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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Article

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Abstract

Background

We used genome-based typing data with the aim of identifying the routes of acquisition of *Pseudomonas aeruginosa* (PA) 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 PA environmental isolates and collected all PA 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 PA transmission.

Results

All sink traps were positive for PA, 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 PA 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 PA originating from sink traps to be infrequent (1.5%) in a MICU with sink trap-bleaching measures, and that only 7.7% of the patients acquired PA originating from another patient.

Introduction

P. aeruginosa (PA) is an opportunistic Gram-negative bacillus that can thrive in a wide variety of niches. Hence, PA is widespread in soil and water and frequently found in the environment, including in the wastewater evacuation network of hospitals (1). PA 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 to 15% of healthcare-associated infections are attributed to this pathogen (1). Such infections consist mostly of ventilator-associated pneumonia or bacteremia, associated with high mortality (4). A high number of such infections are nosocomial, especially among mechanically ventilated patients (5). The PA genome can also readily acquire genetic material and thus gain new antibiotic resistance (6).

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

The distribution of the sources of PA (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 PA by patients in a medical ICU (MICU) in which patients positive for PA were managed according to the recommendations of the French Society of Infection Control 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 PA isolates, for which the genomes were entirely sequenced and compared at the nucleotide level. Such analysis elucidated the precise network of PA transmission in this MICU and identified the routes of PA 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 (Fig. 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 PA.

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).

Bacteriological methods. Patients admitted to the MICU were screened for PA carriage upon admission and twice a week thereafter using nasal swabs, rectal swabs, and tracheal aspirates when intubated. In parallel, we collected all PA isolates retrieved from diagnostic samples. All sink traps were sampled every week for PA detection by collecting 50 ml sink-trap content with a suction catheter and a syringe. The sample was centrifuged 5 min at 5000 x *g* at room temperature and the supernatant discarded. Swabs and pellets were streaked on PA-selective cetrimide agar plates (Bio-Rad) and incubated 48 h at 35°C. All colony phenotypes were identified using a MALDI-TOF mass spectrometer (Maldi Biotyper, Bruker). We assessed the activity of 13 antipseudomonal agents (listed in Supplemental Table 1) from three classes (β-lactams, aminoglycosides, fluoroquinolones) by the agar diffusion method as recommended by the EUCAST (www.eucast.org). Morphologically different colonies of PA 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 carbapenemases) were sought within the genomic data against the ResFinder database (25).

Data. Each isolate collected was associated with its date of isolation, its patient or sink trap of origin, and its antibiotic resistance profile. See Supplemental Material and Methods for details on bacteriological and sequence analysis. We sequenced the full genome of all PA isolates (Supplemental Table 1). Sequencing data are available in the NCBI BioProject PRJNA788732. We first identified the sequence type (ST) of each isolate by MultiLocus Sequence Typing (MLST) (26). Then, isolates for which the genome contained ≤ 30 different genes were clustered into groups with Core Genome MLST (cgMLST) using 3,867 core genes (27). We further measured the genetic relatedness of isolates within each group by the number of single nucleotide polymorphisms (SNPs) between genomes. See Supplemental Fig. 1 for the justification of the threshold.

Definitions. *(i)* Isolates from a given group were defined as clonal when their genomes clustered with a threshold of seven SNPs. (*ii*) Cross-contamination was defined by the identification of clonal isolates in two sampling points (patient or sink trap). (*iii*) In cases of cross-contamination between two sampling points ≥ 7 days apart, we defined the older one as the source. (*iv*) Infections were defined according to Sepsis-3.

Statistical analysis. The data were analyzed with R Studio (v 1.4) using the circlize and vegan libraries. Differences between the distributions of resistance profiles of the two PA 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 PA. This led to the collection of 404 environmental isolates. All sink traps were positive for PA at some point of 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 PA. We collected 115 clinical isolates from these patients during their hospitalization. Three patients (3/65, 4.6%) were infected with PA.

Population structure of **P. aeruginosa**. The 519 isolates 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%), 69 (13.3%), 39 (7.5%), and 32 (6.2%) isolates, respectively, and ST309 was represented by 55 isolates (10.6%) (Supplemental Table 1). 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 PA was 4.1-times richer and 2.6-times more diverse than that retrieved from the sink traps (Supplemental Fig. 2).

Transmission routes of **P. aeruginosa**. The routes of transmission of PA 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 (Supplemental Fig. 3). We combined these genomic data with spatiotemporal data to identify intra- and inter-reservoir transmission events (Table 1, Fig. 2).

Table 1
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	Apr. 14	Unknown	Patient49	A1	Apr. 18
	ST274, group30_1	Wild-type	Patient49[c]	C2	Apr. 12	Unknown	Patient32[c]	C2	Apr. 18
	ST1197, group25_1	Wild-type	Patient1	C1	Aug. 7	Unknown	Patient13	C2	Aug. 12
	ST1238, group27_1	Wild-type	Patient9	B5	Jul. 22	Unknown	Patient48	A1	Jul. 22
	ST3218, group15_1[d]	Wild-type	Patient32[c]	C2	Apr. 15	Unknown	Patient49[c]	C2	Apr. 18
	ST3218, group15_1[d]	Wild-type	Patient8	A3	Oct. 14	→	Patient39	C2	Oct. 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	Feb. 23	→	Sink trap	B3	Mar. 4
	ST234, group33_1	Wild-type	Patient5	A5	May 2	→	Sink trap	A5	Jul. 29
	ST253, group0_3	Wild-type	Patient17	A1	Oct. 23	→	Sink trap	A1	Nov. 4
	ST308, group1_6	Wild-type	Patient54	A2	Jul. 8	→	Sink trap	A2	Jul. 15
	ST309, group2_3	Wild-type	Patient10	B2	Sep. 2	→	Sink trap	B2	Sep. 23
	ST671, group32_1	Wild-type	Patient19	A2	Mar. 30	→	Sink trap	A2	Apr. 8

[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.

Most of the links occurred within a given sink trap, showing that such niches were contaminated with a signature flora 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 (Fig. 2A).

We identified six events of PA cross-transmission between patients, four involving one clonal isolate and one involving two (Table 1, Fig. 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 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 1, Fig. 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 1). We never retrieved this isolate from any sink trap (Supplemental Table 1).

In terms of environment-to-patient contamination, only one transmission of a PA 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 1, Fig. 2B). In addition, we identified six transmission events of PA from patients to the sink traps of their rooms (Fig. 2B). The six STs involved (ST27, ST234, ST253, ST308, ST309, ST671) were transmitted in rooms A1, A2, A5, B2, and B3 (Table 1). Overall, among the 65 patients infected or colonized with PA, one patient (1.5%) acquired a PA isolate from a sink trap and five other patients (7.7%) were contaminated with a PA isolate from another patient.

Resistance profiles of **P. aeruginosa.** The proportion of isolates susceptible to all antibiotics tested was higher for the PA of human origin (74.8%; 86/115) than the PA found in the sink traps (48.0%, 194/404) (Fisher's exact test, $p = 2.8x10^{-7}$; Supplemental Table 1). 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, $p = 1.6x10^{-5}$). The 13 isolates resistant to all antibiotics tested (Supplemental Table 1) were exclusively retrieved from sink traps and clustered within two clones belonging to ST111 (room C2) and ST357 (room B1).

Four of the five PA 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 1, Supplemental Table 1, Supplemental Fig. 3).

Discussion

We investigated the cross-transmission of PA 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 PA, 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 PA 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). Although PFGE can detect local outbreaks caused by PA, comparison at the nucleotide level is required to identify contamination routes of the pathogens (22, 23, 28). 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 PA from another patient (Table 1, Fig. 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 PA isolates retrieved from the two patients were completely identical (Supplemental Fig. 3). Three transmission events involved patients hospitalized in different rooms, indicating transmission by healthcare workers (Fig. 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 1, Fig. 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 PA. All clinical PA transmitted to the sink traps originated from patients occupying the room (Table 1), probably during their bathing.

Among the 65 patients positive for PA, 29 (44.6%) tested negative at admission. Only two (2/29; 6.9%) acquired a PA isolate from another patient. In other words, the vast majority of patients who became positive with PA 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 PA outbreak and no bleach-disinfection of sink traps (22, 25). Although we cannot rule out contamination with PA 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).

Overall, we can assume that the cleaning and disinfection procedures, together with infection control procedures, applied in this MICU limit the risk of PA 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 (29). Additionally, other mechanisms may limit the transmission of PA from sink traps to patients. Hence, sub-inhibitory concentrations of bleach can promote horizontal gene transfer (30), thus favoring the adaptation of pathogens to a harsh environment. Such adaptation, illustrated here by the stability and low diversity of PA populations in sink traps (Fig. 2A, Supplemental Fig. 2), could impair the ability of PA to colonize patients (31).

Our study focused on PA, but other pathogens (*Klebsiella pneumoniae*, *Acinetobacter baumannii, Elisabethkinga meningoseptica*) have already been retrieved from sink traps (32–34). 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 (35, 36).

Outbreaks of multidrug-resistant bacteria specifically linked to drains or sinks are overrepresented in the literature (35). We found all PA 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 (37).

This monocentric study was not designed to assess the effect of infection control procedures on the transmission of PA. 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 PA to patients. Instead, our design focused on breadth and depth sampling to identify the contamination routes of PA to patients. We isolated PA from both sink traps and patients over 10 months, collecting the largest dataset yet used for an epidemiological study (38). As we were aware of the complex flora of the sink traps (20), we sampled all sink traps weekly and analyzed all colonies with various colony phenotypes and resistance profiles in each sample to obtain a complete picture of the PA 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 (25, 39). 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 (27). 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 (Supplemental Fig. 3). This threshold was experimentally optimized (see Supplemental Fig. 2), consistent with previous studies (6–10 SNPs), and is fully compatible with the evolution rate of the bacterial pathogen (40).

Conclusions

Genome-based typing revealed the contamination of patients by PA 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 PA from cross-contamination.

List Of Abbreviations

cgMLST, core genome multilocus sequence typing

ICU, intensive care unit

MALDI-TOF, matrix assisted laser desorption ionization - time of flight

MICU, medical intensive care unit

MLST, multilocus sequence typing

PA, *Pseudomonas aeruginosa*

SNP, single nucleotide polymorphism

Declarations

Ethics approval and consent to participate. This study is exempt from the need for ethical approval under local law as per advise received from the Human Protection Committee, East Area II, Besançon.

Consent for publication. Not applicable

Availability of data and materials. The datasets generated during the current study are available in the NCBI repository as the BioProject PRJNA788732.

Competing interests. The authors declare that they have no competing interests.

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Authors' 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. analyzed the data; and C.C., X.B., B.V., and D.H. wrote the paper.

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Figures

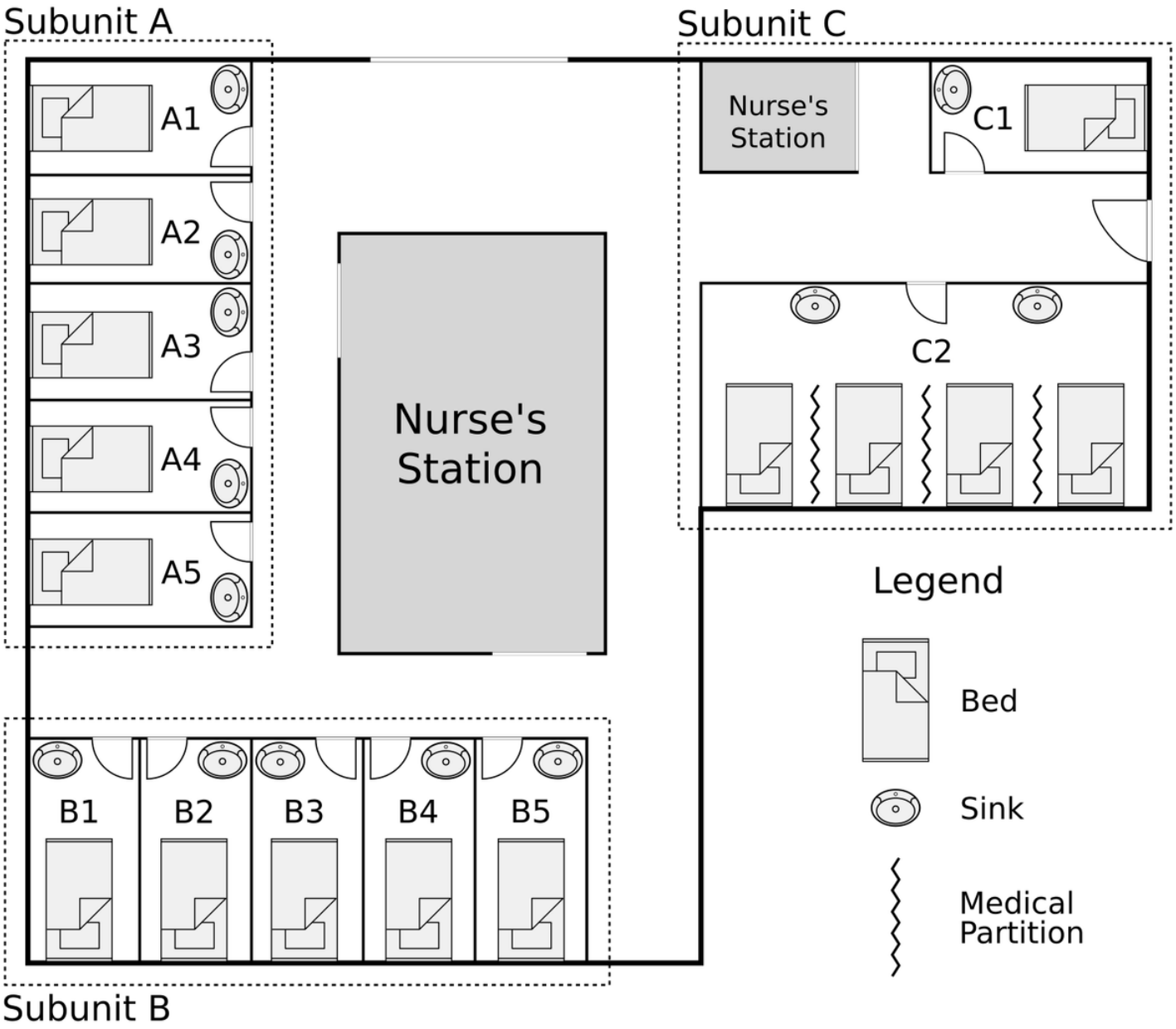

Figure 1

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.

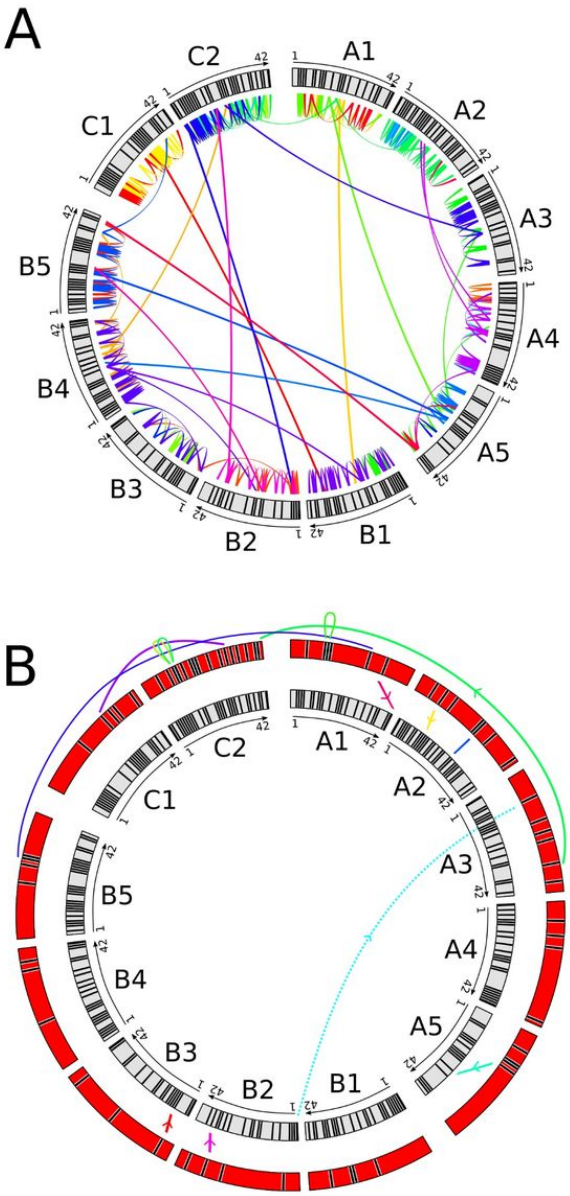

Figure 2

Representation of the transmission routes of *P. aeruginosa* between sink traps and patients in the MICU. Each sector represents a room with the chronology of PA isolation from week 1 to week 42 in a clockwise direction, with the black bars showing PA-positive samples in a given week. The gray circle represents the sink trap isolates and the red circle the human isolates. Each colored link connects two isolates for which the genomes clustered at a threshold of seven SNPs. Isolates from the same cgMLST cluster are connected with a link of the same color. 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 PA within and between sink traps. (B) Transmission of PA 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 PA from the sink trap of room B2. The six links between the outer red and inner grey circles indicate sink traps contaminated with PA of human origin.

Supplementary Files

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- CouchoudetalSupplementaryfile.pdf