

China need to become aware of *R. japonica* disease presentation, so they can administer the appropriate treatment to patients with suspected *R. japonica* infections.

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## ***Burkholderia lata* Infections from Intrinsically Contaminated Chlorhexidine Mouthwash, Australia, 2016**

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*Burkholderia lata* was isolated from 8 intensive care patients at 2 tertiary hospitals in Australia. Whole-genome sequencing demonstrated that clinical and environmental isolates originated from a batch of contaminated commercial chlorhexidine mouthwash. Genomic analysis identified efflux pump–encoding genes as potential facilitators of bacterial persistence within this biocide.

*Burkholderia contaminans* and *B. lata* together form group K of the *B. cepacia* complex (Bcc). These predominantly environmental species are a major cause of pharmaceutical contamination and have been linked to multiple instances of associated opportunistic infection (1). Although both species are capable of causing serious infections in humans (2,3), only *B. contaminans* has been associated with infection outbreaks (3,4). We report a healthcare-associated *B. lata* infection outbreak occurring in intensive care units (ICUs) in 2 tertiary hospitals in Australia.

During May–June 2016, bacterial contamination of chlorhexidine mouthwash (0.2% mg/mL) was associated with an interjurisdictional outbreak in New South Wales and South Australia. Bcc isolates were obtained from blood and tracheal aspirates from 6 ICU patients in hospital A (South Australia) (sample information and isolation protocols detailed in the online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/24/11/17-1929-Techapp1.pdf>). An investigation by the hospital’s infection and prevention control team noted discoloration of a commercial chlorhexidine mouthwash. Bcc isolates were cultured from

all 5 tested bottles from the discolored batch but not from 11 bottles tested from 4 other batches. Isolates were further obtained from the surfaces of hand basins in 3 separate ICU rooms, where bottles of mouthwash from the contaminated batch were in use. Separately, a Bcc isolate was obtained from the sputum of an ICU patient in hospital B (New South Wales), where the same batch of mouthwash was in use. After a nationwide recall of the contaminated mouthwash batch, no further cases were reported.

The genomes of 15 Bcc isolates from the 2 hospitals were sequenced (online Technical Appendix). A closed genome of isolate A05 was generated as a reference, consisting of 3 circular chromosomes of 3.93, 3.71, and 1.15 Mbp (National Center for Biotechnology Information BioProject accession no. PRJNA419071).

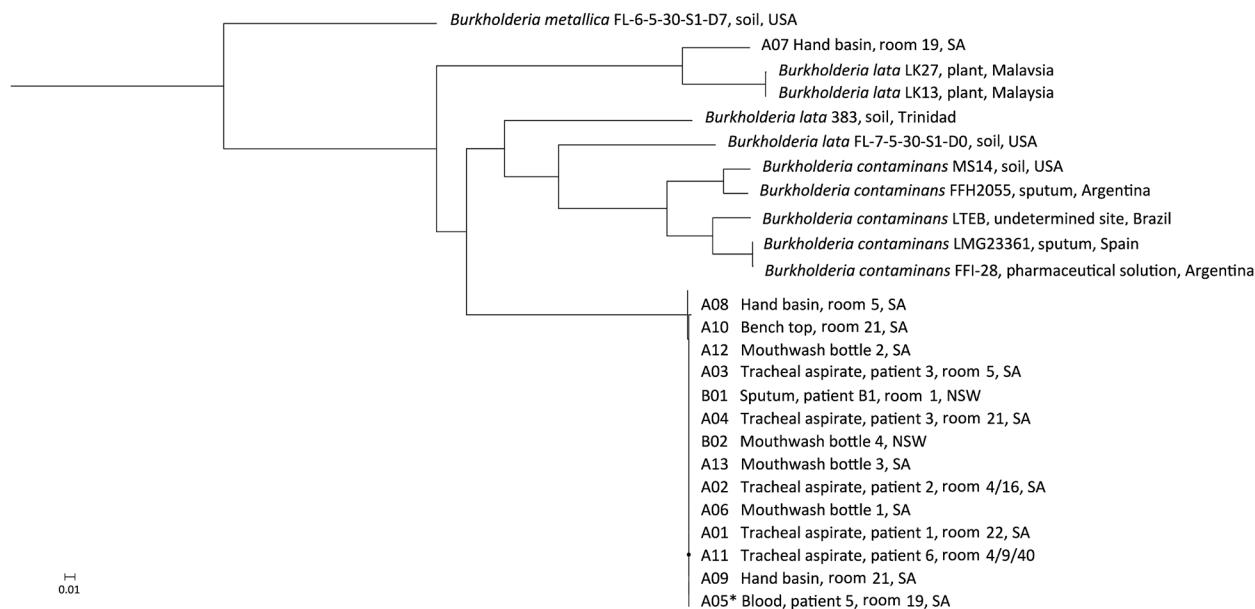
Genome analysis identified an Australasian sublineage of *B. lata* as the cause of the outbreak. Specifically, all isolates were sequence type 103 (ST103), which sits within a subclade of *B. lata* isolates from Australia and New Zealand (online Technical Appendix Figure 1). Two isolates from hand basins (A07 and A08) and 1 from a hospital bench (A10) were of unknown sequence type.

Single-nucleotide polymorphism (SNP)-based phylogenetic analysis identified the outbreak strain as a distinct clonal lineage (0–1 SNPs) within the group K clade (Figure), separated from other members of the group by a minimum of 167,662 SNPs. SNP variation within the clonal sublineage ranged from 0 to 2 SNPs across isolates from contaminated mouthwash bottles and patients. A single SNP distinguished isolates from patient 4 in room 21 (A04)

and the hand basin in the same room (A08) (collected 2 days apart). Three of the taxa isolated from the hospital environment (A07, A08, and A10) had substantial SNP variations compared with contaminated mouthwash and patient isolates (an average of 191,893 SNPs for A07, 655 SNPs for A08, and 1,408 SNPs for A10).

Differences of 0–1 SNPs between isolates from mouthwash in the 2 hospitals, including from unopened bottles, strongly suggests contamination during the manufacturing process. After several previous incidents of contamination of cosmetic, toiletry, and pharmaceutical products by members of Bcc, the US Food and Drug Administration commented that these bacteria very likely are introduced into the manufacturing processes through contaminated water (<https://www.fda.gov/Drugs/DrugSafety/ucm559508.htm>). Direct patient-to-patient transmission of *B. lata* would appear unlikely in this instance, given the use of individual ICU rooms with nonshared facilities and incomplete overlap in periods of care.

Chlorhexidine mouthwash is perhaps a surprising source of *B. lata* infection, given its potent biocidal properties. However, Bcc species have been isolated previously from 0.02% chlorhexidine gluconate (in irrigation apparatuses used for vaginal douching) and from 0.05% preparations (used for storage of suction catheters) (5). The ability of *B. lata* to remain viable in chlorhexidine appears to be a result of a combination of efflux pump activity, biofilm formation, and cell wall impermeability (1,6,7). These mechanisms probably also contribute to the common inefficacy of combination antibiotic therapy



**Figure.** Phylogenetic analysis of isolates implicated in an outbreak *Burkholderia lata* infection from intrinsically contaminated chlorhexidine mouthwash, Australia, 2016. The maximum-likelihood tree is constructed from core genome single-nucleotide polymorphism alignments ( $N = 512,480$ ) of the outbreak genomes, bootstrapped 1,000 times, and archival genomes from *B. cepacia* complex group K, relative to the reference genome *B. lata* A05 (identified by an asterisk). *B. metallica* was included as a comparator.

in the treatment of associated infections (8). Prolonged exposure to microbiocides, including chlorhexidine, has been shown to result in a stable increase in the expression of antibiotic-resistance mechanisms (1,6), and elevated chlorhexidine resistance has been reported in multidrug-resistant strains of *B. cenocepacia* from cystic fibrosis patients (9). Three resistance-nodulation-division (RND) efflux pump genes (RND3, RND4, and RND9) have been shown to be essential for chlorhexidine tolerance in *B. cenocepacia* (9). Examination of the complete genome of *B. lata* isolate A05 revealed the presence of RND3, RND4, and RND9 in each strain ( $\geq 94\%$  sequence identity) (online Technical Appendix Figure 2).

*B. contaminans* is the cause of widespread pharmaceutical product contamination, and infection outbreaks by this species are well-documented (3,10). Our findings suggest that the other member of Bcc group K, *B. lata*, also represents an important opportunistic pathogen of relevance to infection control, particularly given its intrinsic biocide tolerance.

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## Estimating Latent Tuberculosis Infection Using Interferon- $\gamma$ Release Assay, Japan

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We estimated the latent tuberculosis infection (LTBI) rate for foreign-born students at Keio University, Tokyo, Japan, using an interferon- $\gamma$  release assay. The LTBI rate for students from countries with estimated tuberculosis incidence  $>100$  cases/100,000 persons was high (10.0%). Universities should screen for LTBI in students from countries with high tuberculosis incidence.

# *Burkholderia lata* Infections from Intrinsically Contaminated Chlorhexidine Mouthwash, Australia, 2016

## Technical Appendix

### Materials and Methods

#### Sample Collection

Patient information is shown in Technical Appendix Table. Isolates from Hospital A and Hospital B were recovered from clinical samples by SA Pathology (Adelaide, South Australia) and by NSW Health Pathology (Sydney, New South Wales), respectively. Bcc isolates from the contaminated chlorhexidine mouthwash and ICU patients were cultured using Blood agar (under CO<sub>2</sub> and anaerobic conditions), Chocolate agar, and Nalidixic acid colistin agar. Environmental samples (hand-basins and bench tops) were collected using liquid amies flocculated swabs and cultured on Blood, MacConkey and *Burkholderia cepacia* selective agar.

#### Genomic DNA Isolation and Sequencing

Genomic DNA from Hospital A and Hospital B isolates was extracted and sequenced on an Illumina NextSeq500 platform (Illumina Inc, California, USA). A closed genome was obtained from isolate A05 through Single Molecule, Real-Time (SMRT) sequencing on a PacBio RS II platform using P6-C4 chemistry (Pacific Bioscience Inc, California, USA) according to the 20 kb template preparation using BluePippin size-selection protocol (Pacific Bioscience).

#### Genomic Analysis

Illumina sequencing reads were processed using the Nullarbor pipeline (<https://github.com/tseemann/nullarbor>). Single-nucleotide polymorphism (SNP) analysis was performed using Snippy v3.1 (<https://github.com/tseemann/snippy>) with isolate A05 as a reference. The reference genome was assembled as described with indels corrected using Illumina sequencing reads (1). A maximum-likelihood phylogenetic tree was created based on the SNP variations in the core genome using PhyML v3.1 (2), with complete and draft genomes

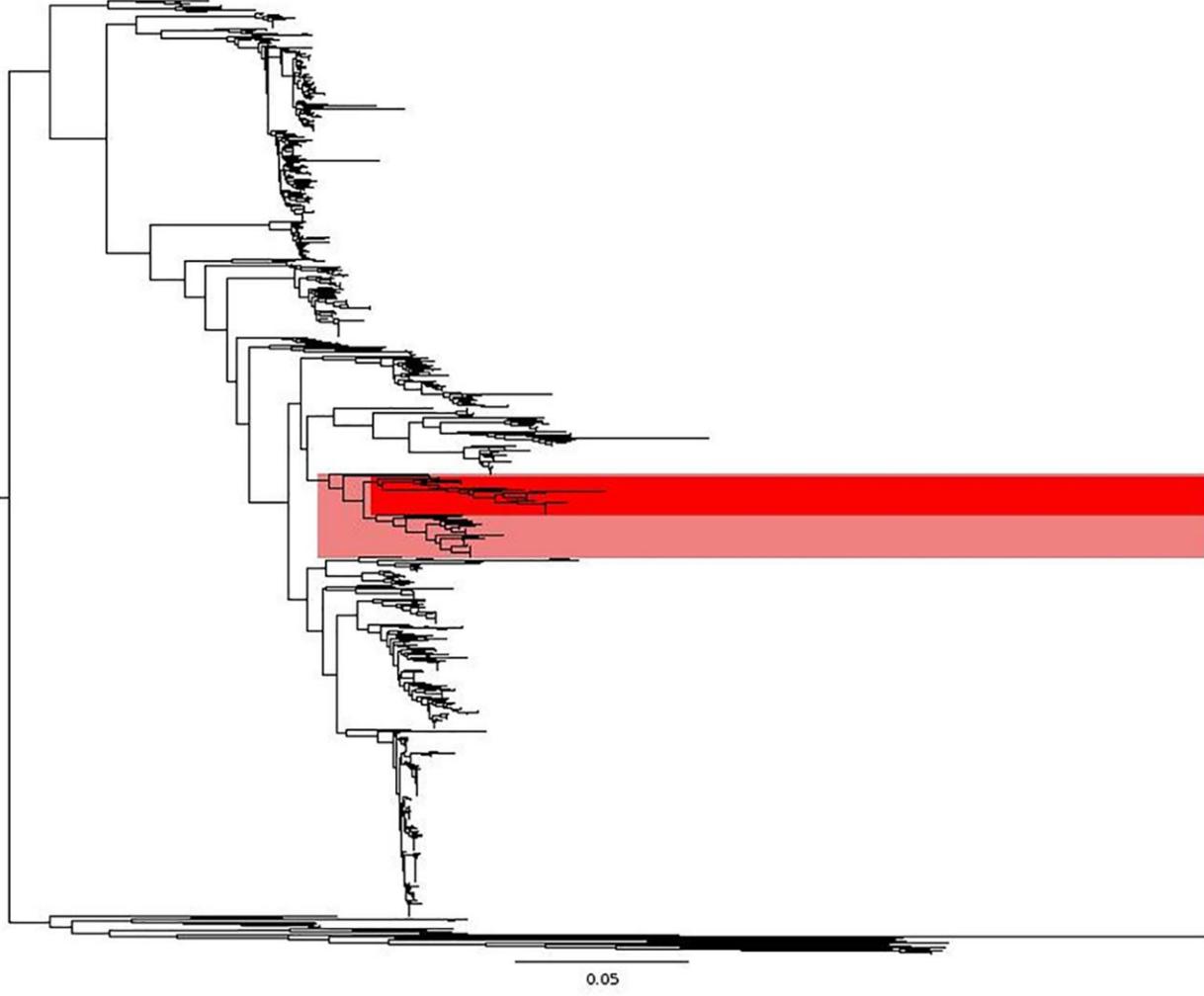
from other Bcc group K strains providing context (*B. lata* strain 383, *B. lata* LK27, *B. lata* LK13, *B. lata* FL-7-5-30-S1-D0, *B. contaminans* MS14, *B. contaminans* FFH2005, *B. contaminans* LTEB, *B. contaminans* LMG23361, *B. contaminans* FFI-28, and *B. metallica* FL-6-5-30-S1-D7). Genes encoding putative efflux pump within the *B. cenocepacia* J2315 genome were used to identify orthologous sequences within Bcc group K (E-value  $\leq 10^{-5}$ ). Multi loci sequence typing (MLST) analysis was performed using MLST (<https://github.com/tseemann/mlst>, version 2.0). To construct a maximum likelihood tree for MLST results, allelic sequences for all Bcc isolates were retrieved from BIGSdb database (3). Orthologous sequences in *B. lata* isolate genomes were identified using HMMer v.3.1b2, aligned to database sequences using MUSCLE (v3.8.1551), and concatenated.

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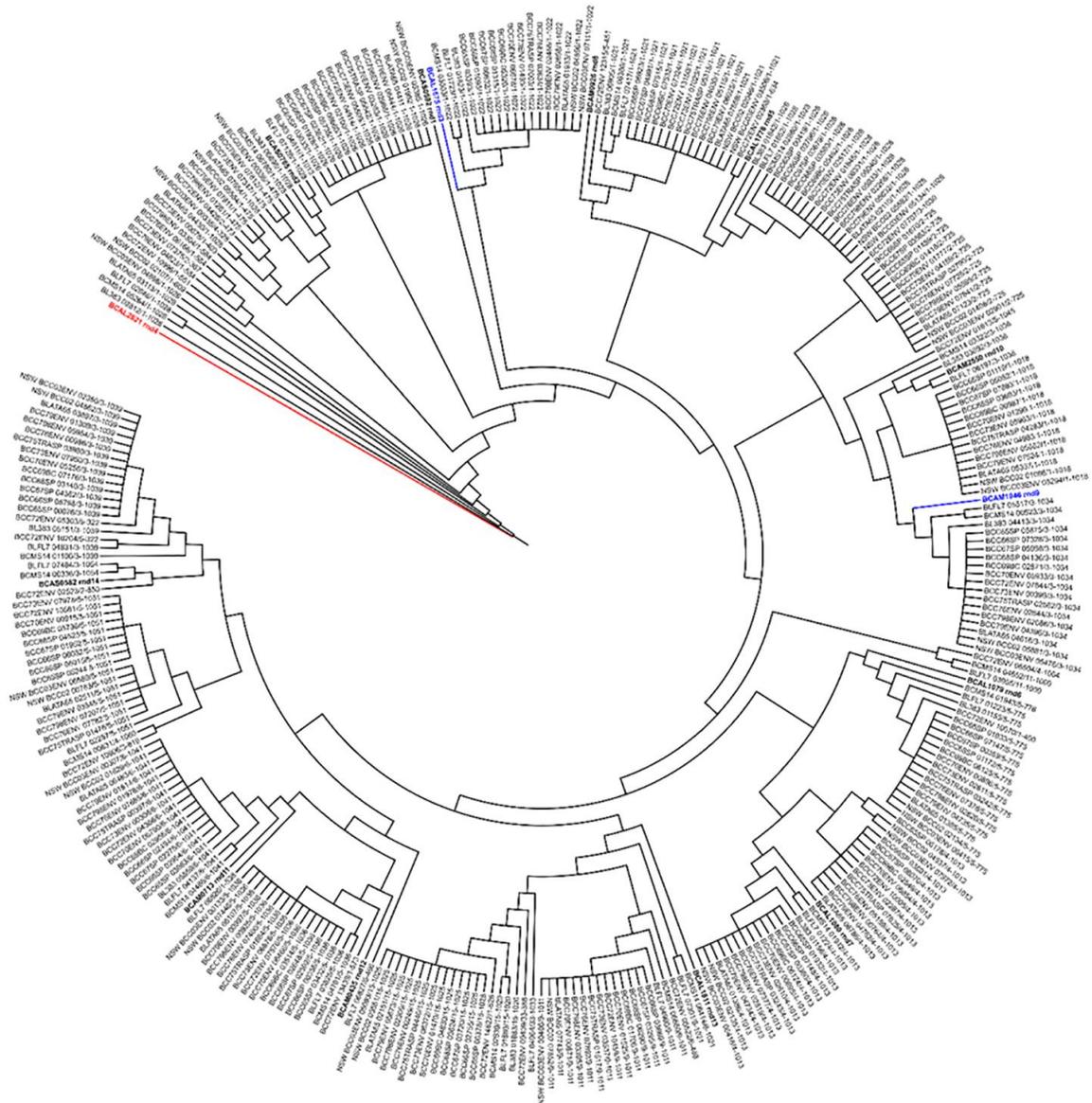
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**Technical Appendix Table.** Outbreak strains of *Burkholderia lata* isolated from clinical samples, contaminated mouthwash bottles and ICU sinks and bench top in two Australian tertiary hospitals

Hospital	Isolate	Room	Specimen	Patient	Initial presentation	Isolation Date
Clinical isolates						
A	01	22	Tracheal aspirate	A1	Pneumonia	15 May 2016
A	02	4,16	Tracheal aspirate	A2	Dissecting aortic aneurysm	14 June 2016
A	03	5	Tracheal aspirate	A3	Aortic dissection	18 June 2016
A	04	21	Tracheal aspirate	A4	Intracranial injury	22 June 2016
A	05	19	Blood	A5	Statin myositis	7 May 2016
A	11	4,9,40	Tracheal aspirate	A6	Angina	26 June 2016
B	01	1	Sputum	B1	Pneumonia	24 June 2016
Nonclinical isolates						
A	06		Mouthwash bottle 1			25 June 2016
A	07	19	Hand basin			20 May 2016
A	08	5	Hand basin			24 May 2016
A	09	21	Hand basin			24 June 2016
A	10	21	Bench top			27 June 2016
A	12		Mouthwash bottle 2			29 June 2016
A	13		Mouthwash bottle 3			29 June 2016
B	02		Mouthwash bottle 4			4 July 2016



**Technical Appendix Figure 1.** MLST phylogenetic tree of the outbreak *Burkholderia lata* from Australasian sublineage. MLST allelic sequences for the all *Burkholderia cepacia* complex were obtained from PUBMLST database {Jolley, 2010 #324}. Aligned allelic sequences were concatenated and maximum likelihood tree was computed using PhyML v3.0 {Guindon, 2010 #325} with parameters of HKY85 as model of nucleotide substitution and BioNJ as initial tree. Group K of the BCC were highlighted in light red, and *B. lata* taxa were highlighted in dark red.



**Technical Appendix Figure 2.** Maximum likelihood tree for the bacterial multidrug efflux pump (RND) proteins of the Bcc Group K. The tree was built with LG as amino acid substitution model against the efflux pumps of *B. cenocepacia* J2135 (highlighted in bold, with RND 3 and 9 in blue, while RND 4 is in red).