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Serratia marcescens Colonization in a Neonatal Intensive Care Unit Has Multiple Sources, with Sink Drains as a Major Reservoir

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ABSTRACT Compelling evidence suggests a contribution of the sink environment to the transmission of opportunistic pathogens from the hospital environment to patients in neonatal intensive care units (NICU). In this study, the distribution of the opportunistic pathogen *Serratia marcescens* in the sink environment and newborns in a NICU was investigated. More than 500 sink drain and faucet samples were collected over the course of five sampling campaigns undertaken over 3 years. Distribution and diversity of *S. marcescens* were examined with a modified MacConkey medium and a high-throughput short-sequence typing (HiSST) method. Sink drains were an important reservoir of *S. marcescens*, with an average of 44% positive samples, whereas no faucet sample was positive. The genotypic diversity of *S. marcescens* was moderate, with an average of two genotypes per drain, while the spatial distribution of *S. marcescens* was heterogeneous. The genotypic profiles of 52 clinical isolates were highly heterogeneous, with 27 unique genotypes, of which 71% of isolates were found in more than one patient. *S. marcescens* acquisition during the first outbreaks was mainly caused by horizontal transmissions. HiSST analyses revealed 10 potential cases of patient-to-patient transmission of *S. marcescens*, five cases of patient-to-sink transmission, and one bidirectional transfer between sink and patient. Environmental and clinical isolates were found in sink drains up to 1 year after the first detection, supporting persisting drain colonization. This extensive survey suggests multiple reservoirs of *S. marcescens* within the NICU, including patients and sink drains, but other external sources should also be considered.

IMPORTANCE The bacterium *Serratia marcescens* is an important opportunistic human pathogen that thrives in many environments, can become multidrug resistant, and is often involved in nosocomial outbreaks in neonatal intensive care units (NICU). We evaluated the role of sinks during five suspected *S. marcescens* outbreaks in a NICU. An innovative approach combining molecular and culture methods was used to maximize the detection and typing of *S. marcescens* in the sink environment. Our results indicate multiple reservoirs of *S. marcescens* within the NICU, including patients, sink drains, and external sources. These results highlight the importance of sinks as a major reservoir of *S. marcescens* and potential sources of future outbreaks.

KEYWORDS health care-associated infections, nosocomial infections, NICU, opportunistic pathogen, outbreak, molecular typing, P trap, HiSST

Hospital-acquired infections (HAIs) are the main challenge in health care delivery, representing 25% of all hospital-treated sepsis cases (1). It is estimated that waterborne infectious disease causes 6,630 deaths in the United States annually, with biofilm-associated pathogens accounting for most hospitalizations and deaths, representing an annual cost of 2.39 billion U.S. dollars (2). Therapeutic and diagnostic developments are leading to

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the care of an increasing number of immunocompromised patients prone to infections with opportunistic pathogens (OPs). HAIs are associated with increases in morbidity, mortality, length of hospital stay, and costs. In intensive care units, up to 30% of patients will be affected by HAI, with up to 52% mortality. This mortality rate is two to three times higher for infections involving multidrug-resistant microorganisms and 3 to 20 times higher in low-income countries, especially among neonates (1, 3). Worldwide, HAIs cause 4 to 56% of mortality in premature infants, with the highest rates occurring in developing countries (3).

According to the Centers for Disease Control and Prevention and the World Health Organization, HAIs are defined as infections that occur after 48 h of admission with no sign of infection detected upon admission. In newborns, HAIs do not include infections acquired in the first 72 h of life or those of maternal origin, such as herpes or syphilis (4–8). Most HAIs are caused either by bacteria from the patient's microbiota or via transmission from other patients, medical devices, health care workers, or visitors or more broadly from the hospital environment (1, 9–12). Interactions between patients and the hospital built environment have gained attention in epidemiological studies aimed at identifying sources of nosocomial outbreaks. The sink environment is an important potential source (13–20), as sink drains represent a recognized reservoir of OPs.

Serratia, a member of the *Enterobacteriaceae*, is the sixth most frequently encountered microorganism in intensive care unit (ICU) pneumonia in Europe (21). This genus is ubiquitous in water, soil, plants, and different hosts, including insects, humans, and other vertebrates (22, 23). *Serratia marcescens* is the most important opportunistic human pathogen among *Serratia* species, often multidrug resistant and involved in outbreaks of HAIs in neonatal intensive care units (NICU) (24–34). A recent study reported an incidence of 2.3 *Serratia* late-onset infections per 1,000 very preterm infants, associated with lower survival and significant morbidity (35). *S. marcescens* is the second most frequently cited species in studies linking sinks with bacterial HAI in NICU, preceded by *Pseudomonas aeruginosa* (20). However, the importance of the sink environment in HAI events is still debated (36). The difficulty in inferring causality between infections and environment may be due to a lack of chronological data on isolates or the lack of resolution of molecular biology methods (20). For outbreak investigation, molecular typing methods are commonly used to examine the relatedness of environmental and clinical isolates (37–43). Nevertheless, these techniques are not tailored for epidemiological surveys involving large numbers of samples, as they are technically demanding due to upstream culture and isolation efforts.

The present study evaluated the sink-patient relationship during five suspected *S. marcescens* outbreaks in a NICU. Our report provides a precise timeline for clinical isolates as well as environmental samples and cutoff criteria to assign isolates to a given clone, as recently suggested by Choquet and Mullié (20). A combination of molecular and culture-based approaches was used to maximize the detection of *S. marcescens*, depending on the complexity of the matrix (i.e., drain samples usually have high concentrations of microorganisms, as opposed to tap water). Extensive patient and sink surveys led to detection of clinical isolates with sequence types (ST) related to those detected in environmental samples.

RESULTS

Adaptation of selective agar for *S. marcescens*. Selectivity and adequacy of culture media are key factors in retrieval of representative samples of culturable clinical and environmental isolates. These features were examined with two media conventionally used to isolate *Serratia*. A first trial was conducted by examining the diversity of bacteria from drain water samples with DNase medium. Of 20 isolates, only one was confirmed as *S. marcescens*; therefore, this medium was considered insufficiently selective. A second trial was done with caprylate-thallous medium, which however inhibited the growth of some environmental *S. marcescens* strain (e.g., strain DB1b-2wD from this study). Given the poor performance of both culture media, we modified MacConkey medium with the addition of antibiotics. This was the most efficient approach allowing for easy identification of *S. marcescens*, with the MacConkey medium becoming colorless and turning yellow-brown

with increasing pH above 6.8, due to the non-lactose-fermenting character of most *S. marcescens* isolates (44). Nevertheless, since some biotypes of *S. marcescens* are able to ferment lactose (45), as indeed was observed during our study (e.g., isolate BD-S17-0028-Sm1 in Table S2 in the supplemental material), we did not use this phenotype as a differential criterion. Selectivity of the medium was examined with a diverse collection of isolates (Table S2). Apart from *S. marcescens*, only *Serratia plymuthica* and *Serratia rubidaea* were able to grow slightly on *S. marcescens*-specific MacConkey medium (Sm-MacConkey), but the medium turned red due to their ability to ferment lactose (46). Sm-MacConkey medium was thus used for the remaining of this project.

High-throughput short-sequence typing (HiSST) of clinical strains. Among the 52 *S. marcescens* clinical isolates collected over the 3 years of this project, 71% were found in samples from more than one patient (37 of 52 clinical isolates). The genotypic profiles were highly heterogenous, with a total of 27 distinct sequence types (STs). The *S. marcescens* ST-46 caused most infections during the first cluster, with five infections (Fig. 1 and Table S1), while ST-51 was involved in five infections and three colonizations during the second cluster, with one potential colonization during the fifth cluster (clinical isolate BB35). On the other hand, there was no dominant genotype among clinical isolates isolated in the next three clusters. ST-48 was isolated from two patients 1 year apart (isolate BB17 in October 2019 and isolate BB25 in September 2020). Isolates BB3 and BB4 (genotype ST-46) and isolates BB44 and BB45 (genotype ST-66) were retrieved from twins. Isolates BB10 to BB12 (genotype ST-51) were isolated from the same patient, as well as isolates BB14 and BB16 (genotype ST-51). *S. marcescens* ST-18 retrieved from the throat of a newborn was found in his mother's breast milk. Ten cases of potential patient-to-patient transmission of *S. marcescens* occurred in shared rooms or rooms located in close proximity in the same corridor (Table 1). The exception was *S. marcescens* ST-54, which was involved in two infections that were not associated with room proximity.

In three cases, pulsed-field gel electrophoresis (PFGE) profiles of several clinical isolates were identical, while HiSST profiles showed different genotypes (Table S1). Whole-genome sequencing (WGS) comparison based on average nucleotide identity (ANI) analysis confirmed the lack of relatedness (ANI based on the MUMmer algorithm [ANIm] $<$ 99.0%) between isolates BB25, BB26, and BB31, isolates BB27 and BB39, or isolates BB41 and BB46, as predicted by the HiSST results (Fig. S1). ANI analysis confirmed the close relatedness between genomes BB17 and BB25 (ANIm $>$ 99.5%) as shown by the HiSST analysis, whereas their PFGE profiles were different. A limitation of the genotype comparison was found for isolate BB35, which had the same ST-51 as isolates BB10-16 and BB18 but a different pulsovar. The genome sequences were found to be similar but not close enough to conclude a clonal relationship (ANIm $>$ 99.2%; 98.9% $<$ ANI based on the BLASTN algorithm [ANIb] $<$ 99.0%).

Epidemiological link between *S. marcescens* clinical strains and the sink environment. Most clinical strains were isolated from gastrointestinal and respiratory tracts, as these were sites sampled for screening. Of a total of five STs found in both patient and sink drain samples, four STs were isolated from gastric tube samples (ST-7, ST-51, ST-59, and ST-62). In addition, two genotypically close STs (2 identical SSTs and 7 single nucleotide polymorphisms [SNPs] at the *gabR* locus) were detected in sink drains and isolated from several clinical sites (Fig. 2), including the respiratory tract, urinary tract, blood (ST-46 and ST-51), and gastrointestinal tract for ST-51. The dominant environmental ST-7 (eST-7) detected in eDNA sample BWD-S9-1292 is genotypically close to ST-51, with 2 identical SSTs and 4 SNPs at the *bssA* locus, which was detected in the same sink, S9, a week later and for the first time in 2020.

The HiSST method was applied to environmental DNA and isolates to examine the distribution and diversity of *S. marcescens*. None of the faucet samples (tap water and faucet aerator) were positive for *S. marcescens*. On the other hand, sink drains were frequently colonized, with an average of 44% positive samples, including 17% (n = 6 sinks), 33% (n = 6 sinks), 33% (n = 18 sinks), 45% (n = 20 sinks), and 55% (n = 39 sinks) positive drains during sampling campaigns 1 to 5, respectively. Overall, the diversity of *S.*

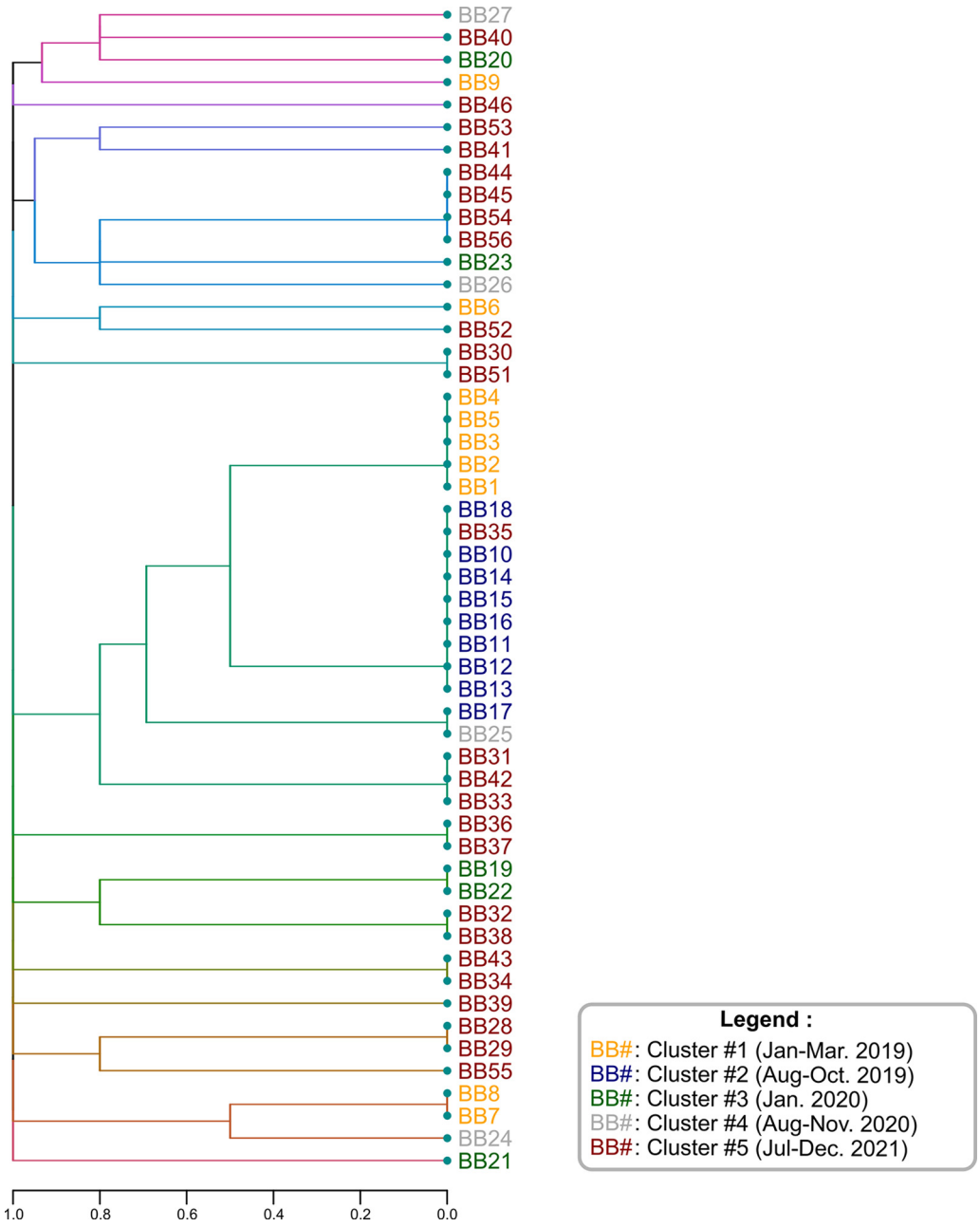

FIG 1 UPGMA dendrogram based on Jaccard distance computed with the HiSST profile of *gabR*, *bssA*, and *dhaM* loci among clinical isolates involved in NICU infections/colonizations.

marcescens was a mosaic of genotypes distributed across the NICU sink drains. Low genotypic diversity was found at the drain, with 1 to 13 different environmental short-sequence types (eSSTs) (average of 2 eSSTs per sample) depending on the locus and sample (Table S3). HiSST profiles are mostly similar between samples from the same room but taken on different dates. For example, the diversity profile of *S. marcescens* in the handwashing station (HWS) sink HWSs was conserved even after almost 2 years, as depicted by the HiSST profile of the sample WD-HWSs-0049, taken in February 2020, and the sample BWD-HWSs-1326, taken in November 2021 (Fig. 3). Moreover, the sinks HWSr and HWSs have the same HiSST profile. HWSr is in the neonatal intermediate care unit, while HWSs is in the NICU, and both are used by health care workers for handwashing at the entrance of each unit. The genotype profile was more similar between sinks sharing the same drain connection, as for sinks S17 and S18 or sinks S26 and S27 (Fig. 4).

TABLE 1 Summary of *S. marcescens* transmissions observed between sampling points from the NICU between 2019 and 2022

Transmission type and HiSST profile	Source 1	Room 1[a]	Date 1[b]	Direction of transmission[c]	Causal score[d]	Source 2	Room 2[a]	Date 2[b]	Findings
Patient to patient									
ST-46	BB4	S1	23 Jan 19	Unknown	0	BB3	S3	24 Jan 19	First acquisition occurred in twins (isolates BB4 and BB3) having shared a double room (S1 and S3) before and during their infection. Then, *S. marcescens* ST-46 spread to patients in rooms located at the top left on the NICU map (Fig. 4), in the same corridor.
	BB3 or BB4	S3, S1	23 Jan 19, 24 Jan 19	→	+	BB2	S4 or S5	16 Feb 19	
				→	+	BB5	S4	17 Feb 19	
	BB2	S4 or S5	16 Feb 19	→	+	BB1	S7	19 Feb 19	
ST-18	BB8	NA (isolated from the mother)	24 Feb 19	→	++	BB7	R6	26 Feb 19	*S. marcescens* ST-18 was transmitted from mother to newborn through mother's breast milk.
ST-51	BB10 = BB11 = BB12	S9 or R2	24 Aug 19, 25 Aug 19	→	+	BB15	S10 or R2	28 Aug 19	*S. marcescens* ST-51 was isolated from a patient in room S9 (isolates BB10 to BB12). ST-51 was detected 4 days later in a patient (isolate BB15), who was in room S10 a few days before sampling. This patient was moved from room S10 in the NICU to room R2 in the intermediate care unit a few days after colonization. One wk later, three new patients positive for *S. marcescens* ST-51 were confirmed in three adjacent rooms (R2, R4, and R5). A patient infected with isolates BB10 to BB12 was in room S30 before the sampling, adjacent to room S29, where a patient was positive with *S. marcescens* ST-51 (isolate BB18) 3 wks after the first colonization.
	BB15	R2 or R5	28 Aug 19	→	+	BB13	R4	4 Sep 19, 23 Sep 19	
	BB13 or BB15	R4 or R5	28 Aug 19, 4 Sep 19	→	+	BB14 = BB16	R5	11 Sep 19, 2 Oct 19	
	BB10 = BB11 = BB12	S30	24 Aug 19, 2 Oct 19	→	+	BB18	S29	13 Sep 19, 23 Sep 19, 2 Oct 19	
ST-48	BB17	S2	2 Oct 19	→	+	BB25	S6	21 Sep 20	Transmission was observed in adjacent rooms S2 and S6.
ST-53	BB19	S28	15 Jan 20	→	+	BB22	S30	21 Jan 20	*S. marcescens* ST-53 was detected in two patients (isolates BB19 and BB22) who shared the same double room before they became positive.
ST-52	BB28	S18	2 Jul 21	→	+	BB29	S20	9 Jul 21	Transmission observed in adjacent rooms S18 and S20.
ST-54	BB51	NA (pediatric ICU)	3 Feb 20	→	0	BB30	S5	8 Jul 21	Strain BB30 was isolated from a NICU patient, while isolate BB51 was detected in a patient from the pediatric ICU.
ST-59	BB31	S15	17 Aug 21	Unknown	0	BB33	S16	17 Aug 21, 11 Sep 21	Transmission observed in adjacent rooms S14, S15, and S16.
				→	+	BB42	S14	18 Oct 21	
ST-60	BB32	S18	17 Aug 21	→	+	BB38	S17	22 Sep 21	Transmission observed in adjacent rooms S17 and S18.
ST-61	BB34	R3	2 Sep 21	→	+	BB43	R2	20 Oct 21	Transmission observed in adjacent rooms R2 and R3.
ST-62	BB36	S16	17 Sep 21	Unknown	0	BB37	S15	17 Sep 21	Transmission observed in adjacent rooms S15 and S16.
ST-66	BB44 or BB45	S15 or S16	2 Nov 21	→	+	BB54	S15	16 Dec 21	*S. marcescens* ST-66 transmission was initiated by two colonizations that occurred in adjacent rooms S15 and S16. One mo later, ST-66 was isolated from a patient in room S15. This patient was moved to room S1, adjacent to room S3, where an *S. marcescens* was isolated a few days later.
	BB54	S15 or S1	16 Dec 21	→	+	BB56	S12 or S3	20 Dec 21	

(Continued on next page)

TABLE 1 (Continued)

Transmission type and HiSST profile	Source 1	Room 1[a]	Date 1[b]	Direction of transmission[c]	Causal score[d]	Source 2	Room 2[a]	Date 2[b]	Findings
Sink drain to patient									
ST-59	BWD-S9-1250-Sm3	S9	7 Sep 21	→	++	BB42	S14	18 Oct 21	ST-59 was found in sink S9 before being involved in a new infection in October 2021.
Patient to sink drain									
ST-46	BB4	S1	23 Jan 19	→	+	BD1b-2wD	S1	9 Apr 19	Strain BD1b-2wD was isolated from sink drain S1 over one mo after patient positivity. Since sink #S1 was not sampled prior to infant colonization, patient to sink drain transmission can only be assumed.
ST-51	BB10 = BB11 = BB12	S9	24 Aug 19	→	+	WD-S9-0042, BWD-S9-1301	S9	11 Feb 20, 28 Oct 21	ST-51 was detected in sink drain S9 almost six mo after colonization, corresponding to the dominants eSST in the sample WD-S9-0042 (Table S3). Then, ST-51 was detected in sink S9 1.5 yr later (corresponding to the dominants eSST of sample BWD-S9-1301). Sink S9 was not sampled prior to infant colonization.
ST-7	BB27	S12	10 Nov 20	→	+	BWD-S12-1236-Sm1	S12	24 Aug 21	ST-7 was detected in sinks S12 and S21 six mo and one yr after clinical detection, respectively. The clinical isolate BB27 and the environmental isolate BWD-S12-1236-Sm1 were closely related, as confirmed by WGS (ANIm > 99.9%) (Fig. S1). Sink drain S12 was negative for *S. marcescens* nine mo to one yr prior to infant colonization, on three sampling dates. Sink 21 was not sampled prior to infant colonization.
				→	+	BWD-S21-1349	S21	15 Dec 21	
ST-59	BB33 or BB42	S16 or S14	17 Aug 21 or 18 Oct 21	→	+	BWD-S16-1313-Sm1, BWD-S16-1326, BWD-S16-2028	S16	9 Nov 21, 22 Nov 21, 28 Jan 22	Two patients were infected by ST-59 in August 2021 (isolates BB31 and BB33). This genotype was found in sink S9 in September 2021, whereas it was negative in 2019 and 2020 prior to infant colonization. Then, ST-59 was involved in a new infection in October 2021 (isolate BB42). Later, two other sink drains were positive for ST-59 (sink S1 and S16 between November 2021 and January 2022). Sink S16 was negative on October 2019, prior to infant colonization. Sink S1 was negative for ST-59 in 2019 and on August 2021 (same day as the clinical sampling). Isolates BB31 and BWD-S9-1250-Sm3: ANIm > 99.9%.
	BB33	S3	17 Aug 21	→	+	BWD-S1-1349	S1	15 Dec 21	
	BB31 or BB33	S15 or S3 or S16	17 Aug 21	→	++	BWD-S9-1250-Sm3, BWD-S9-1278, BWD-S9-1285	S9	7 Sep 21, 5 Oct 21, 12 Oct 21	
ST-62	BB36	S2	17 Sep 21	→	+	BWD-S2-1326, BWD-S2-1330	S2	22 Nov 21, 26 Nov 21	Two patients were colonized (isolate BB36) or infected (isolate BB37) by ST-62, before detecting this genotype in sinks S2 and S3 two mo later. Sink drains were sampled in 2019 prior to infant colonization and was negative for *S. marcescens*.
	BB37	S3	17 Sep 21	→	+	BWD-S3-1326	S3	22 Nov 21	

(Continued on next page)

TABLE 1 (Continued)

Transmission type and HiSST profile	Source 1	Room 1[a]	Date 1[b]	Direction of transmission[c]	Causal score[d]	Source 2	Room 2[a]	Date 2[b]	Findings
ST-2	Strains BWH-35 and 95[e]	NA	2012 and 2015	→	+	WD-S34-9288-Sm2	S34	15 Oct 21	The genotype ST-2, corresponding to *S. marcescens* 95 and BWH-35 isolated in a Boston hospital (USA) from sputum samples, was detected in sink drains S34, S17, S18 and sink S15 (corresponding to the dominant eSST of sample BWD-S15-1313, BWD-S15-1326 and BWD-S15-1330). Pairwise comparison of contiguated genomes WD-S17-9295-Sm, WD-S18-9295-Sm1 and WD-S34-9288-Sm2 (isolates from sinks S17, S18 and S34) confirmed the high degree of similarity with *S. marcescens* 95 and BWH-35 genomes (ANIm > 99.9%) (Fig. S1).
				→	+	WD-S17-9295-Sm	S17	22 Oct 21	
				→	+	WD-S18-9295-Sm1	S18	22 Oct 21	
				→	+	BWD-S15-1313 to BWD-S15-1330	S15	22 Nov 21, 26 Nov 21	

[a]Room(s) (where the patients were admitted) most likely to have transmitted the pathogen due to the proximity of room 1 and room 2. NA, not applicable.
[b]Day the sample was collected.
[c]For transmission between two sampling points >72 h apart, we defined the older one as the source.
[d]Type of evidence for causal relationship, adapted from modified CADDIS (54), scored as follows: 0, evidence for causal relationship is somewhat supported by biological specificity confirmed by HiSST but is neither supported nor weakened by temporal sequence and spatial proximity between source 1 and source 2 in the NICU; +, evidence for causal relationship is somewhat supported by temporal sequence (exposure to contaminated sink drain was present prior to organism acquisition, or vice versa) and/or by spatial proximity between source 1 and source 2 in the NICU (e.g., positive patients in same NICU area or a colonized sink in the same room as a positive patient) and by biological specificity confirmed by HiSST; ++, evidence for causal relationship is strongly supported by temporal sequence (exposure to contaminated sink drain was present prior to organism acquisition, or vice versa), by spatial proximity between source 1 and source 2 in the NICU (e.g., positive patients in same NICU area or a colonized sink in the same room as a positive patient), and by biological specificity confirmed by HiSST and WGS.
[e]Strains 95 and BWH-35 were isolated from Brigham and Women's Hospital by Xiaomin Zhao (BioProject: PRJNA278886).

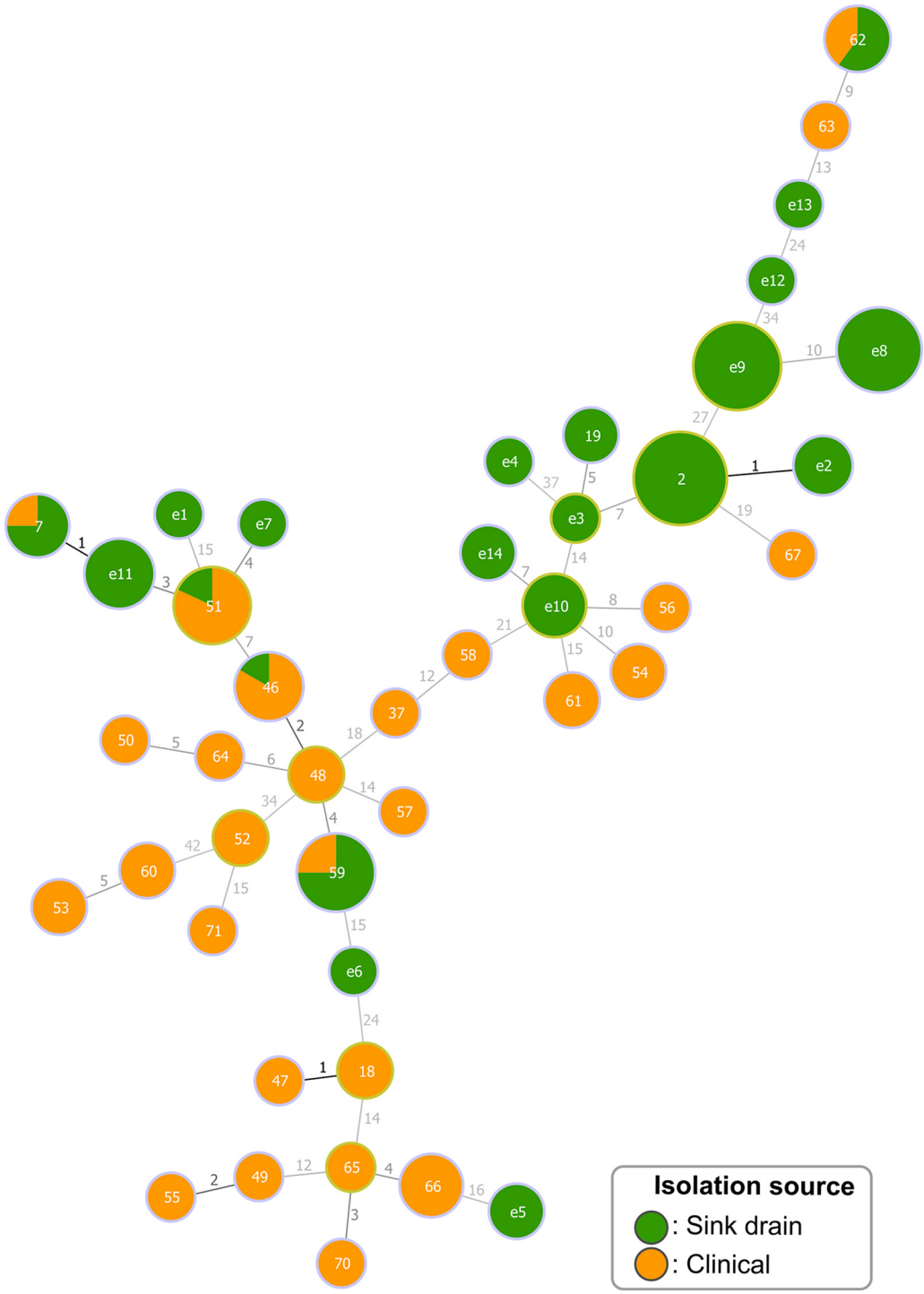

FIG 2 Relationship among the HiSST profile of 52 epidemiologically related isolates and eDNA originating from sink drains. The minimum spanning tree is based on SNP analysis of *S. marcescens* HiSST profiles. The distance labels between two STs correspond to the number of SNPs that differ between them, while the ST nodes are colored based on their sample origins. HiSST profiles of clinical strains are depicted in orange. Green nodes correspond to HiSST profiles generated for the sink drain samples based on the combination of the dominant eSST (i.e., ASV) for each locus from eDNA samples. HiSST profiles found exclusively in eDNA samples, with no match to the HiSST database for *S. marcescens*, are identified by an "e" followed by a number.

The pairwise comparison of HiSST profiles derived from isolates and environmental DNA shows two distinct clades: a clade with only sink drain samples (blue in Fig. 3) and a clade comprising all the clinical isolates and some sink drain samples (yellow in Fig. 3). Four phylogenetic clusters encompassed clinical and environmental samples with identical

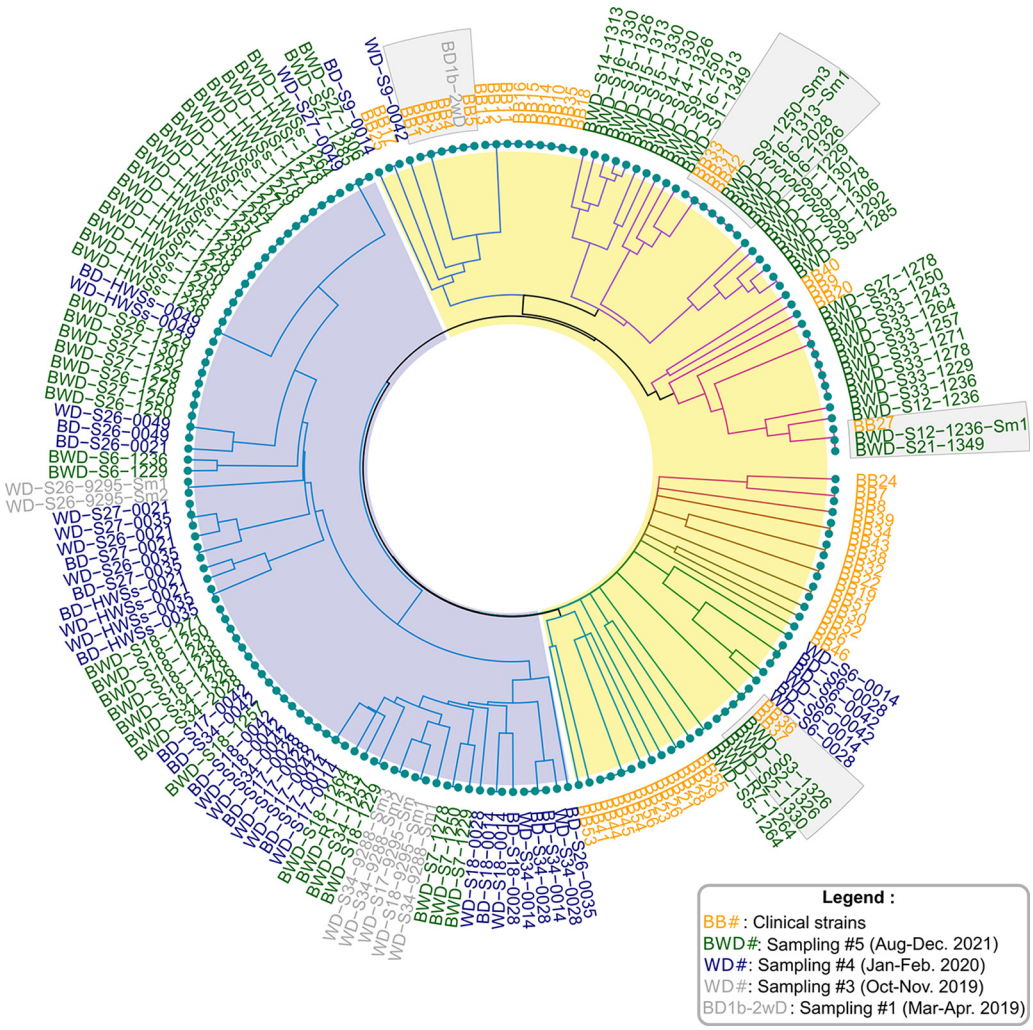

FIG 3 Circular UPGMA dendrogram based on Jaccard distance, computed with the HiSST profile of *gabR*, *bssA*, and *dhaM* loci among all positive clinical and environmental samples obtained from the NICU between 2019 and 2022. A clinical isolate with an HiSST profile close to a sink sample is more likely to colonize that sink. Samples are named as follows: sample type (BD or WD for biofilm or water drain; BWD for biofilm and water drain samples pooled during sampling 5)–room number–date (The first digit represents the year [0 for 2020, 1 for 2021, 2 for 2022], while the next three digits indicate the day number of the year). Isolate are identified by "Sm" numbers at the end of the sample name. Clinical strains are numbered from BB1 to BB56. The clade in yellow highlights the epidemiological link between clinical and sink drain samples' HiSST profiles, while the blue clade gathers sink drain samples without epidemiological links. Phylogenetic clusters in gray correspond to sink drain samples and clinical isolates with identical HiSST profiles.

HiSST profiles. However, some sink drains have several eSSTs within the same locus, and the combination of those eSSTs corresponds to the ST of several clinical strains. For example, sink S9 was likely colonized by two clinical strains involved in HAI during clusters 2 and 5 (genotype ST-51) and during cluster 5 (genotype ST-59).

Five cases of possible transmission events of *S. marcescens* between patients and sink drains were identified (not including genotype ST-2, clinical strains of which were not collected in this study), linked with each other in space and time (Table 1 and Fig. 5). Among them, four suggested *S. marcescens* transfer from a patient to the drain environment and one highlighted potential colonization by clinical strains introduced in the sink environment in the room of a patient colonized with the bacterium. In fact, potential successive transfers of *S. marcescens* ST-59 between patients and sinks were observed during the last cluster of cases (Table 1). ANI analysis of whole genomes confirmed the HiSST analysis of ST-59 for clinical isolates BB31, BB33, and BB42, showing a very close relatedness with the environmental isolates retrieved from sinks S9 and S16 (isolates BWD-S9-1250-Sm3 and BWD-S16-1313-Sm1; ANIm $>$ 99.9%) (Fig. S1). Sink

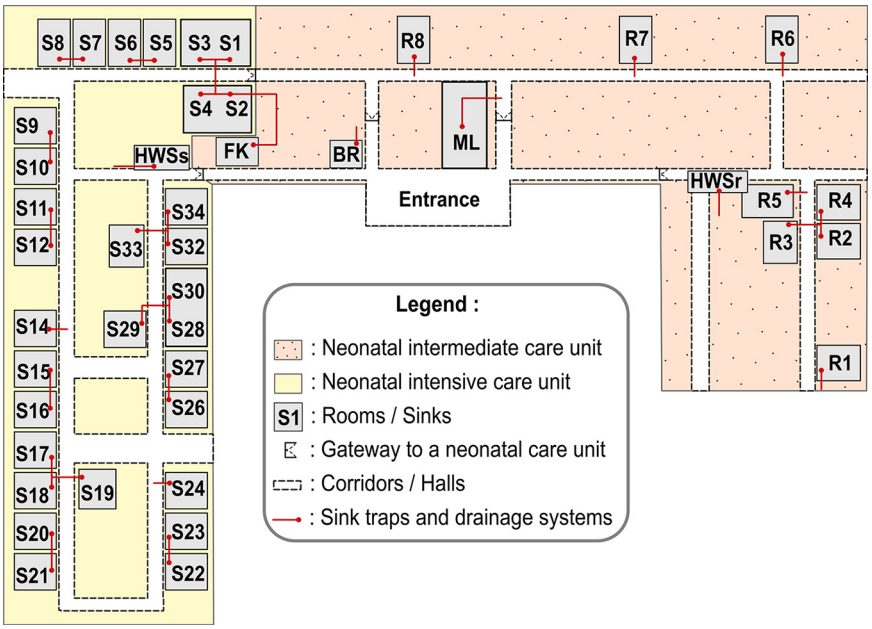

FIG 4 Layout of the sinks surveyed in the investigated neonatal unit. The neonatal intensive care unit area is mapped in yellow, and the neonatal intermediate care unit is mapped in pink. Studied sinks are represented by gray rectangles labeled with the sink numbers. Sinks in patient rooms are identified by "S" for intensive care or "R" for intermediate care. The water drainage systems are represented by red lines, where each red dot corresponds to the sampled P trap. The corridors are delimited by dotted lines. HWSs, handwashing station in ICU; HWSr, handwashing station in intermediate care unit; FK, family kitchen; BR, breastfeeding room; ML, milk laboratory.

drains can remain colonized by clinical strains for long periods of time, ranging from weeks to years (e.g., sink drains S2 and S3 were positive for ST-62 5 weeks after infant colonization but negative 1 week after detection; ST-51 was detected in sink drain S9 over 2 years after infant colonization). Unfortunately, ST-51 was not isolated from drain S9, precluding definitive confirmation by WGS.

DISCUSSION

Our study is a retrospective and prospective investigation of *S. marcescens* transmissions between newborns and sink environments during five clusters of cases in a NICU

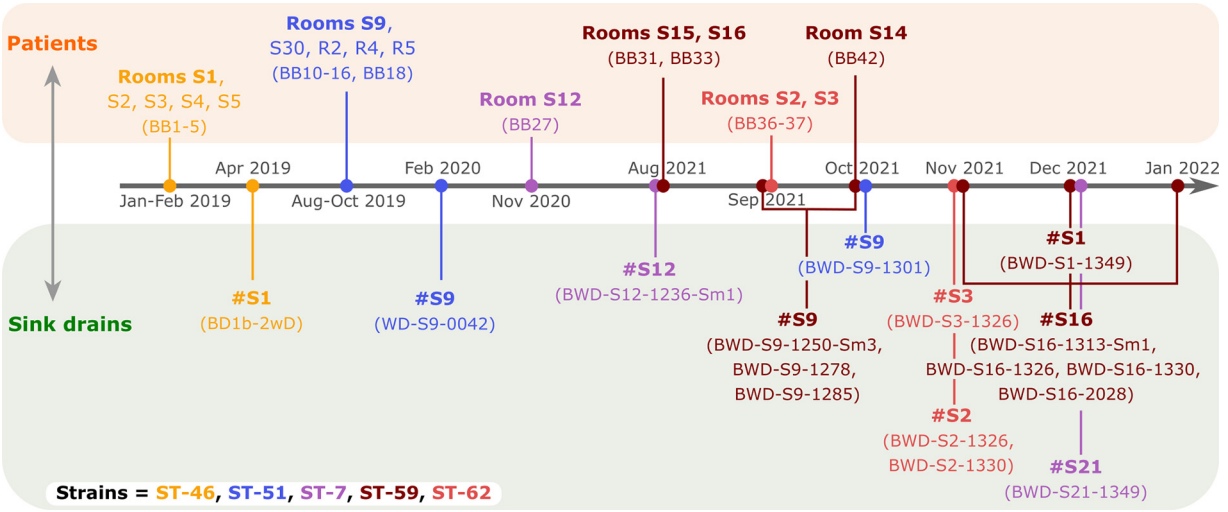

FIG 5 Chronology of potential transmission events of *S. marcescens* between sink drains and patients. Five transmission events are represented by different colors, depending on the ST involved. Rooms where patients were positive for *S. marcescens* are identified in the orange rectangle, and sink drains are identified in the green rectangle. Rooms where sink drains were colonized by the same genotype as a clinical strain are highlighted in bold at the top. Names of positive samples are in parentheses.

between 2019 and 2022. A new epidemiological approach based on ST analysis of eDNA and clinical isolates was applied to more than 500 sink environmental samples and 56 clinical samples. The HiSST method overcomes limitations observed in previous studies in which the resource-intensive nature of environmental source tracking, relying on conventional typing culture-dependent methods, reduces the number of field observations to identify the potential source (34). The presence of several genotypes in the same environmental sample may bias determination of the clonal relationship between clinical isolates and environmental samples. Therefore, the pairwise comparison of HiSST bacterial profiles, expressed as a pairwise Jaccard distance, should be carefully analyzed for samples that have a similar but not identical HiSST profile. Such a result would indicate the likely presence of a clonal strain in these samples and should be confirmed by a culture-dependent approach combined with ANI analysis of whole genomes (e.g., sink S9 was colonized by the clinical strains with genotypes ST-51 and ST-59). Since ANI analysis based on WGS shows a higher specificity for HiSST genotyping than PFGE in our study, HiSST analysis appears to be more reliable for examining the relatedness of environmental or clinical isolates.

Furthermore, HiSST offers the advantages of rapid and accurate identification of bacterial strains directly from eDNA or clinical samples, thus allowing the detection of pathogens present in a viable-but-nonculturable (VBNC) state. Failure to account for VBNC microbes could underestimate the risk of HAIs associated with the sink environment (47). In addition to HiSST, the use of alternative approaches such as targeted 16S rRNA gene amplicon sequencing could provide a more complete picture of microbial diversity within the sink ecosystem, including VBNC bacteria (48).

In this study, the spread of *S. marcescens* was most likely caused by horizontal transmission within the NICU during the first outbreaks. In all, 37 clonal relationships were noted among the 52 clinical isolates of *S. marcescens*, with a total of 27 unique STs. Ten potential cases of patient-to-patient transmission were found, involving 12 unique STs and 65% of the isolates (n = 32 of 49 isolates, not including duplicate isolates from the same patients). Thus, colonized or infected patients from this NICU were the principal source of *S. marcescens* detected in this study, as reported in most previous studies (32), specifically in gastrointestinal tract and respiratory tract samples. During clusters 1 and 2, patients' movements facilitated the transmission of *S. marcescens* through the NICU rooms and between different units of the same hospital. The circulation of *S. marcescens* in different areas of the same hospital and interhospital had been observed in another study (49, 50).

On the other hand, the latest clusters were most likely caused by multiple sources of *S. marcescens*, including sink drains. The high rate of positivity in sink drains (up to 55%) emphasizes that sink drains play an important role as a reservoir for *S. marcescens*, in agreement with a large prospective study recently reported by Valentin et al. (18). Some environmental and clinical isolates were found in sink drains up to 1 year after the first sampling campaign, testifying to the persistence of these strains in drains. The clinical strains displayed diversified genotypes, without a dominant strain involved in the last three clusters. The high genotypic diversity of clinical strains, particularly during the fifth cluster, suggests diverse sources of *S. marcescens* within the NICU. Proximity between sinks and their use (e.g., families' or health care workers' handwashing and medical or nonmedical equipment washing) could explain the presence of the same *S. marcescens* genotype in different sink drains (51, 52). For example, the relationship between sinks S26 and S27, or between sinks S17 and S18, is supported by the proximity of both sinks and a shared drain connection (Fig. 4). Nevertheless, two sinks were colonized with the same genotypes despite their distance: sink HWSr is in the neonatal intermediate care unit, while HWSs is in the NICU. In this case, sinks are linked by their use and location in the unit, as they are located at the entrance of each unit, where health care workers wash their hands before entering or leaving the unit. Handwashing may be the main vector explaining the presence of same genotypes in both sinks (53), but other factors intrinsic to the sink or from the surrounding

environment are important to consider. In fact, the presence of a genotype could depend on specific parameters of sink drains (e.g., microbial diversity, physicochemical parameters, and plumbing material) as well as external event(s) (e.g., a colonized or infected newborn in the room or handwashing by a colonized visitor). Thus, many factors can explain the prevalence of heterogenous genotypes through the NICU even in a given area. The impact of these factors specifically on *S. marcescens* remains unclear.

Furthermore, five cases of potential transmission events of *S. marcescens* from patients to sink drains were identified. Three genotypes (ST-7, ST-59, and ST-62) detected in sink drains were detected exclusively in gastric tube samples, while two other genotypes (ST-46 and ST-51) were detected in multiple clinical specimens and in sink drains (Fig. 2 and Table S1). Overall, eight clinical isolates among five unique STs were detected in sinks mainly after colonization or infection, representing 16% of clinical isolates from this study (not including duplicate isolates from the same patients). In most cases, clinical strains were detected in P traps after the organism acquisition. This shows a host-pathogen interaction, mostly detected as a transmission from patients to sinks. The spatiotemporal distribution profile of strain ST-59 was identified as a potential case of bidirectional transfers between sink and patient. The likelihood of the causal relationship was assessed by applying six evidence areas of the modified CADDIS tool (54). According to this tool, the likelihood of causality of sink drain S9 in patient BB42's infection by strain ST-59 is strongly supported by temporal sequence (exposure to the contaminated sink drain occurred prior to organism acquisition) and by evidence of exposure and biological specificity as confirmed by HiSST and WGS. The manipulation of exposure domain somewhat supports the causal relationship between sink and patient, as an intensive disinfection of sink drains by a thermal process (performed at the end of sampling 5) coincided with the end of colonization or infection events in the NICU during the first months after this intervention (data not shown).

The sampling design included 32% of all the sinks in the neonatal care units (40 of 125 sinks in intensive and intermediate care units), although 89% of sinks in patient rooms were sampled in the NICU. Despite this intensive survey, only rare occurrences of sink-to-patient transmission were discovered. However, limitations of our study include that not all sinks were sampled, and in some cases, sink drains were sampled only after the onset of infections. Also, the sampling method did not technically allow recovery of the entire drain community. In most cases, therefore, this approach did not allow us to verify the presence of the pathogen in sinks prior to patient colonization. Another limitation to the detection of *S. marcescens* in sink drains was the use of a molecular method, which is limited by the PCR sensitivity and by *S. marcescens* genomic databases. Environmental sources are rarely found overall because of the multitude and complexity of the surrounding sources, as reported elsewhere (32, 55, 56). Nevertheless, the proportion of *S. marcescens* potential transmission from sink to patient is similar to that of *Pseudomonas aeruginosa* (1.5%), as reported by Couchoud et al. (19). Since *S. marcescens* can survive in many conditions and colonize both wet and dry surfaces during a long period, other sources should be investigated, such as sink drains in the patient's family home, cleaning material used in rooms, and soap or hydroalcoholic gel used for hand washing (57–60). On the other hand, the increase in *S. marcescens*-positive cases in the NICU may be explained by the increase in *S. marcescens* screening efforts, which is rarely performed in other units. Intensive procedures to prevent surface contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may also select for *S. marcescens* because of its resistance to disinfection and its ability to survive on inanimate surfaces for long periods (60, 61).

Incorporating non-culture-based methods into future studies could improve the understanding of the risks posed by the built environment and inform infection control strategies to reduce the risk of HAIs associated with the neonatal intensive care unit's built environment.

Conclusion. Our results suggest the presence of multiple reservoirs of *S. marcescens* within the NICU, including patients, sink drains, and external sources. The genotype profiles of clinical isolates were highly heterogenous. Ten potential cases of patient-to-patient transmission were found, with 71% isolates found in more than one patient. The diversity of

S. marcescens was highly heterogenous across the NICU sink drains, but low genotypic diversity was found at individual drains. Our results demonstrate that sink drains are an important reservoir of *S. marcescens*, which can cause cross-contamination of patients. Moreover, some clinical isolates can thrive in sink drains and can persist for long periods of time.

This study correlates various types of epidemiological information between patients and sink environments, which is essential to identify the outbreak source(s). The sparse distribution of various genotypes in individual sinks implies that all NICU sinks should be analyzed by HiSST to gain a better understanding of the direction of *S. marcescens* transmission. Indeed, listing all *S. marcescens* HiSST profiles from all NICU sinks would be useful to assess sink involvement in future infection events. Regarding the high rate of transmission between patients and sink drains, systematic disinfection of drains is strongly recommended between newborn hospitalizations to prevent cross-contamination events. These preventive measures should reduce the risk of sink colonization by an infectious strain and thus of cross-contamination.

MATERIALS AND METHODS

NICU description. The neonatal care unit has a capacity of 35 intensive care and 45 intermediate care beds, with mainly single bedrooms and 10 double bedrooms for twins on a single floor in a dedicated hospital area. The nursing staff and personnel are highly compliant with infection control measures: handwashing, disinfection of surfaces and medical devices in the room at patient discharge, and wearing of personal protective equipment, as required. Isolation measures are applied, based on syndrome and etiology, as per hospital protocol. Patients are moved between rooms depending on their health and to facilitate care delivery. Each patient room has its own sink at the room entrance, separated from the corridor by a door. Double rooms have the same configuration as single rooms, with two sinks but without a separation between beds. Sink configuration in the NICU consists of hot and cold water activated through two distinct pedals, with the faucet not aligned with the sink drain inlet, and P traps in chrome-plated brass. The water drainage system of the NICU floor is mainly vertical, connecting directly to the main sewage collectors on the lower floors. Overall, half of the adjacent sinks from patient rooms share a drain pipe before connecting to the main sewage collector pipes on the lower floors. The building's hot- and cold-water system supplies the entire NICU.

Clusters of *S. marcescens* and sampling in the NICU. Five clusters of *S. marcescens* were recorded in the NICU between January 2019 and January 2022 (Fig. 6). As a preventive measure, whenever an *S. marcescens* infection or colonization occurred, patients on the entire unit (intensive and/or intermediate) were screened once and then every 2 to 4 weeks until 2 to 4 weeks after the last known positive patient was discharged. A total of 52 *S. marcescens* isolates were obtained by the infection prevention and control (IPAC) team from 23 reported infections and 29 colonized patients (Table S1). Clinical isolates retrieved from clinical specimens are designated with the prefix "BB." The first cluster of cases was reported in the beginning of 2019, with eight newborns positive for *S. marcescens* in 3 months, and included one strain isolated from the mother of a positive newborn. Between August and October 2019, a second cluster involved nine clinical isolates from six newborns in the NICU. The third cluster happened in January and February 2020, where five isolates were found in newborns upon screening. Between August and November 2020, four new patients were positive for *S. marcescens*, including two confirmed infections. Finally, the largest cluster lasted 6 months, in the second half of 2021, with 25 patients positive for *S. marcescens* (i.e., 8 infections and 17 colonized patients). Patients were mostly premature newborns who were on average 36 days old. In addition, five *S. marcescens* isolates were retrieved from patients hospitalized in the pediatric intensive care unit, aged 81 days old to 3 years old (isolates BB2, BB6, BB40, BB46, and BB51), including one patient who was in the NICU prior to his infection (isolate BB2). One strain was retrieved from the mother (isolate BB8) of a positive newborn (isolate BB7). These clinical strains were isolated from multiple sources, mostly from the gastrointestinal tract ($n = 26$), respiratory tract ($n = 13$), blood ($n = 5$), urinary tract ($n = 3$), and other sources (conjunctiva, $n = 2$; abdomen, $n = 2$; breast milk, $n = 1$). Clinical isolates were included in this study to examine their relatedness with environmental strains and genotypes from the sink environment.

Five sampling campaigns of the sink environment were conducted in the NICU between 2019 and 2022 (Fig. 6). A first sampling was conducted in March and April 2019 to test the sampling methodology and the selective culture medium for *S. marcescens*. Tap water, drain water, and biofilm from six sinks were sampled on three occasions over 2 weeks, including five sinks in rooms occupied by *S. marcescens*-positive patients and one sink in an unoccupied room. A second sampling round was performed in September 2019. Water from six sink drains was sampled, including three sinks in patient rooms, one HWS localized at the entrance of the NICU, one sink with a new drain in an unused room, and one sink equipped with a Kleanik (Surgmed Group, Montréal, QC, Canada) drain self-disinfection system. In October 2019, a third sampling round was done to collect tap and drain water and to swab faucet aerators and drain biofilm in 1 day from 18 sinks in patient rooms. For 2 months at the beginning of 2020, tap water, faucet aerators, drain water, and drain biofilm were sampled from 20 sinks on three dates during a fourth sampling event. Sinks sampled were those in patient rooms, sink HWSs, and sinks in the intermediate unit, including one handwashing station in the intermediate unit (sink HWSr), one sink in the family kitchen (sink FK), one sink in the breastfeeding room (sink BR), and one sink in the milk laboratory

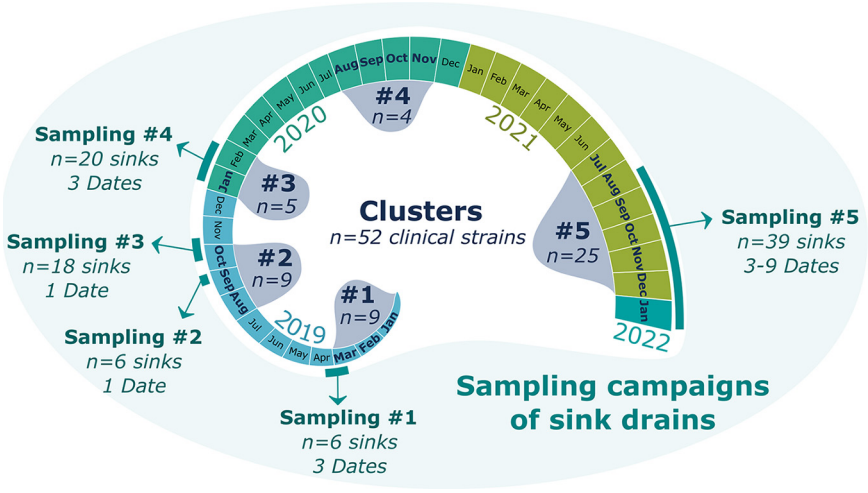

FIG 6 Chronology of sampling campaigns and clusters between 2019 and 2022. Five colonization clusters occurred between 2019 and 2022; the number of clinical strains isolated for each cluster is shown in dark blue. Sampling campaigns are shown in green, with the corresponding number of sinks sampled and sampling dates.

(sink ML) (Fig. 4). One last sampling campaign was performed during the second half of 2021 to January 2022, with 39 sinks sampled on several occasions, including sinks described above and sinks in rooms of positive patients.

Water and biofilm were sampled from the faucet and the drain (i.e., the P trap) of selected sinks. The sampling routine started with drain water collection using a flexible autoclaved plastic tube, attached to a 50-mL sterile syringe. The tip of the tube was inserted at the bottom of the P trap to collect a representative 100-mL drain water sample, which was transferred to a 100-mL sterile plastic bottle. The biofilm from the inner sides of sink drains was sampled from the top of the strainer to the bottom of the P trap with a nylon-flocked swab (Puritan Medical Products, Guilford, ME, USA) extended with a rigid wooden rod. Swab tips were stored in 15-mL sterile tubes with 2 mL phosphate-buffered saline (PBS). Faucet aerators were then sampled using nylon-flocked swabs stored in 2 mL PBS. Blank samples consisting of 2 mL PBS in 15-mL tubes were prepared before each sampling routine to ensure that sterile conditions were maintained during material preparation and sample processing. As a last step, a defined volume of tap water (1 L; hot and cold water, 1:1) was collected and stored in 1-L sterile plastic bottles. Samples were processed within 6 h from sampling. During the last sampling campaigns in 2021 and for molecular detection, faucet aerator samples were pooled with tap water samples from the same sink, just as drain biofilm was pooled with drain water samples, since the prevalence of pathogens was similar between water and swab samples from the same sink part.

Sample processing. Samples were processed for downstream isolation efforts of cultivable *S. marcescens*, PCR analyses, or archive storage. Tap water (500 mL) and drain water (50 mL) were filtered under aseptic conditions on 0.45-μm-pore-size mixed cellulose ester (MCE) sterile membranes (Millipore Sigma-Aldrich, Oakville, ON, Canada). Membranes from tap water samples were put on *S. marcescens*-specific MacConkey medium (Sm-MacConkey; see below) and incubated for 48 h at 30°C. Membranes from drain water samples were stored in 5-mL tubes (Eppendorf) with 4 mL glycerol (16% [vol/vol]) at −80°C. These archived samples were stored for subsequent isolation efforts in samples displaying environmental HiSST (eHiSST) profiles identical to those of clinical isolates. In such cases, the archived tube was thawed, 3 to 5 sterile glass beads were added, and the tube was vortexed for 30 s at maximum speed to homogenize the sample. A defined sample volume (100 μL) was spread on the selective agar plates using 4 or 5 sterile glass beads, before incubation for 48 h at 30°C. Each unique colony morphotype was purified on plates containing Trypticase soy broth (TSB) (Difco Laboratories, Sparks, MD, USA) solidified with agar (15 g/L) (Alpha Biosciences, Inc., Baltimore, MD, USA) at 30°C for 48 h. A single colony of each isolate was inoculated in 2 mL TSB and grown for 48 h at 30°C for subsequent genomic DNA extraction and HiSST analysis. Tap water (500 mL) and drain water (50 mL) samples dedicated to eHiSST genotyping were filtered on 0.22-μm MCE sterile membranes. Membranes were stored in 2 mL lysing matrix A tubes (MP Biomedicals, Irvine, CA, USA) at −80°C. Quality control of the filtration was ensured by three successive rinses of the system with sterile water, 70% ethanol (vol/vol), and sterile water between samples. Funnel and filter plates were changed after two filtrations with new sterile materials, and forceps were sterilized in 70% ethanol (vol/vol) and a flame after each use. A blank was established by filtering 100 mL of sterile water used for the rinse on a sterile MCE membrane placed on an Sm-MacConkey plate. No growth was observed after 48 h of incubation at 30°C.

Biofilm samples from swabs used for faucet aerator sampling and for sink drain sampling were thoroughly mixed (vortex) with 4 or 5 sterile glass beads to detach biomass. Subsamples were then collected for cultivation purposes (100 μL) and environmental genomic DNA (eDNA) extraction (1 mL) conducted according to procedures described above.

Genomic DNA extraction. An environmental sample (1 mL) or bacterial suspension (1 mL) was transferred to 2-mL FastPrep lysing matrix tubes, including 250 mg of 0.5-mm Mini-BeadBeater glass mill

beads and 250 mg of 0.1- to 0.15-mm Mini-BeadBeater zirconia-silicate beads (Cole-Parmer Canada Company, Quebec, QC, Canada). Tubes were centrifuged for 10 min (16,000 × *g*), and the supernatant was discarded. Microbial cells from water samples were resuspended in 1 mL DNA extraction buffer (50 mM Tris-HCl [pH 8], 5 mM EDTA-2Na [pH 8]; 3% SDS [vol/vol]; ultrapure Milli-Q water) supplemented with 20 μg/mL RNase, whereas microbial cells from biofilm samples were subjected to extracellular polymeric substance (EPS) digestion in 1 mL glucoside hydrolase solution to improve DNA extraction yield, as described by Fleming et al. (62). Glucoside hydrolase solution is a 1:1 mixture of molecular-grade α-amylase from barley malt (Sigma Chemical Co., St. Louis, MO, USA) and cellulase (Trichromatic Techno-Chem Inc., QC, Canada). Digestion was conducted for 30 min at room temperature, and the cells were pelleted by centrifugation (10 min, 16,000 × *g*) and suspended in 1 mL DNA extraction buffer supplemented with 20 μg/mL RNase. Cell lysis of water and biofilm samples was then conducted through two successive runs of bead beating (45 s, 6.5 m/s) (Fastprep-24; MP Biomedicals, Solon, OH, USA). Ammonium acetate was added (2 M, final concentration) to precipitate proteins and cellular debris. The mixture was chilled 5 min on ice and centrifuged (21,000 × *g*) 15 min at 4°C. Supernatant was collected for a second precipitation on ice and centrifuged (21,000 × *g*) 15 min at 4°C. One volume of 100% isopropanol was added to the supernatant for overnight precipitation at 4°C, supplemented with 2 μg glycogen (molecular biology grade; Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania). The DNA pellet obtained after centrifugation (21,000 × *g*) for 30 min at 4°C was successively washed with ethanol (70% [vol/vol]) and centrifuged (21,000 × *g*) for 15 min at 4°C twice. Purified genomic DNA was dried for 10 min under aseptic conditions and solubilized in 50 μL of sterile water. DNA extract was quantified (NanoDrop 2000c; Thermo Fisher Scientific), diluted to 25 ng/μL, and stored at −80°C.

Selective culture medium for *S. marcescens*. The specificity of three selective culture media for *S. marcescens* was examined: DNase test agar medium (22, 63) (Difco Laboratories, Sparks, MD, USA), caprylate-thallous agar medium (64), and MacConkey agar medium (Difco Laboratories, Sparks, MD, USA). To make it more specific for *S. marcescens*, we modified the MacConkey medium (referred to as Sm-MacConkey here) with the addition of antibiotics: 5 mg/L colistin, 10 mg/L cephalothin, 5 mg/L ampicillin, and 2.5 g/L amphotericin B (antibiotic selection adapted from the DNase medium preparation [63]).

Tests were performed with 15 clinical and environmental strains of *S. marcescens*. Plates were incubated at 30°C for 48 h. Negative controls, comprising *Serratia liquefaciens* (n = 1), *Serratia plymuthica* (n = 1), *Serratia rubidaea* (n = 1), *Stenotrophomonas maltophilia* (n = 1), *Pseudomonas aeruginosa* (n = 1), *Klebsiella pneumoniae* (n = 1), and *Pseudomonas beteli* (n = 1), were included to evaluate the selectivity of the culture media (Table S2).

***S. marcescens* detection and genotyping.** Species of presumptive *S. marcescens* strains isolated from tap water and faucet aerator samples were verified by PCR targeting the loci *bssA*, *dhaM*, and *gabR*, which were comprised in the HiSST scheme we developed for *S. marcescens* genotyping (65). Presumptive occurrence of *S. marcescens* in water and biofilm from sink drain samples was assessed by PCR targeting only the locus *bssA* to reduce manipulation effort. PCRs were carried out in 25-μL reaction volumes containing 0.6 U Fast-*Taq* DNA polymerase (Bio Basic Inc., Markham, Canada), 1× Fast-*Taq* buffer (Bio Basic Inc., Markham, Canada), a 200 μM concentration of deoxynucleoside triphosphates (dNTPs), 0.4 mg/mL bovine serum albumin (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania), a 0.4 μM concentration of each primer, and 2 ng/μL of extracted template DNA. A 0.5× band sharpener solution (Bio Basic Inc., Markham, Canada) was included for the *gabR* mixture only. Samples displaying positive PCR signals were then subjected to HiSST profiling, with the preparation of *gabR*, *bssA*, and *dhaM* sequencing libraries (65). PCR amplicons were sequenced with the Illumina MiSeq PE-250 platform at the Centre d'expertise et de services Génome Québec (Montréal, Canada). Raw sequencing read processing included primer sequence removal with the software Cutadapt v. 2.10 (66), followed by quality control, paired-end merging, and chimera check using the default parameters specified in the package dada2 v1.8.0 (67), which includes the packages ShortRead v1.48.0 (68) and Biostrings v2.58.0 (69). Reads containing a mismatch in the primer region and unexpected sequences regarding amplicon size were deleted. PCR conditions, primer sequences specific to HiSST loci, and processing of raw sequencing reads were described by Bourdin et al. (65). Filtered sequences displaying 100% identity were clustered into distinct amplicon sequence variants (ASV). ST assignation of chimera-free ASV was done using *gabR*, *bssA*, and *dhaM* reference databases with a 100% identity cutoff. Barcoded primers used for library preparation and the proportion of reads remaining after each step of the bioinformatics pipeline are provided in Table S3. R scripts and databases of the HiSST scheme for *S. marcescens* are available on the GitHub project at https://github.com/TBourd/R_scripts_HiSST_SM-colonizations.git.

HiSST profile analyses and whole-genome sequencing. Quality control for eHiSST was performed by removing sequence reads displaying less than 1% relative abundance in each sample. These rare genotypes were considered potential unspecific reads, especially in the case of reads that were likely unspecific due to sequencing error or background noise, according to sequence comparison in the NCBI database (70). Applying this threshold reduced the risk of misinterpretation caused by possible false positives and low-level contaminant or misassignment (71). High-quality eHiSST matrices were utilized for downstream comparison purposes. The pairwise comparison of eHiSST bacterial profiles was expressed as a pairwise Jaccard distance computed with the presence or absence score for detected or nondetected environmental short-sequence type (eSST), respectively. The UPGMA (unweighted pair group method using average linkages) dendrograms were carried out with R version 4.0.4 (72) using the packages pvclust (73), dendextend (74), tidyverse (75) and circlize (76).

Minimum spanning trees (MST) were generated for the HiSST profile of clinical isolates and eDNA from sink drains, based on the geoBURST algorithm. The data set type "Aligned Sequences (FASTA)" was employed by merging DNA sequences of the three loci (*bssA*, *gabR*, and *dhaM*) associated with each ST,

resulting in the creation of a single sequence per ST. Then, the geoBURST distance algorithm was used to compute the MST, using the PHYLOViZ software, version 2.0a (77). Sink drain samples with a missing locus (i.e., *bssA*, *gabR*, or *dhaM*) were removed to generate the data set type gathering isolates and eDNA HiSST profiles. For eDNA samples, only the dominant allele was conserved for each of the 3 specific loci, allowing us to assign a presumptive environmental sequence type (eST) for the sink drain samples. Indeed, the most straightforward link between eDNA and isolates is the case where the HiSST profile was based on the dominant alleles, as in our previous observations (65) and the sequencing results of the samples from this study. A single allele was dominant (i.e., 70 to 100% relative abundance in a sample) in over 90% of eDNA samples (Table S3). Thus, eSTs were compared to the *S. marcescens* HiSST database, in the same way as for clinical isolates. The R script used to generate the data set for the MST is available at the GitHub project mentioned above.

According to the findings of this study and that by Bourdin et al. (65), eDNA samples with identical STs (i.e., identical *gabR*, *bssA*, and *dhaM* SSTs) are very likely to have been colonized by the same strain. However, it should be noted that the accuracy of this interpretation is constrained by our current knowledge, and the possibility of the presence of distinct strains cannot be excluded. Sink drain samples whose HiSST profiles were similar to STs retrieved from clinical strains were cultured on Sm-MacConkey. The specificity of purified isolates obtained from sink drains was confirmed by PCR, and isolates were typed using the HiSST scheme, as previously described. The whole genomes of environmental isolates and clinical isolates with the same ST were sequenced to ensure relatedness between the sink drains and *Serratia*-positive patients. Moreover, clinical isolates with same ST (HiSST profile) but different pulsovar (PFGE profile; determined by the Laboratoire de Santé publique du Québec), or vice versa, were subjected to WGS by the Microbial Genome Sequencing Center (Pittsburgh, PA, USA) using the Illumina NextSeq 2000 platform. Read trimming and *de novo* genome assembly were performed following the steps described before (65). A similarity analysis at the genomic nucleotide level for assembled genomes was conducted by determining average nucleotide identity based on the MUMmer algorithm (ANIm) within Pyani (78). Only one genome was analyzed for closely related genomes with the same HiSST and PFGE profiles (e.g., for isolates BB10 to BB16 and BB18, which were identified with ST-51 and pulsovar E, only the genome of strain BB16 was included).

Ethical approvals. Ethics approval was obtained from the CHU Sainte-Justine Research Ethics Committee (form number F9H-37164; approval date, 28 November 2018) and Research Ethics Committee of Polytechnique Montreal (certificate number CER-1819-16; approval date, 23 October 2019). The confidentiality and anonymity of all participants were ensured by using a unique number for each patient and removing any identifying characteristics.

Data availability. Raw sequencing reads have been deposited in the Sequence Read Archive of the NCBI in the BioProject PRJNA910571.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.9 MB.

SUPPLEMENTAL FILE 2, XLSX file, 6.6 MB.

SUPPLEMENTAL FILE 3, XLSX file, 0.1 MB.

SUPPLEMENTAL FILE 4, DOCX file, 0.4 MB.

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