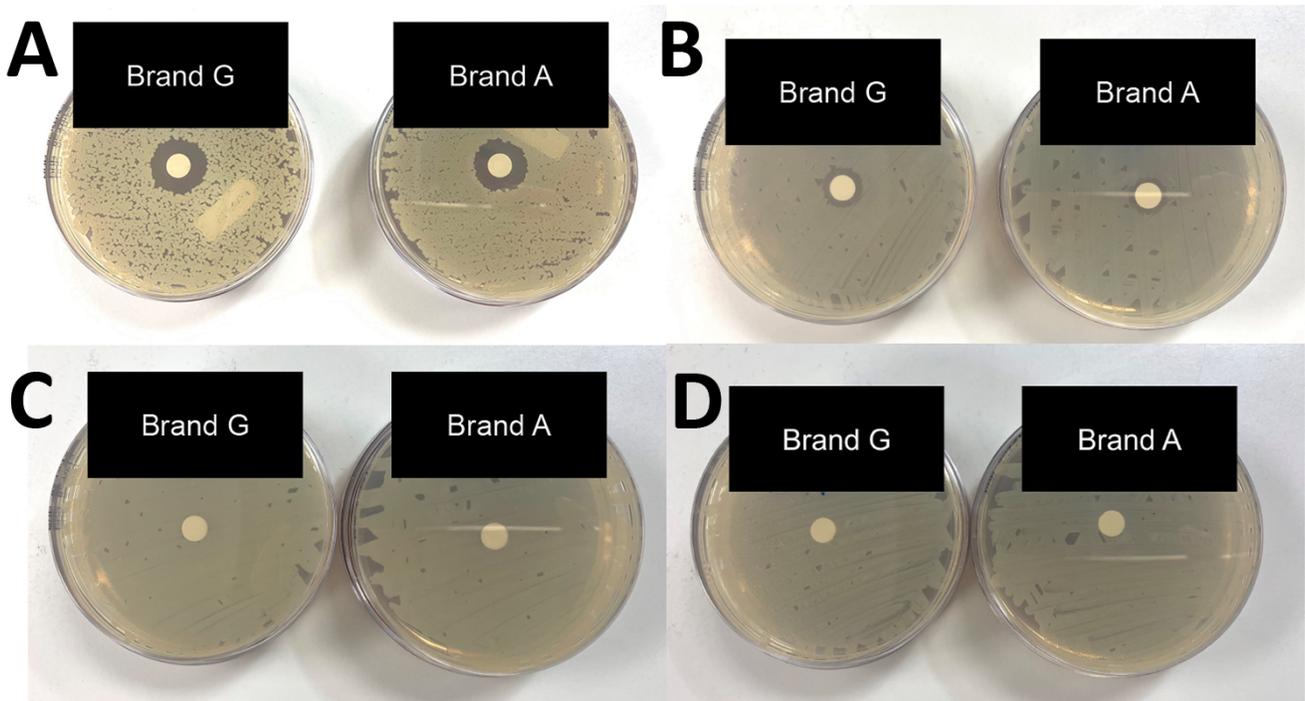
Appendix Table 4. Summary of antiseptic- and medication-related *Burkholderia cepacia* complex outbreaks involving $\geq 50\%$ nonsterile sites*

Year of outbreak, country	Site(s) of BCC isolation	Duration of outbreak, d†	Type of patients involved	No. affected patients	Implicated source (intrinsic or extrinsic contamination)	Multistate or multiple hospital involvement	Reference
1995, United States	Respiratory tract specimen	215	Medical center	42	Nebulized albuterol (extrinsic contamination)	No	(30)
1996, United States	Respiratory specimens	330	Several adult ICUs in a hospital	44	Albuterol nebulization solution (extrinsically contaminated)	No	(31)
2000, United States	Respiratory specimens	699	Adult ICU, ventilated patients	69	Alcohol-free mouthwash (intrinsic contamination)	2 hospitals	(32)
2005, Saudi Arabia	Respiratory (31), blood (21), wound (2), CSF (1), eye (1), others (3) (some patients with >1 positive culture)	336	Tertiary care hospital and a 150-bed hospital	52	Albuterol nebulization solution (intrinsically contaminated)	2 hospitals	(33)
2006, Spain	Respiratory specimens (35), unspecified (2)	365	ICU (35) and non-ICU (2) patients	37	Alcohol-free 0.1% hexetidine mouthwash (intrinsically contaminated)	No	(34)
2006, United States	Respiratory tract specimen	183	Adult acute care facility (hospital A)	18	Contaminated albuterol (extrinsic contamination)	No	(35)
2007, United States	Respiratory specimens (83), urine (33), blood (20), tissue (3)	146	Multiple hospitals, especially ventilated patients.	116	Alcohol-free cetylpyridinium chloride mouthwash (intrinsic contamination)	22 hospitals in 9 states	(36)

Year of outbreak, country	Site(s) of BCC isolation	Duration of outbreak, d†	Type of patients involved	No. affected patients	Implicated source (intrinsic or extrinsic contamination)	Multistate or multiple hospital involvement	Reference
2009, Japan	Vaginal culture	61	Obstetrics and gynecology ward	17	0.025% benzalkonium chloride prepared in hospital pharmacy	No	(37)
2011, United States	4 Sinus and 1 tracheal aspirate	90	Pediatric hospital	5	0.05% oxymetazoline hydrochloride nasal spray (intrinsic contamination)	No	(38)
2013, South Korea	Sputum (10), Blood (4), CSF (1), others^ (3).	92	ICU and general wards	37	Contaminated purified water used for chlorhexidine dilution at hospital site	No	(39)
2014, Ecuador	Respiratory specimens	458	ICU	13	Alcohol-free chlorhexidine 0.12% mouthwash (intrinsic contamination)	No	(40)
2018, Australia	1 Blood and 6 respiratory specimens	61	ICU	7	Alcohol-free chlorhexidine mouthwash (intrinsic contamination)	2 hospitals	(41)
2018, Germany	Respiratory specimens	30	Postcardiac surgery	3	Octenidine mouthwash solution (intrinsic contamination)	No	(42)
2019, New Zealand	Peritoneal dialysis catheter exit sites	377	Peritoneal dialysis patients	9	4% chlorhexidine body wash (extrinsic contamination)	No	(43)

*An outbreak in Lebanon was excluded because the prolonged outbreak duration was attributed to the political instability at the time of outbreak. Only reports where outbreak duration were described are included). BCC, *Burkholderia cepacia* complex; CSF, cerebrospinal fluid; ICU, intensive care unit.

†If exact dates are not specified in the report, the whole month will be counted toward duration of outbreak.



Appendix Figure. Activity of 0.05% aqueous chlorhexidine (brands G and A) against *Escherichia coli* ATCC25922, an outbreak-unrelated *Burkholderia cepacia* isolate, and an outbreak-related *B.* isolate. All plates show bacterial lawns with a 0.5 McFarland standard of the test strain against sterile filter paper disk soaked with 40 μ L of aqueous chlorhexidine and incubated overnight at 37°C. A) *E. coli* ATCC25922 and large zone of inhibition. B) Outbreak-unrelated *B. cepacia* isolate and small zone of inhibition. C) Outbreak-related *B. cepacia* patient isolate, no zone of inhibition. D) Outbreak-related *B. cepacia* isolate from brand A aqueous chlorhexidine, no zone of inhibition.