Transmission of Legionnaires' Disease through Toilet Flushing

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We describe 2 cases of healthcare-associated Legionnaires' disease in patients in France hospitalized 5 months apart in the same room. Whole-genome sequencing analyses showed that clinical isolates from the patients and isolates from the room's toilet clustered together. Toilet contamination by *Legionella pneumophila* could lead to a risk for exposure through flushing.

Legionella pneumophila is a gram-negative bacterium usually found in small amounts in water in both nature and built environments. In larger amounts, it can be responsible for a severe pneumonia known as Legionnaires' disease (LD). Transmission usually occurs when someone inhales contaminated aerosols from showers, cooling towers, faucets, or fountains. Person-to-person transmission is extremely rare (*1*). Researchers have shown evidence of a variety of other uncommon sources of contamination, such as windshield washer fluid (*2*) or dental unit waterlines (*3*). LD transmission through flushing toilets has also been suspected (*4*) but not demonstrated. We report 2 cases of LD in immunocompromised patients in France, potentially caused by *L. pneumophila* transmission through flushing toilets.

The Study

In the first case, an 18-year-old woman who had undergone an allogeneic bone marrow transplant for acute myeloid leukemia in February 2014 in a hospital hematology unit in France was hospitalized in December 2015 in the same unit for 9 days. At that time, she received immunosuppressive agents (steroids, cyclosporine A, and ruxolitinib) for a chronic graft-versus-host disease. She was readmitted to the hematology unit with fever (38.5°C), shivering, and dyspnea 6 days after discharge. Six days later, she was transferred to the medical intensive care unit (MICU) of the same hospital with fever (40°C), bilateral pneumonia, and respiratory and renal failure. The result from a *L. pneumophila* urinary antigen test (BinaxNOW Legionella Urinary Antigen EIA kit; Abbott, https://www.abbott.com) was positive at the time of MICU admission, leading clinicians to consider this LD case to be probably healthcare associated. Physicians successfully treated the patient with spiramycin and levofloxacin, and she was discharged from the MICU after 8 days.

In the second case, a 51-year-old man was admitted to the same hematology unit in May 2016 for an autologous transplant for a recurrent Hodgkin lymphoma. A persistent fever (38.8°C) appeared on the 12th day after admission and a computed tomography scan of the chest showed a multifocal consolidation. The result from a *L. pneumophila* urinary antigen test (BinaxNOW) on day 22 was positive; therefore, this LD case was considered to be definitely healthcare associated. The patient's condition suddenly worsened, and he was transferred to the MICU, where he rapidly recovered after doctors treated him with spiramycin and levofloxacin.

For both patients, a bronchoalveolar lavage procedure found *L. pneumophila* bacteria from serogroup 1 (LP1), pulsed-field gel electrophoresis pulsotype Paris, sequence type (ST) 1, monoclonal antibody subgroup Philadelphia. Both patients had been hospitalized in the same room (room 1) of the hematology unit, 5 months apart. Air filtration systems with HEPA filters were used to control the environment of the unit. Water from the sink in each room and from the shower, shared by all of the unit's patients, was filtered through 0.1-µm pore filters. Both patients were provided only bottled water and did not take

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showers during their hospital stay. In addition, there was no cooling tower within the hospital.

We sampled and analyzed the potential sources of exposure in accordance with the NF T 90-431 standard (Association Française de Normalisation, https://www.afnor.org) for counting *Legionella* spp., after the second LD case. In brief, we sampled 500 mL of water in sterile vials containing 20 mg sodium thiosulfate. First, we inoculated 0.2 mL of water on GVPC (glycine, vancomycin, polymyxin, cycloheximide) plates (Oxoid France, http://www.oxoid.com/fr/blue/). Then, we filtered 10 mL and 100 mL of water through 0.2-µm pore polycarbonate membranes placed on GVPC media. We incubated plates at 36°C (± 2°C) for 8–11 days. We subcultured suspicious colonies on buffered charcoal yeast extract media with and without cystein and identified them by latex agglutination (Oxoid France).

We found no *L. pneumophila* in the hot water from the shared shower (temperature 26.0°C, chlorine 1.12 mg/L), nor in the hot or cold water from the sink in room 1 (temperatures 24°C and 25°C, chlorine 1.11 mg/L and 0 mg/L, respectively). However, sampling of the water from the toilet bowl in room 1 showed contamination (1,100 CFU/L LP1, with a temperature of 22.0°C and no chlorine). LP1 was also in the toilet bowl of the adjacent room (room 2) (100 CFU/L, temperature 24.4°C), the hot water from the sink in the nurses' office (10 CFU/L, temperature 49.8°C, chlorine 0.77 mg/L), and the cold-water inlet of the building (20 CFU/L, temperature 15.0°C). Contamination in the cold-water inlet (20 CFU/L, temperature 9.0°C) had also been detected in March 2016. Tests of samples of toilet water from tanks in nearby rooms did not show any contamination.

The room was closed, and the toilet was disinfected daily with bleach. The toilet water in the room was

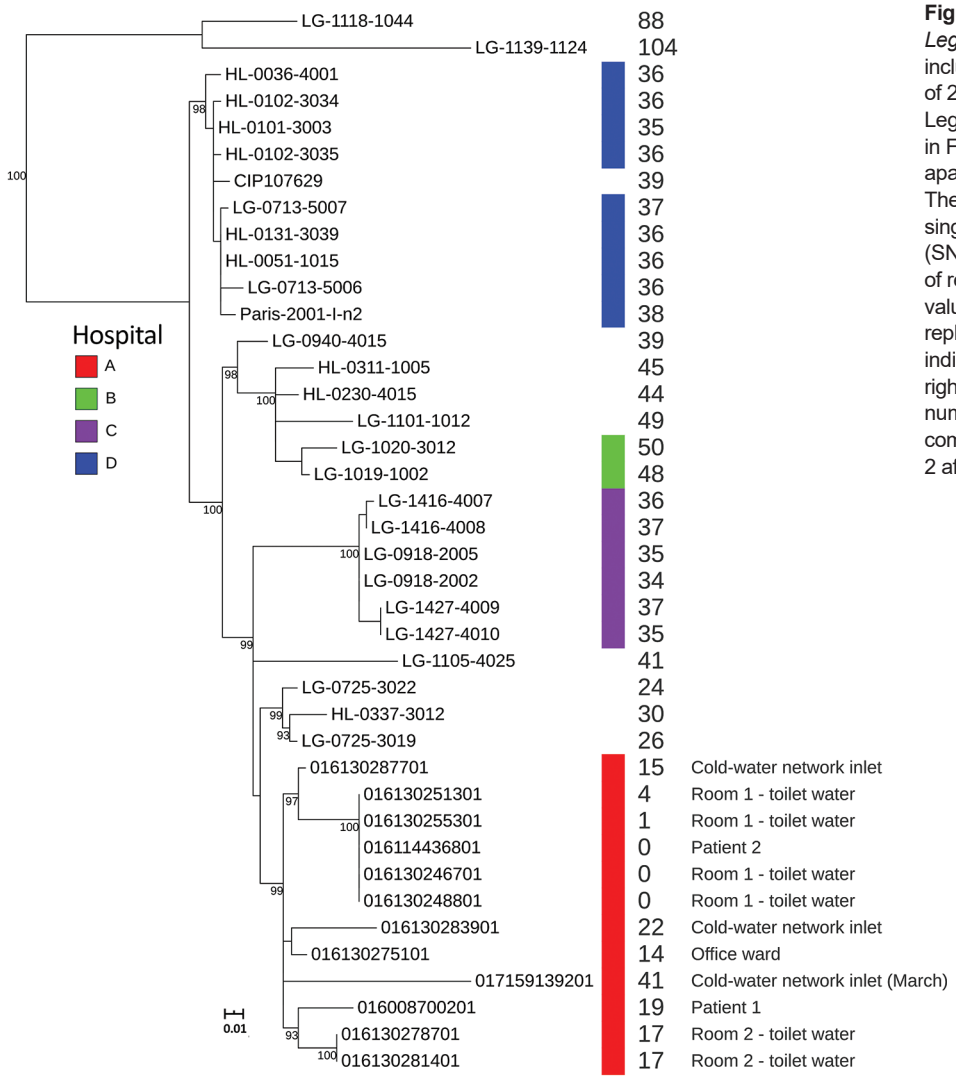

Figure. Maximum-likelihood tree of 39 *Legionella pneumophila* ST1 isolates, including isolates from investigation of 2 cases of healthcare-associated Legionnaires' disease in patients in France hospitalized 5 months apart in the same room (red bar). The tree was constructed using 258 single-nucleotide polymorphisms (SNPs) identified after the removal of recombination events. Bootstrap values were calculated from 500 replicates; only values >90 were indicated on the tree. Values at the right side of the tree represent the number of SNPs between the genome compared with the genome of patient 2 after recombination removal.

monitored through iterative testing; results were negative from 10 successive samples tested between June 2016 and November 2017. To determine the extent of such contamination, 29 toilets in 5 different hospital buildings were analyzed. All samples were negative, suggesting that *L. pneumophila* contamination of toilet water was not common.

We used Nextera XT technology (Illumina, https://www.illumina.com) for whole-genome sequencing of 2 clinical and 10 environmental LP1 strains and deposited raw reads into the European Nucleotide Archive (study accession no. PRJEB32615). We used SPAdes to assemble genomes (*5*) and mompS tool to extract STs from the WGS data (*6*). All 12 strains belonged to ST1. We analyzed these 12 strains in more depth by comparing their genomes to 27 other epidemiologically unrelated LP1 ST1 genomes from France available in the Sequence Read Archive database (Figure; Appendix, https://wwwnc.cdc.gov/EID/article/26/7/19-0941-App1.pdf). We also performed phylogenetic analyses on this genome's dataset, as described by David et al. (*7*).

The 2 clinical isolates were nested within and thus derived from the clade of isolates sampled from the hospital water network. All 12 strains, including the strains isolated in the cold-water inlet, were part of a cluster sharing the same most recent common ancestor (Figure). We observed no difference in single-nucleotide polymorphisms (SNPs) between the 2 isolates from the toilet water from the patients' room and the isolate of the second patient (Figure). The strains present in the toilet water were identical or closely related to the strains infecting the patients, and no other potential contamination source was identified, strongly suggesting that the toilet water was the contamination source. The few differences in SNPs between the first clinical isolate and the isolates found in the environment (0–23 SNPs; see Appendix Table) could be explained by diversity of the ST1 population in the water network or the micro-evolution of the environmental LP1 population during the 5 months between the 2 LD cases (*7*).

Conclusions

We describe 2 cases in which LD was probably caused by *L. pneumophila* transmitted through contaminated toilet water that became aerosolized during flushing. We reached this conclusion because we found little to no detectable difference between whole genomes in isolates obtained from 2 patients hospitalized 5 months apart in the same room and those from the toilet in that room. The other commonly suspected sources, in this case the shower and the sink, tested negative for *L. pneumophila*.

This investigation suggests that transmission of *L. pneumophila* through toilet flushing should be considered when investigating a LD case. However, as previously suggested, there remains a need for a laboratory-based study to explore whether flushing toilets can generate and spread contaminated aerosols (*8*,*9*).

About the Author

Dr. Couturier is a medical biologist at the Saint-Antoine Hospital, and a teaching assistant at the René Descartes Faculty of Pharmacy, Paris, France. Her research interests include microbiology and hospital hygiene.

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Appendix

Phylogenetic Analyses

The genomes were compared by mapping the reads against the reference genome NC_006368.1 of the strain Paris CIP107629. The reads were mapped using snippy (https://github.com/tseemann/snippy) with a minimum depth coverage of 10x. A total of 3,087 core SNPs were identified. SNPs inferred as being recombination events were removed from the full alignment generated by snippy using Gubbins (*1*). The remaining 258 SNPs were used to construct an unrooted maximum likelihood phylogenetic tree based on the GTRGAMMA model using RAxML https://github.com/stamatak/standard-RAxML (*2*). A total of 500 bootstraps were performed to assess the robustness of the tree (Figure). An all-against-all pairwise identity analysis was also performed using the alignment generated by Gubbins. The resulting distance matrix is available in the table below.

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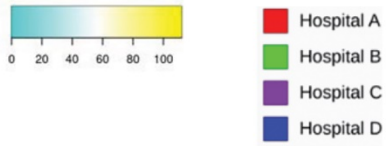
Isolates ID	016008700201	016114436801	016130246701	016130248801	016130251301	016130255301	016130275101	016130278701	016130281401	016130283901	016130287701	017159139201	LG-1427-4009	LG-1427-4010	LG-1416-4007	LG-0918-2005	LG-0918-2002	LG-1416-4008	LG-1019-1002	LG-1020-3012	HL-0131-3039	LG-0713-5006	HL-0051-1015	HL-0102-3034	HL-0102-3035	Paris-2001-I-n2	LG-0713-5007	HL-0101-3003	HL-0036-4001	LG-0725-3022	LG-0725-3019	HL-0230-4015	HL-0311-1005	HL-0337-3012	LG-0940-4015	LG-1101-1012	LG-1105-4025	LG-1118-1044	LG-1139-1124	CIP107629
016008700201	0	19	19	19	23	20	13	12	12	21	18	39	36	34	35	34	33	36	47	49	35	35	35	35	35	37	36	34	35	23	25	43	44	29	38	48	40	87	103	38
016114436801	19	0	0	0	4	1	14	17	17	22	15	41	37	35	36	35	34	37	48	50	36	36	36	36	36	38	37	35	36	24	26	44	45	30	39	49	41	88	104	39
016130246701	19	0	0	0	4	1	14	17	17	22	15	41	37	35	36	35	34	37	48	50	36	36	36	36	36	38	37	35	36	24	26	44	45	30	39	49	41	88	104	39
016130248801	19	0	0	0	4	1	14	17	17	22	15	41	37	35	36	35	34	37	48	50	36	36	36	36	36	38	37	35	36	24	26	44	45	30	39	49	41	88	104	39
016130251301	23	4	4	4	0	3	18	21	21	25	11	40	35	33	34	33	32	35	46	48	34	34	34	34	34	36	35	33	34	22	24	42	43	28	37	47	39	86	102	35
016130255301	20	1	1	1	3	0	15	18	18	22	14	40	38	36	37	36	35	38	49	51	37	37	37	37	37	39	38	36	37	25	27	45	46	31	40	50	42	89	105	38
016130275101	13	14	14	14	18	15	0	11	11	14	13	33	31	29	30	29	26	31	41	43	29	28	29	29	29	31	30	27	29	18	20	37	38	24	32	42	35	81	97	32
016130278701	12	17	17	17	21	18	11	0	0	19	16	38	34	32	33	32	31	34	45	47	33	33	33	33	33	35	34	32	33	21	23	41	42	27	36	46	38	85	101	36
016130281401	12	17	17	17	21	18	11	0	0	19	16	38	34	32	33	32	31	34	45	47	33	33	33	33	33	35	34	32	33	21	23	41	42	27	36	46	38	85	101	36
016130283901	21	22	22	22	25	22	14	19	19	0	20	42	39	37	38	37	36	39	50	52	38	38	38	38	38	40	39	37	38	26	28	46	47	32	41	51	43	89	105	40
016130287701	18	15	15	15	11	14	13	16	16	20	0	34	28	26	27	26	25	28	37	39	27	27	27	27	27	29	28	26	27	15	17	35	36	21	30	40	32	79	95	27
017159139201	39	41	41	41	40	40	33	38	38	42	34	0	49	49	50	47	48	47	56	58	45	50	45	45	45	47	46	43	45	37	36	52	53	40	47	57	51	96	112	46
LG-1427-4009	36	37	37	37	35	38	31	34	34	39	28	49	0	2	5	3	5	4	43	45	31	35	31	31	31	33	32	30	31	23	23	39	40	27	34	44	36	82	98	34
LG-1427-4010	34	35	35	35	33	36	29	32	32	37	26	49	2	0	3	2	3	4	43	45	31	33	31	31	31	33	32	30	31	22	23	39	40	27	34	44	36	82	98	34
LG-1416-4007	35	36	36	36	34	37	30	33	33	38	27	50	5	3	0	3	4	3	44	46	32	34	32	32	32	34	33	31	32	23	24	40	39	28	35	45	37	81	97	35
LG-0918-2005	34	35	35	35	33	36	29	32	32	37	26	47	3	2	3	0	3	2	41	43	29	33	29	29	29	31	30	28	29	20	21	37	38	25	32	42	34	80	96	32
LG-0918-2002	33	34	34	34	32	35	26	31	31	36	25	48	5	3	4	3	0	5	43	45	31	30	31	31	31	33	32	29	31	22	24	39	40	28	34	44	37	82	98	34
LG-1416-4008	36	37	37	37	35	38	31	34	34	39	28	47	4	4	3	2	5	0	41	43	29	35	29	29	29	31	30	28	29	22	21	37	38	25	32	42	34	79	95	32
LG-1019-1002	47	48	48	48	46	49	41	45	45	50	37	56	43	43	44	41	43	41	0	6	30	37	30	30	30	32	31	30	30	33	32	14	15	36	19	19	43	79	92	32
LG-1020-3012	49	50	50	50	48	51	43	47	47	52	39	58	45	45	46	43	45	43	6	0	33	40	33	33	33	35	34	33	33	35	34	16	17	38	22	20	45	82	96	35
HL-0131-3039	35	36	36	36	34	37	29	33	33	38	27	45	31	31	32	29	31	29	30	33	0	7	0	2	2	2	1	2	4	21	20	28	27	24	19	32	32	61	77	5
LG-0713-5006	35	36	36	36	34	37	28	33	33	38	27	50	35	33	34	33	30	35	37	40	7	0	7	9	9	9	8	7	11	24	26	35	34	30	26	39	38	68	84	12
HL-0051-1015	35	36	36	36	34	37	29	33	33	38	27	45	31	31	32	29	31	29	30	33	0	7	0	2	2	2	1	2	4	21	20	28	27	24	19	32	32	61	77	5
HL-0102-3034	35	36	36	36	34	37	29	33	33	38	27	45	31	31	32	29	31	29	30	33	2	9	2	0	2	4	3	2	4	21	20	28	27	24	19	32	32	61	77	5
HL-0102-3035	35	36	36	36	34	37	29	33	33	38	27	45	31	31	32	29	31	29	30	33	2	9	2	2	0	4	3	2	4	21	20	28	27	24	19	32	32	61	77	5
Paris-2001-I-n2	37	38	38	38	36	39	31	35	35	40	29	47	33	33	34	31	33	31	32	35	2	9	2	4	4	0	3	4	6	23	22	30	29	26	21	34	34	63	79	7
LG-0713-5007	36	37	37	37	35	38	30	34	34	39	28	46	32	32	33	30	32	30	31	34	1	8	1	3	3	3	0	3	5	22	21	29	28	25	20	33	33	62	78	6
HL-0101-3003	34	35	35	35	33	36	27	32	32	37	26	43	30	30	31	28	29	28	30	33	2	7	2	2	2	4	3	0	4	20	19	28	27	23	19	32	31	61	77	5
HL-0036-4001	35	36	36	36	34	37	29	33	33	38	27	45	31	31	32	29	31	29	30	33	4	11	4	4	4	6	5	4	0	21	20	28	27	24	19	32	32	61	77	7
LG-0725-3022	23	24	24	24	22	25	18	21	21	26	15	37	23	22	23	20	22	22	33	35	21	24	21	21	21	23	22	20	21	0	5	29	30	9	24	34	26	73	89	24
LG-0725-3019	25	26	26	26	24	27	20	23	23	28	17	36	23	23	24	21	24	21	32	34	20	26	20	20	20	22	21	19	20	5	0	28	29	6	23	33	25	72	88	23
HL-0230-4015	43	44	44	44	42	45	37	41	41	46	35	52	39	39	40	37	39	37	14	16	28	35	28	28	28	30	29	28	28	29	28	0	11	32	17	17	41	77	90	31
HL-0311-1005	44	45	45	45	43	46	38	42	42	47	36	53	40	40	39	38	40	38	15	17	27	34	27	27	27	29	28	27	27	30	29	11	0	33	16	17	42	74	90	30
HL-0337-3012	29	30	30	30	28	31	24	27	27	32	21	40	27	27	28	25	28	25	36	38	24	30	24	24	24	26	25	23	24	9	6	32	33	0	27	37	29	75	92	27
LG-0940-4015	38	39	39	39	37	40	32	36	36	41	30	47	34	34	35	32	34	32	19	22	19	26	19	19	19	21	20	19	19	24	23	17	16	27	0	21	35	68	84	22
LG-1101-1012	48	49	49	49	47	50	42	46	46	51	40	57	44	44	45	42	44	42	19	20	32	39	32	32	32	34	33	32	32	34	33	17	17	37	21	0	46	81	97	35
LG-1105-4025	40	41	41	41	39	42	35	38	38	43	32	51	36	36	37	34	37	34	43	45	32	38	32	32	32	34	33	31	32	26	25	41	42	29	35	46	0	84	100	35
LG-1118-1044	87	88	88	88	86	89	81	85	85	89	79	96	82	82	81	80	82	79	79	82	61	68	61	61	61	63	62	61	61	73	72	77	74	75	68	81	84	0	44	64
LG-1139-1124	103	104	104	104	102	105	97	101	101	105	95	112	98	98	97	96	98	95	92	96	77	84	77	77	77	79	78	77	77	89	88	90	90	92	84	97	100	44	0	80
CIP107629	38	39	39	39	35	38	32	36	36	40	27	46	34	34	35	32	34	32	32	35	5	12	5	5	5	7	6	5	7	24	23	31	30	27	22	35	35	64	80	0



Appendix Figure. Distance matrix from the all-against-all pairwise identity analysis of SNPs between isolates after recombination events removal using Gubbins. Colors of the firsts raw and column represent the different hospitals. Colors gradient represents a heat map according to SNPs number.