



Archived at the Flinders Academic Commons:

<http://dspace.flinders.edu.au/dspace/>

'This is the peer reviewed version of the following article:

Leong, L., Lagana, D., Carter, G. P., Wang, Q., Smith, K.,
Stinear, T. P....Rogers, G. B. (2018). Burkholderia lata
Infections from Intrinsically Contaminated Chlorhexidine
Mouthwash, Australia, 2016. Emerging Infectious Diseases,
24(11), 2109-2111. [https://dx.doi.org/10.3201/
eid2411.171929](https://dx.doi.org/10.3201/eid2411.171929).

which has been published in final form at

<https://doi.org/10.3201/eid2411.171929>

*"Emerging Infectious Diseases is an open access journal in
the public domain"*

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

This study was supported by the National Natural Science Foundation of China (81571963); Science Foundation of Anhui Province of China (1608085MH213); Natural Science Foundation Key Project of Anhui Province Education Department (KJ2015A020, KJ2016A331); and Scientific Research of Anhui Medical University (XJ201314, XJ201430, XJ201503).

About the Authors

Dr. Li is a research coordinator at The First Affiliated Hospital of Anhui Medical University, Hefei, China. His research interests are pathogenic mechanisms of tickborne infectious diseases, including severe fever with thrombocytopenia syndrome, human granulocytic anaplasmosis, and spotted fever group rickettsioses. Dr. Wen Hu is an electron microscope technician at The First Affiliated Hospital of the University of Science and Technology of China, Hefei, China. His research interest is pathogen structure.

References

1. Uchida T, Yu XJ, Uchiyama T, Walker DH. Identification of a unique spotted fever group rickettsia from humans in Japan. *J Infect Dis*. 1989;159:1122–6. <http://dx.doi.org/10.1093/infdis/159.6.1122>
2. Mahara F. Japanese spotted fever: report of 31 cases and review of the literature. *Emerg Infect Dis*. 1997;3:105–11. <http://dx.doi.org/10.3201/eid0302.970203>
3. Chung MH, Lee SH, Kim MJ, Lee JH, Kim ES, Lee JS, et al. Japanese spotted fever, South Korea. *Emerg Infect Dis*. 2006;12:1122–4. <http://dx.doi.org/10.3201/eid1207.051372>
4. Gaywee J, Sunyakumthorn P, Rodkvamtook W, Ruang-areerate T, Mason CJ, Sirisopana N. Human infection with *Rickettsia* sp. related to *R. japonica*, Thailand. *Emerg Infect Dis*. 2007;13:657–9. <http://dx.doi.org/10.3201/eid1304.060585>
5. Uchida T, Yan Y, Kitaoka S. Detection of *Rickettsia japonica* in *Haemaphysalis longicornis* ticks by restriction fragment length polymorphism of PCR product. *J Clin Microbiol*. 1995;33:824–8.
6. Ishikura M, Fujita H, Ando S, Matsuura K, Watanabe M. Phylogenetic analysis of spotted fever group rickettsiae isolated from ticks in Japan. *Microbiol Immunol*. 2002;46:241–7. <http://dx.doi.org/10.1111/j.1348-0421.2002.tb02692.x>
7. Luo LM, Zhao L, Wen HL, Zhang ZT, Liu JW, Fang LZ, et al. *Haemaphysalis longicornis* ticks as reservoir and vector of severe fever with thrombocytopenia syndrome virus in China. *Emerg Infect Dis*. 2015;21:1770–6. <http://dx.doi.org/10.3201/eid2110.150126>
8. Tenquist J, Charleston W. A revision of the annotated checklist of ectoparasites of terrestrial mammals in New Zealand. *J R Soc N Z*. 2001;31:481–542. <http://dx.doi.org/10.1080/03014223.2001.9517666>
9. Lu Q, Yu J, Yu L, Zhang Y, Chen Y, Lin M, et al. *Rickettsia japonica* infections in humans, Zhejiang Province, China, 2015. *Emerg Infect Dis*. 2018 Nov [date cited]. <https://doi.org/10.3201/eid2411.170044>

Address for correspondence: Yan Liu, Anhui Medical University, School of Basic Medical Sciences, Anhui, 230032, China; email: yliu16888@163.com; Bo-Yu Liu; email: centian2004@163.com

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

Lex E.X. Leong, Diana Lagana, Glen P. Carter, Qinning Wang, Kija Smith, Tim P. Stinear, David Shaw, Vitali Sintchenko, Steven L. Wesselingh, Ivan Bastian, Geraint B. Rogers

Author affiliations: South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia (L.E.X. Leong, S.L. Wesselingh, G.B. Rogers); Flinders University, Bedford Park, South Australia, Australia (L.E.X. Leong, G.B. Rogers); Royal Adelaide Hospital, Adelaide (D. Lagana, D. Shaw); University of Melbourne, Melbourne, Victoria, Australia (G.P. Carter, T.P. Stinear); The University of Sydney, Westmead, New South Wales, Australia (Q. Wang, V. Sintchenko); SA Pathology, Adelaide (K. Smith, I. Bastian)

DOI: <https://doi.org/10.3201/eid2411.171929>

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

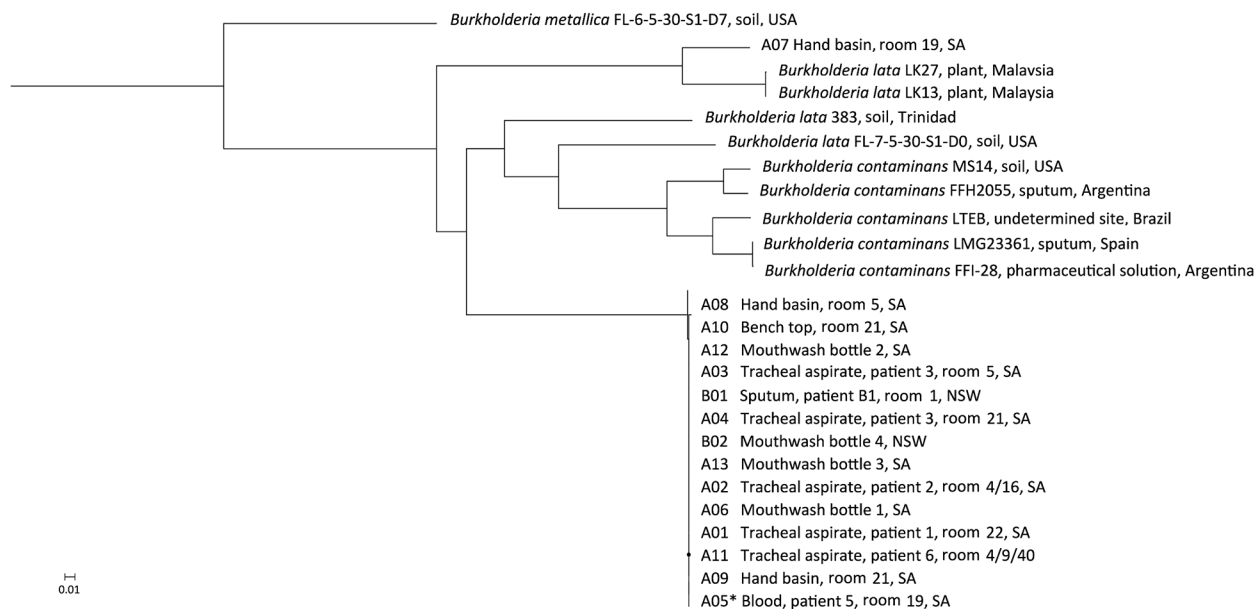


Figure. Phylogenetic analysis of isolates implicated in an outbreak *Burkholderia lata* infection from intrinsically contaminated chlorhexidine mouthwash, Australia, 2016. The maximum-likelihood tree is constructed from core genome single-nucleotide polymorphism alignments ($N = 512,480$) of the outbreak 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.

Acknowledgments

The authors gratefully acknowledge Deborah Williamson and Mark Schultz for their helpful discussions, Trang Nguyen for providing the New South Wales isolates, and microbiological laboratory staff from South Australia Pathology, Pathology North of New South Wales, and Pathology West of New South Wales for their technical input.

About the Author

Dr. Leong is a research scientist at the South Australian Health and Medical Research Institute. His research interests include pathogen genomic epidemiology and infection outbreaks.

References

- Rushton L, Sass A, Baldwin A, Dowson CG, Donoghue D, Mahenthalingam E. Key role for efflux in the preservative susceptibility and adaptive resistance of *Burkholderia cepacia* complex bacteria. *Antimicrob Agents Chemother*. 2013;57:2972–80. <http://dx.doi.org/10.1128/AAC.00140-13>
- Pope CE, Short P, Carter PE. Species distribution of *Burkholderia cepacia* complex isolates in cystic fibrosis and non-cystic fibrosis patients in New Zealand. *J Cyst Fibros*. 2010;9:442–6. <http://dx.doi.org/10.1016/j.jcf.2010.08.011>
- Medina-Pascual MJ, Valdezate S, Carrasco G, Villalón P, Garrido N, Saéz-Nieto JA. Increase in isolation of *Burkholderia contaminans* from Spanish patients with cystic fibrosis. *Clin Microbiol Infect*. 2015;21:150–6. <http://dx.doi.org/10.1016/j.cmi.2014.07.014>
- Martina P, Bettiol M, Vescina C, Montanaro P, Mannino MC, Prieto CI, et al. Genetic diversity of *Burkholderia contaminans* isolates from cystic fibrosis patients in Argentina. *J Clin Microbiol*. 2013;51:339–44. <http://dx.doi.org/10.1128/JCM.02500-12>
- Oie S, Kamiya A. Microbial contamination of antiseptics and disinfectants. *Am J Infect Control*. 1996;24:389–95. [http://dx.doi.org/10.1016/S0196-6553\(96\)90027-9](http://dx.doi.org/10.1016/S0196-6553(96)90027-9)
- Knapp L, Rushton L, Stapleton H, Sass A, Stewart S, Amezcuita A, et al. The effect of cationic microbicide exposure against *Burkholderia cepacia* complex (Bcc): the use of *Burkholderia lata* strain 383 as a model bacterium. *J Appl Microbiol*. 2013;115:1117–26. <http://dx.doi.org/10.1111/jam.12320>
- Drevinek P, Holden MT, Ge Z, Jones AM, Ketchell I, Gill RT, et al. Gene expression changes linked to antimicrobial resistance, oxidative stress, iron depletion and retained motility are observed when *Burkholderia cenocepacia* grows in cystic fibrosis sputum. *BMC Infect Dis*. 2008;8:121. <http://dx.doi.org/10.1186/1471-2334-8-121>
- George AM, Jones PM, Middleton PG. Cystic fibrosis infections: treatment strategies and prospects. *FEMS Microbiol Lett*. 2009;300:153–64. <http://dx.doi.org/10.1111/j.1574-6968.2009.01704.x>
- Coenye T, Van Acker H, Peeters E, Sass A, Buroni S, Riccardi G, et al. Molecular mechanisms of chlorhexidine tolerance in *Burkholderia cenocepacia* biofilms. *Antimicrob Agents Chemother*. 2011;55:1912–9. <http://dx.doi.org/10.1128/AAC.01571-10>
- Martin M, Christiansen B, Caspari G, Hogardt M, von Thomsen AJ, Ott E, et al. Hospital-wide outbreak of *Burkholderia contaminans* caused by prefabricated moist washcloths. *J Hosp Infect*. 2011;77:267–70. <http://dx.doi.org/10.1016/j.jhin.2010.10.004>

Address for correspondence: Lex Leong, South Australian Health and Medical Research Institute, North Terrace, Adelaide, South Australia 5000, Australia; email: lex.leong@sahmri.com

Estimating Latent Tuberculosis Infection Using Interferon- γ Release Assay, Japan

Tomoyasu Nishimura, Masaki Ota, Masaaki Mori, Naoki Hasegawa, Hiroshi Kawabe, Seiya Kato

Author affiliations: Keio University, Tokyo, Japan (T. Nishimura, M. Mori, H. Kawabe); Research Institute of Tuberculosis—Japan Anti-Tuberculosis Association, Tokyo (M. Ota, S. Kato); Keio University School of Medicine, Tokyo (N. Hasegawa)

DOI: <https://doi.org/10.3201/eid2411.171948>

We estimated the latent tuberculosis infection (LTBI) rate for foreign-born students at Keio University, Tokyo, Japan, using an interferon- γ 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.