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High-risk clones of *Pseudomonas aeruginosa* contaminate the drinking water networks of French cities

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Pseudomonas aeruginosa is a major opportunistic pathogen responsible for severe infections in immunocompromised patients. The contamination of drinking water networks (DWNs) with this pathogen is underestimated, as it is mostly in the state of persister cells undetected by the recommended monitoring technique. We collected water samples from eight cities distant from each other and searched for *P. aeruginosa* using a culture-based method that resuscitates persister cells. The genomes of isolates were sequenced. Five DWNs of the eight tested (62.5%) were contaminated with *P. aeruginosa*, of which four were contaminated with high-risk clones (ST308, ST395). Surprisingly, the ST308 isolates retrieved from the four independent and distant DWNs were clonal. Most *P. aeruginosa* isolates shared a genomic island conferring tolerance to copper-ions. The population structure of the collection may result from both a common source of contamination by plumbing supplies and the selection of clones sharing genetic elements that presumably aided their propagation in DWNs.

Pseudomonas aeruginosa is an opportunistic pathogen responsible for severe infections in immunocompromised patients[1–3]. This species is ubiquitous in soil, water, and other moist environments, including drinking water networks (DWNs) and those in hospitals. *P. aeruginosa* often contaminates the distal parts of water distribution systems, such as taps, sinks, showers, and U-bends, also called sink traps[2,4,5], and can thus contaminate patients through water, leading to waterborne nosocomial infections[6–8]. It is responsible for a broad range of diseases, including respiratory infections in mechanically ventilated patients, bloodstream infections, and infections of wounds from burn injuries, which can lead to death[9]. Outside of hospitals, tap water is a source of contamination by *P. aeruginosa* for nebulizers used by cystic-fibrosis patients[10].

European regulations require monitoring for *P. aeruginosa* in DWNs by immediate filtration and culture on agar media[11,12]. However, this method does not detect persister cells[13]. Indeed, water distribution systems are hostile environments for bacterial development, with nutrient-poor conditions and the presence of inhibitors (copper ions, chlorine). This favors the development of persister cells, also called viable but not-culturable, that are no longer culturable on media upon which they are usually able to grow[14,15]. Although not culturable, these cells can be resuscitated and recover their full virulence under suitable conditions[16].

Using a culture-based method that resuscitates persister cells, we previously found that a copper-tolerant high-risk clone of *P. aeruginosa* ST308 contaminated the DWN of a University Hospital in France[5]. This clone was tolerant to copper due to a genomic island (called GI-7) that probably originated from environmental *Pseudomonas* spp[5].

The source of contamination of DWNs with *P. aeruginosa* is still unclear. This pathogen can contaminate drinking water reservoirs[17] but water treatment is usually efficient in removing it before the water enters DWNs[18]. By contrast, *P. aeruginosa* has been repeatedly isolated from water meters prior to their installation[19].

Whether the presence of a high-risk clone in the plumbing system of such premises was an isolated event or a concern for other DWNs is unknown. Here, we searched for *P. aeruginosa* in eight independent and distant DWNs in France, including both healthcare and community DWNs. We determined the population structure of the bacterial isolates using genome-based typing, allowing the identification of genetic elements shared by *P. aeruginosa* that contaminate DWNs and providing clues about the source of contamination.

Results and discussion

Contamination of drinking water networks by *P. aeruginosa* in France

Using a method that resuscitates persister cells, we previously detected high-risk clones of *P. aeruginosa* in the DWNs of a hospital and a long-term care

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Table 1 | Description of water samples and characteristics of the *Pseudomonas aeruginosa* isolates retrieved from eight drinking water networks in France between January 2019 and November 2020

Characteristics of the water samples						Characteristics of the isolates				Reference
City	Location	Sampling point	Date	*N*	Positive samples (n)	ID	Isolation date	ST	SRA	
Amiens	Hosp.	Water inlets 1, 2	Jun.-Sep. 2020	6	0	-	-	-	-	-
		Buildings 1, 2	Jun.-Sep. 2020	30	0	-	-	-	-	
Angers	Hosp.	Water inlets 1, 2	Jun.-Sep. 2020	6	0	-	-	-	-	-
		Building 1	Jun.-Sep. 2020	15	0	-	-	-	-	
		Building 2	Jun.-Sep. 2020	15	3	wPA-Ang1	Jul. 2020	ST27	SAMN25995947	This study
						wPA-Ang2	Jul. 2020	ST27	SAMN25995948	
						wPA-Ang3	Jun. 2020	ST606	SAMN25995949	
Besançon	Hosp.	Water inlet 1	*May-Aug. 2017*	*11*	*2*	*wPA-Bes1*	*Jul. 2017*	*ST308*	*SAMN25995944*	5
						wPA-Bes2	*Aug. 2017*	*ST308*	-	
			Jan.-Mar. 2019	3	0	-	-	-	-	-
		Water inlet 2	*May-Aug. 2017*	*11*	*2*	*wPA-Bes4*	*May 2017*	*ST308*	*SAMN25995943*	5
						wPA-Bes5	*Jul. 2017*	*ST308*	-	
			Jan.-Mar. 2019	3	0	-	-	-	-	
		Building 1	*May-Aug. 2017*	*33*	*2*	*wPA-Bes6*	*May 2017*	*ST308*	-	5
						wPA-Bes7	*Jul. 2017*	*ST308*	-	
			Jan.-Mar. 2019	3	0	-	-	-	-	-
		Building 2	*May-Aug. 2017*	*44*	*2*	*wPA-Bes8*	*Jul. 2017*	*ST308*	-	5
						wPA-Bes9	*Aug. 2017*	*ST308*		
			Jan.-Mar. 2019	3	0	-	-	-	-	-
		Building 3	*May-Aug. 2017*	*22*	*2*	*wPA-Bes10*	*Jul. 2017*	*ST308*	-	5
						wPA-Bes11	*Jul. 2017*	*ST308*	-	
			Jan.-Mar. 2019	3	0	-	-	-	-	-
		Building 4	*May-Aug. 2017*	*11*	*1*	*wPA-Bes12*	*Jul. 2017*	*ST308*	*SAMN25995945*	5
			Jan.-Mar. 2019	3	0	-	-	-	-	-
	LTCF	Water point-of-use	*Oct. 2018*	*35*	*1*	*wPA-Bes13*	*Oct. 2018*	*ST309*	*SRS7847408*	20
						wPA-Bes14	*Oct. 2018*	*ST309*	*SRS7847412*	
	Comm.	House 1	Jan.-Mar. 2019	3	2	- wPA-Bes15	Feb. 2019 Mar. 2019	ST395 ST395	- SAMN25995951	This study
		House 2	Jan.-Mar. 2019	3	1	wPA-Bes16	Mar. 2019	ST395	SAMN25995950	
		Houses 3 to 5	Jan.-Mar. 2019	9	0	-	-	-	-	-
Bordeaux	Hosp.	Water inlets 1, 2	Feb.-Apr. 2019	6	0	-	-	-	-	-
		Buildings 1, 2	Feb.-Apr. 2019	12	0	-	-	-	-	-
	Comm.	Houses 1, 2, 4	Feb.-Apr. 2019	9	0	-	-	-	-	-
		House 3	Feb.-Apr. 2019	3	1	wPA-Bor1	Apr. 2019	ST308	SAMN25995954	This study
		House 5	Feb.-Apr. 2019	3	1	wPA-Bor2	Apr. 2019	ST308	SAMN25995955	
Nantes	Hosp.	Water inlets 1, 2	Sep.-Nov. 2020	6	0	-	-	-	-	-
		Building 1	Sep.-Nov. 2020	15	1	wPA-Nan1	Oct. 2020	ST308	SAMN25995946	This study

Table 1 (continued) | Description of water samples and characteristics of the Pseudomonas aeruginosa isolates retrieved from eight drinking water networks in France between January 2019 and November 2020

Characteristics of the water samples						Characteristics of the isolates				Reference
City	Location	Sampling point	Date	*N*	Positive samples (n)	ID	Isolation date	ST	SRA	
Nîmes	Hosp.	Water inlets 1, 2	Feb.-Apr. 2019	6	0	-	-	-	-	-
		Building 1	Feb.-Apr. 2019	9	3	wPA-Nim1	Apr. 2019	ST3299	SAMN25995952	This study
						wPA-Nim2-	Feb. 2019 Apr. 2019	ST308 ST308	SAMN25995953-	
		Building 2	Feb.-Apr. 2019	9	0	-	-	-	-	-
	Comm.	Houses 1 to 3	Feb.-Apr. 2019	9	0	-	-	-	-	-
Tours	Hosp.	Water inlet 1	Feb.-Mar. 2019	3	0	-	-	-	-	-
		Building 1	Feb.-Mar. 2019	9	0	-	-	-	-	
	Comm.	Houses 1 to 3	Feb.-Mar. 2019	9	0	-	-	-	-	-
Trévenans	Hosp.	Water inlet 1	Jan.-Mar. 2019	3	0	-	-	-	-	-
		Building 1	Jan.-Mar. 2019	9	0	-	-	-	-	
	Comm.	Houses 1 to 5	Jan.-Mar. 2019	14	0	-	-	-	-	-

Isolates retrieved from previous sampling campaigns in 2017 and 2018 are indicated in italics[5]. All isolates were fully susceptible to ticarcillin, ticarcillin-clavulanate, piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, gentamicin, tobramycin, amikacin, and ciprofloxacin. The ID of the isolates consists of the concatenation of wPA (water *P. aeruginosa*), the three initials of the city, and the number of the isolate.
ID identification, *ST* sequence type, *Hosp.* hospital, *LTCF* long-term care facilities, *Comm.*, community.

facility, both located in Besançon (France), in 2017–2018[5,20] (Table 1, Fig. 1). We tested the extent of such contamination throughout the country by broadening our sampling to seven other French cities and retesting the DWNs of Besançon. Among the collected water samples, 5% (12/239) were positive for *P. aeruginosa*. We found *P. aeruginosa* in the DWNs of five of the eight cities tested. This pathogen contaminated three of the eight hospital DWNs and two of the five community networks tested (Table 1). All isolates of *P. aeruginosa* were susceptible to all antibiotics tested. As culture-based methods may fail to detect persister cells of *P. aeruginosa* in water[5], our findings highlight the urgency for new recommendations for the monitoring of drinking water contamination that consider the presence of persister cells.

Most of the *P. aeruginosa* isolates detected in the drinking water networks belong to high-risk clones

The sequence type (ST) of all 12 isolates were first determined by PCR and sequencing. Five isolates belonged to the high-risk clone ST308, three to the high-risk clone ST395, two to ST27, one to ST606, and one to ST3299 (Table 1, Fig. 1). We then sequenced the genome of one isolate representative of each ST per sample site for 10 isolates in total. The DWNs of four cities (Besançon, Bordeaux, Nantes, and Nîmes) were contaminated with the high-risk clones ST308 or ST395. ST308 was found in the community networks of Bordeaux and in the hospital networks of Nantes and Nîmes (Table 1). Of note, ST308 was also retrieved during the 2017 sampling campaign in the hospital network of Besançon[5] (Table 1). ST308 has been responsible for outbreaks in hospitals where water was reported as a potential reservoir of *P. aeruginosa*[6,8]. We also retrieved ST395 isolates from the community DWN of Besançon (Table 1). This ST was responsible for a large hospital outbreak in the same city, as well as in the United Kingdom, where the authors suggested that *P. aeruginosa* ST395 rapidly colonized the plumbing system of a new hospital, before its commissioning and was transmitted to patients by the water[7,21]. Although ST27 and ST606 are not yet classified as high-risk clones, they have emerged in European countries and can produce metallo-ß-lactamases of Ambler class B (VIM-2 and VIM-4 for ST27, and IMP-15 for ST606)[22,23].

The biological features needed to survive in the DWNs (resistance to inhibitors and predators) suggest that *P. aeruginosa* STs contaminating DWNs may have gathered in a clade. We tested this possibility by localizing these STs on the phylogenetic tree of all currently known STs of *P. aeruginosa* (Fig. 2a) and found the six STs (Table 1) to be scattered throughout the tree, suggesting no evidence of clades specifically adapted to this niche.

Seeking a common source of *P. aeruginosa* in drinking water networks

ST308 was the most highly represented ST (4 of 10 isolates) in the 2019–2020 campaign and was also repeatedly found in the DWN of the hospital of Besançon in 2017 (Table 1, Fig. 1). Understanding the population structure of ST308 in French DWNs could help to identify the source of contamination. We compared the genomes of the ST308 isolates found in the DWNs of Nantes (wPA-Nan1), Nîmes (wPA-Nim2), Besançon (wPA-Bes1, wPA-Bes4, wPA-Bes12, for which the genomes were available from a previous study[5]), and Bordeaux (wPA-Bor1) with those of the 95 ST308 strains for which the genomes were deposited at the NCBI. Surprisingly, we found that isolates from the French DWNs grouped together on the phylogenetic tree of ST308 (Fig. 2b), with less than nine genes with different alleles (Fig. 2c).

Such genetic proximity between ST308 isolates collected in 2019–2020 in distant DWNs was unexpected and raised suspicions. Two factors allowed us to rule out cross-contamination at the laboratory. First, water samples positive for *P. aeruginosa* ST308 were processed at different times by the Infection Control laboratory of the UHB (Table 1). Second, given the mutation rate of *P. aeruginosa* (~10–15 SNPs per year), the number of genomic differences between these ST308 isolates was higher than that between the genomes of contaminating strains in the laboratory[24].

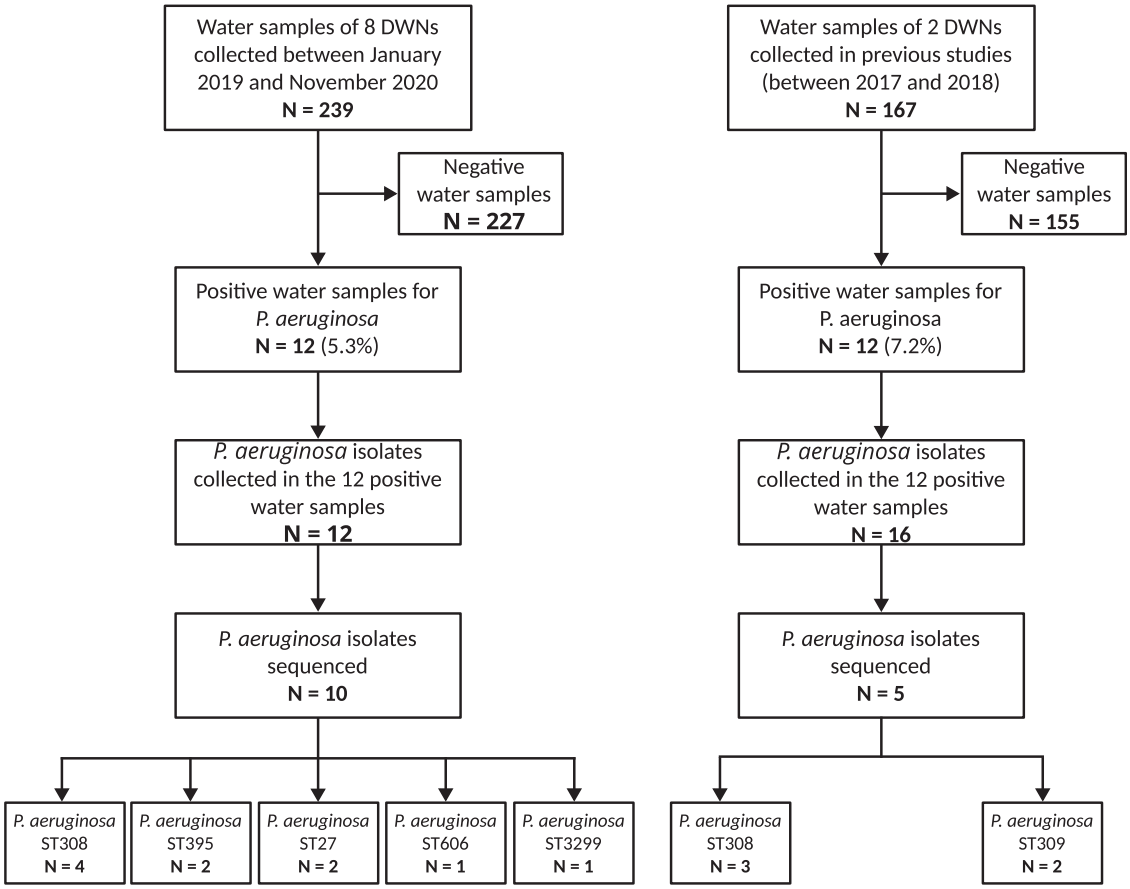

Fig. 1 | Flow chart of the collected water samples and *Pseudomonas aeruginosa* isolates contaminating drinking water networks in France between May 2017 and November 2020. DWNs, drinking water networks.

This clonal distribution strongly suggests a common source of contamination with *P. aeruginosa* ST308 in the DWNs of four distant cities in France (Fig. 3). As the DWNs of these four cities are supplied by independent water sources, it is reasonable to exclude them as a reservoir of *P. aeruginosa* ST308. However, DWNs are built with supplies, such as water meters, provided by a small number of manufacturers, which calibrate water meters on test benches before shipment to the clients. The calibration of water meters has been identified as a high-risk activity for contamination by *P. aeruginosa*. Hence, a technical report in 2016 indicated that 23% of the brand-new water meters from the stock of water supply companies were contaminated with *P. aeruginosa*[19]. It is thus possible that water meters could inoculate a DWN with *P. aeruginosa* that further disseminates and persists for years in the network. This hypothesis needs to be tested by additional studies. However, collections of *P. aeruginosa* isolated from water meters prior to their installation are not available.

Drinking water network: niche speciation of *P aeruginosa* persister cells and a source of contamination for patients?

Previous studies reported that the core genomes of ST308 and ST395 harbor the 37-kb genomic island GI-7, which contains an array of 13 genes encoding copper transporters and helps *P. aeruginosa* to tolerate copper in solution[5,21]. We confirmed the presence of GI-7 in 24 of the 28 isolates (86%) of our collection contaminating DWNs. GI-7-containing isolates all belonged to ST308, ST309, and ST395. However, the tolerance to copper-ions was not fully specific to GI-7[21]. Although scattered throughout the phylogenetic tree of the species (Fig. 2a), these STs share a biological feature (tolerance to copper-ions) that presumably aided their proliferation and propagation in DWNs.

The few clones of *P. aeruginosa* surviving and persisting in the DWNs must have been able to cope with the harsh environment of this niche (e.g., nutrient limitation, predation by free-living ameba, and inhibition by copper-ions and chlorine)[13]. The biocidal properties of copper are exploited for the distribution of water and copper-containing materials are widely used for pipes and the fixtures of plumbing systems, leading to the release of copper-ions into drinking water[25]. The observed speciation may have resulted from the selection of a few niche specialists (ST308, ST309, ST395) that evolved to overcome the harsh conditions and adapt to the DWNs. We were unable to test this possibility, as any attempt to correlate the presence of copper-resistant *P. aeruginosa* with the network material was precluded because all the drinking water systems of the participating hospitals were composite, with portions in copper and others in polyvinyl chloride, galvanized steel, or cross-linked polyethylene.

No outbreaks with these high-risk clones of *P. aeruginosa* were reported during the study period in the tested hospitals. However, UHB experienced an outbreak of *P. aeruginosa* ST395 between 1997 and 2008, probably originating from the contamination of distal parts of the water distribution system by the drinking water[21]. Notwithstanding, wgMLST analysis showed that the two isolates of *P. aeruginosa* ST395 (wPA-Bes15 and wPA-Bes16; Table 1) in the DWN were not clonal with the ST395 clinical isolates coming from the hospitalized patients (data not shown). However, hospital outbreaks with high-risk *P. aeruginosa* ST308 and ST395 have been repeatedly reported[7,8,21,26], with epidemiological investigations, when performed, identifying the DWN as a probable source of contamination[7,21]. In these studies, *P. aeruginosa* was cultured from water samples using the recommended method unsuitable for persister cell recovery. Although not immediately culturable, persister cells can resuscitate and recover their full virulence and cultivability under suitable conditions[16,25]. Persister cells of *P. aeruginosa* contaminating the DWNs could then act as a reservoir for patient contamination by contaminating and thriving in the distal portion of

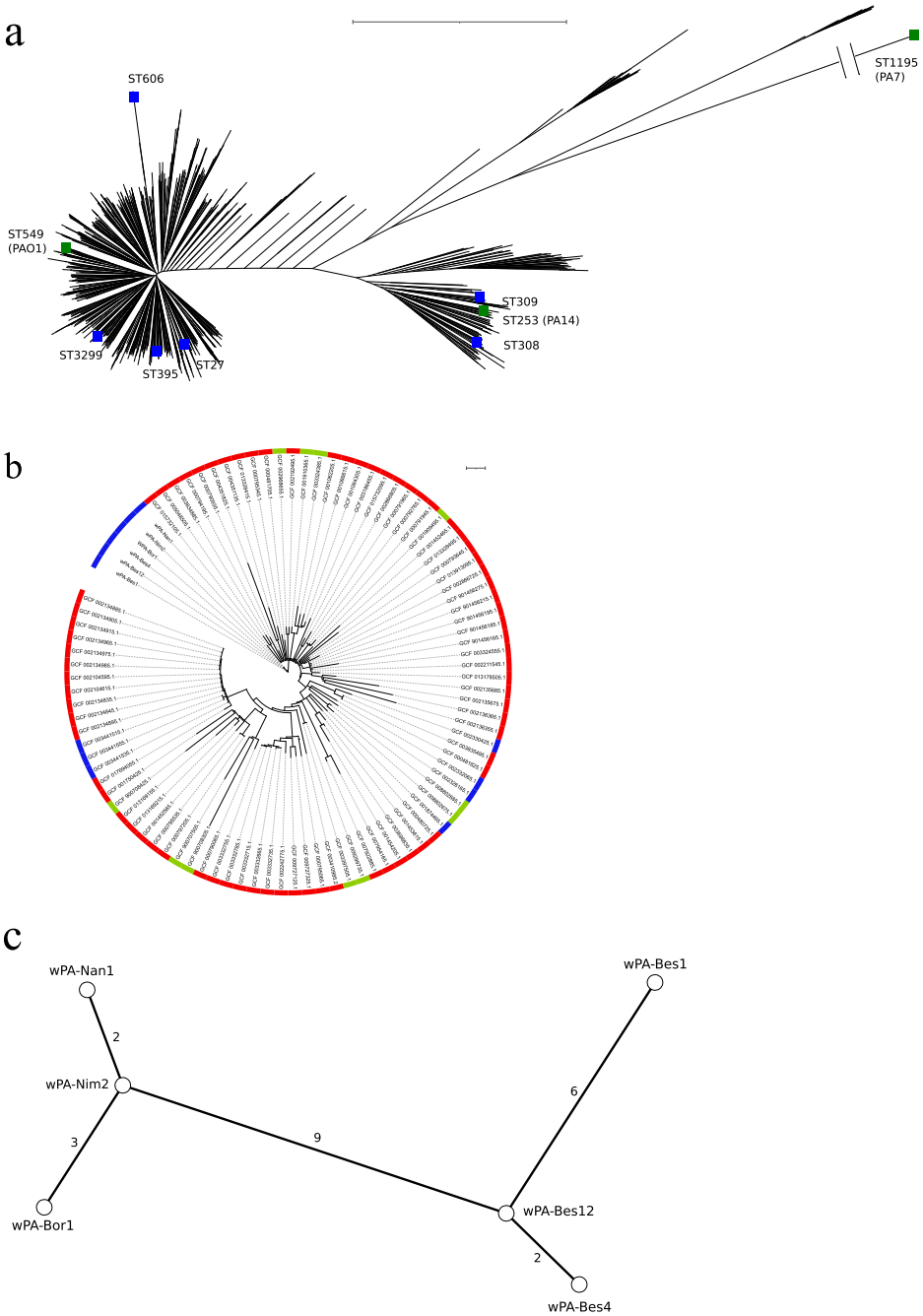

Fig. 2 | Population structure of *Pseudomonas aeruginosa* contaminating drinking water networks in France. a shows the distribution of the STs of the *Pseudomonas aeruginosa* strains isolated from hospital and community drinking water networks (blue squares) on an unrooted tree built using the data of all known STs of *P. aeruginosa* ($n = 753$) and cgMLST data (3786 genes). The scale bar indicates the genetic distance in number of substitutions per site. The green squares represent the ST of reference strains PAO1, PA7, and PA14. Strain PA7 now belongs to the species *Pseudomonas paraeruginosa* and is no longer classified as *P. aeruginosa*[39]. Scale bar refers to a phylogenetic distance of 0.01 nucleotide substitutions per site. **b** is the distribution of *Pseudomonas aeruginosa* ST308 isolated in hospital and community drinking water networks on a phylogenetic tree built from all 95 genomes of ST308 isolates deposited at the NCBI by April 5, 2021, based on the wgMLST of ST308 (2937 genes). The scale is the length of branch that represents 0.00001 nucleotide substitutions per site. The color of the outer circle indicates the origin of the isolates (red for human, blue for environment, green for unknown origin). **c** is the minimum spanning tree (built from wgMLST distances) that grouped the *P. aeruginosa* ST308 strains isolated from the drinking water networks in France.

hospital water distribution systems and then recovering their virulence under more favorable conditions.

Limitations and strengths of the study

Although numerous ($n = 239$), the water samples were distributed within the DWNs of only eight cities in a single country. Moreover, the number of water samples differed between the DWNs tested (Table 1). Further studies should globally test the presence of persister cells of *P. aeruginosa* in DWNs. Water sampling during different seasons was another limitation, as water temperature can affect the presence of *P. aeruginosa* in DWNs, with higher quantities of *P. aeruginosa* being reported in the spring[27]. We did not measure the temperature of the water at the time of water sampling. However, low temperature possibly accounted for the absence of *P. aeruginosa* ST308 in DWNs sampled in the winter (i.e., Tours, Trévenans). Although artificial selection of the specific strains by the method we used for bacterial resuscitation cannot be excluded[25], the major strength of this study was the culture-based method that allowed the resuscitation of persister cells, necessary for genome sequencing and comparison at the nucleotide level. A culture-independent typing method (named high-throughput short sequence typing—HiSST) has been recently developed and used to type uncultured environmental *Serratia marcescens*[28]. Its extension to *P. aeruginosa* would facilitate the detection of high-risk clones of this pathogen in DWNs. We also ensured that all water samples and all bacterial isolates were processed in a central laboratory to limit technical deviation.

Here, we searched for *P. aeruginosa* in the DWNs of eight French cities using a culture-based method that resuscitates persister cells and found five of the networks to be positive. High-risk clones (ST308, ST395) contaminated four of the eight DWNs tested. The close genetic proximity of the ST308 isolates retrieved from four independent and distant DWNs suggests

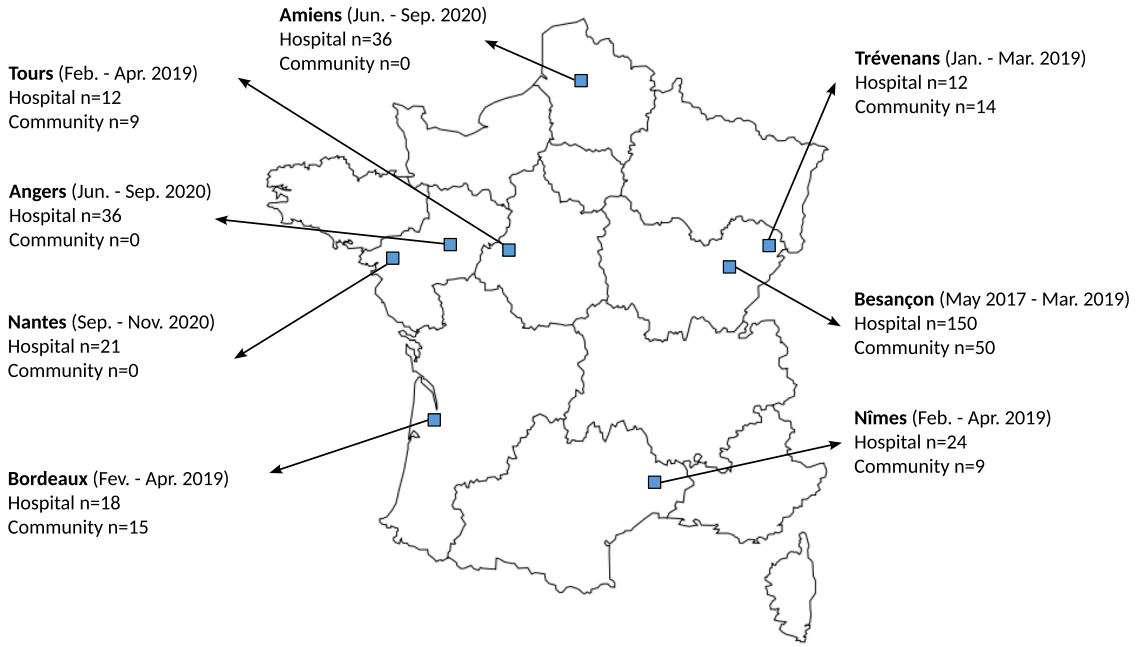

Fig. 3 | Map of France showing the cities for which the drinking water networks were tested between 2019 and 2020 for the presence of persister isolates of *Pseudomonas aeruginosa*. The number of samples collected from hospital and community drinking water networks is indicated, along with the sampling period. The water samples came from the networks of the cities of Amiens (49.875 N, 2.253 E), Angers (47.484 N, 0.556 W), Besançon (47.225 N, 5.963 E), Bordeaux (44.828 N, 0.604 W), Nantes (47.211 N, 1.554 W), Nîmes (43.825 N, 4.321 E), Tours (47.387 N, 0.668 E), and Trévenans (47.577 N, 6.872 E).

the plumbing supplies as the common source of contamination. However, most of the isolates contaminating the DWNs shared a genomic island that confers tolerance to copper-ions. Overall, this suggests that *P. aeruginosa* isolates retrieved from DWNs result from both a common source of contamination and the selection of clones sharing genetic elements that presumably aid their proliferation and propagation in DWNs. More attention should be given to persister cells of *P. aeruginosa* of DWNs, which could contaminate patients after proliferation in water points of use.

Methods

Setting

Between January 2019 and November 2020, we collected 239 water samples from the DWNs of eight French cities (Table 1), for which the geographic location is indicated in Fig. 3. The hospital DWNs in the eight cities were sampled, as well as the community DWNs of four cities (Fig. 3).

Water sampling

One liter of cold-water samples was collected monthly over 3 months for the hospital and community DWNs (Table 1). For the hospitals, we sampled faucets distant from any medical activity to avoid any contamination with *P. aeruginosa* isolates originating from patients or the environment of care units. Faucets were in technical areas, such as medical equipment storage areas or technical facilities. For each hospital DWN, we sampled the main water inlet and three to five water points of use from the building served by this supply (Table 1). We followed the EN ISO 19458 guidelines for water sampling. Briefly, water was sampled in sterile polypropylene bottles after aerator removal, disinfection of the outlet with a gas burner or a swipe soaked with the disinfectant Aniosurf (ANIOS, Lille-Hellemmes, France), and a 1-min pre-flush[29,30]. The bottles contained 20 $mg.L^{-1}$ sodium thiosulphate (VWR, Fontenay-sous-Bois, France) and 100 µM of the copper-ion chelating agent diethyldithiocarbamate (DDTC; Sigma-Aldrich, Saint-Quentin Fallavier, France), which can resuscitate copper-impregnated bacterial cells[25]. Bottled sterile water (Fresenius Kabi, Sevres, France) was used as a negative control.

Microbiological analysis of the water

All samples were sent to (within 24 h and at room temperature) and analyzed by the Infection Control laboratory of the University Hospital of Besançon (UHB). Samples were stored for 14 days at 22 °C in the dark to promote the resuscitation of copper-stressed *P. aeruginosa* induced by the chelator DDTC[5,25]. Then, we filtered three subsamples of 250 mL through 0.45-µm membranes which were then placed on (i) R2A agar (Biokar Diagnostics, Allonne, France) and further incubated for 7 days at 22 °C, (ii) cetrimide-containing agar (Biorad, Marne la Coquette, France) and further incubated for 48 h at 37 °C, or (iii) Columbia agar supplemented with 5% horse blood (Thermofisher Oxoid, Dardilly, France) and incubated for 48 h at 37 °C. *P. aeruginosa* CFUs were identified by MALDI-TOF mass spectrometry (Microflex LT; Bruker Daltonik GmbH, Bremen, Germany) according to the manufacturer's recommendations. We stored each isolate of *P. aeruginosa* in brain-heart infusion broth supplemented with 30% glycerol at −80 °C until further analysis at the Centre de Ressources Biologiques - Filière Microbiologique de Besançon (Biobank BB-0033-00090).

Determination of susceptibility to antibiotics

We tested the activity of nine clinically relevant antibiotics (ceftazidime, cefepime, piperacillin-tazobactam, imipenem, meropenem, aztreonam, amikacin, tobramycin, and ciprofloxacin) against all *P. aeruginosa* isolates by the disk diffusion method on Muller-Hinton agar and interpreted it according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing[31].

Genome sequencing and phylogeny

Bacterial DNA was extracted using a QIAamp DNA Minikit (QIAgen, Hilden, Germany) according to the manufacturer's recommendations. Isolates were first typed by MLST using PCR and sequencing according to the protocol of Curran et al., modified by ref. 32. We sequenced the genomes of all *P. aeruginosa* isolates using Illumina NextSeq high-output v2.5 technology (pair-end, 150 bp and coverage >240X; Microsynth, Balgach, Switzerland). Reads were subsampled to a coverage of 80X before genome assembly using Spades v3.13 with the optimized mode[33]. To build the

phylogenetic tree of the species, we extracted the 5415 genomes of *P. aeruginosa* deposited at the NCBI by April 5, 2021. The sequence type (ST) of the strains was determined using pyMLST software[34]. We randomly selected one complete genome per ST to retain 753 genomes. Then, a core genome multilocus sequence typing (cgMLST) base with 3,867 genes was created. We built a phylogenetic tree from the multiple alignment of a subset of 3,786 genes present in >95% of the genomes using FastTree 2.1.10 with a GTR + G substitution model[35].

To build the phylogenetic tree of ST308, we extracted the 95 genomes of *P. aeruginosa* ST308 deposited at the NCBI by April 5, 2021. We created a whole genome MLST (wgMLST) using genes of the strain Pa58 as a reference[36]. We selected 2937 genes present in 95% of the ST308 genomes and containing at least one variation for Bayesian phylogenetic analysis using, MrBayes v3.2.7a with a GTR + G + I substitution model[37]. We built a minimum spanning tree on ST308 strains described in our study from wgMLST allelic distances using Grapetree (v2.2)[38].

Data availability

All raw reads generated were deposited under the BioProject accession number PRJNA807601 on the National Center for Biotechnology Information (NCBI) database. The data generated and/or analyzed in this study are available from the corresponding author upon reasonable request. All codes used in this study are available from the corresponding author on reasonable request or directly on the GitHub repository of the pyMLST software (github.com/bvalot/pyMLST)[34].

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Author contributions

All authors contributed to the design or conduct of the study. A.J., A.A., and D.H. wrote the study protocol. D.H. and X.B. obtained funding. A.J., A.A., M.T., J.O., H.B., N.V.d.M.M., N.L., D.L., and M.E. collected the samples and epidemiological data. A.H., A.J., A.A., X.B., B.V., and D.H. performed or supervised the microbiological analyses. A.H., A.J., and B.V. provided a standardized procedure and performed genetic analyses for the whole-genome-sequencing. A.H., A.J., X.B., B.V., and D.H. drafted the manuscript and all authors reviewed and contributed to the manuscript. A.H. and A.J.: are considered “co-first author”.

Competing interests

The authors declare no competing interests.

Additional information

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