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Genome-based typing reveals rare events of patient contamination with *Pseudomonas aeruginosa* from other patients and sink traps in a medical intensive care unit

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SUMMARY

Aim: We used genome-based typing data with the aim of identifying the routes of acquisition of *Pseudomonas aeruginosa* by patients hospitalized in a medical intensive care unit (MICU) over a long period in a non-epidemic context.

Methods: This monocentric prospective study took place over 10 months in 2019 in a 15-bed MICU that applies standard precautions of hygiene. Lockable sink traps installed at all water points of use were bleach disinfected twice a week. We sampled all sink traps weekly to collect 404 *P. aeruginosa* environmental isolates and collected all *P. aeruginosa* isolates ($N = 115$) colonizing or infecting patients ($N = 65$). All isolates had their phenotypic resistance profile determined and their genome sequenced, from which we identified resistance determinants and assessed the population structure of the collection at the nucleotide level to identify events of *P. aeruginosa* transmission.

Findings: All sink traps were positive for *P. aeruginosa*, each sink trap being colonized for several months by one or more clones. The combination of genomic and spatiotemporal data identified one potential event of *P. aeruginosa* transmission from a sink trap to a patient (1/65, 1.5%) and six events of patient cross-transmission, leading to the contamination of five patients (5/65, 7.7%). All transmitted isolates were fully susceptible to β-lactams and aminoglycosides.

Conclusions: Genome-based typing revealed the contamination of patients by *P. aeruginosa* originating from sink traps to be infrequent (1.5%) in an MICU with sink trap-bleaching measures, and that only 7.7% of the patients acquired *P. aeruginosa* originating from another patient.

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Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative bacillus that can thrive in a wide variety of niches. Hence, *P. aeruginosa* is widespread in soil and water and frequently found in the environment, including in the wastewater evacuation network of hospitals [1]. *P. aeruginosa* is also one of the most frequent species responsible for nosocomial infection in Europe and the USA [2,3]. In intensive care units (ICUs), 10—15% of healthcare-associated infections are attributed to this pathogen [1]. Such infections consist mostly of ventilator-associated pneumonia or bacteraemia, associated with high mortality [4]. A high number of such infections are nosocomial, especially among mechanically ventilated patients [5]. The *P. aeruginosa* genome can also readily acquire genetic material and thus gain new antibiotic resistance [6].

The rate of colonization by *P. aeruginosa* is low (2.3%) among healthy humans, more frequent among patients admitted to ICUs (4.1—11.6%), and can reach higher rates (57.8%) during hospitalization [7—10]. ICU patients can acquire *P. aeruginosa* from their environment and other patients, directly or via the hands of healthcare workers [8,11]. Hence, *P. aeruginosa* can contaminate respiratory equipment, endoscopes, and sections of the hospital water network, such as taps, shower drains, and sink traps (also known as U-bends or P-traps) [12—16]. The proportion of sink traps contaminated with *P. aeruginosa* varies from 15 to 50% in European ICUs and studies have reported that 7—50% of patients acquire *P. aeruginosa* from water points of use [17,18]. In addition, investigations of hospital outbreaks have identified the water supply system as the source of the *P. aeruginosa* outbreaks [19]. This has led infection control departments to recommend sink trap disinfection or sink removal or redesign in high-risk wards, such as ICUs and haematology units [20,21].

The distribution of the sources of *P. aeruginosa* (endogenous, environmental, other patients) varies greatly between studies because of differing infection control procedures (contact precautions, sink trap disinfection). In addition, discrepancies in study conclusions may also result from differences in sampling protocols and bacterial typing methods. Hence, genome-based typing identifies transmission routes of pathogens with a higher accuracy than older typing methods, which probably overestimate the number of transmission events [22,23].

We aimed to identify the acquisition pathways of *P. aeruginosa* by patients in a medical ICU (MICU) in which patients positive for *P. aeruginosa* were managed according to the recommendations of the French Society of Hospital Hygiene and, additionally, in which sink traps were disinfected with bleach twice a week. We sampled patients and sink traps for 10 months to collect 519 *P. aeruginosa* isolates, for which the genomes were entirely sequenced and compared at the nucleotide level. Such analysis elucidated the precise network of *P. aeruginosa* transmission in this MICU and identified the routes of *P. aeruginosa* acquisition by the patients.

Methods

Study characteristics

This prospective monocentric study took place in a 15-bed MICU between January and November 2019 in a university hospital in France. This MICU has 11 individual rooms distributed within three subunits (A, B, and C) with one water point of use and a four-bed room with two water points of use (Figure 1). The MICU admits ~700 patients/year and all 549 patients admitted during the time of the study were included. Each patient received care from ~10 members of the healthcare staff per day. Healthcare workers were dedicated to a subunit but could help in another subunit when needed. We conducted this study in the absence of an identified outbreak of *P. aeruginosa*.

Infection control procedures

All 13 water points of use were equipped with a lockable sink trap (Geberit, France) that was bleach-disinfected twice a week and at patient discharge. Briefly, locked sink traps were treated 15 min with 20 mL 2.6% liquid bleach and then rinsed with tap water. This unit systematically applies standard precautions of hygiene according to the recommendations of the French Society of Infection Control [24]. French regulations recommend a quarterly control of *P. aeruginosa* contamination of hospital drinking water and recommend a water treatment (e.g., 0.2-μm disposable filter) when the concentration is ≥1 CFU/100 mL [25]. During the study period, all water samples ($N = 162$) collected in the ward were negative for *P. aeruginosa*. As a result, the water points at the MICU were not equipped with filters. All patients were bathed daily with a washcloth impregnated with tap water and mild soap (Laboratoire Rivadis, France). To reduce the risk of pneumonia, ventilated patients had their teeth and gums brushed three times a day with an antiseptic-containing kit (Stryker, IL, USA) and non-ventilated patients benefited from daily mouthwash with a chlorhexidine-containing solution (Laboratoire Pierre Fabre, France).

Bacteriological methods

Patients admitted to the MICU were screened for *P. aeruginosa* carriage upon admission and twice a week thereafter using nasal swabs, rectal swabs, and tracheal aspirates when intubated. In parallel, we collected all *P. aeruginosa* isolates retrieved from diagnostic samples. All sink traps were sampled every week for *P. aeruginosa* detection by collecting 50 mL of sink-trap content with a suction catheter and a syringe. The sample was centrifuged for 5 min at 5000 *g* at room temperature and the supernatant discarded. Swabs and pellets were streaked on *P. aeruginosa*-selective cetrimide agar plates (Bio-Rad) and incubated for 48 h at 35 °C. All colony phenotypes were identified using a matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometer (Maldi Biotyper, Bruker). We assessed the activity of 13 antipseudomonal agents (listed in Supplementary Table S1) from three classes (β-lactams, aminoglycosides, fluoroquinolones) by the agar diffusion method as recommended by the EUCAST (www.eucast.org). Morphologically different colonies of *P. aeruginosa* recovered from the same sink trap sample were retained for further analysis when they showed distinct resistance profiles. The resistance profile of isolates susceptible to all tested antibiotics was considered to be wildtype. Acquired genes encoding β-lactamases (including

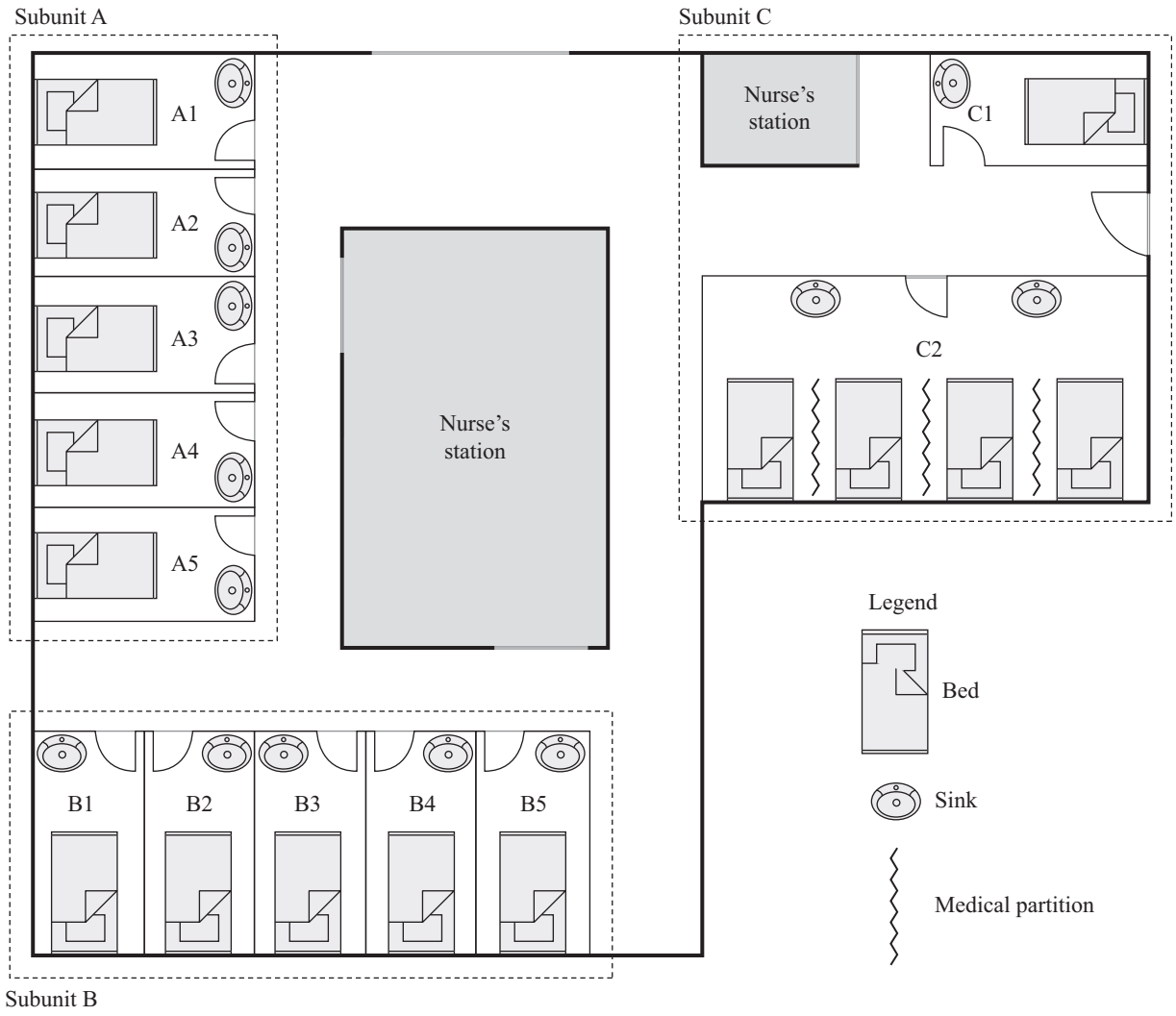

Figure 1. Layout of the medical intensive care unit at Besançon University Hospital (France). Subunits A and B are each composed of five rooms, with each room containing one bed and one water point of use. Subunit C is composed of two rooms, room C1 containing one bed and one water point of use and room C2 containing four beds and two water points of use.

carbapenemases) were sought within the genomic data against the ResFinder database [26].

Data

Each isolate collected was associated with its date of isolation, its patient or sink trap of origin, and its antibiotic resistance profile. See the Supplementary Material and Methods for details on bacteriological and sequence analysis. We sequenced the full genome of all *P. aeruginosa* isolates (Supplementary Table S1). Sequencing data are available in the NCBI BioProject PRJNA788732. We first identified the sequence type (ST) of each isolate by multi-locus sequence typing (MLST) [27]. Then, isolates for which the genome contained $\leq$30 different genes were clustered into groups with core genome MLST (cgMLST) using 3867 core genes [28]. We further measured the genetic relatedness of isolates within each group by the number of single nucleotide polymorphisms (SNPs) between genomes. See Supplementary Figure S1 for the justification of the threshold.

Definitions

(1) Isolates from a given group were defined as clonal when their genomes clustered with a threshold of seven SNPs. (2) Cross-contamination was defined by the identification of clonal isolates in two sampling points (patient or sink trap). (3) In cases of cross-contamination between two sampling points $\geq$7 days apart, we defined the older one as the source. (4) Colonization was defined as the culture of *P. aeruginosa* in patient's samples with no sign of infection, and infections were defined according to Sepsis-3 [29].

Statistical analysis

The data were analysed with R Studio (v 1.4) using the circlize and vegan libraries. Differences between the distributions of resistance profiles of the two *P. aeruginosa* reservoirs (patients and sink traps) were tested using Fisher's exact test at a threshold of 0.01.

Results

Contamination of sink traps and patients by P. aeruginosa

During the 10 months of the study, the 13 sink traps of the MICU were sampled 42 times each, for a total of 546 samples, of which 282 (51.6%) were positive for one or more isolates of *P. aeruginosa*. This led to the collection of 404 environmental isolates. All sink traps were positive for *P. aeruginosa* at some point during the study and could be contaminated with multiple STs (mean: 5, min: 2, max: 10), with clones persisting in each sink trap for long periods of time (mean: 242 days, max: 286 days). Among the 549 patients included, 65 (11.8%) were positive for *P. aeruginosa*. We collected 115 clinical isolates from these patients during their hospitalization. Three patients (3/65, 4.6%) were infected with *P. aeruginosa*, and 62 (62/65, 95.4%) were colonized with this bacterial species.

Population structure of P. aeruginosa

The 519 isolates (115 of clinical origin, 404 of environmental origin) were distributed within 62 different STs, with five STs accounting for 54.9% of the entire collection. Hence, the high-risk clones ST253, ST308, ST298 and ST244 were represented by 90 (17.3%; 81 environmental isolates, nine clinical isolates), 69 (13.3%; 65 environmental isolates, four clinical isolates), 39 (7.5%; 39 environmental isolates, no clinical isolates), and 32 (6.2%; 31 environmental isolates, one clinical isolate) isolates, respectively, and ST309 was represented by 55 isolates (10.6%) (Supplementary Table S1). Isolates from sink traps and patients were distributed within 27 and 48 different STs, respectively, with 13 STs in common. We then compared the clonal diversity of the population of isolates retrieved from the sink traps with that of the clinical isolates and found that the community of clinical *P. aeruginosa* was 4.1-times richer and 2.6-times more diverse than that retrieved from the sink traps (Supplementary Figure S2).

Transmission routes of P. aeruginosa

The routes of transmission of *P. aeruginosa* within the MICU were accurately identified by comparing the genomes of all isolates with a pipeline that allowed variant calling. This method clustered the isolates into 36 groups (Supplementary Figure S3). We combined these genomic data with spatio-temporal data to identify intra- and inter-reservoir transmission events (Table I, Figure 2). Most of the links occurred within a given sink trap, showing that such niches were contaminated with a signature ecosystem that was stable over time. However, we identified 22 cross-transmission events between sink traps of different rooms, with 10 between sink traps of different subunits (Figure 2a).

We identified six events of *P. aeruginosa* cross-transmission between patients, four involving one clonal isolate and one involving two (Table I, Figure 2b). These events involved five non-high-risk clones (ST198, ST274, ST1197, ST1238, ST3218) and nine patients. Isolate ST198 group34_1 was shared by two patients hospitalized in room A1 during the same week, but whose hospitalization period did not overlap. Isolates ST1197 group25_1 and ST1238 group27_1 were transmitted between patients hospitalized during the same week in different rooms (C1 and C2 for ST1197, A1 and B5 for ST1238). Two patients from room C2 shared two isolates (ST274 group30_1 and ST3218 group15_1). The temporal proximity of the finding of these

Table I
Details of the transmission of *P. aeruginosa* isolates involving patients in the medical intensive care unit at Besançon University Hospital (France) between January and November 2019

Type of transmission	Isolate (ST, group)	Resistance phenotype	Reservoir 1	Room	Date (2019)	Direction of transmission[a]	Reservoir 2	Room	Date (2019)
Patient-to-patient									
	ST198, group34_1	FQs[b]	Patient43	A1	April 14	Unknown	Patient49	A1	April 18
	ST274, group30_1	Wild type	Patient49[c]	C2	April 12	Unknown	Patient32[c]	C2	April 18
	ST1197, group25_1	Wild type	Patient1	C1	August 7	Unknown	Patient13	C2	August 12
	ST1238, group27_1	Wild type	Patient9	B5	July 22	Unknown	Patient48	A1	July 22
	ST3218, group15_1[d]	Wild type	Patient32[c]	C2	April 15	Unknown	Patient49[c]	C2	April 18
	ST3218, group15_1[d]	Wild type	Patient8	A3	October 14	→	Patient39	C2	October 22
Sink trap to patient									
	ST253, group0_6	Wild type	Sink trap	B2	May 13	→	Patient14	A3	May 24
Patient to sink trap									
	ST27, group21_1	Wild type	Patient36	B3	February 23	→	Sink trap	B3	March 4
	ST234, group33_1	Wild type	Patient5	A5	May 2	→	Sink trap	A5	July 29
	ST253, group0_3	Wild type	Patient17	A1	October 23	→	Sink trap	A1	November 4
	ST308, group1_6	Wild type	Patient54	A2	July 8	→	Sink trap	A2	July 15
	ST309, group2_3	Wild type	Patient10	B2	September 2	→	Sink trap	B2	September 23
	ST671, group32_1	Wild type	Patient19	A2	March 30	→	Sink trap	A2	April 8

ST, sequence type.
[a] In cases of transmission between two sampling points ≥7 days apart, we defined the older one as the source.
[b] Isolated low-level resistance to fluoroquinolones.
[c] Patient32 and Patient49 shared both isolates ST274, group30_1 and ST3218, group15_1.
[d] ST3218, group15_1 was shared by Patient32 and Patient49 and transmitted from Patient8 to Patient39.

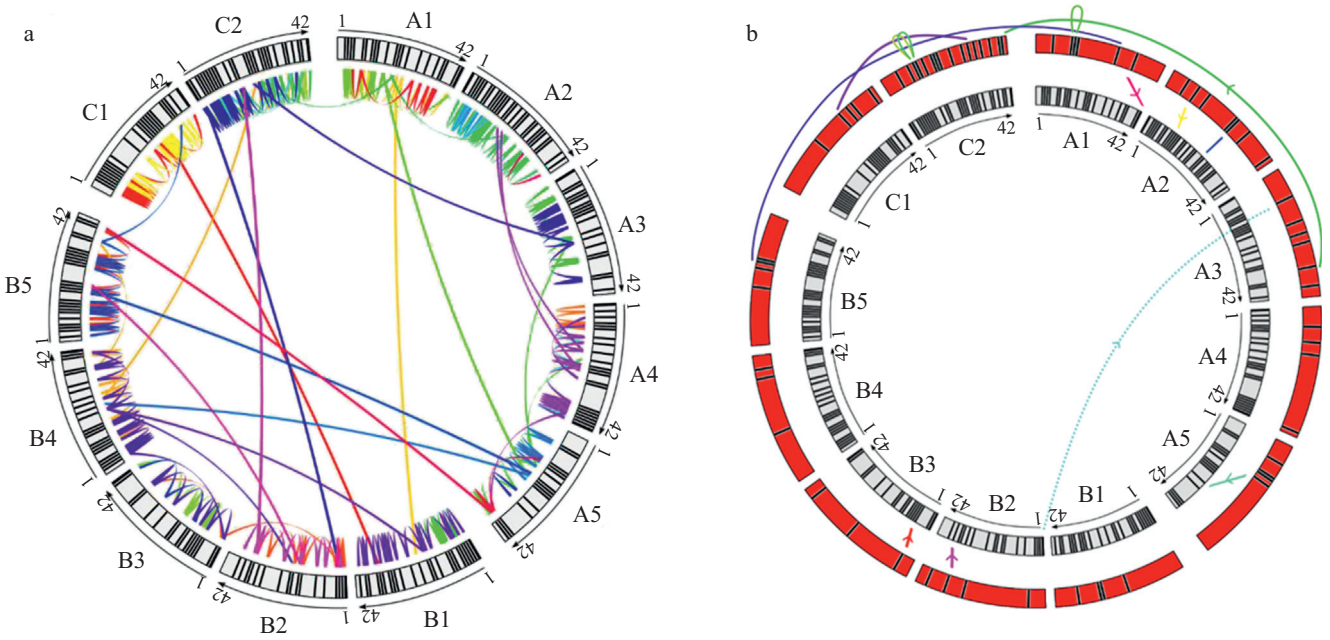

Figure 2. Representation of the transmission routes of *Pseudomonas aeruginosa* between sink traps and patients in the medical intensive care unit. Each sector represents a room with the chronology of *P. aeruginosa* isolation from week 1 to week 42 in a clockwise direction, with the black bars showing *P. aeruginosa*-positive samples in a given week. The grey circle represents the sink trap isolates and the red circle the human isolates. Each coloured link connects two isolates for which the genomes clustered at a threshold of seven single nucleotide polymorphisms. Isolates from the same core genome multi-locus sequence typing (cgMLST) cluster are connected with a link of the same colour. The arrow in the link shows the orientation of the transmission, when determined. When a clone was repeatedly found in a sink trap, we only considered its first appearance to identify the potential links of transmission. (a) Transmission of *P. aeruginosa* within and between sink traps. (b) Transmission of *P. aeruginosa* from and to patients. The dotted link between the inner grey and outer red circles indicates the contamination of a patient in room A3 with a *P. aeruginosa* from the sink trap of room B2. The six links between the outer red and inner grey circles indicate sink traps contaminated with *P. aeruginosa* of human origin.

isolates prevented the identification of the source of contamination. In one case, we could document the direction of contamination of a patient of room C2 with the isolate ST3218 group15_1 from a patient hospitalized in room A3 (Table I, Figure 2b). Overall, four patients shared the isolate ST3218 group15_1: two patients were hospitalized in April 2019 in room C2 and two others six months later in rooms C2 or A3 (Table I). We never retrieved this isolate from any sink trap (Supplementary Table S1).

In terms of environment-to-patient contamination, only one transmission of a *P. aeruginosa* isolate occurred from a sink trap to a patient. A high-risk ST253 clone, repeatedly found in the sink trap of rooms B2 and B3 from January to September 2019, was isolated from a patient hospitalized in May in room A3 (Table I, Figure 2b). In addition, we identified six transmission events of *P. aeruginosa* from patients to the sink traps of their rooms (Figure 2b). The six STs involved (ST27, ST234, ST253, ST308, ST309, ST671) were transmitted in rooms A1, A2, A5, B2, and B3 (Table I). Overall, among the 65 patients infected or colonized with *P. aeruginosa*, one patient (1.5%) acquired a *P. aeruginosa* isolate from a sink trap and five other patients (7.7%) were contaminated with a *P. aeruginosa* isolate from another patient.

Resistance profiles of P. aeruginosa

The proportion of isolates susceptible to all antibiotics tested was higher for the *P. aeruginosa* of human origin (74.8%; 86/115) than the *P. aeruginosa* found in the sink traps (48.0%, 194/404) (Fisher's exact test, $P = 2.8 \times 10^{-7}$; Supplementary Table S1). The only clone that produced extended-spectrum β-lactamase (VEB-1) belonged to ST357 and was represented by eight isolates found in the sink trap of room B1. Isolates non-susceptible to carbapenems were more frequently found in sink traps (152/404, 37.6%) than in patients (19/115, 16.5%) (Fisher's exact test, $P = 1.6 \times 10^{-5}$). The 13 isolates resistant to all antibiotics tested (Supplementary Table S1) were exclusively retrieved from sink traps and clustered within two clones belonging to ST111 (room C2) and ST357 (room B1).

Four of the five *P. aeruginosa* isolates transmitted between patients (ST274 group30_1, ST1197 group25_1, ST1238 group27_1, ST3218 group15_1) displayed wildtype resistance profiles. Of note, isolate ST3218 group15_1 was transmitted on two occasions involving four patients. The fifth isolate (ST198 group34_1) displayed an isolated low level of resistance to ciprofloxacin. Finally, the isolate ST253 group0_6 transmitted from a sink drain to a patient had a wildtype resistance profile to antibiotics (Table I, Supplementary Table S1, Supplementary Figure S3).

Discussion

We investigated the cross-transmission of *P. aeruginosa* between patients and sink traps over 10 months in the MICU of a university hospital in France in the absence of a recognized

outbreak. Among the 65 patients infected or colonized with *P. aeruginosa*, one (1.5%) was contaminated with a clone originating from a sink trap and five (7.7%) from one originating from another patient.

The proportion of patients contaminated with a *P. aeruginosa* isolate previously found in a sink trap (1.5%) was lower than that previously reported (7—50%) [17,18]. Such a discrepancy could be due to the more accurate typing method used here relative to older typing techniques based on DNA restriction, such as pulsed-field gel electrophoresis (PFGE).

WGS-based typing sharply discriminates clonal populations and the sequencing data also allows the identification of resistance determinants, the deciphering of genome plasticity, the dating of the most recent common ancestor, the identification of plasmids, protein prediction, and the portability of the data. PFGE results are easily interpreted because pulsotype clustering is based on the similitude coefficient. The interpretation of cgMLST data is more complicated, for which the number of differences depends on the species. Typing of a bacterial strain by PFGE and by WGS is quoted in France at 135€ and 2206€, respectively. Despite its higher cost and despite the bioinformatics skills needed to interpret the data, WGS-based typing will certainly overtake other typing methods. Although PFGE can detect local outbreaks caused by *P. aeruginosa*, comparison at the nucleotide level is required to identify contamination routes of the pathogen [22,23,30]. Hence, a recent investigation of nosocomial outbreaks of *P. aeruginosa* in an ICU using WGS-based typing have unequivocally identified water points of use as the source of contamination [21].

The implementation of infection control procedures, with improved hand hygiene, presumably accounted for the low transmission rate from the environment to patients. In addition, we identified five patients from among the 65 (7.7%) who acquired *P. aeruginosa* from another patient (Table I, Figure 2b). This is the first quantification of the rate of patient cross-contamination in a non-epidemic context. For each cross-contamination event, the genomes of the *P. aeruginosa* isolates retrieved from the two patients were completely identical (Supplementary Figure S3). Three transmission events involved patients hospitalized in different rooms, indicating transmission by healthcare workers (Figure 2b). Of note, the six cross-transmission events between patients were concentrated in the geographically close rooms A1 (two events) and C2 (four events), which frequently shared healthcare workers (Table I, Figure 1). Hence, the proximity of the beds and the sharing of sinks in the four-bed room C2 could enhance the risk of cross-contamination with *P. aeruginosa*. All clinical *P. aeruginosa* transmitted to the sink traps originated from patients occupying the room (Table I), probably during their bathing.

Among the 65 patients positive for *P. aeruginosa*, 29 (44.6%) tested negative at admission. Only two (2/29; 6.9%) acquired a *P. aeruginosa* isolate from another patient. In other words, the vast majority of patients who became positive with *P. aeruginosa* during their hospitalization acquired an isolate not previously found in the other patients or sink traps. Other studies have reported higher proportions (50.0—93.6%) of patients contaminated with an exogenous isolate in ICUs with no detectable P. aeruginosa outbreak and no bleach-disinfection of sink traps [22,26]. Although we cannot rule out contamination with *P. aeruginosa* isolates originating from unexplored sources (e.g., healthcare workers, invasive devices), our data show that endogenous sources (i.e., patient flora) predominate over exogenous sources in a non-epidemic context [22]. The poor sensitivity of the *P. aeruginosa* detection in a single rectal swab (mostly due to low concentrations of *P. aeruginosa* in faeces and the sometimes low amount of faeces collected) presumably underestimated the proportion of *P. aeruginosa* carriers at admission. Hence, we previously found that repeated swabbing of patients almost doubles the chance of positivity with *P. aeruginosa* [31]. We restricted our environmental sampling to sink traps but the high clonal diversity of the clinical isolates of *P. aeruginosa* (Supplementary Figure S2) advocates against the existence of a common source of contamination.

Overall, we can assume that the cleaning and disinfection procedures, together with infection control procedures, applied in this MICU limit the risk of *P. aeruginosa* transmission. Sinks were cleaned daily and sink traps were disinfected twice a week with 2.6% bleach. Disinfection procedures using bleach, acetic acid, electrochemically activated solutions, or self-disinfecting sink drains fail to sterilize sink traps because bacterial biofilms in wastewater plumbing systems resist disinfectants and are not easy to access. However, such procedures likely limit the inoculum size, which, in turn, reduces the risk of contamination of the surrounding sink area and further transmission [32]. Additionally, other mechanisms may limit the transmission of *P. aeruginosa* from sink traps to patients. Hence, sub-inhibitory concentrations of bleach can promote horizontal gene transfer [33], thus favouring the adaptation of pathogens to a harsh environment. Such adaptation, illustrated here by the stability and low diversity of *P. aeruginosa* populations in sink traps (Figure 2a, Supplementary Figure S2), could impair the ability of the *P. aeruginosa* isolates found in this environmental niche to colonize patients [34].

Our study focused on *P. aeruginosa*, but other pathogens (*Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Elisabethkinga meningoseptica*) have already been retrieved from sink traps [35—37]. One can assume that disinfection also limits the risk of outbreaks with these pathogens. However, the implementation of sink bleaching alone has not been shown to be systematically associated with the cessation of outbreaks and the implementation of a bundle of measures is recommended for infection control [38,39].

Outbreaks of multi-drug-resistant bacteria specifically linked to drains or sinks are overrepresented in the literature [38]. We found all *P. aeruginosa* isolates transmitted from and to patients to be fully susceptible to β-lactams and aminoglycosides. Clinicians and microbiologists should not neglect the potential spread of strains with unspectacular resistance profiles [40].

We identified the limits and strengths of our study. This monocentric study was not designed to assess the effect of infection control procedures on the transmission of *P. aeruginosa*. Hence, the absence of a comparable ICU using different hygiene practices prevented assessment of the efficacy of sink trap disinfection in preventing the transmission of *P. aeruginosa* to patients. Instead, our design focused on breadth and depth sampling to identify the contamination routes of *P. aeruginosa* to patients. We isolated *P. aeruginosa* from both sink traps and patients over 10 months, collecting the largest dataset yet used for an epidemiological study [41]. The sensitivity of detection of *P. aeruginosa* in environmental and respiratory samples is good, but we may have

overestimated the number of negative rectal swabs due to poor performance of culture-based detection methods. As we were aware of the complex ecosystem of the sink traps [20], we sampled all sink traps weekly and analysed all colonies with various colony phenotypes and resistance profiles in each sample to obtain a complete picture of the *P. aeruginosa* population in this environmental niche. The use of isolates collected over 10 months increased the chance of finding clonal isolates that overlapped among patients and sink traps. The genomes of all isolates were fully sequenced and compared at the nucleotide level. This contrasts with typing methods previously used in epidemiological studies [26,42]. We circumvented the absence of a consensus threshold for clonal isolate identification from genome-based data by performing a two-step analysis of the genomes. First, we clustered the isolates with cgMLST and grouped all isolates using a threshold (30 alleles of difference) higher than that found in the literature (15 alleles of difference) to avoid missing any clonal isolates [28]. Second, we called variants between isolates within a group and performed a second clustering to access clonal strains with a threshold of seven SNPs of difference (Supplementary Figure S3). This threshold was experimentally optimized (see Supplementary Figure S2), consistent with previous studies (six to 10 SNPs), and is fully compatible with the evolution rate of the bacterial pathogen [43].

In conclusion, genome-based typing revealed the contamination of patients by *P. aeruginosa* isolates originating from sink traps to be rare (1.5%) in a MICU with sink trap bleaching measures and that 7.7% of the patients acquired *P. aeruginosa* from cross-contamination.

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Author contributions

C.C., X.B., B.V. and D.H. designed the study; C.C., M.B. and B.V. performed the research; B.V. contributed analytical tools; C.C., G.P., B.V. and D.H. analysed the data; and C.C., X.B., B.V. and D.H. wrote the paper.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2023.01.010.

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